Antibiotic Molecules Involved in Increasing Microbial Resistance

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Over the past two decades, the resistance to antibiotics, especially for Gram-negative bacteria, has increased at an alarming rate, requiring constant concern for resolving and controlling this extremely important therapeutic aspect in any medical department but in particular, in Anaesthesia and Intensive Care Units, in units of neonatology, paediatrics, neurosurgery, burned patients and immunosuppressed. Specialists note a particular concern for the resistance of Enterobacteriaceae to third-generation cephalosporins and aztreonam, with a resistance profile frequently associated with the expression of extended-spectrum â-lactamases (ESBL). The Enterobacter genus comprises 14 species, but two are of medical interest, Enterobacter aerogenes and E. cloacae, which are involved in inducing healthcare-associated infections such as urinary tract infections, pneumonia associated with mechanical ventilation, bacteremia, septicemia, etc. The purpose of the study was to highlight the antibiotic molecules in which microbial resistance of some circulating strains of enterobacteria was detected. A descriptive and retrospective study was conducted between 2012-2017, on a batch of 35 patients, admitted to the Sf. Maria Emergency Clinical Hospital for Children of Iasi, from whom various pathological products were collected to highlight the Enterobacter sp strains involved in the production of infections associated with the inpatient medical care. The antimicrobial sensitivity of each strain was determined by diffusimetric method, while the interpretation criteria were considered to be those of the laboratory standards. Most cases were reported in 2017 (31.42%). The majority were registered in newborns (42.85%) and infants (25.71%). The Anaesthesia and Intensive Care Units and Neonatology Anaesthesia and Intensive Care Units departments were the most involved. Microbial antibacterial resistance of Enterobacter sp isolates showed that all manifested resistance to ampicillin, amoxicillin and clavulanic acid, 48.57% were resistant to Cefuroxime, 42.85% resistant to Ceftazidime and Ceftriaxone, 14.28% to ciprofloxacin, 11.42% to ertapenem, 5.71% to Meronem. Although it showed relatively few cases with infections associated with healthcare in which strains of Enterobacter sp. were isolated our study, which was carried out over a period of 5 years, provides useful indications regarding the prevalence of healthcare associated infections with Enterobacter sp in paediatric patients and guidelines for antibiotic therapy.

Keywords: antibiotic, Enterobacteriaceae, infection, child, epidemiology, neurosurgery

From the point of view of financial resources both developed and developing countries face the burden of healthcare associated infections. In a cooperative study of WHO (55 hospitals in 14 countries in four WHO regions), approximately 8.7% of hospitalized patients had a healthcare-associated infection (HCAI). A 6-year surveillance study performed between 2002-2007 that involved intensive care units from Latin American, Asian, African and European regions, using WHO and CDC definitions, revealed higher rates of bacteremia and septicemia associated with central venous line and ventilation pneumonia, but also urinary tract infections associated with urinary probing and catheterization, as compared with rates in other sections. The study also reported higher frequencies of methicillin resistance by part of *Staphylococcus aureus* (MRSA), ceftriaxone resistance by part of *Enterobacter sp,* and *Pseudomonas aeruginosa* resistance to fluoroquinolones. As an increasing recognition of the burden generated by the HCAI, national surveillance systems have been developed in different countries, effective in reducing the incidence of HCAI and microbial antibioresistance [1].

Over the past two decades, antibiotic resistance, especially for Gram-negative bacteria, has increased at an alarming rate, requiring constant concern for resolving and controlling this extremely important therapeutic aspect in any medical department, but in particular, in Anaesthesia and Intensive Care Unit, in units of neonatology, paediatrics, neurosurgery, burns and immunosuppressed. Specialists note a particular concern for the resistance of Enterobacteriaceae to third-generation cephalosporins and aztreonam, with a resistance profile frequently associated with the expression of extended-spectrum β-lactamases (ESBL). This family of enzymes was identified in the 1980s and confers resistance to almost all β-lactam antibiotics, except for carbapenems and cephamycins. Genes encoding ESBL are found on plasmids that also carry other antibiotic resistance genes, which make the ESBL-producing strains to be multidrug-resistant. In addition to ESBLs, the broad-spectrum β-lactam resistance of

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Enterobacteriaceae is increasingly provided by other chromosomal-encoded plasmid enzymes, such as carbapenemases and cephalosporinases AmpC. Such mobile genetic and molecular equipment of resistant bacteria has further complicated the detection of ESBL-producing species. Due to the limitations and variability of the tests and the different reporting, third generation cephalosporin resistance is used as a more reliable indicator for the actual reporting of the prevalence of ESBL strains [2- 5].

