

POTENTIAL RISKS OF THE SPREAD OF ANTIBIOTIC-RESISTANT MICROORGANISMS AND ANTIBIOTIC-RESISTANCE GENES IN POTABLE WATER – HUMAN ORGANISM CHAIN

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ABSTRACT

The aim: Determination of circulation interrelations between antibiotic-resistant microorganisms of *Enterobacteriaceae* family and their resistance genes in clinical strains and potable water samples taken in Uzhhorod and Uzhhorod district.

Materials and methods: We carried out generic identification of the microorganisms isolated from clinical samples of the oral cavity of 64 patients suffering from periodontal inflammatory diseases, and potable water samples taken from sources of public centralized and decentralized water supply; the isolated microorganisms were tested for antibiotic sensitivity by the Kirby-Bauer disc diffusion method according to EUCAST. With the help of molecular-genetic methods, the total DNA of potable water was isolated and tested for the presence of the following genetic resistance determinants: carbapenems *bla*NDM; *bla*OXA48-like; tetracyclines *bla*Tet-M; cephalosporins *bla*CTX-M.

Results: In the microbiota of the clinical material and potable water samples, the same spectrum of microorganisms belonging to *Enterobacteriaceae* family dominated; the isolated bacteria showed a high resistance level to beta-lactam antibiotics and to natural antibiotic preparations. The highest level of resistance was established for microorganisms isolated from well water samples, where genetic resistance determinants to *bla*CTX-M cephalosporins and *bla*Tet tetracyclins were also revealed.

Conclusions: The obtained results proved high probability of the spread of antibiotic-resistant microorganisms and their genetic resistance determinants via potable water.

KEY WORDS: antibiotic resistance genes, oral cavity, potable water, water sources

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INTRODUCTION

The wide spread of the use of antibiotics for various branches of medicine and economy and lack of any legally valid perfect system of regulation of reasonable use of antibiotic preparations have led to a growing level of antibiotic resistance [1].

During the COVID-19 pandemic, the demand for antimicrobial preparations soared up, which fact only worsened the situation with confronting the development of resistance to antibiotics [2-4]. The lack of efficient antimicrobial preparations throws the progress of medicine back for decades, increases the risk of growing of the number of incurable infectious diseases, and makes it impossible to perform many hi-tech surgeries [5, 6].

The spread of resistant strains in environmental objects (surface and soil waters, bottom deposits, wastewaters, soils) where a large-scale exchange with resistance determinants takes place between clinical strains and natural bacteria, attracts increasingly greater attention of researchers [7]. The spread of poly- and multiresistant bacteria, pharmaceuticals in environmental objects may serve as a potential source of entry of genetic resistance determinants to human organisms via food chains [8,9]. The existing out-dated water treatment facilities and imperfect potable water treatment techniques fail to interfere

with the spread of genetic resistance determinants on their way to the customers, which causes heightened academic interest to study the possible spread of resistance genes via potable water [10].

THE AIM

The purpose of the given scientific paper is to determine the circulation interrelations of antibiotic-resistant microorganisms of *Enterobacteriaceae* genes and their resistance genes in clinical strains and potable water samples taken from sources of public centralized and decentralized water supply within the city of Uzhhorod and areas adjacent thereto.

MATERIALS AND METHODS

Samples for microbiological study of tap-water were taken from 2 monitoring sites in Uzhhorod located in different parts of the city (48.632576, 22.283702; 48.622222, 22.250806), and from 3 sources of decentralized water supply (water wells) located in the village of Velyky Berezny (48.885283, 22.452778), on the outskirts of the town of Perechyn (48.729388, 22.448340), and in the village of Storozhnytsia (48.600345, 22.238672). Clinical isolates

were isolated from the oral cavities of patients suffering from periodontal inflammatory diseases (n=213). The microorganisms of *Enterobacteriaceae* family were identified with the study of morphological characteristics on Hottinger's broth, and their further differentiation was carried out on selective media (Ploskirev's, Endo's, bismuth sulphite agar). The generic and specific attribution of the microorganisms was performed with the help of biochemical tests (Enterotest 24 and Enterotest 16) made by Erba Lachema, the Czech Republic. The antibiotic sensitivity of the isolates was determined by the Kirby-Bauer disc diffusion method according to EUCAST (European Committee on Antimicrobial Susceptibility Testing). The isolates were tested for antibiotic sensitivity with the use of the following discs with antibiotics: ceftriaxon (30 mg), ampicillin (10 mg), ceftriaxon (30 µg), meropenem (10 µg), cefuroxim (30 µg), imipenem (10 mg), amycacin (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), levofloxacin (5 µg), norfloxacin (10 µg), gatifloxacin (5 µg), ofloxacin (2 µg), lomefloxacin (30 µg), tetracyclin (30 µg) and doxycycline (10 µg) made by Farmaktyv and HiMedia. As control test culture, *Escherichia coli* ATCC 25922 strain was used to check the quality of the media, discs with antibiotics, and the testing precision.

