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Original Research Article

Antibiotic susceptibility pattern of extended spectrum β-lactamase ESBL producing gram negative bacilli in a tertiary care teaching hospital, Bareilly, India

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ABSTRACT

Background: The increasing prevalence of infections caused by antibiotic-resistant bacteria makes empirical treatment of these infections difficult. Resistance to a wide variety of common antimicrobials has made the proliferation of extended spectrum β-lactamase (ESBL) producing strains a serious global health concern that has complicated treatment strategies and is very alarming. This study was undertaken to identify ESBL production in various gram negative bacilli isolated and to further study the antibiogram of ESBL producers and their contribution towards anti-microbial resistance.
Methods: A total of 2008 samples were taken and studied for positive bacterial growth. Presence of ESBL positivity was detected using Kirby-Bauer sensitivity testing method and their antibiogram was studied. Data was analysed using IBM SPSS version 20. Chi-square test was applied wherever applicable to check the significant difference among the different groups. p value of ≤0.05 was considered to be significant.
Results: A total of 2008 samples were studied. Out of which 655 gave positive bacterial growth and amongst these 312 were ESBL producers. Resistance to multiple classes of antibiotics was observed among ESBL producers and mostly imipenem, colistin and polymyxin B were the antibiotics which were sensitive to most of the strains.
Conclusions: The frequency of ESBL producing strains among clinical isolates has been steadily increasing. Advance drug resistance surveillance and development of newer antibiotics is necessary to guide the appropriate and judicious antibiotic use.

Keywords: Antimicrobial resistance, Colistin, ESBL, Imipenem

INTRODUCTION

β-Lactam antimicrobial agents represent the most common treatment for bacterial infections and continue to be the leading cause of resistance to β-lactam antibiotics among Gram-negative bacteria worldwide and thus emergence of β -lactamase producers have become a matter of serious concern. Extended-spectrum beta lactamases (ESBLs) are enzymes that confer resistance to all β - lactam antibiotics except cephamycins and carbapenems and now some ESBLs are even resistant to carbapenems. Treatment of these multiple drug-resistant organisms is a therapeutic challenge. ESBLs are able to hydrolyze 3rd and 4th generation cephalosporins and monobactams. ESBL producing strains are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam).[1,2] ESBL

isolates were first detected in Western Europe in the mid-1980s. Since then, their incidence has been increasing steadily. In recent surveys, a significant increase in the ESBL rate was reported from all parts of the world.[3-10] *Klebsiella pneumoniae* and *Escherichia coli* remain the major ESBL-producing organisms isolated worldwide, which are recommended to be routinely tested for and reported by the Clinical and Laboratory Standards Institute.[11,12] In addition, bacteria harbouring ESBLs may also acquire and most often exhibit additional resistances to other antimicrobial classes such as the quinolones, tetracyclines, cotrimoxazole, trimethoprim, and aminoglycosides, which further limits therapeutic options and thus pose a therapeutic crisis.[13-16] Prevalence of ESBLs varies from an institute to another. However, there is paucity of scientific information available on antibiotic profile with rate of ESBL production. Keeping in view the above facts, the present study was undertaken to find the antimicrobial susceptibility pattern of ESBL producers and contribution of ESBL towards anti-microbial resistance.

METHODS

The design of this study was cross sectional study and the study conducted at the Department of Pharmacology in collaboration with Department of Microbiology, Shri Ram Murti Smarak Institute of Medical Sciences, Bhojipura, Bareilly. The study period was April 2013 to March 2014

Clinical isolates of various gram positive and gram negative organisms, from sputum, endotracheal tip, tracheal aspirate, urine, pus swab, pus aspirate, bronchial wash, catheter tip, blood, pleural fluid, peritoneal fluid, pericardial fluid, ascitic fluid, sample from shunt tube, corneal swab, intracervical swab, wound tissue, CSF and drainage tip from the inpatients and outpatients of Obstetrics and Gynaecology, Surgery, Medicine, Orthopaedics, Ophthalmology, Paediatrics, Casualty, ENT, ICU wards were taken from Microbiology records.

The study was conducted in compliance with the protocol and the Institutional Ethics Research Committee (IERC).

