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REVIEW ARTICLE

TYROSINE KINASE, AURORA KINASE AND LEUCINE AMINOPEPTIDASE AS ATTRACTIVE DRUG TARGETS IN ANTICANCER THERAPY - CHARACTERISATION OF THEIR INHIBITORS

Joanna Ziemska[*]*, Jolanta Solecka*

National Institute of Public Health – National Institute of Hygiene, Laboratory of Biologically Active Compounds, 24 Chocimska Str., 00-791 Warsaw, Poland

ABSTRACT

Cancers are the leading cause of deaths all over the world. Available anticancer agents used in clinics exhibit low therapeutic index and usually high toxicity. Wide spreading drug resistance of cancer cells induce a demanding need to search for new drug targets. Currently, many on-going studies on novel compounds with potent anticancer activity, high selectivity as well as new modes of action are conducted. In this work, we describe in details three enzyme groups, which are at present of extensive interest to medical researchers and pharmaceutical companies. These include receptor tyrosine kinases (e.g. EGFR enzymes) and non-receptor tyrosine kinases (Src enzymes), type A, B and C Aurora kinases and aminopeptidases, especially leucine aminopeptidase. We discuss classification of these enzymes, biochemistry as well as their role in the cell cycle under normal conditions and during cancerogenesis. Further on, the work describes enzyme inhibitors that are under *in vitro*, preclinical, clinical studies as well as drugs available on the market. Both, chemical structures of discovered inhibitors and the role of chemical moieties in novel drug design are discussed. Described enzymes play essential role in cell cycle, especially in mitosis (Aurora kinases), cell differentiation, growth and apoptosis (tyrosine kinases) as well as G_1/S transition (leucine aminopeptidase). In cancer cells, they are overexpressed and only their inhibition may stop tumor progression. This review presents the clinical outcomes of selected inhibitors and argues the safety of drug usage in human volunteers. Clinical studies of EGFR and Src kinase inhibitors in different tumors clearly show the need for molecular selection of patients (to those with mutations in genes coding EGFR and Src) to achieve positive clinical response. Current data indicates the great necessity for new anticancer treatment and actions to limit off-target activity.

Key words: *tyrosine kinase, Aurora kinase, leucine aminopeptidase, enzyme inhibitors*

STRESZCZENIE

Nowotwory stanowią jedną z głównych przyczyn zgonów na świecie. Dostępne w lecznictwie substancje przeciwnowotworowe charakteryzują się niskim indeksem terapeutycznym jak i wysoką toksycznością. Rozwijająca się oporność komórek nowotworowych na dostępne w terapii leki przyczynia się do konieczności poszukiwania nowych punktów uchwytu/miejsc docelowych (z ang. targets) dla potencjalnych substancji przeciwnowotworowych. Obecnie prowadzonych jest również wiele prac nad nowymi związkami przeciwnowotworowymi o wysokim potencjale terapeutycznym, nowym mechanizmie działania i/bądź wyższym indeksie selektywności. W pracy, autorzy skupili uwagę na trzech grupach enzymów, będących obecnie w obszarze zainteresowań współczesnej medycyny. Omówione zostały kinazy tyrozynowe na przykładzie enzymów EGFR i Src, kinazy Aurora typu A, B i C, a także aminopeptydazy na przykładzie aminopeptydazy leucynowej. Scharakteryzowano klasyfikację enzymów, ich rolę w cyklu komórkowym w warunkach fizjologicznych i procesie nowotworowym. Opisano również inhibitory enzymów, substancje będące w trakcie badań *in vitro*, przedklinicznych i klinicznych jak i leki wprowadzone na rynek farmaceutyczny. Zwrócono uwagę na budowę chemiczną inhibitorów enzymów i tym samym na kierunek poszukiwań nowych leków przeciwnowotworowych. Omówione enzymy w warunkach fizjologicznych odgrywają ważną rolę w cyklu komórkowym, zwłaszcza na etapie podziału mitotycznego. Jednakże w procesie nowotworowym dochodzi do ich nadekspresji. Zjawisko to można zahamować poprzez inhibicję aktywności enzymu. Autorzy omówili wpływ inhibitorów kinaz tyrozynowych, kinaz Aurora czy aminopeptydaz leucynowych na cykl komórkowy i bezpieczeństwo stosowania tych potencjalnych leków u ludzi. Dotychczasowe badania przedkliniczne i kliniczne inhibitorów kinazy tyrozynowej typu EGFR czy Src potwierdziły konieczność selekcji pacjentów, na tych z mutacją w genie kodującym dany enzym. Badania prowadzone na wybranej grupie chorych przynosiły oczekiwany pozytywny wynik. Wiele aspektów dotyczących nowych punktów uchwytu w terapii przeciwnowotworowej wciąż wymaga dalszych prac, aczkolwiek daje również nadzieję na odkrycie skutecznych i selektywnych leków.

Słowa kluczowe: *kinaza tyrozynowa, kinaza Aurora, aminopeptydaza leucynowa, inhibitory enzymów*

[*] **Corresponding author:** Joanna Ziemska, National Institute of Public Health – National Institute of Hygiene, Laboratory of Biologically Active Compounds, 24 Chocimska Str., 00-791 Warsaw, Poland, phone: +48 22 5421423, e-mail: jziemska@pzh.gov.pl

INTRODUCTION

Cancer is a leading worldwide cause of deaths in humans. According to the data of the World Health Organization, 8.2 mln people die from cancer each year, which corresponds to 13% of the total number of deaths noted worldwide [3, 6]. Over the next two decades, a 70% increase of new cancer cases is expected. More than 100 cancer types have been determined to exist, each requiring unique diagnosis and treatment [3, 6]. There is a demanding need for new drugs with novel modes of action and fewer side-effects. Drugs currently used in cancer chemotherapy usually show high toxicity toward normal cells. Another undesired consequence is drug resistance which persists during cancer chemotherapy. Alternative therapeutic options are also still limited. Therefore, the growing trend toward targeted cancer therapy [22, 45], which is based on delivering drugs to particular enzymes, genes, proteins or peptides essential in carcinogenesis or which promote cancer growth. The effectiveness of such therapy lies in minimizing side effects in normal tissues [45].

There is great effort to identify crucial molecular targets of anticancer drug discovery. In general, the approach is based on sequencing various cancer genomes, which is often a complex and heterogeneous activity. Among known key enzyme targets for anticancer drugs are tyrosine kinases, Aurora kinases and leucine aminopeptidases. There are on-going intensive studies to discover the most potent and selective inhibitors against these enzymes. In this review, we present enzymes classification, overall structure, mode of action as well as chemical structures of inhibitors that are currently studied or have just been discovereware involved in the cell cycle, particularly in mitosis. They are involved in checkpoints regulation and their abnormal expression may disturb checkpoints functions [35]. Also, leucine aminopeptidase (LAP) plays a significant role in the cell cycle by promoting G_1 checkpoint progression. Aberrant LAP expression causes proliferation of cancer cells and metastasis [65]. Based on the data above, the current findings demonstrate that these enzymes represent potential "druggable targets" in cancer, controlling key oncogenic pathways.

TYROSINE KINASES

Tyrosine kinases (TKs) are a family of enzymes that transfer the phosphate group from adenosine triphosphate (ATP) to a tyrosine amino acid residue in a protein, subsequently triggering downstream molecular signaling [22, 47]. They are important mediators in signal transduction, leading to cell proliferation, differentiation, migration, metabolism and programmed cell death [47, 49]. TKs can be classified as receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (NRTKs). The function of both is based on regulating other enzymes by phosphorylation. However, while RTKs (e.g. EGFR, PDGFR, FGFR) exist on the surface of the cell as part of cell membrane, NRTKs (e.g. SRC, ABL, FAK or Janus kinase) are located in the cytoplasm [47, 49].

RECEPTOR TYROSINE KINASES (RTKs)

RTKs constitute a protein superfamily that plays an important role in the control of cellular processes, including cell cycle, cell migration, survival, cell proliferation and differentiation [57]. The domain structure of RTKs consists of an extracellular hydrophylic ligand binding domain, which recognizes the ligand, a hydrophobic transmembrane domain and an intracellular domain essential in signal transduction processes [58]. The intracellular domain contains a conserved protein tyrosine kinase core and other regulatory sequences that are subjected to autophosphorylation or phosphorylation by heterologous protein kinases [57]. RTKs are activated by the ligand which binds to their extracellular domain. Ligands are extracellular signal molecules that induce receptor dimerization [39, 47]. There are several strategies in which ligands can achieve stable dimeric conformation – one ligand may bind to two receptors and form a dimer complex or two ligands can bind to two receptors. Then, when the dimer is phosphorylated, it is fully active and various proteins can attach to phosphorylated RTKs. This causes a series of signal transduction event. There are nearly 60 RTKs, divided into 20 subfamilies. Among them, EGFR/ErbB (class I), the receptor for insulin (class II), for PDGF (class III), for FGF (class IV), for VEGF (class V) and HGF (MET, class VI) are strongly associated with carcinogenesis [49, 58]. Many cancers are caused by mutated RTKs which are active without a signal molecule (ligand). In malignant cells, there is excessive production of tyrosine kinase receptors. High levels of receptor expression lead to formation of increased number of binding sites that are available to the ligand. This in effect causes a cascade of excessive signals.

The epidermal growth factor receptor (EGFR) family, called "prototypical" RTKs [40], is regulated in humans by at least seven different activating ligands, including the epidermal growth factor (EGF), transforming growth factor-α (TGF-α), betacellulin (BTC) and others. The EGFR family is comprised of four structurally related receptors, EGFR (ErbB1/HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) [40, 72]. EGFR (ErbB1/HER1) is a multiple domain glycoprotein (170 kDa) that consists of a typical for RTKs extracellular ligand-binding

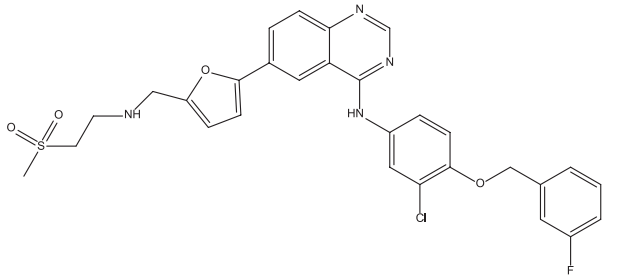
domain and intracellular tyrosine kinase domain separated by a transmembrane region [28, 51]. Endogenous ligands, such as EGF, can bind to EGFR and cause receptor homo- or heterodimerization. The receptor dimerization leads to autophosphorylation of the intracytoplasmic EGFR tyrosine kinase domain. Then, phosphorylated tyrosine kinase stimulates an intracellular signal transduction cascade [51].

Tyrosine kinase inhibitors (TKIs) that target the intracellular tyrosine kinase region include those, which mode of action is to interfere with ATP binding to the receptor, and other compounds which act at the substrate binding region [50].

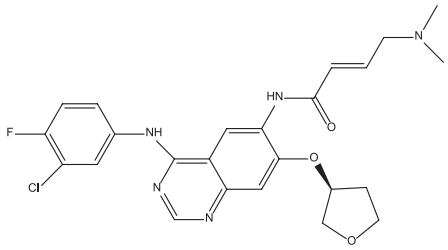
Based on the chemical structure, RTKIs can be divided as those possessing the quinazoline core (e.g. lapatinib, afatinib, gefitinib, erlotinib, etc.) and their derivatives (such as inhibitors with oxazolo [4,5-*g*] quinazolin-2(1*H*)-one moiety).

EGFR and HER2 are frequently overexpressed in breast, ovarian, prostate and colon cancers. It was also observed that dual EGFR/HER2 inhibitors can cause more potent inhibition than solely EGFR and HER2 inhibitors. There are some dual EGFR/HER2 inhibitors available on the market, such as lapatinib (*N*-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(5-(((2-(methylsulfonyl)ethyl)amino)methyl)furan-2-yl) quinazolin-4-amine, Figure 1) and afatinib (*S*,*E*)-*N*-(4-((3-chloro-4-fluorophenyl)amino)-7-(tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-4-(dimethylamino)but-2-enamide) (Figure 1) [72]. The quinoline moiety plays a significant role in EGFR/HER2 inhibitory activity. However, due to the toxicity of marketed drugs, further studies are carried out to discover new, more optimal drugs. In the work of *Yin* et al., authors found a series of novel dual EGFR/HER2 inhibitors with oxazolo[4,5-*g*] quinazolin-2(1*H*)-one moiety (Figure 1). Several newly discovered molecules showed more potent activity against the mentioned enzymes than the reference drug, lapatinib, and exhibited impressive results on cancer cell lines (human lung adenocarcinoma A549 and breast cancer SK-Br3) as well as lower toxicity [72].

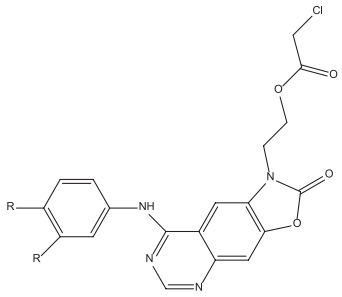
The introduction of EGFR inhibitors, such as gefitinib (*N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholinopropoxy)quinazolin-4-amine, Figure 1), erlotinib (*N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine, Figure 1) and afatinib, to the market has changed the treatment of patients with advanced non-small-cell lung cancer (NSCLC) with mutations in the EGFR gene [53]. These drugs inhibit phosphorylation and tyrosine kinase activity of the intracellular ATP-binding domain of EGFR through competitive binding to this site. The



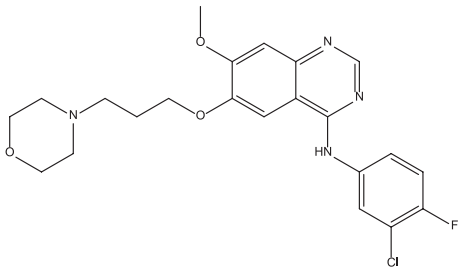
lapatinib



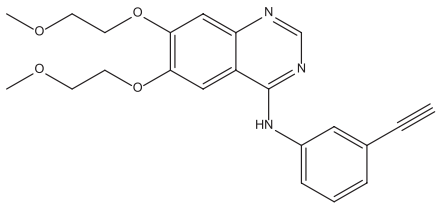
afatinib



oxazolo[4,5-*g*]quinazolin-2(1*H*)-one scaffold



gefitinib



erlotinib

Figure 1. Chemical structures of RTKIs

response to EGFR-TKIs is associated with the presence of activating EGFR mutations in NSCLC. Different studies showed that EGFR-TKIs have high anti-tumor activity in EGFR mutation-positive patients [53].

In the study of *Rossi* et al., efficacy and safety of gefitinib and afatinib were compared in patients of different age. The trial was conducted on a group of NSCLC patients with mutation in exon 19 or 21. This group was divided into patients aged <70 and ≥70 years [54]. The overall results showed that elderly patients gave better response in comparison to the group aged <70 years. However, majority of patients experienced disease progression after first-line TKI treatment and none of them achieved a complete response [54].

At present, there are plenty of TKIs on the market that are widely used in clinical treatment of solid tumors and other types of cancers. In colon cancer, regorafenib is one of the current therapeutic solutions. Imatinib, sunitinib and regorafenib are used in therapy of gastrointestinal stromal tumors and chronic myeloid leukemia (imatinib). Sorafenib shows a positive response in treatment of hepatocellular carcinoma. Melanoma treatment is based on vemurafenib and dabrafenib [66]. As mentioned previously, in non-small cell lung cancer therapy, gefitinib, erlotinib, afatinib, ceritinib and crizotinib are among the therapeutic TKI representatives [51]. The therapeutic potential of pazotinib is described in ovarian cancer.

TKIs, as other medicines, induce side effects, especially gastrointestinal disorders, such as diarrhea, nausea and emesis. Moreover, these drugs tend to develop hypertension and renal disorders. They are metabolized in the liver and usually give interactions through CYP3A4 induction [66].

NON-RECEPTOR TYROSINE KINASES (NRTKs)

The family of NRTKs consists of nine main families, differing in the domain structure. The activation process of NRTKs is more complex in comparison to RTKs, requiring heterologous protein-protein interactions to enable transphosphorylation. The largest group of NRTKs is the Src family. It is divided into three main subfamilies: Lyn-related, Src-related and PTK6/Brk-related [24]. Members of the Src family display a conserved domain organization: a myristoylated N-terminal segment (S4 domain), SH3, SH2, linker, tyrosine kinase domains (SH1 domain containing Tyr416), a short C-terminal regulatory segment containing Tyr527 [20, 46, 52]. The C-terminal region bears an auto-inhibitory phosphorylation site [20]. The activity of Src enzymes is regulated by phosphorylation and intramolecular protein-protein interactions (in SH2 and SH3 regions) [20]. Both sites, Tyr416 and Tyr527, play major role in protein phosphorylation.

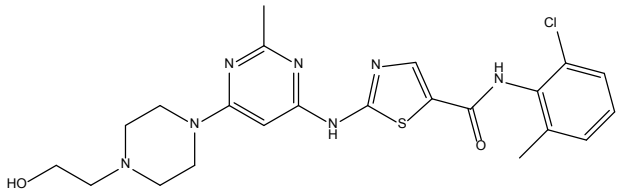
There are several ways in which Src kinases can be activated and many ways in which they can activate the process of carcinogenesis. Src kinases participate in a variety of signaling processes, including cell proliferation, T- and B-cells activation, cytoskeleton restructuring, cell movement and endocytosis [24, 52]. They are activated during the G_2/M cell cycle. Brain, osteoclasts and platelets express higher Src levels than other human tissues [52]. Elevated expression and activity of Src promote tumor growth and stimulate its migratory and invasive potential [20]. Src deregulation and overexpression has been linked to several human cancers, such as melanoma, breast, lung and colon cancer [20, 24]. There is clear evidence that Src elevation has an impact on progression of colon cancer; however, further studies should be conducted to determine this direct association. Similar data suggest elevated expression of Src in ovarian, esophageal, lung, head and neck as well as gastric cancers [20]. Imbalance between phosphorylation and dephosphorylation of the Src protein leads to drastic changes. Among processes that can contribute to carcinogenesis are: dephosphorylation of Tyr527, deletion or mutation of Tyr527, displacement of the SH3- and SH2-mediated intramolecular interactions or phosphorylation of Tyr416 [20].

Oncogenes, in other words, genes involved in biochemical points (called checkpoints) that control transitions in the cell cycle, can mutate or be overexpressed [35]. The v-Src oncogene was first isolated from the transforming virus, Rous Sarcoma Virus. It lacks the cellular protein region (c-Src) that contains Tyr527, what makes it continually active [4]. The v-Src has a growth-promoting effect in fibroblasts. In comparison to normal cells, v-Src cells suppress expression of the cyclin-dependent kinase (CDK) inhibitor p27, leading to more rapid transit of the G_1 phase of the cell cycle and a failure to enter the quiescent state when deprived of serum mitogenes [20].

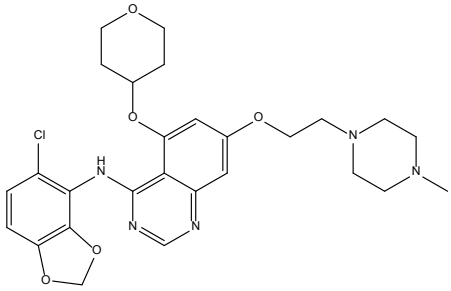
Until now, several Src inhibitors were tested in clinical trials, e.g. dasatinib, saracatinib and bosutinib [74]. Their chemical structures are more diverse than RTKIs and are based on 4-(piperazin-1-yl)pyrimidine (dasatinib), 7-(2-(piperazin-1-yl)ethoxy)quinazoline (saracatinib) and 7-(3-(piperazin-1-yl)propoxy)quinoline (bosutinib). Bosutinib, 4-((2,4-dichloro-5-methoxyphenyl)amino)-6-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy) quinolone -3-carbonitrile (Figure 2) was tested on breast cancer cells. Study of *Vultur* et al. showed that this compound causes decreased cell motility and invasion as well as increased cell-cell adhesion [67]. Phase I clinical trial of bosutinib on patients with breast cancer was completed in 2011. At present, there is an ongoing phase I trial for bosutinib in combination with another anticancer agent (inotuzumab ozogamicin) in the Philadelphia-chromosome (PC) positive acute

lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) [2]. Besides evident activity in pre-clinical studies, candidates for Src inhibitors did not show potent efficacy when tested in monotherapy of various types of solid tumors in clinical trials [74]. The Src inhibitor, saracatinib (AZD-0530, (*N*-(5-chlorobenzo[*d*][1,3]dioxol-4-yl)-7-(2-(4-methylpiperazin-1-yl)ethoxy)-5-(tetrahydro-2*H*-pyran-4-yl)oxy)quinazolin-4-amine (Figure 2), showed promising activities when tested in pre-clinical models of biliary tract carcinomas (BTC) [11]. In the study of *Cavalloni* et al., this compound exhibited reduced cell migration and proliferation *in vitro* and delayed tumor growth in human BTC xenografts *in vivo* [11]. Saracatinib was also tested on patients with colorectal cancer. However, in this trial patients were not selected as those with tumors with activated Src signaling and those without activated Src. This accounts for the unsatisfactory results of the study. Also, in trials with pancreatic cancer patients, Src inhibitors did not show high activity. However, it has been hypothesized that activated Src signaling maybe a biomarker for successful targeting of Src and positive clinical outcomes [74]. The recent study of *Heusschen* et al. showed that Src inhibition by saracatinib limits the development of osteolytic bone disease in multiple myeloma [29]. The *in vitro* data revealed an inhibitory effect of the tested drug on osteoclast differentiation, polarization and resorptive function [29]. There is also an ongoing phase II clinical trial on saracatinib versus placebo in cancer-induced pain SarCaBon [2].

Another chemical moiety, 1*H*-pyrazolo[3,4-*d*]pyrimidine, is a core structure in Src inhibitors, such as PP2. PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine, Figure 2) was also investigated in *in vitro* and *in vivo* research regarding malignant glioma cells U251 [17]. The effect of chemoradiotherapy with PP2 and temolozomide on cancer cells was tested using clonogenic assays and *in vivo* brain tumor model. PP2 enhanced radioselectivity of malignant glioma cells and suppressed invasion and migration of U251 cells. However, the authors did not



dasatinib



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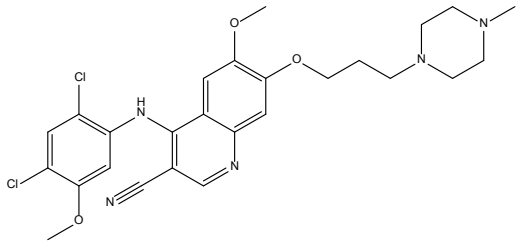
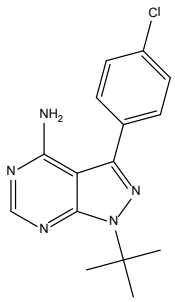


bosutinib



PP2

Figure 2. Chemical structures of NRTKIs

observe a significant decrease in tumor volume [17].

In another study performed by *Formisano* et al., Src inhibitors (saracatinib, dasatinib and bosutinib) were tested on NSCLC models [19]. Obtained results showed that all tested compounds directly inhibited EGFR, which is a well-characterized mutated oncogene in NSCLC. Among the three inhibitors tested in an *in vitro* kinase assay, saracatinib presented the most potent activity towards EGFR, whereas dasatinib (*N*-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-yl)amino)thiazole-5-carboxamide, Figure 2) was least effective. Saracatinib also showed impressive results on erlotinib-sensitive cells containing EGFR-activating mutants and *in vivo* HCC827 tumor xenografts (where it was more active than dasatinib). Saracatinib mode of action was based on Src inhibition and by EGFR activation reduction. Conducted study demonstrated that Src inhibitors may act through various mechanisms in NSCLC [19]. Taking together all of the above, it is clear that Src inhibitors present antiproliferative

activity in various cancers. Disappointing results from a series of clinical trials (resulting mainly from the lack of proper molecular patient selection) delayed the development of Src inhibitors. However, studies on patients with Src-activated signaling should be continued due to the great beneficial effect of these inhibitors.

AURORA KINASES

Aurora enzymes are type serine/threonine (Ser/Thr) kinases, which serve as important mitotic regulators with essential role in the regulation of cell division, from mitotic entry to cytokinesis [21, 33, 35]. In mammals, three enzyme types (A, B and C) are distinguished. They contain two domains: catalytic, at the COOH-terminus, and regulatory, at the NH_2-terminus. Kinases A, B and C have identical catalytic domains, but various regulatory domains. Moreover, each Aurora kinase differs in its subcellular location.

AURORA A KINASE

Aurora A is a 403-amino acid enzyme with a molecular weight of 46 kDa [5, 14], which demands phosphorylation for its activation [7]. The presence of phosphoric groups at residues Ser51, Thr288 and Ser342 of the activation motif is essential for the proper function of this enzyme [7]. Aurora A kinase is found mainly on centrosomes, spindle poles and transiently along the spindle microtubules as cells progress mitosis [14, 33]. The human Aurora A kinase maps to chromosome 20q13.2 [7]. The level of the enzyme is regulated by different factors, such as the anaphase-promoting complex (APC) [10] and phosphorylation [7]. Its production is raised at the G_2/M transition and decreases at M/G_1 transition of cell cycle [10]. The enzyme controls correct development of various phases of mitosis (centrosome maturation and separation, mitotic entry, bipolar spindle assembly, chromosome alignment on the metaphase plate and cytokinesis) [7, 14, 34]. Cell growth in normal conditions depends on the balance between Aurora A and other factors, such as Chfr (a mitotic checkpoint protein), BRCA1 and p53 (tumor suppressor) [21]. Several studies indicated that this enzyme suppresses BRCA1 and BRCA2 [34, 70], which are human genes encoding tumor suppressor proteins engaged in repair of damaged DNA [1]. In certain cancer types, such as ovarian, pancreatic and breast, there is a negative correlation between Aurora A kinase expression and BRCA2 [70]. *Yang* et al. suggested that Aurora A kinase and BRCA2 expression ratio can be a valuable tool in predicting ovarian cancer outcomes [70]. The enzyme is overexpressed in some types of cancer (solid tumors, such as breast, bladder and ovarian cancers as well as gastrointestinal cancers [34]) and is considered to play a crucial role in the inactivation of apoptosis of cancer cells, oncogenic transformation through development of centrosome amplification and chromosomal instability (CIN) [14, 61]. As mentioned, Aurora A kinase plays important role in gastrointestinal cancers. The enzyme was previously described to promote activation of the AKT pro-survival signaling pathway (signal transduction pathway that promotes survival and growth in response to extracellular signals) as well as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells; complex that controls DNA transcription) and STAT3 (signal transducer and activator of transcription) pathways [34, 71]. Recent research proved that dual Aurora A and JAK2 kinase (Janus kinase important in tumor survival) blockade effectively suppresses malignant transformation. Depletion of both of these enzymes is effective at inhibiting anchorage-dependent and –independent growth and invasion and at inducing apoptosis [71].

It is assumed that Aurora A kinase is a 'druggable' target in cancer as it controls essential oncogenic pathways. Its inhibitors can be divided into various groups based on their chemical core, such as *N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl) pyrimidin-4-amines (e.g. tozasertib, ENMD-2076) and 9-chloro-5*H*-benzo[*c*]pyrimido[4,5-*e*]azepines (e.g. alisertib, MLN8054) or *N*-(pyridin-2-yl)thiazol-2-amine (MK-5108). The first registered Aurora A kinase inhibitor was tozasertib (MK-0457, UX-680, *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl) cyclopropane carbo xamide, Figure 3), which showed promising activities towards Aurora kinases: K_i of 0.6 nM against Aurora A, 18nM against Aurora B and 5nM against Aurora C. It was withdrawn from the market due to cardiovascular side effects, such as QTc prolongation [9]. However, several new Aurora A kinase inhibitors were investigated and tested in clinical trials. Among them, MLN 8054 [41] (4-((9-chloro-7-(2,6-difluorophenyl)-5*H*-benzo[*c*]pyrimido[4,5-*e*]azepin-2-yl)amino)benzoic acid, Figure 3), which was shown to inhibit proliferation of various cultured tumor cell lines and demonstrated potent oral antitumor activity in mice bearing human tumor xenografts. It delayed G_2/M progression in cultured human tumor cells [41]. When tested in phase I clinical trial in patients with advanced solid tumors, the drug candidate caused reversible somnolence, which prevented adequate dose establishment [16]. MLN 8054 was further chemically modified (to limit its toxicity) to alisertib (MLN 8237, 4-((9-chloro-7-(2-fluoro-6-methoxyphenyl)-5*H*-benzo[*c*]pyrimido[4,5-*e*]azepin-2-yl)amino)-2-methoxybenzoic acid, Figure 3). At present, the latter compound undergoes several clinical trials [44]. Up to now, therapies with alisertib alone or in combination with known chemotherapeutic agents

have been conducted on patients with hematological and solid tumor malignancies. Also, the efficacy of alisertib is clinically tested on patients with non-small cell lung cancer, advanced solid tumors, lymphoma, ovarian carcinoma, fallopian tube cancer, peritoneal cancer, breast carcinoma, small cell prostate cancer and others [2]. Its activity was also evaluated in soft tissue sarcomas [44]. *Nair* et al. demonstrated that alisertib at nanomolar concentrations is a potent inhibitor of Aurora A and induces apoptosis. At micromolar concentration, it also inhibits Aurora B-induced polyploidy [44]. A novel Aurora A kinase inhibitor, TAS-119, entered clinical trials: one as a monotherapy, second in combination with taxane-based chemotherapy (phase I clinical trials) [2, 14]. Moreover, two other Aurora A kinase inhibitors are in clinical development: ENMD-2076 ((*E*)-*N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-styrylpyrimidin-4-amine) and MK-5108 ((1*R*,4*R*)-4-(3-chloro-2-fluorophenoxy)-1-((6-(thiazol-2-ylamino) pyridin-2-yl)methyl)cyclohexanecarboxylic acid) [2, 34]. Both chemical structures are presented on Figure 3.

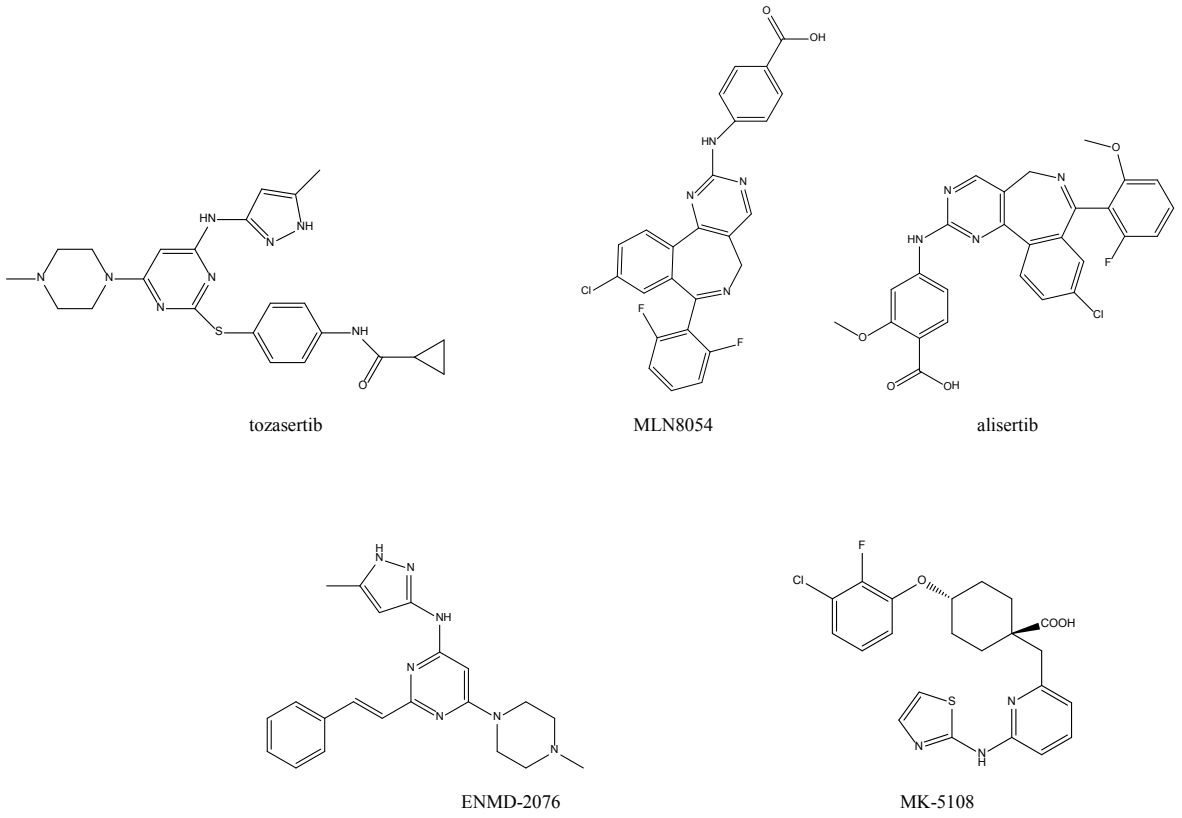


Figure 3. Chemical structures of Aurora A kinase inhibitors

AURORA B KINASE

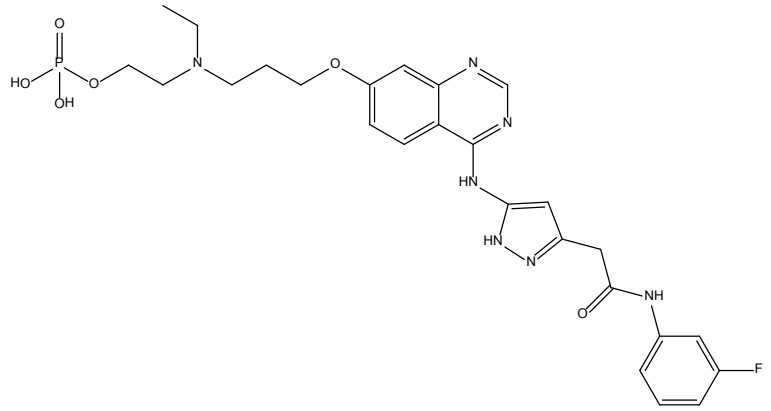
Aurora B, known as a chromosome passenger protein, is 344-amino acid enzyme with a molecular mass of 39 kDa [5]. It is localized in centromeres in early mitosis and then in the spindle mid-zone in anaphase [23, 36]. It is associated with the chromosomal passenger complex (CPC)[33]. The enzyme is activated by auto-phosphorylation of Thr232 in the T-loop and requires interaction with CPC proteins [33]. Aurora B kinase is required for histone H3 phosphorylation, which then helps in chromatin condensation and separation. The enzyme also takes part in chromosome bi-orientation, the spindle assembly checkpoint (SAC) and cytokinesis [15, 23, 36]. It maps to chromosome 17q13 [21]. Overexpression of Aurora B kinase leads to multi-nucleation and polyploidy as well as defects in chromosome segregation and in cytokinesis [21].

Among Aurora B inhibitors, no distinguishable chemical core that is evidently crucial for inhibitory activity has been identified. Inhibitors barasertib (AZD1152, Figure 4) and BI 811283 have been examined in clinical trials. Barasertib (2-(ethyl(3-((4-((3-(2-((3-fluorophenyl)amino)-2-oxoethyl)-1*H*-pyrazol-5-yl)amino)quinazolin-7-yl)oxy)propyl) amino)ethyl dihydrogen phosphate) was shown to be a highly specific inhibitor of Aurora B (0.37 nM). It was tested on patients with large B-cell lymphoma (phase II trial, completed in 2013), patients with AML (phase I trial, completed in 2009) and patients with advanced solid malignancies (phase I trial, completed in 2008) [2]. In phase II trial, the activity of barasertib was compared with low dose cytarabine in patients with AML; however the survival benefit was not achieved [59]. In prior studies, it was shown that barasertib is rapidly converted into AZD1152-HQPA, an active compound with advanced pharmacokinetic properties

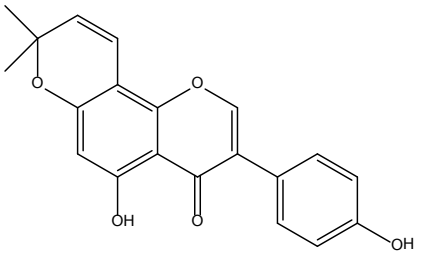
in human plasma [33].

Another Aurora B inhibitor, BI 811283, was tested in phase II in combination with cytarabine in previously untreated AML patients unqualified for intensive treatment (trial completed in 2015 [2]). In contrast, phase I was performed in patients with various solid tumors (completed in 2014) [2].

There is an ongoing search for new inhibitors of Aurora kinases. Recently, a new inhibitor, Derrone, was described by *Hoang* et al. [31]. The chemical structure of this compound, 5-hydroxy-3-(4-hydroxyphenyl)-8,8-dimethylpyrano[2,3-*f*]chromen-4(8*H*)-one, is presented on Figure 4. Derrone was isolated from a natural plant source, *Erythrina orientalis* L. Murr. It showed inhibitory activity towards Aurora kinases A (IC_{50}=22.3 μM) and B (IC_{50}=6μM), being more selective towards the latter. Derrone inhibited phosphorylation of histone H3 on Ser10 (a natural



barasertib



Derrone

Figure 4. Chemical structures of Aurora B kinase inhibitors

substrate of Aurora B, used as an indicator of Aurora B inhibition) both in the kinase assay and at the cellular level [31].

AURORA C KINASE

Aurora C kinase is less known in comparison to the two previously mentioned Aurora kinases, A and B. The human enzyme is a protein comprising 309 amino acids and a molecular mass of 35kDa [5]. It maps to chromosome 19q13 [21]. The enzyme shows 83% similarity to Aurora kinase B and 71% - to enzyme A. However, Aurora C kinase lacks the N-terminal domain, found in both enzymes [48]. The enzyme is essential in mitosis and centrosome function. In comparison to the two other mentioned kinases, which have various localizations in the body, Aurora C kinase is expressed mainly by cells undergoing meiosis (sperm and oocyte) [18, 48]. Normal somatic cells usually show low expression of this enzyme. The localization of Aurora C kinase in spermatophytes and oocytes is dynamic [48]. *Fellmeth* et al. found that human oocytes express three splice variants of the enzyme with different functions, however, all additive in meiosis [18].

There are several studies concerning enzyme overexpression in certain types of cancers cells, such as HeLa, HepG2, MDA-MD-453 and Hu H7. Mutations in Aurora C kinase were described to be linked with male infertility and cancer [18]. However, it has not been proven that Aurora C kinase plays role in carcinogenesis, thus, further investigation is needed [21, 35]. Probably, enzyme overexpression in mitotic cells leads to centrosome amplification and multi-nucleation [48].

LEUCINE AMINOPEPTIDASE (LAP)

Aminopeptidases are a class of zinc metalloenzymes that catalyze the cleavage of amino acids nearby the N-terminus of polypeptides. They are widely distributed in the natural environment, in plants, animals, fungi and bacteria [30, 62] and seem to be important for both, prokaryotic and eukaryotic cells. Aminopeptidases are involved in a variety of biological processes as protein maturation, angiogenesis [56], antigen presentation, neuropeptide and hormone processing, pregnancy and reproduction, protein turnover, memory, inflammation, tumor growth, cancer and metastasis, blood pressure and hypertension [43, 62].

Among medically important aminopeptidases, there are (i) leucine aminopeptidase (α-aminoacyl-peptide hydrolase, LAP, EC 3.4.11.1), which belongs to the M17 family, (ii) alanyl aminopeptidase (also known as aminopeptidase N, APN or CD13; E.C.3.4.11.2), (iii) leucine aminopeptidase 3 (LAP3) and (iv) cystyl aminopeptidase (α-aminoacyl-peptide hydrolase, oxytocinase, EC 3.4.11.3) from the M1 family [43, 55].

Leucine aminopeptidase removes most effectively the leucine amino acid (Leu) and other hydrophobic residues from peptide substrate analogs. In its substrate spectrum, there are also other amino acids, such as phenylalanine (Phe). This cytosolic enzyme is of utmost importance in various biological processes. Its altered activity is observed in pathological disorders, such as cancers, cataracts, cystic fibrosis as well as in ageing [25, 60, 64]. Among LAP enzymes, bovine lens leucine aminopeptidase (blLAP) is one of the best described [60], although leucine aminopeptidase from porcine kidney is the only one commercially available. blLAP is a hexamer, consisting of six identical protomers, 54kDa each (487 amino acids). There are two zinc ions (Zn488 and Zn489) in the blLAP active site and interactions with these ions are substantial for the enzyme catalytic activity, substrate binding and activation as well as inhibitory activity of compounds [25, 60].

Alanyl aminopeptidase (APN) is located in cellular membrane and most favourably cleaves peptide bonds with alanine (Ala) at N-terminus of polypeptide. However, peptides with Leu, Phe, tyrosine (Tyr), arginine (Arg), methionine (Met), lysine (Lys), tryptophan (Trp), glycine (Gly), glutamine (Gln), Ser and histidine (His) are also substrates for APN [8].

Numerous studies have been conducted on the role of leucine aminopeptidases in the cell cycle. They are mostly devoted to cell surface leucine aminopeptidase 3 (LAP3) [26, 65, 68, 73]. LAP3 is a neutral protease which belongs to M1 family. The work of *He* et al. showed that LAP3 could promote proliferation of glioma cancer cells U87 and U251 and inhibition of this enzyme might be involved in cell cycle arrest at G_0/G_1 phase [26]. In the study of *Zhang* et al., authors reported that LAP3 was able to promote G_1/S transition [73]. Overexpression of LAP3 promoted esophageal squamous cell proliferation and migration abilities *in vitro*. It was also observed that LAP controlled cell cycle progression through the activation of cyclin-dependent kinases CDK2, CDK4, CDK6 and cyclin A [73]. *Tian* et al. examined the biological function of LAP and its influence on hepatocellular carcinoma (HCC) [65]. LAP3 promoted HCC cells proliferation and its overexpression accelerated G_1/S phase transition. In another work, LAP3 was suggested to be involved in regulation of tumor invasion [68]. The study of *Wang* et al. showed that inhibition of the enzyme caused up-regulation of the p38 MAPK/Hsp27 pathway and down-regulation of fascin protein expression which resulted in suppression of ovarian cancer cell invasion [68]. The function of the mammalian p38 mitogen-activated protein kinases MAPK is to regulate the cell cycle checkpoints at G_0, G_1/S and G_2/M transitions [13, 27]. The small heat shock protein - HSP27, is believed to be a physiological substrate for these kinases. Phosphorylation of serine residues on HSP27 play a role in actin cytoskeleton remodeling during cellular stress and growth [27]. Fascin, an actin-bundling protein, organizes actin filaments into tightly packed bundles. Its high level is observed in various transformed cells [69]. On the other hand, down-regulation of fascin results in a decrease of cell proliferation.

It is presumed that aminopeptidase inhibition influences mammalian target of rapamycin (mTOR). mTOR is a protein kinase known as a master regulator of protein synthesis, cell growth and proliferation through the control of translation, transcription, mRNA turnover, protein stability, actin cytoskeletal organization and autophagy [32]. It consists of two complexes: mTORC1 and mTORC2. Data suggests that leucine is essential for mTORC1 activation and its deprivation due to LAP inhibition makes mTORC1 inactive [30].

There are two types of LAP inhibitors: amino acid or peptide analogues. Among the first group are: aminoaldehydes, α-aminoboronic acid analogues, phosphonic acid analogues of amino acids, hydroxamate amino acid analogues, chloromethyl ketone amino acid analogues as well as thiol analogues of amino acids. L-leucinal is one of the described aminoaldehyde LAP inhibitors. The crystal structure of the enzyme-inhibitor complex was determined by *Sträter* et al. [60]. Aminoaldehydes are strong LAP inhibitors. L-leucinal was shown to inhibit LAP activity with an inhibition constant of K_i = 60 nM [60].

The second group of LAP inhibitors comprises: bestatin and amastatin analogues, sulfur-containing analogues of bestatin, phosphonic analogues of peptides, ketomethylene peptide analogues as well as other peptide LAP inhibitors. Bestatin, a dipeptide analogue of PheLeu ([(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-(*S*)-leucine, Figure 5) is the first clinically approved aminopeptidase inhibitor (trade name Ubenimex). This drug was originally discovered as an immune-modulating agent [30]. However, further research showed that it strongly inhibits LAP and APN. Bestatin was shown to be a stronger inhibitor towards aminopeptidases with two metal ions in the active center (LAP, K_i= 9 nM) than those with a single metal ion (APN, K_i=3.03 μM) [43]. It binds to each protomer of blLAP [63]. Thus, six bestatin equivalents are bound per LAP hexamer [64]. Amastatin is a tripeptide analogue ([(2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoyl-*S*-valyl-*S*-valyl-*S*-aspartic acid], Figure 5) and it is a strong inhibitor of LAP as well as APN [25].

Among aminopeptidases inhibitors, both amino acid and peptide analogues, are those containing phosphorus (Figure 5). Although the phosphorus/phosphinate group is a rather weak zinc-complexing

moiety, their tetrahedral shape mimics the high energy transition state of the peptide bond hydrolysis [43]. For instance, isosters of hydrophobic aliphatic amino acids such as valine (Figure 5) and leucine are potent LAP inhibitors, K_i of 0.15 and 0.23 μM, respectively [38, 43]. Other, aminopeptidase inhibitors worth mentioning are amino acid-derived hydroxamates.

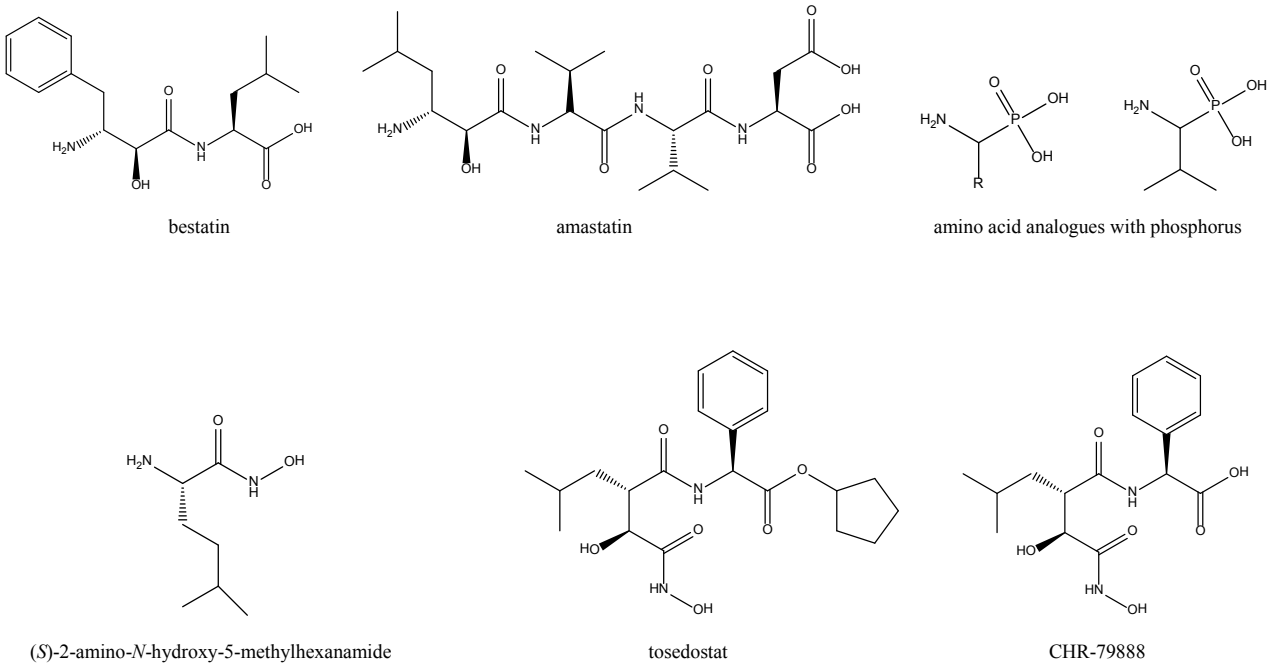


Figure 5. Chemical structures of LAP inhibitors

Hydroxamic acids are analogues of carboxylic acids and amides. They exhibit a close structural similarity to the substrates/products of the peptide bond hydrolysis. They also have metal-complexing properties. One of α-aminohydroxamates, (*S*)-2-amino-*N*-hydroxy-5-methylhexanamide (Figure 5), showed LAP inhibitory activity of K_i= 14 μM [12, 43].

Among the recent developments on aminopeptidase inhibitors is the study on tosedostat (CHR-2797; (*S*)-cyclopentyl 2-((*S*)-2-((*S*)-1-hydroxy-2-(hydroxyamino)-2-oxoethyl)-4-methylpentanamido)-2-phenylacetate, which is currently in phase II clinical trials on acute myeloid leukemia (AML) [42]. Tosedostat is a prodrug converted into a pharmacologically active acid product, CHR-79888 ((*S*)-2-((*S*)-2-((*S*)-1-hydroxy-2-(hydroxyamino)-2-oxoethyl)-4-methylpentanamido)-2-phenylacetic acid) [37]. Chemical structures of tosedostat and CHR-79888 are presented on Figure 5. Tosedostat is a potent inhibitor of various aminopeptidases, including leucine aminopeptidase (IC_{50}=100 nM) (active compound CHR-79888 IC_{50}=30 nM). It exerts antiproliferative activity against histiocytic lymphoma U-937 (IC_{50}=10 nM), acute myelogenous leukemia KG-1 (IC_{50}=15 nM) and HNT-34 (IC_{50}=35 nM), promyelocytic leukemia HL-60 (IC_{50}=30 nM) and myelomonoblastic leukemia GDM-1 (IC_{50}=15 nM) [37]. Currently, there is an ongoing phase II clinical trial of tosedostat with cytarabine or decitabine in newly diagnosed older patients with AML or high-risk myelodysplastic syndrome [42]. Another study recruits patients with metastatic pancreatic adenocarcinoma to be treated with tosedostat with capecytabine [2].

CONCLUSIONS

There is a demanding need for the new molecular drug targets. In the present study, the authors review enzymes, such as RTKs, NRTKs, Aurora kinases and LAP, that are currently actively explored as novel targets, and characterize their potential inhibitors. TK and Aurora kinase enzyme inhibitors present chemical scaffolds mainly comprised of piperazine, pyrimidine, quinoline or quinazoline, whereas LAP inhibitors are mainly amino acid or peptide analogues. First clinical trials on TK or Aurora kinase inhibitors were rather disappointing, indicating that much should be done to explore the role of the enzymes in cancer pathophysiology. A proper clinical study design based on selection of patients with mutations in genes coding for the mentioned enzymes caused positive outcomes. Even when clinical responses to the new drug candidates were rather poor, the combination of such compounds as RTKI, NRTKI, Aurora kinase or LAP inhibitors with other anticancer agents is considered a promising strategy of increasing their antitumor activity. The discovered enzyme inhibitors may serve as model compounds in searching of new "hits" with

optimal activity and physicochemical properties as well as low toxicity.