IDENTIFICATION OF GENETIC RESISTANCE MARKERS FROM METAGENOMIC DNA OF WATER SAMPLES

The water samples (500 ml – 1000 ml) were filtered through a sterilized membrane filter (Millipore, USA) with pore size 0.22 µm. Total DNA was extracted from the membrane filter with the help of modified method from hexadecyltrimethylammonium bromide (CTAB) according to Andronov's method [11]. The isolated metagenomic water DNA was studied with the help of polymerase chain reaction for the presence of resistance genes, using Litex reagent set (Russia) to identify the following genetic determinants: carbapenems – *bla*NDM; *bla*OXA48-like; tetracyclines – *bla*Tet-M; cephalosporines – *bla*CTX-M. The testing was carried out with the use of the DTPrime Amplifier (DNA Technology, Russia), according to the Manufacturer's Manual. The molecular genetic studies were performed on the basis of the private laboratory of Madicover Medical Centre, Lviv. The microbiological studies were carried out on the basis of the Microbiological Laboratory, Department of Genetics, Microbiology and Plant Physiology, Biological Faculty, Uzhhorod National University.

RESULTS

The spread of antibiotics via the food chain is an increasing problem of today. Globalization in the use of antibiotic substances in products of animal origin, migration of antibiotics and leftovers of pharmacological preparations in natural waters lead to the increase of the share of antimicrobial preparations in the environment.

The studies of microbiota of different biotopes of the

oral cavity of Uzhhorod residents showed that *Enterobacteriaceae* family bacteria characterized by resistance to a wide spectrum of antibiotic preparations were isolated in 30% cases. The specific spectrum of the microorganisms isolated from the oral cavity of 64 patients (the total number of the studied samples of biomaterial n=213) suffering from inflammatory diseases of periodontium tissues is presented in Figure 1.

Based on the results of the microbiological water analysis, domination of *Enterobacteriaceae* family bacteria was established for microbial associations of water samples taken from centralized and decentralized water facilities. Microorganisms of *Escherichia spp*, *Citrobacter spp*, and *Enterobacter spp* genera were observed in three out of the 12 water samples taken from centralized water supply systems; bacteria of *Citrobacter spp*, *Escherichia spp*, *Klebsiella spp*, and *Enterobacter spp* genera were found in five out of the 10 water samples taken from wells (Fig.2).

The spectrum of opportunistic microorganisms isolated from potable water samples and human organism indicates to a possibility of existence of common sources of bacterial entry and their migration, which constitutes a menace to the spread of genetic resistance determinants between humans and the environment.

The *Enterobacteriaceae* family microorganisms isolated from the oral cavity manifested a high level of resistance to antibiotic preparations. According to the analysis of the obtained antibiograms, the highest resistance level was established for macrolides, tetracyclines and unprotected beta-lactams (Fig.3).

The highest susceptibility indices were established for III-IV generation cephalosporins and II-III generation phthorcinolons.

The microorganisms isolated from central water supply sources showed the highest resistance to natural antibiotics and, compared with the bacteria isolated from well water, were susceptible to most of the tested antibiotics (Fig.4).

The enteric bacteria isolated from decentralized water supply sources were characterized by a high level of resistance to tetracyclines, aminoglycosides, unprotected beta-lactams and cephalosporins (Fig.5).

Following the research results, a similar generic spectrum of Gram-negative microorganisms was isolated from potable water samples and from the oral cavity, of which the most widely spread were bacteria of *Escherichia spp*, *Klebsiella spp*, *Citrobacter spp* and *Enterobacter spp* genera characterized by a similar antibiotic resistome, which indicated to a possible migration of microorganisms between the human organism and the environment.

Following the results of the molecular genetic studies, metagenomic DNA was isolated from potable water samples and tested for presence of the following antibiotic resistance genes: *bla*NDM; *bla*OXA48-like; *bla*Tet-M; and *bla*CTX-M.

