ANTIBIOTICS IMPLICATIONS ON THE OUTBREAK NOSOCOMIAL INFECTIONS

Alina Banciu[1], Liliana Daniela Niculescu[1], Mihai Nita-Lazar[1], Irina Lucaciu[1]

[1] *National Research and Development Institute for Industrial Ecology - ECOIND, 71-73 Drumul Podu Dambovitei Street, 060652 Bucharest, district 6, Romania*

Abstract
Nosocomial infections are those acquired in or associated with hospitals. They are also known as hospital-acquired or healthcare-associated infectious.
The patients are exposed to a variety of microorganisms during hospitalization. Contact between the patient and microorganism does not by itself necessarily result in the development of clinical disease; other factors influence the nature and frequency of nosocomial infectious: patient susceptibility, environmental conditions, bacterial resistance.
Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements antibiotics promote the emergence of multidrug-resistant bacteria. These kinds of strains are spread in the hospital environment, from surfaces and water dispensers to the wastewater from treatment plant. The bacteriological characteristics of water used in health care institutions must meet local regulations, a special case being purified water. Bacteriological tests may not always give true estimates of contaminations because of the presence of disinfectants or antibiotic resistant microorganisms.
The aim of this paper was to control environmental risks for infection and to highlight the role of antibiotic resistant bacteria from the environmental conditions of hospital in the outbreak nosocomial infections.
In early 2016, it was started monitoring water quality in an emergency hospital, following an agreement of collaboration. There were detected many bacterial strains potential implicated in nosocomial infections: *Escherichia coli, Klebsiella oxytoca, Citrobacter freundii, Proteus mirabilis*. Most of them were coliform bacteria with resistance to broad-spectrum antibiotics, especially β-lactams.
Even if the results have considerably increased monitoring measures for disinfection and sterilization used in the prevention program in the hospital, aims to complement the surveillance program tests Sanitation.

Keywords: *nosocomial infections, healthcare conditions*

Introduction
A nosocomial infection also called hospital acquired infection can be defined as an infection acquired in hospital by a patient who was admitted for another reason than the hospital acquired infection (Duccl G. et al., 2002; WHO, 2002).
Despite progress in the public health and hospital care, infections continue to develop in hospitalized patients and subsequently affecting the hospital staff. Nosocomial infections occur worldwide and affect both developed and/or poor countries.
Organisms causing nosocomial infections could be transmitted to the entire human community through discharged patients, staff and visitors. If organisms are multi-resistant, they may cause significantly disease outburst in the community.
Factors influencing the development of nosocomial infections could be the microbial agent, the patient susceptibility, environmental factors, and bacterial resistance. Health

care settings are an environment where congregate both infected persons and persons at increased risk of infection. Patients with infections or pathogenic microorganisms carriers admitted to the hospital are potential sources of infections for other patients and staff. Microbiota may contaminate objects, devices and materials which subsequently enter in contact with patients. Prevention of nosocomial infections is the responsibility of all individuals and services providing health care. One of the most important aspects of the infections control program is the monitoring of sterilization and/ or disinfection efficiency.
In addition, new infection associated with waterborne bacteria continues to be identified.
The physical, chemical and bacteriological characteristics of water used in health care institutions must meet the local regulations. Drinking water should be safe for oral ingestion (Law nr. 458/2002). Unless adequate treatment is provided, fecal contamination may be sufficient to cause infection through food preparation, washing and even through steam or aerosol inhalation. Even water that conforms to accepted criteria my carry potentially pathogenic microorganisms. Organisms present in water have frequently been implicated in nosocomial infections (WHO, 1993).
The hospital waste waters era a potential reservoir of pathogenic microorganisms and requires appropriate handling and than, the monitoring of waste water should be important (Pruss A et. Al., 1999). Nosocomial infections are often caused by antibiotic resistant microorganisms and this is the reason for specific control measures.
The aim of the project was to control the environmental contamination and to highlight the role of antibiotic resistant bacteria from the natural or hospital environmental conditions in the outbreak nosocomial infections.
In the early 2016, following an agreement of collaboration, we started to monitor the water quality in a emergency hospital,.

