The presence of microorganisms in medicinal plants is a common, though medically unfavorable phenomenon. Microorganisms inhabiting medicinal plants belong both, to saprophytic as well as pathogenic flora. Even saprophytic microorganisms may cause infection in people having contact with them. This effect may be exemplified by *Enterobacter agglomerans*, which causes a number of medication-induced infections, including diseases of the respiratory and urinary tracts as well as wound infections (1).

Severe infections of the genitourinary tract or pneumonia often follow administration of drugs that had been contaminated with saprophytic rods such as *Serratia marcescens*, *Alcaligenes faecalis*, or *Acrobacter cloacae* (2).

The above-mentioned microorganisms are characterized by low pathogenicity, nevertheless they may cause infection in people having decreased natural immunity, especially in those suffering from neoplasms, diabetes mellitus or any other severe conditions, as well as in those subjects who have not developed natural immunological mechanisms yet, as infants and babies.

Pathogenic bacteria can also be found on herbal raw material, and when present in the products, they cause a severe threat to the health of patients, especially the youngest ones. *Escherichia coli* is currently considered as the most common reason of cerebrospinal meningitis and digestive tract infections in neonates (2).

Severe genitourinary infections have been confirmed following administration of drugs contaminated with microorganisms from *Enterobacter*, *Klebsiella*, *Proteus*, or *Pseudomonas* species (1-3).

The toxicity of *Staphylococcus* and *Enterococcus* species is associated with numerous toxins released by them, hemolysins being the most dangerous ones. *Clostridium* species release toxins and enzymes which cause extensive lesions in the infected tissues (2,4).

The presence of fungi in raw material and pharmaceutical products may be equally dangerous. Moulds do not belong to the category of invasive microorganisms, and the risk of them causing infection is usually associated with the status of decreased immunological response of the organism (1,5).

Skin and mucous membranes infections are most commonly caused by moulds such as *Aspergillus spp.*, *Penicillium spp.*, *Mucor spp.* and *Rhizopus spp.*. Apart from the skin, the fungi may invade the conjunctive, nails, mucous membrane of the tonsils and vagina.

Especially dangerous are those moulds which produce mycotoxins, which have a strong toxic effect. In herbal raw material we can find about 7% of moulds which are able to produce strong toxins, e.g. *Aspergillus flavus* isolated from *Salviae folium*, which secretes aflatoxin, a strong hepatocarcinogen (1,3,6).

According to the requirements of Polish Pharmacopoeia VI (7) and European Pharmacopoeia (8), the products containing natural raw material should fulfill relative microbiological norms.

Among pathogenic microorganisms, special attention, according to international norms, should be...
paid to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and anaerobic bacteria from *Clostridium* species. In case of plat raw material subjected to the action of boiling water, the allowable norms for *Escherichia coli* are $10^2$ in 1 g or 1 mL. On the other hand, plant raw material which is not designed to be treated with boiling water must be completely free from *Escherichia coli* and *Salmonella* (7).

The aim of the trial was to determine the degree of microbiological contamination of brewed (tea bags) and instant (granulated) herbal teas.

**EXPERIMENTAL**

**Materials**

The investigation involved twelve kinds of tea in tea bags - so called express tea manufactured in Poland as well as ten granulated teas - instant teas, manufactured by Kruger and Impress companies and Plantex (Lek Polska). All of them were purchased in Wroclaw’s pharmacies and are designed for the youngest patients - infants and small children.

**Methods**

The microbiological assessment was carried out according to methods recommended in Polish Pharmacopoeia VI (7).

**Collection and size of samples**

Samples of preparations for microbiological investigation were collected from three packages under aseptic conditions and thoroughly mixed. The size of powdered sample was from 40 to 45 g.

Microorganisms from *Enterobacteriaceae* species were evaluated on 20 g samples of drug (7).

**Preparation of samples**

10 g of each sample was collected in aseptic conditions and completed to 100 mL with buffer solution at pH 7 containing polysorbat 80 in the amount of 1 g/L of the vehicle.

**Assessment of the amount of microorganisms by means of direct culture**

Samples corresponding to 1 g of the preparation were diluted to obtain 1:10, 1:100, 1:1000, 1:10000, 1:100000 and 1:10000000 solutions. 1 mL of solution from each dilution was measured and PM 2 medium for bacteria and PM 3 medium for fungi was added. The cultures were incubated for 5 days at temperatures from 30°C to 35°C (for bacteria) and from 20°C to 25°C (for fungi).

