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MICROBIOLOGICAL QUALITY OF FOOD SUPPLEMENTS

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Abstract: Many specialists note that the food offered today – as a result of very complex technological processing – is devoid of many components that are important for the organism and the shortages have to be supplemented. The simplest for it is to consume diet supplements that provide the missing element in a concentrated form. In accordance with the applicable law, medicinal products include all substances or mixtures of substances that are attributed with properties of preventing or treating diseases with humans or animals. Permits to admit supplements to the market are issued by the Chief Sanitary Inspector and the related authorities; permits for medicines are issued by the Chief Pharmaceutical Inspector and the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products. Therefore, admittance of a supplement to the market is less costly and time consuming than admittance of a medicine. Supplements and medicines may contain the same component but medicines will have a larger concentration than supplements. Sale of supplements at drug stores and in the form of tablets, capsules, liquids or powders makes consumer often confusing supplements with medicines. Now there are no normative documents specifying limits of microbiological impurities in diet supplements. In Polish legislation, diet supplements are subject to legal acts concerning food. Medicines have to comply with microbiological purity requirements specified in the Polish Pharmacopeia. As evidenced with the completed tests, the proportion of diet supplement samples with microbiological impurities is 6.5%. Sales of diet supplements have been growing each year, they are consumed by healthy people but also people with immunology deficiencies and by children and therefore consumers must be certain that they buy safe products.

Keywords: microbiological purity, Good Manufacturing Practice (GMP), diet supplements

According to the definition included in the Act of 25 August 2006 on the Safety of Food and Nutrition, dietary supplements are "foodstuffs intended to add further nutritional value to the normal diet, representing concentrated sources of vitamins, minerals or other substances having a nutritional or otherwise physiological effect, either individual or combined, marketed in a dosage form" (1).

There are currently no normative documents specifying microbiological contamination limits for dietary supplements. In the Polish legislation, dietary supplements are subject to legal acts regulating food products. Pursuant to the Act on the Safety of Food and Nutrition, all business entities operating on the food market are obliged to comply with requirements of Good Hygiene Practice (GHP) in their production plants, and follow rules embodied in the Hazard Analysis and Critical Control Points (HACCP) system.

The aim of this work was to retrospectively analyze results of studies investigating microbiological purity of dietary supplements produced in pharmaceutical manufacturing plants in the Wielkopolska region.

MATERIALS AND METHODS

The analysis comprised results of microbiological purity tests performed for a total of 1165 samples of the dietary supplements in form of tablets. Tablets were the final forms of dietary supplements in the sales package, from three manufacturing plants. The study was performed within one month from the date of manufacture of the product. The studies were conducted over a period of three years (from 2010 to 2012). Testing performed according to requirements set out by the ordering party involved determination of total aerobic count, total fungal count, presence and count of Gram-negative *Enterobacteriaceae* bacteria, presence of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* in 1 g/mL, and *Salmonella* in 10

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g/mL. Microbiological testing of preparations was carried out in compliance with methods laid down in relevant Polish Pharmacopoeia (PP) monographs (2). For this purpose, 10 gram of product to be examined was dissolved in 100 mL of buffered sodium chloride-peptone solution pH 7.0. From sample prepared in this way, qualitative examination and quantitative enumeration were performed. The plate-count method was used in the study. For the determination of total aerobic microbial count (TAMC) soya bean digest agar was used and for determination of total combined yeast / moulds count (TYMC) Sabouraud – dextrose agar was used. The volume of 0.1 mL of the sample prepared was spread on the surface of the media, at least 2 Petri dishes for each media for each dilution of sample. After incubation (for bacteria – five days in temperature 30–35°C; for yeast / moulds – five to seven days in temperature 20–25°C), the arithmetic mean of the counts per medium and the number of CFU (colony forming unit) in original inoculum was calculated. For qualitative examination, 10 mL of prepared sample was added to 100 mL of casein soya bean digest broth. The further procedure was dependent on the determination of the absence or limited occurrence of specified microorganism that may be detected:

S. aureus – incubation (24 h, 35°C) and transfer on Mannitol Salt Agar;

P. aeruginosa – incubation (24 h, 35°C) and transfer on Cetrimide Agar;

E. coli – incubation (24 h, 35°C) and transfer to MacConkey broth (incubation 48 h , 44°C), then transfer on MacConkey agar;

Salmonella spp. – incubation (24 h, 35°C) and transfer to Rappaport Vassiliadis broth (incubation 24 h, 35°C) then transfer on xylose, lysine, deoxycholate agar;

Enterobacteriaceae – incubation (2–5 h, 20–25°C), transfer to enterobacteria enrichment broth-Mossel (incubation 24–48 h 35°C), transfer on violet red bile glucose agar.

