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DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY OF ESBL POSITIVE ENTEROBACTERIACEAE ISOLATES IN VARIOUS CLINICAL SPECIMENS OF PATIENTS ADMITTED IN CRITICAL CARE AREAS

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ABSTRACT

Extended spectrum *β*-lactamases (ESBLs) pose a major problem in clinical therapeutics as infections caused by the organisms producing such enzymes are associated with a higher morbidity and mortality. Critical care areas (CCA) in a hospital are the high pressure area where the microbes isolated may be multidrug resistant. The present study was undertaken at a tertiary care healthcare setup in New Delhi, India to look for the distribution of ESBLs producing *Enterobacteriaceae* isolates in various clinical specimens of patients admitted in CCA and to study their antimicrobial susceptibility pattern. A total of one hundred *Enterobacteriaceae* isolates from CCA were studied. The strains were identified by API system and their antimicrobial susceptibility was tested by API and VITEK systems. ESBLs production was detected by CLSI, Double Disc Diffusion (DDS) and Vitek methods. The maximum number of *Enterobacteriaceae* isolates was obtained from respiratory tract (50%) but maximum ESBLs positivity was seen in isolates from blood stream infections (85%). ESBLs production was seen in 66% isolates. The antibiotic resistance rate was more for ESBLs positive isolates in comparison to ESBLs negative isolates. Detection of ESBLs producing strains should be routinely done in clinical microbiology laboratory to prevent misuse of antimicrobials leading to large scale benefits to society.

KEY WORDS

ESBL, critical care area, *Enterobacteriaceae*

INTRODUCTION

Antibiotic resistance among nosocomial pathogens is a cause of major concern. Traditionally, beta lactam antimicrobial agents have been the mainstay of our fight against bacterial pathogens. A common mechanism of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamase enzymes that break down the structural beta lactam ring of penicillin-like drugs. Genetic control of beta-lactamase production resides either on plasmids or on the chromosome, while expression is either constitutive or inducible[1].
With the introduction of each generations of beta lactams, selection of strains that have either produced large quantities of beta lactamases or have mutant beta lactamases with extended spectrum has become a norm, especially in Gram negative bacteria. Even the introduction of beta lactamase stable beta lactams has not been able to stop these evolutionary processes. The presence of Extended Spectrum beta lactamases (ESBLs) in Gram negative bacilli have thus been associated with high pressure of antimicrobials especially beta lactams. The ESBLs are penicillinases that possess an extended hydrolysis spectrum directed towards third generation cephalosporins. ESBLs can confer resistance against all beta lactam drugs (penicillins, 1st, 2nd and 3rd generation cephalosporins, monobactams) except carbapenems and cephamycins[2].

Plasmid mediated ESBLs result in cross strain transmission, thereby spreading resistance among related and unrelated Gram negative bacteria. ESBLs pose a major problem for clinical therapeutics. It is necessary to identify the prevalence of these strains in hospitals, and to determine suitable prevention measures and treatment policies.

The various risk factors for the ESBLs production are the severity of illness, time in intensive care unit (ICU), intubation, mechanical ventilation, urinary or arterial catheterization and previous exposure to antibiotics. Critical care areas are such high pressure areas where the patients are at an increased risk of infection with ESBLs producing strains.
The test modalities employed for antimicrobial susceptibility of isolates from clinical specimens may not depict the presence of such ESBLs producing bacteria. Most standardized guidelines for the detection of ESBLs have been from Clinical Laboratory Standards Institute (CLSI) but this lacks commitment to extend these beyond *Escherichia coli*, *Klebsiella* and *Proteus* to a large extent. Many other techniques like Double Disc Synergy (DDS) and automated microbroth (VITEK) have also been described[3,4].
This study was undertaken to look for production of ESBLs in *Enterobacteriaceae* isolates from various clinical samples of patients admitted in critical care areas and to compare the antimicrobial susceptibility pattern of ESBLs positive and negative isolates.