Materials and methods
The current problems of hospital infection pathology required that all medical units should comply with a management program of the nosocomial infections.
The management program provides monitoring of the hygiene level according to the laws, reglemenations.
In the firs half of 2016, we quarterly conducted three sampling campaigns to monitor the microbiological quality of drinking water from drillings, municipal water supply, dispensers used in pediatric wards from Bucharest, Romania as well as the microbiological quality of its waste water.
The water samples were assessed for microbiological contamination level with heterotrophic bacteria (SR EN ISO 6222), fecal bacteria as well as their antibiotic resistance. The quantitative analysis was based on most probable number technique with Colilert-18 (Idexx Mediclim, Romania) for coliform bacteria and membrane filtration method on Slanetz and Bartley Agar (Merck Romania) for Enterococci (SR EN ISO 9308-2; SR EN ISO 7899-2).
The bacterial species were identified with an Omnilog equipment (Biolog, USA) and their resistance was specifically tested with the most commonly used antibiotics in hospital: some beta-lactams (Ceftazidime, Amoxicillin-Clavulanate, Ceftriaxone, Cefepime, Ampicillin, Imipenem) and other antimicrobial drugs such as Kanamycin, Chloramphenicol, Nalidixic Acid and Tetracycline. The antibiograms were performed on Muller-Hinton Agar (BioLab, Romania) by disk-diffusion method and they were interpreted with Scan 500 (Interscience, France) according to CLSI recommendations (CLSI, 2015).

Results and discussions

The first sampling campaign was performed in February 2016 and the results were presented in the figure 1. It could be seen that the values of bacteriological indicators showed not significant overruns. The psychrophilic and mesophilic bacteria values that exceed the Drinking water Law limits caould be especially caused by the biofilms from inside plumbing.

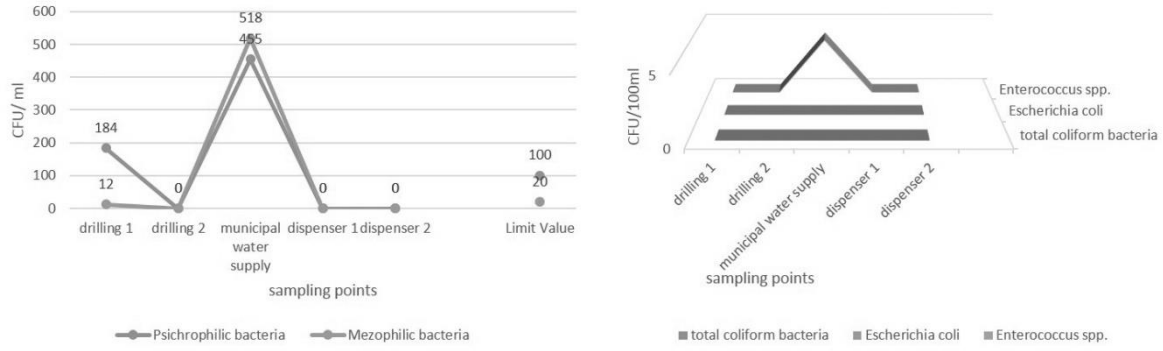

Fig.1. Density of microbiological indicators of drinking water from a health care unit (Bucharest, Romania) in February 2016.

