Prevalence of Extended Spectrum Beta-lactamase (ESBL) producing Enterobacteriaceae from clinical samples in a tertiary care hospital in Mumbai

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Abstract
Aim: The aim of this study was to detect prevalence of Extended Spectrum Beta Lactamase (ESBL) among Enterobacteriaceae isolated from various clinical samples received in a tertiary care hospital of Mumbai.
Materials and Methods: This study was a retrospective study of 13 months (April 2017 to April 2018) done in the section of Microbiology of our institute. All gram negative isolates were identified and their antibiotic sensitivity pattern was studied according to standard microbiological procedures. ESBL activity among Enterbacteriaceae class of bacteria were confirmed by phenotypic confirmatory disc diffusion test according to Clinical Laboratory Standards Institute guidelines (CLSI) 2017. Clinical and demographic data was collected from Hospital Information and Management system (HIMS).
Result: Total Enterobacteriaceae isolates studied were 1194 out of which 32.7% isolates were ESBL producers. ESBL producing *Escherichia coli (E.coli)* accounted to 34.8% of the total *E.coli* isolates and 30.7% of total *Klebsiella pneumoniae (K.pneumoniae)* isolates were ESBL producers. ESBL producing *Klebsiella oxytoca (K.oxytoca*) and *Proteus mirabilis (P.mirabilis*) were 29.7% and 18.6% respectively.
Conclusion: Knowledge of prevalence of ESBL isolates in our institute will play a pivotal role in curtailing the use of unnecessary antibiotics and assist in taking measures to prevent their spread.

Keywords: Extended spectrum beta lactamase, Enterobacteriaceae, Multidrug resistance.

Introduction
ESBL producing strains have become a threat to human health globally.[1] ESBL producing organisms are multi drug resistant.[1] Such isolates show high frequency of polyclonal spread of ESBL genes among bacteria.[2] ESBLs are plasmid associated enzymes that deactivates penicillins, third generation cephalosporins like cefotaxime, ceftazidime, ceftriaxone and aztreonam but are sensitive to carbapenems like meropenem, imipenem and ertapenem.[2] To detect ESBLs, the property of inhibition of this enzyme production by antibiotics like clavulanic acid, sulbactums and tazobactum is utilised.[3]

Materials and Methods
Study Design
It was a retrospective analysis carried out in the Microbiology section from April 2017 to April 2018. Various clinical samples like urine, sputum, blood, stool, pus, wound swabs, body fluids and invasive medical devices received in the laboratory from patients belonging to all age groups, both genders were included in the study. Clinical samples were received from Outpatient Department (OPD), In-patient department (IPD) and Intensive Care Unit (ICU).

Sample Processing
In all, 13,000 samples were processed for bacterial growth. Isolation, identification and antibiotic sensitivity pattern of the bacterial isolate was done by standard microbiological procedures.[4] Samples were inoculated on Blood Agar, Mac Conkey agar and Nutrient agar under sterile conditions and incubated at 37 °C overnight. Growth was identified using standard biochemical tests and also using automated identification on Vitek 2 Compact system, Biomerieux. Antibiotic sensitivity testing was done on Mueller-Hinton agar according to Kirby Bauer's disc diffusion method. This Study was carried out only on isolates of *E. coli, K. pneumoniae, K. oxytoca and P.mirabilis* belonging to Enterobacteriaceae group.

Detection of ESBL
Enterobacteriaceae isolates showing zone of diameter of ≤ 27 mm for cefotaxime (30 mcg), ≤ 22 mm for ceftazidime (30 mcg), ≤ 25 mm for ceftriaxone (30 mcg) and aztreonam (30 mcg) ≤ 27 mm, that is, which had screening test positive were selected for further confirmation of ESBL production by phenotypic screening disc diffusion method (CLSI 2017). Other Enterobacteriaceae were not included in this study.

In Phenotypic confirmatory disc diffusion test, cefotaxime, ceftazidime singly and along with combination antibiotic that is cefotaxime/clavulanic acid (30/10 mcg) and ceftazidime/clavulanic acid (30/10 mcg) were used for confirmation. Antibiotic disc of cephalosporin were placed 20mm apart from cephalosporin-clavulanic acid combination disc (centre to centre) on lawn culture plate of Mueller-Hinton agar. Increase in the zone of inhibition by ≥ 5 mm around the combination discs as compared to that of cephalosporin alone was considered to be confirmatory test for ESBL producer. *K. pneumoniae* ATCC700603 strain was used as positive control and E.coli ATCC25922 was used as negative control in the study.

This study was approved by Scientific and Ethics committee of the hospital.

Statistical Analysis
Data was presented across study group in terms of frequencies as well as percentage to the total. It was a

descriptive explorative study to elaborate the prevalence of ESBL in Enterobacteriaceae group.

