Beta-lactam Resistance Mechanisms in Pathogens Isolated from Patients Admitted to Intensive Care Unit

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Intensive care units (ICUs) are often referred to as the epicentre of infection diseases in a hospital. Many studies highlighted the importance of using local antimicrobial resistance data, to guide empirical antibiotic therapy. As a consequence, the present study is particularly important, especially in the current context, when we are witnessing an ascending trend of antimicrobial resistance. Beta-lactams are the most frequently used class of antibiotics for treating patients infected with various germs. The aim of this study is to analyse the modalities by which microorganisms become resistant to antibiotics of this class, in an intensive care unit of a Romanian university hospital. During the period between January, the 1st 2012 and December the 31st 2013, a prospective study was conducted in the largest ICU from the Western part of Romania. Various resistance mechanisms to beta-lactam antibiotics were detected. Among these, there is great concern regarding the high number of extended-spectrum beta-lactamase producing microorganisms, as in most cases they determine the use of carbapenems, thus increasing the risk of occurrence and dissemination of carbapenemase-producing bacteria.

Keywords: antibiotics, beta-lactams, beta-lactamases

At the time antibiotics were discovered, 25 classes being characterised during a period of around 70 years, many announced the end of infectious diseases. Unfortunately, time proved that microorganisms have the capacity to gain a much higher resistance than our capacity to create new antibiotics. There are not too many novel antibiotic classes discovered since 1987, while multi-resistant pathogens are spreading [1], but currently various substances of vegetable or synthetic origin are tested, to determine their antimicrobial activity [2-7].

Starting with 1928, when Alexander Fleming discovered penicillin, many other antimicrobial substances were added to the class of beta-lactams.

This class includes penicillins, cephalosporins, cephamycins, monobactams and carbapenems, all sharing the same chemical structure i.e. the beta-lactam ring [8]. Depending on their antimicrobial activity, penicillins may be classified into five classes: natural penicillins, penicillinase-resistant penicillins, aminopenicillins, carboxypenicillins and ureidopenicillins.

There are four clinically relevant mechanisms by which bacteria can become resistant to beta-lactam antibiotics [8]:

- destruction of the antibiotics by secreting beta-lactamases;
- incapacity of the antibiotic to penetrate the external membrane of Gram-negative bacteria (GNB) and consequently to reach the target represented by penicillin-binding proteins (PBP);
- efflux of beta-lactams through the external membrane of GNB;
- low affinity for antibiotic binding to PBPs.

The main mechanism by which beta-lactam resistance may occur in the case of GNB is the secretion of beta-lactamases. These are enzymes which hydrolyze the beta-lactam ring of beta-lactamines, that is crucial for inhibition of the PBP targets. The most frequently encountered beta-lactamases are penicillinases, cephalosporinases and more recently, carbapenemases.

Beta-lactamase inhibitors (such as clavulanic acid, sulbactam and tazobactam) increase the antibacterial activity of penicillins only in cases where antimicrobial resistance is the result of beta-lactamase production (fig. 1).

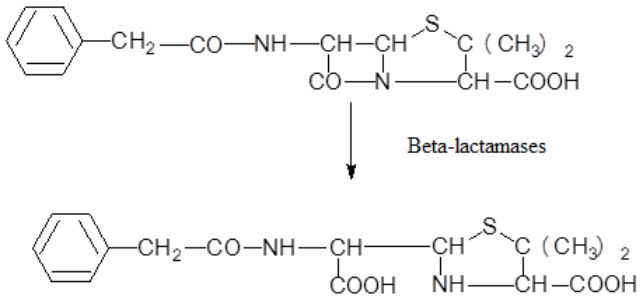

Fig.1. The site of Beta-lactamase attack on the structure of the penicillin [8]

Resistance by penicillinase production has always been widespread among Gram-positive bacteria (GPB). In the case of GNB beta-lactam resistance was installed more slowly, but in 1975, already 15-20% of strains were reported to be resistant due to beta-lactamase production [8].

