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Activity of beta-lactam antibiotics and production of beta-lactamases in bacteria isolated from wound infections in Brazzaville, Congo

MOYEN Rachel[1]*, AHOMBO Gabriel[1], NGUIMBI Etienne[1], ONTSIRA Nina Esther[2], NIAMA Rock Fabien[1], YALA Gatsielé Claudette[3] and LOUEMBE Delphin[1]

[1]Department of Cellular and Molecular Biology, Faculty of Science and Technology, University Marien, NGOUABI, Brazzaville, Congo.
[2]Laboratory of Bacteriology and Virology of University Hospital Center, Brazzaville, Congo.
[3]Centre of Medical Biology (CBM laboratories), Mougali III Brazzaville, Congo.

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To determine the mechanism of bacterial beta-lactam resistance, 165 bacteria isolated from wounds of hospitalized patients composing of: 42 *Staphylococcus aureus*, 37 *Pseudomonas aeruginosa*, 23 *Escherichia coli*, 22 *Proteus*, 12 *Klebsiella,* 10 coagulase-negative staphylococci (CNS), eight *Enterobacter*, six *Citrobacter*, five *Providencia* were tested for their sensitivity to beta-lactams and their production of beta-lactamases. The antibiotic susceptibility was considered by the method of the standard diffusion on agar Mueller Hinton. The rate of production of ß-lactamase in all bacteria was determined using the Strips of nitrocefin. The percentages of resistance to beta-lactams obtained were as follows: *Staphylococcus aureus* (77.90%), *Pseudomonas aeruginosa* (44.14%), *E. coli* (73.8%), *Proteus* (57.4%), Klebsiella (63.6%), CNS (57.15%), *Enterobacter* (56.3%), *Citrobacter* (83.3%), *Providencia* (67.5%). The rate of beta-lactamases were as follows: *S. aureus* (7.34%), *P. aeruginosa* (89.19%), *E. coli* (95.65%), *Proteus* (86.36%), *Klebsiella* (91.67%), CNS (90%), *Enterobacter* (87.5%), *Citrobacter* (66.67), *Providencia* (100%). The studied bacteria produce beta-lactamases which is the primary mechanism of resistance to beta-lactam antibiotics in the majority of the bacteria. Beta-lactamases rates vary from one genus to another. It is extended spectrum beta-lactamase-producing strain.

Key words: Bacteria resistance, beta-lactamases, wounds infections, Brazzaville.

INTRODUCTION

Since the discovery of penicillin 80 years ago, Gram-negative bacteria have become proficient at evading the lethal activity of β-lactam antibiotics, principally through the production of β-lactamases (Rapp and Urban, 2012).

*Corresponding author. E-mail: rmoyen@yahoo.fr. Tel :(242)066671363.

Table 1. Activity of beta-lactam antibiotics on staphylococci.

Bêta-lactam tested	*Staphylococcus aureus*		*Negative coagulase Staphylococci*	
	S (%)	R (%)	S (%)	R (%)
Amoxcillin+acid clavulanic	10 (23.80)	32 (76.20)	2 (20)	8(80)
Penicillin	0	42 (100)	0 (0.00)	10(100.00)
Céfalotin	3 (7.14)	3939(92.86)	1(10.00)	9(90)
Cefotaxime	0	42 (100)	0(0.00)	10(100.00)
Ceftazidime	20 (47.60)	22 (5 2.40)	8(80.00)	2(20)
oxacillin	3 (7.14)	39 (92.86)	9(90)	1 (10)
Imipénème	29 (60.10)	13 (30.90)	10(100.00)	0(0.00)
Total (%)	65 (22.10)	229 (77.90)	30(42.85)	40 (57.15

Beta-lactam antibiotics are a broad class of antibiotics, consisting of all antibiotic agents that contain a β-lactam ring in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), mono-bactams and carbapenems (Holten and Onusko, 2000). They are a family of first-line antibiotics both in terms of availability and in terms of the cost. Since their discovery by Flemming in 1929 with penicillin, they are commonly used in the treatment of various infections (Livermore and Brown, 2001).

