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ANTIBIOTIC RESISTENCE OF NON-PATHOGENIC BACILLUS SPP AS AN INDICATOR OF THE GENERAL CONDITION OF HUMAN COLON AND SOIL MICROBIOMES

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Summary. *The sensitivity to antibiotics of the non-pathogenic strains of bacteria of the genus Bacillus was investigated. Strains were isolated from the feces of healthy individuals, patients with colon dysbiosis, as well as food and pharmacological agents - tincture of Echinacea purpurea. It was established that antibiotic resistance of the non-pathogenic Bacillus spp. depends on the general condition of the colon microbiota and may serve as an indicator of soil contamination by microscopic fungi.*
Key words: antibiotic resistance, non-pathogenic Bacillus spp., microbiomes.

The relevance of the spread of antibiotic resistance among human pathogens is undisputed. This problem is not limited to the spread of this phenomenon among causative agents of the infectious diseases only. It is closely related to the food safety too [1]. Antibiotic resistance has general biological significance. Bacteria and fungi that were able to produce antibiotics and protect themselves against antibiotics' impact existed long before the discovery of penicillin and even before H. sapiens arose. It is well known that between bacteria of the same species and quite remoted in the phylogenetic aspect the exchange of genetic information occurs. Including the one that provides resistance to antibiotics. So the logical question arises about the relationship of antibiotic resistance of human pathogens and spreading of this phenomenon in the surrounding microbiological environment. If such a link exists, it is in principle possible to monitor the epidemiological situation with the spread antibiotic resistance, not only in hospitals and others medical institutions, but to a much wider scale.

When looking for approaches to solving this problem, the first need is choose the object of study. In this regard, the most piece of attention, in our opinion, should be paid to the non-pathogenic bacteria of genus Bacillus.

Genus Bacillus consists of a variety of rod-shaped Gram-positive (or positive only in the early stages of growth) bacteria that move by means of flagella and are aerobes. Previously the genus united together a diverse group of organisms, as evidenced by the wide range of ratios of DNA guanine — cytosine base pairs to the rest of their genomes. This ratio varied from 32 to 69 mole percent, which is much more than accepted levels for members of the same genus. The phylogenetic approach to Bacillus taxonamy has been accomplished largely by analysis of 16S rRNA molecules by oligonuclatide sequencing. As a result, many former members of the genus Bacillus have been moved to new families or genera [2].

Non-pathogenic species of the genus Bacillus — subtilis, licheniformis, pumilus are very close and it is difficult to differentiate them from each other. Historically, they were included into the "subtilis-group" or "subtilis-range". B. subtilis is the typical species of the genus. Historically, before the publication of monographs Smith in 1946 and 1952, under the term B. subtilis all aerobic spore-forming bacteria had been combined. B. subtilis may be distinguished from closely related species of the genus, but it needs to use modern sophisticated methods, for example — pyrolysis-gas chromatography [3].

Bacillus subtilis is a ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue. B. subtilis is often referred to as a 'soil dweller'. Although the actual numbers in existence in the environment for this species has not been determined, bacilli occur at population levels of 10^6-10^7 per gram of soil.

Work in recent years has transformed our view of what B. subtilis can do within the gastrointestinal (GI) tract of animals. Formerly, B. subtilis was thought to be an obligate aerobe that simply traveled through the mostly anaerobic GI tract as a spore. Recent evidence, however, indicates that B. subtilis can complete its entire life cycle within the GI tract, going from

spore to vegetative cell and sporulating again [4 , 5].

Their number in foods is limited only by the rate of total microbial contamination — MAFAnM (mesophilic aerobic and facultative anaerobic bacteria). Permissible contamination by this indicator, depending on the product category, ranging from 500 to 1,000,000 CFU (colony forming units) per 1 gramm.

In conclusion, current data indicate that the apparent ubiquitous spreading B. subtilis is not only a consequence of the stability of spores. B. subtilis is able to grow in a variety of environments including soil, plant roots and in the gastrointestinal tract of animals.