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Conflict of interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

ANTIMICROBIAL RESISTANCE OF *SALMONELLA* SPP. ISOLATED FROM FOOD

Łukasz Mąka[1], Magdalena Popowska[2]*

[1]National Institute of Public Health - National Institute of Hygiene,
Department of Food Safety, Laboratory of Food Microbiology, Warsaw, Poland
[2]University of Warsaw, Faculty of Biology, Institute of Microbiology,
Department of Applied Microbiology, Warsaw, Poland

ABSTRACT

This review summarizes current data on resistance among *Salmonella* spp. isolates of food origin from countries in different regions of the world. The mechanisms of resistance to different groups of antimicrobial compounds are also considered. Among strains resistant to quinolones and/or fluoroquinolones the most prevalent mechanism is amino acid substitutions in quinolone resistance-determining region (QRDR) of genes *gyrA*, *parC* but mechanism of growing importance is plasmid-mediated quinolone resistance (PMQR) associated with genes *qnrA, qnrB, qnrC, qnrD, qnrS* but frequency of their detection is different. Resistance to sulfonamides is mostly associated with genes *sul1* and *sul2*, while resistance to trimethoprim is associated with various variants of *dhfr* (*dfr*) genes. Taking into account *Salmonella* spp. strains isolated from food, resistance to β-lactams is commonly associated with β-lactamases encoding by bla_{TEM} genes. However strains ESBL and AmpC – positive are also detected. Resistance to aminoglicosides is commonly result of enzymatic inactivation. Three types of aminoglycoside modifying enzyme are: acetyltransferases (AAC), adenyltransferases (ANT) and phosphotransferases (APH). Resistance to tetracyclines among *Salmonella* spp. isolated from food is most commonly associated with active efflux. Among numerous genetic determinants encoding efflux pumps *tetA*, *tetB*, *tetC*, *tetD*, *tetE* and *tetG* are reported predominatingly. One of the most common mechanisms of resistance against chloramphenicol is its inactivation by chloramphenicol acetyltrasferases (CATs), but resistance to this compound can be also mediated by chloramphenicol efflux pumps encoded by the genes *cmlA* and *floR*.

It is important to monitor resistance of *Salmonella* isolated from food, because the globalization of trade, leading to the long-distance movement of goods, animals and food products, encourages the spread of resistant pathogens around the world.

Key words: *foodborne pathogens, multiresistance of Salmonella spp., antimicrobial resistance, food safety, food*

STRESZCZENIE

W artykule przedstawiono aktualne dane na temat mechaniznów lekooporności pałeczek *Salmonell*a spp. pochodzących z żywności. Wśród szczepów opornych na chinolony i/lub fluorochinolony najczęściej identyfikowanym mechanizmem są substytucje aminokwasów w obrębie regionów determinujących oporność na chinolony (QRDR-quinolone resistance-determining region) w genach *gyrA* i *parC*, jednak coraz częściej identyfikowane są geny qnr (*qnrA, qnrB, qnrC, qnrD, qnrS*) związane z plazmidami (PMQR - plasmid-mediated quinolone resistance). Oporność na sulfonamidy jest najczęściej związana z genami *sul1* i *sul2*, natomiast różne warianty genów *dhfr* (*dfr*) warunkują oporność na trimetoprim. Biorąc pod uwagę szczepy *Salmonella* spp. pochodzące z żywności, oporność na antybiotyki β-laktamowe związana jest zazwyczaj z produkcją β-laktamaz kodowanych przez geny bla_{TEM}. Jednakże coraz powszechniej identyfikowane są szczepy produkujące β-laktamazy o rozszerzonym spektrum substratowym (ESBL) oraz cefalosporynazy AmpC. Oporność na aminoglikozydy najczęściej wynika z wytwarzania enzymów modyfikujących cząsteczki leku: acetylotransferaz (AAC), adenylotransferaz (ANT) oraz fosfotransferaz (APH). Oporność wobec tetracyklin wśród pałeczek *Salmonella* spp. izolowanych z żywności najczęściej związana jest z mechanizmem aktywnego usuwania leku za pomocą pomp (efflux) kodowanych, najczęściej przez geny *tetA*, *tetB*, *tetC*, *tetD*, *tetE* i *tetG*. Jednym a najczęściej wykrywanych mechanizmów oporności na chloramfenikol jest jego inaktywacja w wyniku działania acetylotransferazy chloramfenikolowej (CAT). Oporność na chloramfenikol może być również związana ze zjawiskiem aktywnego wypompowywania leku. Pompy efflux są kodowane przez geny *floR* (warunkujące oporność także na florfenikol) lub *cml*.

Istotne znaczenie ma monitoring lekooporności wśród szczepów *Salmonella* spp. pochodzących z żywności, ponieważ transport środków spożywczych oraz zwierząt do i z krajów całego świata ułatwia rozprzestrzenianie się szczepów lekoopornych.

Słowa kluczowe: *patogeny żywności, wielolekooporność Salmonella spp., lekooporność, bezpieczeństwo żywności, żywność*

[*]**Corresponding author**: Łukasz Mąka, National Institute of Public Health - National Institute of Hygiene, Department of Food Safety, Laboratory of Food Microbiology, 24 Chocimska str., 00-791Warsaw, Poland, Phone: + 48 22 54 21 381, e-mail: lmaka@pzh.gov.pl

INTRODUCTION

Although new microbilogical hazards are detected in food [76], *Salmonella* spp. remain one of the most common foodborne pathogens worldwide. More than 2600 *Salmonella* serovars have been identified [24]. These bacteria are prevalent in the environment, and are found in both domestic and wild animals as pathogens or commensals. They can infect humans, mainly via the contaminated food: chicken, pork, dairy products, eggs, fruits, vegetables and others [92, 98].

The clinical symptoms of salmonellosis are usually fever, abdominal pain, diarrhoea and vomiting, although the same strain may sometimes cause different symptoms in separate hosts. The nature of the illness can depend on factors including the type of contaminated food, the infecting dose, the gut flora and the immunological condition of host. More severe salmonellosis occurs in immunocompromised people, the very young and the elderly.

Salmonella is a serious problem for food safety and public health, and is one of the most common human foodborne pathogens in the European Union (EU). In 2014, *Salmonella* spp. was most frequently detected in poultry meat and less often in pig or bovine meat. It is one of the major factor of reported foodborne outbreaks. A total of 88'715 confirmed human cases of salmonellosis were reported in the EU in 2014 and of these, 34.4% were hospitalized (hospitalization status was provided for 10.4% of all confirmed cases) [25].

Scallan et al. estimated that each year in the United States, non-typhoidal *Salmonella* spp. cause 1.0 million cases of foodborne illness (11% of all foodborne illnesses). Non-typhoidal *Salmonella* spp. are the leading cause of hospitalizations and mortality due to the consumption of contaminated food in the USA. Infections with non-typhoidal *Salmonella* spp. are also responsible for the majority of deaths among people in the USA who have eaten contaminated food. The costs resulting from salmonellosis in the USA amount to several billions of dollars [85].

In Australia, in 2010, more than one-third of the notified diseases or infections commonly transmitted by food were caused by *Salmonella*. Just over one-third (34%) of all foodborne and suspected outbreaks were due to *S.* Typhimurium [62].

Another serious problem and a major challenge for medicine is antimicrobial resistance among pathogens. Each year, about 25,000 patients die in the EU, Iceland and Norway from infections with antibiotic-resistant bacteria, two-thirds of them Gram-negative. Infections by resistant bacteria result in annual costs due to additional healthcare and lost productivity, of at least EUR 15 billion in the EU [22].

ANTIMICROBIAL RESISTANCE AS A FOOD SAFETY PROBLEM

The growing importance of antimicrobial resistance as a problem for food safety has been recognized by various international organizations [91]. This problem is multifaceted and intersectoral, and cooperation and the exchange of information between the sectors of agriculture, veterinary, food production and public health appear to be essential. The globalization of trade, which depends on the movement of goods, animals and food products, means that resistant bacteria can become widely distributed and transferred to consumers around the world.

Another route of resistance transfer is from the environment contaminated by the disposal of high levels of antibiotics and antibiotic-resistant bacteria. One example is the application of manure from pig farms, where large amounts of antibiotics are used in preventive treatments [74]. The contamination of vegetables and fruits can occur through their contact with contaminated soil or water during growth, and then resistant bacteria are transferred via the fecal-oral route [40, 86].

Resistant bacteria are transferred from food animals to man via the food chain. After the ingestion of contaminated food, commensal and pathogenic bacteria in the gut can exchange mobile genetic elements mediating resistance. Recent epidemiological studies have revealed that human infections with resistant *Salmonella* spp. are associated with prolonged illness, an increased risk of invasive disease and hospitalization, and excess mortality [59].

The spread of resistance to some antibiotics is particularly worrying. Farm animals and meat products often contain resistance genes active against 3rd and 4th generation beta-lactams, which are crucial antibiotics in human medicine. Resistance against these drugs mediated by the AmpC and Extended Spectrum Beta-lactamase (ESBL) families is often found in *E. coli* and *Salmonella* spp. [98]. Genetic analyses of the bacterial strains and resistance genes in farm animals, food and humans have found strong similarities/common genetic features [45]. These studies provide indirect evidence that ESBL genes, mobile genetic elements and resistant strains are transmitted to people via the food chain.

Another widespread problem is the use of fluoroquinolones in the poultry industry. Quinolone-resistant bacteria (*E. coli*, *Salmonella* spp. and *Campylobacter* spp.) spread through the ingestion of contaminated food, have been shown to have an impact on the management of human infections [23, 26].

Table 1. Resistance of *Salmonella* spp. of food origin to various antimicrobial compounds

Country	Year(s)	Source	Antimicrobials (percentage of resistance)														Reference
			AMP	AMC	CRO	CAZ	IPM	TE	CN	STR	NA	CIP	SUL	SXT	W	C	
Morocco	2002-5	food	13	9	nd	0	nd	21	0	6.7	3.8	0	nd	2.8	nd	4	[9]
UK	2003-5	Beef	41.2	nd	nd	nd	nd	58.8	0	64.7	0	0	64.7	nd	5.9	29.4	[48]
UK	2003-5	Lamb	50	nd	nd	nd	nd	50	0	56.3	25	0	56.3	nd	6.3	37.5	[48]
UK	2003-5	Pork	42	nd	nd	nd	nd	76	0	44	6	4	54	nd	26	34	[48]
Senegal	2003	beef	nd	0	nd	0	nd	0.4	0	21.5	0.4	0	14.7	nd	0	0.8	[73]
Ethiopia	2003	food	20	nd	0	nd	nd	16	nd	26	nd	nd	14[a]	nd	nd	0	[97]
Austria	2004*	**	17	nd	nd	nd	nd	33	nd	27	42	9.6	nd	nd	nd	17	[50]
Brazil	2004-6	chicken carcasses	38	nd	6	nd	nd	12	12	78	40	4	58	10	10	6	[54]
Brazil	2005	fresh pork sausage	30.9	nd	nd	nd	nd	71.6	2.5	28.4	24.7	nd	55.6	29.6	nd	30.9	[60]
China	2005	pork	16.7	0	0	8.3	nd	33.3	0	0	50	0	83.3	50	nd	16.7	[92]
China	2005	chicken	47.4	10.5	0	5.3	nd	47.4	31.6	36.8	73.7	42.1	89.5	57.9	nd	42.1	[92]
China	2005	beef	0	0	0	0	nd	0	0	0	6.7	0	86.7	33.3	nd	0	[92]
China	2005	mutton	0	0	0	0	nd	6.7	0	6.7	6.7	0	73.3	26.7	nd	0	[92]
China	2005	seafood	10	0	0	0	nd	10	5	5	15	0	95	65	nd	0	[92]
Turkey	2005-6	chicken carcasses	85.2	nd	nd	nd	nd	67.6	14.7	61.7	nd	nd	nd	nd	nd	10.2	[96]
Spain	2006	chicken	10.5	10.5	nd	nd	nd	21.1	0	36.8	100	0	nd	5.3	nd	5.3	[5]
Iran	2006-7	chicken and beef	4	3.2	nd	0	0	69	0	42	82	0	nd	nd	63	1.6	[16]
India	2006-7	chicken eggs	41.1	nd	nd	nd	nd	0	29.4	0	nd	0	70.6[b]	nd		23.5	[70]
India	2006-8	fish and sprouts	2.8	nd	2.8	nd	nd	62	nd	2.8	5.6	1.4	50.7[b]	nd	2.8	2.8	[43]
Tunisia	2006-8	raw meat	16.2	5	nd	1.2	nd	1.2	0	6.2	1.2	nd	1.2	1.2	nd	0	[2]
Malaysia	2006-9	retail meats	19.7	1.5	0	0	nd	72.7	3	66.6	40.1	3	69.7	19.7	nd	10.6	[79]
Malaysia	2006-9	street foods	9	0	0	0	nd	77.3	0	31.8	54.5	0	45.5	18.2	nd	9	[79]
Canada	2007-8	chicken	31	21	21	nd	nd	49	0	40	0	0	7[a]	0	nd	1	[7]
Canada	2007-8	turkey	29	25	25	nd	nd	54	4	29	0	0	8[a]	4	nd	4	[7]
Canada	2007-8	pork	0	0	0	nd	nd	0	0	33	0	0	33[a]	33	nd	0	[7]
Algeria	2007-8	***	4.8	nd	nd	nd	nd	12.9	nd	16.1	16.1	nd	87.1	4.8	4.8	4.8	[56]
Vietnam	2007-9	pork and chicken	39.8	2.9	nd	0	nd	58.5	17.8	47.3	27.8	5	58.1	nd	34	37.3	[78]
Poland	2008-12	meat and meat products	28.3	16	0	0	0	32.1	6.6	28.3	52.8	0	26.4	3.8	3.8	7.5	[51]
Poland	2008-12	products other than meat	4.9	2.5	0	0	0	1.6	1.6	1.6	35.2	0	6.6	0	0	0.8	[52]

AMP – ampicillin; AMC – amoxycillin/clavulanic ac.; CRO – ceftriaxone; CAZ – ceftazidime; IPM – imipenem; TE – tetracycline; CN – gentamicin; STR – streptomycin; NA – nalidixic ac; CIP – ciprofloxacin; SUL – sulphonamides comp; SXT – trimethoprim/sulphametoxazole; W – trimethoprim; C – chloramphenicol.

Antibiotic resistance in *Salmonella* spp. has led to more frequent hospitalizations, more complicated and prolonged illnesses, treatment failures, a higher risk of invasive disease and a twofold increase in the risk of death in the two years following infection. The growing problem of antimicrobial resistance has resulted in a decrease in the efficacy of antimicrobials and a situation similar to the pre-antibiotic era in some cases [47, 91]. In richer countries, routine laboratory susceptibility testing assists in the selection of the appropriate antimicrobial treatment, but this is not possible in low-income communities, and blind therapy may lead to treatment failure, long-term disability and increased mortality rates. Inappropriate antibiotic therapy can result in *Salmonella* remaining in the host's cells (intracellular) and thus resulting in asymptomatic carriage, which is associated with further complications and the development of resistance [75].

The dissemination of antimicrobial resistance is often via mobile genetic elements such as plasmids, transposons and gene cassettes in integrons [64]. The most common integrons involved in antimicrobial resistance are class 1 integrons that are abundant in the genomes of many bacterial species [4].

Increasing resistance among foodborne pathogens is linked to the excessive use of antimicrobials in animals. *Mellon* et al. [55] estimated that annual non-therapeutic antibiotic use in animals has increased in the USA from 16.1 million pounds in the mid-1980s to 24.6 million pounds in the 2000s. The amounts would be even higher if antimicrobials used therapeutically for animals were included.

According to data collected in 10 European countries, the amounts of veterinary antibacterial agents relative to the sum of the biomass of food-producing animals varies from 18 to 188 mg/kg per country [33]. Overall, tetracyclines accounted for 48% of the sales of veterinary antibacterial agents, sulphonamides and trimethoprim (as sulphonamides or in combination) for 17%, and β-lactams for 16%.

ANTIMICROBIAL RESISTANCE OF SALMONELLA ISOLATED FROM FOOD

Surveys of antimicrobial resistance in *Salmonella* strains isolated from food have been conducted in various countries around the world and have examined a broad spectrum of antimicrobial compounds. To facilitate a useful comparison between the results of these studies, we have chosen to focus on the antimicrobials that are most often used for *Salmonella* tesitng by authors of articles i.e. ampicillin, tetracycline, gentamicin, streptomycin, nalidixic acid, ciprofloxacin, sulfonamides, sulphametoxazole/trimethoprim and chloramphenicol (Table 1).

Salmonella spp. resistant to ampicillin have been frequently isolated from food products. Only among isolates from pork in Canada and those from beef and mutton in China was resistance to this antibiotic not found.

Similar results have been obtained for tetracycline, with reported frequencies of resistance to this antibiotic among *Salmonella* spp. isolates often ≥50.0%: 50.0 – 76.0% among strains isolated from various meats in the UK, 71.6% from pork sausage in Brazil, 67.6% from chicken carcasses in Turkey, 69.0% from chicken and beef in Iran, 62.0% from fish and sprouts in India, 72.7 – 77.3% from foods in Malaysia, 54.0% from turkey in Canada, and 58.5% from pork and chicken in Vietnam. However, it is noticeable that resistance to tetracycline has been less frequently detected among *Salmonella* spp. strains isolated from foods in African countries: only 0.4% among isolates from Senegal and up to 21.0% among those from Morocco.

Susceptibility to aminoglycosides was examined in all surveys and differences between the levels of resistance to gentamicin and streptomycin were found. Only isolates from beef in China and from chicken eggs in India were fully susceptible to streptomycin. The highest incidence of resistance to streptomycin was observed among *Salmonella* strains isolated from chicken carcasses in Brazil (78.0%), retail meats in Malaysia (66.6%), beef in the UK (64.7%) and chicken carcasses in Turkey (61.7%). The frequency of resistance to gentamicin was lower and amounted to no higher than 31.6% among isolates from chicken in China. Moreover, three fifths (15/25) of the results obtained for different origins, reported that 100.0% of *Salmonella* spp. isolates were susceptible to gentamicin.

Almost all of the surveys examined susceptibility to nalidixic acid and ciprofloxacin. Of these antimicrobials, ciprofloxacin is definitely more effective against *Salmonella* spp., and the majority of surveys reported no resistance to this compound. Only isolates from chicken samples collected in China displayed relatively frequent resistance (42.1%) to ciprofloxacin, while 73.7% of these strains were resistant to nalidixic acid. The highest rate of resistance to this quinolone was observed among *Salmonella* isolates from chicken products in Spain (100.0%).

The frequency of resistance to sulfonamides ranged between 1.2% (raw meat in Tunisia) and 95.0% (seafood in China). The proportion of resistant strains was particularly high among *Salmonella* spp. isolates from foods in Asian countries: 45.5% and 69.7% in Malaysia, 58.1% in Vietnam, 73.3 – 95.0% in China.

The highest incidence of resistance to chloramphenicol was reported by *Yan* et al. (2010) for *Salmonella* strains isolated from chicken samples in China. All of the surveys conducted in European countries reported rates of resistance to

chloramphenicol ranging between 5.3% (chicken in Spain) and 37.5% (lamb in the UK). The frequency of resistance to this antibiotic among isolates from African countries was not higher than 4.8% (various meat products in Algeria), while no resistance to chloramphenicol was detected in *Salmonella* spp. from foods in Ethiopia and Tunisia.

Table 2. Number of resistant *Salmonella* spp. strains isolated in different countries.

	number of tested strains	number of resistant strains	% of resistant strains	Reference
	19	19	100	[5]
	250	250	100	[54]
	68	68	100	[96]
	27	27	100	[70]
	71	69	97	[43]
	81	76	93.8	[92]
	62	56	90.3	[56]
	124	105	85	[16]
	88	74	84	[79]
	82	67	82	[60]
	241	189	78.4	[78]
	247	193	78	[73]
	83	64	77	[48]
	110	78	71	[7]
	106	73	68.9	[51]
	52	30	57.7	[50]
	122	52	42.6	[52]
	93	32	34.4	[97]
	105	30	29	[9]
	80	16	20	[2]
Total:	2111	1568	74.3	

Table 2 presents the combined survey results showing the general resistance of *Salmonella* spp. isolates of food origin. The lowest level of antimicrobial resistance was among isolates from raw meat collected from stores in the North African countries of Morocco – 29.0% [9] and Tunisia – 20.0% [2]. Studies conducted on food samples from Spain [5], Brazil [54], Turkey [96] and India [70] reported that all tested *Salmonella* spp. isolates were resistant to at least one antimicrobial compound. The surveys whose results are summarized in Table 2 tested a total of 2111 isolates and 74.3% (1568) showed resistance to at least one antibiotic. This confirms that antimicrobial resistance among *Salmonella* spp. isolated from food is a serious problem for food safety and public health.

MECHANISMS OF RESISTANCE OF *SALMONELLA* SPP. ISOLATED FROM FOOD

Resistance to quinolones and fluoroquinolones

Quinolone resistance in *Salmonella* spp. is usually associated with point mutations in the quinolone resistance-determining regions (QRDR). Such mutations cause amino acid substitutions that modify the targets gyrase (*gyrA*, *gyrB*) and topoisomerase IV (*parC*, *parE*), and make them less susceptible to quinolone binding. Amino acid substitutions in the target enzymes cause increases in the MIC value that may depend on the *Salmonella* serovar. The following alterations are those most frequently reported: GyrA – Ser83→Phe (MIC=256 μg/mL for nalidixic acid, MIC=0.25 – 2 μg/mL for ciprofloxacin), Asp87→Gly, Tyr (256 – 512 μg/mL for nalidixic acid, MIC=0.12 – 0.5 μg/mL for ciprofloxacin); ParC – Ser80→Ile, Arg. Changes in GyrB are not found in many surveys [15, 26].

A new plasmid-mediated quinolone resistance (PMQR) mechanism to nalidixic acid, ciprofloxacin and other fluoroquinolones was reported by *Martinez-Martinez* et al. [49]. This mechanism is based on protection of a quinolone target. Many related *qnr* genes have since been described, i.e. *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* [13, 42, 61]. There are also numerous variants within each family, with the differences between them associated with amino acid substitutions, e.g. QnrB1, QnrB7 and QnrB17 [42]. *Qnr* genes are often located on plasmids that carry multiple resistance determinants,

and particularly those that harbor genes encoding extended-spectrum β-lactamases (ESBL) [61]. The genes *qnrA* and sometimes *qnrB* are frequently found as components of complex *sul1*-type integrons. *Qnr* genes and those encoding extended-spectrum or AmpC-type β-lactamases are often present on the same plasmids [65].

Another mechanism of resistance to ciprofloxacin is the production of an modified aminoglycoside acetyltransferase (AAC(6')-Ib-cr) that reduces the activity of this compound by enzymatic modification [66].

Resistance can also be mediated by efflux due to overproduction of the periplasmic protein AcrAB belonging to the AcrAB-TolC efflux pump. This results in a multiple antibiotic resistance (MAR) phenotype [31]. *Baucheron* et al. [8] reported that fluoroquinolone resistance in *Salmonella* Typhimurium DT104 is highly dependent on the AcrAB-TolC efflux system.

A conjugative plasmid conferring resistance to the antibiotic olaquindox was found in *E. coli* strains isolated from swine. The resistance mechanism was identified as a multidrug efflux pump OqxAB [36, 72]. Quinoxalines are sometimes regarded as growth promotors, but they are used mainly in the prevention of swine dysentery [11]. Another efflux pump, QepA, was identified in an *E. coli* strain isolated from a urine specimen from an inpatient in Japan. It was encoded on a plasmid conferring multiple-resistance against aminoglycosides, fluoroquinolones and broad-spectrum β-lactams [95].

Among 13 nalidixic acid-resistant *Salmonella* spp. strains isolated between 2004 – 2007 in Colombia from foods of animal origin (chicken, sausages and ground meat), four (30.8%) were *qnrB* (*qnrB19* in all cases) positive. All of these strains were susceptible to ciprofloxacin. The QnrB gene was identified in *S*. Infantis, and twice in *S*. Uganda and in *Salmonella* 6,7:d:-. No other quinolone resistance genes (*aac(6')-Ib-cr*, *qepA*, *qnrA* or *qnrS*) were detected [44].

In a study of *Salmonella* Schwarzengrund isolates from humans, food and food animals in Denmark, Thailand and the USA, ciprofloxacin resistance was detected in 29 (24%) of 123 nalidixic acid-resistant strains [1]. Ten ciprofloxacin-resistant isolates tested in this study contained a double mutation in *gyrA* at codons 83 (Ser→Phe) and 87 (Asp→Asn), which resulted in high level ciprofloxacin resistance.

An international collaborative study conducted in 13 European countries showed that among isolates of *Salmonella enterica* of various origin (environment, food, humans, pigs, fowl, reptiles, sheep, turkeys), 59% (288/485) carried PMQR genes. Among the food isolates, the *qnrS1* gene was most prevalent, being detected in 6 (along with the *aac(6')-1b-cr* gene in one isolate), while two isolates were *qnrB19* positive and a single strain carried the *qnrD* gene [82].

Thirty multidrug resistant (MDR) *Salmonella* spp. isolates were recovered from retail meat samples (chicken, pork and lamb) taken in Shaanxi Province, China, in 2007 and 2008. A total of 68 mutations in gyrase subunit A (*gyrA*), topoisomerase IV subunit C (*parC*) and topoisomerase IV subunit E (*parE*) were identified in the 30 *Salmonella* spp. isolates, but no mutation was detected in gyrase subunit B (*gyrB*) [94].

Wong and *Chen* [90] detected *oqxAB* in *Salmonella* spp. isolated from retail meats in Hong Kong. Importantly, this was the first time that two olaquindox-resistant isolates were found to contain the gene combination *oqxAB*, which confers resistance to olaquindox quinolones and chloramphenicol and reduces susceptibility to other antibiotics. Other isolates characterized in this study carried the *qnrS* and *aac(6=)-Ib-cr* genes.

Resistance to sulfonamides and trimethoprim

Due to widespread resistance, the use of sulfonamides is no longer common. The resistance of Gram-negative enteric bacteria to these compounds is mediated by plasmid-borne genes encoding alternative variants of the dihydropteroate synthase (DHPS) that have no affinity for sulfonamides [71]. A second gene encoding "normal" (non-modified) DHPS is present on the chromosome in both resistant and susceptible bacteria. The plasmid-encoded DHPS are 1000-fold less susceptible to sulfonamides compared with that encoded by the chromosomal gene. Plasmid-mediated sulfonamide resistance is often associated with resistance to other chemotherapeutics.

When used in combination with trimethoprim, sulfonamides are bacteriocidal. Like sulphonamides, trimethoprim is a compound which competes with substrates of the essential folic acid pathway in bacteria and inhibits dihydrofolate reductase (DHFR). Raesistance to trimethoprim is mediated by genes encoding dihydrofolate reductase variants (*dhfr* and *dfr*) that have decreased affinity for the antimicrobial agent. This allows folic acid biosynthesis to occur in the presence of trimethoprim [39].

A panel of 73 *Salmonella enterica* strains isolated from food products in Portugal in 2002 and 2003 were screened for the presence of *sul* genes [6]. Of six *sul3*-positive isolates obtained from foods of animal origin, four also carried the *sul1* gene, and one was positive for *sul1*, *sul2* and *sul3*. The association of the *sul3* genes with conjugative plasmids in these isolates could facilitate the spread of this gene to other bacteria. The *sul3* gene was shown to occur in *Salmonella* spp. carrying class 1 integrons with *aadA* and *dfrA* gene cassettes, which allows these strains to survive exposure to a combination of sulfamethoxazole and trimethoprim. Sul3-positive *Salmonella* spp. strains of food origin have also been isolated in Germany [34].

Among *Salmonella* spp. isolates obtained from beef samples collected from retail markets in Vietnam in 2009, resistance to sulfonamides was found in 39.7% (25/63 isolates) and 80.0% of these (20/25) were *sul1* positive [77]. Trimethoprim resistance was detected in 28.6% (18/63) of the isolates and of these, 55.6% (10/18) carried the *dfrA1* gene and 33.3% (6/18) the *dfrA12* gene.

Also in Vietnam, in the years 2007–2009, *Salmonella* spp. strains were isolated from pork and chicken [78]. In this case, 58.1% of isolates were resistant to sulphonamides and 34% to trimethoprim.

Between 2007–2008, 110 *Salmonella* spp. isolates were obtained from meat (chicken, turkey and pork) from retail stores in Canada [7]. Of these, 71% (78/110) showed resistance to sulphonamides. The *sul1* gene was found in 5 isolates, *sul2* in 3 isolates and the *sul3* gene was only found in one (pork) isolate.

Among 88 *Salmonella* spp. strains isolated from retail meats and street foods in Malaysia, 63.6% were sulfonamide-resistant [79]. Of these, 32 were positive for *sul1* and *sul2*, 5 were positive for *sul1*, and 14 were positive for *sul2*. Resistance to trimethoprim-sulfamethoxazole was found in 19.3% of the isolates. The gene cassettes identified in the variable regions included trimethoprim resistance genes *dfrV*, *dfrA1* and *dfrA12*. In addition, the *sul1* gene and *aadA2* gene (encoding resistance to streptomycin) were also identified.

Among *Salmonella* spp. strains isolated from meat products from supermarkets and free markets in Shaanxi Province in China between 2007–2008, 67% were resistant to sulfamethoxazole and 58% to trimethoprim/sulfamethoxazole [93]. Five resistance gene cassettes were identified, which included the determinants *dhfr*, *aadA*, *tetR*, *bla*PSE-1, bla_{DHA-1} and bla_{VEB-1}, encoding resistance to trimethoprim, streptomycin, tetracycline and beta-lactams, respectively. One *S*. Enteritidis isolate from chicken contained two integrons (1.2/1.8) carrying three resistance genes (bla_{PSE-1}/*dhfr*17-*aad*A5).

Chen et al. [14] reported that all sulfonamide-resistant *Salmonella* spp. isolated from retail meats in the USA and in China were *sul1*- and/or *sul2*-positive, and dihydrofolate reductase genes (*dhfr1*, *dhfr12* and *dhfr13*) were detected in each of the trimethoprim-resistant isolates.

Resistance to β-lactams

β-lactamases were widespread before penicillin was widely used therapeutically, which suggests that these enzymes are a mechanism to counter antimicrobial substances produced by other species of bacteria or fungi in the environment.

The production of β-lactamases is the main mechanism of resistance to β-lactams in Gram-negative bacteria. In 1965, *Datta* and *Kontomichalou* [18] described a plasmid-encoded β-lactamases, found in an *E. coli* strain isolated in Greece from a patient named Temoneira, and they named this enzyme TEM-1 [18]. Within a few years, TEM-1 had become widespread in many species representing different families of bacteria. SHV-1 is another common plasmid-encoded β-lactamase [10].

The chromosomal *ampC* gene found in many *Enterobacteriaceae* is usually expressed at a low level and is inducible in response to β-lactam exposure. *Salmonella* spp. are naturally $AmpC^-$, but *ampC* genes may occur on transmissible plasmids [41, 63].

The increased use of antibiotics and the introduction of new compounds have resulted in the increasing occurrence of β-lactamases and the appearance of new forms. In the 1980s oxyimino-cephalosporins were introduced to treat infections caused by Gram-negative bacteria. The use of these new β-lactam antibiotics resulted in the appearance of resistant strains producing extended spectrum β-lactamases (ESBLs). ESBLs are able to hydrolyze penicillins, cephalosporins (excluding cephamycins) and monobactams, and can be inhibited by β-lactam inhibitors.The genes bla_{SHV}, bla_{TEM}, bla_{CTX}, bla_{CMY} and bla_{OXA} are responsible for ESBL-mediated resistance in *Salmonella* spp. [10, 89].

Numerous studies have investigated the occurrence of different β-lactamases in Gram-negative bacteria isolated from human infections, including *Salmonella* spp.. There are fewer reports describing these enzymes in isolates from food animals, with only a small number concerning *Salmonella* spp. isolates of food origin [87, 89]. In some countries, ESBL-producing *Salmonella* spp. have yet to be identified in food, but their appearance in food animals makes their eventual isolation from food samples likely.

Among *bla* genes, presence of bla_{TEM} has been reported most often among *Salmonella* spp. isolated from food. However other genes such as bla_{CTX-M} and bla_{CMY-2} have also been found.

Thai et al. [77] reported that among 20 ampicillin-resistant strains isolated from retail beef in Vietnam, 90% were bla_{TEM}-positive, 5% were bla_{OXA-1}-positive and 5% harbored both genes. According to *Aslam* et al. [7], among 110 *Salmonella* spp. isolates from retail meat in Canada, 17 were bla_{TEM}-positive and 23 were bla_{CMY-2}-positive. The following β-lactamase genes were detected among 7 ceftiofur-resistant *Salmonella* isolates from food in Germany: $bla_{CTX-M-1}$, bla_{TEM-1}, bla_{CMY-2}, bla_{TEM-52} and bla_{TEM-20} [67].

In the study of *Thong* and *Modarressi* [79], of the 6 types of β-lactamase gene tested for ($bla_{TEM,}$ bla_{CMY-2}, $bla_{SHV,}$ $bla_{CTX,}$ $bla_{OXA,}$ bla_{PSE-1}), only bla_{TEM} was detected in 3 ampicillin-resistant *Salmonella* spp. isolated from retail meats and street foods in Malaysia.

Among multiple-resistant *Salmonella* spp. isolated in the USA and China, from meat products [14], bla_{CMY-2} was the β-lactamase gene most frequently found in extended-spectrum β-lactam-resistant strains. However, a bla_{TEM-1}-like gene was also detected. All ampicillin-resistant isolates from meat products in China contained a bla_{TEM-1}-like gene, while a bla_{PSE-1} gene located on a 1.0-kb class 1 integron was identified in two *Salmonella* Typhimurium DT104 isolates displaying the ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphametoxazole, tetracycline) multi-resistant phenotype [14].

Resistance to aminoglycosides

There are various mechanisms of aminoglycoside resistance, including alteration of the ribosomal binding sites, decreased uptake, decreased accumulation in bacteria, and the expression of enzymes which modify and inactivate these antibiotics. Of these mechanisms, enzymatic inactivation seems to be the most important and most common type of aminoglycoside resistance among *Salmonella spp.* isolated from food. There are three types of aminoglycoside modifying enzyme: acetyltransferases (AAC), adenylytransferases (ANT) and phosphotransferases (APH). Some *aph* genes are also known as *strA* or *strB* genes conferring resistance to streptomycin. Aminoglycoside nucleotidyltransferases can confer resistance to gentamicin, tobramycin or streptomycin and include the genes *aad* and *ant* [28].

Another resistance mechanism is rRNA methylation, which is employed by actinomycetes as a means of self-protection against the aminoglycosides they produce. Over the last decade, 16S rRNA methyltransferases have emerged in Gram-negative bacteria. A number of different methyltransferase-encoding genes have been identified in *Salmonella* spp. isolates of different origin: *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE* and *npmA*. Aminoglycoside inactivating enzymes may be encoded by plasmids or associated with transposons, e.g. *armA* is associated with the transposon Tn*1548* [19; 29].

The majority of aminoglycoside methyltransferases have been identified in clinical isolates, but there are occasional reports of this type of resistance mechanism in *Salmonella* spp. of food origin.

The presence of *Salmonella* spp. carrying 16S rRNA methyltransferases in the East of Africa was confirmed by *Granier* et al. [32] who detected an ArmA methyltransferase in an isolate identified as *S.* enterica I.4,12:i:-, obtained from a sample of chicken meat. *Hopkins* et al. [37] reported a strain of *Salmonella* Virchow bearing *rmtC*, isolated from food in the UK. Among 19 streptomycin-resistant isolates - 78.9% contained the *aadA1* gene and 5.3% *aadA2*. All kanamycin resistant *Salmonella* spp. isolated from beef samples collected in Vietnam harbored the *aphA-1AB* gene, and 88.9% of gentamicin-resistant isolates were *aac(3)-IV*-positive [77]. Of the 30 multiresistant isolates obtained by *Chen* et al. [14] from retail meats in the USA and in China, most carried *aadA1* (60%) and the following genes were also detected: *aph(3')-IIa* (13.3%), *aadA2* (10%), *aacC2* (3.3%) and *aac(3)-IVa* (3.3%). In Canada, 42% of all *Salmonella* spp. strains isolated from meat products were *strA*/*B* positive and these were the most common resistance genes detected in this study [7]. Other genes were detected less frequently among the isolates: *aadA* (5%), *aphA2* (4%) and *aphA1* (2%). In a study on *Salmonella* spp. isolated from retail meats and street foods in Malaysia, 45 of the 51 streptomycin-resistant isolates contained both *strA* and *strB* [79]. Among these, 2 contained only *strA*, 3 *S.* Newport isolates contained only *strB*, while 5 *S.* Typhimurium isolates also had an additional *aadA* gene.

Resistance to chloramphenicol

One of the most common mechanisms of resistance against chloramphenicol is its inactivation by chloramphenicol acetyltransferases (CATs). These enzymes are encoded by *cat* determinants that may be chromosomal, carried on a plasmid or associated with a transposon or integron. CatA proteins are encoded by the genes *catA1* and *catA2*. A separate *catB* variant has also been identified in *Salmonella* spp. [3, 14, 83].

Chloramphenicol resistance in *Salmonella* spp. can also be mediated by chloramphenicol efflux pumps encoded by the genes *cmlA* and *floR* [77, 88].

Among *Salmonella* spp. isolates obtained from seafood in India, *Deekshit* et al. [20] identified one chloramphenicol-resistant strain that was positive for the presence of the *catA1* gene. Interestingly, some chloramphenicol-susceptible isolates also possessed this gene.

Thai et al. [77] found that all chloramphenicol-resistant *Salmonella* spp. strains isolated from retail beef in Vietnam carried at least one resistance gene. Among these isolates, 57.1% were *floR* positive, 50% were *cmlA1*-positive and 14.3% were *cmlA1+floR* positive, while none carried the *catA1* gene.

Miko et al. [58] reported that among 154 chloramphenicol-resistant *Salmonella* spp. isolates obtained from food in Germany, the majority were *floR*-positive (90.9%), whereas the *catA* and *cmlA1*-like genes were found in only 3.2% and 2.6%, respectively.

Neither the *cat1* nor the *cat2* gene was detected in nine chloramphenicol-resistant *Salmonella* spp. isolated from meat products and street food in Malaysia [79]. Instead, the *floR* gene was detected in 7 isolates and *cmlA* was detected in 2 isolates.

Resistance to tetracyclines

The most common mechanisms of tetracycline resistance are active efflux and protection of the ribosome. Numerous genetic determinants encoding efflux pumps have been described: *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetH*, *tetI*, *tetJ*, *tetK*, *tetL*, *tetP*, *tetV*, *tetY*, *tetZ*, *tet30*, *tet31*, *tet33*, *tet34*, *tet35*, *otrB* and *tcr3* (*tcrC*). Similarly, multiple tetracycline resistance determinants associated with ribosomal protection have been reported: *tetM*, *tetO*, *tetP*, *tetQ*, *tetS*, *tetT*, *tetW*, *otrA*, *tet32* and *tet36* [46, 57]. Notably, two genes encoding enzymes capable of inactivating tetracyclines have also been identified: *tetX* and *tet37* [21]. However in *Salmonella* spp. isolates, tetracycline resistance is usually mediated by the following determinants: *tetA, tetB, tetC, tetD and tetG* [17].

Deekshit et al. [20] found that the phenotypic expression of tetracycline resistance in *Salmonella* spp. isolated from seafood in India was always accompanied by the presence of the corresponding resistance determinant. Among the isolates analyzed, they detected the *tetA* gene located on a plasmid, plus the *tetB* and *tetG* genes, but none carried the *tetC* or *tetD* genes.

The *tetA* and/or *tetB* genes were detected in each tetracycline-resistant isolate obtained from meat samples collected in the USA and China, whereas the genes *tetC*, *tetD*, *tetE* and *tetG* were not found [14].

More than half (54.3%) of the tetracycline-resistant *Salmonella* spp. isolated from food in Germany carried *tetG*, while *tetA* and *tetB* were detected in 28.7% and 14.3%, respectively [58]. The genes *tetC* and *tetD* were detected occasionally (1.5% and 0.8%, respectively) and none of the tetracycline-resistant isolates harbored the *tetE* gene.

Out of 65 tetracycline-resistant *Salmonella* spp. isolated from food in Malaysia, 62 and 3 were positive for *tetA* and *tetB*, respectively [79].

Resistance and multiresistance among different Salmonella serotypes isolated from food

The frequency of resistance and multiresistance has been found to vary in different *Salmonella* serotypes. *Singh* et al. [70] and *Yildirim* et al. [96] reported that 100% of tested *S*. Typhimurium isolates were multiresistant, while according to *Thong* and *Modarressi* [79] all *S*. Typhimurium strains isolated from food in Malaysia showed resistance to at least one antimicrobial and 78.9% were multiresistant. Lower but still high levels of resistance/multiresistance among isolates of this serotype were reported by *Little* et al.[48] (91.1%/78%), *Mąka* et al. [52] (91%/70%) and *Zewdu* and *Cornelius* [97] (87.5%/42.9%). All 5 multiresistant *S*. Typhimurium isolates tested by *Bouchrif* et al. [9] were the pentaresistant (ACSSuT) strain DT104.

Salmonella Hadar is another serotype which isolates derived from food often display multiresistance profiles. All strains of this serotype isolated by *Dallal* et al. [16] and *Yildirim* et al. [96] were multiresistant. *Bouchrif* et al. [9] and *Thong* and *Modarressi* [79] also reported that 100% of *S*. Hadar isolates were antibiotic resistant, and of these 50% and 28.6% were multiresistant, respectively. *Aslam* et al. [7], *Mąka* et al. [52] and *Zewdu* and *Cornelius* [97] detected similarly high levels of resistance among *S*. Hadar isolates, with respective frequencies of 96.4%, 85.7% and 83.3%.

The resistance profile of *Salmonella* Infantis appears similar to that of the aforementioned serotypes. All strains of this serotype tested by *Zewdu* and *Cornelius* [97] were multiresistant. *Yildirim* et al. [96] found that all *S*. Infantis isolates were resistant to one or more antimicrobial and 90% of them were multiresistant. In contrast, *Bouchrif* et al. [9] reported that among *S*. Infantis isolates, only 16% were resistant.

Although *Salmonella* Enteritidis is considered to be generally susceptible, this has changed in recent years. Studies conducted by *Mąka* et al. [51, 52, 53] have shown the increasing frequency of resistant *S*. Enteritidis isolates in retail foods in Poland. Among strains of this serotype isolated between 2004–2007, the overall percentage of resistance was 13.6% (7% multiresistant) [53]. However, in isolates from the years 2008 – 2012 this value had increased to 54% (5% multiresistant) in strains of this serotype isolated from meat products [51], and to 43.7% (6.7% multiresistant) of strains from foods other than meat [52]. These results are similar to those obtained in Austria by *Mayrhofer* et al. [50] - 36% of *S*. Enteritidis isolates were resistant.

Álvarez-Fernández et al. [5] reported that all *S*. Enteritidis strains isolated from retail poultry were multiresistant. In studies conducted in various countries (e.g. Korea, Turkey) poultry has been shown to represent a major reservoir of multiresistant *Salmonella* spp., which suggests that it can be difficult to achieve successful antimicrobial therapy for salmonellosis caused by strains of poultry origin [96].

Strains of *S*. Newport isolated from food are generally characterized by a high frequency of antimicrobial resistance [5, 51,79, 96]. However, *Little* et al. [48] and *Zewdu* and *Cornelius* [97] reported that all *Salmonella* spp. isolates of this serotype were susceptible to all tested antimicrobials.

In the USA and Canada, *Salmonella* Heidelberg represents one of the major serotypes isolated from retail meats. *Zhao* et al. [98] found that 67% of isolates of this serotype were resistant to at least one antimicrobial, and 16.4% were resistant to at least five (one quarter of resistant isolates). *Aslam* et al. [7] reported that among *S*. Heidelberg strains isolated from retail meats in Canada, 80.6% were resistant and 45% displayed a multiresistant profile (i.e. 56% of resistant isolates).

Table 3. Examples of antimicrobial resistance genes detected in *Salmonella* spp. isolated from food

Resistance gene	Antimicrobial class	Reference
Point mutation in QRDR of *gyrA*, *parC*, *parE* *qnrB*, *qnrD*, *qnrS, oqxAB*	Quinolones and Fluoroquinolones	[44, 82, 90, 94]
sul1, sul2, sul3	Sulfonamides	[6, 34]
dfrA1, dfrA12, dfrV, dhfr1, dhfrV, dhfrA7, dhfr12, dhfr13, dhfr17	Trimethoprim	[14, 20, 77, 93, 79,]
bla_{TEM}, bla_{TEM-1}, bla_{TEM-20}, bla_{TEM-52}, $bla_{CTX-M-1}$, bla_{CMY-2}, bla_{OXA-1}, bla_{PSE-1}	β-lactams	[7, 14, 67, 77]
armA, *rmtC*, *aadA1, aadA2, aadA5, aphA-1AB, aac(3)-IV*, *aph(3')-IIa, aacC2, aac(3)-IVa,aacA4, strA, strB, aadA, aphA2, aphA1*	Aminoglycosides	[7, 14, 32, 37, 77, 79, 80]
catA1, *floR, cmlA1*	Chloramphenicol	[20, 58, 77]
tetA, tetB, tetC, tetD, tetG	Tetracyclines	[14, 20, 58]

GENETIC ELEMENTS AND ANTIMICROBIAL RESISTANCE IN *SALMONELLA* SPP.

In *Salmonella* spp., resistance genes are often located within mobile genetic elements that participate in horizontal gene transfer, i.e. plasmids, transposons, integrons and gene cassettes.

Plasmids are known to play a role in the transfer of genes in *Salmonella* spp.. *Ferguson* et al. [27] showed that antibiotic resistance plasmids can be transferred by conjugation from plasmid-containing strains of *S*. Typhimurium to plasmid-free strains of the same serotype in human epithelial cells. Moreover, multidrug resistant plasmids may be transferred between bacterial species by conjugation, e.g. from *S*. Typhimurium to *E*. *coli* [30]. Using different combinations of donor and recipient strains, *Van* et al. [80] demonstrated that resistance markers can be readily transferred among the same and different species (e.g. *Salmonella* spp. and *E. coli*). These findings demonstrated the importance of plasmids in the dissemination of antibiotic resistance genes in enteric bacteria isolated from food samples.

Karczmarczyk et al. [44] identified a plasmid designated pMK101 (carrying the *qnrB19* gene) in *Salmonella* 6,7:d:- isolated from ground meat in Colombia. This plasmid showed 97% sequence identity to the plasmid pMK100 (also carrying *qnrB19*) found in *S*. Infantis isolated from chicken, and was also highly similar to other *qnrB19*-carrying plasmids, including pSGI15, a small ColE plasmid identified in *S. enterica* serovar Typhimurium isolated in Germany [35], and pPAB19 from an *S*. Infantis clinical isolate recovered in Argentina. The small dissimilarity between pMK101 and the other plasmids is due to the presence of an insertion sequence identical to that found in plasmid pBC633 from *K*. *pneumoniae* strain KN633 (accession number EU176012), a urinary isolate from Colombia displaying carbapenem resistance and containing bla_{KPC-2} [84].

Most of the antimicrobial resistance determinants in the *Salmonella* isolates studied by *Chen* et al. [14], including bla_{CMY-2} and the genes contained in integrons, were present on plasmids and could be transferred to *E. coli* by conjugation. The *E. coli* recipient strain acquired 9 to 11 antimicrobial resistance phenotypes by receiving the plasmid from *Salmonella* Agona and *Salmonella* Typhimurium DT208 via conjugation. This finding indicated that conjugal plasmids can play a significant role in the dissemination of multiple-antimicrobial-resistance.

Of the 23 antibiotic-resistant *Salmonella* spp. isolates tested by *Van* et al. [80], all contained plasmids ranging in size from less than 8 kb to more than 165 kb. Plasmids of > 95 kb were found in 35% of the *Salmonella* spp. isolates, and some contained two large plasmids. These large plasmids were conjugative and carried many antibiotic resistance genes. It was also observed that recipient strains could acquire plasmids from donor strains by conjugation regardless of whether or not the recipients harbored their own plasmids. Antibiotic susceptibility testing of the transconjugants showed that the donors could transfer all or part of their resistance phenotype to the recipients. In addition to antibiotic resistance, high-molecular-weight plasmids are often associated with virulence [68].

The transfer of conjugative plasmids is thought to be the most common mechanism of genetic exchange between bacteria. This process can occur with high frequency, it is capable of co-transferring several resistance genes, and transfer can occur both within and between bacterial species [12].

A recent study of *Salmonella* spp. isolates from India found that the *tetA* gene in tetracycline-resistant strains was located on a plasmid [20]. This gene was identical to *tetA* detected in other *Salmonella* spp. serovars and in other bacterial species including *Escherichia coli*, *Edwardsiella tarda* and *Vibrio*

cholerae. Moreover, some isolates also possessed the *catA1* gene mediating chloramphenicol resistance located on a plasmid that was identical to a *catA1* gene found in *E. coli* (FN554766) and other *Salmonella* spp. serovars.

Deekshit et al. [20] also showed that the presence of a resistance gene does not necessarily result in resistance to the antibiotic in question. Among tested *Salmonella* spp. isolates, 16 chloramphenicol-sensitive strains possessed *catA1* genes, indicating a lack of expression of this gene. This is one of the few studies to show that environmental nontyphoidal *Salmonella* spp. strains can carry silent antibiotic-resistance genes. Similarly, *Thong* and *Modarressi* [79] reported that an isolate of *Salmonella* Agona containing *aadA2* and *sul1* gene cassettes was susceptible to streptomycin and sulfonamides.

Integrons and gene cassettes also play an important role in the dissemination of antimicrobial resistance. Identical resistance gene cassettes have been found in bacteria of the same species and among different bacterial species [38]. Class 1 integrons are the most prevalent among *Salmonella* spp. of animal, food and human origin, whereas class 2 and 3 integrons are detected rarely or not at all [79, 81].