Data was collected from records of Bacteriology Laboratory of Microbiology Department. All the collected data was grouped in two categories i.e. indoor samples and outdoor samples. Further isolates were screened for ESBL production.

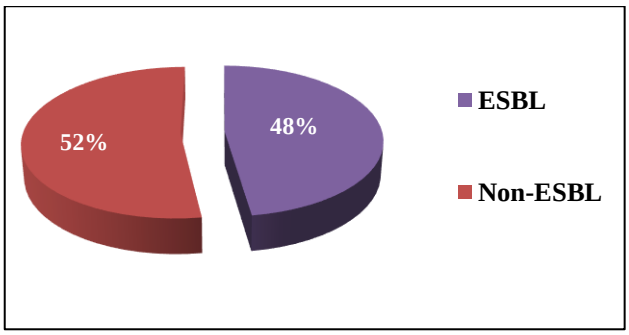
The sensitivity and resistance pattern of ESBL producing bacteria was analysed by Kirby-Bauer sensitivity testing method and was compared between the two groups. Muller Hinton Agar (MHA) media was used. The antibiotic sensitivity was designated 'S' if the drug was sensitive while 'R' was designated to drugs which were resistant. (Note: Intermediate sensitivity was regarded as resistant 'R' in present study as these drugs based on the susceptibility pattern are usually not utilised for treatment).

Statistical analysis

The data was analysed using IBM SPSS version 20. Chi-square test was applied wherever applicable to check the significant difference among the different groups. p value of ≤ 0.05 was considered to be significant.

RESULTS

Present cross-sectional study includes a total of 2008 samples from various clinical departments. Of these 2008 samples, 655 (32.62%) specimen gave significant growth of bacteria while rest were either non-pathogenic or sterile (Figure 1).



Total samples = 2008

Figure 1: Total number of samples.

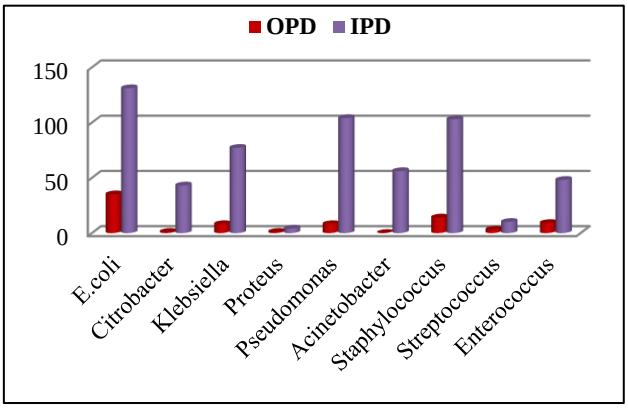


Figure 2: Distribution of organisms in IPD and OPD groups.

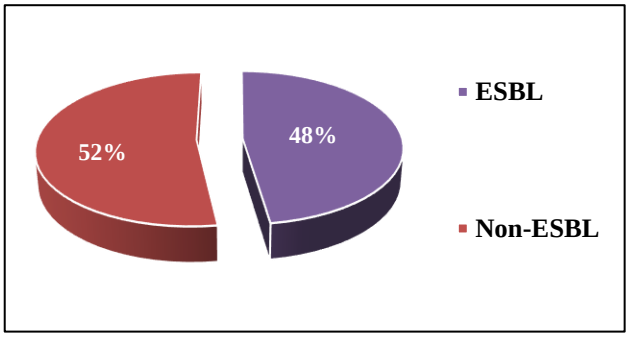


Figure 3: Phenotypically positive ESBL.

Of the total number of positive isolates 576 (87.94%) were from IPD and 79 (12.06%) specimen were from OPD. Majority of the specimen were infected by gram negative bacteria, *E. coli* (25.3%) (Figure 2). Out of total positive samples, 312 (47.63%) isolates were ESBL producers (Figure 3).

Table 1: Antibiotic sensitivity pattern of ESBL producing enterobacteriaceae.