It was established that no resistance genes were revealed in the well water samples from Velyky Berezhny; this site is located in the high-mountain area which is referred to environmentally congenial regions. From total DNA of the well water

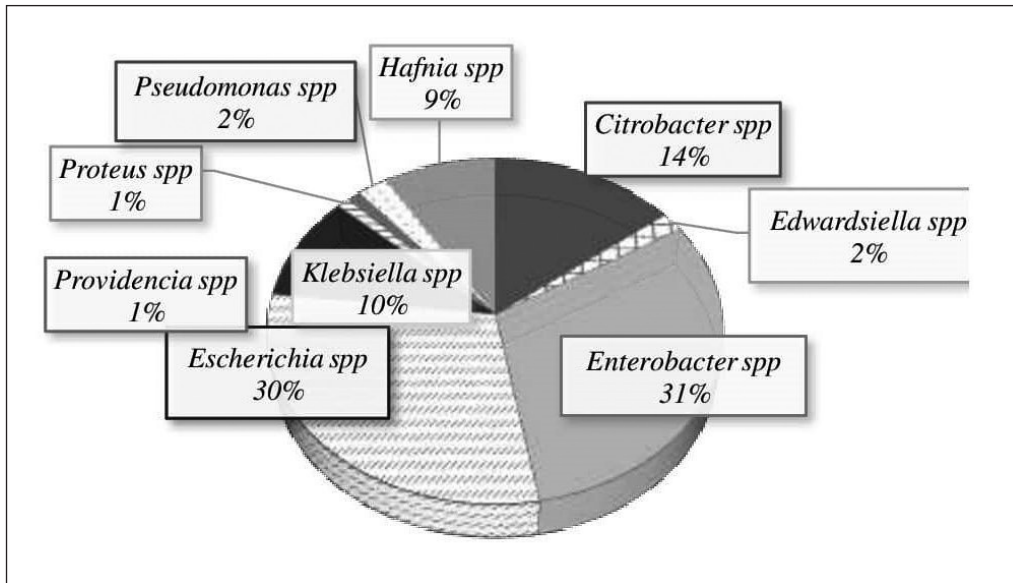


Fig.1. Specific spectrum of the microorganisms isolated from the oral cavity

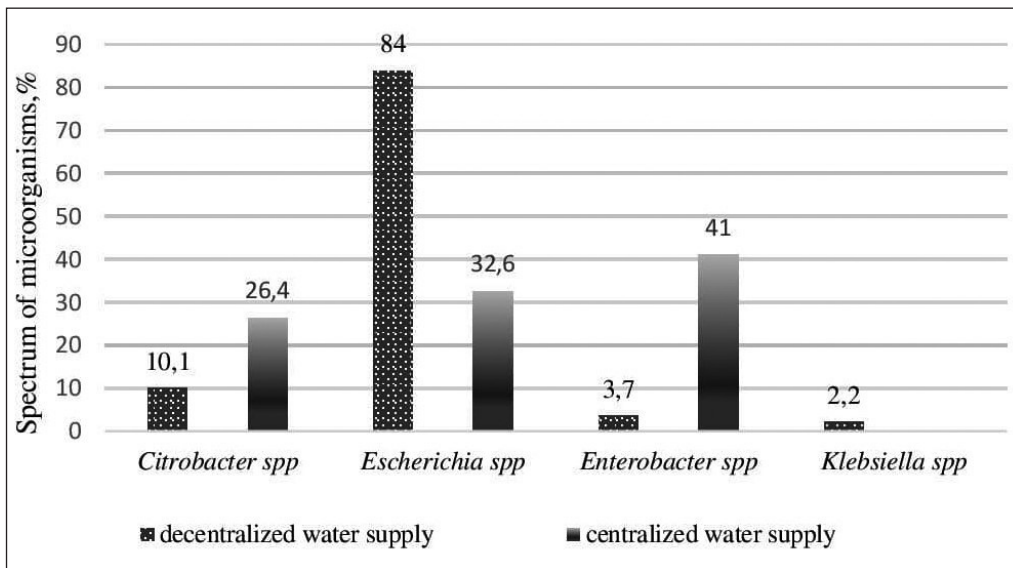


Fig.2. Microorganisms isolated from water samples taken from centralized and decentralized water supply, the city of Uzhhorod.

located in Perechyn, *blaTet* genes were isolated. The presence of genetic resistance determinants in the well water may have been caused by a number of different reasons, including undue sanitary state of the wells, pollution of the adjacent areas, ignoring the need of regular disinfection according to sanitary requirements, incorrect disinfection, etc. It is known that water disinfection with the use of chlorine may work as a trigger, enhancing the development of resistance to medicines [12].

From the well water samples taken in Storozhnytsia, *blaCTX-M*-type genes were isolated; this site is located in a more densely populated area than the previous ones, and it is located in breach of sanitary requirements – first of all, not above the subsurface water flow which causes regular pollution of the well.