The aim of the present study was to analyse the modalities by which microorganisms become resistant to beta-lactams, as these are regarded as important weapons towards the prevention and of emerging bacterial resistance. Knowledge of the modalities by which microorganisms become resistant to antibiotics is crucial for the targeted treatment of infected patients.

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Intensive care units (ICUs) are often referred to as the epicentres of infection diseases in a hospital [9,10], and in addition, many studies highlighted the importance of using local results to guide empirical antibiotic therapy [11,12]; consequently, this study has a particular importance, especially in the present context, when we are witnessing a rising trend of antimicrobial resistance.

Experimental part

During the period between January the 1st 2012 and December the 31st 2013, in the Intensive Care Unit of the Pius Branzeu Clinical Emergency County Hospital Timisoara, a prospective surveillance study was conducted on the modalities by which bacterial strains become resistant to beta-lactam antibiotics.

The approval of the Ethics Committee within Pius Branzeu Timisoara Emergency Clinical County Hospital was requested and granted: no.44346/11.12.2012.

The sensitivity of isolated germs to antiinfectious chemotherapeutic drugs was determined by the automated reading of the minimum inhibitory concentration (MIC) followed by classification into resistance phenotypes, using the Vitek 2 Compact analyser, according to Clinical Laboratory and Standards Institute (CLSI).

In order to test the sensitivity of GNB to beta-lactam antibiotics, we used the AST-GN27, AST-NO93 or AST-NO22 cards. We tested for ampicillin, ticarcillin, piperacillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, ticarcillin/clavulanic acid, cephazolin, ceftazidime, cefotaxime, cefpirome, cefepime, cefoxitin, aztreonam, imipenem, and meropenem. In order to detect extended spectrum beta-lactamase producing strains (ESBL) by AST-GN27 cards, additional tests for cefepime, cefotaxime and ceftazidime, both single and in combination with clavulanic acid were performed. The confirmation of carbapenemase production was done by the modified Hodge test and EDTA - disk synergy test.

For Gram-positive cocci (*Staphylococcus* spp. and *Enterococcus* spp.) the AST-P554 cards were used, these providing cefoxitin screening for detecting meticillin-resistant *Staphylococcus aureus* (MRSA), as well as testing for sensibility to penicillin, ampicillin, ampicillin/sulbactam, oxacillin and imipenem. In Gram-positive cocci, beta-lactamases production was confirmed by the nitrocefin test (CLSI), with the Nitrocef Matchbook stick (Hardy Diagnostics).

The statistical analysis of the database was done with the EpiInfo version 3.2.2 (CDC, 2005). The category variables were characterised by value and percentage. Comparison of category variables was performed by chi-square test and Fisher correction. Two-tailed statistical significance was calculated and the threshold was set at ≤0.05.

Results and discussions

The studied group included 1,596 patients hospitalised in the ICU from 1 January 2012 to 31 December 2013, who received antimicrobial treatment.

The consumption of penicillins and of other beta-lactams represents almost two-thirds of the total amount of antimicrobials used for systemic consumption [12].

Acquired resistance of the bacterial strains to various groups of beta-lactams, as well as the number of corresponding strains, are presented in figure 2.

The strains were classified into resistance phenotypes (table 1). Strains belonging to species naturally resistant to penicillins or to Ist or IInd generation cephalosporins, by producing low inducible penicillinases or cephalosporinases levels, as well as the intrinsic resistance of enterococci to cephalosporins have been excluded.

An increased resistance (69.95% resistant strains) to penicillins (presently less frequently prescribed in ICUs) was found in GNB. Regarding carboxypenicillins and ureidopenicillins, also known as *anti-Pseudomonas penicillins,* 26.27% of the *Pseudomonas* strains have been found to be resistant.

The most frequently detected penicillin resistance mechanism (23.83%) was ESBL production, all the strains resistant to IIIrd generation cephalosporins being confirmed for this phenotype. The same situation was described in Europe, but also in other regions of the world [9,13,14], with 60-100% ESBL producing strains of those resistant to IIIrd generation cephalosporins. ESBL production provides resistance to penicillins, Ist, IInd, IIIrd, IVth generation cephalosporins and monobactams, with conserved sensitivity to carbapenems and cephamycins.

