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Resistance of Enterobacter in a Tertiary Hospital and the Isolation of Enterobacter Amnigenus Multiresistant Strain

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Abstract: ***The aim of this study was to assess the frequency and antimicrobial susceptibility of Enterobacter isolated from clinical specimens of patients in a tertiary hospital. We collected different samples mostly from intensive care units (ICU), including sputum, central venous catheters, samples from infected surgical sites, from endotracheal and urethral catheters. Disk diffusion test was used to study the antimicrobial resistance. The predominant strains were E. cloacae 82 (60.3%), E. sakazacki 29 (21.3%) and E. aerogenes 24 (17.6%), and a strain of the rare E. amnigenus (p<0.01). The antimicrobial resistance of Enterobacter cloacae had increased recently. The change of the antimicrobial resistance should be investigated in order to direct rational drug usage in the clinic and prevent bacterial strain of drug resistance from being transmitted.***

Keywords: ICU, nosocomial infections, *Enterobacter, E. amingenus*

1. Introduction

Hospital-acquired infections (HAI), also called nosocomial infections are associated with an increase in morbidity, mortality and health-care costs (1). Patients requiring intensive care unit are prone to HAI 5 to 7-fold compared on general hospital wards (2). Enterobacter is a gram-negative bacillus that belongs to the Enterobacteriaceae family. Enterobacteriaceae are the most common bacterial isolates recovered from clinical specimens. The normal habitat is the intestine of human and animals and moist environment, especially soil and water. Many different species comprise the genus *Enterobacter*. Some have never been associated with human infections. The most commonly isolated species include *E. cloacae* and *E aerogenes*, followed by *E. sakazakii*, which are important nosocomial pathogens responsible for various infections mainly in critically ill patients in ICU. *Enterobacter* infections are most common in neonates and in elderly individuals, reflecting the increased prevalence of severe underlying diseases at these age extremes. Risk factors for nosocomial infections include invasive procedures, length of hospitalization, treatment with antibiotics and a presence of a central venous catheter. Enterobacter species have a global presence in both adult and neonatal intensive care units. Antimicrobial resistance is a serious problem that affects patients in hospitals worldwide. The production of extended spectrum beta-lactamases (ESBLs) among members of the *Enterobacteriaceae* has become one of the most difficult clinical problems in relation to therapeutics and epidemiology (3). In the last decades, however, hospital-acquired *Enterobacter* bacteremias have been increasingly reported, especially in intensive care units (ICUs) (4). The impact of different neonatal surgery, infectious diseases and internal nosocomial infections has been well documented in medicine. Nosocomial infections have been reviewed in several studies (5).

In addition to that, species of the genus *Enterobacter* show increasing resistance to antimicrobial drugs (6-8). Antimicrobial resistance, especially towards beta-lactam antibiotics, in addition to other risk factors, was associated with several outbreaks caused by species of the genus *Enterobacter* in neonatal ICUs (9). National surveillance programs continually demonstrate that *Enterobacter* species remain a significant source of morbidity and mortality in hospitalized patients. Multidrug resistance increased over time, especially in infections caused by *E cloacae* (10). The aim of this study was to assess the frequency and antimicrobial susceptibility of *Enterobacter* isolated from clinical specimens of patients in a tertiary hospital.

2. Material and Methods

This is a prospective study conducted in the University Hospital 'Mother Theresa" in Tirana, Albania which is a tertiary referral centre for the country. The isolates of *Enterobacter* were collected in various units from hospitalized patients between January 2012 and December 2013. We collected different samples mostly from ICU, including sputum, central venous catheters, samples from infected surgical sites, from endotracheal and urethral catheters and urine.

2.1 Isolation and Identification

The isolation was carried out in blood-agar and Mc Conkey media and identification was performed with enterosystem 18 R LIOFILCHEM-Italy.

The susceptibility testing was carried out with sensi test gram-negative liofil-chem and Disk-Difusion. Antimicrobial agents used in this study, were ampicillin (10 µg), piperacillin-tazobactam(100/10µg) ceftazidime (30µg), cefotaxime (30 µg), imipenem (10 µg), meropenem (10µg), aztreonam (30 µg), µg), amikacin (30 µ), gentamicin (10 µg), tobramycin (10 µg) ciprofloxacin (5 µg), levofloxacin

(5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and Amox/clavula (20/10µg), Cefuroxime (30µg), Netilmicine (30µg).

2.2 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Version16.0). The proportions were compared using the Chi-square and Fishers exact test. A *p*-value <0.05 was considered statistically significant.