Drugs	*E. coli*		p-value	*Klebsiella*		p-value	*Proteus*		p-value	*Citrobacter*		p-value
	IPD (n=113)	OPD (n=19)		IPD (n=59)	OPD (n=2)		IPD (n=2)	OPD (n=0)		IPD (n=33)	OPD (n=0)	
Cip	1.77	5.26	>0.05	16.95	1	>0.05	0	0	N.A.	27.27	0	N.A.
Le	8.85	10.52	>0.05	20.34	2	<0.05	0	0	N.A.	36.36	0	N.A.
Of	1.77	5.26	>0.05	11.86	1	>0.05	0	0	N.A.	27.27	0	N.A.
Nx	0.88	5.26	>0.05	3.39	1	<0.05	0	0	N.A.	-	-	
AK	74.34	63.16	>0.05	35.59	1	>0.05	0	0	N.A.	48.48	0	N.A.
Gen	49.56	42.1	>0.05	23.73	1	>0.05	0	0	N.A.	21.21	0	N.A.
Tb	65.49	57.89	>0.05	32.20	1	>0.05	0	0	N.A.	30.23	0	N.A.
MRP	13.27	10.53	>0.05	27.12	1	>0.05	1	0	N.A.	24.24	0	N.A.
I	88.5	84.21	>0.05	67.8	2	>0.05	2	0	N.A.	75.75	0	N.A.
Tet	0	26.31	>0.05	18.64	1	>0.05	0	0	N.A.	21.21	0	N.A.
C	67.26	47.36	>0.05	30.51	2	>0.05	2	0	N.A.	20.3	0	N.A.
Cot	12.39	10.53	>0.05	3.39	0	>0.05	0	0	N.A.	6.06	0	N.A.
CFS	46.02	57.89	>0.05	32.2	2	>0.05	1	0	N.A.	27.27	0	N.A.
AMC	0.88	5.26	>0.05	3.39	0	>0.05	0	0	N.A.	6.06	0	N.A.
Pit	52.21	57.89	>0.05	40.68	0	<0.05	2	0	N.A.	42.42	0	N.A.
Nit	28.32	52.63	<0.05	5.08	2	<0.001	-			-	-	
Cl	98.23	94.74	>0.05	93.22	2	>0.05	-			96.96	0	N.A.
PB	100	100	>0.05	93.22	2	>0.05	-			100	0	N.A.

Table 2: Antibiotic sensitivity pattern of non - fermenting gram negative ESBL isolates.

Drugs	*Acinetobacter*		p-value	*Pseudomonas*		p-value
	IPD (n=43)	OPD (n=0)		IPD (n=36)	OPD (n=5)	
Cip	2.32	0	N.A.	8.33	0	>0.05
Le	23.25	0	N.A.	8.33	0	>0.05
Of	4.65	0	N.A.	8.33	0	>0.05
AK	6.97	0	N.A.	22.22	0	>0.05
G	4.65	0	N.A.	27.78	0	>0.05
Tb	18.6	0	N.A.	11.11	0	>0.05
Mrp	23.25	0	N.A.	5.56	0	>0.05
I	62.79	0	N.A.	72.22	5	>0.05
Tet	11.63	0	N.A.	-	-	-
C	2.32	0	N.A.	-	-	-
Cot	0	0	N.A.	-	-	-
CFS	6.97	0	N.A.	11.11	0	>0.05
AMC	0	0	N.A.	0	0	N.A.
Pit	2.32	0	N.A.	19.44	0	>0.05
Cl	97.67	0	N.A.	97.22	5	>0.05
PB	100	0	N.A.	97.22	5	>0.05

The frequency of ESBL production was maximum with *Escherichia coli* (86.26% IPD, 54.29% OPD) followed by *Acinetobacter* (70.78% IPD), *Citrobacter* (76.74% IPD), *Klebsiella* (76.62% IPD, 25% OPD), *Proteus* (50% IPD) and *Pseudomonas* (34.61% IPD, 62.5% OPD) (Figure 4). When sensitivity pattern of ESBL positive isolates was done an antibiogram of the isolates was presented in Table 1 and 2 which showed that sensitivity to Aminoglycosides

for ESBL producing *E. coli* and *Klebsiella* ranges between 74% to 49% and 36% to 23% respectively while imipenem was sensitive to all ESBL positive isolates in a range of 68% to 88%.