Chen et al. [14] detected integron amplicons in 54% of tested multi-resistant *Salmonella* spp. isolates. The most common antimicrobial resistance genes carried by these integrons were *aadA1* and *aadA2*, conferring resistance to streptomycin, and *dhfrXII*, conferring resistance to trimethoprim. The bla_{PSE-1} gene, located in a 1.0-kb class 1 integron, was amplified in each of two *Salmonella* Typhimurium DT104 isolates with an ACSSuT antibiogram.

Multidrug resistant *S*. Weltevreden and two strains of *S*. Newport isolated from seafood were found to be integron positive [20], and there was an excellent correlation between the presence of gene cassettes and the corresponding antibiotic resistance phenotype of these isolates.

Among resistant *Salmonella* spp. isolated from meat samples taken in Vietnam, 13% were positive for class 1 integrons [80]. This indicated that the majority of the tested resistant isolates contained resistance elements other than integrons. Moreover, restriction fragment length polymorphism analysis of resistance gene PCR products suggested that isolates giving the same amplicon sizes carried identical gene cassettes. Of the MDR *Salmonella* spp. isolates characterized by *Thong and Modarressi* [79], 28.8% harbored class 1 integrons that were mostly located on plasmids (no class 2 or class 3 integrons were detected), which again indicated that the majority of the resistant *Salmonella* spp. isolates probably contained resistance elements other than integrons. Conjugation experiments were carried out with 14 MDR *Salmonella* spp. isolates containing the integrase gene, but only 4 isolates (three *S*. Typhimurium and one *S*. Corvallis) successfully transferred their resistance genes to *E. coli* J53.

CONCLUSIONS

Antimicrobial resistance in *Salmonella* spp. is a growing problem for food safety. As highlighted in this review, resistant *Salmonella* spp. are becoming more frequent in food in many countries situated in different regions of the world.

To monitor the potential spread and development of resistance, there is the need for further research on antibiotic resistant bacteria in food. Without quantitative estimates it is not possible to increase the quality of risk assessments or develop targeted interventions. In many countries, epidemiological data on antibiotic resistance, from a food safety perspective, are scarce. To permit the comparison of data obtained in many locations around the world, a harmonized approach to monitoring antibiotic resistance should be developed and applied, following international standards and recommendations.

Resistance of *Salmonella* spp. in food is linked to the use of antimicrobials in food animals. The practice of herd treatment of such animals (e.g. broiler chickens) with antimicrobials, leads to their higher exposure to these compounds and consequently promotes the increase in antibiotic resistance. The extensive use of antimicrobials in food production has already resulted in acquiring of resistance by *Salmonella* spp. If current farming practices are not changed, the development and spread of antibiotic resistance will undoubtedly continue.

The use of a single antibiotic may result in the development of resistance to other antimicrobial compounds of the same or different classes. Even in the absence of exposure to a particular antibiotic, resistant bacteria often carry resistance genes for long periods of time and may readily transfer and uptake these genes via horizontal gene transfer. Resistance genes in *Salmonella* spp. are often located on mobile genetic elements like integrons, transposons and sometimes insertion sequences, that promote the spread of resistance determinants.

The potential for the rapid dissemination of resistance among bacteria makes it especially important to monitor antimicrobial susceptibility and mechanisms of resistance of *Salmonella* spp. isolated from food, because new mechanisms of resistance occurring in animals may enter the food chain and be transferred to the consumer. This worrying scenario emphasizes the importance of cooperation between sectors in order to monitor antimicrobial resistance and rapidly identify trends that might further reduce the effectiveness of therapeutic antibiotics.

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Conflict of intrest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

OCCURRENCE OF PESTICIDE RESIDUES IN FRUITING VEGETABLES FROM PRODUCTION FARMS IN SOUTH-EASTERN REGION OF POLAND

Magdalena Słowik-Borowiec[*]*, Ewa Szpyrka, Julian Rupar, Magdalena Podbielska, Aneta Matyaszek*

Institute of Plant Protection – National Research Institute, Regional Experimental Station in Rzeszów, Laboratory of Pesticide Residue Analysis, Poland

ABSTRACT

Background. Considering the fact that pesticides are commonly used in agriculture, continuous monitoring of these substances in food products is of great significance. Residues of these substances can be present in crops after harvest.

Objective. The aim of this study was to evaluate presence of pesticide residues in fruiting vegetables from production farms in south-eastern region of Poland in 2012–2015.

Material and methods. 138 samples were tested using accredited test methods. The monitoring programme covered determination of 242 pesticides. The tests covered tomato, cucumber and pepper crops. The test results were interpreted in accordance with criteria included in the European Commission recommendations published in the document SANCO/12571/2013 (now superseded by Document SANTE 2015), as well as on a basis of the maximum residue levels in force in the EU Member States.

Results. Pesticide residues were found in 47 samples, representing 34% of all tested samples. 17 active substances were found, belonging to fungicides and insecticides. Azoxystrobin (38%), boscalid (28%) and chlorothalonil (21%) were most commonly found in fruiting vegetables testing samples. Non-compliances related to use of plant protection product not authorized for protection of a given crop were observed in 6% of analysed samples. However, pesticide residues of fruiting vegetables in quantities that exceed the maximum residue levels (NDP, ang. MRLs), as well as substances which use for plant protection is forbidden were no found.

Conclusions. Crops monitoring is used to determine to what extent such products are contaminated with pesticide residues, and ensures protection of customer health.

Key words: *fruiting vegetables, pesticide residue analysis, maximum residue levels*

STRESZCZENIE

Wprowadzenie. Ze względu na powszechne stosowanie pestycydów w rolnictwie bardzo ważne jest prowadzenie stałej kontroli tych substancji w produktach spożywczych. Ich pozostałości mogą znajdować się w płodach rolnych nawet po zbiorze.

Cel badań. Celem pracy była ocena występowania pozostałości pestycydów w warzywach owocowych pochodzących z gospodarstw produkcyjnych z terenu Polski południowo-wschodniej w latach 2012–2015.

Materiał i metoda. Przebadano 138 próbek stosując akredytowane metody badawcze. Program kontroli obejmował oznaczenie 242 pestycydów. Badaniami objęto uprawy pomidora, ogórka, papryki i 242 substancje czynne. Wyniki badań interpretowano według kryteriów zawartych w zaleceniach Komisji Europejskiej i opublikowanych w dokumencie SANCO/12571/2013 (obecnie zastąpionym przez dokument SANTE 2015), a także w oparciu najwyższe dopuszczalne pozostałości pestycydów obowiązujących w państwach UE.

Wyniki. Pozostałości pestycydów wykryto w 47 próbkach warzyw owocowych, co stanowi 34% ogółu przebadanych próbek. Wykryto 17 substancji czynnych należących do fungicydów oraz insektycydów. W próbkach warzyw owocowych najczęściej wykrywano: azoksystrobinę (38%), boskalid (28%) i chlorotalonil (21%). W 6% analizowanych próbek stwierdzono nieprawidłowości związane z zastosowaniem preparatów niezarejestrowanych do ochrony danej uprawy. Natomiast w żadnej próbce warzyw owocowych nie stwierdzono pozostałości środków ochrony roślin w ilości, która przekraczałyby najwyższe dopuszczalne poziomy pozostałości (NDP, ang.MRLs), jak również substancji, których stosowanie w ochronie roślin zostało zabronione.

Wnioski. Kontrola płodów rolnych pozwala stwierdzić w jakim stopniu są one zanieczyszczone pozostałościami stosowanych pestycydów oraz zapewnia ochronę zdrowia konsumentów.

Słowa kluczowe: *warzywa owocowe, analiza pozostałości środków ochrony roślin, najwyższe dopuszczalne pozostałości*

[*]**Corresponding author**: Magdalena Słowik-Borowiec, Institute of Plant Protection – National Research Institute, Regional Experimental Station in Rzeszów, Laboratory of Pesticide Residue Analysis, M. Langiewicza Street 28, 35-101 Rzeszów, Poland, phone/fax: +48 178547319, e-mail: m.borowiec@iorpib.poznan.pl

INTRODUCTION

Pesticides are chemicals used in agriculture to protect crops against insects, fungi, weeds and other pests. Although highly selective formulations are used acting on a specific agrophage or a specific group agrophages, in modern agriculture there is a risk that also humans will be affected. According to WHO, pesticides are also potentially toxic to humans. They may induce adverse health effects including cancer, effects on reproduction, immune or nervous systems. Before they can be authorized for use, pesticides should be tested for all possible health effects and the results should be analysed by experts to assess any risks to humans [23]. Considering the fact that pesticides are commonly used in agriculture, continuous monitoring of these substances in food products is of great significance. Their residues can be present in crops even after harvest.

Since 2004, Poland has been participating in the European monitoring system for pesticide residues in food and conducting tests for those substances as a part of official monitoring programme. Such tests facilitate quality assessment of plant products for correct use of plant protection agents in accordance with current legislation, as well as verification whether food products available in the market contain pesticide residues at a level exceeding current maximum residue levels (MRLs) [17], established in the European Commission Regulation No. 396/2005 [12].

The Laboratory of Pesticide Residue Analysis in Rzeszów conducts analyses of pesticide residues in fruit, vegetables and cereals as a part of official monitoring under the long-term programme for 2011–2015. This study covers results of tests conducted in 2012–2015 on a selected group of plants, i.e. fruiting vegetables (cultivated in a field or under shelter).

The aim of this study was to evaluate presence of pesticide residues in fruiting vegetables from production farms in south-eastern region of Poland in 2012–2015.

MATERIALS AND METHODS

Monitoring tests covered fruiting vegetables including tomatoes, cucumbers and peppers. Samples were collected randomly from farms by inspectors of the Inspectorates of Plant Health and Seed Inspection according to the Regulation of the Minister of Agriculture and Rural Development of 27 November 2013 [13]. All tested samples were raw products. The range of delivered samples resulted from the official monitoring programme, considering both production and consumption of relevant products. Vegetables were collected from the south-eastern region of Poland, from Podkarpackie, Małopolskie, Lubelskie and Świętokrzyskie voivodeships.

The monitoring program covered 242 substances. Table 1 presents classification of all active substances according to biocidal effects, with a quantification limit specified.

Accredited methods of gas chromatography and spectrophotometry were used for identification and quantification of tested active substances.

A multiresidue chromatographic method was based on residue extraction with an organic solvent and further purification of the extract with column chromatography. Final determination of residues was performed on gas chromatographs Agilent 7890 and Agilent 6890 equipped with electron capture (ECD) and nitrogen–phosphorus detectors (NPD) [7, 15].

Dithiocarbamate fungicides were analysed by a spectrophotometric method, based on their decomposition to CS_2 in acid environment and transfer to methyl blue, which was then analysed with the spectrometer Unicam Helios [1].

Analytical methods were verified in a validation process. Recovery assays of active substances were carried out on blank samples spiked with target compounds at two concentrations, in five replicates. The methods' trueness and precision parameters in terms of the average recovery and relative standard deviation were calculated and assessed according to the European Union guidelines [2, 3]. The linearity of the chromatographic and spectrophotometric responses were evaluated at five concentration levels. The measurement uncertainty of methods was estimated and was found to be compliant with European Union guidelines [2, 3]. Blank fortified samples were analysed within the framework of quality control/assurance, and methods' repeatability and reproducibility were also verified. Parameters of both methods were acceptable for standard tests of pesticide residues.

The laboratory regularly participates in proficiency tests organised by the European Union Reference Laboratories and by FAPAS, and confirms its analytical competencies for conducted tests.

The test results were interpreted in accordance with the criteria included in the European Commission guidelines published in the Document SANCO/12571/2013 (now superseded by Document SANTE 2015) [2, 3], as well as by comparison with the Maximum Residue Levels (MRLs) in force in EU countries [12]. Verification of proper application of pesticides was conducted on a basis of the current "Register of Plant Protection Products Approved for Marketing and Application" [14]. Non-compliances related to pesticide use were notified in the Rapid Alert System for Food and Feed (RASFF).

Table 1. Scope of analysis with levels of quantifications (mg/kg)

Insecticides, their isomers and metabolites	acetamiprid (0.05), acrinathrin (0.01), aldrin (0.01), alpha-cypermethrin (0.01), azinophos-ethyl (0.01), azinophos-methyl (0.05), beta-cyfluthrin (0.01), bifenthrin (0.01), bromophos-ethyl (0.01), bromophos-methyl (0.01), bromopropylate (0.01), buprofezin (0.01), cadusafos (0.01), carbaryl (0.02), carbofuran (0.02), carbosulfan (0.01), chlorantraniliprole (0.01), chlorfenvinphos (0.01), chlorpyrifos (0.01), chlorpyrifos-methyl (0.01), cyfluthrin (0.01), cypermethrin (0.01), p,p'-DDD (0.01), p,p'-DDE (0.01), o,p'-DDT (0.01), p,p'-DDT (0.01), deltamethrin (0.02), diazinon (0.01), dichlorvos (0.01), dicofol (0.01), dieldrin (0.006), dimethoate (0.02), endosulfan alfa (0.01), endosulfan beta (0.01), endosulfan sulphate (0.01), endrin (0.01), esfenvalerate (0.01), ethion (0.01), ethoprophos (0.01), EPN (0.01), etoxazole (0.02), fenamiphos (0.02), fenazaquin (0.01), fenchlorphos (0.01), fenitrothion (0.01), fenoxycarb (0.05), fenpropathrin (0.01), fenthion (0.01), fenvalerate (0.01), fipronil (0.005), flonicamid (0.01), flubendiamide (0.01), formothion (0.01), HCB (0.01), α-HCH (0.01), β-HCH (0.01), γ-HCH (lindane) (0.01), heptachlor (0.01), heptachlor-endo-epoxide (0.003), heptachlor-exo-epoxide (0.001), heptenophos (0.01), hexythiazox (0.01), indoxacarb (0.02), isocarbophos (0.01), isofenphos (0.01), isofenphos-methyl (0.01), isoprocarb (0.01), lambda-cyhalothrin (0.01), lufenuron (0.02), malathion (0.01), mecarbam (0.01), methacrifos (0.01), methidathion (0.01), methoxychlor (0.01), mevinphos (0.01), parathion-ethyl (0.01), parathion-methyl (0.01), permethrin (0.02), phenthoate (0.01), phosalone (0.01), phosmet (0.01), pirimicarb (0.01), pirimiphos-ethyl (0.01), pirimiphos-methyl (0.01), profenofos (0.01), propoxur (0.05), prothiofos (0.01), pyrethrins (0.1), pyridaben (0.02), pyriproxyfen (0.02), quinalphos (0.01), spirodiclofen (0.02), spiromesifen (0.02), spirotetramat (0.1), tau-fluvalinate (0.01), tebufenozide (0.05), tebufenpyrad (0.01), teflubenzuron (0.01), tefluthrin (0.01), tetrachlorvinphos (0.01), tetradifon (0.01), tetramethrin (0.01), thiacloprid (0.02), triazophos (0.01), triflumuron (0.05), zeta-cypermethrin (0.01)
Fungicides	azaconazole (0.01), azoxystrobin (0.01), benalaxyl (0.05), benthiavalicarb-isopropyl (0.01), bitertanol (0.05), bixafen (0.01), boscalid (0.01), bromuconazole (0.01), bupirimate (0.01), captafol (0.02), captan (0.02), carbendazim* (0.05), chlorothalonil (0.01), chlozolinate (0.01), cyflufenamid (0.02), cymoxanil (0.05), cyproconazole (0.01), cyprodinil (0.01), dichlofluanid (0.01), dicloran (0.01), diethofencarb (0.05), difenoconazole (0.01), dimethomorph (0.01), dimoxystrobin (0.01), diniconazole (0.01), diphenylamine (0.05), dithiocarbamates* (mancozeb, maneb metiram propineb, thiram, zineb, ziram) (0.05), epoxiconazole (0.01), etaconazole (0.01), fenamidone (0.02), fenarimol (0.01), famoxadone (0.02), fenbuconazole (0.02), fenhexamid (0.05), fenpropidin (0.01), fenpropimorph (0.02), fludioxonil (0.01), fluquinconazole (0.01), flusilazole (0.01), fluopicolide (0.01), flutolanil (0.02), flutriafol (0.02), folpet (0.01), fuberidazole (0.05), hexaconazole (0.01), imazalil (0.02), imibenconazole (0.01), iprodione (0.02), iprovalicarb (0.04), isoprothiolane (0.01), krezoxim-methyl (0.01), mepanipyrim (0.01), metalaxyl (0.01), metalaxyl-M (0.05), metconazole (0.02), metrafenone (0.01), myclobutanil (0.01), oxadixyl (0.01), penconazole (0.01), pencycuron (0.05), picoxystrobin (0.01), prochloraz (0.01), procymidone (0.01), propiconazole (0.01), prothioconazole-desthio (0.02), proquinazid (0.02), pyraclostrobin (0.02), pyrazophos (0.01), pyrimethanil (0.01), quinoxyfen (0.01), quintozene (0.01), spiroxamine (0.05), tebuconazole (0.02), tecnazene (0.01), tetraconazole (0.01), thiabendazole (0.05), tolclofos-methyl (0.01), tolylfluanid (0.01), triadimefon (0.01), triadimenol (0.01), trifloxystrobin (0.01), triflumizole (0.1), triticonazole (0.01), vinclozolin (0.01), zoxamide (0.01)
Herbicides	acetochlor (0.01), atrazine (0.01), bromacil (0.01), carfentrazone-ethyl (0.01), chloridazon (0.05), chlorotoluron (0.05), chlorpropham (0.01), clomazone (0.01), cyanazine (0.01), cyprazine (0.01), diflufenican (0.01), dimethachlor (0.02), diuron (0.01), fenoxaprop-P (0.1), fluazifop-P (0.05), flufenacet (0.02), flumioxazine (0.02), flurochloridone (0.01), flurtamone (0.02), haloxyfop-2-etotyl (0.05), haloxyfop-methyl (0.05), isoproturon (0.05), lenacil (0.05), linuron (0.05), metamitron (0.1), metobromuron (0.01), metolachlor (0.02), metribuzin (0.01), metazachlor (0.01), monolinuron (0.05), napropamide (0.05), nitrofen (0.01), oxyfluorfen (0.01), pendimethalin (0.02), pethoxamid (0.01), procyazine (0.01), prometryn (0.01), propachlor (0.01), propaquizafop (0.05), propazine (0.01), propham (0.02), propyzamide (0.01), prosulfocarb (0.01), quinoclamine (0.01), simazine (0.01), S-metolachlor (0.02), terbuthylazine (0.02), terbutryn (0.01), trifluralin (0.01)
Growth retardant	paclobutrazol (0.01)
Plant activator	acibenzolar-S-methyl (0.01)
Acaricide	fenpyroximate (0.05)

* determinated as CS_2 residues

RESULTS AND DISCUSSION

In 2012–2015, 138 samples of fruiting vegetables in total (85 samples of tomatoes, 37 samples of cucumbers and 16 samples of pepper) from primary production were tested. The test results were interpreted in accordance with criteria adapted in Europe included in the European Commission recommendations and published in the document SANCO/12571/2013 (now superseded by Document SANTE 2015) [2, 3], as well as on a basis of a MRLs list for pesticides in force in Poland [12].

Of 242 analysed compounds, residues of 17 active substances were found: 15 fungicides and 2 insecticides (Table 2).

Plant protection agent residues were found in 47 samples, representing 34% of all tested samples.

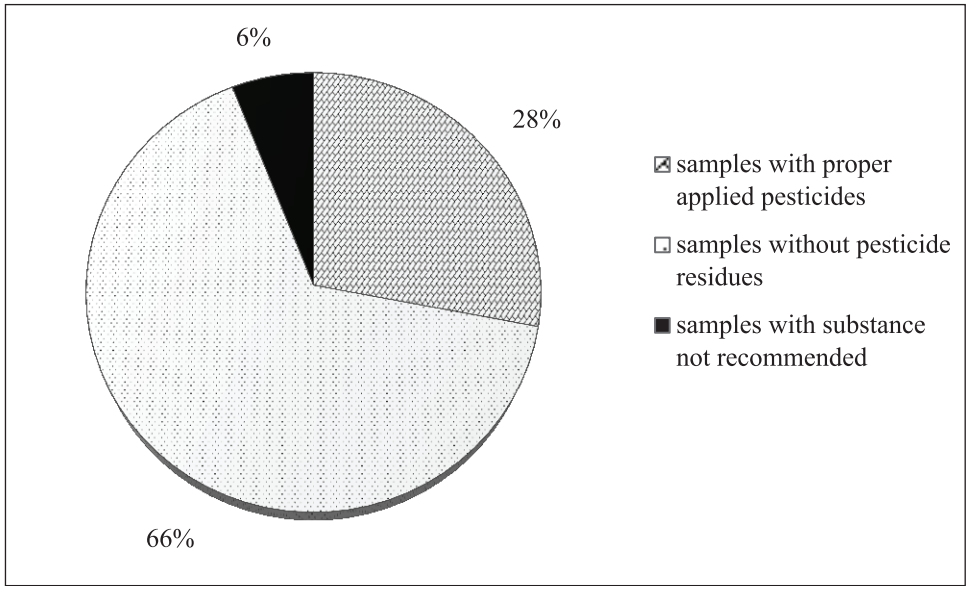

Figure 1. Pesticide residues in fruiting vegetables (2012–2015)

Pesticide residues were most commonly found in tomato – 46% of samples with residues, followed by pepper (25%), while cucumber was a plant in which residues were least common – 12% of samples with residues.

Quantified residue levels were generally low, near or equal to respective quantification limits of analytical methods used.

Of active substances which residues were found in samples of fruiting vegetables, most commonly found included azoxystrobin (as many as 38% of tested samples), boscalid (28%) and chlorothalonil (21%), belonging to fungicides.

Multiple residues were found in analysed vegetables, residues of one compound were found in 26 samples, while residues of two, three and four active substances were found in 13, 5 and 2 samples, respectively. In one sample, residues of as many as five active substances were found.

Non-compliances related to use of plant protection products not authorized for protection of given crops were found in the analysed samples. Azoxystrobin and chlorpyrifos were found in cucumber; chlorpyrifos, as well as fluopicolide and esfenvalerate were found in tomatoes; while boscalid, pyrimethanil and iprodione were found in pepper. Non-compliances related to use of plant protection products containing active substances not approved in the UE were found in practically all crops indicating insufficient number of available agents for their protection. A problem of an insufficient number of plant protection agents for minor uses, including vegetable crops, emerged with withdrawal of many formulations from the market, following the EU review of active substances. It aimed at leaving in the market only those substances that are safe to human health and to the environment. One of the ways to limit use of unapproved pesticides is continuous extending of marketing approvals to small-scale crops [10] and dissemination of information concerning changes in pesticide applications.

No substance was found in any sample which use for plant protection is forbidden [11], in any sample pesticide residues above MRLs were also found. According to other authors in Poland in 2011-2015 the most frequently notified products due to exceeding the MRL were: black currants, tea, Chinese cabbage, lettuce and carrots, but these are marginal percent of all tested samples of food of plant origin [18].

Analysis results and MRL values are listed in Table 2.

Concerning results obtained in previous years in fruiting vegetable samples from the region of south-eastern Poland it can be said that the percentage of detected residues decreased slightly (by ca. 10%) versus years 2010 and 2011 [16, 19]. The obtained results are consistent with monitoring data covering the whole territory of Poland [9, 10, 21] – the comparable level of residues (17%–46%) and the same active substances were detected in the same species of tested fruiting vegetables. The most commonly found substances were fungicides, azoxystrobin, boscalid and chlorothalonil, used to fight mildew, grey mould, alternariosis, white mould or potato blight [14].

Referring to the results obtained from the monitoring encompassing all groups of fruits and vegetables, conducted in previous years (2004 – 2007) [6] and from the central and eastern region of Poland (2014) [20] it can be concluded that in vegetables, especially in fruiting vegetables, pesticide residues were less frequently indicates than in the fruit.

Results of the studies published by The European Food Safety Authority (EFSA) for 2012 and 2013 and concerning monitoring tests for pesticide residues in food products in the European Union Member States

were as follows: 0.9%–1.4% of fruiting vegetable samples exceeded MRLs, 22%–27% contained residues of multiple pesticides (more than one substance in a sample), and 49%–53% of samples did not contain pesticide residues [4, 5]. In Chinese crops overall percentage of samples with pesticide residues was 31%, including 25% and 29% of green peppers and cucumbers, respectively [22]. Nevertheless, more than half (59%) of the cucumbers and tomatoes from the Almaty region of Kazakhstan contained pesticides, while 28% contained pesticide residues above MRLs [8].

When obtained test results are compared to this data, it can be assumed that Polish plant production contained less pesticide residues (66% of residue-free samples) versus EU states and other countries, the percentage of samples with multiple residues (15%) was also lower.

Table 2. Occurrence of pesticide residues in fruiting vegetables (2012–2015)

Fruiting vegetables	Number of samples	Active substance		Samples with residues	Values of found residues [mg/kg]	MRLs [mg/kg]
Greenhouse cucumber	16	azoxystrobin *	F	2	0.02; 0.03	1.0
Field cucumber	21	chlorpyrifos *	I	1	0.03	0.05
		chlorothalonil	F	1	0.01	5.0
		fluopicolide	F	1	0.02	0.5
Greenhouse sweet pepper	15	azoxystrobin	F	1	0.02	1.0
		iprodione *	F	1	0.13	4.0
		pyrimethanil *	F	1	0.02	0.7
Field sweet pepper	1	boscalid *	F	1	0.01	3.0
Greenhouse tomato	82	azoxystrobin	F	15	0.01; 0.01; 0.01; 0.01; 0.01; 0.02; 0.02; 0.02; 0.02; 0.02; 0.03; 0.06; 0.07; 0.09; 0.14	3.0
		boscalid	F	12	0.02; 0.02; 0.03; 0.03; 0.03; 0.04; 0.05; 0.07; 0.08; 0.13; 0.13; 0.21	3.0
		bupirimate	F	1	0.29	2.0
		chlorpyrifos *	I	1	0.11	0.5
		chlorothalonil	F	9	0.01; 0.01; 0.03; 0.03; 0.03; 0.05; 0.07; 0.11; 0.81	6.0
		cyprodinil	F	8	0.01; 0.03; 0.03; 0.03; 0.03; 0.04; 0.04; 0.28	1.5
		difenoconazol	F	2	0.01; 0.02	2.0
		dimethomorph	F	1	0.05	1.0
		dithiocarbamates	F	3	0.07; 0.10; 0.21	3.0
		esfenvalerate *	I	1	0.04	0.1
		famoxadone	F	6	0.02; 0.03; 0.04; 0.05; 0.05; 0.07	2.0
		fenamidone	F	1	0.04	1.0
		fludioxonil	F	8	0.01; 0.01; 0.02; 0.02; 0.03; 0.03; 0.04; 0.04	3.0
		fluopicolide *	F	2	0.02; 0.02	1.0
		iprodione	F	3	0.07; 0.10; 0.98	5.0
		metalaxyl	F	1	0.03	0.2
Field tomato	3	–		–	–	–

MRLs – maximum residues level
* application of the substance not recommended for that crop
– no pesticide residues were found
F –fungicides
I - insecticides

CONCLUSIONS

Agricultural crop monitoring shows to what extent such products are contaminated with pesticide residues ensuring protection of consumers' health and verifying the proper regulatory compliance in the use of the plant protection products. Therefore, these surveys should be continued, and, moreover, this monitoring should be continuously expanded with new active substances.

Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

INFLUENCE OF TEMPERATURE AND BREWING TIME OF NETTLE *(URTICA DIOICA L.)* INFUSIONS ON VITAMIN C CONTENT

Jolanta Wolska[1], Michał Czop[2], Karolina Jakubczyk[1], Katarzyna Janda[1*]

[1]Pomeranian Medical University in Szczecin, Department of Biochemistry and Human Nutrition, Szczecin, Poland
[2]University of Strathclyde, Naval Architecture, Ocean & Marine Engineering, Glasgow, UK

ABSTRACT

Background. Stinging nettle (*Urtica dioica* L.) can be found in temperate climate zones of Europe, Africa and America Nettle may be a source of nutritional ingredients, mineral salts, vitamins and antioxidants.

Objective. The aim of the study was to determine the effect of temperature and brewing time *Urtica dioica* L. infusions from different parts of this plant on vitamin C (ascorbic acid) content.

Material and methods. Infusions of nettle leaf, stem and root were prepared at room temperature, 50°C, 60°C, 70°C and 80°C for 10 minutes. Leaf infusions were also brewed for 5, 10, 15 and 20 minutes at initial water temperature of 60°C. The amount of vitamin C was determined by the spectrophotometric method.

Results. The best temperature of brewing nettle infusions, in terms of vitamin C concentration, is between 50 °C and 60 °C as it is sufficient to extract the substance, yet not high enough to destroy it.

Conclusions. The optimal time of brewing appeared to be 10 minutes as the prolonged exposure to high temperature appeared to be detrimental for ascorbic acid as well.

Key words: *infusion, leaf, stem, root, Urtica dioica, vitamin C*

STRESZCZENIE

Wprowadzenie. Pokrzywa zwyczajna *(Urtica dioica* L.) występuje w strefie klimatu umiarkowanego Europy, Afryki i Ameryki. Może być źródłem składników odżywczych i mineralnych, witamin i antyoksydantów.

Cel badań. Celem badań było określenie wpływu temperatury i czasu przygotowywania naparów z różnych części pokrzywy zwyczajnej na zawartość witaminy C (kwasu askorbinowego).

Materiał i metody. Napary z liści, łodyg i kłączy były przygotowywane w temperaturze pokojowej, 50°C, 60°C, 70°C i 80°C przez 10 minut. Ponadto napary z liści były przygotowane w temperaturze 60°C przez 5, 10, 15 and 20 minut. Zawartość witaminy C określono spektrofotometrycznie.

Wyniki. Optymalna temperatura przygotowywania naparów z pokrzywy, pod kątem uzyskania maksymalnej zawartości witaminy C, mieści się w przedziale od 50°C do 60°C. Jest wystarczająca do przejścia tego związku do naparu i jednocześnie nie jest na tyle wysoka, aby ją zdegradować.

Wnioski. Optymalnym czasem przygotowywania naparów z pokrzywy celem uzyskania jak największego stężenia witaminy C jest czas 10 minut; przedłużanie tego czasu może prowadzić do degradacji kwasu askorbinowego i zmniejszonej jego zawartości w naparach.

Słowa kluczowe: *napar, liście, łodygi, kłącza, Urtica dioica, pokrzywa zwyczajna, witamina C*

INTRODUCTION

Stinging nettle *(Urtica dioica L.*) can be found in temperate climate zones of Europe, Africa and America. Its leaves and stem are covered with small needles that inject substances such as histamine, formic acid or acetic acid, causing irritation and pain when they come in contact with skin, which serves as a protection mechanism. Stinging nettle is associated by most people with red itching bumps it causes and it is often forgotten that nettle is an extremely important plant for medicine. Stinging nettle has a huge variety of properties that are beneficial for human organisms. It can be used to treat arthritis and joint pain. It is also known to have antiulcer, antimicrobial, antioxidant [10, 15, 18, 25] and analgesic properties [4, 11]. It has

[*]**Corresponding author**: Janda Katarzyna, Department of Biochemistry and Human Nutrition, Pomeranian Medical University in Szczecin, Broniewskiego Street 24, 71-460 Szczecin, Poland, tel. +48 91 4414806, e-mail: katarzyna.janda@pum.edu.pl

also been used as a nutritional tea for anaemic people [17]. Stinging nettle is remarkably rich in vitamins A, C and D along with minerals such as iron, manganese, potassium and calcium and is rich in proteins [3]. Nettle leaves contain vitamin C (270 mg%), carotenoids (50 mg%), vitamins: B, K (200 mg%) and E, coumarins, flavonoids, phenolic acids, tannins, phytoncides, glycoside urticin, organic acids, sterols, chlorophyll (up to 5%), alkaloids and minerals. Moreover, nettles contain minerals (especially iron), vitamin C and pro-vitamin A, that is simply digested in the host's small intestine. Nevertheless, to enjoy all the wonders of nettle it must be first turned into an ingestible form. Besides the aforementioned industrial products nettle can be used in home cooking. It is also quite common to prepare nettle in the form of soup or puree. Nettle leaves can also be used as a food additive to other dishes in a similar way as parsley. In spite of the huge variety of possibilities, the most common method of serving stinging nettle is preparing infusions. Nettle is considered to be a nutritive food. Nettle leaf has a long history as a herbal remedy and nutritious addition to the diet [2].

Vitamin C has the molecular formula $C_6H_8O_6$ and the atomic weight of 176.13. It is a water-soluble vitamin as it is polar due to presence of hydroxyl groups. Its structural formula resembles the one of glucose which it is derived from. It is an excellent reducing agent as it can donate one or two electrons forming semi-dehydroascorbic acid and dehydroascorbic acid, respectively. Vitamin C exists only as a solid and as a pure substance it occurs as colourless powder. It is vulnerable to light and air as well as to high temperature. It decomposes completely at approximately 190°C. Vitamin C is considered as one of the most important water soluble vitamins with different important biological functions [7]. Vitamin C is considered to be one of the most active anti-oxidants, which means that it helps the organism to neutralise free radicals. These are elements with unpaired electrons on the valence shell which makes them very reactive species. They are potent oxidation agents, which makes them immensely dangerous as excessive oxidation leads to cell degradation and cancer. Free radicals may be formed in different ways, including digestion of certain types of food, inhalation of tobacco smoke or as a result of being exposed to radiation.

Vitamin C is one of the most important micronutrients postulated to have a beneficial role in health-promoting effects (antioxidant, biosynthesis of collagen, carnitine and hormones, immune response, iron absorption) [5, 12]. According to American National Institute of Cancer vitamin C can be used to improve cancer treatment, yet it is not recognised as a valid method of fighting the disease [8, 16]. Vitamin C is known to boost the immune system, thus its regular ingestion is helpful in protecting oneself from bacteria and viruses which cause flu and other illnesses. Vitamin C is important for maintenance of immunity and stimulation of the interferon synthesis, thus participating in immune modulating processes [1, 9]. People require vitamin C for collagen formation and tissue repair. It is reversibly oxidized to dehydroascorbic acid in the body, which makes it important to oxidation-reduction reactions. Vitamin C is involved in tyrosine metabolism, carbohydrate metabolism, synthesis of lipids and proteins, conversion of folic acid to folinic acid, iron metabolism and helps to improve resistance to infections and cellular respiration [5, 12]. Moreover, vitamin C enhances wound healing and prevents many other diseases such as scurvy [22]. Plants synthesise this compound in order to protect themselves from oxidising stress appearing as a result of photosynthesis, metabolic processes and external pollutants [22].

The aim of the study was to determine the effect of temperature and brewing time of nettle (*Urtica dioica* L.) infusions on vitamin C content.

MATERIALS AND METHODS

Plant material of *Urtica dioica* L. was taken from the ecological farm "Goat Delicacies" in Marwice located in West Pomeranian Voivodeship in northwestern Poland. The nettle was collected in April before blooming. Directly after picking, the nettle was separated into parts: leaves, stems and roots, placed in a refrigerator and stored at constant temperature of -20°C. All measurements were performed within two weeks from plant collection.

Previously frozen parts of *Urtica dioica* L. were unfrozen and then homogenised. 1g of a sample was transferred to a conical flask to which 100 cm^3 of water at given temperature was added. Flask with infusion has been closed and rotated with a speed of 180 rpm for a chosen time. After brewing the plant parts were separated from infusion through filtration. This work shows how the time and temperature of brewing affect the amount of vitamin C present in infusions of three parts of plant. The presented results were obtained in the tests in which the parts of nettle were brewed for 10 minutes at the following temperatures: 25 °C, 50 °C, 60 °C, 70 °C, 80 °C. Infusions from leaves of nettle were also checked for vitamin C presence at constant brewing temperature 60 °C for the following brewing times: 5, 10, 15 and 20 min.

Determination of vitamin C content was carried out according to ISO 6557-2:1984 [13]. In this method 2,6-dichlorophenoloindophenol (2,6-DCPIP) is added to a sample, reacts with vitamin C and after extraction with xylene its excess is determined spectrophotometrically. Absorbance measurements were taken at 500 nm in a 1 cm quartz cuvettes with xylene as a reference.

Measurements were taken on Agilent 8453 UV-VIS spectrophotometer. In this work following chemicals were used: o-xylene of spectrophotometric grade, 98% (SIGMA-ALDRICH), 2,6-dichlorophenolindophenol sodium salt hydrate (SIGMA-ALDRICH), remaining chemicals were of analytical grade. Concentration of vitamin C was expressed in mg of vitamin C per 100 g of infusion (mg/100 g).

Table 1. Statistical parameters of calibration curve for 2,6-dichlorophenoloindophenol from ChemStation

Parameters	2,6-dichlorophenoloindophenol
Number of standards	10
Equation of calibration curve	V = k1*A
Coefficient k1 [cm^3]	2.3216
SD of k1 [cm^3]	0.0404
SD of calibration curve [cm^3]	0.0680
Correlation coefficient R^2	0.9973

The results were analysed statistically using the Statistica 10 software (StatSoft, Poland). Vitamin C measurement for every type of infusion has been repeated five times. From obtained data the mean value and standard deviation (SD) were calculated. *Pearson* coefficient has been used in order to interpret correlation of calibration curve. In order to assess the statistical significance of *Pearson* coefficient P-value has been calculated alongside the Pearson coefficient. Normal distribution of data has been checked with *Shapiro-Wilk's* test. Statistically significant differences between mean values of obtained results were determined by *Student's* t-test for independent samples. Tests were evaluated at the significance level $\alpha = 0,05$.

RESULTS AND DISCUSSION

Vitamin C is unstable and decomposes during the collection of plant material. It is sensitive to light, heat and pH, therefore some amount of this vitamin decomposes during preparation of infusions. Content of vitamin C in infusions depends on various factors: time and temperature of brewing, phase of development of the plant used for preparation of infusions [6].

In Figure 1 the amount of vitamin C with SD in investigated infusions of nettle parts obtained for constant brewing time (10 min) but at different temperatures is presented.

Statistical analysis (*Student's* t-test) implied that between vitamin C contents in all investigated nettle's parts, for almost all temperatures, there is a significant difference ($p < 0,01$). Only one deviation, for leaf vs. stalk at 70 °C, has been found. There is no statistically significant difference between vitamin C amount in infusions made of nettle's leaves and stalk at 70 °C ($p = 0.2878 > \alpha$). Mean values of vitamin C in infusions of leaf and stalk at 70 °C are almost similar (1.88 mg/100 g and 1.85 mg/100 g, respectively). There is observed an increase in vitamin C content along with the increase of temperature of brewing up to 60 °C and then a decrease at higher temperatures: 70 °C and 80 °C. Infusions prepared from leaves of nettle showed higher amount of vitamin C comparing to infusions from other parts of tested plant. At brewing temperature of 60°C the infusions prepared from leaves contained 3 mg/100 g, whereas the infusions from stalk and root contained 1.9 and 2.2 mg/100 g of vitamin C, respectively. Surprisingly infusions from roots of nettle obtained at brewing temperature from 25°C to 60 °C contained more vitamin C than nettle's stalk. Influence of time of brewing on vitamin C concentration in infusions has been investigated at 60°C for nettle's leaf infusions. Those conditions have been chosen due to the highest content of vitamin C.

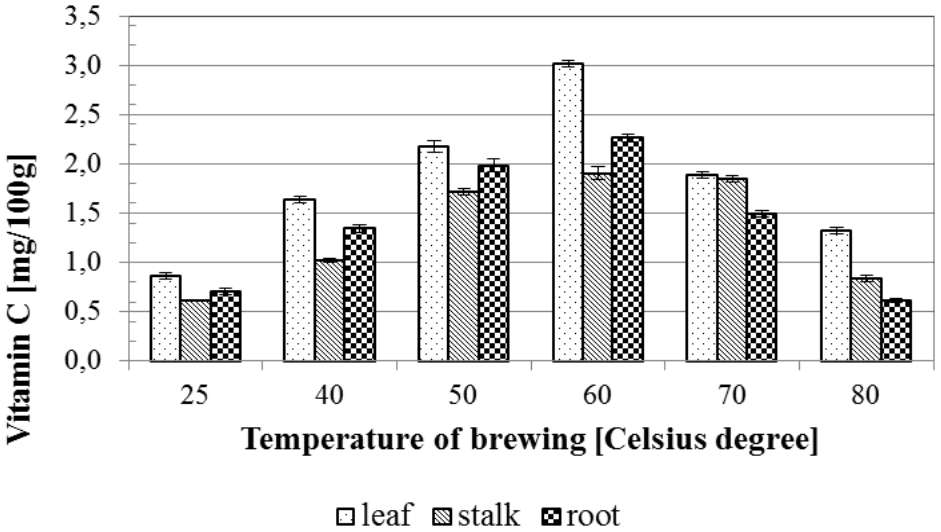

Figure 1. Amount of vitamin C present in infusions from different parts of nettle depending on temperature of brewing

In Figure 2 the results obtained for nettle's leaves infusions at constant temperature (60°C) but for different brewing times are presented.

There is a statistically significant relationship of amount of vitamin C in infusion on time of brewing, which has been confirmed by *Student's* t-test ($p < 0.00001$). It is also clearly visible that 10 minutes is the most efficient time of brewing. The concentration of vitamin C after 10 minutes of brewing is 3 mg/100 g, whereas when the brewing time is extended to 15 and 20 minutes, the amount of vitamin C decreases to 1.3 and 1.0 mg/100 g, respectively. At the same time when the brewing time was 5 minutes the amount of vitamin C in infusion was about 2 mg/100 g.

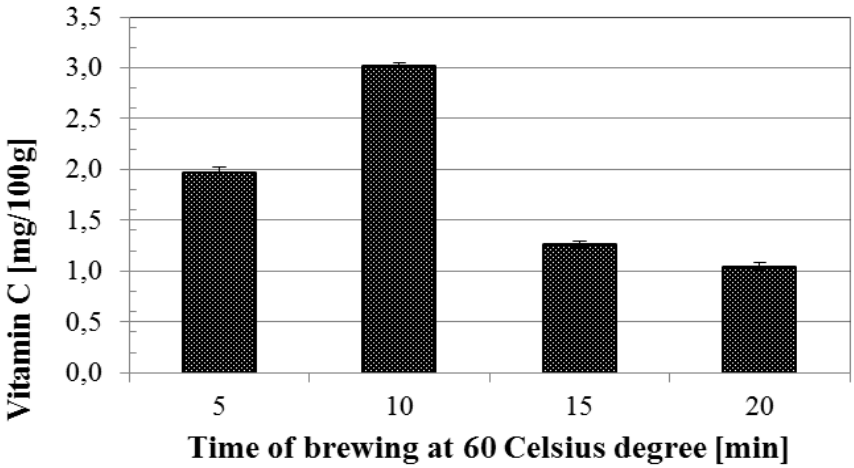

Figure 2. Amount of vitamin C present in nettle's leaves infusions for different time of brewing at constant temperature of 60°C

According to many authors, temperature is one of the main factors that significantly influence the stability of vitamin C in solution [12, 14, 20]. *Njoku* et al. [19] showed that along with the increase of temperature from 20 °C to 80 °C the amount of vitamin C in fruit's juices decreases significantly reaching minimum value at 80 °C. *Suntornsuk* et al. [23] proved, that even storing juices at low temperature of 4°C caused losses in vitamin C concentration. *Skalozubo*va & *Reshetova* [21] determined amount of vitamin C in infusions made of nettles' leaves with iodimetric titration and titration by 2,6-dichlorophenolindophenol sodium salt. Investigated infusions were prepared at 40 °C and possessed small amount of vitamin C – 0.0032 mg%. Our results showed concentration of vitamin C on the level of 1 mg% for 1% infusions made of nettles' leaves. This significant dissimilarity could have been caused by the following differences: chosen method of determination of vitamin C, temperature and procedure of preparation of infusions, source of plant and also degree of fragmentation of nettle.

CONCLUSIONS

In this study it was found that both temperature and time of brewing affects the amount of vitamin C in infusions of leaves, stalk and root of *Urtica dioica* L. Amount of vitamin C present in infusions made of leaves of nettle on average is 1.6 times greater than in infusions from stalks and 1.3 times greater than in infusions from nettle's roots. It appeared that the most effective conditions of brewing, on account of the highest vitamin C concentration in infusion, are 60 °C and 10 min. Based on infusions made from leaves of nettle has been established that while increasing the time of brewing above 10 min, the concentration of vitamin C decreases 2.2 to 2.9 times for 15 and 20 min, respectively. Presented results have proved, that infusions made of different parts of nettle are significant source of vitamin C. Nettles' infusions added to everyday diet, besides pro-health qualities, can be also a valuable replacement of vitamin C for present on the market synthetic L-ascorbic acid.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

NATURAL MINERAL BOTTLED WATERS AVAILABLE ON THE POLISH MARKET AS A SOURCE OF MINERALS FOR THE CONSUMERS. PART 2: THE INTAKE OF SODIUM AND POTASSIUM

Anna Gątarska[1], Joanna Ciborska[2], Elżbieta Tońska[1]*

[1]Department of Commodity Science and Food Research, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland
[2]Human Nutrition Department, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT

Background. Natural mineral waters are purchased and consumed according to consumer preferences and possible recommendations. The choice of appropriate water should take into account not only the general level of mineralization but also the content of individual components, including electrolytes such as sodium and potassium. Sodium is necessary to ensure the proper physiological functions of the body. It is defined as a health risk factor only when its excessive intake occurs. Potassium acts antagonistically towards sodium and calcium ions, contributes to a reduction of the volume of extracellular fluids and at the same time reduces muscle tension and permeability of cell membranes. The demand for sodium and potassium is of particular importance in people expending significant physical effort, where an increased electrolyte supply is recommended.

Objective. The aim of the study was to estimate the content of sodium and potassium in natural mineral waters available in the Polish market and to evaluate the intake of those components with the commercially available mineral waters by different groups of consumers at the assumed volume of their consumption.

Material and Methods. The research material consisted of natural mineral waters of forty various brands available on the Polish market. The examined products were either produced in Poland or originated in other European countries. Among the products under examination, about 30% of the waters were imported from Lithuania, Latvia, the Czech Republic, France, Italy and Germany. A sample for analyses consisted of two package units of the examined water from different production lots. Samples for research were collected at random. The study was conducted with the same samples in in which calcium and magnesium content was determined, which was the subject of the first part of the study [6].

The content of sodium and potassium was determined using the emission technique (acetylene-air flame), with the use of atomic absorption spectrometer – ICE 3000 SERIES – THERMO – England, equipped with a GLITE data station, with wavelengths of 589.0 nm and 766.5 nm, respectively.

Results. The obtained research results indicate a high differentiation of the content of both sodium and potassium in natural mineral waters available on the Polish market, particularly in medium- and highly-mineralized waters. The consumption of 1 liter of low-mineralized natural mineral water ensures recommendations concerning the amount of sodium intake only in a limited scope, while in case of products of medium and high level of mineralization, it is much diversified. On the other hand, potassium supply with one liter of natural mineral water may ensure no more than several percent of the recommended daily intake of this component.

Conclusions. The high diversification of sodium content in natural mineral waters available on the Polish market should encourage the analysis of their composition to avoid health disorders in a given group of consumers. The natural mineral waters examined in the study, at the assumed volume of their daily consumption, are not a good source of potassium for the population groups under analysis.

Key words: *natural mineral water, minerals, sodium, potassium, daily intake*

STRESZCZENIE

Wprowadzenie. Naturalne wody mineralne są nabywane i spożywane przez konsumentów według ich preferencji oraz ewentualnych zaleceń. Dobór odpowiedniej wody powinien uwzględniać nie tylko ogólny stopień mineralizacji, lecz również zawartość poszczególnych składników, w tym elektrolitów takich jak sód i potas. Sód jest niezbędny dla prawidłowego fizjologicznego funkcjonowania organizmu, jako czynnik stanowiący ryzyko zaburzeń zdrowia definiowany jest dopiero wówczas, gdy odnotowuje się jego nadmierne spożycie. Potas działa antagonistycznie do jonów sodu i wapnia, powoduje

[*]**Corresponding author**: Anna Gątarska, Uniwersytet Warmińsko-Mazurski w Olsztynie, Wydział Nauki o Żywności, Katedra Towaroznawstwa i Badań Żywności, Pl. Cieszyński 1, 10-957 Olsztyn, Poland, phone: +48 895234139, Fax +48 89 5233554, e-mail: gicz@uwm.edu.pl 10-957 Olsztyn, Poland, gicz@uwm.edu.pl

obniżenie objętości płynów zewnątrzkomórkowych i jednocześnie zwiększa napięcie mięśniowe i przepuszczalność błon komórkowych. Zapotrzebowanie na sód i potas ma szczególne znaczenie w grupie osób wykonujących znaczący wysiłek fizyczny, gdzie wskazana jest większa podaż elektrolitów.

Cel. Celem badań było oszacowanie rzeczywistej zawartości sodu i potasu w naturalnych wodach mineralnych występujących na polskim rynku oraz ocena pobrania tych składników z badanych produktów przez różne grupy konsumentów przy założonej ilości ich spożycia.

Materiał i metody. Materiał badawczy stanowiły naturalne wody mineralne 40 różnych marek dostępne na polskim rynku. Badane produkty były wyprodukowane w Polsce oraz pochodziły z innych krajów europejskich. Wśród badanych produktów około 30% stanowiły wody pochodzące z Litwy, Łotwy, Czech, Francji, Włoch i Niemiec. Próbkę do badań stanowiły dwa opakowania badanej wody z różnych partii produkcyjnych. Próbki do badań pobierane były losowo. Badania przeprowadzono na tym samym materiale badawczym, na którym dokonano oznaczeń wapnia i magnezu, co było przedmiotem pierwszej części pracy [6].

Zawartość sodu i potasu oznaczono techniką emisyjną (płomień acetylen-powietrze), przy użyciu spektrometru absorpcji atomowej – ICE 3000 SERIES - THERMO - Anglia, wyposażonego w stację danych GLITE, stosując odpowiednio długości fali: 589,0 nm, 766,5 nm.

Wyniki. Uzyskane wyniki badań wskazują na duże zróżnicowanie zawartości zarówno sodu, jak i potasu w naturalnych wodach mineralnych występujących na polskim rynku, szczególnie tych średnio- i wysokozmineralizowanych. Spożycie 1 litra naturalnych wód mineralnych niskozmineralizowanych pozwala w niewielkim stopniu realizować zalecenia dotyczące ilości spożycia sodu, a w przypadku produktów o średnim i wysokim stopniu mineralizacji jest to bardzo zróżnicowane. Natomiast dostarczanie potasu z taką ilością naturalnej wody mineralnej może stanowić co najwyżej kilka procent zalecanej dziennej ilości tego składnika.

