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Prevalence of Extended Spectrum Β-Lactamase (ESBL) Producing Bacteria among the Clinical Samples in and around a Tertiary Care Centre in Nerul, Navi Mumbai, India

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A B S T R A C T

To study the prevalence of extended spectrum β-lactamase (ESBL) producing bacteria and its sensitivity pattern to commonly used antibiotics in a tertiary care centre in Navi Mumbai. The study was conducted from June 2016 to July 2017 in Microbiology Department of our tertiary health care centre. A total of 2850 sample was studied, out of which 812 (54.79%) ESBL producing bacteria was detected by using Clinical Laboratory Standard Institute (CLSI) guidelines that described the phenotypic confirmatory test along with routine antibiotic susceptibility testing. ESBL production was confirmed in 812 (54.79%) isolates. The isolates of *E. coli* (45.50%) were the most common ESBL producers. Maximum ESBL isolates were obtained from urine samples (44.3%) and male patients (56.4%). Surgical ward showed highest prevalence (21.1%) and age group between 51 and 60 were mostly affected (19.9%). This study conducted in D Y Patil Hospital, Nerul, Navi Mumbai shows high prevalence of ESBL production among Gram negative bacteria. E.coli showed highest prevalence i.e. 45.5%. Colistin showed 100% sensitivity followed by Imipenem which showed 98.2%. Prevalence of ESBL producers was more prevalent in urine sample among males than females. Timely administration of sensitive antibiotic and avoiding antibiotic abuse will help to lessen the burden of ESBL

Introduction

Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor clinical outcomes. Community and hospital-acquired ESBL-producing Enterobacteriaceae are prevalent worldwide[1]. Reliable identification of ESBL-producing organisms in clinical laboratories can be challenging, so their prevalence is likely underestimated.

Beta-lactamases are enzymes that open the beta-lactam ring, inactivating the antibiotic. The first plasmid-mediated beta-lactamase in gram-negative bacteria was discovered in Greece in the 1960s. It was named TEM after the patient from whom it was isolated (Temoniera)[2]. Subsequently, a closely related enzyme was discovered and named TEM-2. It was identical in biochemical properties to the more common TEM-1 but differed by a single amino acid with a resulting change in the isoelectric point of the enzyme. These two enzymes are the most common plasmid-mediated beta-lactamases in gram-negative bacteria, including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. TEM-1 and TEM-2 hydrolyze penicillins and narrow spectrum cephalosporins, such as cephalothin or cefazolin. However, they are not effective against higher generation cephalosporins with an oxyimino side chain, such as cefotaxime, ceftazidime, ceftriaxone, or cefepime. Consequently, when these antibiotics were first introduced, they were effective against a broad group of otherwise resistant bacteria. [3-5] Bacteria producing ESBL are spread mostly through hospital staff like doctors, nurses or other healthcare professionals. They are vastly responsible for causing infections such as UTI, diarrhoea, skin infection and pneumonia. Symptoms of an ESBL infection depends on the site of bacterial colonization, such as burning micturation in case of UTI, loss of appetite and presence of blood in stool in case of GIT infection and rashes in case of skin infection. Since these bacteria are highly resistant, antibiotics should be administered only after performing an antibiotic sensitivity testing according to CLSI guidelines.

Materials and Methods

The study was conducted in microbiology department at D. Y Patil Medical College and Hospital. The hospital is an 800 bedded multi-speciality hospital with latest advances, catering to more than 1000 patients in a day. All the clinical samples collected in a sterile container for bacteriological study were included. For isolation and identification of the bacteria, following media were used: Blood agar, Chocolate agar, MacConky agar along with series of biochemical test like Indole, Citrate, Urease and TSI. The bacterial isolates were then tested for antimicrobial susceptibility test by the disc diffusion method according to the guidelines of Clinical and Laboratory Standard Institute (CLSI) [6]. The following antibiotics discs were used in the sensitivity test: Imipenem (10 mcg), Colistin (10 mcg), Amikacin (10 mcg),Gentamicin (10 mcg), Cefotaxime (30 mcg), Ceftrioxone (30 mcg), Ceftazidime (30 mcg), Cefepime (30 mcg), Ciprofloxacin(5 mcg), Nitrofurantoin (100 mcg), Cotrimoxazole (25 μg), Tobramycin (10 mcg), Norfloxacin (10 mcg), PipracillinTazobactum (100/10mcg), and Ampicillin (10mcg).