3. Results and Discussion

A total 136 clinical specimens were isolated from hospitalized patients. The median age of study participants was 54.6 years, according to gender 102 (60.7%) were males and 66 (39.3%) females. The frequency of isolates among different hospital wards are presented in table 1. Forty four (32.4%) isolates were collected in sputum & endotracheal catheters, 36 (26.5%) isolates in urine & urethral catheters, 26 (19.3%) isolates central venous catheter, 23 (16.5%) isolates in surgical site infection, and 7 (5.3%) isolates from blood ($p<0.01$) (figure 1). The predominant strains were E. cloacae 82 (60.3%), *E. sakazacki* 29 (21.3%), *E. aerogenes* 24 (17.6%), and a strain of the rare *E. amnigenus* ($p<0.01$). (figure 2). The rare enterobacter strain was isolated in the Central ICU from a central catheter tip. *Enterobacter amnigenus* manifested a high degree of resistance to all cepfalosporines, to carbapenems to fluoroquinolones and fortunately was sensible to Amikacin, Minocycline and Trimetoprim/Sulfamethoxasole (TMP-SMZ) (11,12). The susceptibility testing was carried out with Vitek 2. The case was a woman of 66 years old with myasthenia and dyspnea admitted in ICU and was intubated. She was treated with five sessions plasmaferesis for myasthenia. Later she appears with temperature under 38 for several days. The blood culture showed no growth while the central catheter tip showed Enterobacter amnigenus. The patient was treated with 960mgx2 TMP-SMZ per os and the remission-healing was obvious. The most important in this strain is the resistance to carbapenems which was not encountered the previous years. The blood culture showed no growth while the central catheter tip showed *Enterobacter amnigenus*. In literature there is an example with a port-a cath infection with *E. amnigenus* and some cases with fatal sepsis and lower limb infection with this rare human pathogen. This pathogen displays a high natural resistance to antibiotics. *E. amnigenus* a motile gram (-) aerobic bacilli. Have been isolated from water and soil and also from various clinical specimens including blood, feces, sputum, wounds. E. amnigenus has been grown in samples from a few patients with sepsis. *Enterobacter* species are notorious for their drug resistance, which is thought to have been amplified by the use of broad spectrum cephalosporins in hospitals (13). The most resistant strains were from pediatric intensive care with *Enterobacter Sakazacki* and *Enterobacter cloacae* isolated from bronchial aspirates being resistant to cephalosporines, aminoglucosides, and sensible to carbapenems and fluoroquinolones (13-15). Also, the *Enterobacter Cloacae* from bronchial aspirates in ICU of neurosurgery and urinary tract in central ICU with almost the same resistance patterns being sensible to carbapenems. The isolates of patients from pediatric and alergology wards were almost sensible to all antibiotics whereas the *Enterobacter* isolated from pediatric ICU was resistant to cephalosporines, aminoglucosides and sensible to carbapenems. One possible mechanism of the resistance to carbapenems might be the loss of the outer membrane (through mutations) which reduce the carbapenem uptake which is most seen in *Enterobacter spp*. or it might be carbapenemases.

We have found Enterobacter to be a frequent gram-negative in asthmatic patients in alergology ward in the first days of admition. They were chronic patients with several hospital admissions. So *Enterobacter* might be community or hospital acquired. In both cases in those patients we dealed with almost sensible strains. In *Enterobacter* the AmpC gene encoding for beta-lactamases is repressed. Derepression can be induced by beta-lactams such as ampicillin, amoxicillin, cephalosporins of first and second generation and cephamycins which are strong inducers. Inducible AmpC is shown by elliptic view around Cefotaxime-CTX (fig. 3). Organisms considered susceptible with in vitro testing can become resistant by the following events: a) induction of AmpC beta lactamases b) mutation among the induced strains c) hyperproduction of AmpC beta lactamases by mutants and selection of the resistant mutant. E. aerogenes has been the most common carrier of ESBL plasmid DNA. ESBL in enterobacter is difficult to detect due to Amp.C (16-18). Only the resistance to cefoxitine can show the diference.

4. Conclusion

The antimicrobials most commonly indicated in *Enterobacter* infections include carbapenems, fourth-generation cephalosporins, aminoglycosides, fluoroquinolones, and TMP-SMZ. Carbapenems continue to have the best activity against *E cloacae, E aerogenes,*and other *Enterobacter* species. They are not affected by ESBLs. Imipenem and meropenem are used most often. First-generation and second-generation cephalosporins are inactive against *Enterobacter* infections. Third-generation cephalosporins frequently show good in vitro activity against these organisms, but, as explained above, a significant risk of developing full resistance during therapy exists and are not indicated for the treatment of severe *Enterobacter* infections. Resistance develops much less frequently with fourth-generation cephalosporins because they are relatively stable to AmpC beta-lactamase less frequently produce ESBLs.

Fluoroquinolones such as Ciprofloxacin and levofloxacin have the best activity against gram-negative bacilli and should generally be selected over the newer fluoroquinolones if clinically indicated. The change of the antimicrobial resistance should be investigated in order to direct rational drug usage in the clinic and prevent bacterial strain of drug resistance from being transmitted.

Important practices such as hand-hygiene, environmental decontamination, hospital surveillance of antibiotic resistance, controlled use of antibiotics and aseptic insertion of catheters and implanted devices will reduce transmission of the organism.