Their use is of therapeutic importance, in the improvement of the infectious diseases treatment, the reduction of mortality and the eradication of the once formidable diseases. However, since their introduction in medicine, bacteria have developed mechanisms that have resulted in the emergence of the multi resistance. Several biochemical or genetic mechanisms are responsible for this resistance, including the production of enzymes (Andremont et al., 1997; Zogheib and Dupont, 2005). The infections caused by extended-spectrum beta-lactamase (ESBL) producing bacteria, constitute severe problems (Kuzucu et al., 2011).

The purpose of this study was to assess the rate of resistance of these bacteria to beta-lactams and determine the possible mechanism of inhibition of these antibiotics.

MATERIALS AND METHODS

Strains and used antibiotics

Samples were cultured and bacterial isolation was done by conventional methods (Esmaeili et al., 2014). 165 bacteria isolate composing of: 42 strains of *Staphylococcus aureus*, 10 coagulase-negative staphylococci (CNS), 23 *Escherichia coli*, 22 *Proteu*s, 12 *Klebsiella*, 8 *Enterobacter*, 6 *Citrobacte*r and 5 *Providencia* were tested for their sensitivity to beta-lactam antibiotics.

In staphylococci, seven (7) beta-lactam antibiotics were tested: penicillin G (Oxoid 10 IU), oxacillin (Oxoid 5 µg), amoxicillin + clavulanic acid (Oxoid 30 µg), cefalotin (oxoid), ceftazidime (bioMérieux), cefotaxime (oxoid) and imipeneme (bioMérieux).

In enteric bacteria, eight (8) antibiotics of this family were tested: amoxicillin (oxoid) amoxicillin + clavulanic acid (Oxoid 30 µg), cefalotin (oxoid), ceftazidime (oxoid), cefotaxime (oxoid), cefuroxime (oxoid) and imipeneme (bioMérieux).

For the *Pseudomonas* three (3) antibiotics were tested: carbenicillin (oxoid), ceftazidime (bioMérieux) and imipeneme (bioMérieux).

Strains identification

Gram negative bacteria which belong to the enteric bacteria group were identified and used in a leminor galery which comprise urea-indole, Kligler Hajna, citrate Simmons. The identification was confirmed by the tests of ONPG and sugar fermentation.

For pyocyanic bacilli, enhancing pigment production galery was assayed with the King A and B media.

In the genus staphylococcus, Gram positive bacteria were isolated and identified by using cultural and biochemical characters using Chapman media (production or not of yellow pigment). Identification was confirmed by the tests of catalase and coagulase.

Strains susceptibility

Betalactam antibiotics susceptibility was determined by disk diffusion on Mueller Hinton (Sanofi pasteur) medium (Carret et al., 2001; Esmaeili et al., 2014). Discks of beta-lactam antibiotics have been used in natural resistance to each type or kind of bacteria. The method, Etest was also used as described by Saito et al. (2013).

The beta-lactamases were detected using a chromogen substrate, laboratories Oxoid nitrocefin. A colony of a growing strain was put in contact with the substrate on the strip of nitrocefin. After 5 to 10 min of contact, two (2) drops of reagent were added to the nitrocefinase. Hydrolysis by beta-lactamase substrate results in the development of a red color with a blue ring on the disk, which reveals the complex enzyme-substrate (Spicier, 2001). The beta-lactamase distribution was analyzed by excel.

RESULTS

The activities of the beta-lactam antibiotics tested on the staphylococci are given in Table 1 and those of the *Enterobacteriaceae* are given in Table 2. For the *Pseudomonas*, the rate of resistance to beta-lactams obtained is 44.14% with total inhibition of carbenicillin. Ceftazidime and the imipeneme were respectively

Table 2. Activity of beta-lactams in Enterobacteriaceae.