The most frequently used beta-lactams in the studied ICU were IIIrd and I In GNB, IVth generation cephalosporins. For this reason, the resistance levels are concerning, as 23.83% of GNB were found to be resistant to these cephalosporins.

Other 132 strains (13.58%) were classified in the penicillinase-producing phenotype, 120 (12.34%) were cephalosporinase secretors, and 113 (11.62%) were both penicillinase and cephalosporinase producers.

A number of 190 GNB (19.54%) were resistant to carbapenems, with five *Klebsiella pneumoniae* strains confirmed as carbapenemase-producing by the Hodge test. Metalloenzymes were detected in 24 *Pseudomonas aeruginosa* and 85 *Acinetobacter baumannii* strains, respectively. The emergence of such carbapenemase-producing strains was also revealed by the most recent study conducted by the European Centre for Disease Prevention and Control (ECDC) [14], presenting a serious menace to the patient safety and healthcare in European hospitals. This represents an alarm signal, carbapenems

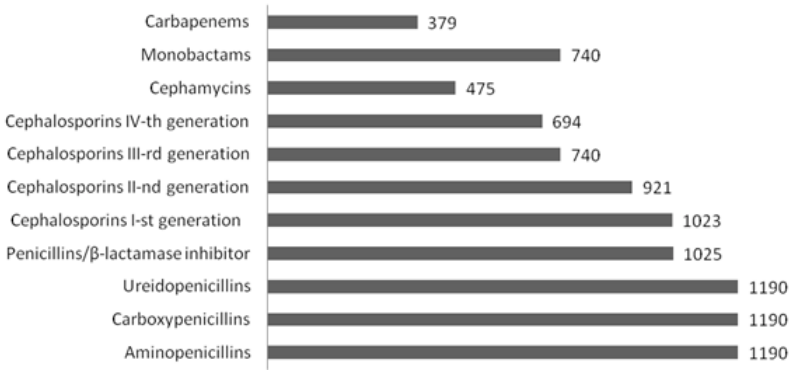

Fig.2. Acquired resistance to beta-lactam antibiotics of the bacterial strains isolated during the study

Acquired resistance phenotypes	Identified value [n (%)]
LPASE	21 (1.58)
HPASE	111 (8.39)
CASE	74 (5.59)
CHN	46 (3.48)
PACA	113 (8.55)
ESBL	315 (23.83)
Carbapenem resistant GNB	190 (14.37)
peniR metiS Staphylococci	129 (9.75)
MRSA	129 (9.75)
MRCNS	53 (4.01)
Enterococci with modified PBP	7 (0.53)
Penicillinase-producing Enterococci	2 (0.15)
Total bacterial strains	1322 (100)

Abbreviations: LPASE – low penicillinase level; HPASE – high penicillinase level; CASE - cephalosporinase; CHN – increased level of cephalosporinase; PACA – penicillinase and cephalosporinase; ESBL - extended-spectrum beta-lactamases; GNB – Gram-negative bacilli; PeniR – penicillin resistant; MetiS – meticillin sensitive; MRSA – meticillin resistant S. aureus; MRCNS - meticillin resistant coagulase negative staphylococci; PBP - penicillin-binding proteins.

Table 1
RESISTANCE PHENOTYPES OF ISOLATED STRAINS WITH INDIVIDUALLY IDENTIFIED VALUES

Table 2
MOST FREQUENTLY ISOLATED BACTERIAL SPECIES AND THEIR ANTIBIOTIC RESISTANCE. COMPARISON BETWEEN VALUES OBTAINED IN THE PRESENT STUDY IN 2012 (R1) AND THOSE IN THE REPORT PROVIDED BY ROMANIA IN 2012 (R2)