MATERIAL AND METHODS

This study was conducted at the department of Microbiology, Indraprastha Apollo Hospitals, New Delhi. A total of 100 isolates of gram negative bacilli belonging to family *Enterobacteriaceae* isolated from different clinical specimens i.e. blood, urine, respiratory tract secretions etc, of patients admitted in Critical Care Areas (CCA) were studied in the year 2004-2005. Critical Care Area comprises of Intensive care units (ICU) and High Dependency Units (HDU). The selection criteria

of strains were: (a) Patient had been exposed to critical care area (ICU and/or HDU) for 48 hrs. (b) Repeat isolates of the same organism from the same type of sample (e.g. both isolates from blood obtained within 72 hrs.) were not taken. (c) Isolates from consecutive samples obtained that day were not taken. (d) Similar isolates from central venous pressure (CVP) or internal jugular (IJ) or other intravenous (I/V) access device and blood cultures; foley's tip and urine culture were not taken into consideration if obtained within 72hrs of each other. Strains were identified by API system using rapid ID 32 E strip. Antimicrobial susceptibility test was done by API using rapid ATB E 4 strip and VITEK using GNS card. Production of ESBLs was detected through disc diffusion tests using CLSI and Double Disc Synergy (DDS) method. Standard method of CLSI was used using Ceftazidime (30µg) and Ceftazidime/Clavulanic acid (30/10µg) discs. Similarly, standard DDS method was used using Cefotaxime (30µg) and Amoxycillin/Clavulanic acid (20/10 µg) discs giving a centre-to-centre distance of 15mm. ESBLs production in *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* was also detected by using Vitek system. *Escherichia coli* ATCC35218 was used as a negative control and *Klebsiella pneumoniae* ATCC700603 was used as a positive control. The Vitek based minimum inhibitory concentration (MIC) of both beta lactam and non-beta lactam antimicrobial agents were also evaluated for ESBLs positive and negative isolates.

RESULTS AND DISCUSSION

The total number of ESBLs positive isolates was 66 (66%). The distribution of various ESBLs positive isolates amongst various samples is given in table-1. The resistance to various antimicrobial drugs in major ESBLs positive and negative *Enterobacteriaceae* isolates from CCA is given in table-2. The minimum inhibitory concentration (MIC) values of various drugs based on Vitek method is given in table-3.

Table-1

Distribution of various ESBLs positive isolates amongst various clinical samples in CCA

Organism	Respiratory tract		Urinary tract		Blood stream		Pus/wound swab		Body Fluid	
	Total number	ESBLs positive (%)	Total number	ESBLs positive (%)	Total number	ESBLs positive (%)	Total number	ESBLs positive (%)	Total number	ESBLs positive (%)
Klebsiella pneumoniae	15	11 (73.3)	4	4 (100)	11	10 (90.9)	2	2 (100)	1	1 (100)
Escherichia coli	8	5 (62.5)	8	5 (62.5)	2	1 (50)	3	2 (66.7)	-	-
Enterobacter cloacae	8	4 (50)	1	0 (0)	1	1 (100)	1	0 (0)	-	-
Serratia marcescens	6	3 (50)	1	1 (100)	4	3 (75)	-	-	-	-
Proteus mirabilis	4	3 (75)	4	2 (50)	-	-	-	-	-	-
Klebsiella oxytoca	2	2 (100)	1	1 (100)	1	1 (100)	-	-	-	-
Citrobacter freundii	2	0 (0)	1	0 (0)	-	-	-	-	-	-
Klebsiella ornithinolytica	1	1 (100)	1	0 (0)	1	1 (100)	-	-	-	-
Morganella morganii	1	0 (0)	1	0 (0)	-	-	-	-	-	-
Pantoea spp.	2	1 (50)	-	-	-	-	-	-	-	-

Providencia rettgerii	-	-	1	1 (100)	-	-	-	-	-	-
Providencia stuartii	1	0 (0)	-	-	-	-	-	-	-	-
Total	50	30 (60)	23	14 (60.9)	20	17 (85)	6	4 (66.7)	1	1