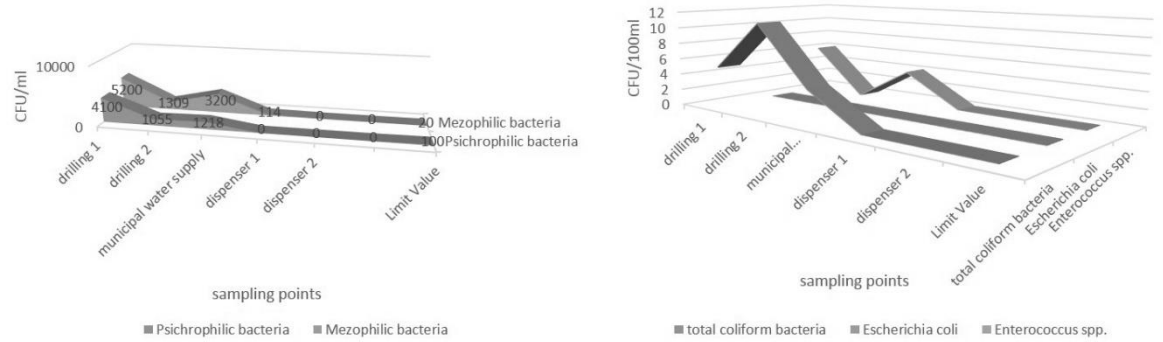

Fig.2. Density of microbiological indicators in water samples intended for human consumption from a health care unit (Bucharest, Romania) in May 2016. (for corrections see above Fig 1)

In the second campaign from May 2016, the results showed significantly higher values compared to previous results (February 2016). It is possible that the hot temperature during spring had favored the development and breeding microbiota in wells. In the figure 2, it could be observed the coliform bacteria presence in the drilling water. These bacteria were identified and tested for antibiotics susceptibility. Therefore, in the municipal water supply the detected *Serratia liquefaciens* (fig. 3.) were resistant to beta-lactams and kanamycin, but*Serratia marcescens* (fig. 4.) were only resistant to beta-lactams.

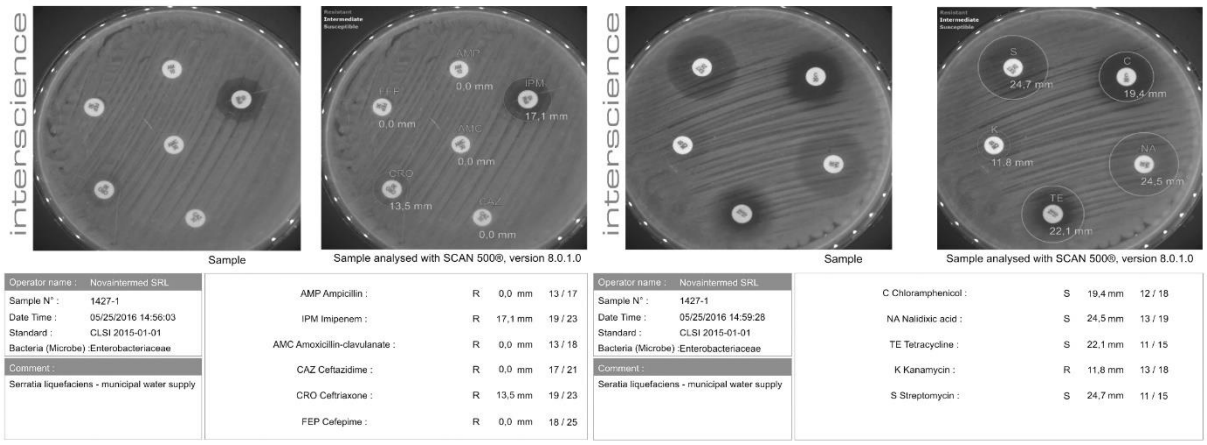

Fig. 3. Antibiotic susceptibility testing for *Serratia liquefaciens* isolated from municipal water supply – health care unit from Bucharest, Romania

