



Detection of Gram Negative Bacilli Producing Extended Spectrum Beta Lactamase on Intensive Care Unit at Tertiary Care Hospital

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Antimicrobial resistance is a budding threat worldwide. The every class of antibiotic agents must have resistance mechanisms. The principal mechanism for resistance to the β -lactam antibiotics in gram-negative bacteria is the production of β -lactamase. The creation of extended-spectrum β -lactamases (ESBLs) is a vital mechanism which is responsible for the resistance to the cephalosporins. During the last 2 decades, ESBL producing gram-negative bacilli have arose as a major problem in many settings. Resistance to 3rd generation cephalosporins by attainment and manifestation of extended spectrum beta lactamase (ESBL) enzymes among gram-negative bacilli is on a rise. To isolate the ESBL strains from various clinical samples in ICU. To find out the prevalence of ESBL producing gram negative bacilli during the period of December 2017 - December 2018 in the Intensive care unit of Sree Balaji Medical College and Hospital. Totally 27 out of 139 gram negative bacilli (19.42%) were found to be ESBL producers. ESBL triggering gram negative bacilli spiteful the biological sample like blood, urine, wound swab, sputum were 36.36%, 16.00%, 10.00%, 9.09% individually Though Meropenem is 100% sensitive to all ESBL beginning gram negative bacilli, but still sensitivity also witnessed with some cheaper drugs like Cotrimoxazole (33.33%), Amikacin (48.14%), Gentamicin (40.7%), Ciprofloxacin (22.22%). Hence we care and will provide an analysis and treatment for affected patients with Extended Spectrum Beta Lactamase producing organisms.

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Keywords: ESBL; extended spectrum beta lactamase; Bacilli; blood; sputum, wound swab and ceftriaxone bacteria.

1. INTRODUCTION

WHO predictable the burden of incidence of multiple joint pathologies which present with pain and swelling infection globally around 7 -12%, the statistics from India are distressing, it is very difficult to come to a diagnosis with a frequency of occurrence varying between 11% to 83% for diverse kinds of infections that are acquired in the hospital [1]. Determined the most of diseases can be well-known in the initial stages by adding a few more invasive tests such as synovial fluid analysis and synovial tissue biopsy There is an escalating trend of bacterial infections for serious morbidities that are associated with impaired immunity, increased usage of intrusive diagnostic procedures, delay in sterilization and disinfection, and random usage of antibiotics. ICU from Beta lactam antibiotics are commonly used antibiotic, because of wide spectrum activity and squat toxicity [2].

In 1928, Alexander Fleming exposed the first beta lactam antibiotic Penicillin, which remained antibiotic of choice for many years. But later bacteria learnt new inventive mechanisms by which they can resist the antibiotics acting on them. Production of enzymes called beta lactamases. In young adolescent and adult monoarticular immersion of hip or knee with or without constitutional symptoms often poses analytical problem between tubercular, rheumatoid and non-specific pathology.. But in due course of time these beta lactam antibiotics developed resistance by mutation of enzymes continuously due to pressure that is imposed selectively by the antibiotic usage [3].

To overcome this, novel class of antibiotics belonging to beta-lactam group, third generation cephalosporin was developed which have greater activity against the gram negative bacilli causing infections. Due to inadvertent use of third generation cephalosporins like cefotaxime, ceftazidime, ceftriaxone bacteria soon developed resistance by new beta lactamase. These new beta lactamases are called as (ESBL) extended spectrum beta lactamases because of their extended range of activity against third generation cephalosporins and monobactams. ESBL are enzymes secreted by bacteria and they are efficient in hydrolyzing all beta lactam drugs including third generation cephalosporins and monobactams except cephamycins and

carbapenems. This resistance is encoded by transferable conjugative plasmid [4]. Extended spectrum beta lactamases were first identified in 1983 [5]. The first among these is SHV-2 which are efficient in hydrolyzing newer beta lactams, was found in single strain of Klebsiella ozonae isolated in Germany. TEM-1 was the first beta-lactamase mediated through plasmid in gram negative bacilli that has been elucidated in early 1960s [3].

ESBL producing organisms there is higher chance of infection or colonization due to prolonged antibiotic exposure, increased duration of ICU stay, severe illness, and instrumentation or catheterization [6]. There are diverse varieties of ESBL which vary with geographical locations. ESBL are byproducts of TEM and SHV type enzymes. ESBL are derivatives of Temoniera or Sulfhydryl variety type of enzymes. There are major two type such as >90 TEM type and 25 SHV type enzymes, along with OXA type enzymes and inhibitor tough beta lactamases [3]. ESBL producing organisms has played a major role for causing several outbreaks which has been observed worldwide since 15 yrs [7]. Hence this study was done to find out the prevalence of ESBL producing gram negative bacilli during the period of December 2017 -December 2018 in the Intensive care unit of Sree Balaji Medical College and Hospital.