Table-2

Antimicrobial resistance percentage (%) of ESBLs positive and negative Enterobacteriaceae isolates in CCA

Antibiotic	*Klebsiella pneumoniae*		*Escherichia coli*		*Enterobacter cloacae*		*Serratia marcescens*		*Proteus mirabilis*		*Klebsiella oxytoca*	
	Pos (28)	Neg (5)	Pos (13)	Neg (8)	Pos (5)	Neg (6)	Pos (7)	Neg (4)	Pos (5)	Neg (3)	Pos (4)	Neg (0)
Ampicillin	100	100	100	100	100	100	100	75	100	100	100	-
Amoxyclav	78.3	0	92.3	100	100	83.3	100	75	20	33.3	50	-
Ticarcillin+ clavulanic acid	57.4	0	53.8	75	60	83.3	14.2	25	0	0	50	-
Piperacillin+Tazobactam	25.7	0	7.7	25	20	50	14.2	25	0	0	0	-
Ceftriaxone	96.4	0	100	62.5	100	83.3	100	25	100	0	100	-
Ceftazidime	78.5	0	100	62.5	100	83.3	100	25	100	0	100	-
Cefepime	60.7	0	84.6	62.5	100	66.7	100	25	100	0	50	-
Imipenem	0	0	0	0	0	0	0	0	0	0	0	-
Meropenem	0	0	0	0	0	0	0	0	0	0	0	-
Gentamicin	60.7	0	69.2	87.5	100	66.7	85.7	50	100	0	25	-
Netilmicin	64.2	0	84.6	87.5	100	50	100	50	100	0	50	-
Tobramicin	53.5	0	92.3	100	100	66.7	100	50	100	0	50	-
Amikacin	64.2	0	38.4	25	40	50	85.7	0	100	0	25	-
Ciprofloxacin	60.7	0	100	75	40	50	14.2	50	100	0	75	-
Ofloxacin	64.2	0	100	75	20	50	28.5	50	100	0	75	-
Cotrimoxazole	57.1	20	61.5	87.5	80	33.3	100	50	100	33.3	100	-

Table-3

MIC values of ESBLs positive and negative Enterobacteriaceae isolates for various antimicrobial agents

Drug		MIC (µg/ml)	No. of ESBL positive isolates	No. of ESBL negative isolates
Ampicillin	S	≤8	-	-
	I	16	-	1
	R	≥32	66	33
Piperacillin	S	≤16	4	17
	I	32-64	6	3
	R	≥128	56	14
Cephalothin	S	≤8	-	11
	I	16	-	2
	R	≥32	66	21
Cefotaxime	S	≤8	15	22
	I	16-32	9	1
	R	≥64	42	11
Ceftazidime	S	≤8	2	22
	I	16	16	1
	R	≥32	48	11
Cefepime	S	≤8	28	26
	I	16	5	-

	R	≥32	33	8
Ampicillin +Sulbactam	S	≤8/4	4	10
	I	16/8	9	5
	R	≥32/16	52	19
Piperacillin+Tazobactam	S	≤16/4	34	20
	I	32/4-64/4	11	8
	R	≥128/4	21	6
Imipenem	S	≤4	66	34
	I	8	-	-
	R	≥16	-	-
Meropenem	S	≤4	66	34
	I	8	-	-
	R	≥16	-	-
Gentamicin	S	≤4	18	21
	I	8	-	2
	R	≥16	48	11
Ciprofloxacin	S	≤1	18	22
	I	2	-	-
	R	≥4	48	12
Cotrimoxazole	S	≤2/38	15	22
	I	-	-	-
	R	≥4/76	51	12

Key: S=Susceptible, I=Intermediate, R=Resistant

The isolation of various gram negative bacilli from CCA of a health care set up has been variable in different studies. Our study demonstrated *Klebsiella pneumoniae* to be the most prevalent (33%) of the *Enterobacteriaceae* isolates followed by *Escherichia coli*. This has been corroborated by other studies also[5,6]. Also, there are studies which show *Escherichia coli* to be the most prevalent nosocomial isolate followed by *Klebsiella pneumoniae*[7,8,9]. Variable isolation can be attributed to derivation of *Enterobacteriacaeae* from different sources. *Escherichia coli* is almost invariable derived from gut of the patient whereas *Klebsiella* has been derived primarily from gut but also from environmental sources. Preponderance of *Klebsiella pneumoniae* in our study cannot be attributed to such sources as our study did not relate to environmental influences.