Wnioski. Duże zróżnicowanie zawartości sodu w naturalnych wodach mineralnych występujących na polskim rynku powinno skłaniać do analizy ich składu w celu uniknięcia zaburzeń zdrowia u danej grupy konsumentów. Badane naturalne wody mineralne w założonej wielkości dziennego spożycia nie są dobrym źródłem potasu dla analizowanych grup populacyjnych.

Słowa kluczowe: *naturalna woda mineralna, składniki mineralne, sód, potas, dzienne pobranie*

INTRODUCTION

Numerous studies conducted in various countries indicate a high diversity in the composition of natural mineral waters [1, 3, 5, 14]. The importance of bottled waters in a diet results from the occurrence of such mineral components in their content as calcium, magnesium, sodium, potassium or the presence of carbohydrates, which regulate the acid-base balance in the body. Additionally, the ionized form of mineral components in water affects their good assimilability [15, 18, 16, 17]. Evaluation of natural bottled waters available in the Polish market as a source of calcium and magnesium was the subject of the first part of the study in this regard [6]. As the results of that part of the study indicate, natural mineral waters available in the market are characterized by a varied content of calcium and magnesium and even a very high level of product mineralization does not always guarantee significant amounts of these components. Although water can be the source of many mineral components, those occurring in the amounts above 15% of the recommended daily allowance are of significant importance [25, 26]. Natural mineral waters are purchased and consumed according to consumer preferences and recommendations. The choice of appropriate water should take into account not only the general level of mineralization, but also its content of individual components, including electrolytes such as sodium and potassium.

The concentration of K^+ and Na^+ ions in intracellular and extracellular spaces is a particularly important element for maintaining water and electrolyte homeostasis and the acid-base balance of the body. Changes in the concentration of sodium cations, the concentration of which is lower inside the cell than in the extracellular fluid, are a crucial factor for water and electrolyte balance disorders. Na^+ ions are the most significant and the dominant part of mineral components of blood serum and their normal concentration is estimated within the range of 136-145 mmol/L. Both too high and too low sodium content in blood serum can be detrimental to human health. A sodium content below 120 mmol/L can cause hyponatraemia, which is accompanied by nausea, lack of appetite, headaches, orientation disorders and, in extreme cases, tremors and coma. The health condition of the body in which the sodium concentration in blood serum is higher than 145 mmol/L is described as hypernatraemia and an increase in sodium concentration up to 160mmol/l can result in death [8, 9, 10, 13].

Sodium ions are a necessary element of water exchange between cells and intercellular substances, they also actively participate in the transport of nutrients (amino acids, glucose) through cellular membranes.

Sodium is necessary for the proper physiological functioning of the body and is defined as a risk factor for health disorders only when its excessive intake is recorded. It has been demonstrated that

a lowering of blood tension as a result of reducing the amount of sodium in the diet is a linear relation, proven in the interventional U.S. study known as DASH (Dietary Approaches to Stop Hypertension), evaluating the effect of sodium intake on blood pressure. The results of that study helped to prepare dietary recommendations for the prevention and treatment of blood hypertension [22].

Potassium acts antagonistically to sodium and calcium ions, contributes to a reduction of the volume of extracellular fluids while reducing muscle tension and the permeability of cellular membranes. The concentration of K^+ ions is much higher inside the cell than in the extracellular fluid. The extracellular fluid contains only 2% of the systemic potassium and the concentration of K^+ ions in body cells and extracellular spaces is precisely regulated since it guarantees proper nervous and muscular conduction. K^+ ions participate in the regulation of osmotic pressure and pH of the cell and they are also responsible for sending nervous impulses and for muscle contractions, as well as for proper metabolism of proteins and carbohydrates [9, 13, 23]. Low potassium level (hypokalaemia) is diagnosed when the potassium concentration in blood serum is below 3.4 mmol/l, which results in weakness, disorders of the nervous and muscular system functions, general fatigue, constipation, abnormal heart rhythms and kidney dysfunctions. Chronic potassium deficiency can lead to higher blood pressure and diseases of cardiovascular and urinary systems [2, 9]. Hyperkalaemia – an elevated concentration of potassium in blood serum – describes the condition when the concentration of K^+ ions is higher than 5.5 mmol/l. It results in irregularities in the functioning of the muscular and nervous systems and slowed heart rate [8, 9, 13].

The demand for sodium and potassium is of particular importance among people expending significant physical effort, where an increased electrolyte supply is recommended. The increased secretion of sweat, particularly when a physical effort is made at high temperatures, can lead instead to changes in the water content and the concentration of electrolytes in body fluids, resulting in an increase in their osmotic pressure. Sodium prevents rapid dehydration of the body. It is also worth emphasizing that an excessive supply of hypertonic fluids, provided during intensive physical effort, provokes the occurrence of hypertonic overhydration, characterized by an increased concentration of sodium ions in blood plasma, followed by an increase in osmolality and the volume of extracellular fluid and a reduction of the volume of intracellular fluid. The intake of chlorine-sodium waters during physical effort and at high temperatures allows for a reduction of dehydration and has a favourable effect on the performance and well-being of the body [8, 9, 23].

Early childhood is a period when proper dietary habits are developed, including the important habit of consuming water. For infants and children below the age of 3, for whom reduced sodium intake is advised, bottled spring waters or natural mineral waters with low sodium, low mineral and low sulphate level are recommended [23, 24]. Among the youngest consumers and young people, an excessive sodium consumption is often recorded [7, 11, 19, 20], which additionally indicates the need to consume low-sodium waters.

The aim of the study was to estimate the real content of sodium and potassium in natural mineral water available on the Polish market and to evaluate the intake of those components with the examined products by different groups of consumers at the assumed volume of their consumption.

The aim of the study was achieved by determining:

- the real content of sodium and potassium in the examined products,
- the percentage of intake of sodium and potassium with natural mineral water in the recommended daily intake in various age groups.

MATERIAL AND METHODS

The study material consisted of natural mineral waters of 40 various brands available on the Polish market. The examined products were produced in Poland or originated from other European countries. 30% of the examined products were waters imported from such countries as Lithuania, Latvia, the Czech Republic, France, Italy and Germany. A sample for the tests consisted of two package units of the examined water from different production lots. Samples for the research were collected at random. The research was conducted using the same research material for which calcium and magnesium was determined, which was the subject of the first part of the study [6].

The content of sodium and potassium was determined using the emission technique (acetylene-air flame), with the use of an atomic absorption spectrometer – ICE 3000 SERIES – THERMO – England, equipped with a GLITE data station, with wavelengths of 589.0 nm and 766.5 nm, respectively. Determinations were carried out in the AAS laboratory, in which the above methods were validated. The applied concentrations of Na and K standard solutions provided the measurement range of the analytical method, which was characterized by the linearity of calibration curves. The limits of the measurement range (µg/ml) assumed for sodium and potassium, the formula ($y = ax + b$) and the coefficient of calibration curve (R^2 regression), respectively, were as follows:

Na	0.5-4.0	y=22.284+11.985	0.9982
K	2.0-20.0	y=4.6449x+9.2497	0.9891

The determinations used:

- Na and K models: standards of concentration 1 mg/cm^3 dissolved with 0.1 M solution of HNO_3 – by *J.T. Baker*, Holland,
- de-ionized water with resistivity >18.2 MΩ. cm obtained with the use of a water de-ionizer by SYNERGY - MILLIPORE – France.

The reliability of the analytical methods was assessed based an analysis of the certified reference material (Institute of Nuclear Chemistry and Technology). The following results were obtained for:

- sodium (mg/kg) - certified value -24.7±3.2, determined content - 25.1±2.9, recovery (%) - 101.6
- potassium (g/kg) - certified value -17.0±1.2, determined content - 16.8±1.4, recovery (%) - 98.8.

The intake of mineral waters was evaluated according to the norms of sodium and potassium intake, established at the level of adequate intake (AI) for and sex (Table 1) [16]. Additionally, the daily consumption of natural mineral water was assumed at the level of one liter. The evaluation did not take into account factors affecting the bioavailability of components in the body, as it was not the subject of the research.

The statistical analysis was carried out using Statistica 12 software (StatSoft, USA).

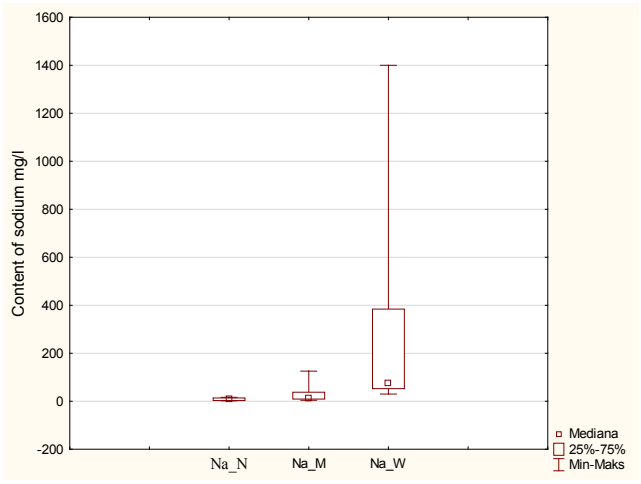
Table 1. Norms for electrolytes established at the level of adequate intake (AI) [Jarosz, 2012]

Group (sex, age/years)	Potassium (mg/d)	sodium (mg/d)
	AI	AI
Children		
1-3	2400	750
4-6	3100	1000
7-9	3700	1200
Boys		
10 – 12	4100	1300
13 – 15	4700	1500
16 - 18	4700	1500
Men		
19 – 30	4700	1500
31 – 50	4700	1500
51 – 65	4700	1400
66 – 75	4700	1300
≥ 75	4700	1200
Girls		
10 – 12	4100	1300
13 – 15	4700	1500
16 - 18	4700	1500
Woman		
19 – 30	4700	1500
31 – 50	4700	1500
51 – 65	4700	1400
66 – 75	4700	1300
≥ 75	4700	1200

RESULTS AND DISCUSSION

Sodium and potassium content in natural mineral waters available on the market

The natural mineral waters available in the Polish market are characterized by a varied level of mineralization, which is determined by the general content of mineral component. While analysing the dietary quality of this group of products, one criteria is the content of individual components, including the amount of sodium and potassium. As regards the group of low-mineralized natural mineral waters (which accounted for about ¼ of the examined products) all of them were characterized by low sodium content (Table 2). The amount of this component ranged from about 2 to about 16 mg in one liter. The physiological effects of such waters on the human body, as one of the criteria for classifying them as natural mineral waters, results exactly from the low content of sodium (below 20 mg/l). Diversification of the sodium content in this group of products was strongly lower than in medium- and highly-mineralized waters [Figure 1a]. The waters with a low mineralization level also featured the lowest diversification of potassium content, with values ranging from 0.59 to 3.42 mg/L (Table 2).



N – low-mineralized natural mineral water
S – medium-mineralized natural mineral water
W – high-mineralized natural mineral water

Figure 1a. Content of sodium in natural mineral water

The content of sodium in products with medium and high levels of mineralization was definitely higher (Table 2, Figure 1a), with sodium levels ranging from 4.6 to 808.40mg/l and from 31.38 to 1400.05 mg/L, respectively. These waters, as compared to low-mineralized waters, were also characterized by a high diversification of the potassium content (Table 2, Figure 1b). Among these waters, it is also possible to find products with a potassium ion content below 1mg/L, as well as products for which this value exceeded 50

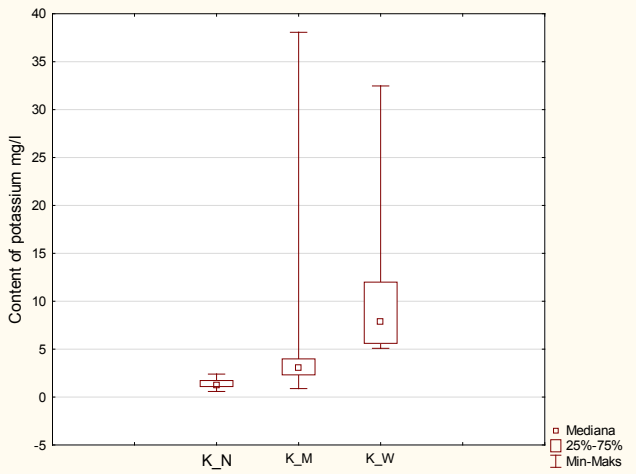
mg/l. The previous characteristics of natural mineral waters available on the market also indicate the high variability of sodium and potassium ions in the group of cations found in natural mineral waters [12].

Table 2. Sodium and potassium content in natural mineral waters, (mg/L)

Trade name of product	Content of minerals, mg/L	
	Na	K
Low-mineralized natural mineral waters		
Artic	7.65	1.53
Bolle	3.43	1.14
Dobrowianka	1.94	1.06
Evian	6.35	1.18
Fonta de Medici	16.32	1.92
Kropla Beskidu	11.70	1.29
Neptunas	2,48	0.59
Salinger	15.91	3,42
Vilsa	16.09	2.40
Medium-mineralized natural mineral waters		
Augustowianka	125.41	10.68
Cisowianka	9.91	4.06
Everest	11.02	3.02
Food & Joy Natural Mineral Water	38.53	4.10
Grodziska Tesco	15.08	2.90
Jurajska	9.22	3.91
Kinga Pienińska	4.06	3.82
Krystynka	808.40	13.07
Magnesja	4,50	1.52
Nałęczowianka	8.94	3.70
Naturalna Woda Min. Sudety (Carrefour)	37.88	3.06
Naturalna woda mineralna Kaufland	37.68	1.92
Perrier	10.11	0.89
Polanica Tesco	36.36	3.16
Polaris	11.92	2.79
San Pellegrino	28.86	2.66
Ustronianka	5.19	1.32
Veroni Mineral	8.13	2.88
Wielka Pieniawa	57.00	38.07
Wielka Wieniecka	15.18	1.98
Zdroje Piwniczna	82.01	5.11
High-mineralized natural mineral waters		
Borjomi	1400.05	32.47
Darida	1071.5	13.91
Kryniczanka	52.72	6.50
Muszyna Skarb Życia	31.38	5.61
Muszyna Tesco Cechini	30.10	5.09
Muszynianka	73.41	7.88
Naturalna Woda Mineralna Piotr i Paweł	63.65	9.19
Staropolanka	110.55	53.09

Percentage of sodium and potassium intake with natural mineral water in the daily intake of these components in different population groups.

The evaluation of body demand for sodium and potassium is difficult due to a lack of clear data and research results which would make it possible to determine the recommended daily allowance (RDA) or estimated average requirement (EAR). Consequently, the norms of sodium and potassium intake were established at the level of appropriate intake (AI).



N - low-mineralized natural mineral water
S – medium-mineralized natural mineral water
W- high-mineralized natural mineral water

Figure 1b. Content of potassium in natural mineral water

The analysis of sodium intake with low-mineralized waters (Table 3) showed that for all population groups under analysis, this type of natural mineral water is not a significant source of sodium in a diet. An adequate intake of sodium was provided to the highest extent in the group of children aged 1-3 years (Vilsa, Salinger, Fronta de Medici waters) and when determined as a percentage of AI coverage it amounted to 2.12-2.18. For other population groups, it was low and provided about 1% of the referential values.

The intake of 1 liter of medium-mineralized mineral water (Table 4) ensures, depending on the product, very good or poor satisfaction of the recommendations concerning dietary sodium intake. A high sodium content was found in "Krystynka" water, the consumption of which, pursuant to the research assumptions, provides almost 54% of AI for women and men. An equally high percentage of meeting the recommendations was observed in population groups of girls and boys, and among children aged 1-3, the supply of 1 litre of this water provides almost 108% of AI, for children aged 4-6 years it provides 81%, and for 7-9-year-old children it provides 67.37%. Other medium-mineralized mineral waters examined satisfy the recommendation at the level between a few and twenty percent (Augustowianka,

Zdroje Piwniczna, Wielka Pieniawa) and in an amount equal to or less than 1% AI established for individual population groups (Veroni Mineral, Ustronianka, Magnesja, Jurajska).

Table 3. Percentage of daily intake of sodium with low-mineralized natural mineral water at the assumed consumption of 1 liter of water, %.

Trade name of product	Children			Girls			Boys			Woman			Men		
	Age (years)														
	1-3	4-6	7-9	10-12	13-15	16-18	10-12	13-15	16-18	19-30	31-50	51-65	19-30	31-50	51-65
Artic	1,02	0,77	0,64	0,59	0,51	0,51	0,59	0,51	0,51	0,51	0,51	0,54	0,51	0,51	0,54
Bolle	0,46	0,34	0,29	0,26	0,23	0,23	0,26	0,23	0,23	0,23	0,23	0,25	0,23	0,23	0,25
Dobrowianka	0,26	0,19	0,16	0,15	0,13	0,13	0,15	0,13	0,13	0,13	0,13	0,14	0,13	0,13	0,14
Evian	0,85	0,63	0,53	0,49	0,42	0,42	0,49	0,42	0,42	0,42	0,42	0,45	0,42	0,42	0,45
Fonta de Medici	**2,18**	1,63	1,36	1,26	1,09	1,09	1,26	1,09	1,09	1,09	1,09	1,17	1,09	1,09	1,17
Kropla Beskidu	1,56	1,17	0,98	0,90	0,78	0,78	0,90	0,78	0,78	0,78	0,78	0,84	0,78	0,78	0,84
Neptunas	0,33	0,25	0,21	0,19	0,16	0,16	0.19	0,16	0,16	0,16	0,16	0,18	0,16	0,16	0,18
Salinger	**2,12**	1,59	1,33	1,22	1,06	1,06	1,22	1,06	1,06	1,06	1,06	1,14	1,06	1,06	1,14
Vilsa	**2,14**	1,61	1,34	1,24	1,07	1,07	1,24	1,07	1,07	1,07	1,07	1,15	1,07	1,07	1,15

Table 4. Percentage of daily intake of sodium with medium-mineralized natural mineral water at the assumed consumption of 1 liter of water, %.

Trade name of product	Children			Girls			Boys			Woman			Men		
	Age (years)														
	1-3	**4-6**	**7-9**	**10-12**	**13-15**	**16-18**	**10-12**	**13-15**	**16-18**	**19-30**	**31-50**	**51-65**	**19-30**	**31-50**	**51-65**
Augustowianka	**16,72**	**12,54**	**10,45**	**9,65**	**8,36**	**8,36**	**9,65**	**8,36**	**8,36**	**8,36**	**8,36**	**8,96**	**8,36**	**8,36**	**8,96**
Cisowianka	1,32	0,99	0,83	0,76	0,66	0,66	0,76	0,66	0,66	0,66	0,66	0,71	0,66	0,66	0,71
Everest	1,47	1,10	0,92	0,85	0,73	0,73	0,85	0,73	0,73	0,73	0,73	0,79	0,73	0,73	0,79
Food&Joy Natural Mineral Water	5,14	3,85	3,21	2,96	2,57	2,57	2,96	2,57	2,57	2,57	2,57	2,75	2,57	2,57	2,75
Grodziska Tesco	2,01	1,51	1,26	1,16	1,01	1,01	1,16	1,01	1,01	1,01	1,01	1,08	1,01	1,01	1,08
Jurajska	1,23	0,92	0,77	0,71	0,61	0,61	0,71	0,61	0,61	0,61	0,61	0,66	0,61	0,61	0,66
Kinga Pienińska	0,54	0,41	0,34	0,31	0,27	0,27	0,31	0,27	0,27	0,27	0,27	0,29	0,27	0,27	0,29
Krystynka		**80,84**	**67,37**	**62,18**	**53,89**	**53,89**	**62,18**	**53,89**	**53,89**	**53,89**	**53,89**	**57,74**	**53,89**	**53,89**	**57,74**
Magnesja	0,60	0,45	0,38	0,35	0,30	0,30	0,35	0,30	0,30	0,30	0,30	0,32	0,30	0,30	0,32
Nałęczowianka	1,19	0,89	0,75	0,69	0,60	0,60	0,69	0,60	0,60	0,60	0,60	0,64	0,60	0,60	0,64
Natural mineral water Sudety (Carrefour)	5,05	3,79	3,16	2,91	2,53	2,53	2,91	2,53	2,53	2,53	2,53	2,71	2,53	2,53	2,71
Natural mineral water Kaufland	**5,02**	**3,77**	**3,14**	**2,90**	**2,51**	**2,51**	**2,90**	**2,51**	**2,51**	**2,51**	**2,51**	**2,69**	**2,51**	**2,51**	**2,69**
Perrier	1,35	1,01	0,84	0,78	0,67	0,67	0,78	0,67	0,67	0,67	0,67	0,72	0,67	0,67	0,72
Polanica Tesco	4,85	3,64	3,03	2,80	2,60	2,60	2,80	2,42	2,42	2,42	2,42	2,60	2,60	2,60	2,60
Polaris	1,59	1,19	0,99	0,92	0,79	0,79	0,92	0,79	0,79	0,79	0,79	0,85	0,79	0,79	0,85
San Pellegrino	3,48	2,89	2,41	2,22	1,92	1,92	2,22	1,92	1,92	1,92	1,92	2,06	1,92	1,92	2,06
Ustronianka	0,69	0,52	0,43	0,40	0,35	0,35	0,40	0,35	0,35	0,35	0,35	0,37	0,35	0,35	0,37
Veroni Mineral	1,08	0,81	0,68	0,63	0,54	0,54	0,63	0,54	0,54	0,54	0,54	0,58	0,54	0,54	0,58
Wielka Pieniawa	**7,60**	**5,70**	**4,75**	**4,38**	**3,80**	**3,80**	**4,38**	**3,80**	**3,80**	**3,80**	**3,80**	**4,07**	**3,80**	**3,80**	**4,07**
Wielka Wieniecka	2,02	1,52	1,27	1,17	1,01	1,01	1,17	1,01	1,01	1,01	1,01	1,08	1,01	1,01	1,08

Table 5. Percentage of daily intake of sodium with high-mineralized natural mineral water at the assumed consumption of 1 liter of water, %.

Trade name of product	Children			Girls			Boys			Woman			Men		
	Age (years)														
	1-3	**4-6**	**7-9**	**10-12**	**13-15**	**16-18**	**10-12**	**13-15**	**16-18**	**19-30**	**31-50**	**51-65**	**19-30**	**31-50**	**51-65**
Borjomi	**186,73**	**140,00**	**116,67**	**107,70**	**93,34**	**93,34**	**107,70**	**93,34**	**93,34**	**93,34**	**93,34**	**100,00**	**93,34**	**93,34**	**100,00**
Darida	**142,87**	**107,15**	**89,29**	**82,42**	**71,43**	**71,43**	**82,42**	**71,43**	**71,43**	**71,43**	**71,43**	**76,54**	**71,43**	**71,43**	**76,54**
Kryniczanka	**7,03**	**5,27**	**4,39**	4,06	3,51	3,51	4,06	3,51	3,51	3,51	3,51	3,77	3,51	3,51	3,77
Muszyna Skarb Życia	4,18	3,14	2,62	2,41	2,09	2,09	2,41	2,09	2,09	2,09	2,09	2,24	2,09	2,09	2,24
Muszyna Tesco Cechini	4,01	3,01	2,51	2,32	2,01	2,01	2,32	2,01	2,01	2,01	2,01	2,15	2,01	2,01	2,15
Muszynianka	**9,79**	**7,34**	**6,12**	**5,65**	4,89	4,89	5,65	4,89	4,89	4,89	4,89	5,24	4,89	4,89	5,24
Naturalna Woda Mineralna Piotr i Paweł	**8,49**	**6,37**	**5,30**	**4,98**	4,24	4,24	4,98	4,24	4,24	4,24	4,24	4,55	4,24	4,24	4,55
Staropolanka	**14,73**	**11,06**	**9,21**	**8,50**	**7,37**	**7,37**	**8,50**	**7,37**	**7,37**	**7,37**	**7,37**	**7,90**	**7,37**	**7,37**	**7,90**
Wysowianka	**51,20**	**38,40**	**32,00**	**29,53**	**25,60**	**25,60**	**29,53**	**25,60**	**25,60**	**25,60**	**25,60**	**27,43**	**25,60**	**25,60**	**27,43**
Źródła Muszyny	**15,93**	**11,95**	**9,96**	**9,19**	**7,96**	**7,96**	**9,19**	**7,96**	**7,96**	**7,96**	**7,96**	**8,53**	**7,96**	**7,96**	**8,53**

Table 6. Percentage of daily intake of potassium with low-mineralized natural mineral water at the assumed consumption of 1 liter of water, %.

Trade name of product	Children			Girls			Boys			Woman			Men		
	Age (years)														
	1-3	**4-6**	**7-9**	**10-12**	**13-15**	**16-18**	**10-12**	**13-15**	**16-18**	**19-30**	**31-50**	**51-65**	**19-30**	**31-50**	**51-65**
Artic	0,06	0,05	0,04	0,04	0,03	0,03	0,04	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Bolle	0,05	0,04	0,03	0,03	0,02	0,02	0,03	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Dobrowianka	0,04	0,03	0,03	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Evian	0,05	0,04	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Fonta de Medici	0,08	0,06	0,05	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Kropla Beskidu	0,54	0,04	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03

The highly-mineralized waters examined (Table 5) are a generally good or very good source of sodium (Borjomi, Darida waters) satisfying 70-100% of the recommendations or even significantly exceeding them in population groups of children aged 10-12. It should also be emphasized that highly-mineralized waters can be low in sodium and ensure specific AI at a level below 5% for most population groups. The study by *Rychlik* [21] carried out with the participation of 11-13-year-old students of Warsaw schools proved that mineral and spring waters consumed by the youth covered sodium intake norms specified for this group at the level of 0.9-1.2%. The research carried out among students of the Warsaw University of Life Sciences and the Cardinal Wyszyński University in Warsaw [4] demonstrated that sodium intake with bottled water was at the level of about 1% of the referential value and potassium intake was about 0.04% AI, regardless of the university type.

The examined natural mineral waters in the assumed amount of daily consumption are not a good source of potassium for all population groups under analysis. For highly-mineralized waters (Table 8), the highest satisfaction of recommendations can be obtained through the supply of Staropolanka and Borjomi waters (from 0.69% AI for women, men, girls and boys aged 13-15 and 16-18 years old to 1.35% and 2.21% AI for children aged 1-3), while among medium-mineralized waters (Table 7), the best source of potassium is Wielka Pieniawa water, satisfying

1.58% of the recommended levels of AI for 1-3-year-old children, to 0.81 – 0.93% of the referential values for women, men, girls and boys.

Low-mineralized natural mineral waters (Table 6), at the assumed daily intake of 1 liter, satisfy dietary recommendations concerning potassium supply in a diet in the amount of tenths or hundreds of a percentage of referential values for individual population groups. The highest values (obtained for Salinger water) were at the level of 0.07% with reference to AI for women, men, boys and girls and up to 0.14% with reference to AI for children aged 1-3.

Table 7. Percentage of daily intake of potassium with medium-mineralized natural mineral water at the assumed consumption of 1 liter of water, %.

Trade name of product	Children			Girls			Boys			Woman			Men		
	Age (years)														
	1-3	**4-6**	**7-9**	**10-12**	**13-15**	**16-18**	**10-12**	**13-15**	**16-18**	**19-30**	**31-50**	**51-65**	**19-30**	**31-50**	**51-65**
Augustowianka	0,44	0,34	0,29	0,26	0,23	0,23	0,26	0,23	0,23	0,23	0,23	0,23	0,23	0,23	0,23
Cisowianka	0,17	0,13	0,11	0,10	0,08	0,08	0,10	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
Everest	0,13	0,09	0,08	0,07	0,06	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
Food&Joy Natural Mineral Water	0,17	0,13	0,11	1,00	0,09	0,09	1,00	0,09	0,09	0,09	0,09	0,09	0,09	0,09	0,09
Grodziska Tesco	0,12	0,09	0,08	0,07	0,06	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
Jurajska	0,16	0,13	0,11	0,09	0,08	0,08	0,09	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
Kinga Pienińska	0,15	0,12	0,10	0,09	0,08	0,08	0,09	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
Krystynka	0,54	0,42	0,35	0,31	0,28	0,28	0,31	0,28	0,28	0,28	0,28	0,28	0,28	0,28	0,28
Magnesja	0,06	0,05	0,04	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Nałęczowianka	0,15	0,12	0,10	0,09	0,08	0,08	0,09	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
Natural mineral water Sudety (Carrefour)	0,13	0,10	0,08	0,07	0,06	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
Naturalna woda mineral. Kaufland	0,08	0,06	0,05	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Perrier	0,03	0,03	0,02	0,02	0,01	0,01	0,02	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Polanica Tesco	0,13	0,10	0,09	0,07	0,06	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
Polaris	0,12	0,09	0,08	0,07	0,06	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
San Pellegrino	0,11	0,09	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
Ustronianka	0,05	0,04	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Veroni Mineral	0,12	0,09	0,08	0,07	0,06	0,06	0,07	0,06	0,06	0,06	0,06	0,06	0,06	0,06	0,06
Wielka Pieniawa	**1,58**	**1,23**	**1,03**	0,93	0,81	0,81	0,93	0,81	0,81	0,81	0,81	0,81	0,81	0,81	0,81
Wielka Wieniecka	0,08	0,06	0,05	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04

Table 8. Percentage of daily intake of potassium with high-mineralized natural mineral water at the assumed consumption of 1 liter of water, %.

Trade name of product	Children			Girls			Boys			Woman			Men		
	Age (years)														
	1-3	**4-6**	**7-9**	**10-12**	**13-15**	**16-18**	**10-12**	**13-15**	**16-18**	**19-30**	**31-50**	**51-65**	**19-30**	**31-50**	**51-65**
Borjomi	**1,35**	**1,05**	0,88	0,79	0,69	0,69	0,79	0,69	0,69	0,69	0,69	0,69	0,69	0,69	0,69
Darida	0,58	0,45	0,38	0,34	0,30	0,30	0,34	0,30	0,30	0,30	0,30	0,30	0,30	0,30	0,30
Kryniczanka	0,27	0,21	0,18	0,16	0,14	0,14	0,16	0,14	0,14	0,14	0,14	0,14	0,14	0,14	0,14
Muszyna Skarb Życia	0,23	0,18	0,15	0,13	0,12	0,12	0,13	0,12	0,12	0,12	0,12	0,12	0,12	0,12	0,12
Muszyna Tesco Cechini	0,21	0,16	0,14	0,12	0,11	0,11	0,12	0,11	0,11	0,11	0,11	0,11	0,11	0,11	0,11
Muszynianka	0,33	0,25	0,21	0,19	0,17	0,17	0,19	0,17	0,17	0,17	0,17	0,17	0,17	0,17	0,17
Naturalna Woda Mineralna Piotr i Paweł	0,38	0,30	0,25	0,22	0,19	0,19	0,22	0,19	0,19	0,19	0,19	0,19	0,19	0,19	0,19
Staropolanka	**2,21**	**1,71**	**1,43**	**1,29**	**1,13**	**1,13**	**1,29**	**1,13**	**1,13**	**1,13**	**1,13**	**1,13**	**1,13**	**1,13**	**1,13**
Wysowianka	0,50	0,39	0,32	0,29	0,26	0,26	0,29	0,26	0,26	0,26	0,26	0,26	0,26	0,26	0,26

CONCLUSIONS

1. The high diversification of the sodium content in natural mineral waters available on the Polish market should encourage an analysis of their composition in order to avoid health disorders in a given group of consumers.
2. The examined natural mineral waters in the assumed amount of daily consumption are not a good source of potassium for the population groups under analysis.

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Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

DETERMINANTS OF THE USE OF DIETARY SUPPLEMENTS AMONG SECONDARY AND HIGH SCHOOL STUDENTS

*Karolina Gajda, Monika Zielińska, Anna Ciecierska, Jadwiga Hamułka**

Department of Human Nutrition, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (SGGW), Warsaw, Poland

ABSTRACT

Background. All over the world, including Poland, the sale of dietary supplements is increasing. More and more often, people including children and youths, use dietary supplements on their own initiative and without any medical indications or knowledge in this field.

Objectives. Analysis of the conditions of using the dietary supplements with vitamins and minerals among secondary school and high school students in Poland.

Material and methods. The study included 396 students aged 13-18 years (249 girls and 147 boys). Authors' questionnaire was used to evaluate the intake of dietary supplements. The use of cluster analysis allowed to distinguish groups of students with similar socio-demographic characteristics and the frequency of use of dietary supplements.

Results. In the studied population of students three clusters were created that significantly differed in socio-demographic characteristics. In cluster 1 and 2, were mostly students who used dietary supplements (respectively, 56% of respondents and 100%). In cluster 1 there were mostly students coming from rural areas and small city, with a worse financial situation, mainly boys (56%), while cluster 2 was dominated by girls (81%) living in a big city, coming from families with a good financial situation and who were more likely to be underweight (28.8%). In cluster 3 there were mostly older students (62%), not taking dietary supplements. In comparison to cluster 2, they had lower frequency of breakfast consumption (55% vs. 69%), but higher frequency of the consumption of soft drinks, fast-food, coffee as well as salt use at the table.

Conclusions. The results show that the use of dietary supplements in adolescence is a common phenomenon and slightly conditioned by eating behaviors. This unfavorable habit of common dietary supplements intake observed among students indicates the need for education on the benefits and risks of the supplements usage.

Key words: *dietary supplements, vitamins, minerals, students, nutritional behaviors*

STRESZCZENIE

Wprowadzenie. Na całym świecie, również w Polsce rośnie sprzedaż suplementów diety. Coraz częściej sięgają po nie osoby, w tym również dzieci i młodzież bez odpowiednich zaleceń (wskazań medycznych) oraz wiedzy w tym zakresie.

Cel. Analiza uwarunkowań stosowania suplementów diety zawierających witaminy i składniki mineralne przez uczniów gimnazjum oraz szkół ponadgimnazjalnych w Polsce.

Materiał i metody. Badaniami objęto 396 uczniów w wieku 13–18 lat (249 dziewcząt i 147 chłopców). Do oceny spożycia suplementów diety zastosowano autorski kwestionariusz ankiety. Zastosowanie metody analizy skupień pozwoliło na wyodrębnienie grup uczniów o podobnych cechach sojco-demograficznych oraz częstotliwości stosowania suplementów diety.

Wyniki. W badanej grupie uczniów wyodrębniono trzy skupienia różniące się między sobą istotnie w obszarze cech socjo-demograficznych. W skupieniu 1 i 2 dominowali uczniowie stosujący suplementy diety (1 - 56%; 2 - 100% badanych). W skupieniu 1 przeważali uczniowie pochodzący ze wsi i mniejszego miasta, o słabszej sytuacji finansowej, głównie chłopcy (56%), natomiast w skupieniu 2 przeważały dziewczęta (81%) mieszkające w dużym mieście, pochodzące z rodzin o dobrej sytuacji materialnej oraz, u których częściej występowała niedowaga (28,8%). W skupieniu 3 przeważali uczniowie starsi (62%), nie stosujący suplementów diety. Charakteryzowali się oni, w porównaniu do uczniów ze skupienia 2, niższą częstotliwością spożywania I śniadań (55% vs. 69%), natomiast wyższą konsumpcją napojów słodzonych, dań typu *fast-food*, kawy oraz częstszym dosalaniem potraw.

Wnioski. Uzyskane wyniki wskazują, że stosowanie suplementów diety w okresie młodzieńczym jest zjawiskiem powszechnym i w niewielkim stopniu uwarunkowanym zachowaniami żywieniowymi. Zaobserwowany niekorzystny zwyczaj przyjmowania suplementów przez uczniów wskazuje na konieczność edukacji w zakresie korzyści i zagrożeń wynikających z bezpiecznego stosowania suplementów diety.

Słowa kluczowe: *suplementy diety, witaminy, składniki mineralne, młodzież szkolna, zachowania żywieniowe*

*__Corresponding author__: Jadwiga Hamułka, Chair of Nutritional Assessment, Department of Human Nutrition, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (SGGW), Nowoursynowska Street 159C, 02-776 Warsaw, Poland, phone +48 22 59 37 112, fax +48 22 59 37 123, e-mail: jadwiga_hamulka@sggw.pl

INTRODUCTION

The source of nutrients should be well balanced diet reach in vegetables and fruits and with limited amounts of highly processed foods. Research shows, however, that lifestyle, rush, exposure to stress, low physical activity, use of stimulants and poor diet can lead to vitamin and mineral deficiencies, and numerous diseases [3, 7, 17, 21]. A group particularly vulnerable to the effects of poor nutrition, including nutrients deficiencies, are children and youth. Other nutritional strategies to overcome nutrients deficiencies, beside improving the diet *per se*, are to consume fortified food products or dietary supplements [1, 4, 15]. However, the usage of dietary supplements should be undertaken consciously and should be justified from the health perspective. Numerous studies indicate, that supplementation is coming more and more popular, even among children and adolescents [6, 8, 11, 23]. Daily diet is supplemented, on average, by 10 to 80% of people [9, 11, 18, 20, 26], and taking dietary supplements stems mainly from the conviction that they complement inadequate diet, or reduce the risk of serious health complications [13]. On the other hand, one should keep in mind the unjustified usage of supplements without consultations with a doctor or dietitian may impair balance in the organism, lead to hyperalimentation or numerous diseases. Therefore, it is recommended that the taken amounts of supplements would not exceed doses recognized as safe, according to the current knowledge, in order to eliminate the effects of poor nutrition [1, 4, 22].

The increasing number of dietary supplements available on the market in Poland, more and more aggressive advertising in the media and increasing percentage of people taking supplements indicate the need of continues monitoring of this phenomenon and of inclusion this question into nutritional studies. Hence the aim of this study was to analyze the conditions of use of dietary supplements containing vitamins and minerals among secondary school and high schools students.

MATERIAL AND METHODS

For this study, students were selected from secondary and high schools, that principals had agreed to conduct the research in their schools. The purposive sampling was used for participants selection, and the study was carried out after obtaining the written consent from the student's parents or guardians. Finally, in the study 396 students in the aged of 13-18 years old participated, including 249 girls (63%) and 147 boys (37%), from Warsaw and surrounding areas. The survey was conducted in years 2013-2014, in autumn-winter period. Data were collected by direct interview by the questionnaire. The questionnaire contained questions about socio-demographic situation of students, selected elements of their lifestyle, including diet, usage of stimulants and dietary supplements, and physical activity. On the basis of data on height and weight BMI z-score was calculated for the appropriate age and the classification by *Kułaga* et al. [16] was applied to assess the accuracy of body weight. BMI z-score ≤-2SD was used as a threshold value for the weight deficiency, ≥+1SD for overweight and ≥+2SD for obesity [16].

Statistical analysis was performed with STATISTICA ver. 10. In order to distinguish clusters of students differing in the use of dietary supplements as well as socio-demographic characteristics, self-organizing *Kohonen's* network model was used. The analysis was conducted using a random sampling method; sample size parameter equaled 100% for the training set and the 0% for test and validation sets; the epoch number was set at 200.

The input variables for analysis were: usage of dietary supplements and selected socio-demographic determinants like gender, age, BMI category, place of residence, self-estimated material situation and parents' level of education. Characteristics of each cluster was also carried out in terms of some features of diet and lifestyle, including consumption of stimulants, and physical activity. Chi^2 test was used to assess the differences between clusters and p-value ≤0.05 was applied as the level of significance for all statistical analysis.

RESULTS

The characteristic of the population in terms of socio-demographic features is presented in Table 1. Youths 16-18 years old, girls, coming from families of average financial situation, whose parents had mainly secondary or higher education predominated in the studied group. The majority of respondents (85%) had normal body mass index as BMI z-score was in the range between -1,0 and +1,0, with the highest percentage (89%) fund in cluster 2. Excessive body weight was observed in 14% of the whole population.

In the present study more than 40% of youths took dietary supplements, more girls (53%) than boys (37%; p≤0.001), and the percentage was higher among older students and as well as coming from a big city.

The *Kohonen's* network model analysis has allowed to separate 3 clusters differing significantly in the use of dietary supplements as well as socio-demographic characteristics (Table 1), selected eating behaviors (Table 2) and certain aspects of lifestyle (Table 3).

Table 1. Socio-demographic characteristic of study group according to cluster classification (percentage of respondents)

Demographic features	Total n=396	Cluster 1 n=86	Cluster 2 n=139	Cluster 3 n=171	p-value
Total (%)	100	21.7	35.1	43.2	-
Age (years):					
13 – 15	32.1	15.1	35.3	38.0	≤ 0.001
16 – 18	67.9	84.9	64.7	62.0	
Gender:					
girls	62.9	34.9	81.3	62.0	≤ 0.001
boys	37.1	65.1	18.7	38.0	
Place of residence:					
village	30.6	72.1	18.0	19.9	
city <100 thousand inhabitants	21.0	15.1	23.0	22.2	≤ 0.001
city >100 thousand inhabitants	48.5	12.8	59.0	57.9	
Household size (number of person):					
2 – 3	26.6	19.8	26.6	30.4	
4	36.0	39.5	36.0	32.2	NS
≥5	37.4	40.7	37.4	37.4	
Self- assessments of socioeconomic status:					
poor	25.3	72.1	7.9	15.8	
average	49.7	10.5	68.3	54.4	≤ 0.001
very good	17.7	8.1	20.1	20.5	
difficult to say	7.3	9.3	3.6	9.4	
Parental education:					
higher	36.9	2.3	48.2	45.0	
incompete higher	10.9	4.7	13.7	11.7	≤ 0.001
secondary	37.9	70.9	26.6	30.4	
vocational	14.4	22.1	11.5	12.9	
BMI z-score:					
underweight	0.8	0	1.4	0.6	
normal	85.1	82.6	89.2	83.0	NS
overweight and obesity	14.1	17.4	9.4	16.4	
Dietary supplements users:					
yes	47.2	55.8	100	-	≤ 0.001
no	52.8	44.2	-	100	

Distinguished clusters can be characterized as follows:

– *Cluster 1* (n=86; 22%) – in this cluster, the use of dietary supplements was declared by 56% of the students, and not taking them by 44%. In comparison to clusters 2 and 3, there was the highest proportion of young people aged 16-18 years old, male, living in rural areas, with poor financial situation, and with excessive body weight. Parents of those students had lower education level.

– *Cluster 2* (n=139; 35%) – all students in this cluster used dietary supplements. When comparing to other clusters, there can be found the highest percentage of girls, habitants of cities > 100.000 residents, students of average financial situation, whose parents had higher education level. Besides, in this cluster there was the highest percentage of students with normal weight, and the lowest percentage of those being overweight or obese.

– *Cluster 3* (n=171; 43%) – none of the member of this cluster used dietary supplements. There were mostly students aged 16-18 years old, girls, living in cities > 100.000 residents, with an average financial situation, whose parents had higher education level. Excessive body weight had 16.4% of students.

Analysis of data on eating behavior has also showed a high percentage of youths who declared the consumption of fortified foods (62%), and it was slightly higher in cluster 2 than in other ones (differences not statistically significant). In addition, a high percentage of adolescents exhibited inadequate eating habits, including irregular consumption of meals (69%) with 1-2 meals throughout the day (30%), snacking between meals (68%), frequent consumption of sweetened drinks (everyday consumption declared by 19%) and fast-foods. In contrast, a relatively low percentage (51%) of young people declared eating fruit and vegetables every day (Table 2).

Table 2. Characteristic of study group in terms of selected eating habits according to cluster classification (percentage of respondents)

Characteristics	Total n=396	Cluster 1 n=86	Cluster 2 n=139	Cluster 3 n=171	p-value
Consumption of fortified food:					
yes	62.1	60.5	64.7	60.8	
no	10.4	14.0	11.5	7.6	NS
not sure	27.5	25.6	23.7	31.6	
Number of meals during the day:					
1 – 2	29.6	29.0	27.3	31.6	
3	59.1	64.0	59.7	56.1	NS
4	11.4	7.0	12.9	12.3	
Regularity of meals:					
yes	31.1	34.9	33.8	26.9	NS
no	68.9	65.1	66.2	73.1	
Snacking between meals:					
yes	67.7	58.1	71.9	69.0	NS
no	32.3	41.9	28.1	31.0	
Regularity of breakfast consumption:					
yes	61.4	61.6	69.1	55.0	0.04
no	38.6	38.4	30.9	45.0	
Frequency of consumption of fruits and vegetables:					
everyday	50.8	44.2	56.8	49.1	
2-3 x per week	37.4	39.5	38.1	35.7	NS
few times per month	10.4	14.0	5.0	12.9	
never	1.5	2.3	-	2.3	
Frequency of consumption of fast-food:					
everyday	2.3	3.4	0.7	2.9	
2-3 x per week	16.4	17.4	15.1	17.0	NS
few times per month	64.1	62.8	64.0	64.9	
never	17.2	16.3	20.1	15.2	
Frequency of consumption of soft drinks:					
everyday	18.7	15.1		22.8	
2-3 x per week	36.4	43.0	34.5	34.5	NS
few times per month	31.1	29.1	33.1	30.4	
never	13.9	12.8	16.5	12.3	
Adding salt to food:					
yes	45.5	40.7	45.3	48.0	NS
no	54.4	59.3	54.7	52.0	

Except for regular consumption of breakfast, what was declared by 69% of young people in cluster 2 (compared to 55.0% in cluster 3 and 61.6% in cluster 1; p=0.04), there were no statistically significant differences found for other features of eating habits. However, there was a tendency to exhibit better dietary behaviors in cluster 2 in terms of frequency consumption of fruit and vegetables, fast-foods and sweetened beverages. In cluster 3, there were mostly students who had the lowest frequency of eating breakfasts. Besides, in this cluster greater percentage of students consumed everyday sweetened beverages and added salt to dishes at the table 2.

A similar correlation was also observed in relation to the use of stimulants (Table 3). The lowest percentage (11%) of youths reported smoking cigarettes was found in cluster 2, in which all persons used dietary supplements, while the highest percentage was in cluster 3 (81.9% $p \leq 0.001$) in which no one took dietary supplements. Energy and isotonic drinks were less popular in cluster 2, in which higher percentage of young people spending their free time actively were found. Alarming is, however, a high proportion of young people (59%; comparable figure in all clusters), that declared the consumption of alcohol.

The most common cause for the use of dietary supplements was a period with increased incidence of colds (autumn-winter period – 52.4%). Taking more than one dietary supplement at the same time was declared by 31.0% of respondents, and adherence to the manufacturer's instructions declared 86.6%. Pharmacies, followed by hypermarkets were the most common places for purchasing the dietary supplements, namely 85.6% and 20.9%, respectively. Buying online declared 5% of students. Most of young people took dietary supplement in the form of tablets (66%), of powder (23%), gummy (17%), the capsules (14%) or syrup (12%). The source of information on dietary supplements were mainly TV advertisements (55%) and the Internet (48%), and to less extent – doctors (17%), leaflets (16%) and popular magazines (15%).

Table 3. Characteristic of study group in terms of selected lifestyle factors and to cluster classification (percentage of respondents)

Characteristics	Total n=396	Cluster 1 n=86	Cluster 2 n=139	Cluster 3 n=171	p-value
Consumption of tea:					
yes	89.4	87.2	89.9	90.1	NS
no	10.6	12.8	10.1	9.9	
Consumption of coffee:					
yes	47.7	45.3	45.3	50.9	NS
no	52.3	54.7	54.7	49.1	
Consumption of energy dring:					
yes	48.6	50.0	44.2	51.5	NS
no	51.4	50.0	55.8	48.5	
Consumption of isotonic drinks:					
yes	31.1	38.4	27.5	30.4	NS
no	68.9	61.6	72.5	69.6	
Consumption of alcohol:					
yes	59.1	60.5	59.0	58.5	NS
no	40.9	39.5	41.0	41.5	
Cigarette smoking:					
yes	18.2	30.2	10.8	81.9	≤ 0.001
no	81.8	69.8	89.2	18.1	
Self-assessment of physical activity:					
low	25.8	26.7	28.1	23.4	
moderate	50.8	46.5	47.5	55.6	NS
high	23.5	26.7	21.1	21.1	
Spending of leisure time:					
active	48.0	47.7	50.4	46.2	NS
passive	52.0	52.3	49.6	53.8	

DISCUSSION

Nutrition and dietary habits of young people is one of the most important environmental factors determining the growth, development, psychophysical activity, learning ability and good health in adulthood. Modern model of nutrition widespread among young people do not allow to meet the requirements of certain nutrients and differs remarkably from the dietary guidelines. Deficiencies of vitamins and minerals conduce to develop of a number of diet-related diseases. Undoubtedly, the use of dietary supplements is becoming more and more common among the actions to correct those abnormalities [11, 13]. It is important, however, that the dietary supplementation is adequate to the needs and correct the nutritional mistakes properly. Inadequate intake of nutrients in the form of dietary supplements and also fortified foods may cause the excessive intake, beyond the recommended levels, which in turn may lead to, i.a. serious health consequences [20, 22]. The use of dietary supplements is a common phenomenon conditioned by many factors, derived from nutrition, socio-demographic as well as the desire to maintain or improve the health status.

Given the socio-economic factors, in present study and in others we demonstrated the relationship between the level of parental education and the use of dietary supplements by children [5, 10, 26]. Parents with higher and secondary education level gave the supplements to their children more often. In the survey of *Jeżewska-Zychowicz* [12], the most important reasons for the use of dietary supplements by young people were: the desire to maintain good health, the need for diet improvement, care about the physical appearance and improper nutrition. Similarly, in a study conducted in post-secondary school students and university students, the desire to: increase the intake of deficient nutrients, enhance immunity, support the nervous system, improve memory and concentration, improve of hair, skin and nails conditions, and reduce weight body, were mainly reported reasons for the use of dietary supplements [14, 29]. Also in the research of the *Bailey* et al. [2] it has been shown that the most common causes of dietary supplements intake were improving and maintaining health status, improving the diet and improving the immunity system.