Further identification was carried out using automatic Vitek 2 system Compact (bioMerieux). Results were evaluated in accordance with criteria listed in Table 1.

RESULTS

A total of 1165 samples of dietary supplements of different compositions were assessed. Most of them (67.5%) were supplements containing ingredients of natural (herbal) origin. The analysis showed that 6.5% of all samples under study failed to comply with the requirements in place.

The samples were found to have exceeded the maximum acceptable microbial count. Pathogenic microorganisms were also detected (Table 2). None of the samples under study contained *S. aureus* or *Salmonella* spp.

The most common nonconformity, identified in a half of all non-compliant samples, was the exceeding of the maximum acceptable aerobic bacterial count. Excessive fungal counts were identified less frequently. A small amount of samples was found to contain excessive counts of Gram-negative *Enterobacteriaceae* bacteria. The presence of *E. coli* was confirmed in six samples (Table 2).

Table 1. Limits for microbiological contamination.

Document	Total aerobic microbial count (CFU/g)	Total yeast/moulds count (CFU/g)	Specified microorganisms
Ministry of Health Regulation (from 13. 01. 2003). Dietary products	10^4 - 10^5	10^2 - 10^3	Absence of *Salmonella* (10 g) coli group 10^a - 10^a CFU/ g *S. aureus* 10^a-10^a CFU/ g
Regulation commission UE number 1441/2007. Food	-	-	*Listeria monocytognes* 10^a CFU/ g
Criteria according to the customer	10^4	10^2	Absence of *Salmonella* (10 g) Absence of *E. coli* (1 g) Absence of *S. aureus* (1 g) Not more than 10^a *Enterobacteriaceae* and certain other Gram-negative bacteria (1 g)

Among 381 taken under examination samples not containing natural ingredients, microbiological contamination was detected in only two products . The cause of contamination was exceeding maximum levels of mould counts.

DISCUSSION

Dietary supplements are foodstuffs and must therefore conform to the definition of food contained in Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002, which provides that: "food" (or "foodstuff") means any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be, ingested by humans"(3).

According to the definition contained in the Act of 25 August 2006, dietary supplements are marketed in dosage forms including capsules, tablets, coated tablets and similar forms such as powder-containing sachets, ampoules with liquid, drop bottles and other forms intended for consumption in small measured amounts (4). As the above shows, dietary supplements are available in forms typical for pharmaceutical products, i.e., items which are required to satisfy microbiological purity criteria defined in the Polish Pharmacopoea. Under EU laws, each Member State decides on its own whether a given substance is to be classified as a medicinal product or a dietary supplement. Supplements are intended specifically to make up for nutritional deficiencies or to maintain adequate levels of nutrients in the diet. They are foodstuffs which are associated by patients with caring for their health. Since dietary supplements are routinely purchased in pharmacies, where they stand on the shelf next to medicinal products, they should be as scrupulously controlled in terms of microbiological contamination as medicinal products.

Dietary supplements are purchased as well as by healthy people and those who are ill, or suffering from compromised immunity. The presence of pathogenic microorganisms in such products may thus be dangerous and potentially lead to infections. Another point to consider is the fact that there are supplements formulated specifically for children and infants. Microbiological contamination can also reduce the quality and stability of the finished product.

According to a report published by the European Commission from 2008, the number of substances used in the production of dietary supplements equals ca. 400. In terms of composition, they can be classified into several groups: vitamins and minerals, amino acids, enzymes, pro- and prebiotics, unsaturated fatty acids and supplements containing herbal ingredients (5). In the studies reported here, dietary supplements based on herbal ingredients constituted 67.5% of study samples. They were formulated with a variety of ingredients including European blueberry fruit, hawthorn fruit, raspberry fruit, ginkgo biloba, Jerusalem artichoke tubers and cranberry fruit. Some of these ingredients are registered as stand-alone herbal medicinal products and therefore, they must comply with microbiological requirements set out in the Polish Pharmacopoeia.

