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RESEARCH ARTICLE

Detection of ESBL Producing Gram Negative Bacteria and its Sensitivity Pattern in Intensive Care Unit

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ABSTRACT

Extended Spectrum of Beta Lactamase (ESBL) producing organisms are the major problem and great challenge to clinicians, microbiologists and infection control professionals. These enzymes compromise the efficacy of all beta lactums by hydrolysing the beta lactum ring. In this study, total of 95 gram negative isolates were isolated from various clinical samples from ICU patients and subjected for antibiotic susceptibility testing. These isolates were screened for ESBL and confirmed by combined disc diffusion method. Out of 95 GNB, 37 were ESBL producers. They are highly sensitive to imipenem, followed by ofloxacin, cefoperazone-sulbactum, amikacin and amoxyclav. Proper antibiotic usage and promoting awareness will help in preventing the multidrug resistance among the organisms.

KEYWORDS

ESBL, GNB, ICU

INTRODUCTION

The emergence of Extended Spectrum Beta Lactamase (ESBL) after the successful use of 3rd generation cephalosporin in medical field, further limits the therapeutic options for the clinicians[1]. ESBLs are encoded by plasmids and they have the ability to hydrolyse the penicillins, cephalosporins and monobactams[2].

The drug resistance among bacterial pathogens are of major concern, because of the limited availability of newer antibiotics as well as dissemination of these resistances to the other group of antibiotics such as, aminoglycosides, trimethoprim, sulphonamides, tetracycline and chloramphenicol, as genes for these antibiotics also located on large plasmids codes for ESBL[3,4].

Intensive care unit (ICU) is a favourable and potential place for infections due to the immunocompromised population, frequent use of invasive procedures and nursing pave the way for the growth and transmission of infections and antibiotic resistance[5].

This study was done to isolate the ESBL strains from various clinical samples and to work on its antibiotic susceptibility pattern against various antibiotics in Intensive care unit patients.

MATERIAL AND METHODS

The study was carried out in Microbiology department, at Stanley Medical College, Chennai. Total of 110 samples were collected (urine, tracheobroncheal aspirates, blood, wound

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swab and drainage tube tip) from patients admitted in intensive care unit (ICU).

Ethical committee clearance was obtained from the Institute and informed consent was obtained from all the patients. All the samples were inoculated onto nutrient agar, blood agar and incubated at 37°c overnight. Using standard biochemical tests for gram negative bacilli, colonies were confirmed[6,7].

The antibiogram was performed by Kirby Bauer disc diffusion technique on Muller Hinton Agar with commercially available discs (Hi-Media) Gentamycin (10mcg), Amikacin (30mcg), cotrimoxazole, Ciprofloxacin (5mcg), Ofloxacin (5mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefotaxime (30mcg), Piperacillin (10mcg), Piperacillin Tazobactam (100/10mcg), Amoxyclav20/10 (30mcg), Cefoperazone-Sulbactam (75/15mcg) and Imipenum (10mcg).

Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[8]

Phenotypic Detection of ESBL – Screening Method[8]

ESBLs production was performed according to the CLSI Screening procedures, using indicator cephalosporins, ceftriaxone (30µg), ceftazidime (30µg), and Cefotaxime (30µg). Isolates exhibiting zone size ≤ 25mm with ceftriaxone ≤ 22mm for ceftazidime and ≤ 27mm with cefotaxime were considered as ESBLs producer.

ESBL- Combined Disc Diffusion Method[8]

From the colonies of gram negative bacilli, 0.5 McFarland's turbidity standard suspension was prepared. Lawn culture was made on Muller Hinton Agar plate with this inoculum. Discs of Ceftazidime and Ceftazidime + Clavulanic acid (30 mcg/10 mcg) were placed aseptically on the surface of MHA. The distance of 15mm was kept between the disc and overnight incubation was done at 37°C. An increase of ≥ 5mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the ESBL production by the organisms.

RESULTS

Table 1: Distribution of gram negative bacteria and specimens

Isolates (n=95)	***Percentage***	***E.coli***	***Klebsiella spp***	***Proteus spp***	***Pseudomonas aeruginosa***	***Acinetobacter spp***
Urine (n=48)	50.5	22	12	10	3	1
Drainage tube tips (n=16)	16.8	7	3	3	3	0
Wound swab (n=13)	13.7	3	2	3	5	0
Blood (n=9)	9.5	2	6	0	0	1
Tracheo broncheal aspirates (n=9)	9.5	2	6	1	0	0
Total		36 (37.9%)	29 (30.5%)	17 (17.9%)	11 (11.6%)	2 (11.6%)

Among the 95 isolates of gram negative bacilli, *Escherichia coli* and *Klebsiella* spp accounts the predominance of 37.9% & 30.5%.

