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SCREENING OF RESISTANT BACTERIA FROM HOSPITAL EFFLUENTS WITH AN IMPACT ON THE NATURAL AQUATIC ECOSYSTEM

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Introduction

The hospitals wastewaters are a special kind of effluent, being a carrier for antibiotic resistant bacteria. In Romania, most of the hospitals does not pre-treat the effluent, being taken over directly by the municipal wastewater treatment plant (WWTP). Here, the bacteria can transform the WWTP in "hot spot", were especially the pathogens can acquire resistance genes and become "superbugs". Hospitals in Romania are known for the high incidence of nosocomial infections, *Escherichia coli* being one of the most isolated strain from the Gram-negative family.

Materials and methods

Three effluents from hospitals in Bucharest were collected between January-April 2021. The wastewater samples were mixed with an equal volume of peptone water and incubated 24 hours at 37°C. With this mixture, plates with CASO Agar and Eosin Methylene Blue Agar were inoculated and incubated for 24h at 37°C. Isolated colonies were identified with the Omnilog automatic system (Biolog, USA), using IF-A medium and GEN III plates. The Kirby-Bauer method was used to identify the antibiotic resistance, using Mueller-Hinton medium. Diameters were recorded using Scan 500 (Interscience, France) and the result were interpreted using CLSI 2019. For the test, antibiotics used were: ampicillin (AMP), amoxicillin-clavulanate (AMC), gentamicin (GEN), ceftazidime (CAZ), chloramphenicol (C), trimethoprim-sulfamethoxazole (SXT), tetracycline (TE), ceftriaxone (CRO), imipenem (IPM), nalidixic acid (NA), ciprofloxacin (CIP).

Results and conclusions

During the analysis of the samples, more than 40 strains were identified using the Omnilog system, mostly Gram-negative bacteria that were tested for antibiotic susceptibility. The most common bacteria are showed in Figure 1, *Escherichia coli* being identified for 11 times.

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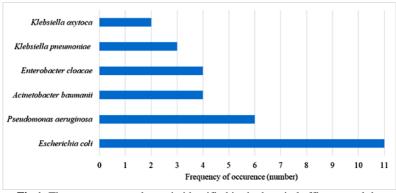
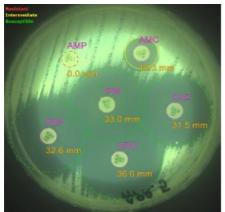


Fig.1. The most common bacteria identified in the hospital effluents and the frequency of occurrence (number)

The Kirby-Bauer test showed that 12 strains were multidrug-resistant to antibiotics, meaning that they were resistant to at least 3 antibiotics used in the clinical settings. Also, 14 strains were susceptible to all of the antibiotics, the bacteria of genus *Pseudomonas* sp. being the ones that showed the highest susceptibility.

Seven out of eleven *E. coli* strains detected were resistant to beta-lactam antibiotics (Figure 2), the most common being the resistance to ampicillin. It was observed that the resistance extends to other antibiotics of this class, the strains having an intermediate resistance to AMC, or even being resistant to it.



	Antibiotics tested	Resistance speci	tra Re	corded value	es CLSI limit
A۱	MC Amoxicillin-clavulanate):	I	14.3 mm	13 / 18
	CAZ Ceftazidime :		S	31.5 mm	17/21
	CRO Ceftriaxone :		S	36.0 mm	19 / 23
	FEP Cefepime :		S	32.6 mm	18 / 25
	AMP Ampicillin :		R	0.0 mm	13 / 17
	IPM Imipenem :		S	33.0 mm	19 / 23

Fig.2. An example of *E. coli* strain with AMP resistance (I-intermediate, S-sensitive, R-resistant)

In conclusions, the hospital effluents represent an important source for dissemination of multidrug resistant bacteria, especially for beta-lactam antibiotics. This type of effluent should be treated before entering the municipal waste water treatment plant, to diminish the resistance genes chances of spreading that could be the cause of the antibiotic resistance.