# Surveillance of Antibiotic Resistance Among Enterobacteriaceae Strains Isolated in an Infectious Diseases Hospital from Romania

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The aim of the present study was to characterize the antibiotic resistance profile of enterobacteriaceae strains isolated in Infectious Diseases Hospital Galati, Romania, during 2016, in order to guide the local antibiotic stewardship strategy. There are 597 biological samples with positive cultures for enterobacteriaceae, related to invasive and non-invasive infections. The main bacterial genus were E. coli 62%, Klebsiella spp 15%, Proteus spp 11% and Salmonella spp 6%. Over a half of isolated strains have one or more antibiotic resistance. The resistance level depends on bacterial genus, with highest level found among the rare isolates: Enterobacter spp, Citrobacter spp, Morganella spp and Serratia spp. The rate of MDR was 17.,6% for E. coli, 40.9% for Klebsiella spp and 50.7% for Proteus spp. while the rate of strains producing Extended Spectrum of Beta Lactamase are 7.2% for E. coli, 28.4% for Klebsiella spp and 12.3% for Proteus spp. The carbapenem resistant strains were found in 1.1% cases.

Keywords: antibiotic resistance, multidrug resistance, ESBL, enterobacteriaceae

The World Health Organization has recognize a list of pathogenic germs that require to intensify the efforts to research and develop new antibiotics. Prioritization has taken into account the mortality, morbidity, effectiveness of existing therapies, the level and rhythm of antibiotic resistance development. Priorities are classified as *critical*, high and medium. Enterobacteriaceae resistant to cephalosporins and carbapenems are on the critical list along with Acinetobacter baumanii and Pseudomonas aeruginosa resistant to carbapenems[1]. Enterobacteriaceae are Gram negative bacteria belonging to the commensally flora of the gut, although are able to be involved in urinary, respiratory, skin and soft tissues infection or in invasive infection. The Enterobacteriaceae Family includes Escherichia coli, Klebsiella spp., Proteus spp., Morganella spp., Providentia spp., Enterobacter spp., Serratia spp., Salmonella spp., Shigella spp. and Yersinia spp. The common microbiological features are short rods, non-sporulating, facultative anaerobes, mobile (except Klebsiella spp. and Shigella spp.), catalase positive, oxidase negative and usually able to reduce Nitrate to Nitrite [2].

The *Enterobacteriaceaee* are naturally susceptible to quinolones, aminoglycozides, betalactam drugs, colistin, fosfomycin, nitrofurantoin and trimethoprimesulfamethoxazole [3].

Beta-lactam antibiotics are the preferred options for many *Enterobacteriaceae* infections. Commonlly, the mechanism of resistance to  $\beta$ -lactamines is enzymatic inactivation by hydrolysis of  $\beta$ -lactam ring (fig.1).

More than 890  $\beta$ -lactamases were identified, applying the bacterial DNA sequencing technology[4]. According to the functional characteristics and enzyme substrate, there are recognized four classes of  $\beta$ -lactamases: A, B, C and D. An updated classification comprise two categories: serine- $\beta$ -lactamases with 2 groups (group 1-class C, group 2-class A and class D) and metal- $\beta$ -lactamases, with the 3rd group - class B[5].



The resistance could be developed one or more antibiotic categories. According to the European Center for Disease Control (ECDC), the antibiotic resistance is classified as *multidrug-resistant* (MDR), *extensively drugresistant* (XDR) and *pandrug-resistant* (PDR). Multidrug resistance (MDR) means the lack of susceptibility to at least one antibiotic of three or more classes, while PDR implies the loss of susceptibility to all antibiotics, in all antimicrobial categories. Extensively drug-resistance (XDR) indicate non-susceptibility to at least one agent in all antimicrobial categories, while susceptibility is preserved to only one or two antimicrobial categories [6]. Since antibiotic testing use is variable in different sites, there is no consensus on the interpretation of XDR and PDR[7].

Assessment of local antibiotic resistance is crucial required for the most efficient therapeutic decisions to patients with infection diseases.