Potentially pathogenic enterobacteria have developed multiple antibiotic resistance, chromosomal or plasmid-mediated resistance, becoming feared enemies in infections associated with inpatient healthcare. Genus *Enterobacter* comprises 14 species, two are of medical interest, *Enterobacter aerogenes* and *E. cloacae*, which are involved in the induction of healthcare associated infections such as urinary infections, pneumonias associated with mechanical ventilation, bacteremia, septicemia, etc. Infections caused by potential or occasionally pathogenic enterobacteria occur due to the alteration of the natural defence mechanisms, of the soil on which the bacteria are implanted and due to the extreme age of the patients.

Although receptivity is general, it is increased in long-term hospitalized individuals for chronic conditions and in immunosuppressed patients. The prevention of these health care associated infections includes measures similar to those instituted for such pathologies, considering that these isolates are frequently MDR [6,7].

The aim of the study was to highlight the antibiotic molecules in which case the microbial resistance of some circulating strains of enterobacteria was detected.

Experimental part

Materials and methods

The descriptive and retrospective study was carried out in the period 2012-2017, on a batch of 35 patients, admitted to the Sf. Maria Emergency Clinical Hospital for Children of Iasi, to whom various pathological products were collected to highlight the *Enterobacter sp* strains involved in the production of infections associated with the inpatient medical care. The data were collected from the patients' observation sheets and from the statistical reports, and the results of the antibiograms performed from the existing reports, according to the hospital's microbiology laboratory. The antimicrobial sensitivity of each strain was determined by diffusimetric method, and the interpretation criteria were considered those of the laboratory standards. The data were statistically processed using the computer software SPSS v.22.0.

Results and discussions

The distribution by years of study shows that most cases were reported in 2017 (11 cases - 31.42%). The quarterly distribution of patients showed that in the first and third quarters most cases were reported (respectively 12 patients - 34.28%), in the fourth quarter 9 cases (25.71%) were reported, and in the second quarter, the least (2 cases - 5.71%) ($p < 0.01$).

The age distribution of patients with infections in whom strains of *Enterobacter sp* were isolated showed an average age of approximately 2.6 years. Most cases were registered in newborns, up to 1 month (15 cases - 42.85%), then in infants up to 12 months (9 cases - 25.71%), then in children between 2 -5 years old (7 cases - 20.00%) and in children between 12-18 years old (4 cases - 11.43%).

Gender distribution showed that there were more cases in boys than in girls, and the ratio M: F = 24/9 (2.67).

The distribution on the departments in which the patients were hospitalized revealed that the Anaesthesia and Intensive Care Unit and Anaesthesia and Intensive Care Unit neonatology departments were the most involved, with 9 cases admitted in each section, thus 18 total Anaesthesia and Intensive Care Unit (51.42%). In the paediatric acute therapy department, 6 cases (17.14%) were diagnosed. Other sections that reported associated infections were: paediatric surgery and burns section, 3 cases (8.57%), departments of neurosurgery, orthopaedics, plastic surgery, oncology and nephrology, with 1 case reported (2.85%).

The pathological products collected from the patients were wound secretion (9 isolates - 25.71%), urine culture (5 isolates - 14.28%), hemoculture (5 isolates - 14.28%), catheter tip culture (3 isolates - 8.57%) burn secretion (3 isolates - 8.57%), tracheal aspirate (3 isolates - 8.57%), gastric aspirate (2 isolates - 5.71%), hypopharyngeal aspirate (2 isolates - 5.71%), peritoneal fluid (2 isolates - 5.71%), pleural fluid (1 isolate - 2.85%) (fig. 1).

The most commonly diagnosed types of infections were wound infection (8 isolates - 22.85%), skin infection (7 isolates - 20.00%), urinary tract infection (5 cases - 14.28%), sepsis (5 cases -14.28%), pneumonia associated with mechanical ventilation (4 cases - 11.42%), catheter infection (3 isolates - 8.57%), digestive infection (2 cases - 5.71%) and respiratory infection (1 isolate - 2.85%) (fig. 2).