In the water samples taken from centralized water supply sources, no genetic resistance determinants were revealed.

The obtained results have proved a significant spread of genetic resistance determinants in potable water used for household needs.

DISCUSSION

The increasing spread of antibiotic resistant microorganisms is causing a global menace to public health. The excessive use of antimicrobial preparations in various fields has caused a significant growth in the spread of antibiotics in different environments. The obtained data of microbiological studies indicated to the domination of the same spectrum of microbiota belonging to *Enterobacteriaceae* family in potable water and clinical samples. The isolated representatives of Gram-negative microorganisms (*Citrobacter spp*, *Escherichia spp*, *Klebsiella spp* and *Enterobacter spp*) were opportunistic bacteria, and as such they could be agents of infectious diseases; their availability in potable water sources indicates to the imperfection of the techniques used to treat potable water. The out-dated and worn water pipelines, and violation of the rules of arrangement and maintenance of water wells and individual water boreholes increase the risk of bacteria's entering potable water. Suchlike circulation of opportunistic microorganisms is

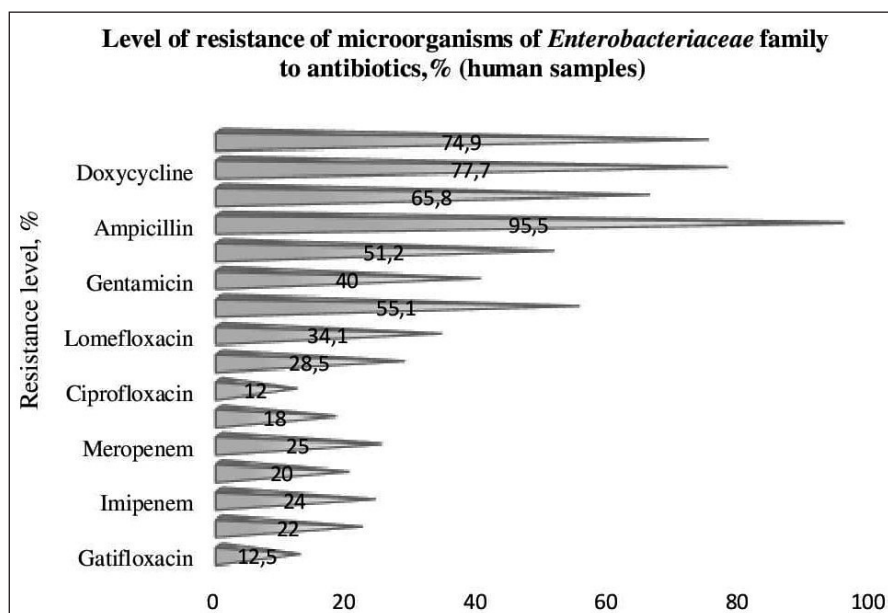


Fig. 3. Level of resistance of microorganisms isolated from human oral cavities

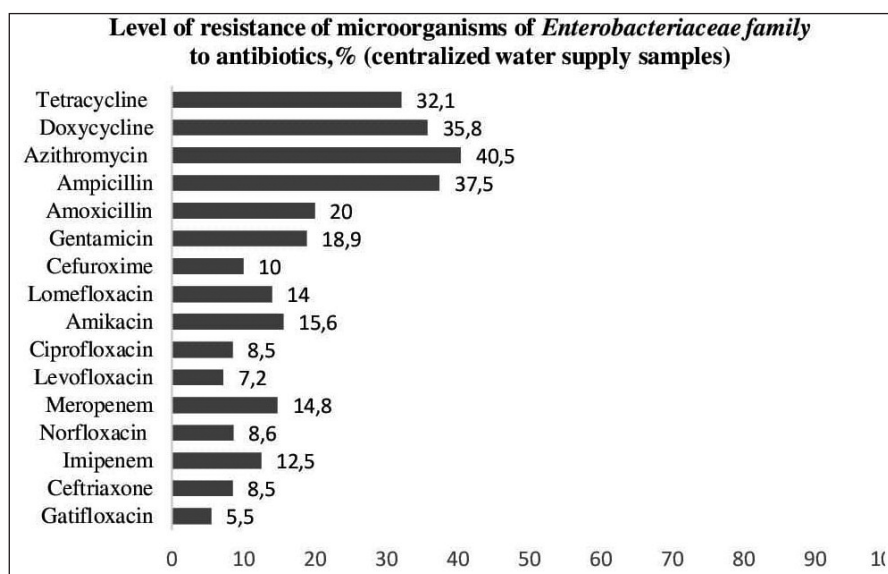


Fig. 4. Level of resistance of microorganisms isolated from centralized water supply samples.