Even though initially *Enterobacter* may appear susceptible to antimicrobials the resistance can be developed early in the course of treatment and clinicians should be aware of that and use a combined treatment scheme.

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Table 1: Distribution of isolates of *Enterobacter* from different hospital wards

Ward	No. of specimens	n (%)
Pediatric ICU	18	13.2
Pediatric ward	14	10.3
Infectious diseases ward	15	11.0
Neurosurgery ICU	19	14.0
Allergology, ward	14	10.3
Central ICU	15	11.0
Heart-surgery, ICU	13	9.6
Urology, ICU	28	20.6

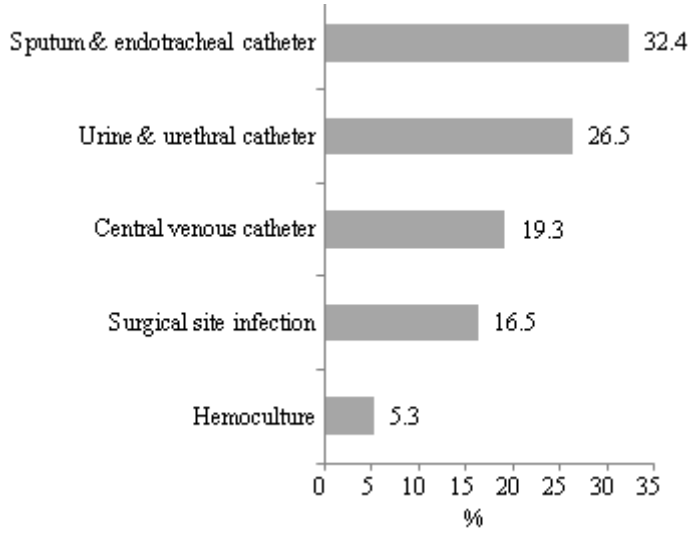

Figure 1: The type of specimen collected

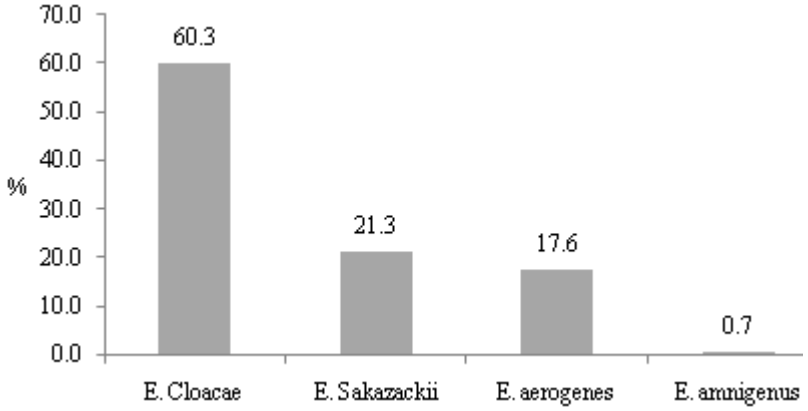

Figure 2: The type of strains isolated

Table 2: Resistant strains of *Enterobacter* in ICU and other hospital wards

Antibiotics	E. Cloacae (n=79)		E. Sakazackii (n=28)		E.aerogenes (n=23)	
	ICU (%)	Non ICU (%)	ICU (%)	Non ICU (%)	ICU (%)	Non ICU (%)
Amikacin	68	33	45	7	60	20
Ampicillin	82	46	77	14	100	48
Amox/clavula	85	20	82	8	87	15
Pipera/Tazob	38	0	42	5	82	0
Gentamicin	85	10	78	5	88	12
Tobramicin	58	0	40	0	0	12
Ceftazidime	75	25	74	15	85	18
Cefuroxime	81	49	69	18	74	26
Cefotaxime	85	35	72	28	90	30
Ciprofloxacin	68	20	48	26	85	12
Imipenem	0	0	10	10	0	0
Meropenem	0	0	0	0	0	0
Netilmicine	38	10	31	10	33	18
Trimet/Sulfa	75	40	62	38	80	42

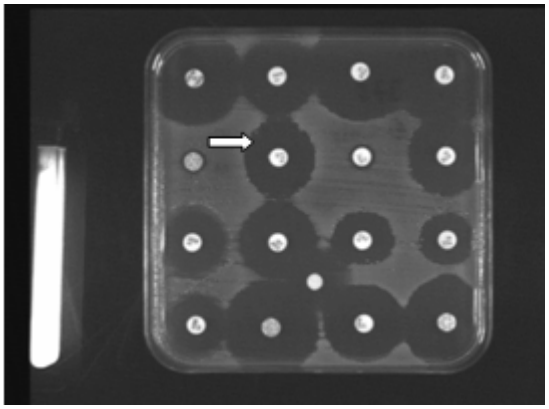

Figure 3: The antibiogram of *Enterobacter* from a patient in central ICU. The arrow points to induction phenomenon by cefoxitin and amoxicillin/clavulanic acid