The transfer of gene sequences between strains of B. subtilis has been demonstrated when the strains were grown together in soil. In addition, Klier et al. (1983) demonstrated the ability of B. subtilis and B. thuringiensis to exchange high frequency transfer plasmids [6]. Other studies have shown that B. subtilis has the ability to express and secrete toxins or components of the toxins that were acquired from other microorganisms through such transfers of genetic material. B. subtilis expressed subunits of toxins from Bordatella pertussis [7] as well as subunits of diphtheria toxin and pneumolysin A pneumococcal toxin. Although B. subtilis does not appear to possess indigenous virulence factor genes, it is theoretically possible that it may acquire such genes from other bacteria, particularly from closely related bacteria within the genus.

Purpose

Investigate the ability to monitor the epidemiological situation with the spread of antibiotic resistance among bacteria — causative agents of infectious human diseases by determining antibiotic resistance of the non-pathogenic species of the genus Bacillus, isolated from food.

Research objectives:

1. To reveal dissemination of antibiotic resistance among non-pathogenic Bacillus spp. which are a part of the GI tract microflora of healthy people and ones with dysbacteriosis of the colon.
2. To investigate dissemination of antibiotic resistance among non-pathogenic Bacillus spp., which are a part of the microflora of food and water.

Materials and methods

Strains of Bacillus spp. were isolated from the feces of healthy individuals (7 strains), feces of the patients with dysbiosis of the colon (11 strains), also from Echinacea tincture (14 strains) and food of plant origin (5 strains). To Bacillus spp. were attributed Gram-positive bacteria with rod-shaped form, which produced catalase, capable of growth under aerobic conditions in conventional nutrient media (meat-peptone agar, yolk-salt agar) which were able to form endospores. Sensitivity to antibiotics was determined by diffusion method. Given the fact that Bacillus spp. are Gram-positive bacteria, and sensitivity to antibiotics in many respects defined by the structure of the cell wall, we used a set of antibiotics, which is recommended by the directive of the Ministry of Health of Ukraine № 167 (from 05.04.2007) to determine the sensitivity of the staphylococci.

Obtained results and discussion

1. Antibiotic resistance of the non-pathogenic Bacillus spp., which are a part of the microflora of the human GI tract.

Non-pathogenic strains of the Bacillus spp. were isolated of the specimens of feces of healthy people in amounts of $4 \cdot 10^2$ to $7{,}5 \cdot 10^4$ CFU in 1 g. From feces of the patients, who had colon dysbiosis Bacillus spp. were isolated in quantities of $6 \cdot 10^2$ to $4{,}0 \cdot 10^5$ CFU in 1 g. Average values of the logarithm totaled 3,70±0,21 for healthy and 3,91±0,25 for patients with dysbiosis. Although from patients with colon dysbiosis Bacillus spp. were allocated in more larger quantities, but the difference is minor and not statistically likely.

Data on the sensitivity of isolated strains to antibiotics are given in table 1 and in figure 1. As seen in Figure 1, the average diameter of the zones of stunted growth strains Bacillus spp., which were isolated from the feces of patients with dysbiosis are smaller than the corresponding values for the strains isolated from healthy individuals. This difference is statistically significant for penicillin, oxacillin, gentamicin, amikacin, clindamycin and azithromicyn (table 1). This strongly suggests that non-pathogenic strains of Bacillus spp., which are part of the human GI tract microflora change their properties depending on the general condition of the colon microflora. Unlikely that all patients with colon dysbiosis have got it as a result of antibiotic treatment (medical histories, unfortunately, were not collected). Antibiotic resistance of the microorganisms of the GI tract develops not as a simple reaction to the antibiotic treatment. Rather, antibiotic resistance has wider significance for the survival of certain species in microbiome and can be a quality that contributes to the survival (under dysbacteriosis condition) and vice versa — to be a kind of the burden that decreases the chances of survival of the species (under eubiotic condition).