Testing for the ESBL production

The ESBLs detection was carried out by modified double disc synergy test using Cefepime along with the third generation Cephalosporins [7]. All the strains which will show a diameter of less than 27 mm for Cefotaxime and less than 25 mm for Ceftriaxone were selected for checking the ESBLs production. The ESBL production was tested by the Modified Double Disc Synergy Test by using a disc of Amoxicillin-clavulanate (20/10 μg) along with four Cephalosporins (Cefotaxime, Ceftriaxone, Cefpodoxime and Cefepime). A lawn culture of the organisms was made on a Mueller-Hinton agar plate, as recommend by CLSI [8]. A disc which contained Amoxicillin-clavulanate (20/10 μg) was placed in the centre of the plate. The discs of third generation cephalosporin and fourth generation cephalosporin was placed 15 mm and 20 mm apart respectively, centre to centre to that of the Amoxicillin-clavulanate disc [9]. Any distortion or increase in the zone towards the disc of Amoxicillin-clavulanate was considered as positive for the ESBLs production.

Results and Discussion

A total of 2850 samples were obtained out of which 1482 gave positive bacterial growth (Fig. 1). Prevalence of ESBL producers were estimated to be 54.79% i.e. 812 out of the total 1482 were ESBL producing bacteria (Fig. 2).

Prevalence was seen more among the male patients than female patients. 56.4% of the males showed to be infected with ESBL whereas female ratio was estimated to be 43.5 % (Fig. 3). The age group of 51-60 showed maximum infection with EBSL (19.9%) followed by age group of 21-30 (19.7%). The least affected age group was 11-20 (0.05%) (Fig. 4). Patients admitted to surgical ward was mostly affected (21.10%) followed by MICU (20.5%) (Fig. 5).

Maximum number of ESBL producers were isolated from urine sample (44.30%) followed by pus (32.2%) (Fig. 6). Among the different gram negative bacteria obtained and studied, *E. coli* showed the maximum number of ESBL production i.e. 45.5% (Fig. 7).

All the ESBL producing organisms were studied for their antibiotic sensitivity pattern and organisms showed 100% sensitivity to Colistin and 98.2% sensitivity to Imipenem. The sensitivity percentage to the antibiotics is listed in the table 1. Other antibiotics including Ceftazidime, Cefepime, Nitrofurantoin and Ampicillin showed complete resistance along with Cefotaxime and Ceftriaxone.