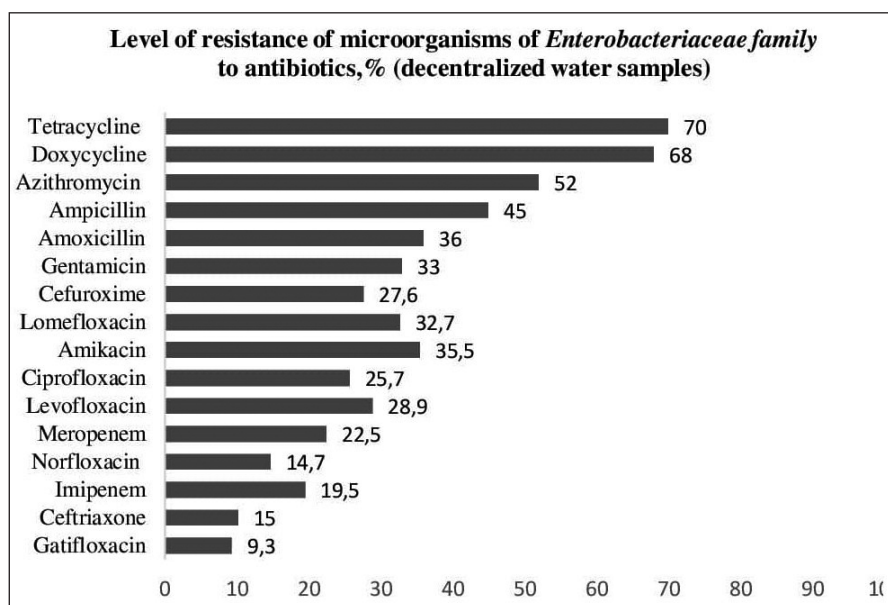


Fig. 5. Level of resistance of microorganisms isolated from decentralized water supply samples.

a source for the spread of genetic antibiotic resistance determinants.

According to the research results, the microorganisms isolated from clinical samples and water showed significant resistance to natural antibiotics, such as tetracycline, ampicillin and gentamycin, as well as to beta-lactam antibiotics. The highest susceptibility to antibiotics was registered among the enteric bacteria isolated from well water characterized by a high level of resistance to phthorcinolons (levofloxacin, ciprofloxacin, lomefloxacin), beta-lactam antibiotics, and to natural antimicrobial preparations. The growth of the share of microorganisms resistant to new-generation antimicrobial preparations in decentralized water supply sources may be caused by the violation of disinfection processes, in particular incorrect calculation of application of disinfectants. An excessive amount of disinfectants, among which bleach powder is the most frequently used agent, may create favourable conditions for the exchange of resistance genes between bacteria by means of natural transformation [13, 14].

The obtained results of molecular genetic studies proved these assumptions, because it was in the water samples taken from the wells that genetic determinants of *bla*CTX-M and *bla*Tet resistance whose spread constitutes serious risks for human health were revealed.

Our previous studies indicated to the circulation of antibiotic resistant isolates with the water from natural ecosystems, in the doctor – patient chain, and via patients' and doctors' personal hygiene items [15,16]. The revealed regularities are of importance for the development of a perfect antibiotic resistance prevention system, and rational application of antibiotics and antiseptics [17] with due regard to their susceptibility, and search for alternative antimicrobial means [18].

The circulation of genetic resistance determinants in the food chain, in particular in water, opens the door for the humankind to return back to the pre-antibiotic era [19-21].

CONCLUSIONS

- 1) A spectrum of microorganisms similar to *Enterobacteriaceae* family was established for the contents of microbiota of potable water and clinical material samples, viz.: *Citrobacter spp*, *Escherichia spp*, *Klebsiella spp* and *Enterobacter spp*.
- 2) The reviewed resistomes of representatives of *Enterobacteriaceae* family revealed a high level of antibiotic resistance in decentralized water supply sources and in clinical samples.
- 3) According to the results of molecular genetic studies, no genetic resistance determinants were established for water samples taken from centralized water supply sources, but cephalosporin – *bla*CTX-M and tetracycline – *bla*Tet resistant genes were revealed in well water samples.
- 4) The obtained results proved the spread of antibiotic resistant microorganisms and their genetic determinants via potable water, and justified the expediency of strengthening

of control over the spread of genetic resistance determinants in potable water supply sources.

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Conflict of interest:

The Authors declare no conflict of interest.

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