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

Sofia C. Patel1*, Sunayana. M. Jangla2, Raji Pillai3, Uma Chaturvedi4, Umesh Gami6, Bhupesh Macchi6

1Scientific Officer, 2Microbiologist, 3pathologist, 4Technical Officer, 6Scientific Assistant, Dept. of Pathology, Bhabha Atomic Research Centre, BARC Hospital, Pathology Unit, Microbiology Laboratory, Mumbai, Maharashtra, India

*Corresponding Author: Sofia C. Patel
Email: sofiap@barc.gov.in

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% 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.3 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 cefalosporin were placed 20mm apart from cefalosporin-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 cefalosporin 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).

**Discussion**

Prevalence of ESBL strains was more in female population. This was in accordance with study by WRPLI Wijesooriya et al.5 The commonest sample received in our laboratory was urine. The prevalence of urinary tract infection is higher in females.6,7 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 al7 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 al9 and another by Pawan et al10 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 al11 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 al13 which showed a prevalence of 38.2%. Also, study by Basavaraj M C et al14 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 prevalence17,18 (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      | 201                                             | 66[32.8%]                                                |
| Devices                  |                                                 |                                                          |
| 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. pneumoniae[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     | Total        | 31                 | 30             | 01               | 02               |
| Devices                 | 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

| Urine Samples          | Total no of isolates | ESBL positive isolates |
|------------------------|----------------------|------------------------|
| 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 enterobacteriaceae 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    | 2018       | 27.9%                                           |
| Sangeetha K et al  [17] | 2017-18   | 47.4%                                           |
| Andrews B et al    | 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.

References
1. Andrew B, Kagirita A, Bazira J. Prevalence of Extended-Spectrum Beta-Lactamases-Producing Microorganisms in Patients Admitted at KRRH, Southwestern Uganda. Int J Microbiol 2017;2017:3183076.
2. Wollheim C, Guerra I M F, Conte V D V, Hoffman S P. Nosocomial and Community acquired infection due to class A extended-spectrum β-lactamases (ESBLA) - producing Escherichia coli and Klebsiella spp in Southern Brazil”. Braz J Infect Dis 2011;15(2):138-43.
3. Clinical and Laboratory Standards Institute (CLSI), Document M100-S25. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-sixth Informational Supplement. Wayne, Pennsylvania, USA Clinical and Laboratory Standards Institute; 2017:118-121.
4. Bailey and Scotts, Diagnostic Microbiology Twelfth edition. Mosby Elsevier, St Louis, Missouri 63146.
5. WRPLI Wijesooriya, YB Herath, RAL. S, A Weerawardhana, Ediriweera, D. S. Antibiotic sensitivity pattern for non-beta lactam antibiotics and carbapenems in extended-spectrum beta-lactamases (ESBL) producing urthropathogens versus non-ESBL producing urthropathogens. Sri Lanka J Infect Dis 2017;7(2):92-9.
6. Kaur J, Chopra S, Sheevan, Mahajan G. Modified Double Disc Synergy Test to Detect ESBL Production in Urinary Isolates of Escherichia coli and Klebsiella pneumonia. J Clin Diagn Res 2013;7(2):229-33.
7. Pamba S G, Saldanha D R M. Prevalence of Extended Spectrum Beta Lactamases [ESBL] producing Klebsiella species and Escherichia coli in a tertiary care hospital. J Int Med Dent 2017;4(1):06-12.
8. Bharata T, Sharma A, Gur R, Duggal S D, Jena P P, Kumar A. Comparative analysis of extended-spectrum beta-Lactamases producing urthropathogens in outpatient and inpatient departments. JIHAS 2018;7:45-50.
9. Andrews B, Joshi S, Swaminathan R, Sonawane J and Shetty K. Prevalence of Extended Spectrum B-Lactamase (ESBL) Producing Bacteria among the Clinical Samples in and around a Tertiary Care Centre in Nerul, Navi Mumbai, India. IJCMS 2018;7(3):3402-9.
10. Pawan K, Tiwari Y K, Saraf G, Pundir S, Patidar V. Identification of ESBL producing Escherichia coli from Urine samples at Tertiary Care Hospital in Jhalawar. Research and Reviews: J Microbiol Virol 2017;7(3):38-45.
11. Shobha K. L, Rao G, Rao S, C K Sreeja. Prevalence of Extended Spectrum Beta -Lactamases in Urinary Isolates of Escherichia coli, Klebsiella and Citrobacter Species and their antimicrobial susceptibility pattern in a tertiary care Hospital. Indian J Practising Doctor 2007;3(6):2007-01-2002.
12. Kumar A, Singh R. To Study Prevalence of Extended Spectrum Beta Lactamase Production in Isolates of E coli in Urinary Tract Infection. Indian J Res 2018;7(1):306-8.
13. Segar L, Kumar S, Joseph N M, Sivaraman U. Prevalence of Extended Spectrum Beta-Lactamases among Enterobacteriaceae and their Antibiogram pattern from various clinical samples. Asian J Pharm Clin Res 2015;8(5):220-3.
14. Basavaraj M, Jyothi P, Basavaraj V. The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India. JCDR 2011;5(3):470-5.
15. Dutta H, Nath R, Saikia L. Multi-drug resistance in clinical isolates of Gram-negative bacilli in a tertiary care hospital of Assam. Indian J Med Res 2014;139:643-5.
16. Kannaiyan M, Abebe G M, Kanimozhhi C, Thambidurai P, Selvam S A, Raja V et al. Prevalence of Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae Members Isolated From Clinically Suspected Patients. AJPCR 2018;11(5):364-9.
17. Sangeetha K.T, Hittinahalli V, P. R Lyra. Study on Phenotypic Detection of ESBL in Gram Negative Bacterial Isolates in a Tertiary Care Hospital in Bangalore. Int J Microbiol Res 2018;10(3):1049-51.
18. Rawat D, Nair D. Extended-Spectrum Beta-lactamases in gram Negative Bacteria. J Glob Infect Dis 2010;2(3):263-74.
19. Choudhary R, Panda S, Singh DV. Emergence and dissemination of antibiotic resistance: A global problem. Indian J Med Microbiol 2012;30(4):384-90.
20. Chaudhary P, Bhandari D, Thapa K, Thapa P, Shrestha D, Chaudhary HK Shrestha A, et al. Prevalence of Extended Spectrum Beta-Lactamase Producing Klebsiella pneumoniae Isolated From Urinary Tract Infected Patients. Nepal Health Res Coun 2016;14(33):111-5.
21. Kaviyarasan G, Rajamanikandan KCP, Sabarimuthu M, Ramya S, Prasanth A D. Antimicrobial Susceptibility and Detection Methods for the Extended- Spectrum β Lactamases producing Enterobacteriaceae from Clinical Samples. Asian J Pharm Clin Res 2018;11(5):139-42.
22. Giddi S, Dasari S, Suryakumari C. Prevalence of Extended Spectrum Beta –Lactamase Producers among Various Clinical Samples in a Tertiary Care Hospital: Kurnool District, India. Int J Curr Microbiol App Sci 2017;6(8):2857-63.

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