In our study maximum isolates were from respiratory tract (50%) although urine and blood constitute the maximum samples sent to our laboratory. Similar picture has been revealed by Orrett and Heinberger *et al* who found that the most common source for all aerobic gram negative bacilli isolates in ICU was respiratory tract[6,9]. However, this is at variance with the study of Shah *et al* on nosocomial and outdoor isolates where they found maximum isolates from urine[7]. In our study *Klebsiella pneumoniae* was the most common amongst the *Enterobacteriaceae* implicated in respiratory tract and blood stream infections. This is in contrast to the finding of the international SENTRY Antimicrobial Surveillance Programme in which it was found that the most frequently occurring bacterial pathogen causing pneumonia and blood stream infections was *Escherichia coli* [10,11]. This could be a depiction of this organism being the overall predominant isolate in our study. This is an independent finding and interrelationship between *Klebsiella pneumoniae* derived from respiratory tract and causing blood stream infections in the same patients within 72 hrs. of isolation of each other has largely been excluded by the defined criteria.

ESBLs production:

In our study the prevalence of ESBLs was found to be 66%. About 85% of *Klebsiella pneumoniae* and 62% of *Escherichia coli* were ESBLs positive in CCA. An increasing trend of ESBLs production

have been observed by 'The India Antimicrobial Resistance Study group' and Daoud *et al* [12,13]. This once again brings out the significance of high pressure exerted by 3rd or 4th generation cephalosporins on the survival of microbes in critical care units.

In our study, although the most common source of *Enterobacteriaceae* isolates was respiratory tract but the maximum ESBLs positive isolates were obtained from blood Stream (85%). This is in contrast to studies by Shah *et al* and Coudron *et al* who reported maximum ESBLs positivity in urinary isolates amongst nosocomial group patients[7,14]. However, a higher rate of ESBLs producing *Enterobacteriaceae* strains has been experienced in our study from all the clinical samples i.e. urinary tract (60.9%), respiratory tract (60%), pus/wound swabs (66.7%). This again emphasizes the high antimicrobial pressure experienced by microorganisms in critical care area.

ESBLs production was looked for in *Klebsiella* and *Escherichia coli* isolated from clinical specimens by CLSI, DDS and Vitek methods. CLSI and DDS methods were also extended to other *Enterobacteriaceae*. In our study, for *Escherichia coli* and *Klebsiella spp.*, CLSI and Vitek both were in 100% agreement. However, DDS was less sensitive as compared to CLSI and Vitek, missing out three *Klebsiella* and one *Escherichia coli* strains. Extension of DDS and CLSI methods to other *Enterobacteriaceae* revealed a very good correlation between the two methods. However, DDS missed one *Enterobacter* strain which was positive by CLSI. This has been corroborated by the studies of Datta *et al* and Shukla *et al*[5,15]. They also found DDS method to be less sensitive than CLSI method.