Fig.1 Shows total bacterial growth obtained in the study

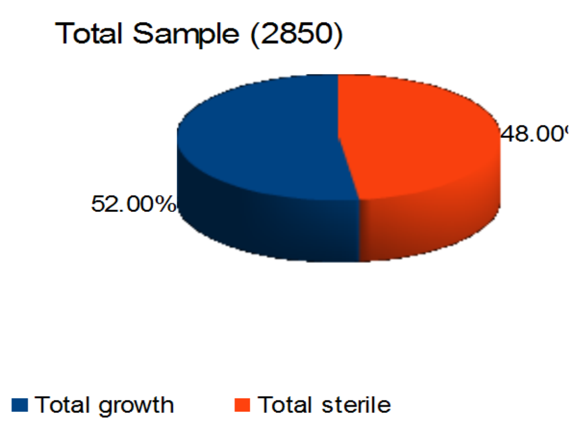

Fig.2 Shows total number of ESBL producers obtained

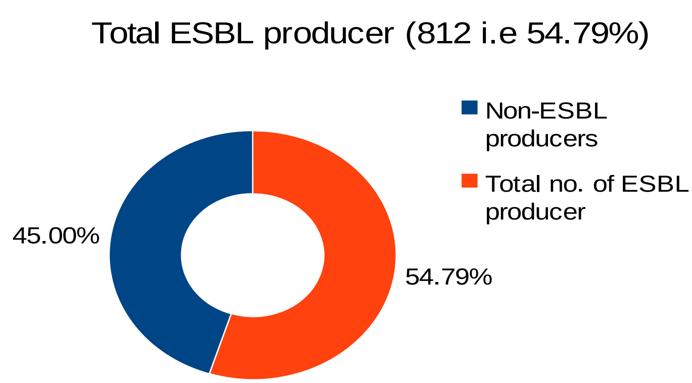

Fig.3 Shows the prevalence of ESBL among male and female patients

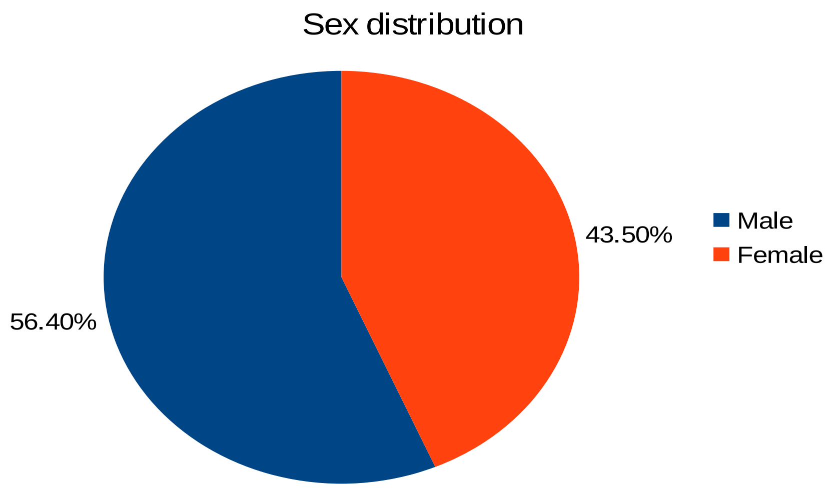

Fig.4 Shows age wise distribution of ESBL. 1 – 10: 8.30%, 11- 20: 0.05%, 21-30: 19.7%, 31-40: 14%, 41–50: 13.7%, 51–60: 19.9%, 60–70: 12%, 71–80 and above: 16.4%

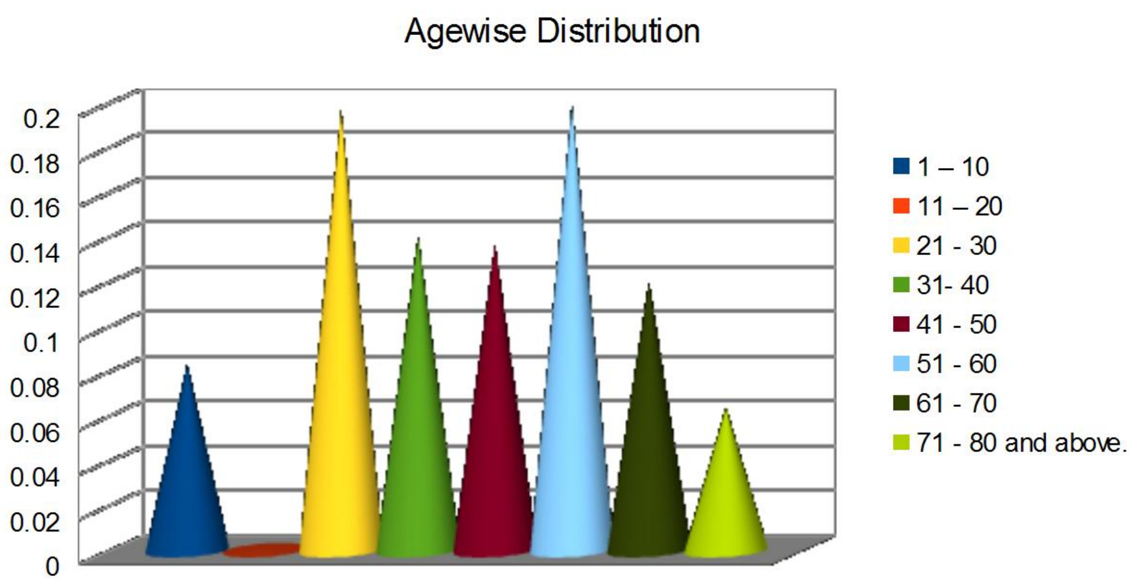

Fig.5 Shows ward wise distribution among ESBL. Outpatient department: 14.9%, Surgical ward: 21.1%, OBGY: 5.9%, Peads: 6.1%, MICU: 20.5%, Medical ward: 16.5%, Urology: 1.4%, Ortho: 4.9%, PICU: 0.61%, Medicine: 6.2%, Nephrology: 0.49%, Ophthalmology: 0.49%, Skin: 0.24%