As results of surveys indicate, TV advertising, Internet and medical consultation are the most

popular sources of information that have meaningful impacts on the consumer decisions to purchase dietary supplements [24]. Data from our study showed that the use of supplements is often a consequence of own decision taken without a consultation with a doctor, dietician or nutritionist. It was also demonstrated that dietary supplements were rarely used because of doctor's recommendation (only 2.2%) in the survey conducted in 7-12 years old children [15]. In the foreign studies 15% were observed, where a small group of supplements was used by the children in accordance with doctors' recommendations [2]. Undoubtedly, insufficient knowledge of the parents on dietary supplements poses a risk of excessive or insufficient intake of vitamins or minerals. For example, in the study conducted in children 11-12 years old, higher than recommended intake of minerals were found for copper (236% of RDA), iron (114% of RDA) and zinc (111% of RDA) in the form of pharmaceutical preparations [9]. American research demonstrated that the upper level (UL) had been exceeded for zinc, folic acid, vitamin A in children aged 9-13 years, and for iron, zinc and folic acid in youths 14-18 years old. At the same time, in the diets of children inappropriate, lower than recommended, amounts of magnesium, phosphorus, vitamin C, and vitamin E were observed [1]. Improper use of dietary supplements, i.e. combining several preparations at the same time, the intake of excessive doses and simultaneous consumption of fortified foods predisposes to disturbance of body homeostasis and appearance of side effects. For instance, high doses of iron can impede the absorption and utilization of zinc, calcium and copper, and high doses of zinc can hinder the absorption of vitamin E [22]. On the other hand, due to the poor nutrition and too low nutrient density of the diets observed among children and adolescents, the use of dietary supplements becomes reasonable, but there is a need to pay attention to the appropriate proportions of vitamins and minerals in the diet.

The present study revealed many incorrect nutritional habits among young people. Those results confirm observations obtained by other researchers. In the study of *Sitko* et al. [21] more than 50% of secondary and high school students admitted that they did not apply to the dietary guidelines, and 30% of them indicated some abnormalities in their body weight. Their diets were characterized by many nutritional errors, i.e.: irregular meals, snacking between meals (mainly sweet and salty snacks) and regular drinking of soft drinks. Similarly, numerous inadequacies were found in the diets of adolescents 13-15 years of age [25]. In turn, irregularities in the structure of food groups consumption among school children were also recorded [3]. They concerned mainly low consumption of dairy products, vegetables and fruit, grain products, seeds and legumes, while a high intake of fast-foods, sugar and sweets, meat and meat products.

Analyzing factors that may determine the use of dietary supplements, it was observed that young people taken them had proper both nutrition and body mass index (BMI) more often. Besides, we also observed a tendency that in the group of adolescents taking dietary supplements, mainly girls, in spite of a normal body weight and better eating habits the vitamin and mineral preparations were used. In the study of *Bylinowska* et al. [5] more children characterized by better nutrition were given supplements than children with poor nutrition. Moreover, it was found that more children being on special diets, like therapeutic, weight reducing or vegetarian, received dietary supplements. Supplementation was also more frequent in underweight than obese children. Similarly, in the study of *Wojtyła-Buciora* and *Marcinkowski* [27] a desire to reduce body weight among young people with adequate BMI and a good nutrition knowledge was demonstrated. Undoubtedly, it is related to the impact of advertising and nutritional trends to improve the physical appearance during adolescence.

In this study no significant relationship between physical activity and the use of dietary supplements was found, wherein the number of students with high physical activity was too low. The survey of *Bylinowska* et al. [5] showed, however, that supplements received more children participating in extracurricular sport activities. Also *Seidler* and *Sobczak* [18] found that students of sport mastery school used dietary supplementation due to a chance to achieve satisfactory sport results much faster. This is also confirmed by the results of research conducted in the United States, which revealed the relationship between the level of physical activity and the use of supplementation [19].

CONCLUSIONS

1. The use of dietary supplements was declared by 47% of the students, and it conditioned by gender, place of residence, the family's financial situation and parental education level.
2. Eating habits and selected elements of lifestyle, except for breakfast eating and smoking, slightly affected the use of dietary supplements by youths.
3. It is essential to monitor continuously eating behaviors of students, including the use of dietary supplements, as well as to undertake among youths and their parents the broad educational activities on the benefits and possible risks of voluntary decisions on the safe use of dietary supplements with vitamins and/or minerals, and other bioactive substances.

Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ESTIMATION OF DIETARY SUPPLEMENTS INTAKE IN A SELECTED GROUP OF WOMEN OVER 50 AND THE RISK ASSESSMENT OF INTERACTIONS BETWEEN THE INGREDIENTS OF DIETARY SUPPLEMENTS AND DRUGS

Joanna Sadowska[*]*, Magda Bruszkowska*

Zakład Fizjologii Żywienia Człowieka, Wydział Nauk o Żywności i Rybactwa, Zachodniopomorski Uniwersytet Technologiczny w Szczecinie, ul. Papieża Pawła VI 3, 71-459 Szczecin, Poland

ABSTRACT

Background. Concurrent use of dietary supplements and drugs may result in complications of pharmacotherapy due to possible interactions between their ingredients.

Objectives. The aim of the survey was to estimate the intake of dietary supplements in a group of women over 50 and to analyse the risk of interactions between the ingredients of dietary supplements and drugs taken by the women.

Material and Methods. The study was carried out among 146 women over 50 years of age. Questionnaire included detailed questions on the type of prescription drugs, OTC (over-the-counter) drugs, and dietary supplements taken. The risk of interactions was determined on the basis of chemical composition of the drugs and supplements specified by the manufacturer, by comparing the obtained data with literature reports on known interactions.

Results. The analysis has shown that 88.4% of respondents constantly took prescription drugs, 44.5% of them took OTC drugs, and 66.4% of respondents took dietary supplements throughout the survey period. It has been found that 71.3% of surveyed women taking prescription drugs, took dietary supplements as well. Among women taking supplements and drugs, 36.9% of respondents were taking them concurrently, 60.9% kept such an interval, but only 21.8% of them waited for at least two hours. It has been found that the drug-supplement interactions might occur in 35.8% women under the survey.

Conclusions. The analysis of the obtained results has revealed that taking dietary supplements by the group under survey was frequent, and the risk of interactions between dietary supplements and drugs was significant. It is recommended that doctors ask their patients about taken supplements during regular check-ups, and inform them about possible interactions between dietary supplements and drugs. Moreover, appropriate would be to change the labelling of dietary supplements, so that the packaging provides information on possible interactions between their ingredients and drugs.

Key words: *dietary supplements, drugs, interaction, women over 50*

STRESZCZENIE

Wprowadzenie. Jednoczesne spożywanie suplementów diety i leków może być przyczyną powikłań farmakoterapii, ze względu na możliwość wystąpienia interakcji pomiędzy ich składnikami.

Cel. Celem przeprowadzonych badań była ocena spożycia suplementów diety w wybranej grupie kobiet po 50 roku życia oraz analiza ryzyka wystąpienia interakcji pomiędzy składnikami suplementów diety a przyjmowanymi przez te kobiety lekami.

Materiał i metody. Badania przeprowadzono wśród 146 kobiet po 50 roku życia. W ankietach zawarto szczegółowe pytania dotyczące rodzaju zażywanych leków przepisanych przez lekarza, przyjmowanych leków bez recepty oraz spożywanych suplementów diety. Ryzyko wystąpienia interakcji określono na podstawie składu stosowanych przez badane leków oraz suplementów, odnosząc uzyskane dane do informacji o interakcjach podanych w literaturze.

Wyniki. Analizując uzyskane wyniki stwierdzono, że 88,4% badanych przyjmowało na stałe leki przepisane przez lekarza 44,5% stosowało leki bez recepty, a 66,4% badanych w okresie badania zażywało suplementy diety. Stwierdzono, że 71,3% badanych kobiet, przyjmujących leki przepisane przez lekarza, stosowało także suplementy diety. Spośród kobiet stosujących suplementy i leki 36,9% przyjmowało je w jednym czasie, przerwę stosowało 60,9% badanych, jednak tylko u 21,8% badanych była ona co najmniej 2 godzinna. Stwierdzono, że interakcja lek - suplement mogła zachodzić u 35,8% badanych kobiet.

Wnioski. Analizując uzyskane wyniki stwierdzono, że przyjmowanie suplementów diety w badanej grupie osób było częste, a ryzyko wystąpienia interakcji pomiędzy przyjmowanymi suplementami diety a lekami było znaczne. Wskazane byłoby wprowadzenie rutynowych pytań podczas wizyty lekarskiej o stosowaniu suplementacji i informowanie pacjenta

[*]**Corresponding author**: Joanna Sadowska, Zakład Fizjologii Żywienia Człowieka, Wydział Nauk o Żywności i Rybactwa, Zachodniopomorski Uniwersytet Technologiczny w Szczecinie, ul. Papieża Pawła VI 3, 71-459 Szczecin, tel. 91 4496572, e-mail: joanna.sadowska@zut.edu.pl

o możliwości interakcji pomiędzy składnikami suplementów diety a lekami. Celowa wydaje się także zmiana oznakowania na opakowaniach suplementów diety, uwzględniająca informacje o możliwości zajścia interakcji pomiędzy składnikami suplementów i przyjmowanymi lekami.

Słowa kluczowe: *suplementy diety, leki, interakcja, kobiety powyżej 50 roku życia*

INTRODUCTION

Reduced level of sex hormones observed in women undergoing menopause results in deteriorated physiological functions in a number of internal organs, and leads to unfavourable changes in skin appearance [22]. This is often followed by mood deterioration, and contributes to the search for ways of effective health maintaining. One of commonly observed behaviours is taking dietary supplements [11], particularly in older age groups [16].

Menopause and geripause are the time when many factors accumulate that increase the risk of disease. This risk is still enhanced by the modern lifestyle change connected with inadequate nutrition and the lack of physical activity, which leads to more frequent and earlier onset of chronic diseases, previously typical to older age. Therefore, the age of chronic sufferers is decreasing, while increasing is the number of people taking drugs for chronic diseases, such as arterial hypertension or type 2 diabetes [27].

Concurrent use of dietary supplements and drugs may result in complications of pharmacotherapy due to possible interactions between their ingredients. It has been proved that many ingredients of dietary supplements may alter various stages of drug pharmacokinetics and hamper the healing process, as well as cause adverse effects leading to serious complications [1]. Despite this knowledge, both doctors and patients do not pay attention to the necessity of the time interval between taking drugs and dietary supplements. The available literature lacks detailed test results regarding the behavior of the related in a group of elderly people who often use pharmacotherapy.

The aim of the survey was to estimate the intake of dietary supplements in a group of women over 50 and to analyse the risk of interactions between the ingredients of dietary supplements and drugs taken by the women.

MATERIAL AND METHODS

The study was carried out in spring 2015, as a survey among 146 women over 50 years of age, attending the University of the Third Age in a city with over a hundred thousand inhabitants. Some 250 questionnaires were handed out, that included detailed questions on the type of prescription drugs, OTC (over-the-counter) drugs, and dietary supplements taken. The women were asked about taking dietary supplements in the past 6 months, and currently (in the term of interview). They were also asked to write down the names of all taking dietary supplements. The questions also concerned the reasons for taking dietary supplements, and expected results. Some 152 questionnaires were returned, 146 of which were filled in correctly and completely. 96% of respondents lived in the city (above 100 thousand inhabitants), in which the survey was carried out, 4% of respondents commute from nearby towns. In the self-assessment 76% of respondents identified their health as good (but describing it also as "typical for the age"), the other person declared a general malaise. The occurrence of chronic diseases was declared by 86% of respondents.

The risk of interactions was determined on the basis of chemical composition of the drugs and supplements specified by the manufacturer, by comparing the obtained data with literature reports on known interactions [9, 24, 25].

RESULTS

The analysis has shown that 88.4% of respondents constantly took prescription drugs (mainly those for arterial hypertension and hypothyreosis), 44.5% of them took OTC drugs (mainly painkillers and cardiovascular drugs), and 66.4% of respondents took dietary supplements throughout the survey period (while 11.6% of them were not aware of the fact because they classified supplements as OTC drugs in the questionnaire) – Table 1.

Table 1. The percentage of women using prescription drugs, non-prescription drugs and dietary supplements in the term of interview, n=146

Type of preparation	Women	
	n	%
Prescription drug	129	88.4
Non-prescription drug	65	44.5
Dietary supplement	97	66.4
Dietary suplements and drugs (together)	97	66.4

It has been found that 71.3% of surveyed women taking prescription drugs, took dietary supplements as well, while 94.8% of woman taking supplements took prescribed drugs as well. The respondents were taking one to fifteen drugs a day (most frequently 1-2), one to

five OTC drugs (most frequently 1) and one to seven dietary supplements (most frequently 2-3 different supplements a day).

The administered supplements mainly included essential fatty acids, lutein, and/or vitamins and minerals (Table 2).

Table 2. Composition of dietary supplements used by surveyed women, n=97 [1, 2]

Component	Women	
	n	%
One vitamin	21	21.6
Several vitamins	30	30.9
One mineral	23	23.7
Several minerals	10	10.3
Vitamin and mineral formula	19	19.6
Fatty acids of n-3	45	46.4
Lutein	25	25.8
Herbal extracts (e.g. ginseng, ginkgo, echinacea)	10	10.3
Vitamin + Mineral formula + Herbal extracts	15	15.4
Glucosamine, chondroitin	11	11.3
Phytoestrogens	1	1.0
Other	13	13.4

[1] - as 100% accept women using supplements
[2] - the respondents could select more than one answer

The main reasons for taking supplements were: to boost immunity (40.2% of respondents), to support the cardiovascular system (28.9% of respondents), and to improve general health (26.8% of respondents) –Table 3.

Table 3. The expectations of surveyed women to supplements, n=97 [1,2]

Designed	Women	
	n	%
Boost of immunity	39	40.2
General health improvement	26	26.8
Support cardiovascular systems	28	28.9
The enhancement of memory and concentration	25	25.8
Vision improved	25	25.8
Improve the condition of the hair, skin, nails	25	25.8
Improving the joints	23	23.7
Improving digestive processes, liver function	20	20.6
Enhancing sleep, mood, anti-stress	20	20.6
Slim products	8	8.2
For diabetics	5	5.1
Other	7	7.2

[1] - as 100% accept women using supplements
[2] - the respondents could select more than one answer

The decision on taking supplements was made mainly due to the belief that supplements are required to stay healthy (37.1% of respondents), every fourth respondent claimed to feel better as a result of taking supplements, but 21.6% of them decided to use supplements because it was fashionable and advertised (responses: "they are recommended", "all people take them", "I was encouraged by advertisement") – Table 4.

Table 4. The reason for applying dietary supplements given for surveyed women, n=97 [1,2]

Reason	Women	
	n	%
To stay healthy	36	37.1
I feel much better after use them	24	24.7
My diet is poor	20	20.6
They are recommended to people of my age	10	10.3
All people use supplements	5	5.1
Encouraged by advertising	6	6.2
Other	12	12.4

[1] - as 100% accept women using supplements
[2] - the respondents could select up to 3 answers

66% of respondents declared they had discussed taking dietary supplements with their physician (Figure 1).

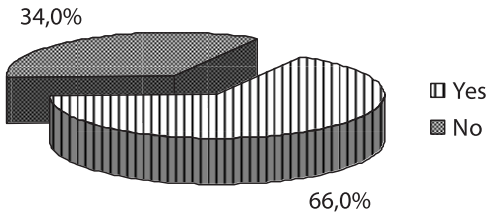

Figure 1. Percentage of respondents which consults intake of dietary supplements with physician, n=97 [1]
[1]- as 100% accept women using supplements

Among 92 people taking supplements and drugs, 36.9% of respondents were taking them concurrently. As to the time interval between taking dietary supplements and drugs, 60.9% kept such an interval, but only 21.8% of them waited for at least two hours (Table 5).

Table 5. The use of the time interval between these medicines and dietary supplements, n=92[1]

Break	Women	
	n	%
No	34	36.9
Yes	56	60.9
<2 h	36	39.1
≥2 h	20	21.8
Non-response	2	2.2

[1]- as 100% accept women using supplements and drugs who replied about the time when they taking drugs and supplements

Less than half of respondents were aware of the fact that supplement ingredients may alter the effects of drugs, one third of respondents were convinced such an alteration is not possible, and every fifth respondent answered "do not know" (Figure 2).

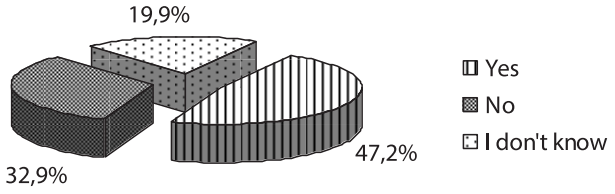

Figure 2. Distribution of answers to the question whether dietary components can alter the effect of drugs, n=146

Taking into consideration the chemical composition of the administered drugs and supplements, irrelevant time intervals between taking them, and not consulting the supplementation with a general practitioner, it has been found that the drug-supplement interactions might occur in 33 women (35.8%) under the survey (Table 6).

Table 6. The risk assessment of interactions between supplements and medicines, n=92 [1]

Behaviour	Women	
	n	%
Taking in the same time drugs and, dietary supplement including drugs + supplement containing ingredients with a proven track interaction	34 22	36.9 23.9
Taking drug and supplements in an interval of <2 h including drugs + supplement containing ingredients with a proven track interaction	36 11	39.1 11.9
In total, women exposed to interactions dietary supplements - drugs	33	35.8

[1]- as 100% accept women using supplements and drugs

The most frequently taken combinations were: calcium/iron supplements along with ß–blockers and levothyroxine, vitamin C with acetylsalicylic acid, ginseng extract with metformin/amlodipine, and magnesium/iron supplements with captopril (Table 7).

Table 7. The most common combined intake of medicines and supplements with the risk of interactions used without consulting a doctor by the test group of women, n=33 [1]

Component	Women	
	n	%
Calcium / iron + levothyroxine	13	45.5
Calcium + β- blockers	5	15.1
Calcium / iron + bisphosphonates	2	6.1
Magnesium / iron + captopril	3	9.1
Vitamin C+ acetylsalicylic acid	4	12.1
Niacin + lovastatin	2	6.1
Ginseng + metformin / amlodipine	4	12.1
Ginkgo biloba + acetylsalicylic acid	2	6.1

[1]- as 100% accept women using in the same time supplements and drugs containing ingredients with a proven track interaction

DISCUSSION

There are reports available that the prevalence of dietary supplementation varies depending on the region, age of respondents, and the method of collecting data, nevertheless it concerns a significant percentage of respondents [11, 16]. In this survey, 66% percent of women were taking supplements throughout the survey period. Similar results were reported by *Kałuża* et al. [10], as well as *Saran and Duda* [17]. Dietary supplementation concerns ca. 30% of general population, however, it has been shown it is more often applied by older people, better educated individuals, and those living in big cities [15, 16].

Analysis of reasons for taking supplements has revealed that the main reason for supplementation was to boost immunity and improve health. Similar reasoning was confirmed by surveys carried out in Poland by *Pietruszka* et al. [14] and by *Sebastian* et al. [19], in the USA.

The taken supplements mainly included vitamins and minerals. Regarding the age of the surveyed women, it is surprising that only one of them was taking phytoestrogen supplements which, due to their ability to activate oestrogen receptors, may be used to prevent menopause symptoms such as bone mass decrease and hot flushes. In case of some women, they seem to be a safe alternative to the hormone substitutive therapy [2].

It has been found, based on the obtained data, that almost all the respondents who took dietary supplements, took also prescription drugs. The fact that chronically ill people often take dietary supplements and drugs at the same time has been confirmed by literature reports [10].

The study confirmed that some surveyed women were taking a number of various drugs and supplements every day (5.5% of respondents were taking ≥10 pills a day, most frequently 2-3 prescription drugs, 2-3 OTC drugs, and 3-4 dietary supplements), and remembered should be that except for active ingredients the drugs also include binding, glazing, dyer, and anti-oxidant agents such as: cellulose, lactose, starch, talc, silicon dioxide, polyvinylpyrrolidone, polyethylene glycol, hydroxypropyl cellulose, magnesium stearate, beeswax, Carnauba wax, titanium dioxide, red iron oxide, indigo carmine, and butylhydroxytoluene. Some of them are xenobiotics, and their detoxification burdens the liver metabolism [7].

The survey has revealed that 66% of respondents declared they had consulted taking dietary supplements with their general practitioners, however, only less than half of them were aware that supplement ingredients may interact with drugs, one third of respondents were convinced such an interaction is not possible, and one fifth answered “do not know”. The problem may be in the labelling of dietary supplements - although

they look like drugs, they are just foodstuffs and their packages lack any information on undesirable effects, contraindications, or interactions that may occur when taking a given supplement along with drugs. In many cases, patients do not inform their doctors about taking dietary supplements as they believe such information is irrelevant. Information about supplement – drug interaction should be included in drug leaflets, but it seems that they were not carefully analyzed, or were incomprehensible and too vague.

Clinical observations indicate that undesirable effects and a number of complications in pharmacotherapy occur in ca. 20-50% of patients, in 5% of patients the complications are so life-threatening that they require hospitalisation [9]. One of the reasons for complications may be combining drugs with certain foods and/or dietary supplements.

The survey has shown that 76% of respondents were taking drugs with supplements at a time interval shorter than 2 hours. Taking into consideration the chemical composition of drugs and supplements, combined with irrelevant time interval between taking supplements and drugs, and the lack of any consultation about dietary supplementation with a general practitioner, it has been found that the drug-supplement interaction might occur in 1/3 of respondents. The most frequently taken combinations were calcium/iron supplements taken together with β-blockers or levothyroxine, and magnesium/iron supplements taken with captopril.

There are reports available that calcium carbonate reduces absorption of atenolol (beta-adrenergic blocker), and magnesium hampers captopril assimilation [13].

The reference data confirm frequent combination of levothyroxine and calcium supplements. It mostly concerns women after menopause [12], and may cause decreased efficiency of hypothyroidism treatment. *Singh* et al. [20], found that in patients taking levothyroxine along with calcium carbonate, the concentration of free and bound thyroxine significantly deceases. In vitro tests have shown that in the acidic environment levothyroxine bounds calcium carbonate which may limit its bioavailability. Calcium supplements and levothyroxine should be taken separately at intervals of at least 4 hours [21]. *Campbell et al.* [3], revealed that also taking iron supplements and levothyroxine at the same time leads to an interaction as ferric sulphate and thyroxine form insoluble complexes.

Also the interaction between vitamin C and acetylsalicylic acid is vital. It may seem that the effect of such an interaction, namely increased excretion of vitamin C in urine, does not pose any danger to health, however, it increases the risk of renal calculi. Vitamin C is metabolised to oxalates and may increase their excretion in urine [5]. Excessive excretion of oxalate is observed at high doses of vitamin C, i.a. delivered from dietary supplements, which contains up to 1000 mg of vitamin C per tablet, which is half the dose of Upper Level. Moreover, excess of vitamin C acidifies urine, which results in intensified crystallization of urates and cysteine salts.

An interaction also occurs between active compounds of ginseng (*Panax ginseng*) and metformin/amlodipine. The ginseng root is one of the most common plant ingredients in dietary supplements [18]. A potential danger of taking it along with drugs is caused by the biological impact of its active compounds on the activity of the CYP3A4 isoenzyme in the cytochrome P450 system which is responsible for drug metabolism [26]. As to anti-diabetic drugs, their effect may be enhanced by the hypoglycaemic impact of ginseng. While in the presence of anticoagulants, ginseng acts as an antagonist, namely it reduces blood concentration of these agents [8].

Moreover, interactions were found from taking ginkgo (*Ginkgo biloba*) supplements with tranquilizing drugs (diazepam). Active compounds of ginkgo and diazepam are both the substrates for the cytochrome P450 isoenzyme CYP3A4. Moreover, it is suggested that *G. biloba* extract may interact with benzodiazepines as a result of an antagonistic effect on GABA-A receptors. Ginkgo biloba supplements may increase the plasma concentration of nifedipine as well. *Yoshioka* et al. [23], reported that the peak plasma concentrations of the drug in tested volunteers increased from 30 to 60% after administering *G. biloba* extract. Moreover, more frequent headaches, dizziness, hot flushes and palpitation were observed in patients taking ginkgo extract along with drugs. Moreover, long-time taking of ginkgo supplements and acetylsalicylic acid exerts an adverse effect as they irritate the stomach mucosa and may cause gastrointestinal bleeding [6].

Interactions between drugs and dietary supplements are indeed a problem as they hamper the pharmacotherapy of many diseases. The effects of such interactions, which are so difficult to foresee, pose a real risk to health, and in some cases are even life-threatening. Therefore, it is of vital importance that, on one hand, general practitioners know about supplements taken by their patients, and on the other hand, that the patients know about possible interactions, particularly due to the increasing number of new supplements of diverse chemical composition.

CONCLUSION

The analysis of the obtained results has revealed that taking dietary supplements by the group under survey was frequent, and the risk of interactions between dietary supplements and drugs was significant.

It is recommended that doctors ask their patients about taken supplements during regular check-ups, and inform them about possible interactions between dietary supplements and drugs. Moreover, appropriate would be to change the labelling of dietary supplements, so that the packaging provides information on possible interactions between their ingredients and drugs.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSESSMENT OF WATER INTAKE FROM FOOD AND BEVERAGES BY ELDERLY IN POLAND[1)]

Małgorzata E. Drywień, Katarzyna Galon*

Warsaw University of Life Sciences, Chair of Nutritional Assessment, Department of Human Nutrition, (WULS-SGGW), Warsaw, Poland

ABSTRACT

Background. Fluid intake in elderly is more important than in younger individuals, because compromised homeostatic mechanisms such as loss of the thirst sensation can result in dehydration.

Objective. The aim of the present study was the assessment of water intake from food and beverages by free-living elderly in Poland.

Materials and Methods. The study was conducted on 138 volunteers (women and men) at the age of 60 to 90, recruited from Warsaw and Płock Universities of the Third Age and different informal groups from the same cities. Food and beverages consumption data were collected using the method of records for 3 days, including two weekdays and one week-end day, in the period April – June 2012.

Results. Average values of total water intake in the present study indicated that women meets of the European Food Safety Agency recommendations (2000 mL/day), but men did not (less about 200 mL/day than the recommended 2500 mL/day). Taking into account the criterion of water per energy intake (mL/kcal) 51% of women and 75% of men did not meet the recommendation.

Conclusions. Continuation of the careers and/or participation in Universities of the Third Age contributed to less intake of water from beverages, what in turn affected the total water intake. The elderly leading an active life (working, studying) may be a risk group vulnerable to dehydration, so monitoring is needed.

Key words: *elderly, nutrition, water intake, water, food, beverages*

STRESZCZENIE

Wprowadzenie. Spożycie wody u osób starszych jest znacznie ważniejszym problemem niż u osób młodych, ponieważ zaburzenia w utrzymaniu homeostazy, takie jak utrata odczuwania pragnienia, mogą doprowadzić do odwodnienia.

Cel. Celem badań była ocena spożycia wody z produktami spożywczymi i napojami przez osoby starsze z wybranych rejonów w Polsce.

Materiał i Metoda. Badania przeprowadzono z udziałem 138 ochotników (kobiet i mężczyzn) w wieku 60-90 lat, uczestników Uniwersytetów Trzeciego Wieku oraz osób niezrzeszonych z terenów Płocka i Warszawy. Dane o spożyciu żywności i napojów zbierano z zastosowaniem metody 3-dniowego bieżącego notowania (2 dni powszednie, 1 dzień świąteczny), w okresie kwiecień-maj 2012.

Wyniki. Badania wykazały, że kobiety spożywały wodę na poziomie zalecanym przez Europejski Urząd Bezpieczeństwa Żywności (EFSA) (2000 mL/dz), natomiast mężczyźni spożywali o około 200 mL wody dziennie mniej od tych zaleceń (2500 mL/dz). Przyjmując kryterium spożycia wody w przeliczeniu na jednostkę energii (mL/kcal), okazało się, że 51% kobiet i 75% mężczyzn nie realizuje tych zaleceń.

Wnioski. Kontynuacja pracy/aktywności zawodowej i/lub udział w Uniwersytetach Trzeciego Wieku przez osoby starsze może przyczyniać się do zmniejszenia spożycia wody z napojami, a tym samym całkowitego jej spożycia. Osoby starsze prowadzące aktywny tryb życia (praca, edukacja) mogą być grupą ryzyka szczególnie narażoną na odwodnienie, a więc wymagającą monitoringu.

Słowa kluczowe: *osoby starsze, sposób żywienia, spożycie wody, żywność, napoje*

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***Corresponding author**: Małgorzata Ewa Drywień, Wydział Nauk o Żywieniu Człowieka i Konsumpcji, Katedra Żywienia Człowieka, SGGW w Warszawie, Nowoursynowska 159C, 02-776 Warsaw, Poland; Tel.: +48 22 59 37 121, Fax: +48 22 59 37 123; e-mail: malgorzata_drywien@sggw.pl

INTRODUCTION

Water is one of the most important nutrient for the maintenance of health and life. Fluid intake in elderly is more important than in younger individuals, because compromised homeostatic mechanisms such as loss of the thirst sensation can result in dehydration. Fluid intake is critical for maintaining vascular volume, regulating body temperature, removing waste from the body, and supporting homeostasis [9, 16]. Fluid deprivation and repletion studies comparing younger adults with the older population have demonstrated that despite physiological needs, older people do not consume adequate amounts of fluids to maintain ideal plasma electrolyte concentration [1]. Diminution of liquid intake and increase in liquid losses are both involved in causing dehydration in the elderly [14]. Despite the physiological importance of water to life, little is known about water intake and excretion patterns in free-living elderly [9] and the situation is different in European countries and depends on climate, dietary habits and socio-demographic factors [10, 18, 25]. Population of elderly in the world keeps increasing, that is why more and more stress should be put not only on their treatment but also on their lifestyle and nutritional habits, including water intake and its determinants [20, 25].

The aim of this study was the assessment of water intake from food and beverages by elderly in Poland.

MATERIALS AND METHODS

The study was conducted on 138 volunteers (women and men), at the age of 60 to 90, recruited from Warsaw and Płock Universities of the Third Age (Warsaw UTA group and Płock UTA group, respectively) and different informal groups from the same cities (marked as Warsaw group and Płock group). Food and beverages consumption data were collected using the method of records for 3 days, including two weekdays and one week-end day, in the period April – June 2012. Prior to recording consumption, the survey participants were trained on how to adequately describe the foods and beverages, amounts consumed, cooking methods, etc. At the end of the recording period, the record was thoroughly reviewed with the subject to make records more accurate by clarifying the entries and by adding any omitted items and amounts [4]. Amounts of food and beverages consumed were converted on the amounts of water, energy and selected nutrients using Dieta 5D program created by the National Food and Nutrition Institute (Warsaw, Poland). Intake of water was assessed according to the AI (Adequate Intake) nutritional standards for Polish population: men from 51 to 75 and older – 2500 mL/day; women – from 51 to 75 and older – 2000 mL/day, and 1.5 mL/kcal of energy intake, and 30 mL/kg body weight [5, 13]. The percentage of individuals who did not follow the recommendation of the European Food Safety Agency [5] for total water intake was calculated. Demographic data, information related to health status and lifestyle factors as well as body mass index (BMI), to investigate their influence on water intake, were evaluated with a self – reported questionnaire.

The informed consent for participation in the study was obtained from each subject. The study protocol has been approved by the Institutional Review Board in Warsaw University of Life Sciences and European Hydration Institute (Madrid).

Statistical analysis

All data are presented as means and standard deviations, minimum and maximum. The distribution of the selected characteristics among groups was compared using analysis of variance (ANOVA). The *Tukey post-hoc* test was used to correct for multiple comparisons. For non-parametric variables the *Pearson* χ^2 analysis was used. Statistical significant was set at $P \leq 0.05$. All statistical analyses were performed using Statistica version 10 (StatSoft, Poland)

RESULTS

Characteristics of a study group

In the study group (n=138) the average age was between 65-75 years (Table 1). The youngest female group was from UTA in Płock, and the youngest male group was from Warsaw. There was a significant difference between age of women from Płock UTA and Warsaw ($p<0.05$). In men such difference has not been observed. Surveyed people lived in two-person households. The least professional active subjects were among those from Warsaw UTA group, and the most active were from the Płock UTA group. A few people reported lack of physical activity. Most subjects had secondary or vocational education. The highest percentage of subjects using healing diets studied at Warsaw UTA, the lowest percentage at Płock UTA. Percentage of smoking individuals was rather similar in all groups. The greater part of the elderly using supplements was reported in Warsaw. Average body size, BMI (Body Mass Index) and WHR (Waist-Hip Ratio) were not significantly different among the study groups.

Selected health factors

Average blood pressure in all groups was on the pre-hypertension (high normal) level and more than half of the population used the medication for hypertension (Table 2). In each study group the android obesity was dominant in women while in men the two types of obesity occurred in similar

proportions. Cardiovascular diseases prevailed between chronic diseases in every group and more than 40% of respondents indicated "other" chronic diseases (Table 2). The main medication was associated with hypertension and "other" diseases, more than 20% of respondents took anti-inflammatory drugs.

Selected indicators of nutrition

The study individuals had regularly meals (4-5 per day) including snacking (data not shown). The average daily intake of energy and macronutrients (protein, fats, carbohydrates, cholesterol, alcohol and dietary fibre) was similar for all study groups. There were significant differences between groups in sodium (without added salt) and potassium intake (Table 3).

Table 1. Socio-demographic and anthropometric characteristics of the studied population

	Płock (n=48, W=33, M=15)	Płock UTA (n=33, W=33, M=0)	Warsaw (n=29, W=24, M=5)	Warsaw UTA (n=28, W=24, M=4)
Average age (years)				
women	70.5±5.8^{ac} (62-87)	65.3±3.7^{b} (60-72)	73.4±8.7^{c} (60-90)	66.8±4.7^{ab} (62-81)
men	69±4.7^{a} (64-81)	nd	75.8±8^{a} (63-82)	73.3±12.1^{a} (64-91)
Average number of persons per household	2	2	2	2
physical activity (%)	81	88	93	100
professional activity (%)	6	42	21	7
Education (%) primary	33	3	14	15
secondary	23	52	52	39
vocational	42	12	10	20
higher	2	33	24	26
Use of healing diet (%)	17	6	24	14
Smoking (%)	13	15	14	11
Use of supplements (%)	13	18	38	54
Average height (m)	1.64±0.73^{a} (1.50 – 1.80)	1.62 ±0.52^{a} (1.53 – 1.70)	1.61 ±0.77^{a} (1.48 – 1.77)	1.65 ±0.66^{a} (1.52 – 1.80)
Average weight (kg)	75.9 ±16.1^{a} (50 – 120)	69.2 ±10.7^{a} (50 – 97)	67.9±13.6^{a} (50 – 95)	70.3 ±11.1^{a} (46 – 98)
Average BMI (m/kg^2)	28.2±5.1^{a} (21.0 – 28.2)	26.5±3.6^{a} (18.8 – 37.9)	26.2±4.7^{a} (19.5 – 36.4)	25.9±3.5^{a} (18.9 – 32.6)
Average WHR – women	0.84±0.1^{a} (0.72 – 1.18)	0.92±0.15^{a} (0.69 – 1.36)	0.83±0.07^{a} (0.71 – 0.98)	0.87±0.12^{a} (0.76 – 1.32)
Average WHR – men	0.99±0.09^{a} (0.84 – 1.15	nd	0.99±0.06^{a} (0.93 – 1.07)	0.97±0.08^{a} (0.87 – 1.07)

Mean ± standard deviation; (Minimum - Maximum), ^{a,b,c} – means in rows marked with the different letter differ significantly at p≤0.05; W - women; M - men; BMI- body mass index; WHR – waist-hip ratio; nd - not determined

Water intake in the elderly

The average daily water intake for women in most groups meets the EFSA recommendation, and the lowest water intake was in Płock UTA group (Table 4). There were no statistically significant differences in water intake among men, but only Warsaw UTA group meets the recommendation. For the average daily water intake with solid foods were not statistically significant differences, but the highest water intake was in Warsaw UTA groups of women and men (Table 4). The statistically significant lowest water intake from beverages was in Płock UTA group of women. There were no significant differences for water intake from beverages among groups of men. The water intake in relation to the energy was significantly lowest in women of the Płock UTA group, but average intake for women population meets the recommendation. Men in all study groups consumed water at a similar level in relation to the unit of energy (Table 4). Average water intake vs. body weight was similar in women and

men, although this parameter was significantly lower in women of Płock UTA group (Table 4). The highest intake of water was with lunch meal, and then were breakfast and II breakfast, independently of the study group, and in Płock and Warsaw UTA groups snacking was also the important source of water. Taking into account the average values for study population, the largest volume of water provide lunch and breakfast, and with other meals water was consumed in similar amounts (Table 4).

Table 2. Characteristics of the studied elderly by health factors

	Płock n=48 (W=33, M=15)	Płock UTA n=33 (W=33, M=0)	Warsaw n=29 (W=24, M=5)	Warsaw UTA n=28 (W=24, M=4)
Average blood pressure (mmHg)	132/80	130/79	133/80	124/76
Gynoid obesity (%)				
women	33	12	38	17
men	47	nd	60	50
Android obesity (%)				
women	67	88	63	83
men	53	nd	40	50
Occurrence of chronic diseases (%)				
renal	4	3	10	0
cardio-vascular	73	61	83	61
lung	6	12	10	7
food allergy	0	6	3	0
osteoporosis (women)	8	9	14	4
other	44	27	48	43
Medication (%)				
for hypertension	67	48	69	57
anti-inflammatory	25	24	31	4
laxative	4	12	0	25
diuretic	8	9	14	7
anti-asthmatic	6	9	0	0
other	46	27	52	46

nd - not determined

Table 3**.** Average daily intake of energy and selected nutrients in studied elderly

Component	Płock	Płock UTA	Warszawa	Warszawa UTA	Average
Total energy (kJ)	6600±2146 [a] (3429 – 14096)	5809±1613 [a] (2725 – 8566)	6196±1870 [a] (2414 – 10151)	6522±2259 [a] (2861 – 12032)	6265±2002 (2414 – 14096)
Total energy (kcal)	1575±513 [a] (817 – 3368)	1387±384 [a] (649– 2046)	1299±446 [a] (577 – 2603)	1555±539 [a] (682 – 2869)	1495±478 (577 – 3368)
Energy (women) (kcal)	1399±264 [a] (817 – 1963)	1387±384 [a] (649 – 2046)	1355±330 [a] (577 – 2040)	1510±511 [a] (682 – 2722)	1397±374 (577 – 2722)
Energy (men) (kcal)	1964±699 [a] (1089 – 3368)	Nd	2071±484 [a] (1435 – 2603)	1829±707 [a] (1371 – 2869)	1964±639 (1089 – 3368)
Protein (g)	72,2±21,9 [a] (37,1 – 144,9)	67,5±21,2 [a] (23,3 – 108,1)	57,0±21,7 [a] (23,8 – 126,2)	76,9±21,7 [a] (34,8 – 130,5)	70,0±21,8 (23,3 – 144,9)
Fats (g)	50,7±22,2 [a] (21,6 – 126,5)	44,9±17,9 [a] (16,6 – 90,2)	42,1±22,7 [a] (14,0 – 108,6)	56,2±33,1 [a] (21,8 – 138,3)	49,5±24,1 (14,0 – 138,3)
Carbohydrates (g)	216,9±67,7 [a] (117,7 – 463)	194,9±55,4 [a] (86,7 – 310,4)	186,6±69,9 [a] (93,6 – 434,5)	198,9±64,2 [a] (98,0 – 335,9)	205,5±64,7 (86,7 – 463,0)
Sodium (mg)*	3578±1008 [b] (1763 – 6158)	2965±916 [a] (1369 – 4737)	2385±846 [a] (1287 – 4513)	3083±981 [ab] (1354 – 5711)	3127±997 (1287 – 6859)
Potassium (mg)	2654±641 [a] (1330 – 4894)	2868±829 [a] (1203 – 5198)	2781±974 [a] (786 – 5216)	3306±907 [a] (1480 – 5026)	2843±845 (786 – 5216)
Cholesterol (mg)	251,4±134,2 [a] (111,2 – 735)	244,4±114,1 [a] (89,7 – 474,6)	203,3±95,1 [a] (46,5 – 465,4)	253,9±139,6 [a] (66,0 – 587)	244,2±122,4 (46,5 – 735)
Alcohol (g)	3,4±11,7 [a] (0 – 164)	0	0,4±2,0 [a] (0 – 13,2)	2,6±5,0 [a] (0 – 74,1)	1,8±7,4 (0 – 249,0)
Dietary fibre (g)	16,9±5,4 [a] (7,1 – 33,1)	18,6±6,0 [a] (7,4 – 30,8)	15,5±6,5 [a] (5,9 – 30,4)	19,3 ±6,0 [a] (9,5 – 37,4)	17,8±5,9 (5,9 – 37,4)

Mean ± standard deviation; (Minimum - Maximum), [a,b,c]– means in rows marked with the different letter differ significantly at $p \leq 0.05$; nd - not determined; * Sodium only from products, without salting

Table 4. Average daily water intake in the studied elderly females and males

	Płock	Płock UTA	Warszawa	Warszawa UTA	Average
			Total (mL/day)		
Women	2367±531 [a] (1411 – 3821)	1523±526 [b] (553 – 2889)	2006±426 [ab] (1109 – 2751)	2283±716 [a] (1104 – 3541)	2032±650 (553 – 3821)
Men	2372±722 [a] (1222 – 3469)	Nd	2167±576 [a] (1525 – 3074)	2536±653 [a] (2075 – 3502)	2360±663 (1267 – 3502)
Average	2368±589 [a] (1222 – 3831)	1523±526 [b] (553 – 2889)	2033±447 [a] (1109 – 3074)	2319±701 [a] (1104 – 3541)	2085±661 (553 – 3821)
			With solid food (mL/day)		
Women	658±128,0 [a] (376,6 – 879)	692±202,1 [a] (301,0 – 1141)	669±248,2 [a] (325,5 – 1387)	763±187,1 [a] (359,5 – 1150)	692±193,3 (301 – 1387)
Men	875±331,7 [a] (571 – 1894)	Nd	751±225,3 [a] (415,1 – 990)	908±158,6 [a] (671 – 1004)	855±286,6 (415,1 – 1895)
Average	725±233,0 [a] (376,6 – 1893)	692±202,1 [a] (301,0 – 1141)	683±242,6 [a] (325,5 – 1387)	783±187,9 [a] (359,5 – 1150)	720±220,1 (301,0 – 1895)
			With beverages (mL/day)		
Women	1615±556 [a] (583 – 3211)	766±471,8 [b] (148,3 – 2171)	1234±355,9 [a] (649 – 1939)	1453±707 [a] (253,0 – 2667)	1255±628 (148,3 – 3211)
Men	1389±583 [a] (252 – 2363)	Nd	1285±407,0 [a] (937 – 1988)	1502±596 [a] (915 – 2310)	1387±535 (252,2 – 2363)
Average	1545±568 [a] (252,2 – 3211)	766±471,8 [b] (148,3 – 2171)	1243±357,9 [a] (649 – 1988)	1460±682,7 [a] (253,0 – 2667)	1278±613 (148,3 – 3211)
			(mL/kcal)		
Women	1,8±0,5 (0,9 – 2,9)	1,1±0,4 (0,6 – 1,9)	1,6±0,5 (0,8 – 2,8)	1,7±0,9 (0,5 – 4,4)	1,5±0,6 (0,5 – 4,4)
Men	1,3±0,5 (0,8 – 2,3)	nd	1,1±0,3 (0,7 – 1,5)	1,4±0,2 (1,2 – 1,7)	1,3±0,4 (0,7 – 2,3)
Average	1,6±0,6 (0,8 – 2,9)	1,1±0,4 (0,6 – 1,9)	1,5±0,5 (0,7 – 2,7)	1,7±0,8 (0,5 – 4,4)	1,5±0,6 (0,5 – 4,4)
			(mL/kg body weight)		
Women	33,8±9,4 (18,6 – 54,1)	22,6±9,5 (10,4 – 49,2)	31,5±8,5 (18,3 – 48,8)	34,1±11,9 (15,7 – 58,9)	30,2±10,9 (10,4 – 58,9)
Men	28,8±9,7 (12,2 – 51,0)	nd	27,6±9,1 (19,6 – 43,3)	31,4±4,0 (27,6 – 35,7)	29,0±8,7 (12,2 – 51,0)
Average	32,2±9,7 (12,2 – 54,1)	22,6±9,5 (10,4 – 49,2)	30,9±8,6 (18,3 – 48,8)	33,7±11,1 (15,7 – 58,9)	29,9±10,5 (10,4 – 58,9)
			(mL/meal)		
Breakfast	400,0±154,2 [ab] (90,1 – 1038)	319,9±172,4 [a] (84,1 – 827)	428,2±145,8 [ab] (201,3 – 739)	464,4±210,0 [b] (149,0 – 1070)	399,8±175,2 (84,1 – 1070)
II breakfast	316±211,6 [a] (0 – 1021)	207,6±158,7 [a] (0 – 853)	311,6±150,5 [a] (0 – 696)	275,2±190,6 [a] (0 – 776)	280,8±187,0 (0 – 1021)
Lunch	630±165,4 [a] (267,7 – 998)	530,5±170,0 [a] (210,3 – 920)	596±163,3 [a] (293,6 – 989)	563±193,3 [a] (197,9 – 999)	585±174,2 (197,9 – 999)
Tea-time	277,1±144,6 [a] (0 – 749)	175,4±160,6 [b] (0 – 633)	224,6±141,0 [ab] (0 – 497,5)	212,5±151,1 [ab] (0 – 468,7)	228,6±152,7 (0 – 749)
Dinner	285,8±148,6 [ab] (0 – 692)	219,9±157,6 [a] (0 – 642)	325,0±149,1 [ab] (0 – 748)	377,8±177,7 [b] (64,9 – 810)	296,9±164,7 (0 – 810)
Snacking	459,1±503,3 [a] (0 – 2166)	75,6±171,7 [b] (0 – 707)	99,4±162,8 [b] (0 – 667)	425,5±545,4 [a] (0 – 1833)	285,0±436,3 (0 – 2166)

Mean ± standard deviation; (Minimum - Maximum), [a,b,c] – means in rows marked with the different letter differ significantly at $p \leq 0.05$; nd - not determined;

More than half of the studied population, both women and men, consumed daily less water than recommended. Over 80% of women in Płock UTA group and men in Warsaw group did not meet the EFSA recommendations (2000 mL/day for women; 2500 mL/day for men). All males in the Warsaw group consumed insufficient amount of water in relation to 1 kcal of energy. Only in the Płock group more than 70% of females meet the recommendation (Table 5).

Table 5. Percentage of individuals who did not meet the recommendations

	Płock	Płock UTA	Warszawa	Warszawa UTA	Average
	for total water intake (mL/day)				
Women	27	82	46	46	51
Men	54	nd	80	75	58
Average	33	82	48	54	52
	for water intake relative to energy (mL/kcal)				
Women	30	82	42	46	51
Men	72	nd	100	50	75
Average	44	82	52	46	55
	for water intake relative to weight (mL/kg body weight)				
Women	42	88	54	38	57
Men	53	nd	80	50	58
Average	46	88	59	39	57

Table 6. Coefficients of the *Pearson's* correlation between selected factors and water intake among study population of elderly (at P≤0.05; at P≤0.1*)

Factors	Total water intake	Water intake with solid foods	Water intake with beverages
Participation in UTA	-0.35	0.15*	-0.37
Gender (women)	-0.17*		
Age	0.18*	-0.18*	0.19
Height	0.16*	0.19	
Weight	0.17*	0.25	
BMI		0.18*	
Professional activity	-0.29		-0.27
Use of supplements	0.18		0.20
Smoking	-0.24	-0.17*	-0.19
Other diseases		-0.24	
Medication	0.21		0.20
Anti-inflammatory medication		-0.21	
Other medication	0.21		0.22
Number of meals per day	0.21	0.26	0.19
Warm meals		0,26	
Soups		0.19	
Energy intake	0.26	0.34	
Protein intake	0.26	0.43	
Fats intake		0.22	
Carbohydrates intake	0.27	0.29	
Dietary fiber intake	0.20	0.36	
Sodium intake	0.28	0.39	
Potassium intake	0.27	0.52	
Total water intake		0.24	0.94
Breakfast	0.62		0.59
II breakfast	0.54		0.53
Lunch	0.47	0.28	0.33
Tea-time	0.41		0.38
Dinner	0.47	0.26	0.41
Snacking	0.55		0.57

Blank cells indicate no significant correlations

No significant correlations was found between occurrence of chronic diseases (renal, cardio-vascular, lung, food allergy, osteoporosis), education, blood pressure, physical activity, and healing diet and water intake. The correlation coefficients indicated strong relationship between selected factors and water intake in the study population of elderly (Tab. 6). Participants of the University of the Third Age (UTA groups) consumed less water in total and from beverages but consumed more water from solid food then individuals gathered in informal groups. The negative correlation was observed between gender (women) and total water intake. Water intake from beverages increased with the age of respondents, and body sizes (height and weight) affected water intake from the solid products. Older individuals consumed more water in total but less from solid food. Professional activity among elderly and smoking resulted in lower total water consumption and water from beverages. The use of supplements and most medications favoured a greater intake of water from beverages, what in turn resulted in higher total water intake. However the anti-inflammatory medication was negatively correlated with the intake of water of solid food.

The greater number of meals per day, the higher water consumption with any type of food. Moreover the presence of warm dishes and soups increased water intake from solid products. Intake of energy, macronutrients, sodium and potassium positively correlated with total water consumption and water of solid products. A strong relationship has been established between the presence of each meal and the consumption of total water and water from beverages, although only eating of lunch and dinner positively correlated with water from solid food.

DISCUSSION

The main objective of this study was to assess the water consumption and its determinants among a selected population of elderly with particular emphasis on the educational activity in old age. The elderly in Poland has not been studied in details as far as diet and especially water consumption concerns. The later one is a key component to their well-being and health.

There is a big change in a lifestyle when people retire because they can cease some activity or undertake a new one (eg. learning, physical activity). Some of them continue their careers. The changes in activity substantially influence the way of nutrition.

The diet of the population in this study was characterized by great regularity and the occurrence of snacking. This is confirmed by the study of *Kołajtis-Dołowy* et al. [15], who found on past and present habits of centenarians, that with age the frequency of breakfast consumption increases (from 86% of the respondents before the age of sixty to 97% at present), second breakfast (from 24% to 35%) and dinner (from 72% to 93%), afternoon tea (from 7% to 24%), supper (from 76% to 83%) and snacking (from 17% to 31%). The study of *Łągiewka* and *Sznajder* [17] also indicated a similar relationship between age and eating habits. They have established that 62% people aged over 64 years dined three times a day, and 30% - more often. Over 50% of the study population ate at fixed time and more than 90% consumed at least one warm meal per day. These results show a better quality of nutrition among elder people, and the greater stress put on a regular diet, and are similar to results of the present work.