Microbial antibacterial resistance of *Enterobacter sp* isolates showed that all isolates exhibited resistance to ampicillin and amoxicillin and clavulanic acid (100%), 17 isolates (48.57%) were resistant to Cefuroxime (CXM), 15 isolates (42.85%) resistant to Ceftazidime (CAZ) and Ceftriaxone (CRO), 11 isolates (31.42%) resistant to

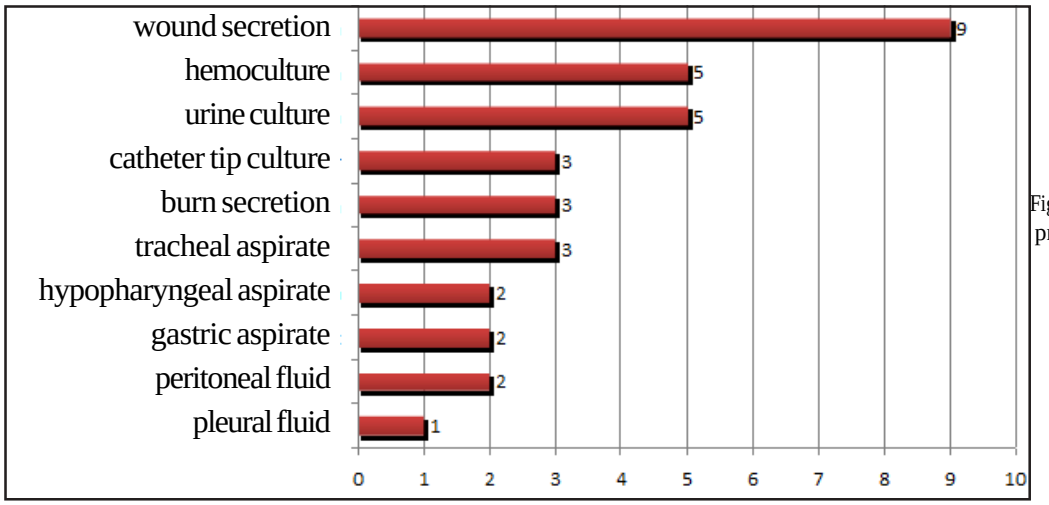


Fig. 1. Types of pathological products taken as samples

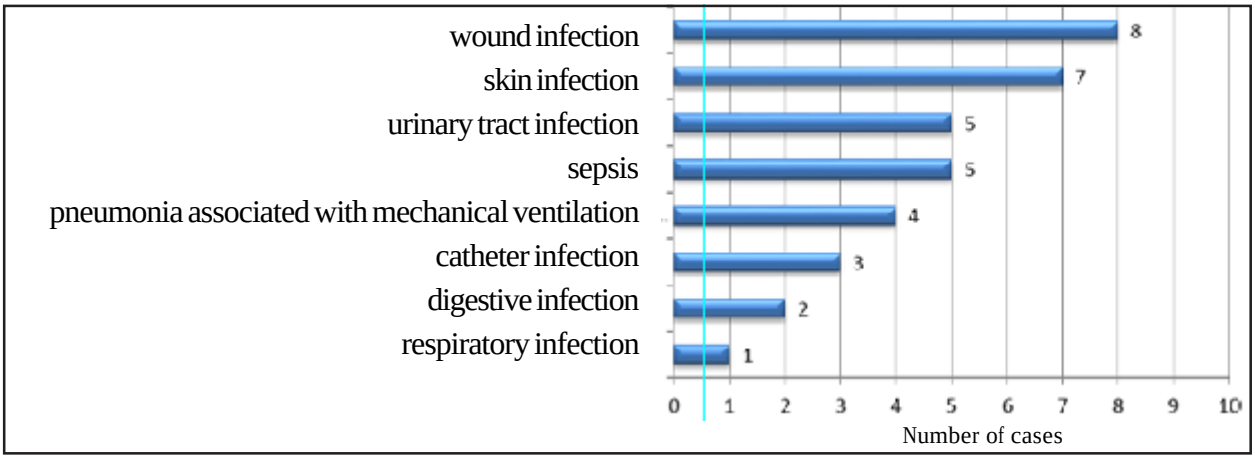


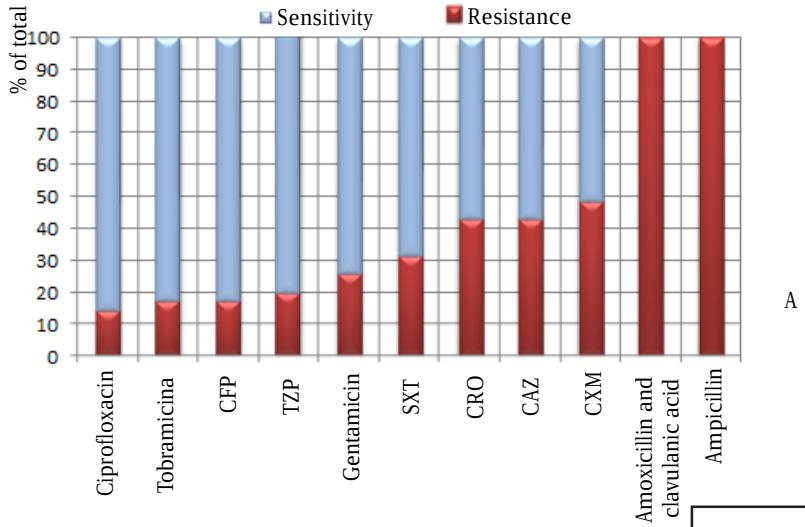
Fig. 2. Types of infection with *Enterobacter sp.*

Trimethoprim-Sulfamethoxazole (SXT), 9 isolates (25.71%) resistant to gentamicin, 7 (20.00%) resistant to Tazobactam-Piperacillin (TZP), 6 isolates (17.14%) resistant to Cefoperazone (CFP) and tobramycin, 5 (14.28%) to ciprofloxacin, 4 (11.42%) to ertapenem, 2 (5.71%) in Meronem (MEM) (fig. 3).

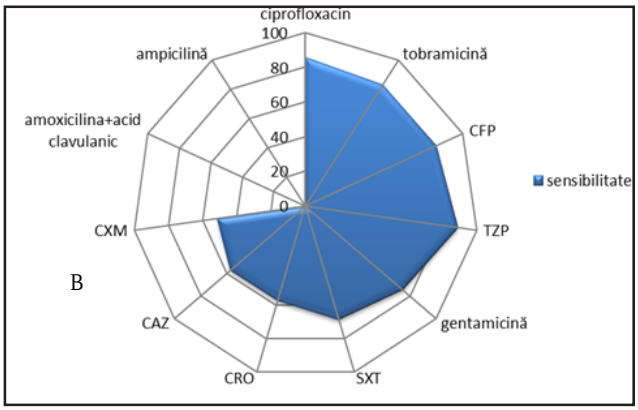
The types of strains were *Enterobacter sp* - 20 isolates (57.14%), *E. cloacae* - 11 isolates (31.42%) and *E. aerogenes* - 4 isolates (11.42%). Of these, 4 isolates were found to produce beta-lactamases ESBL (11.42%) and one CRE (Carbapenem-resistant *Enterobacteriaceae*) (2.85%).

The time interval from hospitalization to diagnosis of the healthcare associated infection was, on average, 27.6 days, with a minimum of 3 days and a maximum of 94 days. Most cases were diagnosed 15-30 days after