## **Experimental part**

À retrospective study on antibiotic resistance among Enterobacteriaceea strains isolated from biological samples of hospitalized patients between 1 January and 31 December 2016, was performed in the Infectious Diseases Hospital Galai. Collection the biological samples, bacterial culture and biochemical identification of bacterial

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strains were performed according to classical methodology[8]. Bactec 9050 automated analysis equipment was used for bacterial growth in hemoculture (HC). Other biological samples, as urine, stool, sputum, pus from wounds or leg ulcers, vomiting, catheters, otic and genital secretions, were inoculated in solid media using streak plate technique and were incubated at 37°C. Microbial identification was performed by Vitek 2 Compact Automated System. Antibiotic susceptibility was tested by Kirby Bauer diffusion method [9], according to CLSI recommendations [10]. We have tested the antibiotics according to the type of biological sample and bacterial genus.

The Enterobacteriaceae isolates were systematically tested to Ampicillin (Amp), Amoxiciline-Clavulanat (AmpC), Cefuroxime (CFX), Cefotaxime (CTX), Gentamycine (Ge), Trimethoprime-Sulfamethoxazole (TMP-SMX) and Norfloxacine (Nor). Supplementary tests to Nitrofurantoine (Nf) and Fosfomicyne (Fos) were achieved for the germs from urine culture (UC). The results of antibiogram were categorised as susceptible (S), intermediar (I) or resistant (R). The strains producing *Extended Spectrum Beta-Lactamase* (ESBL) were identified by Double Disc Synergy Test [8]. The MDR strains were identified according ECDC definition[6]. Depending on the clinical significance and the clinician's requests, several MDR strains were supplementary tested for salvage antibiotics, such carbapeneme (Ertapenem, Imipenem/ Meropenem). The quality controls in antibiotic susceptibility testing used E. coli reference strain ATCC 25922.

The results were collected in the hospital's antibiotic resistance database. The frequency and charts analyses were performed by Microsoft XL software.

### **Results and discussions**

During 2016, there were identified 1074 bacterial strains in the Microbiology Laboratory of the Galati Infectious Diseases Hospital. Gram negative rods were prevalent, with 64.3% (691/1074). The Enterobacteriaceae Family represented 86.3% (597/691) among the Gram negative bacilli and 55.5% (597/1074) of the isolated bacterial strains (fig. 2).

Most *Enterobacteriaceae* were isolated from urocultures (73.3%), leg ulcers and other skin lesions (15.7%), coprocultures (6.3%), followed by hemocultures (1.6%) and other biological products (7%). No more than 10 positive haemocultures were found, with identified 5 *E. coli*, 4 *Klebsiella spp.* and 1 *Proteus spp.* Most coprocultures were positive for *Salmonella spp.* (94.7%). *E. coli* prevailed



Fig. 2. Overview of Enterobacteriaceae isolates in Infectious Diseases Hospital Galati

in urocultures (76.4%). The largest variety of species was found in skin lesions (leg ulcers and other wounds), with higher frequency for *Proteus spp*. (fig. 3).



Fig. 3. Distribution of *Enterobacteriaceae* isolates according to the type of biologic sample

UC: uroculture; VU: ulcer of the leg; CC: coproculture; HC: hemoculture

*E. coli, Proteus spp.* and *Klebsiella spp.* characterize 88% of Enterobacteriaceae isolates. Antibiotic resistance by producing ESBL was variable with bacterial genus: 35% among *Enterobacter spp,* 28.4% *Klebsiella spp,* 12.3% *Proteus spp* and 7.2% *E. coli* (table 1).

The highest rate of MDR was found for *Proteus spp* (50.7%), followed by *Enterobacter spp* (45%), *Klebsiella spp* (40.9%), *E coli* (17.6%) and *Salmonella spp* (11%). *Morganella spp, Citrobacter spp and Serratia spp.* cumulate simply 2.5% of *Enterobacteriaceae*, however majority were MDR. Over a half of isolated strains have one or more antibiotic resistance (table 1).

	Ν	≥lR	≥lR	ESBL	ESBL	MDR	MDR	CRE
		(n)	(%)	(n1)	(%)	(n <sub>2</sub> )	(%)	
E.coli	373	235	63%	27	7.2%	66	17.6%	0
Proteus spp.	65	54	83%	8	12.3%	33	50.7%	1
Klebsiella spp.	88	58	65.9%	25	28.4%	36	40.9%	4
Salmonella spp.	36	19	52.7%	0	-	4	11%	0
Enterobacter spp.	20	19	95%	7	35%	9	45%	1
Citrobacter spp	11	10	-	5	-	7	-	1
Morganella spp.	3	3	-	2	-	2	-	0
Serratia spp	1	1	-	0	-	1	-	0
Total	597		-	75	12.56%	158	26.4%	7