The study women did not meet recommendations for energy intake, but the men did [13]. Similar results were obtained by *Wyka* and *Biernat* [26] in Wroclaw, and *Różańska* et al. [23] in small town Twardogóra, among the elderly. Moreover, it has been shown that the diet has changed over the last twenty years. The energy intake from daily consumption has significantly decreased from 2130 kcal in 1990 to 1455 kcal in 2006. A similar consumption for men in these years decreased by 1000 kcal. Similarly the study on nursing home residents demonstrated an energy deficit in the diet of both women and men; despite care they had [22]. However *Grochowska-Niedworok* et al. [12] have shown significant excess in energy intake in residents of four nursing homes in Silesia. Based on the results of present study and the some reports of other authors it can be concluded that after 2006 the energy consumption among the elderly over the age of 60 stabilized at about 1400 kcal for women, about 1900 kcal for men. The energy intake in women can also be affected by the place of residence. This study found that women living in Warsaw and Płock consumed less energy (about 300 kcal/day) than women living in rural areas [24]. However the place of residence did not have the same effect on male subjects.

In the study population of Płock and Warsaw protein intake exceeded the recommended values. However the amount of fats, carbohydrates and fibre was too low. In the study of elder people living in nursing home *Pysz-Izdebska* et al. [22] showed, in turn, proper protein intake among women and men, and too high fat intake among men, and too low carbohydrate and dietary fibre intake in both gender groups. The study conducted between 2002 and 2005 on people aged 25 - 64 by *Bolesławska* and *Przysławski* [3] confirmed this trend for protein, carbohydrate and fibre intake, however in the case of fat results were inconsistent. Only *Grochowska-Niedworok* et al. [12] reported too high intake of protein and fat in institutionalized elder persons in Silesian region. In terms of cholesterol studied subjects from Płock

and Warsaw showed adequate consumption of this nutrient (about 240 mg). Observed differences could result from a different age of the population, but it can be concluded that elder people pay more attention to the consumption of atherogenic food ingredients. The tendency to reduce intake of fats and carbohydrates may result from desire to reduce energy consumption, as demonstrated by the study of *Górecka* et al. [11] concerning the sanatorium patients in Ciechocinek. It has been found that elder people who are overweight (especially women) chose low fat products (especially cottage cheese, milk, low fat yogurt, and sugar-free drinks such as tea and coffee) unlike people of normal body weight.

It is disturbing in our study that sodium intake was two-fold higher than the AI standard [13], the more that it was the only sodium from products without salting. Potassium intake was less than half of AI. The proportions of sodium to potassium from the appropriate 57:120 Mmol to 152:66.7 Mmol in daily diet been heavily modified, which may strongly affect the management of electrolytes in the organism. This problem requires a separate study in terms of the treatment of hypertension.

Average values of total water intake in the present study indicated that women meets the EFSA recommendations, but men did not (less about 200 mL/day). However the percentage of women and men who did not meet the EFSA recommendations was similar (51% and 58% respectively). These data can be considered satisfactory, since the previous obtained by *Pietruszka* and *Krajewska* [21] showed that only 14 (for n = 206) of respondents carries out recommendations. Taking into account the comparative data from Sweden we can find better hydration of the elderly in Poland [7]. It can be considered that the situation of the elderly in Poland in terms of hydration is slowly improving, but still diet of older people in Poland is not well recognized. Regardless of the total volume of water consumed about 35% came from the solid food and 60% or more - from beverages. Similar proportions was stated in Germany among people over 65 years [19], however data for general population indicate that 70-80% of water came from beverages and the rest from solid food [6, 10, 18]. Despite this the results of *Gibson* and *Shirreffs* [10] helped confirm that age affects the proportions in supplying water by beverages and solid food, from 77% to 73% and from 23% to 27% respectively. This regularity is strongly emphasized in the present studies and it may be an important finding. It can probably be explained by an increased consumption of dishes with high water content i.e. warm vegetable and milk soups, popular in polish diet, especially among elderly, what was confirmed by correlation coefficients. Factors influencing this phenomenon in other countries should be studied.

Taking into account the criterion of water per energy intake (ml/kcal) 51% of women and 75% of men did not meet the recommendation. This is disturbing because it indicates a high risk of poor hydration and this indicator seems to be more sensitive than EFSA's AI or water volume intake per 1 kg of body weight, and could be more applicable across age and gender groups. *Gibson* and *Shirreffs* [10] also paid attention to the importance of this measure. The second important finding of the present study is the confirmation, that total water intake in older people should be assessed in the context of energy consumption, especially that older tend to its limitation.

In the studied population most water was consumed at lunch and breakfast. These are the most important meals in the diet of elderly in Poland and the lunch is the main meal. Respondents consumed approximately 20% of total daily water during breakfast, mostly from beverages and approx. 30% - during lunch. Similar results were obtained for Spanish seniors [8] and French adults [2]. Including second breakfast more than 60% of total daily water was consumed before 15.00 h (lunch time), and so in time of greatest activity of respondents, when the water consumption should be the highest. The *Pearson* correlations indicated a strong influence of beverages here. These observations are similar to [10]. From the point of view of the elderly, such regularity is beneficial, regardless of the type of the activity that the majority of respondents showed.

The physical activity increases water losses what should exceed the water intake [14] and in older people too [8]. In the present study this regularity was not observed, although the over 80% respondents declared practice exercises. However the third important finding of our study is the determination of the adverse effects of other activities of the population studied on the water consumption. Continuation of the careers and/or participation in UTA's contributed to less intake of water from beverages, what in turn affected the total water intake. In interviews, respondents emphasized that they have a lot of activities during the day and it prevents them from frequent and regular consumption of beverages. Meal time is essential for consuming beverages. On this basis, it can be assumed that any activity recommended for the elderly improving their overall well-being [16] may adversely affect the status of their hydration.

The main limitation of our study may be a number of respondents but sometimes it is difficult to get a large number of adequately reliable data in this age group. Despite this, the current results showed an improvement in hydration of elderly people in Poland over the last 10 years.

CONCLUSIONS

1. This study reports that approximately half of the studied elderly women and men aged over 60 years did not meet the EFSA recommendations for total water intake.
2. Small value of the water vs. energy intake ratio is even more worrying, because it concerns a higher proportion of elderly, particularly males.
3. The recommendations for the elderly concerning nutrition and various forms of activity should include detailed information on a regular and proper hydration. This is particularly important in the context of age-related diseases and medication, which are often associated with water-electrolyte balance.
4. The elderly leading an active life (working, studying) may consist a risk group vulnerable to dehydration, so monitoring is needed.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

EVALUATION OF SUSCEPTIBILITY OF POLYMER AND RUBBER MATERIALS INTENDED INTO CONTACT WITH DRINKING WATER ON BIOFILM FORMATION

Maciej Szczotko[*]*, Agnieszka Stankiewicz, Małgorzata Jamsheer-Bratkowska*

National Institute of Public Health – National Institute of Hygiene,
Department of Environmental Hygiene, Warsaw, Poland

ABSTRACT

Background. Plumbing materials in water distribution networks and indoor installations are constantly evolving. The application of new, more economical solutions with plastic materials eliminates the corrosion problems, however, do not fully protect the consumer against secondary microbial contamination of water intended for human consumption caused by the presence of a biofilm on the inner surface of materials applied. National Institute of Public Health - National Institute of Hygiene conducts research aimed at a comprehensive assessment of this type of materials, resulting their further marketing authorization in Poland.

Objectives. Evaluation and comparison of polymer and rubber materials intended to contact with water for the susceptibility to biofilm formation.

Materials and Methods. Plastic materials (polyethylene, polypropylene, polyvinyl chloride) and rubber compounds (EPDM, NBR), from different manufacturers were evaluated. The study was carried out on 37 samples, which were divided into groups according to the material of which they were made. The testing was conducted according to the method based on conditions of dynamic flow of tap water. The level of bioluminescence in swabs taken from the surface of the tested materials was investigated with a luminometer.

Results. Evaluation of plastic materials does not show major objections in terms of hygienic assessment. All materials met the evaluation criteria established for methodology used. In case of rubber compounds, a substantial part clearly exceeded the limit values, which resulted in their negative assessment and elimination of these materials from domestic market.

Conclusions: High susceptibility to the formation of biofilm in the group of products made of rubber compounds has been demonstrated. Examined plastic materials, except for several cases, do not revealed susceptibility to biofilm formation, but application of plastics for distribution of water intended for human consumption does not fully protect water from secondary, microbiological contamination. Complete verification of plumbing materials including biofilm formation test before their introduction into the domestic market should be continued.

Key words: *biofilm, plumbing materials, hygienic assessment, drinking water*

STRESZCZENIE

Wprowadzenie. Materiały wykorzystywane w sieciach wodociągowych oraz instalacjach wewnątrz budynków ulegają ciągłym zmianom. Wprowadzenie nowych bardziej ekonomicznych rozwiązań z zastosowaniem materiałów z tworzyw sztucznych eliminuje problemy związane z korozją, jednak nie zabezpiecza konsumenta przed wtórnym mikrobiologicznym zanieczyszczeniem wody przeznaczonej do spożycia powodowanym występowaniem biofilmu na wewnętrznej powierzchni rur i przewodów instalacyjnych. W Narodowym Instytucie Zdrowia Publicznego - Państwowym Zakładzie Higieny, prowadzone są badania mające na celu kompleksową ocenę tego typu materiałów, czego efektem jest wydawanie Atestów Higienicznych i dopuszczenie materiałów do obrotu na krajowym rynku.

Cel. Celem badań było porównanie i ocena materiałów z tworzyw sztucznych i gumy przeznaczonych do kontaktu z wodą do spożycia w zakresie ich podatności na tworzenie biofilmu.

Materiał i metody. Ocenie poddano materiały z różnych tworzyw sztucznych (polietylen, polipropylen, polichlorek winylu) oraz mieszanki gumowe pochodzące od różnych producentów. Badania wykonano dla 37 próbek, które zostały podzielone na grupy w zależności od rodzaju materiału z jakiego zostały wykonane. Badania prowadzone były zgodnie z metodyką własną, w dynamicznych warunkach przepływu wody, z wykorzystaniem urządzeń przepływowych (UPE). Za pomocą luminometru oznaczano poziom bioluminescencji w wymazach pobranych z powierzchni testowanych materiałów.

[*]**Corresponding author**: Maciej Szczotko, National Institute of Public Health - National Institute of Hygiene, Department of Environmental Hygiene, 24 Chocimska street, 00-791 Warsaw, Poland, tel.: +48 22 5421 382, fax: +48 22 5421 287, e-mail: mszczotko@pzh.gov.pl

Wyniki. Ocena zbadanych materiałów z tworzyw sztucznych nie budziła większych zastrzeżeń pod względem higienicznym. Wszystkie materiały spełniały kryteria oceny określone w stosowanej metodyce badawczej, przy czym w kilku przypadkach oznaczone wartości były bliskie dopuszczalnego limitu. W przypadku mieszanek gumowych, w znacznej części stwierdzono wyraźne przekroczenia dopuszczalnych wartości, co skutkowało negatywną ich oceną oraz eliminacją tych materiałów z obrotu na krajowym rynku i możliwością ich wykorzystywania w kontakcie z wodą do picia.
Wnioski. Wykazano znaczną podatność zbadanych produktów wykonanych z mieszanek gumowych na tworzenie się biofilmu. Materiały tworzywowe jak polietylen, polichlorek winylu i polipropylen, w znacznej większości nie wykazywały podatności na tworzenie się biofilmu, jednak ich zastosowanie do dystrybucji wody przeznaczonej do spożycia przez człowieka nie zabezpiecza jej w pełni przed wtórnym, mikrobiologicznym zanieczyszczeniem. W celu pełnej weryfikacji materiałów, przed ich wprowadzeniem na krajowy rynek, niezbędna jest dalsza ich ocena, z uwzględnieniem ich podatności na tworzenie się biofilmu.

Słowa kluczowe: *biofilm, materiały instalacyjne, ocena higieniczna, woda przeznaczona do spożycia*

INTRODUCTION

To the end of the 90's of the last century the water supply systems in Poland were made of traditional materials like cast iron. They accounted for over 50% of the network and their technical condition due to the long lifetime (over 50 years) was of low quality [6]. However, these installations underwent successive repairs and upgrades, which entailed also change of materials used for this purpose. Since the beginning of the twenty-first century, it was observed a significant part of different material solutions like polyethylene (PE) and polyvinyl chloride (PVC) as well as an increasing use of a new generation of iron, so-called ductile iron [15, 16, 18]. Currently, a significant share in the materials structure have pipes made of polymer materials (PE, PVC and others), which in some cases account for more than a half of the length of water supply networks. Generally, it can be said, that the water supply systems are built mainly of pipes made of cast iron, steel protected against corrosion usually by zinc, cementitious coating, PVC and PE. The pipes made of these materials account for about 90% of the network length [14, 17]. In fact, it does not differ much from the standards in other European Union countries, as well as in the United States [25]. Plastics intended for use in the construction industry, especially those that can be applied in contact with drinking water must meet a number of strict requirements, not only technical like strength, flexibility and corrosion resistance, but primarily hygienic requirements, which apply to both, the physicochemical and microbiological parameters. All over the world the research is carried out focused on the acceptance of different products/materials contacting with water intended for human consumption. These tests include so-called migration tests, performed to proof that the product/material has no negative influence on the quality of water contacting with it, and microbiological tests, including biofilm formation test. The hygienic assessments of products intended to contact with drinking water, are performed, among others, in the research centers in Germany (Gelsenkirchen Institute), France – Pasteur Institute (ACS, AFNOR), Great Britain (WRAS), the Netherlands (KIWA), United States (NSF) and in many other countries, including Poland. Here in the National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) hygienic assessment of materials is conducted during procedure of Hygienic Certificate issuing [3, 7, 12, 27, 30]. These centers conduct physicochemical and microbiological research, but only some of them carry out tests on microbial growth (biofilm formation test) for plastic and rubber products. Increasing number of metallic materials is replaced by polymer materials because of their high chemical resistance, good mechanical properties, which can be improved through the addition of modifiers like chalk, carbon black, chopped glass and elastomers. The use of polymer materials eliminates corrosion, known from occurrence in traditional materials (like cast iron, steel, concrete), provides channels tightness, even in critical situations (deflection instead of cracking) and guarantees proper attention to economy of the solutions. However, an attention should be payed to possible interactions that occur between the microorganisms present ~~ce~~ in tap water, and the material from which the network is made. In water environment, even inside the pipes and lines, which supply drinking water to consumer, there are two main forms of the presence of microorganisms. They can float freely in unbound form or bound with molecules of organic or inorganic matter suspended in water (planktonic form) or they can form complex agglomerates permanently colonizing the inner surfaces of the pipes - biofilm. Both these forms are not mutually exclusive, but microorganisms forming biological membranes are more frequently observed [22]. Biofilm, also defined as a biological membrane, is a three-dimensional, spatially complex structure, arising at the phases border, including different kinds of organic and inorganic surfaces contacting with water. The composition of the biofilm can vary. It can be a monoculture or a cluster of bacteria, very diverse morphologically and physiologically [4, 21]. Biofilm formed in plumbing installation is a very common phenomenon. Once produced, becomes very

difficult to remove and can cause many problems of technological importance by intensifying processes of biological corrosion and causing significant hydraulic losses inside the network and, above all, it may cause the health problems to consumer [13, 26]. Biofilm creates very favorable living conditions for microorganisms, provides them with a greater availability of nutrients [5, 23] and enables long-lasting and very stable settlement of diverse solid substrates, including construction materials contacting with water. Their presence has an impact on the quality of water delivered to the consumer. Frequent detachment of fragments of the mature biofilm inside water network causes ejection of a large number of bacteria, including pathogens and potential human pathogens such as *Legionella pneumophila*, *Pseudomonas* sp., *Aeromonas* sp., *Campylobacter* sp., *Escherichia coli*, *Salmonella* sp., *Shigella* sp. as well as microscopic fungi [9, 10, 19, 29]. During exploitation of the water network, a permanent growth of the biofilm is observed, wherein the biomass of bacteria can reach 95% and only 5% of bacteria is present in the water in the form of so-called phytoplankton [20].

Therefore, it is important to choose the appropriate materials, which are used to build a water supply system at the first stage of its design. It enables avoiding the subsequent operational problems associated with the metabolic activity of microorganisms. Due to the extension of the hygienic certification procedure conducted in NIPH – NIH on biological laboratory tests allowing to perform complete assessment of the materials intended to contact with drinking water. It is possible to verify the quality of certified materials and to eliminate those, that due to the high susceptibility to biofilm formation, can cause secondary microbial contamination of water supplied to the consumer.

The aim of this study was the evaluation and comparison between some polymer materials and elements made of rubber intended to contact with drinking water on their susceptibility to biofilm formation.

MATERIALS AND METHODS

The study was carried out on 37 samples, which were divided into several groups according to the material of which they were made, enabled the general characteristics of each group.

Tested materials

During the research the following polymer materials were used: polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and rubber compounds (EPDM, NBR and others). Polymers as main compounds of plastic materials are safe for human health. However, the remains of unreacted monomer and modified additives can be volatile and toxic. Modifiers usually in the form of low molecular weight compounds can penetrate from the plastic to the water and deteriorate its quality, thus providing nutritional compounds for microbes in tap water. Among the huge amount of rubber compounds the largest part of the sanitary products are those made of EPDM and NBR. They are characterized by a high chemical resistance including a resistance to atmospheric agents. They meet the specific requirements associated with the flow of hot water (high temperature resistance), especially in the case of EPDM and low deformation, which translates into prolonged use. Unfortunately, materials like rubber compounds and polymers contacting with drinking water may cause the secondary pollution both, chemical and microbiological.

All tests were performed using two control reference materials. Positive control (susceptible to biofilm formation) was glass plate coated with paraffin wax layer. Negative control (unsusceptible to biofilm formation) was plate made of stainless steel.

Continuous flow reactor

Specific reactors - UPE (Polish specific name) supplied from cold tap water were used in this investigation (Figure 1). The cylindrical body of UPE (inside diameter was 150 mm, high was 550 mm) was made of high quality stainless steel with Teflon seal. On top of the reactor was a removable cover with a venting valve. Water inflow tube was made of Teflon and water pressure was regulated by a ball valve. Inside the reactor, at the bottom, a conical diffuser with two partitions was located. A special sample stand made of stainless steel was placed in vertical position inside the cylinder. The water outflow with water meter was located on the side of the UPE about 50 mm below the top. The water flow proceeded from the bottom to the top of reactor so that the reactor could be filled with water evenly and flow direction was protected against the mixing of inlet and outlet water.

The testing method applied in the Department of Environmental Hygiene Laboratory of NIH-NIPH involves the measure of bioluminescence level in swabs taken from the surface of material contacting with drinking water by using luminometer. Exposition of tested material lasts from eight (polymers) to ten weeks (rubber) and is performed inside the continuous flow reactor (UPE) in conditions of dynamic water flow. The crucial element of the testing method is the fact, that all living cells include the universal chemical compound - adenosine triphosphate (ATP) which functions as a carrier of free energy. This energy is used in most of the life processes requiring the energy input. During biochemical reaction of enzymatic decomposition of ATP energy is emitted in form of light (bioluminescence). The measure of this energy enables indirect assessment of ATP concentration in swab sample taken from the surface of tested material.

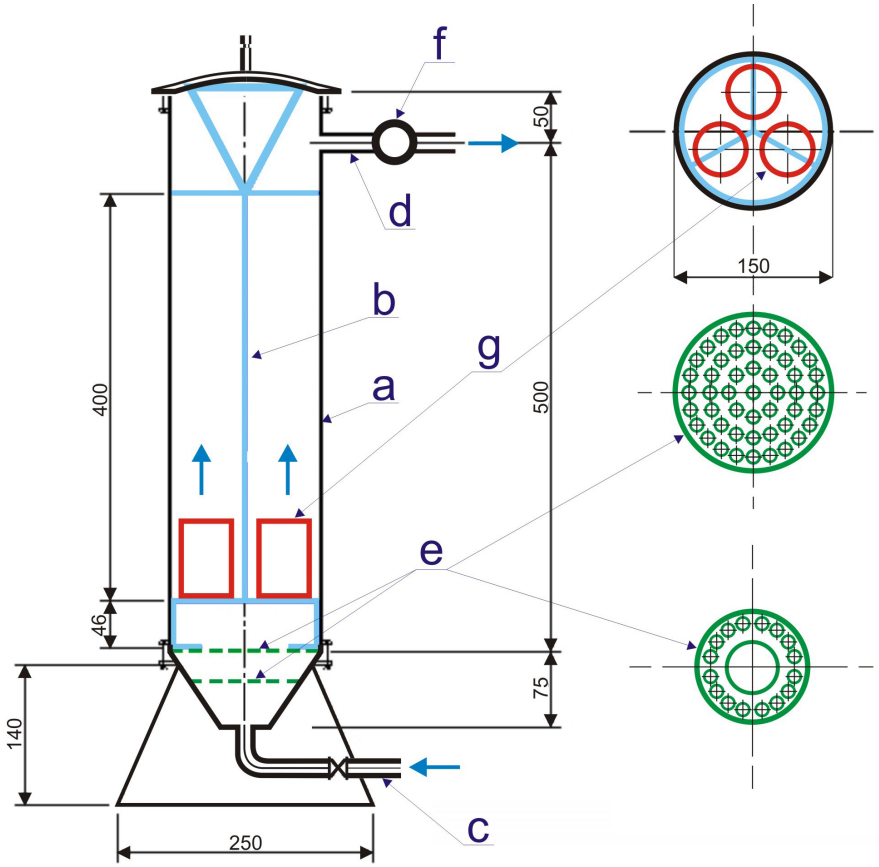

Figure 1. Continuous flowing reactor's diagram. a – steel cylinder, b – samples stand, c – water inflow, d – water outflow, e – diffuser, f – water meter, g – sample of material intended to contact with water.

HY-LiTE® (MERCK) test systems to *in vivo* application were used to bioluminescence level measures. The test system consists of hygiene swabs free of ATP and the "pens" with proper dose of liquid reagents to dilution, buffering and neutralization of the sample, as well as lyophilisate of reaction complex luciferin-luciferase. This test system was adapted to operate with luminometer HY-LiTE 2® (MERCK). The sensitivity of applied tests was 1,4 x 10^{-14} mols ATP.

RESULTS

The tests results on biofilm formation process for various types of materials intended for contact with drinking water from different producers were presented in Tables 1, 2 and 3.

Ten different products made of polyvinyl chloride intended to contact with drinking water were tested and evaluated. The results obtained for all PVC samples were similar (Table 1). In each case values expressed in RLU/cm^2 did not exceed the acceptable limit determined pursuant to ten-time values observed on the negative control surface (10 x K-). Sample number 5 characterized the highest bioluminescence values, however the values were below acceptable limit during full time of investigation. In all cases presented above, examined materials obtained positive test assessment, what confirmed their proper quality and resulted in issuing of the Hygienic Certificate and further marketing authorization in Poland.

Results for tested seven different products made of polypropylene from different producers are presented in Table 2. The bioluminescence level on the surface of six of them was low (<500 RLU/cm^2). In case of one material (sample 2) the bioluminescence level, measured in the measuring period, exceeded slightly 2000 RLU/cm^2, however the negative control sample, tested in parallel, showed results 253 RLU/cm^2. Thus, working on the assumption of the assessment criteria for the method (10 x K-), enabled to positive assessment of this material sample made of polypropylene. All samples made of polypropylene obtained positive test assessment, what confirmed their proper quality and resulted in issuing of the Hygienic Certificate and further marketing authorization for direct contact with water intended for human consumption.

Ten different products intended to contact with drinking water and made of polyethylene from different producers were tested. The results for all samples presented above were similar (Table 3). In case of eight of them, during eight week examined period, the bioluminescence was observed on the level very close to negative control, so in the range from

approximately 100 RLU/cm^2 to 300 RLU/cm^2. In three cases (samples no. 3, 4 and 10) the values obtained were higher and amounted above 1000 RLU/cm^2. They still did not exceed the unacceptable value limit. All examined polyethylene materials obtained positive test assessment, what confirmed their proper quality and resulted in issuing of the Hygienic Certificate and further marketing authorization in Poland.

Table 1. Bioluminescence on the surface of different polyvinyl chloride (PVC) materials expressed in RLU*/cm2

PVC	Subsequent weeks of the study								
	0	I	II	III	IV	V	VI	VII	VIII
sample 1 limit value**	36 170	358 750	721 1100	900 1350	800 1410	870 1630	950 1540	970 1490	1090 1510
sample 2 limit value	18 140	29 650	39 1050	29 2250	62 920	175 1250	457 1020	321 950	125 820
sample 3 limit value	29 130	93 1450	65 770	85 1880	142 2010	162 2070	150 1540	127 1120	110 790
sample 4 limit value	20 310	159 1100	107 1620	187 1160	225 720	188 1210	153 1420	137 1350	73 1470
sample 5 limit value	21 300	155 1100	171 810	166 1400	266 610	411 750	633 1000	670 870	600 900
sample 6 limit value	19 370	56 660	89 1210	74 2100	151 2580	234 3090	287 2740	211 3660	178 3530
sample 7 limit value	19 140	67 800	98 1250	165 1600	170 1400	260 850	210 910	180 1110	160 780
sample 8 limit value	11 90	45 330	90 560	125 800	160 1000	230 1200	250 1450	240 1600	260 1700
sample 9 limit value	11 120	56 450	94 800	144 900	187 1320	156 1600	210 1650	183 1910	162 1800
sample 10 limit value	9 120	40 450	66 800	97 900	110 1320	121 1600	106 1650	120 1910	115 1800

* - Relative Light Units

** - acceptable limit of RLU/cm2 determined pursuant to ten-time values observed on the negative control surface (10 x K-)

Table 2. Bioluminescence on the surface of different polypropylene materials expressed in RLU/cm2

PP	Subsequent weeks of the study								
	0	I	II	III	IV	V	VI	VII	VIII
sample 1 limit value	29 370	95 660	134 1210	195 2100	254 2580	312 2090	378 2340	472 2660	456 2530
sample 2 limit value	30 370	150 660	317 1210	1000 2100	1356 2580	1389 2090	2389 2340	2100 2660	2433 2530
sample 3 limit value	37 370	156 660	140 1210	143 2100	158 2580	165 3090	156 2740	147 3660	166 3530
sample 4 limit value	29 370	98 660	123 1210	167 2100	197 1950	211 2210	356 2740	287 3000	311 2470
sample 5 limit value	25 140	25 650	26 1050	57 2250	248 920	48 1250	350 1020	190 950	160 820
sample 6 limit value	37 310	170 1100	110 1620	120 1160	138 720	145 1210	126 1420	147 1350	136 1470
sample 7 limit value	9 120	64 450	159 800	264 900	350 1320	445 1600	474 1650	450 1910	463 1800

Table 3. Bioluminescence on the surface of different polyethylene materials expressed in RLU/cm^2

PE	Subsequent weeks of the study								
	0	I	II	III	IV	V	VI	VII	VIII
sample 1	37	180	130	140	138	175	156	127	146
limit value	300	1110	980	1330	810	750	930	960	740
sample 2	36	106	182	274	286	311	246	198	233
limit value	230	560	890	1740	1470	1620	1850	1770	1810
sample 3	30	130	217	736	1416	1311	2367	1976	2133
limit value	370	660	1210	2100	2580	3090	2740	3660	3530
sample 4	21	97	196	311	560	710	900	732	1162
limit value	150	350	220	900	1640	1870	1680	2100	1640
sample 5	25	75	110	166	148	136	250	121	100
limit value	170	1000	1520	1700	1650	1480	1800	1300	950
sample 6	25	64	134	157	183	155	110	124	131
limit value	170	1000	1520	1700	1650	1480	1800	1300	950
sample 7	34	58	132	167	190	210	150	110	102
limit value	190	480	1200	1110	980	1300	1570	900	840
sample 8	32	77	86	74	103	94	124	151	133
limit value	300	450	630	900	800	1200	1100	1000	930
sample 9	32	303	433	457	683	225	203	165	177
limit value	170	3130	2830	3720	8670	3470	3920	5480	8980
sample 10	30	150	317	1000	1356	1389	2389	2100	2433
limit value	370	660	1210	2100	2580	3090	2740	3660	3530

Table 4. Bioluminescence on the surface of different rubber materials expressed in RLU/cm2

Rubber	Subsequent weeks of the study										
	0	I	II	III	IV	V	VI	VII	VIII	IX	X
sample 1	37	262	1373	3633	5283	13133	12666	13000	13783	12666	11166
limit value	300	1110	980	1330	810	750	930	960	1330	1240	1110
sample 2	15	60	51	1354	2400	4297	5711	4963	5342	5140	5336
limit value	150	250	220	560	810	700	960	900	1200	1200	1330
sample 3	42	360	1100	3100	4955	6133	5678	4200	3400	3700	3263
limit value	330	870	1000	1230	790	1090	2300	2370	1900	2000	2230
sample 4	37	262	373	2633	5283	23166	12666	3000	13783	12644	13121
limit value	470	980	370	530	1310	1160	850	960	3180	2310	2090
sample 5	14	746	1666	1433	950	846	963	1117	1063	945	1022
limit value	120	760	1220	2590	3170	2730	1430	2110	3630	4000	3890
sample 6	19	470	3100	7500	10600	12300	12150	11200	11630	10700	10500
limit value	140	800	1250	1600	1400	850	910	1110	780	980	930
sample 7	13	1750	4600	13700	16100	19500	25300	28000	29100	33000	31300
limit value	70	650	1410	2100	3600	3900	4800	4500	5100	6100	5500
sample 8	12	654	1890	3750	6570	8590	9100	9600	9500	11900	12300
limit value	90	450	630	1140	1500	1560	1680	1700	1680	1520	1460
sample 9	10	850	3600	7800	8500	7900	9100	8900	9500	12400	14500
limit value	90	330	560	800	1000	1200	1450	1600	1700	2200	3100
sample 10	13	900	1540	1800	2200	2230	2160	2300	2500	2350	2400
limit value	120	940	1090	1300	1740	1690	2200	2000	1900	2300	2500

Ten samples made of rubber compounds intended to contact with drinking water were tested. All of them were intended for production of seals and flexible hoses. The testing period was prolonged to ten weeks, what enabled more precise observation of dynamic changes of bioluminescence level determined in swabs coming from the samples surface. Only in two cases (samples no. 5 and 10) the assessment of the material proceeded without reservations. The bioluminescence values from the surface of the remaining 8 samples were significantly higher than analogical values from the surfaces of polymeric materials (PE, PP and PVC). In the course of the results interpretation the application of the final product, was taken into consideration, including the area of the materials surface, which contacts with drinking water in the real application. In case of the products like sealing and rubber pads this surface is usually of the minimal degree and possible microbial growth covers only the connection points inside the installation sealed with these materials. Despite these materials did not meet the requirements for materials contacting with drinking water they were conditionally allowed for that particular kind of application. In cases when the rubber compound was destined for flexible hoses production – the product with significantly larger surface contacting with drinking water than sealing elements – the samples did not obtain positive assessment and they were not allowed to contact with water intended for human consumption.

DISCUSSION

All tested samples of polymeric materials (polypropylene, polyethylene, polyvinyl chloride) demonstrated a low susceptibility to the biofilm formation. The bioluminescence from their surface were within the predetermined acceptable limits, which means they did not exceed ten times the level of bioluminescence determined in swabs taken from the negative control surface. Despite that fact, in some cases, a temporary growth of determined values was observed, which could be related to the migration of the substances like stabilizers and hardeners added to products during their production from the material's surface. These substances may be a potential source of organic carbon for microorganisms, resulting in enhanced growth of their numbers on the materials surface. The studies conducted by *Traczewska* and *Sitarska* [31] also confirmed that the materials of this type due to the emission of the substances stimulating the growth of microorganisms may be susceptible to biofilm formation, and because of that fact, their use in new water supply networks does not provide protection against secondary microbial contamination and corrosion. This particular fact is important in case of the use of poor quality materials characterizing by high emissions of organic substances. The possibility of appearing in the water network microorganisms, that are capable of metabolic degradation of the polymers should additionally be taken into account. However, such a phenomenon occurs at the moment of formation of the biofilm mature form, which is frequently related to poor quality of the material, which contributes to the promotion of microbial colonization of its surface [8]. Also, comparison of the results of the assessment of various organic materials susceptibility, obtained by using a parallel classical microbiological techniques, indicates such character of the most of the polymeric products and materials [1]. However, it should be taken into consideration, that only a small part of this type of materials available on the market was tested. Among them, it can be stated a considerable variety of additives purposed to prolong the life of polymers (plasticizers), giving them a specific physicochemical properties (stabilizers), and other chemical compounds such as dye additions. Each of these substances undergo a migration from the material into the water inside the installation, which can be a potential source of nutrients for microorganisms [24].

The second group of tested materials was rubber, which is generally used for production of sealing elements in fittings and other products in contact with drinking water. Due to complex chemical composition, as well as a very large number of organic additives, which undergo intensive migration to the water, the materials from this group represent usually the largest percentage among all materials and products evaluated negatively. A final assessment of those materials was expanded to include fact, that the sealing elements are usually small in size and their contact with water inside the network is very limited. In spite of this, only two of the tested rubber materials samples have been evaluated without reservations as appropriate to contact with drinking water. In all other cases, the materials were evaluated negatively. In one case, the regular supplier of raw material (a blend of EPDM) has changed its chemical composition without informing about that fact of the manufacturer of the final product. Changes in the chemical composition caused the increased emission of organic substances, which enhanced the growth of microorganisms on the product`s surface, manufactured from raw material of inadequate quality.

Bressler et al. [2] in their study also included the blend of EPDM to materials, which promote the growth of microorganisms, and biofilms formed on the surface of products from these materials which caused secondary microbial contamination of the water network, and additionally might constitute a reservoir of potentially pathogenic bacteria for instance *Pseudomonas aeruginosa.* Similar observations

published *Kilb* et al. [11], who described the parts of the installation made of soft rubber materials such as EPDM or NBR as a potential source of secondary microbial contamination of the network inside the building. This is also confirmed by the results of the tests comparing the number of microorganisms in the network supervised by the water producer before it is delivered to the consumer and at the consumer's directly. *Pepper* et al. [28] observed that microbiological contamination of water is generally higher in water samples collected from the consumer, which is the result of the indoor plumbing materials, which promote the growth of microorganisms.

Biofilm formation on materials used for distribution and storage of water intended for human consumption is determined by many factors. Among them the main role play complex relations between water quality and the way of its treatment, and the technical and operating conditions of water distribution systems. The specific technical and chemical properties of the materials and construction products used in the water network are one of the factors that could significantly affect the increase of the phenomenon. The assessment of organic materials used in the storage and distribution of water with regard to their susceptibility to biofilm formation is important for practical reasons. This phenomenon should be taken into consideration in the process of hygienic evaluation of the materials prior to their use in practice. It can be helpful for reducing the scale of the risk associated with biofilm formation and, as a consequence, inadequate microbiological quality of water consumed.

CONCLUSIONS

1. Significant susceptibility to biofilm formation in the group of products made of rubber compounds, including NBR and EPDM compounds, was found.
2. Tested polymeric materials such as polyethylene, polyvinyl chloride, polypropylene, except for several cases, do not revealed susceptibility to biofilm formation.
3. Application of polymer materials for distribution of water intended for human consumption does not fully protect water from secondary microbiological contamination.
4. Further assessment of materials and products contacting with water intended for human consumption is necessary in terms to their susceptibility on biofilm formation.

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Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

QUANTITATIVE ASSESSMENT OF NUTRITION IN PATIENTS WITH THE POLYCYSTIC OVARY SYNDROME (PCOS)

Małgorzata Szczuko[1], Magdalena Skowronek[1], Marta Zapałowska-Chwyć[2], Andrzej Starczewski[2]*

[1]Department of Biochemistry and Human Nutrition, Pomeranian Medical University in Szczecin, Poland
[2]Clinic of Gynecology and Urogynecology, Pomeranian Medical University in Szczecin, Poland

ABSTRACT

Background. PCOS (polycystic ovary syndrome) is called a pathology of the XX century and affects at least 10-15% women of childbearing age. The therapy involves pharmacotherapy of hormonal imbalance, as well as the change of lifestyle, including the diet.

Objective. Performing the quantitative assessment of components of diets of women with PCOS, comparing the results with current dietary standards for Polish people and defining dietary requirements for the patients.

Material and Methods. The study was performed on 54 women of childbearing age (average age 26.03± 5.52) with PCOS syndrome diagnosed according to on the Rotterdam criteria. Anthropometric measurements of the patients were made and BMI and WHR calculated. Quantitative assessment of women's diets was performed based on the analysis of 3-day food diaries and food records taken from the previous 24h with the interview method. The data were introduced to a dietary software DIETA 5.0, calculating the average intake of the energy, nutrients, vitamins, minerals, cholesterol and dietary fibre. The obtained results were compared to Polish dietary guidelines.

Results. Examined group was characterized by increased waist circumference (98.71± 13.6 cm) and an average WHR was 0.92± 0.08. An increased average value of BMI was also shown (28.91± 5.54 kg/m^2). The patients consumed, on average, 1952.5±472.7 kcal daily, and the risk of insufficient intake of protein was determined in 36.7% of examined women. The highest risk of deficiency in minerals in women with PCOS was related to calcium (634 mg), potassium (3493 mg) and magnesium (250.1 mg), whereas with reference to vitamins deficiency as much as 70% of tested women were at risk of insufficient intake of folic acid, 36.7% of them - vitamin C, and 26.7% - vitamin B12. The average consumption of vitamin D was at the level of 3.4 µg. Test group was characterized by excessive average consumption of total fat (50%), SFA (70.4%) and saccharose (50%). The percentage of people with excessive average intake of cholesterol was at the level of 40.74%. As much as 83.3% patients consumed too low amounts of dietary fibre in their diets (<25g).

Conclusions. In diet therapy of women with PCOS there should be higher intake of folic acid, vitamins D and C, cobalamin, dietary fibre and calcium. The consumption of total fats, saturated fatty acids and cholesterol should be reduced, as through facilitating the development of diabetes and cardio-vascular diseases, they affect the dysfunction of ovaries. The diet of some of the patients should be also supplemented by potassium, magnesium and zinc. The introduction of a properly balanced diet should be the key in the treatment of women with PCOS diagnosed according to Rotterdam criteria.

Key words*: polycystic ovary syndrome, PCOS, nutrition, diet, nutrients, nutritional deficiencies*

STRESZCZENIE

Wstęp. Syndrom policystycznych jajników (PCOS) nazywany jest patologią XX wieku i dotyka co najmniej 10–15% kobiet w wieku reprodukcyjnym. Terapia dotyczy zarówno leczenia farmakologicznego zaburzeń hormonalnych, metabolicznych jak również zmiany stylu życia, w tym sposobu żywienia.

Cel. Ocena składu ilościowego jadłospisów kobiet z PCOS, porównanie go z obowiązującymi normami żywienia dla ludności polskiej oraz sprecyzowanie zaleceń żywieniowych dla pacjentek.

Materiał i metody. Badaniami objęto 54 kobiety w wieku rozrodczym, z rozpoznanym, według kryteriów Rotterdamskich, zespołem PCO (średni wiek to 26,03± 5,52 lat). Wykonano pomiary antropometryczne oraz obliczono wskaźniki BMI i WHR. Oceny ilościowej sposobu żywienia kobiet dokonano w oparciu o analizę trzydniowych dzienniczków oraz jadłospisu zebranego metodą wywiadu o spożyciu z ostatnich 24 godzin. Dane wprowadzono do programu dietetycznego DIETA 5.0, obliczając średnią podaż energii, składników odżywczych, witamin, składników mineralnych, cholesterolu oraz błonnika. Uzyskane wyniki porównano z obowiązującymi w Polsce normami żywienia.

[*]**Corresponding author**: Małgorzata Szczuko, Department of Biochemistry and Human Nutrition, Pomeranian Medical University in Szczecin, Broniewskiego street 24; 71-460 Szczecin; tel. +48 91 4414810; fax. +48 91 4414807, e-mail: malgorzata.szczuko@pum.edu.pl;

Wyniki. Badana grupa charakteryzowała się zwiększonym obwodem pasa 98,71± 13,6 cm, a średnia WHR była równa 0,92± 0,08. Wykazano zwiększoną średnią wartość wskaźnika BMI (28,91± 5,54 kg/m^2). Pacjentki spożywały średnio 1952,5±472,7 kcal dziennie, a zagrożenie niedostatecznym spożyciem białka stwierdzono u 36,7% badanych kobiet. Największe ryzyko wystąpienia deficytu składników mineralnych u kobiet z PCOS dotyczyło wapnia (634 mg), potasu (3493mg), magnezu (250,1 mg) natomiast wśród witamin aż 70% kobiet badanych było zagrożonych niewystarczającym spożyciem folianów, 36,7% niedoborem witaminy C a 26,7% witaminą B12. Średnie spożycie witaminy D kształtowało się na poziomie 3,4 µg. Badana grupa charakteryzowała się nadmiernym średnim spożyciem tłuszczu ogółem (50%) NKT (70.4%) i sacharozy (50%). Odsetek osób z nadmiernym średnim poborem cholesterolu był na poziomie 40.74%. Aż 83.3% pacjentek miało zbyt niską podaż błonnika w diecie (<25g).
Wnioski. W dietoterapii kobiet z PCOS należy zwiększyć podaż folianów, witaminy D i C, kobalaminy, błonnika oraz wapnia. Powinno ograniczyć się spożycie tłuszczu ogółem, nasyconych kwasów tłuszczowych i cholesterolu które pogłębiając rozwój cukrzycy i chorób sercowo-naczyniowych wpływają na dysfunkcję jajnika. Dietę części pacjentek z PCOS należałoby również uzupełnić w potas, magnez, cynk. Wprowadzenie prawidłowo zbilansowanej diety powinno być kluczem w leczeniu kobiet z PCOS rozpoznawanych kryteriami Rotterdamskimi.

Słowa kluczowe: *zespół policystycznych jajników, PCOS, żywienie, dieta, składniki pokarmowe, niedobory żywieniowe*

INTRODUCTION

It is estimated that PCOS occurs in ca. 10-15% women of childbearing age [12]. It is called a pathology of the XX century, since it can cause numerous health issues. The symptoms occurring in this syndrome are mainly related to lower fertility, menstruation disorders and hyperandrogenism, which is manifested by acne and hirsutism.

Several concepts of the development of PCO syndrome are considered, including ovarian, insulin-dependent and gonadotropic concepts. Improper dietary habits are often noticed among the women with PCO syndrome, and those are related to the intake of food of low nutritional value and high energy content, which seems to be in accordance with the concept of insulin-dependent cause of PCOS. As reported in the literature, insulin increases the production of androgens, what affects ovarian theca cells, and leads to higher concentration of those hormones in women with PCOS, as compared to healthy ones [26]. It has been proposed that insulin activates the expression of CYP-17 gene, which takes part in androgens production [40]. Insulin sensitizes ovarian granulosa cells to LH too quickly, thus stops the maturation of the follicle. LH acting synergistically with insulin leads to the activation of androstenedione synthesis and hormonal imbalance [40].

It also seems that insulin indirectly affects steroid genesis via insulin-like growth factor (IGF-1). Its elevated concentration in follicle fluid contributes, together with insulin, to increased androgenesis, as well as to premature ovarian follicle atresia. The reciprocal effect of insulin-like growth factor (IGF-1) strengthens the above mentioned symptoms [14]. Moreover, the studies showed that the increased concentration of testosterone in blood of women with PCOS is also affected by decreased production of SHBG (sex hormone binding globulin) by the liver, caused by inhibiting activity of insulin [19].

Women eating habits are determined, among others, by the habits taken from family home, economic status, culture (religion, tradition), education, origin and age. The relation between level of nutritional education and the risk of breast cancer was shown by [17]. According to *Chavarro et al.* [10], fertility can be improved by lowering the consumption of trans fats with simultaneous increase in consumption of monounsaturated fatty acids, limiting the intake of animal proteins in exchange for plant proteins, diet rich in dietary fibre and products with low glycemic index (GI), consumption of full fat dairy products and proper amount of minerals and vitamins in a diet. In studies of *Chavarro et al.* [11] it was shown that both the reduction in animal proteins in favour of plant protein and the increase in the consumption of plant protein resulted in lowering the risk of infertility caused by ovulation disorders. This fact justifies reduced secretion of insulin and higher sensitivity to it after the consumption of products being the source of plant proteins. Some sources report the consumption of plant proteins influences the decrease of the concentration of insulin-like growth factor 1 (IGF-1), which is involved in the development of PCOS [33]. However, no negative effect on fertility was shown after the consumption of fish and eggs proteins. Taking into consideration other risk factors, the increase in the consumption of trans fatty acids and carbohydrates by 2% of the energy intake, instead of polyunsaturated fatty acids (omega 6), causes increased risk of infertility among women by 76% and 73%, respectively [11] as well as obesity increases risk of hypercholesterolemia in women in reproductive age [31]. Metabolic and hormonal parameters in the group of women with PCOS are improved by cis fatty acids, in comparison to trans fatty acids, which was proved in the study of *Kasim-Karakas et al*. [20]. Trans isomers of fatty acids lower the activity of peroxisome proliferator-activated receptors γ (PPAR), leading to increased ovulation disorders [7]. The consumption

of dietary fibre is equally important. As shown in the studies of [13], the increased consumption of dietary fibre by 10g, in group of women aged 32 and above, lowered the risk of fertility disorders due to ovulation disorders by 44%. It is also known that vitamin B6 influences the maintenance of the proper concentration of progesterone in blood, therefore proper consumption of the products rich in this vitamin (lean meat, milk, eggs, grain products, dry legumes) is justified. The deficiency in vitamin B12 can contribute to the disorders in ovulation and embryo implantation. This vitamin is essential to produce red and white blood cells. In diet, the sources of vitamin B12 are products of animal origin, mainly eggs, meat and fish. Thus women with PCOS being on a vegetarian diet should take supplements being the source of group B vitamins, due to risk in their deficiencies [25]. Inositol also belongs to the group of B vitamins. There are nine stereoisomers of inositol (chemical compounds, which atoms are bounded in the same sequences differing by spatial arrangement), including myo-inositol (MYO) and D-chiro-inositol (DCI). Everyday diet supplies ca. 1g of inositol, mainly in form of myo-inositol. Data from the literature confirm, that in women with PCO syndrome the insulin resistance is affected by the deficiency in inositol. The intake of myo-inositol sensitizes the tissues to insulin, thus leading to decrease in insulin resistance. Inositol influences the process of maturation of an egg cell, because it lowers the concentration of LH, prolactin and testosterone [22]. Inositol phosphates are the molecules built from myo-inositol. They are classified as auxiliary antioxidants, the so called synergistic antioxidants. They do not directly stop the oxidation chain reaction, but they can enhance the activity of main antioxidants. The sources of inositol phosphate are dry legumes, seeds of oil plants and grains.

Proper intake of antioxidants in a diet, including vitamins C and E, β-carotene, zinc, copper, selenium and coenzyme Q10, protects against free radicals (lipid peroxides). Their adverse effect on the organism is based on triggering oxidative stress, and, as a consequence, the damage of, among others, cellular membranes [28]. Additionally, vitamin E takes part in reproductive processes and regulates the activity of internal secretion. Low concentration of vitamin E (its sources are plant oils, leafy vegetables, legumes) correlates with ovulation disorders [1]. Proper intake of vitamin C (wild rose fruits, cabbages, berries, yellow and green vegetables and fruits) and bioflavonoids can also prevent miscarriages. Among the antioxidants used in PCOS treatment the commonly mentioned is also coenzyme Q10 (ubiquinone), which takes part in ATP metabolism in mitochondria as a catalyst of metabolic processes [23]. Ubiquinone synthesis requires vitamin B12 and folic acids, which are often deficient in diets of women with PCOS. Moreover, folic acid takes part in homocysteine metabolism [30]. It has been proved that too high concentration of homocystein in blood lowers reproductive capabilities and leads to pregnancy complications. The deficiency in folic acid correlates with higher risk of high blood pressure and cardio-vascular diseases, which coexist in PCOS [36]. Its sources are green vegetables, dry legumes, wholegrain products, liver and eggs. Considering mineral elements, an appropriate consumption of zinc is important, which is essential for proper metabolism of estrogens, progesterone and androgens affecting ovulation and the development of an embryo [37]. Similarly, the deficiency in magnesium can influence the reduction of progesterone level and thus cause menstruation disorders. Numerous dietary mistakes of women with PCOS often cause the extensive development of fatty tissue, especially the visceral one, and difficulties with body mass reduction. Polycystic ovary syndrome increases the risk of type 2 diabetes, blood hypertension, cardio-vascular diseases and cancer of reproductive organs. According to the current knowledge, 35%-50% of women with PCOS also have insulin resistance and/or type 2 diabetes. Moreover, the studies report that in every fourth obese women with PCOS the type 2 diabetes and insulin resistance occur before the age of 30 [27]. It is known, that the development of the disease is influenced by genetic, hormonal and environmental factors. there are studies confirming that PCOS is family-related and occurs more often in women having at least one member of the family suffering from type 2 diabetes [15]. The study determining the molecular causes of PCOS development are still being performed. In current literature there are no reports related to the qualitative and quantitative assessment of diets of women with PCOS enabling to apply complex diet therapy.

MATERIALS AND METHODS

Tested group

Screening tests were performed in the Clinic of Gynecology and Urogynecology, Pomeranian Medical University (PMU) in the Independent Public Clinic Hospital No. 1 in Police.

The selection of women for the study was deliberate. PCOS was diagnosed according to Rotterdam's criteria from 2003, which require the diagnosis of 2 out of 3 following criteria: rare ovulations or lack of thereof, and/or biochemical symptoms of hyperandrogenism, and/or image of polycystic ovaries in USG (polycystic ovaries morphology in transvaginal USG - the presence of 12 follicles or more in one or both ovaries, and/or increased volume of ovary >10ml). The image was obtained using Ultrasound Voluson 730 (GE,

Switzerland). Criteria for exclusion from the test were: pregnancy, age above 40, change in dietary habits just before the study (3 months) and co-existence of other diseases linked to hyperandrogenism (Cushing's syndrome, tumours releasing androgens, congenital or acquired adrenal hyperplasia). The research has been approved by the Bioethical Commission of the Pomeranian Medical University in Szczecin, No. KB-0012/134/12, with the annex to the permission No. KB-0012/36/14.

Assessment of nutritional status

To assess nutritional status of the patients the following anthropometric tests were used: body weight (± 0.1 kg), body height, waist circumference and hip circumference - using anthropometric measuring tape (± 0.5 cm). On the basis of those data the Body Mass Index (BMI - Body Mass Index) was calculated and the type of body built (WHR - Waist Hip Ratio) was determined [38].