Bacterial species		R1%	R2%	P	OR [CI95%]	RR
Escherichia coli	ESBL	16.42	25.79	0.118	0.57 [0.26-1.22]	0.64 [0.35-1.15]
	PASE	50.75	60.54	0.163	0.67 [0.37-1.22]	0.84 [0.64-1.09]
	Carbapeneme resistance	0	0	/	/	/
Klebsiella pneumoniae	ESBL	57.98	61.76	0.560	0.85 [0.48-1.52]	0.94 [0.76-1.17]
	Carbapeneme resistance	15.96	15.69	0.954	1.02 [0.47-2.24]	1.02 [0.55-1.87]
Pseudomonas aeruginosa	ESBL	21.81	53.85	**0.001**	0.24 [0.09-0.64]	0.41 [0.23-0.72]
	Tzp resistance	41.81	52.27	0.300	0.66 [0.27-1.57]	0.80 [0.53-1.22]
	Carbapeneme resistance	29.09	61.36	**0.001**	0.26 [0.10-0.65]	0.47 [0.29-0.76]
Acinetobacter baumannii	Carbapeneme resistance	85.93	86.27	0.958	0.97 [0.30-3.15]	1.00 [0.86-1.15]
Staphylococcus aureus	Meticillin resistance	51.16	54.50	0.244	1.33 [0.80-2.23]	1.33 [0.93-1.37]
Enterococcus faecium	Aminopenicillins Resistance	42.85	91.2	**0.009**	0.07 [0.01-0.71]	0.47 [0.20-1.11]
Enterococcus faecalis	Aminopenicillins Resistance	33.33	3.64	0.149	13.12 [0.15-345.88]	9.17 [1.12-74.89]

Abbreviations: PASE - production of penicillinase; ESBL - extended-spectrum beta-lactamases; TZP - piperacillin/tazobactam

being increasingly prescribed at present for the treatment of infections caused by multidrug-resistant germs.

As for GPB, penicillin-resistant staphylococci which exhibit sensitivity to Cefoxitin, are sensitive to penicillins associated to beta-lactamase inhibitors and to penicillinase-resistant penicillins, with 38.5% of the *Staphylococcus* spp. strains being classified into this phenotype.

Meticillin-resistance for staphylococci involves resistance to all beta-lactamines, except Vth generation cephalosporins, also known as *anti-MRSA cephalosporins.* A number of 182 (54.32%) strains were meticillin-resistant, two by beta-lactamase production (1.09%), and the remaining by modified PBP (98.9%).

In the case of enterococci, penicillin resistance by beta-lactamase production is relatively rare, the main

mechanism for the onset of this resistance being the modification of target proteins (PBP), which also incurs resistance to penicillins/ beta-lactamase inhibitors associations and carbapenems. In the present study, for the isolated *Enterococcus* strains, the penicillinase-producing phenotype was detected in two strains (13.33%), the one acquired by modified PBP in 7 strains (46.66%), the remaining enterococci being sensitive to beta-lactams (40%).

A portion of results, namely those obtained during the year 2012 (R1), were compared to those presented in the *Report on antibiotic consumption, antibacterial resistance and nosocomial infections in Romania in 2012* [15], with 10 participating hospital laboratories (R2). The comparison between the two studies is presented in the table 2.

Analysing the reported resistance for strains isolated in 2012, statistically significant differences are only observable for *Pseudomonas aeruginosa* and *Enterococcus faecium* strains for which national data showed higher values than in the present study.

In our previous studies, we investigated bioactive molecules used in this type of infections by some instrumental protocols reported by UMFT study group [16-22].

Conclusions

In the studied ICU, the most frequently prescribed antibacterial drugs were IIIrd generation cephalosporins (especially Ceftriaxone). The wide use of these for prophylactic purposes (in orthopaedic, plastic and reconstructive surgery – the Altemeier class I, in neurosurgery, cardiovascular surgery, gastro-duodenal and gynecologic surgery), may be one of the explanations for the high density of ESBL producing strains in this ICU.

The high number of ESBL producing microorganisms is of great concern because it determines the increased use of carbapenems, which are the second choice for the respective patients. This use increases the risk of emergence and dissemination of carbapenemase-producing bacteria.

Thus, antimicrobial resistance is a serious threat to public health in Romania, but also worldwide, and the detection of increased resistance to certain key groups of antimicrobials is of great concern. Prudent antimicrobial use, infection prevention and control strategies are recommended in order to increase the effectiveness of antimicrobial therapies and to prevent transmission and selection of resistant bacteria.

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