Annex I to the Commission Regulation (EC) No. 1441/2007 of 5 December 2007 specifies microbiological criteria for different groups of foodstuffs, however without identifying a separate group of dietary supplements. These can only be classified as belonging to one of the groups: 1.3. Ready-to-eat foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (6). Furthermore, Annex 7 to the Regulation issued by the Minister of Health, Republic of Poland on 13 January 2003, establishes maximum acceptable limits of microbiological contamination for foodstuffs, however without address-

Table 2. Microbiological contamination of food supplement.

Number of samples of incompatibility (n = 1165)	Frequency of the causes of nonconformities (n = 76)	Causes of incompatibility
38 (3.3%)	50.0%	Exceeded number of bacteria
19 (1.6%)	25.0%	Exceeded number of fungi
6 (0.5%)	8.0%	Presence of *E. coli*
13 (1.1%)	17.0%	Exceeded number of bacteria species of *Enterobacteriaceae*
76 (6.5%)	100%	Total

ing dietary supplements directly. These can only be classified as dietary products for which contaminant limits are provided (7) (Table 2). Consequently, manufacturing plants may – but are not formally required to – test dietary supplements to determine their microbiological contamination levels. As it is, companies may also adopt their own criteria for microbiological contamination and for the presence of pathogenic bacteria. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 sets maximum levels for certain contaminants in foodstuffs (e.g., nitrates, metals, dioxins, mycotoxins) (1).

Mycotoxins are produced by some species of mould (including genera: *Aspergillus, Penicillium* and *Fusarium*), which can be contamination of dietary supplements. The toxins can be carcinogenic and mutagenic substances. They can also cause acute and chronic poisoning, allergies, diseases of the respiratory and digestive systems, and liver damage (8, 9). As demonstrated by studies, out of 1165 tested dietary supplements, maximum acceptable limits for mould were exceeded in 19 cases. Tournas and et al. (10) reported that 78% of the ginseng herb supplements, 100% of the Siberian, 56% of the Chinese and 48% of the American ginseng root samples showed fungal contamination. Fungi found in the ginseng herb were *Alternaria alternate*, *Aspergillus niger*, *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Rhizopus* spp. and yeasts (10). In addition, in another study, it was indicated that 60% of samples of milk thistle dietary supplements were contaminated with fungi (11).

As laid down in Commission Regulation (EC) No. 1441/2007, foodstuffs should not contain microorganisms, their toxins or metabolites in amounts that pose unacceptably high risks to human health. Unfortunately, however, maximum acceptable limits are not specified (6). Tests showed 13 samples to contain Gram-negative *Enterobacteriaceae* bacteria, while 6 samples were contaminated with *E. coli*. The samples in which Gram-negative bacterial contamination limits were exceeded and *E. coli* was detected were dietary supplements containing herbal ingredients (Jerusalem artichoke tubers and European blueberries). The contaminants may have their source in the natural environment (water, soil). Crops may also become indirectly contaminated through poorly composted organic fertilizers. Ruminant feces may be a source of contamination with *E. coli* bacteria which form a part of their natural intestinal flora.

The Chief Sanitary Inspector keeps a register of products covered by the notification of the first market placement (including dietary supplements) at the territory of Republic of Poland. Data from this registry show an increase in the number of registered dietary supplements. In the period 2007–2012 the number was respectively 791, 773, 1906, 1260, 983, 1444 (12). The products are used not only by healthy individuals but also patients with immune deficiencies and children. As the tests showed, the percentage of microbiologically contaminated samples of dietary supplements was 6.5%. Patients taking dietary supplements should be able to feel confident that they ingest products that are safe and pose no risk to their health.

The obligation to ensure the safety of dietary supplements should rest with the manufacturer, as such products should not pose a health risk to consumers. The dietary supplements are produced by various manufacturing sites: pharmaceutical companies using GMP systems in their manufacturing facilities but also by food industry cooperatives. An appropriate legal framework should, however, be adopted to precisely define maximum acceptable microbiological contamination limits in dietary supplements.

Acknowledgment

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