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MULTIPLE B LACTAMASE ENZYMES PRODUCING CLINICAL ISOLATES OF GRAM NEGATIVE BACTERIA IN A TEACHING HOSPITAL

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ABSTRACT

Context: *Gram negative bacilli producing Beta lactamases have been increasingly reported worldwide and infections with such bacteria are difficult to treat. It is also not unusual to find a single isolate that expresses multiple beta lactamase enzymes further complicating the treatment options.* ***Aims:*** *The present study was designed to investigate the coexistence of different beta lactamase enzymes in clinical isolates of gram negative bacilli.* ***Material and Methods:*** *A total of 321 isolates of gram negative bacilli obtained from various clinical specimens were included in the study. Antimicrobial susceptibility testing was performed for all the isolates in accordance with CLSI guidelines. All bacterial strains were tested for ESBL, Amp. C & MBL production.* ***Statistical analysis used:*** *Descriptive statistics was used and the percentage of ESBL, Amp C and MBL carrying gram negative bacilli isolates were calculated.* ***Results:*** *ESBL production was seen in 100 (31.1%) isolates with maximal incidence in Citrobacter species (52.1%), followed by P. aeruginosa (30.4%). Amp C production was detected in 67 (27.8%) isolates with highest percentage (25.4%) among non-fermenters.* ***Conclusions:*** *Early detection of these multiple β lactamase producing isolates in a routine laboratory could help to avoid treatment failure, as often such isolates show a susceptible phenotype in routine sensitivity testing. Unless strict measures to limit the indiscriminate use of cephalosporins and Carbapenems in the hospitals are undertaken, the multiple β lactamase producing pathogens would spread with no treatment options left to treat nosocomial infections with such pathogens.*

KEY WORDS

ESBL, Amp C, MBL.

INTRODUCTION

Gram negative bacilli account for the majority of bacterial pathogens isolated from clinical specimens.[1]The incidence of infections due to Gram negative bacilli resistant to β lactam agents has increased in recent years. Till 2006, ESBL production by GNB was considered as the most important threat to clinical therapeutics. [2, 3].This led to a parallel increase in the usage of β Lactam/ β lactamase inhibitor combinations, monobactams and carbapenems. Eventually, in the last few years, reports from worldwide show resistance to these drugs as well. [4, 5]

The resistance to monobactams and carbapenems is due to the production of Amp.C and Metallo beta lactamases respectively. The genes coding for these β lactamases are carried on plasmids, facilitating rapid spread between micro-organisms and often are co-expressed in the same isolate.[6] The treatment options for such infection are limited and hence of great concern. Hence the present study was designed to investigate the presence of different classes of β lactamase enzymes in the clinical isolates of gram negative bacilli.

MATERIAL AND METHODS

The study was conducted in the department of microbiology of our medical college hospital. A total of 321 consecutive, non-duplicate isolates of gram negative bacilli obtained from various clinical specimens were included in the study. The isolates were characterized by using standard microbiological techniques.[7] Antimicrobial susceptibility testing was performed for all the isolates by using the commercially available discs [Himedia, Mumbai, India] in accordance with CLSI guidelines.[8] The antibiotics which were tested include, Piperacillin 100µg ([PIP), Ceftazidime 30 µg (CAZ), Imipenem 10 µg (IPM), Ciprofloxacin 5 µg (CIP), Gentamycin 10 µg (GEN), Amikacin 30 µg (AK) and Aztreonam 30 µg (ATM). Quality control was achieved using standard strains of E.coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853.

All bacterial strains were tested for ESBL, Amp. C & MBL production by the following methods.

ESBL detection method: ESBL status of the isolates was detected by combined disc diffusion using Cefotaxime 30 µg [CTX] &Ceftazidime 30 µg [CAZ] disc alone and in combination with Clavulanic acid [CEC & CAC] as per CLSI recommendations.

Amp C detection method: The isolates were tested for Amp C production by the disc antagonism test. A test isolate [with a turbidity equipment to that of 0.5 McFarland standard) was spread over a Mueller Hinton agar plate. Cefotaxime 30µg (CTX) and Cefoxitin 30 µg (Fox) discs were placed 20mm. apart from centre to centre. Isolates showing blunting of CTX zone of inhibition adjacent to the Fox disc were taken as Amp C producers.

MBL detection method: The isolates were screened for the presence of MBLs by the combined disc test (CDT). Two Imipenem 10 µg discs were placed on the surface of an agar plate with bacterial inoculum and 5 µl EDTA was added to one of them to obtain a concentration of 750 µg. The zones of inhibition of IPM alone and IPM-EDTA were compared after 16-18 hours incubation in air at 35^{o}C. An increase in zone size of >7mm was taken as positive.

Descriptive statistics was used and the percentage of ESBL, Amp C and MBL carrying gram negative bacilli isolates were calculated.

RESULTS

A total of 321 bacterial isolates were included in the study. **Table 1** depicts the different bacterial species tested and their resistance pattern.

ESBL production was noticed in 100 (31.1%) isolates with maximal incidence in Citrobacter species (52.1%, n=12), followed by P. aeruginosa (30.4%, n=32).