During microbiological studies of the tincture of Echinacea purpurea to determine compliance of this drug substance to the sanitary requirements were found that non-pathogenic strains of Bacillus spp. are a key component of its flora. This can be explained by the fact, that the tincture contains ethyl alcohol in its composition, which kills all asporogenous bacteria. According to our observations, when storing the drug substance in a plastic bottle at room temperature, the non-pathogenic strains of Bacillus spp. have been preserving the viability during 2.5 years at least. Starting from 16.02.2011, of the different batches of

Table 1

Sensitivity to antibiotics of the non-pathogenic strains of Bacillus spp., isolated from feces

Antibiotics	The diameters of zones of growth retardation (mm)		Student's t - criterion	Probability of errors (P)
	eubiosis	dysbiosis		
	M ± m	M ± m		
Penicillinum	26,43 ± 4,12	15,27 ± 2,6	2,29	< **0,05**
Oxacillinum	28,86 ± 3,83	19,82 ± 1,51	2,20	< **0,05**
Vancomycin	23,86 ± 0,34	22,82 ± 0,54	1,63	> 0,05
Gentamicin	37,71 ± 1,41	32,64 ± 1,06	2,87	< **0,05**
Amikacin	30,86 ± 1,14	27,18 ± 1,20	2,22	< **0,05**
Ofloxacin	31,86 ± 1,28	30,0 ± 1,15	1,08	> 0,05
Ciprofloxacin	34,14 ± 1,78	32,09 ± 1,52	0,89	> 0,05
Levomycetin	34,00 ± 1,56	32,74 ± 1,35	0,61	> 0,05
Azithromicyn	21,29 ± 1,13	14,97 ± 2,95	2,22	< **0,05**
Clindamycin	22,57 ± 2,31	15,09 ± 2,17	2,36	< **0,05**
Tetracycline	25,57 ± 2,43	24,36 ± 1,18	0,45	> 0,05

tincture Echinacea were isolated 14 strains of Bacillus spp. and their sensitivity to antibiotics was determined.

2. Antibiotic resistance of the non-pathogenic Bacillus spp., isolated from pharmaceutical substances and food.

As shown in Figure 2, the average value of the diameters of zones of growth retardation of the strains isolated from tincture Echinacea almost indistinguishable from the corresponding values for the strains isolated from humans with eubiosis of the colon. It should be noted that spores Bacillus spp. could get into tincture Echinacea with plant material that has been used for the preparation of the drug substance. In turn, the bacteria got into the plant from the soil, which is the natural environment for them. It can be assumed that susceptibility to the antibiotics of the non-pathogenic strains Bacillus spp. isolated from