admission (10 cases - 28.57%), followed by an interval of 31-90 days (9 cases - 25.71%), between 8-14 days - 8 patients (22.85%) and between 0-7 days - 6 cases (17.14%), and over 90 days - 2 cases (8.57%) (fig. 4).

Enterobacter species have many characteristics common to those of the genus *Klebsiella*, but are easily distinguished by their motility, although non-motile variants occur occasionally. *E. cloacae* is clinically the most important species. *E. aglomerani*, an anaerogenic, yellow pigmented organism, formerly known as *Erwinia herbicola*, is also occasionally encountered. The strains of *E. amnigenus* and *E. asburiele* were also isolated from pathological products taken as samples from infected patients. *Enterobacter* strain colonies may be slightly mucoid. In general, their fermentative activity is more limited than that of typical enterobacteria. Phage typification serotypes and patterns have been developed to characterize E. cloacae strains, with numerous types of O and H currently recognized. In epidemics, the strains were characterized using gel electrophoresis. The normal habitat of *Enterobacter sp* is probably soil and water, but organisms are occasionally found in human faeces and in the respiratory tract, ways in which the infection of



A



B

Fig. 3. Resistance / Sensitivity to antibiotics tested for *Enterobacter sp;*
A. Resistance and sensitivity
B. Sensitivity