Result

Prevalence of ESBL isolates in female and male population were 53.45% (209/391) and 46.54% (182/391) respectively (Fig. 1). ESBL prevalence in age group of < 15yrs, 16-30 years age group, 31-45 years, 46-60 years and > 61 years were 4.3% (17/391), 4.8%(19/391), 9.2%(36/391), 17.9% (70/391) and 63.7% (249/391) respectively (Table 1). ESBL positive samples received from OPD were 51.4% (201/391) and IPD samples were 48.6% (90/391) (Fig. 2). Among urine samples, ESBL positive strain of E.coli were 36.4% (177/486), K.pneumoniae were 28.1% (56/199), K.oxytoca were 47% (8/17) and P.mirabilis were 30.4% (7/23). Among non-urine samples, ESBL positive strain of E.coli were 31.5% (75/238), K. pneumoniae were 33.5% (64/191), K. oxytoca were 15% (3/20) and only 5% (1/20) P.mirabilis strain were ESBL positive (Table 2c). Comparison of ESBL species to total of 1194 Enterobacteriaceae shows 21.1% ESBL E.Coli, 10.05% ESBL K. pneumoniae, 0.92% ESBL K. oxytoca and 0.67% ESBL P.mirabilis respectively. Out of 1194 Enterobacteriaceae, percentage of ESBL prevalence was 32.7 (391/1194) (Table 3). Comparison of our results with that of other studies is shown in (Table 4).

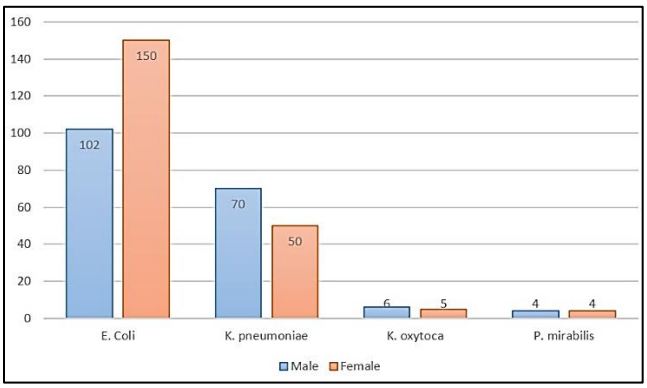

Fig. 1: Distribution of ESBL strains according to gender

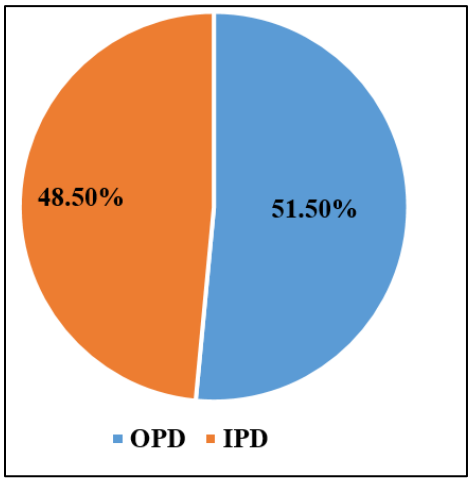

Fig. 2: Distribution of ESBL strain according to OPD/IPD

Discussion

Prevalence of ESBL strains was more in female population. This was in accordance with study by WRPLI Wijessooriya et al.[5] The commonest sample received in our laboratory was urine. The prevalence of urinary tract infection is higher in females.[5,6] Hence this could be the reason for higher ESBL prevalence in female population in our study. Isolates from geriatric population showed highest number of ESBL producers. Study by Phamba S G et al[7] also showed similar findings. This is because maximum samples in our study were from geriatric age group and minimum from patients below 15 years. In our set up OPD patients were more compared to IPD similar to study by Bharara et al.[8] Highest number of samples in our study was urine and commonest organism was *E.coli* followed by *K.pneumoniae*. Study by Andrews B et al[9] and another by Pawan et al[10] also showed dominance of ESBL isolates among *E.coli* followed by *K.pneumoniae* from urine samples. Among urine samples ESBL prevalence was 36.4% among *E.coli* and 28.1% among *K.pneumoniae*. This was in accordance with studies by Shobha K L et al[11] and Kumar A et al.[12] Both showed ESBL prevalence of 32% in *E.coli* from urine samples. Among non-urine samples maximum were sputum samples and highest prevalence of ESBL was seen in *K.pneumoniae* isolates (33.5%) followed by *E coli* (31.5%). Isolates of *K. oxytoca* and *P.mirabilis* were less in number and their ESBL activity noted was 15% and 5% respectively.