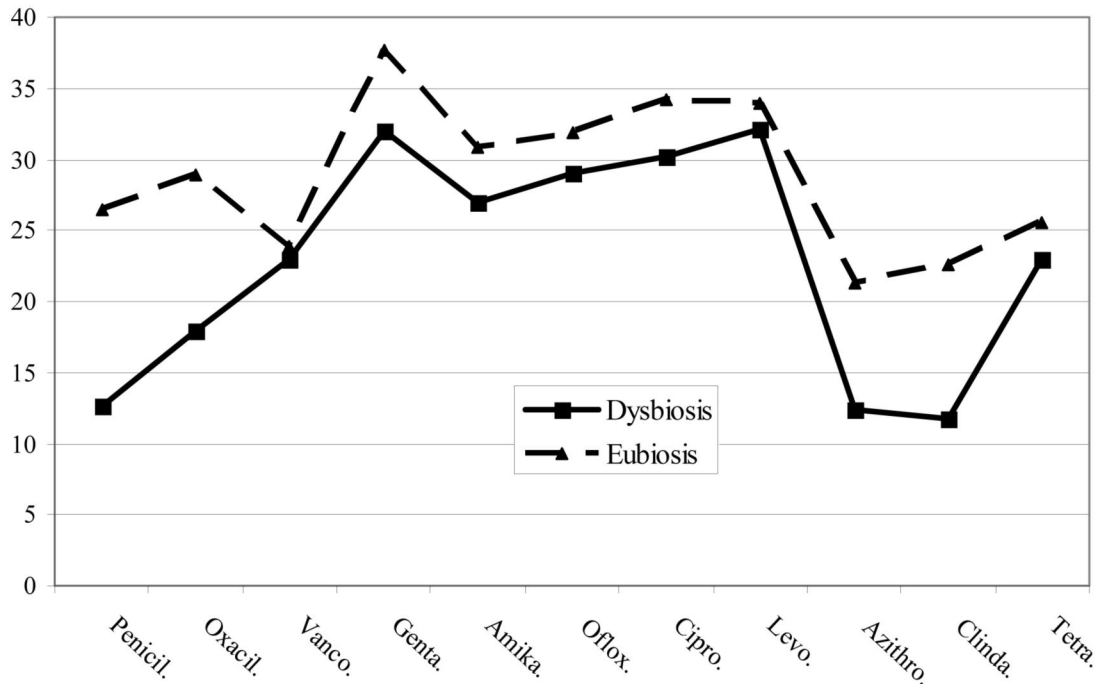

Figure 1. Diameters of the growth retardation zones of the Bacillus spp. isolated of the faeces of healthy people and pacients with colon disbiosis

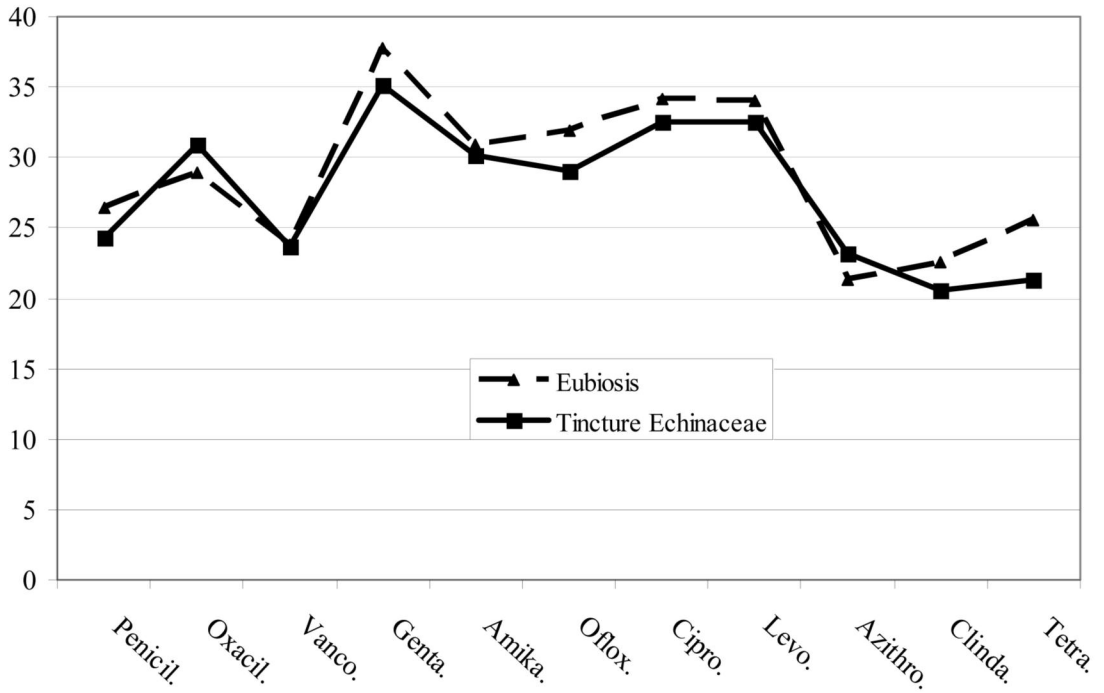

Figure 2. Diameters of the growth retardation zones of the Bacillus spp. isolated of the tincture Echinacea and food.

Echinacea tincture is just optimal for the existence of these bacteria in the environment. The fact that the antibiotic susceptibility bacteria, isolated from the feces of people with eubiotic microflora of the colon does not differ of the susceptibility of the soil strains may indicates on the equal suitability both habitats for Bacillus spp.