Quantitative dietary assessment

The information on the consumption of products and meals were collected using two methods: food records method (food diaries) from 3 days and one-day food record from the last 24-hour dietary interview. Records from the food diary and the interview included: ingredients, quantity, mode of preparation and time of consumption of every meal. Menus were taken from two weekdays (Thursday and Friday) and two weekend days (Saturday and Sunday). Altogether 216 menus from women with PCOS were analysed. The sizes of consumed portions were determined according to the **"***Album of photographs of food products and dishes*" of the Food and Nutrition Institute [32]. Next, the energy and nutrients were calculated using computer software Dieta 5 (National Food and Nutrition Institute, Warsaw, Poland), whose database was based on the "*Tables of the contents and nutritional value of food*" [21].

Statistical analysis

The results were statistically analyzed using the software package Statistica 10.0 (Statsoft, Tulsa, Oklahoma, USA). The arithmetical mean, standard deviation and the significance of differences were calculated using ANOVA.

RESULTS

Nutritional status: In the group of 33 women with PCOS the average age of patients was 26.31 ± 5.52, and the average body weight was 80.98 ± 16.06 kg. The average BMI value was 29.16 ± 5.8 kg/m^2. The characteristics of anthropometric parameters of tested women are presented in Table 1.

Table 1. Anthropometric characteristics of the study group

Parameter	$\bar{x}$	SD
Age (years)	26.31	5.52
Waist circumference (cm)	99.18	14.82
Hip circumference (cm)	108.45	9.39
Body weight (kg)	80.98	16.06
Height (m)	1.67	0.06
BMI - Body Mass Index (kg/m^2)	29.16	5.8
WHR- Waist-Hip Ratio (cm/cm)	0.91	0.08

SD- Standard Deviation

Table 2. The average content of nutrients in diets

Discriminant	Average content	± SD	Range
Energy (kcal)	1952.5	472.7	1353.8-2956.8
Protein (g)	76.1	18.2	46.9-106.8
Fat(g)	72.9	21.9	38.4-122.2
SFA* (g)	26.1	8.3	13.4-47.6
MUFA** (g)	27.3	10.9	13.3-61.9
PUFA*** (g)	12.6	4.9	5.4-28.267
Cholesterol (mg)	301.5	172.2	90.4-1044.7
Carbohydrates (g)	265.0	82.2	170.9-428.6
Saccharose (g)	43.0	24.6	10.6-103.2
Dietary fibre (g)	19.7	7.5	9.7-44.8
Vitamin A (µg)	954.4	356.9	358.7-1682.7
Vitamin D (µg)	3.4	3.8	0.5-21.2
Vitamin E (mg)	11.6	4.6	3.8-26.4
Vitamin B1 (mg)	1.4	1.1	0.6-7.2
Vitamin B2 (mg)	1.6	0.6	0.7-4.3
Niacin (mg)	4.3	15.4	9.1-26.1
Vitamin B6 (mg)	2.1	0.8	0.9-4.1
Folate (µg)	271.8	79.7	123.4-458.0
Vitamin B12 (µg)	3.3	2.2	0.9-13.0
Vitamin C (mg)	98.3	77.1	25.1-421.7
Sodium (mg)	2903.1	805.8	1329.7-5073.8
Potassium (mg)	3493.4	1724.5	1559.0-8430.1
Calcium (mg)	634.3	228.9	301.9-1278.2
Phosphorus (mg)	1392.9	502.5	665.1-2852.5
Magnesium (mg)	250.9	85.9	176.8-583.9
Iron (mg)	11.7	4.4	6.5-23.3
Zinc (mg)	9.0	2.1	5.3- 14.8
Copper (mg)	1.1	0.4	2.4-0.6

* SFA-saturated fatty acids;
**MUFA-monounsaturated fatty acids;
***PUFA-polyunsaturated fatty acids.

The largest percentage of the patients (76%) were women with BMI above the standard (BMI ≥ 25 kg/m^2). The group of obese women (BMI ≥ 30 kg/m^2)

comprised as much as 39% of studied population. Among the participants of the study there were none with BMI showing underweight (BMI < 18,5 kg/m^2). Significant percentage of tested women (73%) had waist circumference ≥ 88 cm, which shows on high risk of metabolic syndrome occurrence among the respondents. In the test group of overweight (BMI ≥ 25 kg/m^2) and obese (BMI ≥ 30 kg/m^2) women, a large majority, i.e. 95.65%, represented android body type.

The results of the study provide the information about the average consumption of energy at the level of 1952.5± 472.7 kcal, and the average content of nutrients in 216 diets are shown in Table 2.

After performing the quantitative assessment of the intake of nutrients using AI level it was observed that there was high probability of insufficient intake of calcium (634 mg), potassium (3493 mg) and vitamin D (3.4 μg) (Table. 3).

Table 3. The assessment of the consumption using AI level

Discriminant	AI level	Average intake ± SD
Sodium (mg)	1500	2903.1 ± 805.8
Potassium (mg)	4700	3493± 1724.5
Calcium (mg)	1000	634±228.9
Iron (mg)	8	11.6 ± 4.4
Vitamin D (μg)	5	3.4± 3.8
Vitamin E (mg)	8	11.6± 4.6

The evaluation of the consumption in the test group using Estimated Average Requirements (EAR) threshold showed, that 70% of examined women were at risk of insufficient intake of folic acid. In 36.7% of examined patients there was a risk of insufficient consumption of protein, but also vitamin C (36.7%), vitamin B12 (26.7%) and magnesium (23.3%). The results showed low risk of deficiency in niacin in 13.3% of examined women (Table 4).

The assessment of the consumption of other nutrients showed, that the intake of total fat, and consequently also SFA, was too high in 70.4% of women. Too high intake of cholesterol was observed in 40.74% of test group. Too low consumption of total carbohydrates was not determined, however, 50% of examined women consumed excessive amounts of saccharose (>40d/day). It was also observed that 83.3% of patients were characterized by too low intake of dietary fibre in their diets (<25 g) (Table 5).

Table 4. The assessment of the consumption using EAR threshold

Determinant	Standard level		Percentage of women	
	EAR	UL	<EAR	>UL
Protein (g)	80	N/A	36.7	-
Phosphorus (mg)	580	4000	0	0
Magnesium (mg)	255	350[1]	23.3	0
Zinc (mg)	6.8	N/A	10,0	-
Copper (mg)	0.7	N/A	6.7	-
Vitamin A (μg)	500	3000	10.0	0
Vitamin B1 (mg)	0.9	N/A	10.0	-
Vitamin B2 (mg)	0.9	N/A	3.3	-
Niacin (mg)	11	35	13.3	0
Vitamin B6 (mg)	1.1	100	6.7	0
Folate (μg)	320	1000	70.0	0
Vitamin B12 (μg)	2.0	N/A	26.7	-
Vitamin C (mg)	60	2000	36.7	0

Table 5. Percentage of the realisation of the standard nutrient requirements

Determinant	Applied standard	Percentage of women >standard	Percentage of women <standard
Energy (kcal)	1802.5-2846.4	13.0	51.85
Fat (%)	<30 (>35)**	18.82	(50,0)**
SFA* (%)	<10	70,4	29,6
Cholesterol (mg)	200-300	40.74	37.04
Carbohydrates (%)	<45	92,6	7.4
Saccharose (g)	40	50	50
Dietary fibre (g)	25	16.7	83.3

*SFA-saturated fatty acids; **intake more than 35% energy from fat

DISCUSSION

Randomized studies carried out in Australia [35], London [18] or in Italy [2] confirmed that overweight or obesity and the excess of abdominal fatty tissue around waist are often the characteristics of women with PCOS. BMI values in our study were similar to those observed by other researchers: 29.7±4.8 kg/m^2 [13] and 27.4±7.3 kg/m^2 [5]. Average WHR value in our study was higher (0.92±0.08) in comparison to the results of *Wright et al.* [39] (0.837±0.097), which could be caused by the selection of the patients according to different criteria (one of the required symptoms was clinical or biochemical proof of hyperandrogenism).

The available literature does not provide the assessment of the diet of women with PCOS.

In the meantime, since the introduction of Rotterdam Criteria (2003) the increasing number of women with normal androgen levels is qualified for this disease. In these women were found polycystic ovary syndrome based on ultrasound and menstrual disorders. The authors of this study show that one of the causes of ovarian dysfunction PCOS (except hormonal disorders) is the poor diet of women.

In this study the energy requirements were properly fulfilled. Only 13% of the examined women consumed higher amount of energy than the required intake. Even if we assume, that some of women with PCOS underestimated their dietary ratios, which was reported in obese people *Chandon* & *Wansik* [9], then we can conclude that the problem is related to the expenditure of consumed energy, i.e. too little physical activity of the patients. The percentage of the energy coming from protein in own study and the studies of *Altieri* and *Wright* [2,39] was similar, and amounted to 15.59, 14.61 and 16.62, respectively. The percentage of the energy in diets coming from carbohydrates was appropriate, unfortunately the major share came from simple sugars, including saccharose. They contribute to the decrease in the HDL cholesterol fraction, the increase in the concentration of triglycerides and insulin resistance. The average content of fat in diets was not high, but 50% of examined women consumed excessive amounts of fat. When analyzing its quality, we determined high consumption of SFA and cholesterol, with simultaneous low intake of dietary fibre, which increases the risk of metabolic diseases related to dyslipidemia, cardio-vascular diseases and type II diabetes. It was previously reported in the literature that there is a positive correlation between the concentration of androstendion, testosterone and free testosterone in blood and the consumption of total fat, SFA, MUFA and cholesterol. Moreover, cholesterol is a precursor of steroid hormones, including androgens distorting hormonal balance in polycystic ovary syndrome.

Despite the fact that PCOS was recognized 80 years ago, no studies have been carried out on the components of diets of women with PCOS, considering the content of vitamins and minerals. The intake of antioxidative vitamins (A, C, E) was at an appropriate level, therefore their deficiency should not occur in PCOS. However, the data from the literature suggest, that the excess in vitamin A may contribute to menstrual disorders and cause scanty menstruations. On the other hand, vitamin D was consumed in too small amounts. Proper intake of this vitamin is crucial, because the studies showed the correlation between its deficiency and insulin resistance, hirsutism and infertility, which are characteristic symptoms of PCOS [34]. The deficiency in vitamin D were reported in people with abdominal obesity, diabetics and in the group of people at risk of insulin release disorders. It was also shown that, due to the presence of VDR (vitamin D receptor) in epithelial cells of uterine glands, the administration of vitamin D significantly affected the thickness of endometrium in women with PCOS, which contributed to higher chances of success of intrauterine insemination [3]. In this study we observed insufficient consumption of group B vitamins, especially folic acid. Only 30% of women consumed it at EAR. In the test group the highest deficiencies were noted in case of folic acid - average intake 271 µg (EAR 320 µg). Folic acid is one of the coenzymes taking part in homocysteine metabolism. Its deficiency inhibits proliferation and growth of cells on nucleic acids level and leads to the increase in the concentration of homocysteine in blood. It was shown that hyperhomocysteinaemia has a negative effect on reproduction, leads to pregnancy complications and disorders in foetus development. High concentration of homocysteine in follicular fluid may distort the interaction between an ovum and a sperm cell, decreasing the chance of fertilization. Moreover, the excessive amount of homocysteine disturbs the mechanism of implantation of fertilized egg and has a negative influence of embryogenesis, which can result in the inhibition of foetus development, embryonic death and miscarriage [36]. High deficiencies in folic acid in women can lead to disorders in the occurrence of proper mucous symptom. Moreover, proper intake of folic acid contributes to the increase in the number of maturating egg cells [29]. Studies performed by other authors suggest that the frequency of ovulations and births may be higher with the intake of folic acid at the level of 700 ug/day (with the general daily intake of 400 ug/day) [10].

Considering the consumption of mineral elements, we noted too low intake of calcium (634.3± 228.9 mg) and potassium (3493.4± 502.5 mg) in the diets of women with PCOS. Proper contents of calcium is very important, because its deficiency has an inhibiting effect on the maturation of ovary follicles and lowers the number of developing follicles [8]. Moreover, crystal structure studies not only revealed calcium-binding sites in the human SHBG but also demonstrated that SHBG is a zinc binding protein [4]. It had previously been shown that the presence of calcium is essential for maintaining homodimer stability and steroid-binding activity [6], but the presence of a zinc molecule was found to help orient the exposed loop over the entrance to the steroid-binding site and to alter the binding affinity of estrogens versus androgens [16]. The contents of other nutrients in the study (magnesium 300.9 mg, iron 11.7 mg, zinc 9.0 mg, copper 1.1 mg) did not pose the risk

of deficiencies, however, as far as dietary procedure is concerned, it seems proper to supplement the diet with zinc. The intake of sodium was too high. Sodium, together with chlorine, increases blood pressure and lowers insulin sensitivity, which is crucial for frequent coexistence of type II diabetes and high blood pressure in PCOS [24]. Thus it seems reasonable to limit the consumption of salt and products containing it.

CONCLUSIONS

1. Too high intake of total fat, saturated fatty acids, cholesterol and saccharose, and low consumption of dietary fibre observed in the study, may contribute to ovaries dysfunction through down regulation of metabolic processes.
2. It may be necessary to supplement the diets of some women with PCOS with potassium, zinc and magnesium. Because of deficiencies and excesses of nutrients in the diet, come to mind also additional implications:
3. One of the most important dietary reasons for disorders in reproductive processes is the deficiency of folic acid in a diet.
4. Proper intake of vitamin D and calcium may positively affect the improvement of menstruation cycle regularity and maturation of ovarian follicle.
5. High consumption of cholesterol, as a substrate for androgens' synthesis, may contribute to hormonal imbalance in PCO syndrome.

Conflict of interest

All authors declare no conflict of interest. Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE

EVALUATION OF COMMUNICATION AND ACCEPTANCE OF THE PATIENTS BY MEDICAL PERSONNEL

Anna Włoszczak-Szubzda[1,2], Mirosław J. Jarosz[1,2], Mariusz Goniewicz[3], Krzysztof Goniewicz[4,5]*

[1]Institute of Rural Health in Lublin, Department of Biostatistics, Demography and Epidemiology, Lublin, Poland
[2]University of Economics and Innovation, Department of Nursing, Lublin, Poland
[3]Medical University of Lublin, Emergency Medicine Department, Lublin, Poland
[4]Polish Air Force Academy, Faculty of National Security and Logistics, Dęblin, Poland
[5]Medical University of Warsaw, Department of Disaster Medicine, Warsaw, Poland

ABSTRACT

Background. The low level of patient satisfaction recorded in many studies and, at the same time, the level of frustration and burnout, disclosed by medics in the perception of the patient as a 'problem', incline to look for the causes of inadequate relationship between physician and patient.

Objective. The aim of this study was to evaluate the level of acceptance of the patient by the medical personnel. The research problem was the acceptance level which was within the range of the communication skills of the nurses and doctors. Another aim was to discover the factors determining this level of acceptance.

Material and Methods. Two methods were used in the research process: 1) a diagnostic survey regarding the medical, professional communication skills; 2) testing of professional self-esteem from the medical aspect. The study population consisted of a total of 1,244 respondents divided into the following groups: registered nurses and doctors (729), students of nursing and medical faculties (515).

Results. The results of the research showed that in most cases the acceptance of the patient by the medical staff was 'conditional', which translated into the level of frustration or lack of satisfaction with their profession, and ultimately into the level of burnout. The level of patient acceptance by medical staff (unconditional acceptance), depended primarily on age, followed by their profession. However, the relationship between this acceptance and gender and work experience was statistically insignificant.

Conclusions. As the method to improve this situation, the expansion of education in the field of interpersonal communication is proposed, adding issues related with both the conditional and unconditional acceptance of the patient, as well as issues regarding how to deal with the patient from the aspect of disease and the psycho-socio-spiritual area.

Key words: *patient satisfaction, communication, personal satisfaction, frustration, medical staff*

STRESZCZENIE

Wprowadzenie. Niski poziom satysfakcji pacjenta odnotowany w wielu badaniach naukowych, jak również frustracja oraz wypalenie zawodowe wśród personelu medycznego skłania do poszukiwania przyczyn tych problemów w nieprawidłowej relacji pomiędzy pacjentem a lekarzem.

Cel. Celem badań była ocena poziomu akceptacji pacjenta przez personel medyczny. Badaniem objęto poziom akceptacji, który mieścił się w zakresie umiejętności komunikacyjnych pielęgniarek i lekarzy. Celem wtórnym było odkrycie czynników determinujących ten poziom akceptacji.

Materiał i metoda. W procesie badań naukowych zostały wykorzystane dwie metody: 1) badanie diagnostyczne dotyczące medycznych, zawodowych umiejętności komunikacyjnych; 2) badanie samooceny zawodowej z punktu widzenia medycznego. Badana populacja składała się z 1244 respondentów podzielonych na grupy: pielęgniarki i lekarze (729), studenci pielęgniarstwa oraz nauk o zdrowiu (515).

Wyniki. Wyniki badań wykazały, że w większości przypadków akceptacja pacjenta przez personel medyczny jest „warunkowa", co przełożyło się na poziom frustracji i brak satysfakcji z wykonywanego zawodu, a ostatecznie na poziom wypalenia zawodowego. Natomiast bezwarunkowa akceptacja pacjenta przez personel medyczny zależała przede wszystkim od wieku, a następnie zawodu pacjenta. Związek między akceptacją a płcią i doświadczeniem zawodowym był statystycznie nieistotny.

[*]**Corresponding author**: Krzysztof Goniewicz, Medical University of Warsaw, Department of Disaster Medicine, Zwirki i Wigury street 81A, 02-091 Warsaw, Poland, tel.: +48 22 57 20 545, fax: +48 22 57 20 543, e-mail: krzysztof.goniewicz@wum.edu.pl

Wnioski. W celu poprawy relacji interpersonalnych na linii pacjent – personel medyczny w zakresie edukacji z komunikacji interpersonalnej proponuje się dodanie do edukacji zawodowej zagadnień związanych zarówno z warunkową jak i bezwarunkowa akceptacją chorego, jak również zmianę dotyczącą sposobu postępowania z pacjentem począwszy od aspektu chorobowego aż do obszaru psycho-społeczno-duchowego.

Słowa kluczowe*: satysfakcja pacjenta, komunikacja, satysfakcja osobista, frustracja, personel medyczny*

INTRODUCTION

Categorization of patients into good and bad, popular and unpopular, or desirable and undesirable used by medical professionals has been present in research in the field of ethics, sociology, and medical psychology since the 50s of the last century [11, 21, 22]. The starting point for research in this area was the phenomenon of the disease (its seriousness), individual behaviours of patients, or their social origin [2, 5, 19].

According to this scheme, a 'good' patient is one who has fairly simple health problems and behaves very peaceful in the physician-patient relationship. A 'bad' or 'problem' patient, firstly shows emotions (complains, grumbles), secondly presents dependence on medical staff (asks for painkillers, does not agree to all treatments), and thirdly, and often most importantly, the health condition of the patient is relatively serious. However, no matter how seriously ill the patient is, in the case of the patient described as being a 'problem', the medical staff regard the patient's behaviour as unjustified ('you should not feel such pain', 'it does not hurt that much', 'do not be afraid', 'please do not get upset', etc.) [18, 23, 31]

The phenomenon of dividing patients by the medical personnel into good or bad, was also considered from the aspect of the personality traits of individual medical professionals, as well as the social interaction between patients and medical professionals (doctors/nurses / physiotherapists, etc.) [14].

Based on interviews conducted with medical staff and patients, physicians emphasized that the 'problem' patients divest them of the sense of the effectiveness of treatment and care, and in physicians' opinions the need for information and involvement of the patient in the treatment process undermines their competences. 'Good' patients thought that when in contact with the medical staff, they should show a passive attitude and acceptance. 'Problem' patients demanded full knowledge concerning their health status, medical interventions undertaken, and demanded cooperation with the medical staff in the treatment process [25, 26].

Considering previous research experiences, in the presented article individual attitudes of medical personnel have been assessed, as well as the social interaction between patients and the staff based on interpersonal communication [34, 35, 36].

Such a categorization of patients by medical personnel is associated with the psychological mechanism of conditional or unconditional acceptance. This conditional acceptance is related with the assessment of others. In such a relationship with another person we feel either accepted, or we realize that the given person - without any words, and very often unconsciously, poses conditions or requirements, which means limiting or denying unconditional acceptance. In each one of us, there is a subjective need for making assessments as character landmarks in our social life. The assessment is mostly comparison with an ideal standard, which is not universally achievable. Unfortunately, an acceptance is often considered as the lack of assessment or false assessment of the state of affairs (e.g. the patient, as such).

Conditional acceptance in the physician-patient relationship, leads to the situation when medical personnel imposes conditions on the patient ('accept the patient if ...' is clean, pleasant, complies with orders, etc.), and in contact with the patient, initially checks whether these conditions are met, and secondly: heals, nurtures, rescues and rehabilitates. Emotional help on both sides of the physician-patient relationship would be the unconditional acceptance of the patient (which does not mean a non-assertive attitude of the medical staff).

In practice, to accept others, means to let them be as they are, with everything that composes their physical and mental traits, this applies to not only to mood - serenity, but also, for example, to sombre discouragement or mistrust. Such unconditional acceptance may be developed only based on good communication with the patient. There are many ways of showing acceptance, primarily by devoting some time to the patient, during which respect can be shown by gesture and word. The simplest sign of respect is to listen carefully to what the patient is saying. An important element of acceptance is emphasizing personal freedom, respect for this freedom (patient's right to decide about health and even life), focusing attention on the patient and on things that he/she experiences (taking into account the fears before surgeries, procedures, or effects of the disease), as well as refraining from expressing assessments of the patient's emotions - each experienced and manifested emotion is appropriate for the patient [24].

The authors of the presented study examined the extent to which medical staff (nurses, doctors and students) accept a patient conditionally and unconditionally.

The rationale for selecting the research problem were the results of studies investigating patient satisfaction with medical services, which show that the cause for negative assessment are, among others, inadequate communication skills of medical personnel.

The aim of this study was to evaluate the level of acceptance of the patient by the medical personnel (a test of the state). The research problem was the acceptance level which was within the range of the communication skills of the nurses and doctors. The aim of the study was also to discover the factors determining this level of acceptance, such as gender, age, occupation, education.

MATERIAL AND METHODS

Research problems:

1) What is the level of patient's acceptance by medical personnel? - lack of acceptance, conditional acceptance, unconditional acceptance.
2) Does the level of acceptance depend on the age, gender, work seniority (professional experience) and medical professions (nurses vs. physicians)?

Research hypotheses:

1) Acceptance of the patient by medical personnel is often located at the level of conditional acceptance.
2) The level of acceptance of the patient by medical staff (unconditional acceptance), depends primarily on age and seniority, and less on gender or type of employment within the health professions.

The diagnostic survey regarding medical, professional communication skills was applied in the research process, and the questionnaire designed by the authors was used as a research instrument. As part of the standardization of the research instruments, the condition of objectivity was fulfilled by ensuring the independence of the studied respondents. All respondents expressed their written consent to participate in the study. The conditions for conducting the study were standardized for all groups examined, and the questionnaire contained precise and clear instructions. The reliability and validity of the instruments were verified by pilot studies, evaluated by competent judges test, *Kendall's* coefficient of concordance for assessing agreement among rates, 'test – retest' examining stability and reliability of the instrument, as well as *Student's* t-test for dependent variables, which explores the significance of the difference of each pairs of questions, having at the same time statistically significant positive correlation, in the test and retest.

Plans for the research and the research instruments were assessed by Local Bioethical Commission of Medical University in Lublin (acceptance number: KE-0254/221/2008), and received a positive assessment.

The research was conducted from October 2009 to January 2014 at: the Medical University (students of nursing and medical faculties), University of Economics and Innovation in Lublin (students of nursing and professional nurses), the 'Novum' Association for Education and Training of Nurses and Midwives (professional nurses), Institute of Rural Health in Lublin (vocational training for family doctors), Clinical Hospitals of the Medical University in Lublin, and the Cardinal Stefan Wyszynski Provincial Specialist Hospital in Lublin (professional nurses and doctors).

The study covered a total of 1,244 respondents in the following groups: 1) professional nurses and doctors (729); 2) students of nursing and medical faculties (515), including: 982 females and 229 males (imbalance due to the feminization of the nurse profession in Poland) in the following age groups: up to 25 (315 respondents), 26-30 (169 respondents), 31-35 (223 respondents), 36-40 180 respondents), 40 and over (345 respondents).

The obtained data were statistically analysed (IBM SPSS) using: descriptive statistics, contingency tables and testing of hypotheses. The existence of relationships between variables was investigated using the *Pearson Chi*-square and *Fisher's* exact test. The level $p \leq 0.05$ was considered significant, whereas the level $p \leq 0.01$ was considered 'very significant', and $p \leq 0.001$ – 'highly significant'.

RESULTS

The great majority of respondents showed a lack of acceptance of patients, with a similar percentage of females (91.3%) and males (89.4%). The relationship between gender and acceptance of patients was investigated using the *Chi*-square test. Analysis showed no statically significant relationship between variables (Table 1).

Table 1. Acceptance of the patient according to gender

			Acceptance of the patient		Total
			Lack	Present	
Gender	Female	n	878	104	982
		% within gender	89.4%	10.6	100.0
	Male	n	209	20	229
		% within gender	91.3%	8.7	100.0
Total		n	1087	124	1211
		% within gender	89,8%	10.2	100.0

Test: *Pearson Chi*-square=0.697; df=1; p=0.404 (*Fisher's* exact=0.468)

Source: Own research.

The biggest number of respondents in the study group were females (81.09%) due to the significant

feminisation of the nurses' profession. In the group, the nursing profession represented 59.91% of the total. The results indicate no significant differences in the area of patient acceptance between females and males.

In the analysed age groups, the highest prevalence of unconditional acceptance of the patient was observed among respondents aged 31-35 (13.9%) and 41 and over (13.3%). The following tendency was clearly observed: the older the group, the higher the percentage of respondents declaring acceptance of the patient. It can be assumed that this is directly related with the professional and life experience of the respondents (Table 2).

Table 2. Acceptance of the patient according to age of medical staff.

			Acceptance of the patient		Total
			Lack	Present	
Age	≤ 25	n	299	16	315
		% within age	94.9	5.1	100.0
	26-30	n	157	12	169
		% within age	92.9	7.1	100.0
	31-35	n	192	31	223
		% within age	86.1	13.9	100.0
	36-40	n	159	21	180
		% within age	88.3	11.7	100.0
	≥41	n	299	46	345
		% within age	86.7	13.3	100.0
Total		n	1106	126	1232
		% within age	89.8	10.2%	100.0%

Test: *Pearson* Chi-square=18,202; df=4; p=0.001
Source: own research.

In the analyzed age groups, the lowest level of patient acceptance was demonstrated by the respondents from the youngest group, aged up to 25 years. The great majority in this group were students, who may have a lower tendency to empathize because of the insufficient experience. It noteworthy that the intensity of the examined trait was relatively lower among respondents in group aged 36-40. *Chi*-square test confirmed a significant correlation between respondents' age and acceptance of the patient (Table 2).

In the analysed material, the work experience variable was observed in two categories: students of medical fields of studies, and working medical personnel. It should be noted that a distinct difference in the presence of unconditional acceptance of the patient was found between both groups. In the category of students, only 8.3% declared such approval. In the category of workers, such a declaration was made by 11.7% of respondents. This indicates that the work experience gained by the professionally active respondents can influence the level of unconditional acceptance of the patient (Table 3). Statistical analysis did not confirm a significant relationship between 'acceptance of the patient' and 'experience of medical personnel', at the required level of significance, although it was at a level very close to significance (Table 3).

Table 3. Acceptance of the patient according to work seniority (professional experience) of medical staff.

			Acceptance of the patient		Total
			Lack	Present	
Professional experience	Student	n	472	43	515
		% within professional experience	91.7	8.3	100.0
	Employee	n	644	85	729
		% within professional experience	88.3	11.7	100.0
Total		n	1116	128	1244
		% within professional experience	89.7	10.3	100.0

Test: *Pearson Chi*-square=3.583; df=1; p=0.058 (*Fisher's* exact=0.059)
Source: own research.

In the study group, nearly half of the respondents were representatives of the nursing profession, both male and female. Representatives of the nursing profession were an occupational group who significantly more often indicated unconditional acceptance of the patient (12.4%). Doctors declared such acceptance only at the level of 8.1% (Table 4). Statistical analysis showed a statistically significant relationship between patient acceptance and profession performed at the level of significance p = 0.013.

Table 4. Acceptance of patient according to professional groups of medical personnel

			Acceptance of the patient		Total
			Lack	Present	
Profession group	Nurse	n	551	78	629
		% within profession	87.6	12.4	100.0
	Physician	n	565	50	615
		% within profession	91.9	8.1	100.0
Total		n	1116	128	1244
		% within profession	89.7	10.3	100.0

Test: *Pearson Chi*-square=6.144, df=1, p=0.013 (*Fisher's* exact=0.015)
Source: own research.

Analysis of the collected material confirmed significant differences in the declared level of acceptance of the patient in studied groups. Tests showed the statically significant effect of the variables of profession and age of the respondents. On the other hand, the differential impact of gender and level of education in terms of professional activity of the respondents was not confirmed. It should be pointed out that the level of declared patient acceptance among medical personnel was generally quite low.

DISCUSSION

Among the members of medical staff, there are increasing numbers who claim (e.g. on online forums) that the cause of abnormal physician-patient relationship are inappropriate attitudes of patients. According to the doctors, a 'demanding attitude' of patients discourages physicians from good communication. At the same time, in the study on patient satisfaction, the patients claim that interpersonal relations and communicating with medical personnel are the weakest aspects. The study showed that acceptance of the patient, required in the process of proper communication, leaves much to be desired.

Gender

Theories about social attitudes relate, *inter alia*, to psycho-social predispositions of gender. The communication dichotomy connected with gender, which divides channels for communication, for 'male' and 'female' was pointed out by *Shem* and *Surrey*. In their opinion, males embody the concreteness of communication, language is less emotional and devoid of personal connotation, while females in communication use primarily emotionality, and thus, a male is more likely to accept conditionally and females unconditionally [10, 27, 29].

Man learns very early about gender stereotypes, knows how a 'real woman' and 'real man' should behave [17]. In the created 'perfect image', women have much more efficient verbal abilities (an expanded speech centre and improved communication between the right and left hemisphere) and also identify themselves with the caller, which implies kindness for the interlocutor [28]. The presented study showed no significant differences in the way of acceptance of the patient, between males and females. Both genders presented a low level of patient's acceptance.

Age

The literature on interpersonal attitudes indicates a relationship between communication competences and age. Knowledge of the human social system is the factor which, among others, enables human communication skills and the appropriate attitudes towards others (the patient), increasing with age. The wider experience of social objects (knowledge of self, parents, spouses, children, institutions, relationships, social events, etc.), the greater the motivation, skill, and consequently, knowledge about the relationship with others. Mature communicate with people is neither innate nor spontaneous. It requires improvement and knack that can only be developed over time – and increases with age [4, 15].

At the same time, researchers recognize a barrier in human communication in the form of stiffness of beliefs, which are intensified with age. Physiological changes due to aging can cause personality changes, depressed mood, irritability, anxiety, aggression, and belief in infallibility. All these qualities together can reduce openness in relation to another human being. Simultaneously, psychological theories proclaim that human personality traits do not change with age. According to this criterion, there are human traits which are consistent, these are human self-confidence (an important element of interpersonal communication), internal heat (understood in communication as kindness, openness, empathy) and cognitive interests (essential in dealing with human curiosity in others, and openness to otherness). At the core of any changes there are specific life experiences so important to the entity that causes metamorphosis [1, 3, 16, 20, 37].

Our own research confirms the tendencies in worldwide studies concerning the lowest acceptance for the patient in the youngest age group. Starting from the middle age of respondents, the level of patient's acceptance significantly increases, although, at the same time, the 36-40 age group had the lowest level of acceptance of the patient, among the middle and oldest age groups (31-35 years old; 41 years and over). This may indicate burnout symptoms which are characteristic of occupations requiring high responsibility, among which the medical professions are definitely included. The result was highly statistically significant.

Professional experience

Experience, or lack of it, arising from the profession, could also be a communication barrier. This appears when people present a different level and range of experiences, which makes them differ in the way of thinking or receiving the surrounding reality, and hence mutual understanding. Communication theories assume that broader horizons (through experience) mean a greater openness to the diversity of attitudes. The meta-communication approach, a reference going beyond the message itself (contextuality), assumes the reference to information about the views and experiences of the sender and recipient [3, 12, 13, 30, 33].

In this study, a big difference was observed in the unconditional acceptance of the patient, between medical students with little experience and medical staff with more work experience. However, it was not statistically significant.

Profession

As with the previous independent variables, affiliation with a particular occupational group influenced the social attitudes within the range of communication skills. According to the literature, the issue that we deal with is feedback. People are predisposed to a particular occupation by their personality, and influence its development through the professional environment.

The choice of profession is a derivative of personal interests connected with the possibilities offered by the world of work, and the professional environment consists of people with a certain type of personality, specific problems, as well as demands specific for the given profession. People in certain occupational groups have similar patterns of personality and react in a similar way to many situations. If the search for the professional environment ends in the wrong choice, we have to deal with internal conflict and frustration. Such a person has low achievement and low motivation to work, as well as a wrong interpersonal attitude [6, 8, 9, 32]. In the presented study, two occupational groups differed from each other in the approach to the patient. Nurses more often accepted the patient unconditionally, but doctors often presented requirements to the patient as a person, limiting the acceptance as conditional. The result was statistically significant.

The number of complaints and low level of satisfaction of patients, recorded in world studies and, at the same time, the level of professional frustration of medical staff, which is manifested in the perception of most patients as 'demanding', as well as the level of burnout of medical staff, show the importance of the issues undertaken. The independent variable - age, which in the studies is identified as the most statistically significant when it comes to the way of acceptance of a patient by medical staff (conditionally / unconditionally), and at the same time, the lack of statistical significance of variables related to work seniority and way of acceptance of the patient, shows that in order to maintain a proper (benefiting both parties relation) attitude towards the patient, work experience is not enough, life experience is also necessary; however, this cannot be obtained by young people / students, in ways other than simply by proper education in the area of communication. Appropriate knowledge, proper motivation and skills training are essential.

The correct way of education is also the basis of the differences in relation to the patient between the nurses and doctors. Separately trained, medical staff acquire other educational experiences, and their understanding of the role of a patient is very different. Proper education should teach the procedure of communication with the patient, not only in the area of physical illness, but also show patients as human beings in all dimensions of their lives (physical, mental, social and spiritual). It should also show benefits for medical staff (individual and group) which are the results of a proper relationship with the patient.

CONCLUSIONS

In the presented study an acceptance of the patient by medical personnel in most cases is conditional. Only a few respondents understood the relationship between imposing conditions on the patient, and their own, interpersonal attitude towards the patient (checking whether the patient met the conditions). At the same time, this translates to the level of frustration or satisfaction with their profession, and ultimately to the level of burnout. In the conducted research, the level of acceptance of the patient by medical staff (unconditional acceptance) depended primarily on age and secondly on profession. Gender and work seniority were not statistically significant when dealing with the way of accepting the patient by medical personnel.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

PUBLIC HEALTH PHYSICIANS AND DENTISTS IN POLAND: RESULTS FROM PUBLIC HEALTH WORKFORCE PILOT STUDY

Dorota Cianciara[1], Katarzyna Lewtak[1], Maria Piotrowicz[1], Małgorzata Gajewska[1], Ewa Urban[1] , Katarzyna Brukało[2]*

[1]National Institute of Public Health-National Institute of Hygiene, Department of Health Promotion and Postgraduate Education, Warsaw, Poland
[2]Medical University of Silesia, Department of Health Policy, Department of Public Health in Bytom, Poland

ABSTRACT

Background. Monitoring public health workforce is one of the essential functions of the public health system.
Objective. The aim of the study was to identify the specialities for physicians and dentists related to public health in the years 1951-2013, and analyse of available data on physicians and dentists certified as public health specialists (PHS) in 2003-2015.
Material and Methods. The historical analysis covers a relevant regulations of a minister in charge of health. The data on PHS were obtained from the Centre of Medical Exams and included: the number of specialists and their demographic characteristics, professional background, spatial distribution. Density was also calculated.
Results. The public health specialty was introduced in 1999. Before there were specialties in disciplines related to public health. In the years of 2003-2015, 360 physicians and dentists were certified as PHS. The majority of them had former background in another discipline, mostly related to clinical medicine. The average age of specialists was 47.2. Currently, the average age of specialists is ca. 57.6 years, with a prevalence of people aged 61-70 years (36.9%). PHS tend to be older than specialists in other disciplines. Over three fourths of PHS were certified in 2004. With the exception of that year, the public health specialist title was annually obtained by an average of 9 persons. The density of PHS in Poland was 0.94 per 100 thousand inhabitants, ranging between 0.16 and 3.12 in a given voivodeship.
Conclusions. The analysis has revealed numerous obstacles in estimation of the number of PHS and indicated a lack of relevant mechanisms aimed at workforce development. A relevant policy for developing public health workforce is urgently needed.

Key words: *public health, specialty, manpower, physicians, dentists*

STRESZCZENIE

Wprowadzenie. Monitorowanie zasobów kadrowych zdrowia publicznego (ZP) stanowi jedną z podstawowych funkcji systemu ZP.
Cel. Analiza specjalizacji przeznaczonych dla lekarzy i lekarzy dentystów w dziedzinach związanych ze zdrowiem publicznym w latach 1951-2013 oraz danych dotyczących lekarzy i lekarzy dentystów specjalistów ZP w latach 2003-2015.
Materiał i metody. Historyczna analiza rozwoju objęła przegląd rozporządzeń ministra właściwego ds. zdrowia, które regulowały tę kwestię. Aktualne dane dotyczące lekarzy uzyskano z Centrum Egzaminów Medycznych (CEM). Uwzględniono: liczbę lekarzy i lekarzy dentystów specjalistów ZP oraz ich demograficzną charakterystykę, doświadczenie zawodowe, dystrybucję przestrzenną oraz liczbę specjalistów na 100 tys. mieszkańców.
Wyniki. Szkolenie specjalizacyjne w zakresie ZP dla lekarzy dostępne jest od 1999 r. W latach wcześniejszych lekarze mogli uzyskiwać specjalizacje w dziedzinach pokrewnych. W okresie 2003-2015 tytuł specjalisty ZP uzyskało 360 lekarzy i lekarzy dentystów. Większość lekarzy i lekarzy dentystów specjalistów ZP posiadała wcześniejsze doświadczenie zawodowe i inne specjalizacje, głównie kliniczne. Średni wiek, w którym lekarze uzyskali tytuł specjalisty w tej dziedzinie wyniósł 47,2 lat. Obecnie średni wiek lekarza specjalisty ZP wynosi 57,6 lat, dominują osoby w grupie wieku 61-70 lat (36,9%). Lekarze specjaliści ZP są starsi niż specjaliści w innych dziedzinach medycyny. Ponad trzy czwarte specjalistów zdało egzamin specjalizacyjny w 2004 r. Wyłączając ten rok, tytuł specjalisty ZP uzyskuje każdego roku przeciętnie 9 lekarzy. W przeliczeniu na 100 tys. mieszkańców liczba lekarzy specjalistów ZP wyniosła 0,94 i wahała się w granicach 0,16-3,12 w poszczególnych województwach.
Wnioski. Uzyskane wyniki dowodzą licznych trudności w oszacowaniu liczby lekarzy zajmujących się szeroko rozumianym zdrowiem publicznym i wskazują na brak mechanizmów rozwoju kadry. Niezbędne jest pilne opracowanie polityki rozwoju zasobów kadrowych ZP.

Słowa kluczowe: *zdrowie publiczne, specjalizacja, zasoby kadrowe, lekarze, dentyści*

[*]**Corresponding author**: Maria Piotrowicz, National Institute of Public Health-National Institute of Hygiene, Department of Health Promotion and Postgraduate Education, Chocimska 24, 00-791 Warsaw, Poland, tel. +48 22 5421363, fax: +48 22 5421375, e-mail: mpiotrowicz@pzh.gov.pl

INTRODUCTION

In this article to describe physicians and dentists who obtained the public health specialisation (were certified as relevant specialist) the term "public health specialists" (PHS) is used.

Workforce is the foundation of the public health system. PHS make up an important, yet not the sole professional group in this sector. Monitoring and strengthening public health workforce resources, both quantity- and quality-wise, is among the key functions of the public health system [40].

Recently, WHO and international studies have increasingly stressed the need for all physicians to be educated in issues related to public health in parallel to their primary medical education [23, 11, 9, 32, 39, 15]. Health advocacy and promotion of social, economic, educational and political changes affecting health are expected from this professional group at large. Thus, physicians should be equipped with relevant competences that would allow them to lend support to and execute social and health system related changes. Also, their role in reducing health inequalities is highlighted [38, 25, 5, 10]. Undergraduate medical education in Poland currently incorporates certain elements of public health, whereas in the period between 1951 and 1957 an independent undergraduate medical education programme known as sanitary and hygienic studies used to exist.

More substantial expectations are held as regards PHS. Currently, specialisation in public health is only available on the postgraduate level. Apart from the public health specialty, there are a few other ones available that are also related to the public health field, such as epidemiology. Under the regulations currently in force, PHS are referred to as individuals who have acquired knowledge, skills and competences allowing them to shape the health policy, efficiently manage the health system, as well as plan, implement, monitor and assess the effectiveness of health-promoting interventions [21].

The study is aimed at overview of specialties (specialisations) related to public health in 1951-2013 and analysis of public health workforce in Poland as regards physicians and dentists certified as public health specialists in the period from 2003 to 2015.

MATERIAL AND METHODS

The regulations of the minister in charge of health were reviewed to analyse the public health related specialties released in the period of 1951-2013 in terms of professions allowed to specialization, specialty levels and disciplines.

The data on PHS certified between 2003 and 2015 were collected from the Centre of Medical Exams (*Centrum Egzaminów Medycznych* – CEM) database in Łódź. The analysis covered: (1) the number of PHS and their demographic characteristic (i.e. age, sex); (2) their professional route (i.e. the year of certification and professional/specialty background), (3) their spatial distribution. Based on Central Statistical Office data on population size in 2015 the density of PHS was calculated. The data obtained from the CEM do not allow the identification of two distinct professional groups i.e. physicians and dentists, hence the analysis has covered both groups jointly (in Poland the dentists have the professional title of doctor).

RESULTS

The overview of specialities related to public health (1951-2013)

The system of certifying has over the years evolved frequently in regards to the professions, specialty levels and disciplines. Information on physicians and dentists are presented in Table 1. Up until 1966, regulations specified the principles of certifying only physicians. In 1973, the ordinance of the Minister specified the specialties to choose for physicians, dentists and pharmacists. Subsequent regulations were concerned with the specialties available to physicians and dentists. Separate regulations, in turn, governed other medical professions such as nurses, midwives, pharmacists, and laboratory assistants (referred to as laboratory diagnosticians in Poland) [27, 28].

In 1951-2013, some changes affecting the specialty levels were introduced (see Table 1). Up until 1966, first (I) and second (II) degree specialty were in force. In 1973, primary specialties were introduced in lieu of the former I degree specialties, whereas "derivative" specialties replaced the former II degree specialties [18]. In the same year, sub-specialties emerged, as a one-time occurrence only, such as health education, or health education pedagogy. In the years of 1999-2013 specialties were divided into primary and detailed specialties for physicians and primary for dentists. In 2013 education system has been changed again and introduced the distinction into 77 medical and 9 dental specialties [26].

Public health specialty was launched in 1999 and has since been available to physicians and dentists alike [30]. In the history of postgraduate education of physicians in the field of public health, however, numerous other related specialties existed. Epidemiology and hygiene as specialties were introduced in 1951, and communicable diseases in 1953. Those have been continued ever since but other disciplines (as hygiene) underwent many changes throughout the years. Occupational medicine recognized as one of the major areas of public health has been released as separate specialty since 1973. Other specialties, such as transport medicine and industrial medicine were available at given periods of time (see Table 1).

Table 1. Specialties related to public health for physicians and dentists (1951-2013) in Poland

Name of specialty (specialisation)	Year of introduction of specialty (specialisation)										
	1951[1]	1953[2]	1958[3]	1962[4]	1966[5]	1973[6]	1999[7]	2001[8]	2005[9]	2013[10]	Type of specialty (specialisation)
Epidemiology	■										
Epidemiology		■									I and II degree
Epidemiology			■	■	■	■					II degree
Epidemiology								■			Medical and dental, detailed specialties
Epidemiology									■		Medical, primary specialties
Epidemiology										■	Medical and dental
General hygiene and epidemiology			■	■	■						I degree
Hygiene and epidemiology							■				Medical and dental, primary specialties
Hygiena	■										
General hygiene		■	■								II degree
Hygiena				■	■						II degree
School hygiene		■									II degree
School hygiene				■	■						I i II degree
School medicine						■					II degree
Occupational hygiene		■									II degree
Occupational hygiene					■						II degree
Occupational medicine							■	■	■		Medical, primary specialties
Occupational medicine						■					I degree
Occupational medicine										■	Medical
Communal hygiene		■									II degree
Environmental hygiene						■					II degree
Food and nutritional hygiene		■				■					II degree
Maritime hygiene		■									II degree
Industrial hygiene			■								I i II degree
Industrial medicine				■	■						I i II degree
Industrial medicine						■					II degree
Transport medicine							■		■		Medical, primary specialties
Transport medicine								■			Medical, detailed specialties
Sport medicine							■	■	■		Medical, detailed specialties
Sport medicine										■	Medical
Maritime and Tropical Medicine										■	Medical
Communicable diseases		■	■	■	■						I i II degree
Communicable diseases						■					II degree
Communicable diseases							■	■	■		Medical, primary specialties
Communicable diseases										■	Medical
Public health							■	■	■		Medical and dental, primary specialties
Public health										■	Medical and dental
Healthcare organization			■								I i II degree
Healthcare organization						■					II degree
Social medicine						■					I degree
Health education						■					Sub-specialty („derivative" from II degree specialty)
Medical pedagogy						■					Sub-specialty („derivative" from II degree specialty)

Note: the names of particular specialty are created as rough (direct) translation from Polish

1 Regulation of the Polish Minister of Health. Monitor Polski 1951, No. 103, item 1507.
2 Regulation of the Polish Minister of Health. Monitor Polski 1953, No. 70, item 852.
3 Regulation of the Polish Minister of Health. Monitor Polski 1958, No. 45, item 263.
4 Regulation of the Polish Minister of Health and Social Care. Monitor Polski 1963, No. 2, item 4.
5 Regulation of the Polish Minister of Health and Social Care. Monitor Polski 1966, No. 34, item 176.
6 Regulation of the Polish Minister of Health and Social Care. Dz. Urz. Min. Zdr. i O.S. 1973, No 7, item. 33.
7 Regulation of the Polish Minister of Health and Social Care. Dz.U. 1999, No. 31, item 302.
8 Regulation of the Polish Minister of Health. Dz.U. 2001, No. 83, item 905.
9 Regulation of the Polish Minister of Health. Dz.U. 2005, No. 213, item 1779.
10 Regulation of the Polish Minister of Health. Dz.U. 2013, item 26.

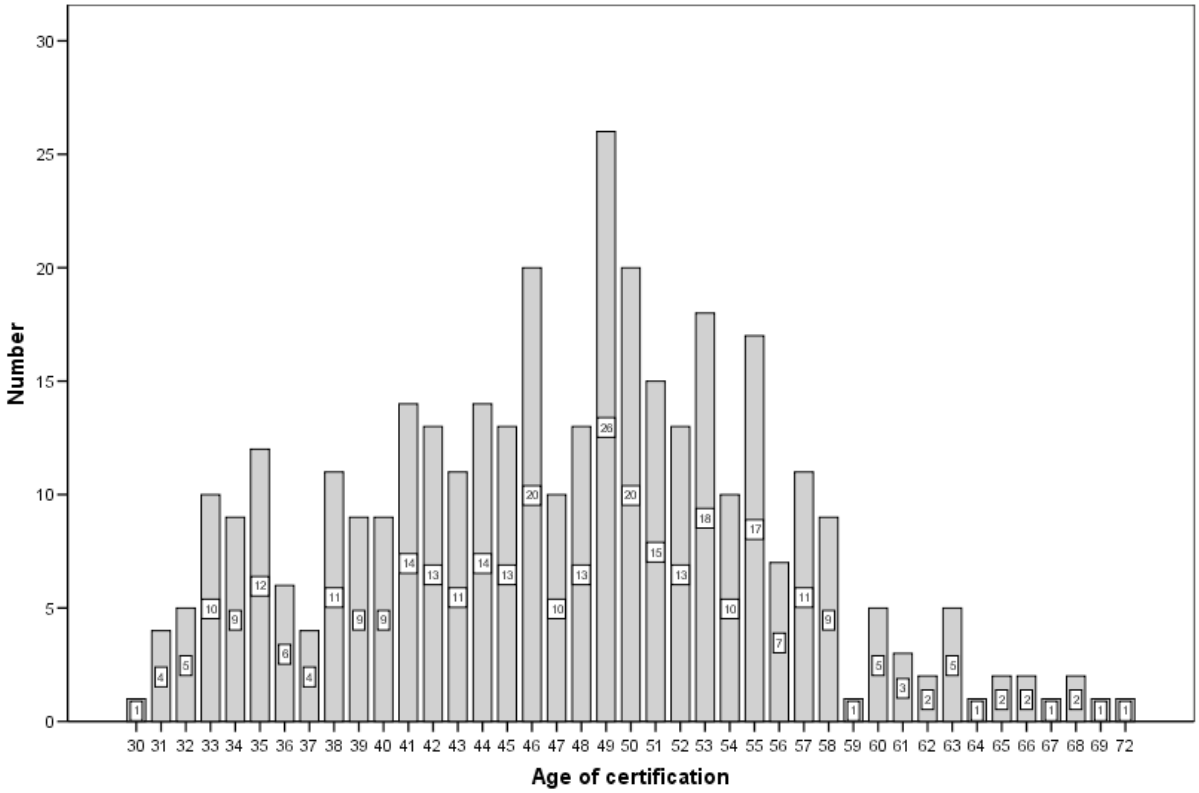

Figure 1. Public health specialists by age of certification

The regulation of 1958 (in force until 1961) allowed physicians to obtain certification in a discipline referred to as "healthcare organisation". Later, this specialty resurfaced again as late as in 1973 beside such specialties as social medicine, health education, or health education pedagogy. All of them were discontinued in 1999. So far, no system has been created that would allow physicians to transfer their "old" specialties into the currently valid ones.