Antimicrobial susceptibility:

In our study, all the ESBLs positive *Escherichia coli*, *Enterobacter cloacae*, *Serratia marcescens*, *Klebsiella oxytoca* and *Klebsiella ornithinolytica* showed resistance for ceftriaxone and ceftazidime. Eighty five percent of ESBLs positive *Escherichia coli* were resistant to cefepime also. In our study, 96%, 79% and 61% of ESBLs positive *Klebsiella pneumoniae* were found to be resistant to ceftriaxone, ceftazidime and cefepime. This has been seen in various other studies also[5,12,15,16]. In *Klebsiella pneumoniae,* we found 26% resistance to piperacillin+tazobactam amongst ESBLs positive isolates while no resistance in

ESBLs negative isolates. Babini *et al* in 1997-98 in their follow up survey of 1994 of ICUs reported a significantly higher piperacillin+tazobactam resistance amongst ESBLs positive isolates. They found that the prevalence of ESBLs in *Klebsiella* did not change significantly as compare to their previous survey of 1994 but the proportion of ESBLs producers resistant to piperacillin+tazobactam had risen from 31% to 63% ($p<0.001$)[17].

The results of our study show that ESBLs-producing organisms are truly multidrug resistant. Our study clearly demonstrated clear differences in susceptibility patterns between ESBLs positive and ESBLs negative *Enterobacteriaceae* isolates. ESBLs positive *Enterobacteriaceae* were found to be more resistant to all the aminoglycosides and cotrimoxazole in comparison to ESBLs negative *Enterobacteriaceae* isolates. This was found to be highly significant on applying chi-square test. p-value was found to be <0.05 for gentamicin, tobramicin, netilmicin and amikacin. Molecular and susceptibility characterization of 52 selected ESBLs producing strains by the India Antimicrobial Resistance Study Group *et al* also showed a high level of co-resistance with aminoglycosides and fluoroquinolones[12]. The results of our study demonstrated that amongst the aminoglycosides, amikacin was statistically invitro more effective in ESBLs producing *Enterobacteriaceae* than netilmicin > gentamicin > tobramicin.

In our study, about 74% of the ESBLs positive isolates and 22% of the ESBLs negative isolates were found to be resistant to co-trimoxazole. This was also found to be statistically significant by chi-square test, p-value=0.007 ($p<0.05$). Thus co-trimoxazole is likely to be more effective in non-ESBLs producing *Enterobacteriaceae* than in ESBLs

producers. We found that ESBLs producing *Klebsiella* spp showed 61% resistance to ciprofloxacin and 64% to ofloxacin as compared to no resistance for both ciprofloxacin and ofloxacin by ESBLs negative isolates from this area. Thus, there was a significant correlation between quinolone resistance and ESBLs production amongst the *Enterobacteriaceae* in our study (p value <0.05). However, Shukla et al found that ciprofloxacin (89.63%) was more effective amongst the antimicrobials in ESBLs producing *Klebsiellae*[15].

Thus, ESBLs producers were statistically found to be significantly associated with multidrug resistance in our study. This could be because of location of ESBLs genes on plasmids which often carry resistance to aminoglycosides as well as chloramphenicol and co-trimoxazole, amongst these organisms and subjecting the patients to other antimicrobials as well, simultaneously. The results of our study showed that all the isolates (ESBLs positive and ESBLs negative) were susceptible to carbapenems. This is in accordance to various other studies[12,16,18,19]. A high degree of resistance to various non-beta lactam drugs is also corroborated by Vitek based MIC in our study.

CONCLUSION

In conclusion, as predicted a high prevalence of ESBLs positive *Enterobacteriaceae* was found in CCA. Although, molecular techniques are required for the definitive detection of ESBLs but if we correlate the antimicrobial susceptibility pattern of our ESBLs positive and negative isolates, we may definitely say that the methods used by us for detection of ESBLs are worthwhile for a clinical microbiology laboratory for detection of ESBLs and this could be of great help to stop misuse of antimicrobials in any hospital. It should also be noted that we have conducted this study some time back i.e. 2004-2005. Newer resistance mechanisms and a combination e.g. ESBLs+ AmpC could be working together. As per 2010 CLSI guidelines, new interpretive criteria for cephalosporins including cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone and aztreonam are suggested and ESBLs detection is said to be no longer necessary to edit results for cephalosporins, aztreonam or penicillins to be resistant[20]. But, there are controversies coming up in this regard[21]. So, we recommend further clinical studies comparing the previous and current guidelines to reach at a definitive conclusion

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