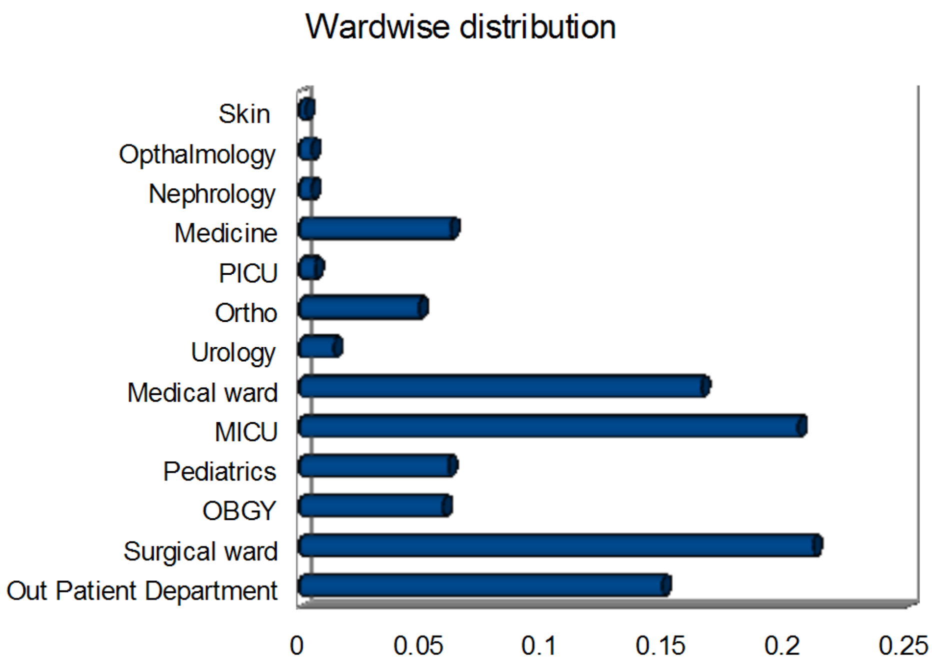

Fig.6 Shows the growth of ESBL producers among the different samples. Sputum: 4.1%, Pus: 32.2%, Urine: 44.3%, Tips: 11.8%, Blood and tissue: 0.73%, Other body fluids: 4.43%, Stool: 2.21%

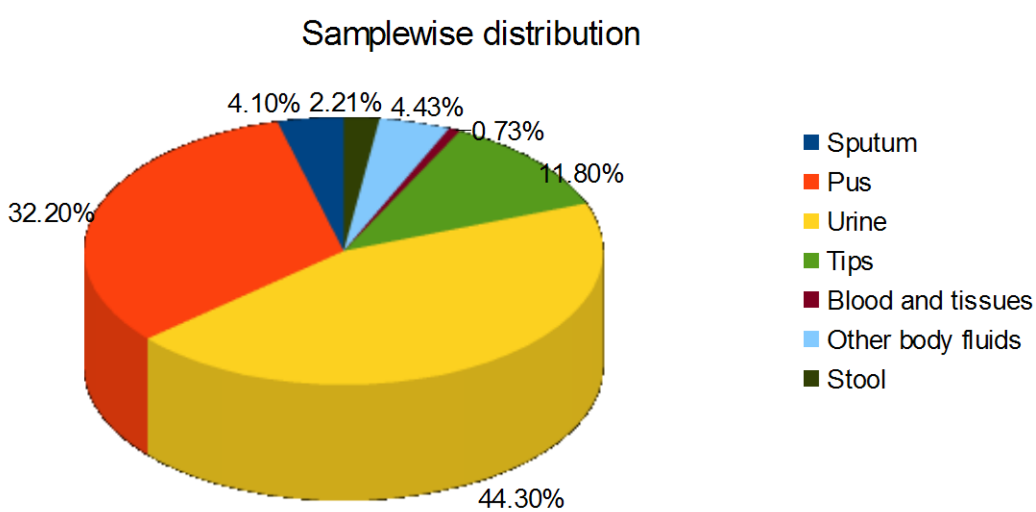

Fig.7 Shows the ESBL production among the different gram negative bacteria obtained in the study. *E. coli: 45.5%, Klebsiella pneumoniae: 24.1%, Klebsiella oxytoca: 9.6%, Pseudomonas species: 2.9%, Pseudomonas aeruginosa: 4.4%, Proteus vulgaris: 2.2%, Proteus mirabilis: 0.98%, Citrobacter freundii: 2.9%, Citrobacter koseri: 1.2%, Acinetobacter species: 5.1% and Enterobacter species: 0.9%*