Physicians and dentists certified as public health specialists

The number and demographic characteristics

According to the data from the CEM 360 PHS obtained the public health specialist title in the years 2003-2015 and 130 of them were women. The average age of PHS was 47.2 years (SD = 8.4), with the youngest aged 30, and the oldest 72 (Figure 1). The largest group of PHS were 41-50 years old (42.8%). Men prevailed across all age groups (Figure 2).

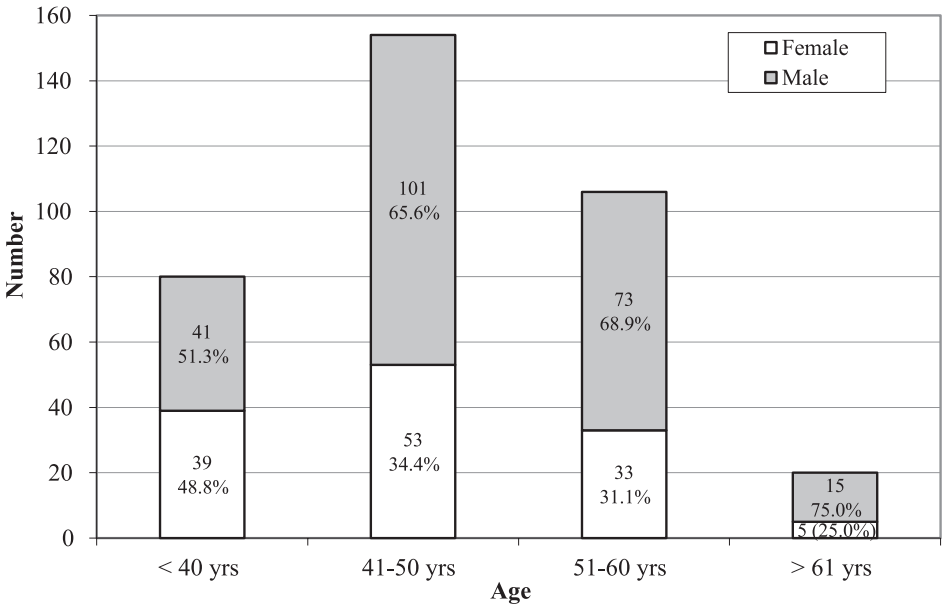

Figure 2. Public health specialists by age of certification and sex

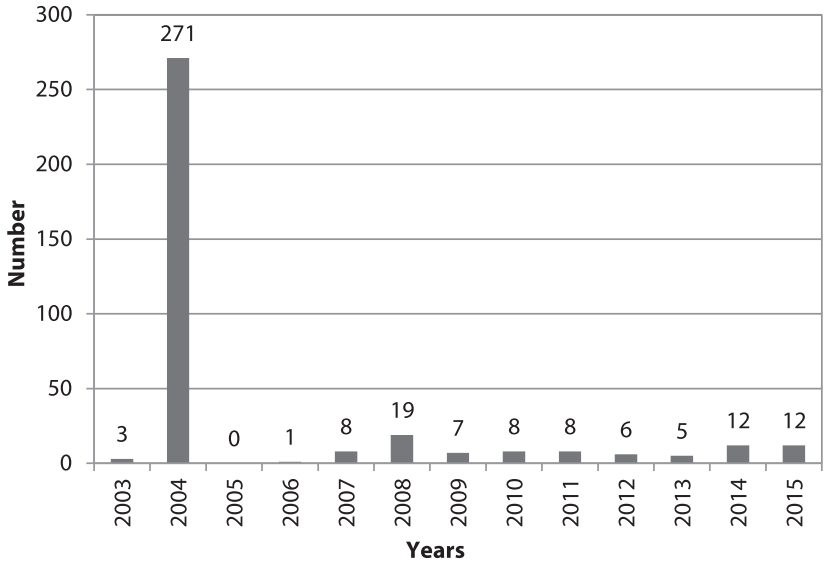

Figure 3. Public health specialists by year of certification

Professional route

Over three fourths of PHS were certified in 2004. In the remaining years, the number of specialist ranged between 1 and 19 (9 per year on average) (Figure 3). In the course of 13 years, a total of 41 exam sessions were held (including 22 in 2004).

More than three fourths of PHS (79.7%) had previous professional experience that is specialty in one or more disciplines. A vast majority (71.1% of total, 89.2% of experienced people) had specialties related to clinical medicine as internal medicine, general surgery, etc. – including medical specialty exclusively (50.3% and 63.1% respectively) or medical speciality together with specialty related to public health (20.8%, 26.1%). Only every tenth PHS (8.6%, 10.8%) had former speciality exclusively related to public health field (including hygiene and epidemiology, occupational medicine, epidemiology, organization of health care, and social medicine). The most common specialties formerly held were: internal medicine (88 people), organization of health care (61), social medicine (49), general surgery (36), paediatrics (32), obstetrics and gynaecology (25), epidemiology (19), occupational medicine (15), hygiene and epidemiology (9).

Table 2. Public health specialists by year of certification and place of residence

Voivodeship / Year	Dolnośląskie	Kujawsko-Pomorskie	Lubelskie	Lubuskie	Łódzkie	Małopolskie	Mazowieckie	Opolskie	Podkarpackie	Podlaskie	Pomorskie	Śląskie	Świętokrzyskie	Warmińsko-mazurskie	Wielkopolskie	Zachodniopomorskie	Total number
2003	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	**3**
2004	12	6	55	12	25	17	48	5	3	14	6	31	2	4	19	11	**271**
2006	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	**1**
2007	0	0	2	0	2	0	1	0	0	0	1	1	0	0	1	0	**8**
2008	0	0	2	0	0	0	11	0	0	1	2	0	0	0	3	0	**19**
2009	0	0	1	0	0	2	3	0	0	1	0	0	0	0	0	0	**7**
2010	0	0	0	0	0	0	1	0	2	1	1	1	0	1	0	1	**8**
2011	0	0	2	0	2	1	1	0	0	0	0	2	0	0	0	0	**8**
2012	0	0	0	0	0	1	2	0	0	1	0	1	0	0	0	1	**6**
2013	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	**5**
2014	0	0	2	0	1	0	2	0	0	0	1	4	0	0	0	2	**12**
2015	1	0	2	0	0	1	4	0	0	1	0	1	0	0	1	1	**12**
Total	**13**	**6**	**67**	**13**	**30**	**22**	**75**	**5**	**5**	**21**	**12**	**42**	**2**	**5**	**24**	**17**	**360**
% of PHs certified in 2004	**92.3**	**100.0**	**82.1**	**92.3**	**83.3**	**77.3**	**64.0**	**100.0**	**60.0**	**66.7**	**50.0**	**73.8**	**100.0**	**80.0**	**79.2**	**64.7**	

Spatial distribution and density

Taking into account the place of residence of PHS, the largest number of them were living in voivodeships Mazowieckie, Lubelskie and Śląskie, whereas the smallest – in Podkarpackie, Warmińsko-Mazurskie, Opolskie and Świętokrzyskie (Table 2). Approx. 65% of PHS (234 persons) were located in cities which are voivodeship capitals.

Three fourths of the total number of the PHS were certified in 2004 (Table 2), with the largest group from Lubelskie (55 people; 20.3% certified in 2004), Mazowiecke (48 people; 17.7%) and Śląskie (31 people; 11.4%). The share of PHS certified in 2004 among all PHS in a given voivodeship ranged from 50% (Pomorskie) to 100% (Kujawsko-Pomorskie, Opolskie and Świętokrzyskie).

Assuming all PHS to be still professionally active, there were 0.94 PHS per 100 thousand inhabitants in Poland in 2015. The number of PHS in particular voivodeships varied. The largest ratios were found in voivodeships Lubelskie, Podlaskie and Mazowieckie, whereas the smallest – in Świętokrzyskie, Kujawsko-Pomorskie and Podkarpackie (Figure 4).

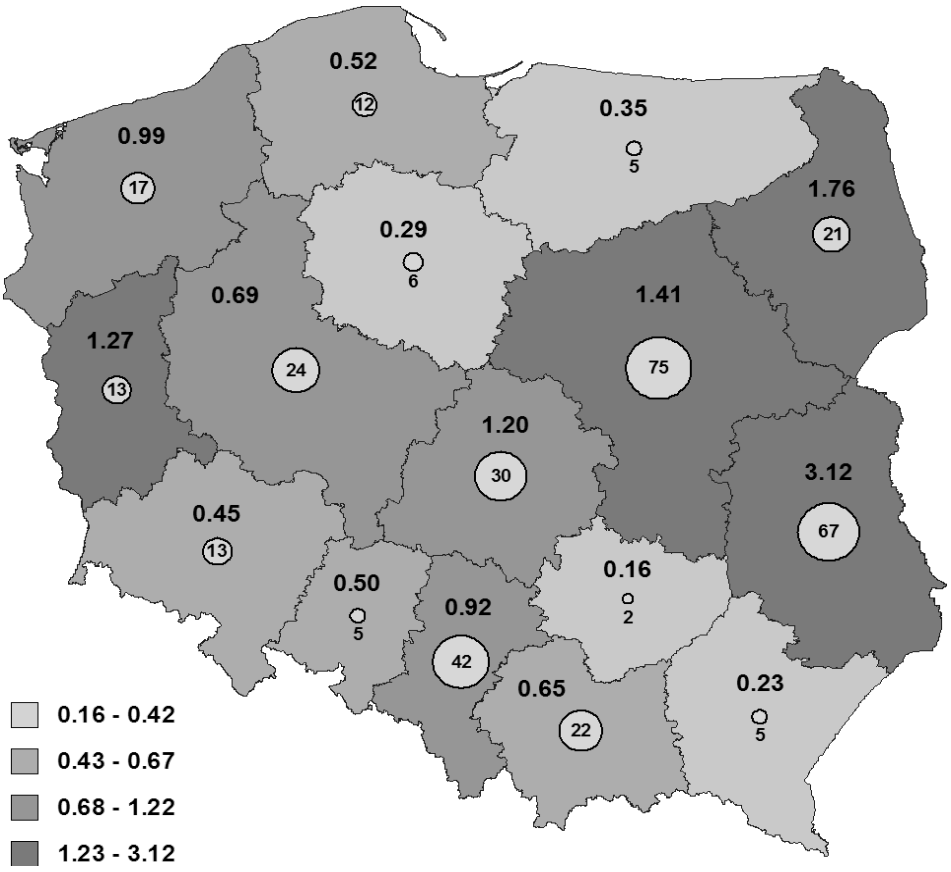

Figure 4. Density of public health specialists by voivodeship (number per 100 thousand inhabitants)

DISCUSSION

There is an inextricable link between medicine and public health, with medicine identified as the source of clinical knowledge, but also the "front-line window" for the identification of public health threats, and the grounds for the practical measures to be executed such as immunizations or screening [17]. Public health domain, in turn, is crucial for community-based measures, preventive services to specific groups and healthy public policy.

Public health history abounds with examples of physicians committed to taking active role on the population level, as was, for instance, the case with John Snow during the cholera outbreak in London's Soho in 1854, or with William Gorgas, a United States Army physician who initiated in 1904 mosquito control in the area of the Panama Canal construction [12, 8].

Public health as a medical specialty originated in England, where, in 1846, Liverpool Sanitary Act mandated the appointment of the Medical Officer of Health. In 1847, doctor William Henry Duncan was appointed the first ever Medical Officer of Health, with an annual salary of £ 750. Concurrently, James Newland was appointed the Borough Engineer and Thomas French - the Inspector of Nuisances [3]. In 1872, the Medical Officer of Health post was introduced throughout the UK [34]. In 1888, the Local Government Act mandated all Medical Officers

of Health practicing in districts with more than 50 thousand citizens to be physicians certified in sanitary science, state medicine or public health. In 1980s, Cambridge University instituted the first ever Diploma of Public health [37].

In the UK, the first school of public health, namely the London School of Hygiene and Tropical Medicine was founded in 1924. In 1970s, the first university specialty programme in public health was launched. In 1972, the UK Faculty of Community Medicine (at present UK Faculty of Public Health) developed training standards for public health specialists. It was only in 1990s that professional education in public health was available to non-medical applicants [6].

In the US, the first medical school to offer postgraduate education in public health was the University of Pennsylvania in 1909, soon followed by Harvard in 1910. The majority of medical schools in the USA, however, did not incorporate public health related content into their primary medical programme. Yet, in spite of the distinct routes of education in medicine and public health, physicians constituted a majority on Public Health Boards.

In 1918, with the substantial support of the Rockefeller's Foundation, John Hopkins University School of Hygiene and Public Health was founded, and in 1922, so was Harvard School of Public Health. The most eminent medical authorities of the times as well as public health leaders solidified the distinct teaching routes for medicine and public health. This situation lasted into second half of a century, when physicians were ultimately recognized as requiring expertise and skills related to public health. The outbreaks of multi-drug resistant TB, Legionnaires' disease, swine influenza, or AIDS that all emerged in 1970s and 1980s showed that physicians need to be adequately qualified in public health related issues to tackle the upcoming health threats [31]. At present, in the US physician education in the public health is conducted in three variants, either as an undergraduate education programme (via combined Medical Doctor or Master of Public Health programs), a residency program (such as General Preventive Medicine), or by mid-career completion of Master of Public Health program available to physicians [16].

Current studies indicate that public health specialty does not enjoy particular popularity among physicians beginning their specialty education programmes either in Poland or in other countries. In a survey carried out among Australian medical students who were asked to indicate a specialty that facilitates combining professional and private life public health was ranked in the third position, following dermatology and general practice. But it occupied the last, nineteenth, position where the prestige of given medical specialties was rated (by male and female physicians alike) [7]. Studies concerning the perceived prestige of given medical specialties have shown an informal hierarchy to exist [33, 24, 13]. The factors affecting the position of a given specialty within the hierarchy have been found to include the following: systems and organs associated with specialty [1]; doctor-patient relationship [33]; social respect [24]; and the use of advanced technologies [13]. According to *Hinze,* surgery and internal medicine are associated with the highest status, whereas paediatrics, psychiatry and general practice are perceived as low-status specialties. In Norway, in turn, physicians and medical students rank neurosurgery as the top-, and geriatrics as the bottom-status specialties [2].

In Poland, education of physicians and dentists in the public health was introduced on the postgraduate level as a specialty programme as late as in 1999. Before that, physicians could be certificated in related disciplines, such as hygiene, epidemiology, communicable diseases, and occupational medicine (or occupational hygiene) and dentists in few of them. However, multiple people still have former certifications in disciplines no longer taught as separate path, such as social medicine, or organization of health care. No system of transfer of the "old" into the currently valid specialties has been developed. Thus, counting all people active in public heath filed is highly difficult if not altogether impossible. According to the information provided by the Supreme Medical Chamber (*Naczelna Izba Lekarska*), the total number of doctors who declared public health specialty totaled 1352 (as of 9.02.2016), whereas the total number of practicing doctors in Poland was 163 756 [20]. In view of these figures, doctors specializing in public health represent 0.8% of doctors population [the Supreme Medical Chamber], while estimates based on CEM data suggest that PHS account 0.2%.

In 2008 in EU alone, specialties related to public health were identified in 21 countries. The names of the specialties in question vary depending on a particular country, including, apart from "public health", other ones, such as preventive medicine, social medicine, or community medicine [35]. The number of physicians specializing in public health in selected countries has been presented in Table 3. In 2014, in Scandinavian countries the percentage of physicians specialising in public health among the total number of physicians ranged from 0.1 % to 1.6%, and among the total number of specialists in all disciplines of medicine ranged from 0.2% up to 3.1 % [36]. For comparison, in Canada, Australia or Japan, the percentage of physicians specializing in public health among the total number of physicians was 0.6% [17, 18, 14].

Table 3. Physicians specializing in public health in various countries

Country	Year	Specialty name	Number of specialists	% of all physicians	% of all specialists
Denmark	2014	Samfundsmedicin (Community Medicine – Public health)	137	0.6	1
Norway	2014	Samfundsmedicin (Community Medicine – Public health)	374	1.6	3.1
Finland	2014	Hälsovård	98	0.5	0.8
Iceland	2014	Socialmedicin	12	1.1	1.4
Sweden*	2014	Socialmedicin	50	0.1	0.2
Canada	2016	Public health & Preventive Medicine	488	0.6	1.3
Australia	2007	Public health Medicine	416	0.6	Not available
Japan	2006	Not available**	1822	0.7	Not available

*members of the Swedish Medical Association. who account for approx. 80% of all physicians in Sweden
** physicians in public health administration agencies; with different specialties related to public health

Data concerning public health workforce in the USA are cited by *Beck* et al. In 2012-2013, a total of 2891 physicians specializing in public health were employed at the local level (namely the National Association of County and City Health Officials (NACCHO) and the Association of State and Territorial Health Officials (ASTHO)). As regards the federal level, in 2013 the Office of Personnel Management (OPM, Federal) employed 6700 public health physicians [4].

As regards the CEM data, assuming all PHS to be professionally active, their current average age would be 57.6 years (SD=9.9), with persons 61-70 years old prevailing in the group. According to the Supreme Medical Chamber data, as of 2015 the average age of a practicing specialist was 54.5. PHS tend to be, therefore, older than specialists in other disciplines of medicine [19]. The data from other countries show these specialists to be relatively younger than it is the case in Poland. In Australia, for instance, the average age of a physicians' specialising in public health was 49 years in 2007 [18]. In contrast to the largely feminized medical profession as a whole in Poland, majority of PHS were men, whereas data from Canada and Australia indicate women to account for approximately half of the total number of these specialists [17, 18].

Three fourths of all PHS were certified in 2004, owing to the fact that a special fast-track specialty line was launched in that year for people with proven achievements in the field [48]. In Poland, the density of PHS (0.94 per 100 thousand inhabitants, in the range of 0.16 - 3.12) is similar to the relevant values in other countries. In Canada, for instance, in 2015 the number of public health physicians per 100 thousand inhabitants was 1.4, and ranged between 0.4 (Newfoundland) and 2.5 (in Quebec) [22].

CONCLUSIONS

1. Between 2003-2013 there was very limited interest in the public health specialty among physicians and dentists. Except 2004, an annual average of 9 physicians (including dentists) were certified as public health specialists.
2. There was a varying number of public health specialists lived in particular voivodeships (from 0.16/100 thousand inhabitants in Świętokrzyskie to 3.12/100 thousand in Lubelskie).
3. The majority of public health specialist had previous background in another discipline of medicine, and was certified in another specialty.
4. The average age of a public health specialist was higher than the average age of a specialist practicing in Poland. Among public health specialists men prevailed.
5. The obtained results indicate multiple obstacles in estimating the number of physicians and dentists practicing in the field of public health, but also point out a lack of relevant mechanisms for the development of workforce in the field. A comprehensive policy of public health workforce development in Poland is indisputably needed.

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Conflict of interest
The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ANALYSIS OF CHANGES IN CANCER HEALTH CARE SYSTEM IN POLAND SINCE THE SOCIO-ECONOMIC TRANSFORMATION IN 1989

Dorota Dudek-Godeau[1], Anna Kieszkowska-Grudny[2,3], Katarzyna Kwiatkowska[1], Joanna Bogusz[1], Mirosław J. Wysocki[1], Magdalena Bielska-Lasota[1]*

[1]National Institute of Public Health – National Institute of Hygiene, Department of Health Promotion and Postgraduate Education, Warsaw, Poland
[2]National Intelligent Specialties, Ministry od Economic Development, Warsaw, Poland
[3]Minds of Hope, Warsaw, Poland

ABSTRACT

Background. The transformation period in Poland is associated with a set of factors seen as 'socio-economic stress', which unfavourably influenced cancer treatment and slowed down the progress of the Polish cancer care in the 90's. These outcomes in many aspects of cancer care may be experienced till today. The results of the international EUROCARE and CONCORD studies based on European data prove evidence that there is a substantial potential for improvement of low 5-year survival rates in Poland. Since high survivals are related to notably efficient health care system, therefore, to improve organization and treatment methods seems to be one of the most important directions of change in the Polish health care system. Till today, cancer care in Poland is based on a network outlined by Professor Koszarowski in the middle of the last century, and is a solid foundation for the contemporary project of the Comprehensive Cancer Care Network (CCCN) proposed in the frame of CanCon Project.

Objective. Analysis of the structure of health care system and the changes introduced within the network of oncology in Poland since the beginning of the post-commuinist socio-economic transformation in 1989.

Materials and Methods. This study was conducted based on the CanCon methods aimed at reviewing specialist literature and collecting meaningful experiences of European countries in cancer care, including the main legal regulations.

Results. The analysis provided evidence that the political situation and the economic crisis of the Transformation period disintegrated the cancer care and resulted in low 5-year survival rates. A step forward in increasing efficiency of the cancer treatment care was a proposal of the *'Quick Oncological Therapy*' together with one more attempt to organize a CCCN. With this paper the Authors contribute to the CanCon Project by exploration, analysis and discussion of the cancer network in Poland as an example of existing net-like structures in Europe as well as by preparation of guidelines for constructing a contemporary CCCN.

Conclusions. (1) 'Socio-economic' stress adversely affected the efficiency of oncological treatment, both by reducing safety and slowing down the development of modern oncology. (2) Changing the current system into the contemporary form - CCCN could be an important step forward to optimise the oncological health care in Poland. (3) Introduction of the mandatory monitoring of organizational changes with the use of health standardized indicators could allow for the assessment of the effectiveness of implemented solutions and their impact on better prognosis for cancer patients. (4) Optimising the organization of the health care system is possible only by implementing necessary legislative corrections .

Key words: *'socio-economic stress', oncology network in Poland, 5-year survival rate, Comprehensive Cancer Centres Network, CanCon*

STRESZCZENIE

Wprowadzenie. Okres Transformacji w Polsce wiąże się z wieloma czynnikami postrzeganymi jako „stres społeczno-gospodarczy", które niekorzystnie wpłynęły na efekty leczenia nowotworów oraz spowolniły postęp w polskim lecznictwie onkologicznym w latach ,90, co w wielu jego aspektach jest odczuwane do dziś. Wyniki międzynarodowych badań EUROCARE i CONCORD dowodzą, że wskaźniki 5-letnich przeżyć w Polsce mogą być znacząco wyższe. Wysokie wskaźniki 5-letnich przeżyć zależą od efektywnego systemu ochrony zdrowia, dlatego, poprawa organizacji i leczenia nowotworów, jest jednym z najważniejszych kierunków zmian w polskim systemie opieki zdrowotnej. System opieki onkologicznej w Polsce jest oparty na modelu sieci onkologicznej, której budowę w połowie ubiegłego stulecia rozpoczął Profesor Koszarowski. Stanowi on nadal solidny fundament do rozwoju nowoczesnej koncepcji sieci wielodyscyplinarnych centrów onkologii (*ang.* Comprehensive Cancer Care Network - CCCN) zaproponowanej w ramach projektu CanCon.

[*]**Corresponding author**: Katarzyna Kwiatkowska, Department of Health Promotion and Postgraduate Education, National Institute of Public Health – National Institute of Hygiene, Chocimska Street 24, 00-791, Warsaw, Poland; phone: +48 22 54 21 377, fax: +48 22 54 21 375, e-mail: kkwiatkowska@pzh.gov.pl

Cel. Analiza struktury lecznictwa i zachodzących zmian w ramach sieci onkologicznej w Polsce po rozpoczęciu w 1989 postkomunistycznej transformacji społeczno-ekonomicznej po 1989 roku.
Materiał i metody. Badanie zostało przeprowadzone w oparciu o metody przyjęte przez CanCon, tj. przegląd literatury specjalistycznej i analizę doświadczeń krajów europejskich w zakresie opieki onkologicznej, ze szczególnym uwzględnieniem polskich regulacji prawnych.
Wyniki. Analiza wykazała, że sytuacja polityczna i kryzys gospodarczy w okresie Transformacji przyczyniły się do dezintegracji leczenia onkologicznego, a w efekcie złych wskaźników 5-letnich przeżyć. Analiza ta stanowi wkład Autorów do projektu CanCon poprzez analizę i omówienie sieci onkologicznej w Polsce, jako przykładu istniejących „podobnych do sieci" struktur lecznictwa w Europie. Zostanie ona wykorzystana przy opracowaniu wytycznych dotyczących współczesnej koncepcji CCCN. Postępem w zakresie organizacji leczenia onkologicznego w Polsce jest wdrożenie tzw. „*Szybkiej Terapii Onkologicznej*" oraz ponowna próba zorganizowania lecznictwa onkologicznego w ramach CCCN.
Wnioski. (1) Stres społeczno-gospodarczy niekorzystnie wpływa na rokowanie u chorych na raka, zarówno poprzez obniżenie poczucia bezpieczeństwa socjalnego, jak i spowolnienie rozwoju nowoczesnej onkologii. (2) Zmiana obecnego systemu w nowoczesną formę - CCCN byłaby istotnym krokiem w stronę optymalizacji lecznictwa onkologicznego w Polsce. (3) Wprowadzenie obowiązku monitorowania zmian organizacyjnych, przy użyciu standaryzowanych wskaźników zdrowotnych w lecznictwie onkologicznym pozwoliłoby na ocenę skuteczności zastosowanych rozwiązań i ich wpływu na poprawę rokowania u pacjentów chorych na raka. (4) Optymalizacja organizacji systemu opieki zdrowotnej nie jest możliwa bez wprowadzenia koniecznych zmian legislacyjnych.

Key words: *„stres społeczno-ekonomiczny", sieć onkologiczna w Polsce, wskaźnik 5-letnich przeżyć, Sieć Wielodyscyplinarnych Centrów Onkologicznych, CanCon*

INTRODUCTION

The results of the international EUROCARE and CONCORD studies clearly show that in the countries of Eastern Europe, Poland including, 5-year survival rates, in most cancer cases, are significantly lower than the European average [1, 8]. However, high 5-year survival rates in the countries of Western Europe prove that there is a substantial potential for improvement of these rates in Poland.

High survivals are related to a notably efficient health care system, therefore, to improve the organization and treatment methods is one of the most important directions of change in the Polish health care system requiring urgent strategic political decisions. While seeking for optimal solutions to provide patients with appropriate health care, the medical community and politicians turned their attention to an idea of a network of comprehensive cancer centres that was created by Professor Tadeusz Koszarowski, and was gradually adapted in the 50's [14].

In 1980 Professor Koszarowski represented Poland during the First Annual Meeting of the Organization of European Cancer Institutes (OECI), the association of directors of European cancer centres and cancer institutions [17]. According to the OECI, setting up the Comprehensive Cancer Center (CCC) is the most efficient operational structure in the multidisciplinary approach to diagnosis, treatment, screening tests implementation and cancer education.

The idea of the CCC as well as the project of a close, formalized cooperation between cancer centres proposed once by Professor Koszarowski lie at the heart of the contemporary project of the Comprehensive Cancer Care Network (CCCN) proposed in the frame of CanCon project [5]. The CCCN is an answer to a more and more frequent need by cancer service providers for a comprehensive cooperation between cancer care centers based on reference guidelines outlined according to the competences of each centre. Currently proposed, a multidisciplinary comprehensive cooperation between cancer centres in the frame of the CCCN and evidence-based public health would greatly facilitate diagnosis and treatment and would reduce disparities in the access to high quality cancer services in a way required by experts.

A Cancer Control Joint Action Project (CanCon) [4] was launched as a result of the international cooperation between the Ministries of Health of the European countries. CanCon is currently working on the recommendations for building a network structure for a modern, comprehensive cancer care system. The *'European Guide on Quality Improvement in Comprehensive Cancer Control'* will include the recommendations in order to: 1) address treatment disparities in Europe, 2) improve the quality of cancer care, 3) improve the quality of life for patients with cancer, and their families.

There is no single universal European model of the CCCN. In every country it has to comply with its specific epidemiological, health and social situation as well as take historical aspects of socio-economic changes into consideration. It seems to be most favourable when a new model starts functioning on the basis of some existing health care background. Such changes need to be implemented following the modern idea of management and monitoring of health indicators.

Building a new model of the CCCN does not only require specialist knowledge on the current situation

in cancer care, but also on the mechanisms that were used to shape it. The Transformation period in Poland is associated with a set of factors seen as 'socio-economic stress', which unfavourably influenced cancer treatment as well as slowed down the progress of the Polish cancer care in the 90's, whose outcomes in many aspects of cancer care may be experienced till today.

Due to the growing interest in health indicators in Poland, their conditioning, and the participation of the Ministry of Health in the CanCon project, a synthetic review of the most significant reasons and organizational processes of decision-making in cancer treatment after Transformation in Poland was prepared.

With this paper the Authors contribute to the CanCon project by exploration and discussion of the cancer network in Poland as an example of existing net-like structures in Europe for its possible incorporation into the modern model of cancer care organization in the European countries.

The aim of this paper was the analysis of the structure of health care and the changes introduced within the network of oncology in Poland after the socio-economic transformation in 1989 began.

MATERIAL AND METHODS

This study was conducted based on the CanCon methods aimed at reviewing literature and collecting meaningful experiences of European countries in cancer care.

Due to lack of data on Poland available for the authors of the CanCon, the research was conducted, also in the Polish specialist literature, with the use of key words whose meaning involved: 'cancer care network', 'comprehensive cancer centres', 'net-like structures'. Table 1 presents the Polish legal acts. The English names of the Polish legal acts were either officially translated or were translated for the purpose of this Paper, if not available otherwise [10].

RESULTS AND COMMENTARY

Changes in Health Care after 1989

Along with the reforms of administration, education and retirement system, the health care system reform was one of the four significant reforms in Poland. The legislative changes in health care introduced at the beginning of the 90's were to decentralize the budget, develop private health care centres and specialist medical practice as well as modernise the infrastructure of public health care providers.

The most important changes were seen in public hospitals in 1991 after the Act on Health Care Institutions coming into force (Table 1, p. 1). The reform allowed for health care providers to become legal entities, which brought them more autonomy, independence and freedom in taking decisions and funding their activities.

Decentralization of the Health Care System Financing - Sickness Funds

The most significant effects of the health care reform became visible in the next decade, after the Act on Universal Health Insurance coming into force in 1999 (Table 1, p. 2). It decentralized the previous health care system and replaced it with a system of financing from health contributions based on the social health insurance. A system of health insurance institutions, the so-called Sickness Funds, was established. There were 16 Sickness Funds, one for each voivoidship, and a separate Sickness Fund for the uniformed services. Sickness Funds guaranteed all the insured the equal access to health care funded by the state through contracts with regional health care providers that complied with required standards of treatment. If a contracted service was inaccessible in the voivodship the insured patient lived in, he/she was entitled to treatment in another one.

Moreover, this reform allowed for extra funding from the state's budget, being at the disposal of the Ministry of Health and Welfare, which funded highly specialised services such as heart transplant service or other expensive procedures. The Act on Universal Health Insurance made it possible to individually purchase services that were not guaranteed by the health insurer, e.g. plastic surgeries or selected modern therapies.

Unfortunately, the health care system reform coincided with the economic slowdown at the end of the 90's, which resulted in lack of funds for expected modernization of health care and planned investments.

Re-centralization of the Health Care System Financing - the National Health Fund (NHF)

The new 2003 Act on Universal Health Insurance in the National Health Fund was supposed to provide conditions to complete the tasks which failed earlier during the reform. It brought back the central funding, and the National Health Fund (NHF), with its regional branches, became the successor to previous Sickness Funds (Table 1, p. 3). Till today, the NHF is the only public payer to health service providers in Poland, which is still not an optimal solution.

The advantage of the 2003 Act on National Health Fund was to introduce uniform contracted procedures. However, this Act did not solve a problem of disparities that had grown over the years in infrastructure, staffing, equipment and organizational procedures between the regional branches of the NHF, which resulted in patient overloading in some health care institutions

responsible for providing patients with the highest quality services, and led other health care providers to debts.

Problems that resulted from lack of financial stability appeared in the health care system. A programme raising health insurance in order to bring income to the health care system was introduced, and a discussion on expenditure cuts and introduction of so-called 'procedures guaranteed from the NHF's budget' began.

Furthermore, the Polish Constitutional Tribunal declared the 2003 Act on National Health Fund unconstitutional and called for drawing new comprehensive regulations of the conditions and range of health care services for Polish citizens (Table 1, p. 4).

The contested Act was replaced by the 2004 Act on Health Care Services Financed from Public Sources (Table 1, p. 5), which with its amendments described the rules for guaranteed health care services. Other legislative regulations aimed at defining guaranteed health care services, tackling corruption, ensuring patients' rights, improving the quality of health care services, introducing complementary insurances and facing lack of personnel due to migration flow to other European labour markets.

The Act on Therapeutic Activity came into force in 2011 – a key act of law for the present health care system, whose significant intention was to improve financial efficiency of hospital management and reduce hospital debts (Table 1, p. 6). It allowed to transform public hospitals into commercial code companies. The 2011 Act on Therapeutic Activity replaced the term 'health care institution' (Polish '*zakład opieki zdrowotnej*') with 'medical entity' (Polish'*podmiot leczniczy*'). The 2011 Act continued earlier efforts to commercialize public hospitals. The model of cancer treatment also experienced significant changes throughout those years.

Cancer Control Programmes

Today, cancer care in Poland is based on a network outlined in the Second Cancer Control Programme in the middle of the last century, and implemented between 1952-1974 [14]. The Programme was born due to the establishment of the Maria Skłodowska - Curie Memorial Institute of Oncology in 1952, with its headquarters in Warsaw and two branches, in Cracow and Gliwice.

One of the main objectives of the Programme, apart from research studies, was to build a three-level reference structure of the oncology network that consisted of cancer centres and outpatient clinics under the supervision of the Institute of Oncology. Moreover, in the same year, in order to obtain epidemiological data on cancer, according to the regulation of the Ministry of Health and Welfare, every cancer case in Poland had to be compulsory reported to the regional cancer registries. By the end of the decade a basic oncology network was established in Poland, and the National Cancer Registry was developed [12, 13].

The main objective of the next edition of the National Cancer Control Programme (1976-1990) was to improve 5-year survival rates of cancer patients from about 25% at the time of the Programme implementation to about 50% after the period of 15 years that followed up [14]. A further development of oncology network was planned by establishing other regional Comprehensive Cancer Centres (CCCs) and increasing the number of regional cancer outpatient clinics as well as optimizing the quality of their activities.

Thanks to the effort of Professor Tadeusz Koszarowski, a doyen of Polish oncology, one of the biggest investments of this Programme was constructing a new modern CCC in Warsaw, which merged with existing structures of the Institute of Oncology and created The Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology. This institution has quickly become and still is the biggest CCC in Poland. It has a unique structure of treatment organization in organ-specific clinics that specialize in multidisciplinary cancer treatment. Such organisation facilitates the modern administration of the cancer centre, the development of its competences and the scientific research. In the 90's those CCCs which were equipped with basic radiotherapy machines served as the centres of reference.

Unsatisfactory treatment results at the beginning of the 90's as well as a rapid increase in cancer cases in Poland required coordinated actions on the national level [24]. After a 15-year-long break, a new multi-year National Cancer Programme (NCP) for years 2006-2015 was established (Table 1, p. 7). It was the fourth programme on the national level, which ensured stable funding, treatment quality monitoring and modes of its provision. Still, one of the main objectives of the Programme was to achieve the average European cancer survival rates in treatment efficiency. At the moment, a project for the new edition of NCP has begun (Table 1, p. 8).

Organization of the Cancer Care System

In practice, till 1998 standard cancer treatment was fully funded by the public sources, and the decision upon the treatment was taken by a doctor responsible for the patient. At that time the most crucial problem was the limited access to drugs. The reforms and changes of the 90's related to the decentralisation of the funding system - the introduction of the regional Sickness Funds did not significantly improve the access to treatment.

Once the Sickness Funds were liquidated and the central funding system was brought back, the NHF

was established. Cancer service providers started to operate on the basis of individual contracts with the NHF, including services financed from public sources at the NHF disposal. As a consequence of the 2014 Act, contracts themselves as well as contracted cancer treatment were limited, being the result of insufficient funds in the NHF budget. One way to make the health care system more efficient was to introduce the '*Oncological Package*' and '*Waiting List Package*' on 1 January with the Act of 22 July 2014 (Table 1, p. 9). Those Acts and the regulations of the Minister of Health, among others, were followed by other important documents: The Act amending the Act on Professions of Nurse and Midwife (Table 1, p. 10), The Act amending the Act on Consultants in Health Care (Table 1, p. 11), The Regulation of the Minister of Health on the Guaranteed Medical Services in Hospital Treatment (Table 1, p. 12) as well as two Decrees of the President of the National Health Fund dedicated to out-patients and hospital treatment (Table 1, p. 13, p. 14).

Oncological Package

The *'Oncological Package*' consists of legal regulations that introduce a new systemic solution – the '*Quick Oncological Therapy*' without cancer treatment limits. Its objective is to guarantee complex cancer care to every patient in a way outlined by the NHF, mainly by improving early diagnosis and shortening long queues of patients waiting for treatment, so the final diagnosis and the beginning of the treatment should not exceed 9 weeks [22].

One of the advantages of the '*Oncological Package*' is that the cancer patient detailed documentation on diagnosis and treatment of the disease is gathered in a standard, electronic way, so called '*DiLO* card' (*the Diagnosis and Oncological Treatment card*) [22] (Table 1, p. 15). Every doctor contracted with the NHF has online access to '*DiLO* card' system.

The 'Q*uick Oncological Therapy*' is followed by the patient in two stages. The first stage - confirmation of cancer diagnosis, is most often coordinated by the primary health care doctor. If the cancer diagnosis is confirmed, the patient is referred to a specialist whose task is to perform further diagnostic tests as the basis for the treatment planned by a council of specialists, if necessary. Until recovery the patient is followed by a coordinator responsible for the efficient treatment process. Having completed the treatment, the patient returns to his/her primary health care doctor.

The objective of the '*Oncological Package'* provided a crucial role of the primary health care doctor who refers the patient to the '*Quick Oncological Therapy'* and coordinates long-term care after the cancer treatment has been completed.

According to the Ministry of Health, the '*Oncological Package*' contributed to a more orderly way of certain aspects of the diagnosis and treatment process [18]. However, since the *'Oncological Package'* has been introduced only recently, the most important epidemiological factors and the treatment costs cannot be yet assessed.

Due to the complexity of problems in oncology, we may still observe a number of deficiencies that impede the treatment process itself, but also unfavourably influence patients' comfort. Already, at the early stage of the *Package* implementation, a limited access to drugs available on the market was observed, which in particular made it difficult for the breast cancer patients to be treated according to the ESMO (European Society for Medical Oncology) recommendations [11].

The main criticism of the '*Oncological Package*' came from the medical community concerned about the lack of prior and careful preparation and organization of the health service providers as well as the lack of proper funding of such a complex initiative on the national level. In the opinion of critics, many difficulties and misunderstandings could have been avoided through the progressive introduction of this reform in the form of a pilot study. Experts and cancer interest groups still cooperate in order to facilitate the functioning of the '*Oncological Package'*. The biggest needs are related to the implementation of diagnostic and treatment standards as well as the rules of referral for cancer centres. Legislative corrections in this respect are expected to be introduced soon.

Despite those problems, the idea of the *'Oncological Package'* is seen favourably.

Paediatrics Oncology

The '*Quick Oncological Therapy'* in children cancer treatment has been criticised by pediatricians and children hematologists. According to pediatricians, actions that follow the recommendations of the '*Oncological Package*' meet potential delays in treatment initiation due to overregulation. Currently, the cooperation between specialists in different children oncological specialisations bases on individual contacts between the doctors. Therefore, diagnosis and treatment initiation of a child patient may start even the same day.

In Poland today, there are 11 regional reference children cancer and hematology centres, with several specialist hospitals, units and wards [15]. Children cancer centres are located in such as a way that the distance between a cancer centre and the patient's place of living is no longer than 120 kilometers. There is an interdisciplinary cooperation between the centres, which allows for quick solutions of diagnostic and treatment problems of young cancer patients, and guarantees highly specialised medical staff. Not only are there doctors who treat children with cancer, but also qualified nurses, psychologists, educators, social workers and occupational therapists.

Table 1. Polish legal instruments used in the study

No	Name of Act	Date	Reference
1.	Ustawa o Zakładach Opieki Zdrowotnej *Act on Health Care Institutions*	30 August 1991	Dz.U. 1991, nr 91, poz. 408
2.	Ustawa o powszechnym ubezpieczeniu zdrowotnym *Act on the Universal Health Insurance*	6 February 1997	Dz.U. 1997, nr 28, poz. 153
3.	Ustawa o powszechnym ubezpieczeniu w Narodowym Funduszu Zdrowia *Act on the Universal Health Insurance in the National Health Fund*	23 January 2003	Dz.U. 2003, nr 45, poz. 391
4.	Wyrok Trybunału Konstytucyjnego sygn. akt K 14/03 *The Constitutional Tribunal Act K14/03*	7 January 2004	Dz.U. 2004, nr 5, poz. 37
5.	Ustawa o świadczeniach opieki zdrowotnej finansowanych ze środków publicznych *Act on Health Care Services Financed from Public Sources*	27 August 2004	Dz.U. 2004, nr 210, poz. 2135
6.	Ustawa o działalności leczniczej *Act on Therapeutic Activity*	15 April 2011	Dz.U. 2011, nr 112, poz. 654
7.	Ustawa o ustanowieniu programu wieloletniego na lata 2006-2015 „Narodowy program zwalczania chorób nowotworowych” *Act on Establishing the Multi-Year "National Cancer Control Programme" for years 2006-2015*	1 July 2005	Dz.U. 2005, nr 143, poz. 1200
8.	Uchwała nr 208 Rady Ministrów w sprawie ustanowienia programu wieloletniego na lata 2016–2024 pod nazwą „Narodowy Program Zwalczania Chorób Nowotworowych” *Resolution No 208 of the Council of Ministers on Establishing the Multi-Year "National Cancer Control Programme" for years 2016-2024*	3 November 2015	M.P. 2015, poz. 1165
9.	Ustawa o zmianie ustawy o świadczeniach opieki zdrowotnej finansowanych ze środków publicznych oraz niektórych innych ustaw *Act Amending the Act on Health Care Services Financed from Public Sources*	22 July 2014	Dz.U. 2014, poz. 1138
10.	Ustawa o zmianie ustawy o zawodach pielęgniarki i położnej oraz niektórych innych ustaw *Act Amending the Act on Professions of Nurse and Midwife*	22 July 2014	Dz.U. 2014, poz. 1136
11.	Ustawa o zmianie ustawy o konsultantach w ochronie zdrowia *Act Amending the Act on Consultants in the Health Care*	22 July 2014	Dz.U. 2014, poz. 1135
12.	Rozporządzenie Ministra Zdrowia w sprawie świadczeń gwarantowanych z zakresu leczenia szpitalnego *Regulation of the Minister of Health on Guaranteed Hospital Services*	20 October 2014	Dz.U. 2014, poz. 1441
13.	Zarządzenie nr 79/2014/DSOZ Prezesa NFZ w sprawie określenia warunków zawierania i realizacji umów w rodzaju ambulatoryjna opieka specjalistyczna *Regulation No 79/2014/DSOZ of the President of the National Health Fund (NFZ) on Conditions of Concluding and Performing Contracts for Outpatient Specialist Care*	5 December 2014	http://www.nfz.gov.pl/zarzadzenia-prezesa/zarzadzenia-prezesa-nfz/zarzadzenie-nr-792014dsoz,6342.html(Accessed 26.09.2016)
14.	Zarządzenie nr 81/2014/DSOZ Prezesa NFZ w sprawie określenia warunków zawierania i realizacji umów w rodzaju leczenie szpitalne *Regulation No 81/2014/DSOZ of the President of the National Health Fund (NFZ) on Conditions of Concluding and Performing Contracts for Outpatient Specialist Care*	5 December 2014	http://www.nfz.gov.pl/zarzadzenia-prezesa/zarzadzenia-prezesa-nfz/zarzadzenie-nr-812014dsoz,6344.html (Accessed 26.09.2016)

Data on childhood cancers is collected in the National Cancer Registry in ICD-10, and since 1999 also in ICCC-3 in the Polish Cancer Childhood Registry [16]. Information from the Registry is used by the National Consultant in Pediatric Oncology and Hematology in the national specialist supervision and monitoring.

Breast Units

In order to improve the functioning of cancer care across Europe there is a tendency to set up cancer specialist diagnostic and treatment centres. Such examples are diagnostic and treatment breast cancer centres – Breast Units (BUs), which operate in compliance with the European standards and hold the accreditation granted by the Senologic International Society (SIS) [23, 28]. Currently, in Poland, there are three operating BUs.

More BUs are needed to satisfy the country's needs. According to the Polish Chamber of Physicians the main obstacles are: understaffing and lack of equipment [20].

DISCUSSION

Health Care System and Health Indicators

During the transformation period the post-communist countries had to reorganise their structures in social, political and economic aspects, which resulted in the significant slowdown of their economic development in comparison to the countries that did not experience such changes.

After 1989 the Polish health care system faced a number of challenges, among which the process of change from the centrally planned funding into the market economy funding was the most crucial one. The economic crisis of the end of the last century and the Article 68 of the Polish Constitution, which guaranteed every citizen the equal access to health care, impeded the implementation of necessary legal regulations to come into effect [26]. The consequence was a worse access to health care and worse population health status indicators, mainly with a drop in the rising trend of life expectancy of Poles [29].

The 5-year Survival Rates in the Context of Cancer Programmes in Poland

The problems of the transformation period resulted in slowing down of the Polish oncology, which reflected in low survival rates for malignant cancers. In Eurocare 3 research Project (1990-1994), 5-year survival rates for all cancers in Poland were approximately 15% lower than the European average and amounted to 29% [6]. Such low survival rates in Poland were the consequence of the unsuccessful implementation of one of the main objectives of the National Cancer Programme (NCP) in years 1976-1990.

It turned out that the long-term objective formulated by Professor Koszarowski – curability at the level of 50%, was in 1990 reachable only for some countries of Western Europe (e.g. Finland), whereas the European average was lower, approximately 45% [14, 6]. At the end of the 90's survival rates in Europe showed at 52%, whereas in Poland they rose only to 42% [6]. In the first decade of the 21st century there was still a rising trend of 5-year survival rates in Europe (in 2007 they showed at 55%), though in Poland the improvement was minimal, and did not exceed 1% (in the same year it showed at 43%) [6].

It is necessary to pinpoint the fact that those unfavourable disparities for Poland were in preventable cancers, whose methods of early diagnosis and optimal treatment were widely used in the countries of Western Europe [3]. Nonetheless, some favourable changes in certain cancers, e.g. leukemia and childhood cancers were observed [2, in prep.].

A critical analysis of research methodology on cancer curability and a multi-year observation of cancer trends proved that persisting disparities in cancer curability between European countries are significant, and particularly unfavourable for post-communist countries [7].

European Recommendations Concerning Cancer Control Programmes

This observation led to the formulation of the recommendations within the European Partnership for Action Against Cancer (EPAAC), which reflected the recommendations of the European Commission for a joint response to prevent and control cancer [9, 27].

According to these recommendations cancer control is the most successful when implemented systematically within a multi-year regional or national cancer control programme which follows well-defined priorities, has stable funding, and its health performance indicators are monitored in accordance with scientific methods.

The recommendations which refer to the third NCP formulated by Koszarowski in the 70's laid the foundations for the fourth NCP for years 2006-2015 [14]. Again, one of the main objectives of this Programme was to improve cancer survival rates in order to reach the European average. However, the health results of the third NCP are not available yet.

The currently started NCP for years 2016-2024 continues to follow the objectives of its previous editions, and, as earlier, is financed from public funds. The fact that the health effects of the previous 10-year-long NCP have not yet been published is a disadvantage when taking strategic decisions in current cancer control programme. Legal regulations for data collecting and

processing to allow for health indicators evaluation and assessment of health effects at every stage of cancer control are being elaborated on.

Health effectiveness of any intervention (set of procedures) ought to be the subject to surveillance. That requires clearly defined specific indicators which allow for comparison between the countries involved in the same programme. The surveillance system requires funds to be properly allocated. It is estimated that the costs of population screening program evaluation will amount to at least 10-20% worth of the total costs of all interventions performed [27].

New-old Solution – Oncology Network

So far, the performance of the tasks outlined in the NCPs was made possible as a consequence of unofficial contacts between doctors and their mutual cooperation in taking diagnostic and treatment decisions. Therefore, it seems that in the current situation in Poland, coming back to the old idea of oncology network in its modern formula might be beneficial - a network built around the contemporary idea of the CCCN that formalizes the cooperation between the already existing cancer centres. According to the CanCon definition, which is essentially similar to Koszarowski's idea, CCCN is characterized by through integration, commonly agreed protocols, common IT, and a formal agreement for common governance. It covers all the components of cancer care: from cancer prevention and organised screening programmes through standard diagnostic and treatment procedures to follow-up plans. Specialised rare tumours-focused care as well as palliative care is also included.

According to the CanCon best experience it is essential that an oncology network is built in a gradual, multidimensional way based on the already existing cancer care system, which ought to be modernized. In the Czech Republic, the cooperation within the CCCN was initiated with a pilot study covering two regions [25]. At the same time, favourable trends in cancer curability were also observed [19, 8].

The Polish National Oncology Network and Cancer Institutes (*unofficial English translation),* which currently consists of several hospitals from every region in Poland, is at the moment holding talks to establish a national CCCN [21].

Summing up, the present cancer care system in Poland faces many challenges, including: a rapid rise in the number of cancer patients due to demographic changes, low effectiveness of population screening, lack of trained personnel or enough funding.

A long process of change into the market-oriented health care system as well as its frequent changes do not bring expected stabilization. A new amendment to facilitate the '*Quick Oncological Therapy*' is waiting to be launched.

Thus, the idea of the oncology network proposed in the middle of the last century by Professor Koszarowski has come full circle.

CONCLUSIONS

1. 'Socio-economic' stress in Poland after the socio-economic transformation began in the 90's of the last century adversely affected the efficiency of oncological treatment, both by reducing the sense of safety and slowing down the development of modern oncology.
2. Upgrading the current system into the contemporary form – the Comprehensive Cancer Centers Network (CCCN) could be an important step forward to optimise the oncological health care in Poland.
3. Developing and introduction of the mandatory monitoring system of organizational challenges by applying standardized health indicators could allow for the assessment of the effectiveness of implemented solutions and their impact on better prognosis of cancer patients.
4. Optimising the organization of the health care system is possible only by implementing the necessary legislative corrections.

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Conflict of interest

The authors declare no conflict of interests.

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