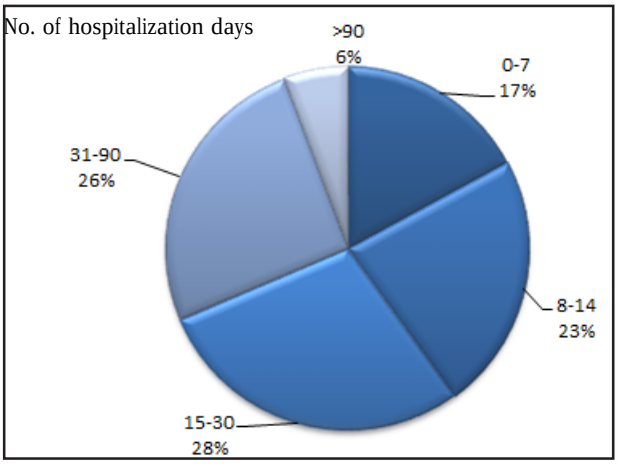


Fig.4 The time interval between hospitalization and the onset of *Enterobacter sp.*infection

hospitalized patients, especially urinary tract infections, takes place. *Enterobacter sp* are also an important cause of bacteremia, but much less than *Klebsiella sp.* The pathogenic mechanisms are poorly understood. In common with certain *Klebsiella* strains, they express type 1 and type 3 fimbriae. Most strains also express an aerobactin-mediated iron uptake system, which is generally associated with extra-intestinal human pathogens. The strains can produce an alpha-hemolysin similar to the alpha-hemolysin produced by *E. coli* strains. Very rarely, strains hybridize with gene probes for verocytotoxin-1. An external membrane protein, called OmpX, may be a pathogenic factor for *E. cloacae* strains. This protein appears to reduce porin production, leading to decreased sensitivity to â-lactam antibiotics and could play a role in host cell invasion. *Enterobacter* strains produce a chromosomal â-lactamase with cephalosporinase activity and are almost always extremely resistant to penicillins and many cephalosporins. Many are also resistant to tetracyclines, chloramphenicol and streptomycin, although most are sensitive to other aminoglycosides, including gentamicin. Most strains are susceptible to fluoroquinolones, co-trimoxazole and carbapenems. *Enterobacter* strains differ from *Serratia* strains in polymyxin sensitivity [8-10].

In numerous studies carried out in hospitals with a paediatric profile, frequent infectious episodes caused by ESBL-producing bacteria have been reported. Regarding *E. cloacae*, the strains of this bacterium are important pathogens that cause bacteraemia or sepsis associated with intra-hospital care and can be a serious cause of Gram negative bacteremia that may evolve as outbreaks in paediatric intensive care units. Other locations include lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, bacterial endocarditis, osteomyelitis and ophthalmic infections associated with healthcare. *E. cloacae* strains are known to have inherent resistance to ampicillin and narrow-spectrum cephalosporins due to a mutation that makes them resistant to broad-spectrum cephalosporins, and carbapenems are generally antibiotics used as a treatment for MDR microorganisms, some studies on paediatric patients mention, as we also noted in our study.

ESBL strains contain enzymes encoded in the plasmid that hydrolyses the β-lactam ring and cause anti-resistance to β-lactam preparations, such as third-generation cephalosporins, ceftriaxones, ceftazidime, cefotaxime and aztreonam. Bacterial strains expressing these β-lactamases frequently lead to major therapeutic challenges due to multiple antibioresistance. In recent years there has been a significant increase in the incidence and prevalence of ESBL-producing bacteria, as it was also observed in the present study [11,12].

Conclusions

Our study, carried out over a period of 5 years, showed relatively few cases of healthcare associated infections in which strains of *Enterobacter sp.* were isolated. However, given that some of these isolates were ESBL producing strains, in the general context of microbial multidrug resistance, these are of great importance for hospital management, for the prevention and control of these infections. Our study provides some useful insights into the prevalence of healthcare-associated infections with *Enterobacter sp* in paediatric patients and guidelines for antibiotic therapy. The isolated *Enterobacter* strains were completely resistant to ampicillin and amoxicillin + clavulanic acid. For second- and third-generation cephalosporins, resistance was lower, but for over one-third of isolates. These findings highlight the fact that strains isolated from paediatric patients, particularly from the Anaesthesia and Intensive Care Unit Department neonatology and neurosurgery, have significant antibioresistance and should be considered for the management of healthcare associated infections and hospital guidelines.

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