Non-pathogenic strains of Bacillus spp. are quite often isolated of the foods of plant origin, including the spices, flour semis, and more. In result of investigation antibiotic susceptibility of the strains isolated from products produced in the EU was revealed that it is close to the sensitivity of strains isolated of tincture Echinacea (figure 3). In the same time, strains isolated from products of Ukrainian origin had a sensitivity similar to strains isolated from feces of patients with colon dysbiosis (figure 4). It should be noted that all of the products, of which Bacillus spp. were isolated,

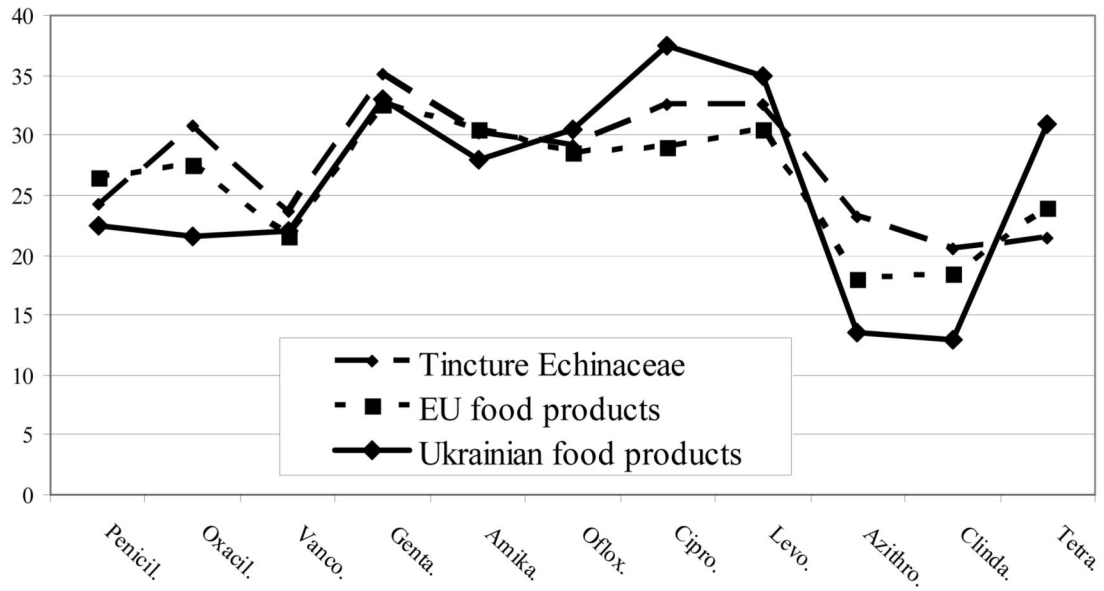

Figure 3. Diameters of the growth retardation zones of the Bacillus spp. isolated of the tincture Echinacea and food products of different origin.