Beta-lactam tested	*E.coli*			*Proteus*			*Klebsiella*			*Enterobacter*		*Citrobacter*		*Providencia*	
	S(%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S(%)	R (%)	S (%)	R (%)	S(%)	R (%)
Amoxcillin	0		23 (100)	2 (9.0)		20 (91)	0	2 (16.7)	12 (100)	0	8 (100)	0	6 (100)	0	5 (100)
Amoxicillin + acid clavulanic	2 (8.7)	1 (4.3)	20 (87.0)	1 (4.5)	2 (9)	19 (86.5)	2 (16.7)		8 (66.6)	1 (12.5)	7 (87.5)	0	6 (100)	0	5 (100)
Céfalotin	2 (8.7)	1 (4.3)	20 (87.0)	13 (59)		9 (41)	2 (16.7)		10 (83.3	2 (25.0)	6 (75.0)	0	6 (100)	0	5 (100)
Ceftazidime	16 (69.7)		7 (30.4)	17 (77.3)		5 (22.7)	10 (83.3)		2 (16.7)	6 (75.0)	2 (25.0)	2 (33.3)	4 (66.7)	4 (80.0)	1 (20.0)
Céfuroxime	1 (4.3)		22 (95.7)	2 (9.0)		20 (91)	1 (8.3)		11 (91.7)	Not tested	-	Not tested	-	Not tested	-
Céfotaxime	3 (13.0)		20 (87.0)	7 (31.8)		15 (68.2)	2 (16.7)		10 (83.3)	Not tested		0	6 (100)	0	5 (100)
Imipénème	22 (95.7)		1 (4.3)	19 (86.4)		3 (13.6)	8 (66.6)	1 (8.4)	3 (25)	7 (87.5)	1 (12.5)	4 (66.7)	2 (33.3)	5 (100)	0
Céftriaxone	6 (26.1)		17 (73.9	12 (54.5)		10 (45.5)	7 (58.3)		5 (41.7)	5 (62.5)	3 (37.5)	2 (33.3)	4 (66.7)	4 (80.0)	1 (20.0)
Total (%)	25.2	8.6	73.8	41.5	1.1	57.4	33.3	3.1	63.6	43.7	56.3	16.7	83.3	32.5	67.5

inhibited by 21.62 and 10.82% of strains. The numbers of positive strains to the test of nitrocefin for each group of bacteria as well as the rate of beta-lactamases are respectively given in Table 3 and Figure 1.

DISCUSSION

Resistance of Staphylococci to tested beta-lactam antibiotics is not very high. These results are consistent with those given in the literature by several authors such as Aryam et al. (2005) and Tronel et al. (2002). These results can be explained by their use as first-line antibiotic.

The meticillino-observed resistance is due to the acquisition and integration of the mecA gene, which induces the synthesis of the PLP2a which is able to assemble Peptidoglycan with low affinity for oxacillin (methicillin) and other beta-lactam antibiotics (Fatholahzadeh et al., 2008; Benoit et al., 2013).

In enteric bacteria, the imipeneme and ceftazidime have been the most active beta-lactams. These results are consistent with that of Sotto et al. (2001) and Lagacé-Wiens et al. (2014). The sensitivity rates of these strains to ceftazidime, were compared with those obtained by Petra et al. (2007).

The association of amoxicillin with clavulanic acid has been completely inactive in some Enterobacteriaceae, and the other has presented a high resistance. This result can be explained by the modification of penicillin (PLP) binding protein which confers resistance to most of the beta-lactam antibiotics (Sotto et al., 2001). The cephalosporin's resistance may be due to the production of cephalosporinases as suggested by Bertrand and Talon (2001). In most cases, the mechanism of resistance to beta-lactams in Enterobacteriaceae is the production of beta-lactamases (Bedenic, 2004).

With regards to *Pseudomonas*, among the tested beta-lactam antibiotics, total resistance to carbenicillin was observed. This result is different from those obtained by Yu et al., (2006) which demonstrated that carbapenems remain the drugs of choice for serious infections caused by

Table 3. Distribution of positive beta-lactamase strains.