Out of total Enterobacteriaceae isolated, 32.7% tested positive for ESBL activity. This was comparable to studies by Segar et al[13] which showed a prevalence of 38.2%. Also, study by Basavaraj M C et al[14] reported 32.1% isolates of Enterobacteriaceae to be ESBL producers from various clinical samples. Similar studies by Dutta H et al and Kannaiyan et al showed ESBL prevalence to be 27.3% and 27.9% respectively.[15,16] However some studies have shown a comparatively higher ESBL prevalence[17,9] (Table 4). Critical patients with longer duration of hospital stay are more prone to get infected with an ESBL producing isolates.[18] ESBL associated multidrug resistant gene transfer among bacteria increases several folds in hospital environment.[19] But in our set up OPD patients were more compared to IPD and hence overall percentage of ESBL associated infection are not very high. Also, several studies on ESBL prevalence across the world showed that ESBL prevalence varies significantly across continents, countries and hospitals.[20] All ESBL producing isolates were resistant to penicillins, cephalosporins, aminoglycosides and quinolones. ESBL positive strains isolated from various clinical samples showed maximum sensitivity to carbapenems. This was also observed by other authors.[21,22]

Table 1: Age wise distribution of ESBL producers

S. No.	Age group	Male	Female	Total
1	<15yrs	5	12	17(4.3%)
2	16-30yrs	8	11	19(4.8%)
3	31-45yrs	8	28	36(9.2%)
4	46-60yrs	33	37	70(17.9%)
5	>61yrs	128	121	249(63.8%)

Table 2A: Sample wise distribution of enterobacteriaceae among 13000 samples processed

Samples	Total no of samples received during study period	Total no of samples showing growth of enterobacteriaceae
1] Urine	7000	724[10.3%]
2] Sputum	2000	108[5.4%]
3] Wound Swab	530	112[21.1%]
4] Pus	420	77[18.3%]
5] Blood	2251	26[1.1%]
6] Stool	350	54[15.4%]
7] Body Fluids	108	03[2.7%]
8] Invasive Medical Devices	201	66[32.8%]
9] Tissue	140	24[17.1%]
Total	**13000**	**1194**

Table 2B: ESBL verses total number of enterobacteriaceae isolates in various samples received

Sample		*E.coli[724]*	*K.pneumoniaea[390]*	*K.oxytoca[37]*	*P.mirabilis[43]*
1] Urine	Total	486	199	17	23
	ESBL	177	56	08	07
2] Sputum	Total	20	80	08	00
	ESBL	06	30	02	00
3] Wound Swab	Total	56	40	04	12
	ESBL	21	09	01	01
4] Pus	Total	44	24	05	04
	ESBL	10	10	00	00
5] Blood	Total	20	06	00	00
	ESBL	06	03	00	00
6] Stool	Total	51	02	01	00
	ESBL	15	00	00	00
7] Body Fluid	Total	02	02	00	00
	ESBL	00	00	00	00
8] Invasive Medical Devices	Total	31	30	01	02
	ESBL	10	11	00	00
9] Tissue	Total	14	07	01	02
	ESBL	07	01	00	00

Table 2 C: Compact form of table 2 A and 2 B

	Total no of isolates	ESBL positive isolates
Urine Samples		
E.coli	486	177(36.4%)
K pneumoniae	199	56 (28.1%)
K oxytoca	17	08 (47%)
P. mirabilis	23	07 (30.4%)
Non urine Samples		
E. coli	238	75 (31.5%)
K. pneumoniae	191	64 (33.5%)
K .oxytoca	20	03 (15%)
P. mirabilis	20	01 (5%)

Table 3: Distribution of ESBL enterobacteriacea according to isolates

Organism	Total no. of isolates (urine + non urine sample)	Total no of ESBL isolates (urine + non urine sample)	Percentage occurrence of ESBL among each species	Percentage occurrence of ESBL out of 1194 Enterobacteriaceae
E. coli	724	252	34.8%	21.1%
K. pneumoniae	390	120	30.7%	10.05%
K .oxytoca	37	11	29.7%	0.92%
P. mirabilis	43	8	18.6%	0.67%
Total	1194	391	32.7%	32.7%

Table 4: Comparision of ESBL prevalence in few studies

Authors	Year	Percentage of ESBL positive Enterobacteriaceae
Segar et al [13]	2015	38.2%
Basavaraj M et al [14]	2009-10	32.1%
Dutta H et al [15]	2011-12	27.3%
Kannaiyan et al [16]	2018	27.9%
Sangeetha K et al [17]	2017-18	47.4%
Andrews B et al [9]	2016-17	54.79%
Current study	2017-18	32.7%

Conclusion

ESBL production is generally accompanied by multi-drug resistance. Hence knowledge of their prevalence in clinical samples will aid in averting the inessential use of antibiotics especially the third generation cephalosporins. Such information will also highlight the importance of taking steps to curtail their spread in this institute.

Conflict of Interest: None.

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