Table 1ANTIBIOTIC RESISTANCEPROFILE OFENTEROBACTERIACEAESTRAINS

Legend: ≥1R: at least one antibiotic resistance; ESBL: Extended Spectrum Beta Lactamase; MDR: multidrog-resistant; CRE: Carbapenem Resistant Enterobacteriaceae; N: total number; n: partial number. Frequency analysis of ESBL producing strains and MDR strains according to the type of biological sample proved the highest antibiotic resistance related to the dermatological infections, followed by the urinary infections. Excepting one strain of E. coli ESBL producing and MDR, the other Enterobacteriaceae isolated from hemocultures had not antibiotic resistance problems (fig.4).



Fig. 4. Frequency of ESBL+ and MDR+ strains according the type of biological sample

#### UC: uroculture; VU: ulcer of the leg; HC: hemoculture; CC: coproculture

The sensitivity to Ampicillin ranged between 50% for *Proteus spp* and 72% for *Salmonella spp* (fig.5). The susceptibility to Trimethoprim-Suphamethoxazole was evidenced for 97% of Salmonella spp strains. This antibiotic could be the first option for the treatment of invasive infectious diarrhea, given that *Salmonella spp*. has been isolated in almost all (36/37) positive coprocultures.

*E. coli* was identified in 76% of urinary infections and proved to be susceptible 93% to Nitrofurantoin and 90% to Fosfomicyne. Consequently, Nitrofurantoin or Fosfomicyne could be the first choice for uncomplicated urinary infections. Association of Gentamicine and cephalosporin or a quinolone could be the first-line treatment of complicated urinary infections, that are more commonly associated with *Klebsiella spp*, *Proteus spp* or other enterobacteria with high risk of resistance (fig.5).



Fig. 5. Antibiotic sensitivity of *Enterobacteriaceae* strains in Infectious Diseases Hospital Galati

The high rate of MDR and the variable etiology of dermatological infections emphasize the difficulty of chose the first-line treatment and the need for systematic test of salvage antibiotics. Management of dermatological infections requires clear differentiation between infection and colonization, judicious antibiotic prescription and prevention of germs transmission, paying special attention to hand hygiene. Analysis of antibiotic susceptibility indicates the maintenance of carbapenem sensitivity for most strains of *E. coli, Klebsiella spp., Proteus spp. and Enterobacter spp.* (over 90%), mentioning that carbapenem testing was not performed systematically. Being salvage antibiotics, carbapenemes are still valid options for the treatment of MDR strains of *Enterobacteriaceae*. Understanding the local situation of antibiotic resistance for *Enterobacteriaceae Family* is useful to choice the first-line therapy in different types of infections and to manage the risks of health care associated infections.

All over the world, grown number of antibiotic resistant strains over the past few years is alarming, in both healthcare and community settings. In the United States, over 2 million infections with resistant germs to one or more antibiotics and above 23000 deaths caused by these infections are recorded every year. *Enterobacteriaceae* producing ESBL are responsible for 26000 (19%) of healthcare associated infections and 1700 deaths [11].

The surveillance report of antibiotic resistance in 2016, achieved by the European Center for Disease Prevention and Control based on invasive bacterial strains isolated from hemocultures or cerebrospinal fluid, highlights the variability of antibiotic resistance rates in European countries. Romania reported the 31.4% resistance rate, ranking third place among the European countries, following Greece (66.9%) and Italy (33.9%) [12]. The MDR frequency in Romania is 11.7% for E. coli and 55.2% for Klebsiella spp.[12]. These national data are worrying and require rigorous measures to limit antibiotic resistance in hospitals, as well as in the community environment. Comparative with the national data, the current study demonstrate higher MDR rate for E coli (17.6%) and lower rate for Klebsiella spp. (40.9%) (table I). The differences should be explained considering the variety of biological samples evaluated in our study, the low risk for invasive procedures in the infectious diseases ward and the local particularity of hospital and community antibiotic resistance.

## Conclusions

This study highlights the local situation of antibiotic resistance in an infectious diseases hospital from the South East of Romania, for Enterobacteriaceae involved in the community infections as in the health care associated infections. Regional particularities of antibiotic resistance should be considered to develop the treatment protocols and the local antibiotic stewardship strategy.

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