Bacteria tested	Total number of strains	Number of positive strains
S. aureus	42	3
CNS	10	9
E. coli	23	22
Proteus	22	19
Klebsiella	12	9
Enterobacter	8	6
Citrobacter	6	4
Providencia	5	5
P. aeruginosa	37	33

CNS = Coagulase negative Staphylococci.

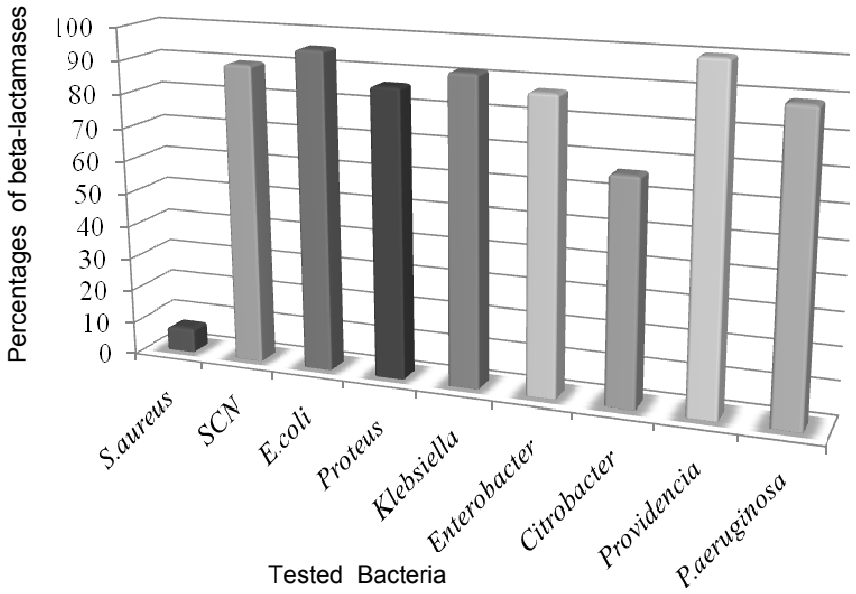

Figure 1. Distribution of beta-lactamases rates.

ESBL-producing organisms.

The ceftazidime was inactivated by 21.62% of strains and two strains of intermediate sensitivity were observed. This level of sensitivity presents a slight difference with the data of Sevillano et al. (2006). The appearance of resistance to ceftazidime in *Pseudomonas* may be a marker of the multi resistance for hyper-producing beta-lactamases strains which represents a factor of risk for the emergence of these strains (Cavallo et al., 2003).

The imipeneme has been the most active antibiotic with only 10.82% of resistant strains. These results are consistent with those obtained by Sinave (2003) and Soussy (2001). The development of resistance to the imipeneme is a risk factor for emergence of strains of *Pseudomonas* multi resistant strain (Rossilini and Mantengolie, 2005). However, high rates of resistance to the imipeneme and ceftazidime on hospital strains were obtained by Sekowska et al. (2005).

Most of the tested bacteria had shown high levels of beta-lactamases. These results have confirmed the work of Susić (2004), Akujobi (2005) and Bedenic (2004). The low rate obtained in *Staphylococcus aureus*, is explained by the resistance to oxacillin linked to the acquisition of the mec A gene. With regards to the strains of *Enterobacter*, beta-lactamases rates are comparable to those reported by Petra et al. (2007); Koren and Vaculíková (2006). These beta-lactamases can spread more often by the interspecific transfer of resistance between the cocci Gram positive and bacilli Gram negative by conjugation.

Conclusion

The results show that many bacteria present high resistance to the beta-lactam antibiotics. All the studied bacteria are beta-lactamase-producing. There is therefore a concordance between the resistance to these antibiotics and beta-lactamase production. The inhibition of these antibiotics is a major problem in the immediate support of the sick, because of their use as first line antibiotics.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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