**Investigation for the presence of *Escherichia coli***

The investigation was carried out according to methods recommended in Polish Pharmacopoeia VI (7) and European Pharmacopoeia (8).

20 mg of the preparation was added to 100 mL of PM 6 medium and incubated for 2 h at 35°C - 37°C in order to reactivate bacteria. After incubation, a sample corresponding to 1 g was transferred from PM 6 to PM 9 and incubated for 24 h at 43°C. After incubation, the sample was sieved onto PM 10 selective medium and incubated for 24 h at 43°C.

If bacterial growth was found on Mc Conkey's agar, selective medium COLI ID was used for culture. It contains two chromogenic substrates: one for β-glucuronidase staining *Escherichia coli* colonies red and the other for β-galactosidase staining the colonies of *Escherichia coli*-like bacteria blue. Combination of both those substrates enables simultaneous detection of *Escherichia coli* and *Escherichia coli*-like bacteria.

The samples were incubated at 35°C to 37°C. After 24 h the plates were identified against white background. The growth of Gram-positive bacteria and fungi on this medium is inhibited (8).

**RESULTS AND DISCUSSION**

As shown in data presented in Table 1, five out of twelve investigated teas contained increased levels of bacteria in comparison to pharmacopoeic norms (10 000 000 w 1 g). All of them are compound teas containing several herbs. The highest level of contamination, i.e. 15.200.000 bacteria per 1 g of the product was found in Nervinum tea bags. Slightly less, i.e. 14.000.000 bacteria in 1 g was cultured from Bronchial, and 12.000.000 bacteria were found in Bobofen and Fito-Mix-9 each. The remaining products, which were tea bags from single herbs, revealed significantly lower levels of bacterial contamination. The amount of cultured bacteria ranged from 2.100.000 per 1 g of Anthodium Chamomillae to 7.900.000 per 1 g of Folium Menthae piperitae and did not exceed the pharmacopoeic norms (10.000.000 per 1 g) in either of the case.

The presence of Gram-negative bacteria was confirmed in five teas in tea bags, the amount in Flatuvit being so high that it made counting of the bacterial colonies impossible. The amount of microorganisms in Bronchial was 22 per 1 g, in Inflorescentia Tiliae - 10 per 1 g, and in Folium Menthae piperitae - 6 per 1 g. Nevertheless, final identification of Gram-negative bacteria on COLI ID medium did not confirm the presence of *Escherichia coli* in either of the samples.

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Table 1 also presents the level of fungal contamination of tea bags. According to the data, the presence
of fungi was confirmed in five preparations, in two of them the level being higher than allowed according to pharmacopoeic norms (over 100,000 per 1 g).

The highest level of fungi, i.e. about 300,000 per 1 g was cultured from Flatuvit preparation, slightly less, i.e. 200,000 per 1 g - from Fructus Foeniculi. On the other hand, Folium Menthae piperitae, Fructus Myrtylli and Nervinum mixture contained about 100,000 fungi per 1 g. This is the upper limit of the pharmacopoeic norm.

Table 2 presents the level of microbiological contamination of granulated herbal teas.

According to the data, only one preparation contained the level of bacteria that exceeded the pharmacopoeic norm. Impress Chamomile tea contained 17,000,000 bacteria per 1 g of the product.

Out of ten investigated instant teas, only one (Impress herbal tea) contained Gram-negative bacteria, which, however, were not identified as Escherichia coli.

On the other hand, all the investigated granulated teas contained increased levels of fungi. In case of Hipp Chamomile tea, the level was 7 times higher than the pharmacopoeic norm. Impress chamomile tea as well as in Hipp digestive tea the allowable norm according to Polish Pharmacopoeia VI (7) was 6 times exceeded.

The lowest levels of fungi were found in Hipp and Impress Fennel Tea, Hipp Sedative Tea, Impress Herbal tea. Each of them contained about 200,000 fungi per 1 g of the product, which still is twice as much as allowable norms.

The high content of fungi in granulated teas is probably due to the specific conditions during the process of granulation, such as increased temperature and relatively high humidity, which are favorable for fungal growth.