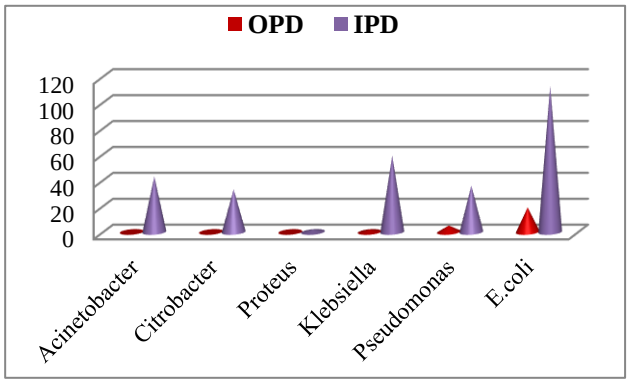


Figure 4: Distribution of ESBL in IPD and OPD.

β-lactamase inhibitors were mostly sensitive to the ESBL producing enterobateriaceae except non-fermenting gram negative ESBL isolates. Colisitin and polymyxin B, the peptide antibiotics, were the only antibiotics sensitive (almost 100%) to all ESBL positive isolates. ESBL positive *Acinetobater* and *Pseudomonas* were mostly resistant to all antibiotics including fluoroquinolones, aminoglycosides and broad-spectrum antibiotics except Imipenem, colistin and polymyxin B.

DISCUSSION

ESBLs have become a widespread serious problem. These enzymes are being increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBL compromise the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. Although the prevalence of ESBL producer varies from country to country, it is more in Asia.[17] The current study described the antibacterial resistance pattern of ESBL producing organisms including role of ESBL contributing towards antibacterial resistance.

In the present study, 47.63% of the gram-negative organisms were detected as ESBL producer. This number is less than that previously reported by Dalela et al, and Narayanswamy et al, while in a study conducted in Bangladesh, only 16.07% were detected as ESBL producer.[18-20] This variation could be due to the geographical changes.

Out of the total ESBL producers, maximum frequency was observed with *Escherichia coli* (79.52%) in the current study which is in accordance to a study conducted in Chennai which showed ESBL production among 75.5% *Escherichia coli* isolates while it was variable in other studies conducted abroad.[20-22]. The reports presented by different authors clearly indicate that the prevalence of ESBL producing organisms among clinical isolates vary greatly geographically and rapidly changing over time.[23,24]

According to Gales et al, and Mathur et al, *Klebsiella* was observed to be the second most common ESBL producer but in present study *Acinetobacter* was found to be the second most common organism producing ESBL with 76.79% while 71.76% *Klebsiella* were ESBL producers showing geographical variation i.e. in this region *Acinetobacter* is more commonly resistant to β-lactam antibiotics as compared to *Klebsiella*.[25,16] The antibiogram pattern of isolates in the present study shows a higher degree of resistance in ESBL producers. It also revealed that most of the gram negative isolates were largely resistant to fluoroquinolones (73 - 100%) which might be indicative of higher selective pressure for fluoroquinolones being prescribed which is similar to a study done by Aruna et al, and Jobayer et al.[20,27] Present study also shows that aminoglycosides are also resistant to *Acinetobacter* and *Pseudomonas* which is similar to a study done by Mansury et al.[28] Sensitivity was maximum with colisitn and polymyxin B followed by imipenem in all ESBL producers in our study which is similar to various other studies done by Solatni et al, Mansury et al, Jobayer et al, Sharma et al.[20,22,28,29] ESBL strains are usually multi-drug resistant. Because these strains become resistant to available antibiotics, there is a need of emergence of newer antibiotics. Antimicrobial therapy has played an important role in the treatment of human bacterial infections, but the drug resistance that has emerged in the treatment of bacterial infections due to ESBL enzymes degrades all beta lactam antibiotics and thus bacteria become multidrug resistant.[30]

This study was conducted in a limited area and thus may not represent the whole population.

CONCLUSION

The findings of this study emphasize the need for a continuous surveillance to detect the resistant strains, strict guidelines for the antibiotic therapy and demand the development of newer antibiotics and thus reduce the increasing burden of antibiotic resistance.

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