Research of antibiotic resistance of microorganisms isolated from fruits and vegetables

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Abstract. The present study is devoted to the study of the antibiotic resistance of microorganisms present on the surface of vegetables and fruits. These microorganisms can enter the consumer's body and form antibiotic resistance in the commensal microflora. In the course of the research, we isolated various types of bacilli (43.7%), aspergillus (35.1%), penicilli (37.7%), micrococci (15.4%), staphylococci (19.8%) and streptococci (12.2%). Escherichia coli bacteria were found in 11.3% of the samples. Yeast cells were present on all vegetables and fruits, but they were found in greater numbers on sweet fruits (persimmons, bananas, grapes). Molds were more often sown in washes from potatoes and cauliflower. Gram-positive and gram-negative flora accounted for 59.5% and 40.5%, respectively. All microorganisms isolated from the surface of vegetables and fruits had multi-resistance to antibiotics. Of the isolates tested, the greatest resistance was observed to claritomycin, penicillin, levofloxacin, tetracycline, and ampicillin.

1. Introduction
The consumption of fresh fruits and vegetables in various countries is increasing, which stimulates their production, which has increased by more than 30% worldwide in recent years [1]. But as a result of anthropogenic activities, vegetables and fruits are often exposed to active bacterial contamination. The survival of microorganisms is facilitated by nutrients that are released from damaged tissues. Contamination of vegetables and fruits can occur during cultivation, during harvesting, transportation and in the consumer's kitchen. The soil can also be a source of pollution, which is often filled with manure containing various microorganisms. Contamination of vegetables and fruits can be facilitated by rainfall or irrigation in agricultural fields [2, 3, 4]. Therefore, fruits and vegetables can be infected with both pathogenic and opportunistic microorganisms, and various saprophytes. E. coli, salmonella, shigella, listeria and cocci are most often found on the surface of vegetables and fruits. Moreover, different groups of bacterial communities differ significantly depending on the type of product. For example, enterobacteria are abundant in strawberries, peppers, tomatoes, lettuce, and spinach, while actinobacteria, bacteroids, and proteobacteria are predominant in foods such as grapes, peaches, apples, and mushrooms [5, 6].

On the surface of fruits and vegetables, microorganisms form a biofilm, which protects them from various environmental stressors, including antibiotics [7, 8]. The uncontrolled use of antibiotics contributes to the emergence of resistant strains of microorganisms, which can serve as a means of horizontal transmission of resistance genes to the human gut microbiome. The formation of transmissible antibiotic resistance in the microflora of fruits and vegetables is a potential threat to human health [9, 10, 11].
2. Research methods
As objects of research, we selected the most popular vegetables and fruits among consumers: potatoes, cucumbers, cauliflower, persimmons, plums, bananas, grapes and tangerines (n = 15). Washes from the surface of vegetables and fruits, quantitative registration of microorganisms (colony forming units - CFU) were carried out with sterile swabs soaked in sterile saline solution. The scope of the research included the determination of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), as well as bacteria of the Escherichia coli group (BGKP). Identification of isolated microorganisms (isolates) was carried out in the following sequence: description of cultural characteristics; obtaining a clean daily culture; Gram stain and microscopy. Determination of the sensitivity of bacteria to antibiotics was carried out by the diffusion method using discs with antibiotics (table 1).

Table 1. List of antibiotics used.

| №  | Name         | Notation | №  | Name         | Notation |
|----|--------------|----------|----|--------------|----------|
| 1  | Ofloxacin    | ОF       | 7  | Levofloxacin | ЛFC      |
| 2  | Clarithromycin| KTM      | 8  | Phosphomycin | FOS      |
| 3  | Benzylpenicillin| PEN     | 9  | Tobramycin  | TOB      |
| 4  | Ciprofloxacin | CIP      | 10 | Optokhin    | OP       |
| 5  | Novobiocin   | NB       | 11 | Ampicillin   | AMP      |
| 6  | Doxycycline  | DOC      | 12 | Tetracycline | TETR     |

Statistical processing of the research results was carried out using classical methods of mathematical statistics and a Microsoft Excel spreadsheet.

3. Results and discussion
The greatest number of microorganisms was present on the surface of potatoes, persimmons, bananas, cauliflower and grapes (from 7.8 CFU log / cm2 to 4.9 log / cm2 CFU). On the surface of mandarin, the total number of microorganisms was less, which can be explained by the increased concentration of acids. Yeast prevailed in washes from persimmons, bananas, grapes and cauliflower (from 7.3 CFU log / cm2 to 4.3 CFU log / cm2). Representatives of Enterobacteriaceae on the surface of vegetables and fruits were much less common (figure 1).

In the microflora of samples of vegetables and fruits, various types of spore-forming microorganisms predominated (43.7%), followed by aspergillus and penicilla molds (35.1% and 37.7%, respectively). Among the isolated microorganisms, the proportion of staphylococci, micrococci and streptococci was lower and amounted to 19.8%, 15.4% and 12.2%, respectively (figure 2).

Gram-positive microflora (59.5%) is represented by saprophytic bacilli, micrococci, staphylococci and streptococci, and gram-negative microflora (40.5%) is represented by coliform bacteria. The presence of coliform bacteria in fresh vegetables and fruits indicates faecal contamination, which can be hazardous to the health of the consumer. Coccal microflora is represented by common saprophytic or commensal bacteria, which could be introduced into vegetables and fruits through cross-contamination. But under certain conditions, cocci can exhibit the properties of an opportunistic microflora, especially in people with weakened immunity.

Gram-positive microflora (59.5%) is represented by bacteria from the genera Bacillus spp., Micrococcus spp., Staphylococcus spp., Streptococcus spp., And gram-negative microflora (40.5%) - and BGKP. The presence of large amounts of Bacillus spp. in fresh vegetables and fruits, as well as the presence of BCG, can be hazardous to the health of the consumer. Micrococcus spp. are common saprophytic or commensal bacteria that can be introduced into fresh vegetables and fruits through cross-contamination. Under certain conditions, micrococcus can manifest itself as an opportunistic microflora, especially of people with weakened immunity.
Figure 1. The content of various representatives of microflora on the surface of vegetables and fruits.

Figure 2. Frequency of microorganism’s occurrence isolated from the surface of vegetables and fruits.

All studied samples of bacterial isolates isolated from the surface of vegetables and fruits had multi-resistance to antibiotics. The microflora of potatoes and cucumbers had a higher resistance to penicillin (figure 3).

Many bacterial isolates have shown high sensitivity to ofloxacin and optoquinone. Of the tested drugs, microorganisms showed the greatest resistance to claritomycin, penicillin, levofloxacin,
tetracycline and ampicillin. Most often, microorganisms resistant to the tested antibiotics were present on potatoes, cucumbers and cauliflower (figure 4).

Figure 4. The frequency of various representatives antibiotic resistance of vegetables and fruits.

4. Conclusions
The largest number of microorganisms was present on the surface of potatoes, persimmons, bananas and grapes.
The microflora of vegetables and fruits was dominated by spore microorganisms, mold fungi, micrococci, staphylococci and streptococci.

The presence of enterobacteriaceae on vegetables and fruits indicates their fecal contamination and potential danger to the consumer.

All studied samples of vegetables and fruits contained relatively equally microorganisms on their surface with multi-resistance to antibiotics.

Many isolates have shown increased sensitivity to ofloxacin and to optoquinone. Of the drugs tested, claritomycin, penicillin, levofloxacin, tetracycline, and ampicillin most often did not have an antimicrobial effect.

The results of this study provide new information for studying the problem of bacterial antibiotic resistance.

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