Table 2: Distribution of ESBL producers among the GNB

Species	Total number	ESBL producers	Percentage (%)
E.coli	36	16	43.3
Klebsiella spp	29	13	35.1
Proteus spp	17	4	10.8
Pseudomonas aeruginosa	11	4	10.8
Acinetobacter spp	2	0	0
Total	95	37	38.9 %

Among the 95 GNB, 38.9% were ESBL producers. 43.3% of *E.coli* & 35.1% of *Klebsiella* spp were predominant ESBL producers.

Table 3: Antibiotic Sensitivity pattern of ESBL producing GNB

Antibiotics (mcg)	Number of isolates (n=37)	Percentage (%)
Imipenem (10)	36	97.3
Ofloxacin (5)	27	72.9
Cefoperazone-Sulbactam (75/15)	25	67.6
Amikacin (30)	24	64.8
Amoxyclav (30)	24	64.8
Piperacillin-Tazobactam	15	40.5
Gentamycin (10)	9	24.3
Ciprofloxacin (5)	9	24.3
Piperacillin (100)	8	21.6
Cotrimoxazole	5	13.5

100% sensitivity was observed with Imipenem. Ofloxacin, Cefoperazone-Sulbactam, Amikacin, and Amoxyclav showed sensitivity of 72.9%, 67.6%, 64.8% and 64.8 % respectively.

DISCUSSION

Infections in ICU along with drug resistant organisms are dangerous and are associated with increased morbidity and mortality (10).Initially it was confined to *Klebsiella* spp but now it is disseminated to all members of enterobacteriaceae and other bacteria also[11].

In our study conducted in ICU, among the total 95 GNB isolates, 37 (38.9%) were ESBL producers. 35.5%, 35.5% and 39.8% were the ESBL producers in the studies of, Loveena Oberon *et al*, Anago *et al* and Kashyap *et al*. Our study is in accordance with these reports.

Slightly higher rate of ESBL production, 52.49% and lower rate, 13.66% were observed with the studies of Meeta Sharma *et al* and Tammana *et al*, respectively.

A very high rate, 74% of ESBL production was noted in ICU patients by Harakuni *et al*. Various Indian studies revealed the rate of ESBL production varies between 25 to 80%. It has been proved that the prevalence of ESBL among clinical isolates varies from country to country and institution to institution with in the same country.

Urine was the predominant sample (51.4%) of isolation of ESBL strains in this study. It is concordant with the studies of Ndugulile *et al*, Loveena Oberon *et al*., Shanthi M *et al* & Tammanah *et al*, whereas respiratory tract samples & blood were the predominant samples of isolation in the reports of Meeta Sharma *et al* & Vemula *et al*, respectively.

Among the 37 ESBL strains of GNB isolated in our study, *Escherichia coli* (43.3%) were the predominant species, followed by *Klebsiella pneumoniae* (35.1%). *E.coli* and *Klebsiella* were the predominant species of ESBL producers, reported in various studies. It was 46.51% & 44.44 % ESBL producers in the study of Variya *et al*, India. In Pakistan, the prevalence rate reported were, 41% in *E.coli* and 36% in *Klebsiella pneumonia*, by Jabeen *et al*. Similar predominance were also observed by Anago *et al*, Ndugulile *et al* and Tammanah *et al*. Whereas *Klebsiella* was the predominant species of isolation in the study of Meeta Sharma *et al* and 72 % were Acinetobacter spp in the study of Ali *et al*.

In our current study, imipenem was found to be the highly sensitive drug to ESBL producing strains. Similar high susceptibility rate to imipenem were reported by Anago *et al*, Meeta Sharma *et al*, Muvunyi *et al* and Mohammedi-Mehr *et al*. This could be due to the rationale use, stability and high activity of carbapenem against most β lactamases.

Moderate sensitivity of 72.9%, 67.6%, 64.8 % and 64.8% was observed to ofloxacin, cefoperazone-sulbactum, amikacin and amoxyclav, respectively. Increased and inappropriate use of this group of drugs may lead to resistance in future.

In our study, ESBL strains isolated in ICU were found to less sensitive to gentamycin, ciprofloxacin, pipercillin and cotrimoxazole. Easy availability, indiscriminate use and inappropriate prescription may be the reason behind this.

CONCLUSION

In ICU, the prevalence of ESBL producing gram negative bacteria emphasizes the need for an early screening for beta lactamases which could be useful in providing an appropriate treatment and helps in preventing the development of multidrug resistant strains. Based on the epidemiological data and institutional guidelines, every health care centres must concentrate on its own antimicrobial policies to improve the patient outcome and cost effective therapy. Continuous surveillance of ICU and proper implementation of infective control measures are very essential.

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