Communications to the editor

β-LACTAMASES OF GRAM-NEGATIVE BACTERIA: SENSITIVITY OF AMPICILLIN, CEPHALORIDINE AND DICLOXACILLIN TO β-LACTAMASES PRODUCED BY ENTEROBACTERIACEAE

Sir:

β-Lactamases better known as penicillinases are bacterial enzymes, capable of hydrolizing the β-lactamic ring of penicillins. These enzymes may be produced by various bacterial species; three β-lactamases with different hydrolytic activity and immunological properties are produced by *Staphylococcus aureus*. *B. cereus* also produces two β-lactamases[1].

In addition to such enzymes excreted by Gram-positive bacteria, there are others, with different biological properties, produced by Gram-negative species. These enzymes can be divided into three groups[2].

1) β-Lactamases produced by certain strains of *E. coli*, *Proteus*, *Pseudomonas* and *Mycobacteria* with activity on penicillin G and to an even greater extent on cephalosporins.

2) β-Lactamases produced by some strains of *Klebsiella* and *Proteus mirabilis* with activity on penicillin G, ampicillin and cephalosporins.

3) β-Lactamases by *E. coli* strain R 1818, is active on all the penicillins as well as on cephalosporins; consequently they have the same characteristics as β-lactamases II of *B. cereus*.

The production of β-lactamases by Gram-negative bacteria therefore, has particular importance in connection with the resistance of these strains to broad spectrum penicillins such as cephalosporins, antibiotics recently used in human therapy. Consequently we proposed to carry out research on the production of β-lactamases by Gram-negative strains recently isolated from clinical cases.

One hundred and thirty-six strains of Gram-negative bacteria (*E. coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus* and *Pseudomonas*) were studied. All strains were isolated during 1969 from patients, with various diseases caused by these organisms under antibiotic treatment. After isolation the strains were typed and their sensitivity spectrum to the most common antibiotics was determined.

For determining possible β-lactamase activity of these strains, the following method was used: Bacteria were cultivated for 24 hours at 37°C in B.B.L. Nutrient Broth, whereupon the cultures were centrifugated for 10 minutes at 4,000 r.p.m. To three portions of 1 ml of the supernatant, was added 1 ml of a solution of ampicillin, respectively cephaloridine or dicloxacillin.[3,4]

Dicloxacillin was used because this semisynthetic penicillin is particularly resistant to staphylococcal penicillinase, though it has only a low activity against Gram-negative bacteria.[5]

After 1 hour in a 37°C water-bath, the tubes were heated to 80°C in order to destroy remaining β-lactamase activity. Finally a microbiological titration of remaining antibiotic activity was carried out. This titration for all the three antibiotics was made using *Sarcina lutea* ATCC 9341 as a test organism at a pH 6.6, on a solid medium, according to the GROVE and RANDALL technique[6].

Among 136 strains of Gram-negative bacteria examined 61 strains (44%) exhibited

Table 1.

136 strains of Gram-negative bacteria were divided in β-lactamases producers (+) and no producers. The β-lactamases (+) strains were further subdivided with respect to their capacity to neutralize ampicillin (A), cephaloridine (C) and dicloxacillin (D). Values are expressed as percentage of each class and total number of bacterial strains.

	A	C	D	% (61)	% (136)
15	+	+	+	25	11
10	+	+		16	7
2	+		+	3	1
5	+			8	4
29		+		48	21
61				100	44
	32			52	23
		54		88	40
			17	28	12

Table 2.

Strains of Gram-negative bacteria investigated in the present study divided in respect to their production of β-lactamases active on ampicillin (A), cephaloridine (C) and dicloxacillin (D).

	Tentative identification	No. of strains
A C D + + +	*Escherichia coli*	8
	Escherichia coli freundii	3
	Proteus mirabilis	1
	Proteus rettgeri	2
	Shigella sp.	1
	Total	15
A C D + +	*Escherichia coli*	1
	Klebsiella pneumoniae	1
	Total	2
A C D + +	*Shigella* sp.	1
	Proteus mirabilis	2
	Providencia gr.	2
	Klebsiella pneumoniae	3
	Escherichia coli	2
	Total	10
A C D +	*Proteus mirabilis*	3
	Escherichia coli	1
	Salmonella sp.	1
	Total	5
A C D +	*Shigella* sp.	4
	Escherichia coli	4
	Pseudomonas aeruginosa	1
	Proteus rettgeri	7
	Klebsiella pneumoniae	2
	Providencia gr.	1
	Escherichia coli freundii	3
	Proteus morganii	2
	Proteus vulgaris	5
	Total	29

different degrees of β-lactamase activity on ampicillin, cephaloridine and dicloxacillin. In fact (see Table 1) among these 61 strains, 15 (25 %) have the capacity of neutralizing all three antibiotics; 10 (16 %) act on ampicillin and cephaloridine and 2 (3 %) on ampicillin and dicloxacillin, where as 5 strains (8 %) neutralize only ampicillin and 29 (48 %) act only on cephaloridine. Examining Table 1 by another viewpoint, we may observe that among the 61 β-lactamase-producing strains, 32 (52 %) inhibit ampicillin, 54 (88 %) cephaloridin and 17 (28 %) dicloxacillin. It is evident that among these three antibiotics cephaloridine, is the one most commonly inactivated by Gram-negative β-lactamases.

It is very curious that certain β-lactamases (see Table 1) possessing considerable activity on ampicillin and on dicloxacillin, are totally inactive on cephaloridine.

This suggests that the β-lactamases produced by one particular strain may represent more than one type and with different degrees of affinity and activity on the three antibiotics. Many Gram-negative bacterial species (Enterobacteriaceae) are able to produce these β-lactamases: in fact (see Table 2) *E. coli*, *Proteus*, *Klebsiella*, *Salmonella*, *Providencia* group and *Pseudomonas* produce distinct types of enzymes.

The number of bacterial strains examined is insufficient for a statistical study in order to determine the characteristic presence of one or two species. On the other hand, the capacity of Enterobacteriaceae to transmit genetic characters by conjugation of one species with another, also in biological fluids, makes typing of the strains and the resolution of this question difficult.[7,8,9]

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