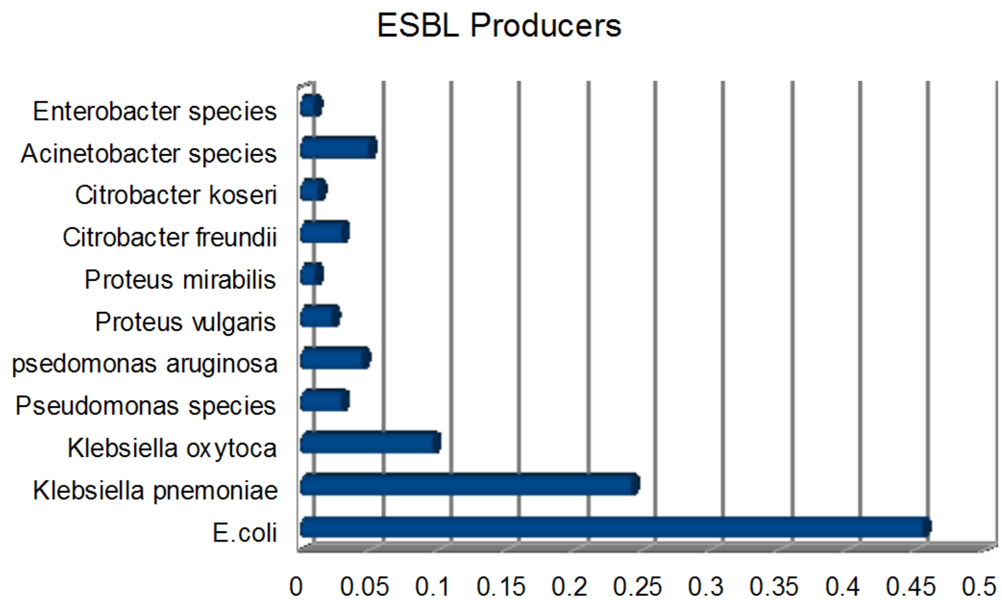

Table.1 The sensitivity percentage to antibiotics

SR.	Name of the antibiotic	Sensitivity percentage
1	Colistin	100.00%
2	Imipenem	98.20%
3	Amikacin	94.90%
4	Gentamycin	93.34%
6	Ciprofloxacin	74.20%
7	Co-trimoxazole	73.80%
8	Tobramycin	72.00%
9	Piperacillin-Tazobactum	70.50%
10	Norfloxacin	50.20%

Moland and colleagues have shown that ESBL-producing isolates were found in 75% of 24 medical centers in the United States (23 Moland *et al.*, 2002) [10] while in other studies around USA, 4.2 44% of gram negative bacteria were found to be ESBL producers (24-26 Saurian s *et al.*, 2000; Mathai *et al.*, 2001; Winokur *et al.*, 2001) [11-13]. Spain has seen a prevalence of 20.8% (27 Romero *et al.*, 2007)[14], Taiwan 28.4% (28 Kuo *et al.*, 2007)[15], Turkey 78.6% (29 Hos, oglu *et al.*, 2007)[16], Algeria 20% (30 Messai *et al.*, 2008)[17] and China 51% (31 Xiong *et al.*, 2002)[18]. The studies conducted in India also show high prevalence of ESBL producers. (59.9%, Telangana, Hema bindu *et al.*, 2015)

[19] In our study, we have also reported a prevalence of 54.97% of ESBLs which is of a very high concern. *E. coli* has shown a prevalence of 45.5%, majority of it being in the urine samples. This could be because of the injudicious use of drugs for urinary tract infections.

This study along with the other studies concludes that prevalence of ESBL producing bacteria is rising in an alarming way. The resistance to the antibiotic is ever increasing among the bacteria due to antibiotic abuse and hence timely reporting of ESBL producing organisms and administration of sensitive antibiotic along with proper awareness is need of the hour.

The study was aimed to understand the prevalence of ESBL producing Gram Negative Bacteria among the microbial samples received in the microbiology department of D.Y Patil Medical College and Hospital in Nerul, Navi Mumbai. The data shows 54.97% (812/1482) prevalence of ESBL producers among the positive samples. The prevalence among the sexes was 56.4% and 46.5%, more common in men than in women respectively. The infection rate was 19.9% among the patients belonging to the age group of 51–60. Surgical ward showed highest prevalence i.e. 21.1% of ESBL producers. Maximum number of ESBL producing bacteria i.e. 44.3% was isolated from urine sample and *E. coli* was identified to be the highest producer of beta- lactamase enzyme which is 45.5%. Colistin showed 100% sensitivity to all the ESBL producing bacteria.

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