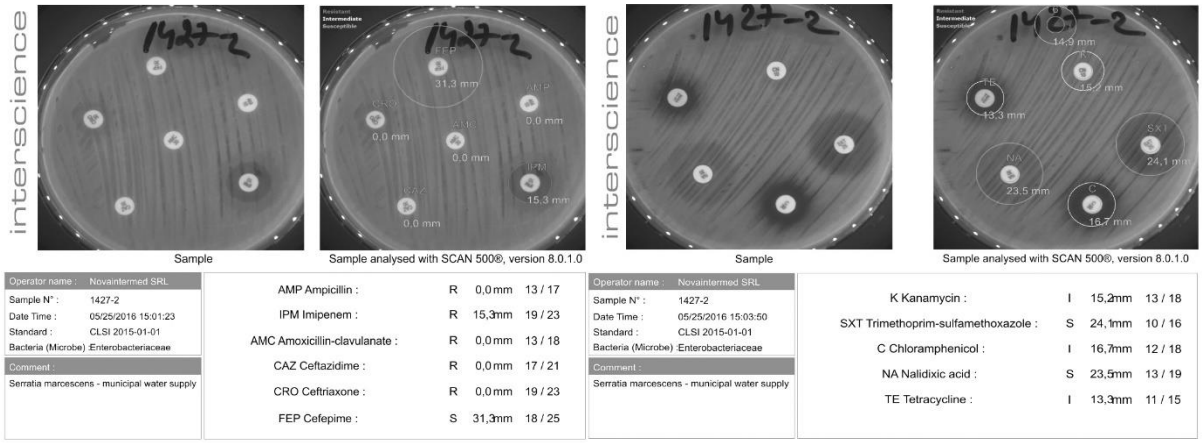

Fig. 4. Antibiotic susceptibility testing for *Serratia marcescens* isolated from municipal water supply – health care unit from Bucharest, Romania.

We hypothesize that in the water quality from the city network met the microbiological standards, and the antibiotic resistant bacteria originated entirely from the hospital environment.

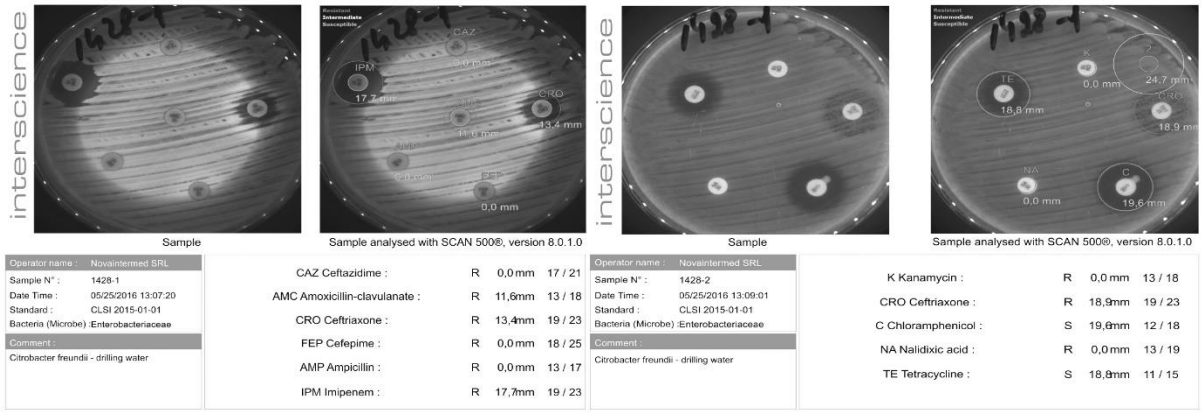

Fig. 5. Antibiotic susceptibility testing for *Citrobacter freundii* isolated from drilling water – health care unit from Bucharest, Romania.

Citrobacter freundii (fig. 5.) resistant to beta-lactams and kanamycin, Ceftriaxone and Nalidixic Acid and *Klebsiella oxytoca* (fig. 6.) only resistant to some beta-lactams were identified in the drillings water.