The pharmacopoeic norms of bacterial and fungal content concern directly oral preparations of natural origin submitted to the action of boiling water and in this context they are surpassed in many instances. However, under the effect of boiling water which is used to prepare infusions and decoctions, the majority of aerobic microorganisms as well as all the fungi, both moulds and yeast-like die (9).

This means that significantly lower levels of microorganisms and fungi get to the patient’s organ-

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**Table 1. Microbiological contamination of tea in tea bags.**

<table>
<thead>
<tr>
<th>Name of preparation</th>
<th>Batch and manufacturer</th>
<th>The level of bacteria in 1 g</th>
<th>The level of fungi in 1 g</th>
<th>The level of Gram(-) in 1 g</th>
<th>The level of E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bobofen Herbapol</td>
<td>10120022L</td>
<td>12.100.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fructus Myrtylli</td>
<td>30102002L</td>
<td>5.400.000</td>
<td>100.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bronchial Herbapol</td>
<td>10322002L</td>
<td>13.900.000</td>
<td>0</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Fito-Mix 9</td>
<td>2022002L</td>
<td>12.600.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flatuvit PhytoPharm</td>
<td>2065211</td>
<td>10.600.000</td>
<td>300.000</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Fructus Foeniculi</td>
<td>2022003L</td>
<td>3.900.000</td>
<td>200.000</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anthodium Chamomillae</td>
<td>101131111 PhytoPharm</td>
<td>2.100.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inflorescetia Tiliae</td>
<td>10041706 PhytoPharm</td>
<td>3.400.000</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Folium Melissae</td>
<td>6032002B</td>
<td>4.200.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Folium Menthae pip.</td>
<td>4032003B</td>
<td>2.400.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Folium Menthae pip.</td>
<td>1004805 PhytoPharm</td>
<td>7.900.000</td>
<td>100.000</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Nervinum Herbapol</td>
<td>2022002L</td>
<td>15.200.000</td>
<td>100.000</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+ uncountable (too many)
Table 2. Microbiological contamination of granulated teas.

<table>
<thead>
<tr>
<th>Name of preparation</th>
<th>Batch and manufacturer</th>
<th>The level of bacteria in 1 g</th>
<th>The level of fungi in 1 g</th>
<th>The level of Gram(-) in 1 g</th>
<th>The level of E. coli in 1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon with vit. C Impress</td>
<td>L-91472</td>
<td>1.400.000</td>
<td>600.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Foeniculi Impress</td>
<td>L-93142</td>
<td>1.500.000</td>
<td>200.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Foeniculi HIPP</td>
<td>L-181731</td>
<td>1.500.000</td>
<td>200.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fruit with vit. C Impress</td>
<td>L-00731</td>
<td>4.800.000</td>
<td>500.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Orange with vit. C Impress</td>
<td>L-92771</td>
<td>1.200.000</td>
<td>500.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chamomillae HIPP</td>
<td>L-181771</td>
<td>1.800.000</td>
<td>700.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chamomillae Impress</td>
<td>L-92651</td>
<td>17.300.000</td>
<td>600.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sedative HIPP</td>
<td>L-187861</td>
<td>3.8000.000</td>
<td>200.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Digestive HIPP</td>
<td>L-181932</td>
<td>1.300.000</td>
<td>600.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Herbal Impress</td>
<td>L-00041</td>
<td>2.300.000</td>
<td>200.000</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Plantex Lek Polska</td>
<td>753806</td>
<td>6.500.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+ uncountable (too many)

ism than it would seem on the basis of microbiological contamination of the plant raw material. In case of aerobic bacteria, after brewing their level does not generally exceed 100,000 per 1 mL, regardless of the initial contamination of the preparation.

However, it should be remembered that the presence of bacteria in raw material is associated with the presence of bacterial spores, which as a rule are resistant to physical and chemical factors. They achieve especially favorable conditions for growth during production of granulates, as well as in macerates and in improperly stored herbal infusions and decoctions.

Adequate microbiological purity of drugs is an important problem, as it directly affects the safety of using preparations from this group. Already a single dose of contaminated drug may cause disease, especially when administered to a weakened or immature organism. This may affect the content of active bodies in plant drugs as well as their quality.

REFERENCES


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