Amp C production was detected in 67 (27.8%) isolates. Majority of P.aeruginosa strains (28.4%, n=30) produced Amp C β lactamases. imipenem resistance was seen in a mere 11(30.9 %) strains, whereas resistance to ciprofloxacin was seen in 108 (95.5 %) strains (**Table 2**).

Co-production of Amp C β lactamase and ESBL was seen in 17.1% (n=55) strains and MBL production was detected in 11 isolates. Co-production of Amp C and Metallo β lactamases was found in 1.2% (n=4) isolates with maximal occurrence among Acinetobacter species (2.3 %) isolates. (**Table 3**)

Table 1: Resistance pattern of bacterial isolates:

Isolate	No.	PIP	CIP	GEN	AK	ATM	CAZ	IPM
E.coli	60	40	39	60	14	9	19	1
Klebsiella species	72	72	30	24	16	12	23	4
Citrobacter species	23	23	23	23	23	2	14	1
Proteus species	18	2	6	4	--	--	1	--
Pseudomonas species	105	92	84	75	53	34	32	3
Acinetobacter	43	41	35	40	31	10	11	2
Total	321	270	217	226	137	67	100	11

Table 2: ESBL & Amp C production in bacterial isolates.

Clinical isolate	No. of isolates	ESBL producers (%)	Amp C producers	Both ESBL & Amp c
E.coli	60	16 (26.6%)	9(15%)	5 (8.3%)
Klebsiella species	72	20(27.7%)	11 (15.2%)	8(11.1%)
Citrobacter species	23	12 (52.1%)	2(8.6%)	6 (26.0%)
Proteus species	18	1(5.5%)	- (-)	- (-)
Pseudomonas species	105	32 (30.4%)	30 (28.4%)	21 (20.0%)
Acinetobacter	43	11 (25.5%)	9 (20.9 %)	9(20.9%)
Total	321	100 (31.1%)	67 (20.8%)	55(17.1%)

Table 3: MBL & Amp C production

	MBL producers	Amp C	Both MBL + Amp C
E.coli	1 (1.6)	9(15%)	-
Klebsiella species	4 (5.5)	11 (15.2%)	-
Citrobacter species	1(4.3)	2(8.6%)	-
Proteus species	-- (-)	- (-)	-- (-)
Pseudomonas species	3(2.8)	30 (28.4%)	2 (1.9)
Acinetobacter	2(4.6)	9 (20.9 %)	2 (2.3)
Total	11(3.4)	67 (20.8%)	4(1.2)

DISCUSSION

Gram negative bacterial isolates show a multiplicity of resistance mechanisms. ESBL producing strains of GNB have emerged as a major problem in hospitalized on well as community based patients.[9] The incidence of ESBL in major hospitals of India has been reported as high as 60%-80%.[10,11] Their prevalence worldwide has been non-uniform. US hospitals have reported 40% of Klebsiellapneumoniae isolates as ESBL producers whereas reports from Taiwan show 94% of Klebsiella species as ESBL producers. [12,13]

In our study 31.1% of total GNB included, showed ESBL production with the highest incidence in Citrobacter species (52.1%) followed by P.aeruginosa (30.1%). Higher percentages of ESBL producing GNB were shown by other studies [14,15]whereas reports from Chennai [16] and Hyderabad [17] show lower percentages of ESBL producers.

Shortly after ESBLs, Amp C β lactamase emerged which were resistant to 3rd generation Cephalosporin including β-lactam/ β lactamase inhibitor (in contrast to ESBL) but sensitive to 4th generation cephalosporins. In 2003, 20.7% Amp C producers were reported from Delhi, [18] 37% from Chennai.[19] The numbers of Amp C producers has been increasing over the years.

In our study 27.8% of GNB isolates showed Amp C production with highest percentage (25.4%) among non-fermenters. Some hospitals have reported high percentage (up to 80%) of Amp C producers.[20]

The only β lactam active against Co-Amp C and ESBL producers are Carbapenems, however, recently resistance to Carbapenems has been increasing, which is mostly due to production of MBL.[21] Our findings showed 3.4% of the bacterial isolates produced MBLs and 1.2% strains produced both Amp C and MBLs. Carbapenemases have been reported in E.coli, Klebsiella species. Pseudomonas species and Acinetobacter species from different parts of the globe. The percentage various widely with some centres reporting low figures (48% in Acinetobacter) where as others showing upto 80% (Acinetobacter species). [22] Lower resistance to imipenem in our centre may probably due to the reserved use of thus drug.

An interesting finding was that 2 isolates were sensitive to imipenem by routine disc diffusion method but showed MBL production by CDT (IPM-EDTA).

These carbapenem susceptible isolates carrying hidden MBL genes, may spread unnoticed and may lead to untoward infection control problems. As there is no single method proven as ideal method for MBL detection in all the isolates, we used the CDT, which is recommended by CLSI and proven by many other studies.

CONCLUSION

The present study emphasizes the prevalence of gram negative bacilli producing β lactamase enzymes of diverse mechanisms. Early detection of these multiple β lactamase producing isolates in a routine laboratory could help to avoid treatment failure, as often such isolates show a susceptible phenotype in routine sensitivity testing. Unless strict measures to limit the indiscriminate use of cephalosporins and Carbapenems in the hospitals are undertaken, the multiple β lactamase producing pathogens would spread with no treatment options left to treat nosocomial infection with such pathogens.

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