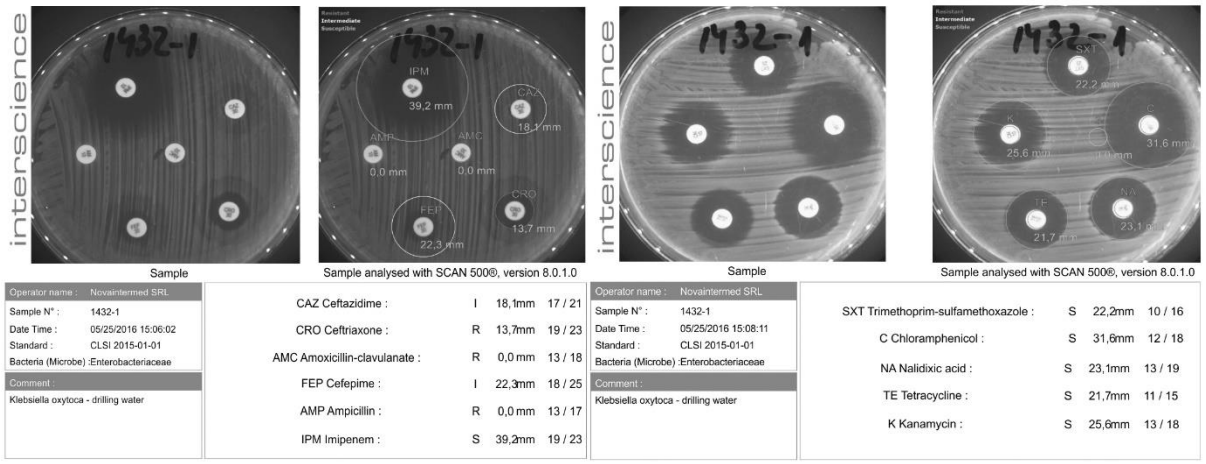

Fig. 6. Antibiotic susceptibility testing for *Klebsiella oxytoca* isolated from drilling water- health care. The enterococci density maintained its values during both analysis sessions. Their presence could indicate a contamination to the consumer and no contamination of the water supply.

Some measures for disinfection have been taken for the contaminated points based on the previous results (February and May 2016 campaign). Other microbiological tests were carried out in June 2016 and it could be seen (fig. 7.) that in decontaminated points, the values of bacteriological indicators didn't exceed the permissible limits, but bacterial density from the water dispensers significantly increased, where has not been acted disinfectants

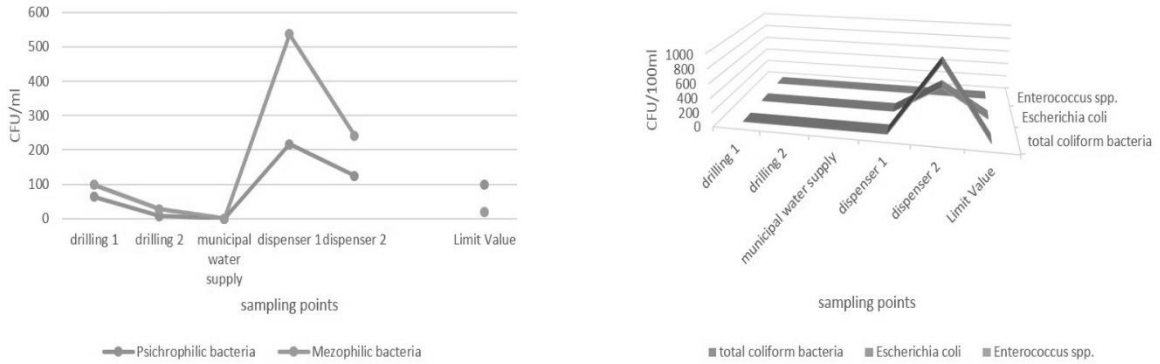

Fig. 7. Density of microbiological indicators in water samples intended for human consumption from a health care unit (Bucharest, Romania) in June 2016.

In these samples, we identified some bacterial strains such as *Serratia liquefaciens* and *Klebsiella oxytoca . These strains were also* presentin the previous analysis. *Pseudomonas aeruginosa* and *Escherichia coli* were other resistant bacteria frequently identified in water samples. The waste water analysis indicated a high density of

fermentative bacteria such as *Raoutella planticola (fig. 8.), Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Klebsiella oxytoca* and *Serratia liquefaciens*.