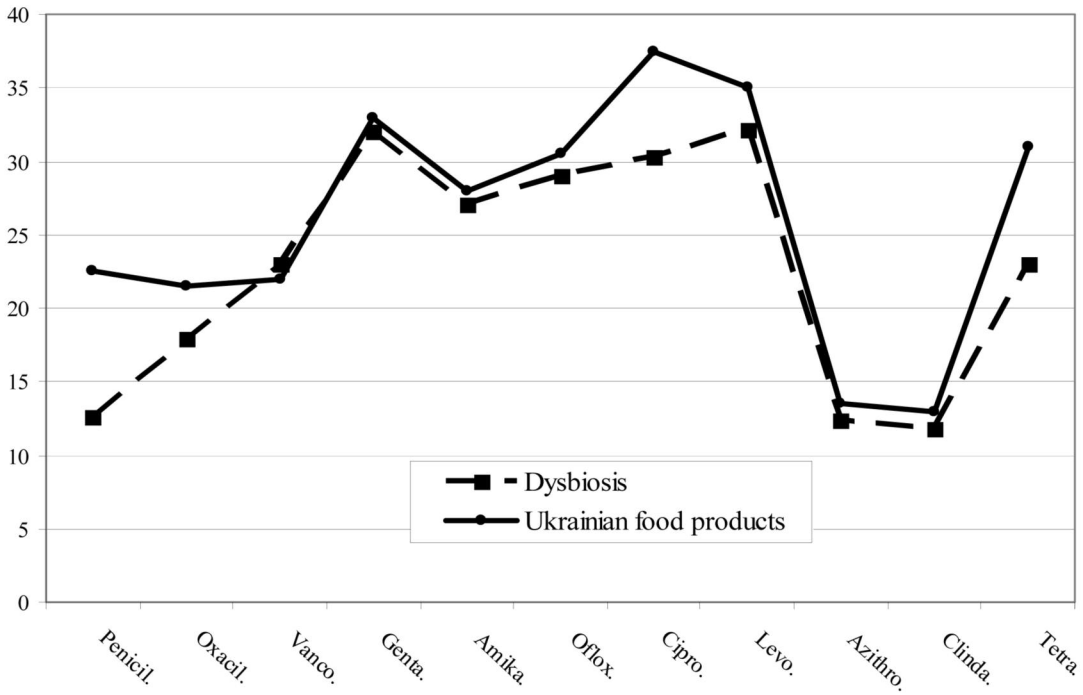

Figure 4. 4 Diameters of the growth retardation zones of the Bacillus spp. isolated of the faeces of patients with colon dysbiosis and of the Ukrainian food products.

meet sanitary requirements for microbiological parameters. This is a sufficient evidence, that the bacteria isolated of the food products of Ukrainian origin, did not originate of the GI tract of the patients with colon dysbiosis. It can be assumed that the change in antibiotic susceptibility of the Bacillus spp. isolated of the products of Ukrainian origin depends on changes in their natural microbiome — soil. Soil microflora is one of the most complex. The question of what is the norm for soil microflora, and what is abnormal for this microbiota was studied not enough. One of the biggest problems of the agriculture is the growing soil contamination by fungi — plant parasites. It is well known that fungi are capable of producing antibiotic substances. Then for survival in this microbiocenosis bacteria must possess antibiotic resistance. It is possible that antibiotic resistance of the non-pathogenic strains of Bacillus spp. may be an indicator of changes of soil microflora — namely, to point out the excessive multiplication of microscopic fungi. A difference in antibiotic susceptibility of strains isolated from foods of various origins reflects the general state of farming in the country of manufacture.

In summary, the findings lead to the conclusion that antibiotic resistance of non-pathogenic strains of the genus Bacillus not directly linked to the spread of antibiotic resistance among causative agents of the infectious diseases of humans. This is due to the fact that antibiotic resistance in these bacteria evolving in response to changes in micro-environment and possibly associated with other qualities that are necessary for survival in adverse conditions. Antibiotic resistance may serve as a marker of general condition of microbiocenosis of the human GI tract and soils.

Conclusions

1. Antibiotic resistance of the non-pathogenic strains Bacillus spp. of the human GI tract is defined by a total state of this microbiome.
2. Antibiotic resistance of non-pathogenic strains Bacillus spp., which are isolated from foods of plant origin may reflect contamination of soil by microscopic fungi — plant parasites.
3. Antibiotic resistance of non-pathogenic strains of genus Bacillus is not directly linked to the spread of antibiotic resistance among causative agents of the infectious diseases of humans.

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Антибіотикорезистентність непатогенних штамів Bacillus spp. як показник загального стану мікробіоценозів товстої кишки людини та ґрунту (англійською мовою)

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Резюме. Вивчено чутливість до антибіотиків непатогенних штамів бактерій роду Bacillus, які виділені з фекалій здорових людей, пацієнтів з дисбактеріозом товстої кишки, а також з харчових продуктів та фармакологічного препарату — настоянки ехінацеї пурпурової. Встановлено, що антибіотикорезистентність непатогенних Bacillus spp. залежить від загального стану мікробіоценозу товстої кишки і може слугувати індикатором забрудненості грунту мікроскопічними грибами.
Ключові слова: антибіотикорезистентність, непатогенні Bacillus spp., мікробіоценоз.

Антибиотикорезистентность непатогенных штаммов Bacillus spp., как показатель общего состояния микробиоценозов толстой кишки человека и ґрунта (на английском языке)

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Резюме. Изучена чувствительность к антибиотикам непатогенных штаммов рода Bacillus, выделенных из фекалий здоровых людей, пациентов с дисбактериозом толстой кишки, а также из пищевых продуктов и из фармакологического препарата — настойки эхинацеи пурпурной. Установлено, что антибиотикорезистентность непатогенных Bacillus spp. зависит от общего состояния микробиоценоза толстой кишки и может служить индикатором загрязненности грунта микроскопическими грибами.
Ключевые слова: антибиотикорезистентность, непатогенные Bacillus spp., микробиоценоз.

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