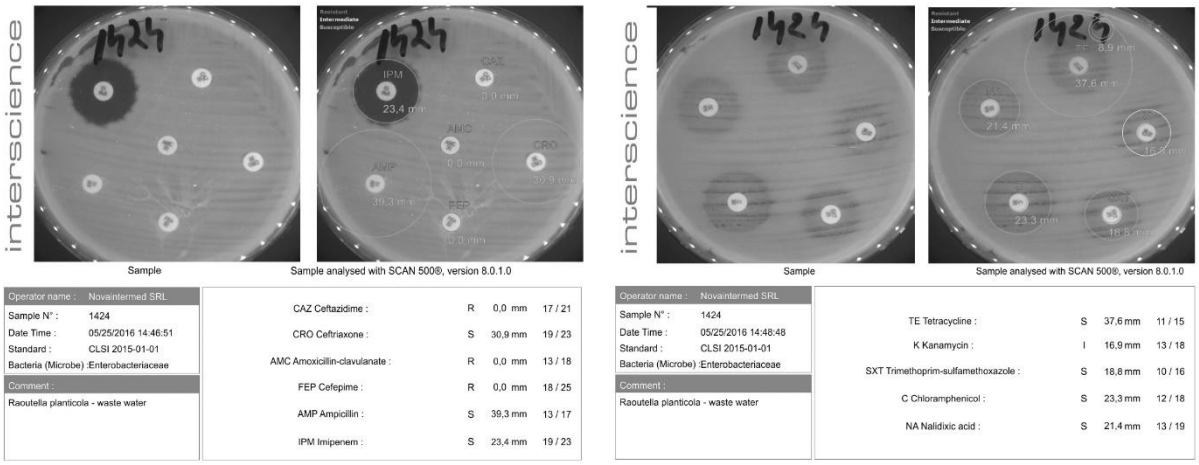

Fig. 8. Antibiotic susceptibility of the *Raoutella planticola* , isolated from the waste water of a health care unit from Bucharest, Romania.

All these microorganisms presented resistance of beta-lactams, a commonly used drugs in the hospital.

Conclusions

Any common infection could also occur in hospital, but there are many factors in the hospital environment that led to a particular spectrum of infective issues. One of them are the nosocomial infections which had been common in the last ten years.
Water quality had been an important role in the disinfection program because it is an environmental factor for breeding and transmission of bacteria. Many nosocomial infections were triggered by the antibiotic resistant microorganisms and this study demonstrated the presence of beta-lactams resistant bacteria in water sources of an emergency hospital.
The microbiological experiments demonstrated the importance and effectiveness of the disinfection process of water sources and hospital equipment.
The results indicated a correlation between antibiotics commonly used in the hospital and the presence of the antibiotic resistant microorganisms.
The use of antibiotics in the community and hospitals had added more dimensions to the crisis. Mechanisms such as antibiotics control programs, better hygiene and using of agents with improved antimicrobial activity need to be adopted in order to limit bacterial resistance. Preventing the spread diseases in the health care units required a hygiene management based on using proper antibacterial reagents at a at key moments in time. Monitor the sterilization conditions and water quality in the hospital settings is a compulsory measure for prevention of nosocomial infections.

References (alphabetical order)

Clinical and Laboratory Standards Institute, 2015, Performance Standards for antimicrobial susceptibility testing, Twenty-Fifth Informational Supplement.
Duccl G. et al., 2002, Guide pratique pour la lute contre l'infection hospitaliere, WHO/Bac/79.1.

Pruss A., Giroult B. Rushbrook P., Safe management and wasters from health care activities, 1999, WHO Geneva.
SR EN ISO 6222, Water quality, Enumeration of culturable microorganisms – Colony count by inoculation in a nutrient agar culture medium.
SR EN ISO 7899-2, Water quality, Detection and enumeration of intestinal enterococci, Part 2, Membrane filtration method.
SR EN ISO 9308-2, Water quality, Enumeration of Escherichia coli and coliform bacteria, most probable number method.
WHO, Guidelines for drinking water quality, 1993, vol.1, Recommendations 2 nd edition.
WHO/CDS/CSR/EPH, 2002, Prevention of hospital acquired infections, A practical guide, 2nd edition.