2. MATERIALS AND METHODS

The present study was carried out through among patients who were admitted in Intensive Care Unit from December 2017 to December 2018 at Sree Balaji Medical College and Hospital, Chennai. A total of 377 patients from both sexes male and female in age group between 20 to 60 years admitted in Intensive Care Units were incorporated in the study. Separates gained from various clinical samples like pus, blood, tracheobronchial aspirates, urine, sputum, and endotracheal drainage tube tips taken from Intensive medical and intensive surgical care units were incorporated in the study.

2.1 Inclusion Criteria

Presence of sustained infections not responding to treatment with routine drugs used in ICU like Ciprofloxacin, Gentamycin, Amikacin and Cefotaxime, Ceftriaxone.

a) E-test strips: source Himedia

Ezy MICTM Strips Ceftazidime / Ceftazidime + Clavulanic acid (Ceftazidime - Clavulanic acid: 0.064 -4 µg/ml, Ceftazidime: 0.5 -32 µg/ml).

Urine – In non-catheterized patients, Clean-catch midstream urine was collected. In case of catheterized patients, freshly voided urine can be collected by clamping the tube off above the port. The catheter port /Wall of the tubing were cleaned with 70% ethylalcohol, and urine was aspirated via syringe and needle; entry of micro-organisms into the bladder was prevented by maintaining the integrity of the closed drainage system. Routine blood inquiries to rule out common joint pathologies were done such as levels of erythrocyte sedimentation rates, serum uric acid levels, total counts and differential counts, rheumatoid factor were done for all the patients. 5 ml of blood was Collected in 50 ml of Brain Heart Infusion broth to give a dilution of one in ten and incubated at 37deg C. Wound Swab - Wound area was wiped with sterile saline or 70% alcohol. Swab was rolled along leading edge of the wound. Endotracheal

Aspirate - was collected under strict aseptic precautions in a sterile container. Drainage Tube Tips – were collected in a sterile container.

3. RESULTS

The present study included patients of both sexes and ages between 20 -80 yrs. Various Samples were obtained from 377 hospitalized patients admitted in Intensive care unit to find the prevalence of Extended Spectrum Beta Lactamase producing gram negative bacilli during the period from December 2017 to December 2018. Samples included Blood, Sputum, catheter tip and endotracheal drainage tube tip and wound swabs.

Out of 377 samples collected, 227 samples showed growth .139 were gram negative isolates, 56 were found to be gram positive isolates. Gram negative separates were known as Klebsiella, Klebsiella oxytoca, Escherichia coli, pneumonia, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter baumannii by biochemical reactions like IMVIC.

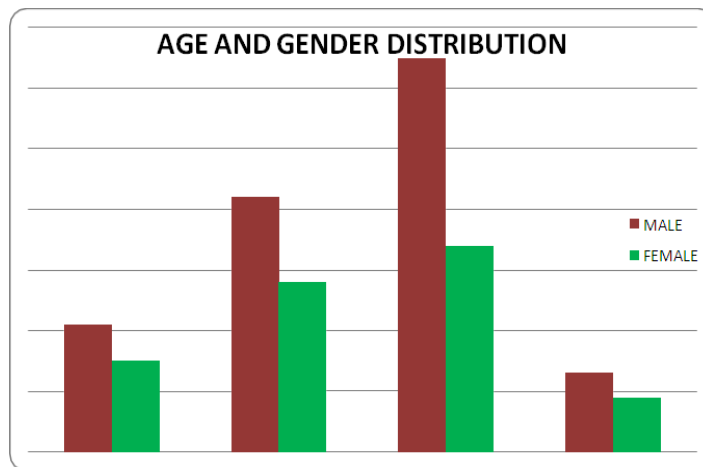


Fig. 1. Age and gender distribution

Table 1. Growth pattern of isolates obtained from various specimens from intensive care units (n=377)

S. no	Growth	No. of samples	% Of samples
1.	CULTURE POSITIVE	227	60.20%
2.	CULTURE NEGATIVE	150	39.80%

Out of 377 samples obtained, 227 samples were culture positive and 150 samples were culture negative.

Table 2. Resistant strains among gram negative bacilli

No.of isolates	Resistant to third generation cephalosporins
139	67

Table 3. Esbl producing gram negative organisms obtained from various specimens

Specimens	Total isolates	ESBLproducers	Percentage
Urine	44	16	36.36%
Blood	50	8	16.00%
Sputum	10	1	10.00%
Wound swab	22	2	9.09%
Drainage tubetip	13	0	0.00%
Total	139	27	19.42%

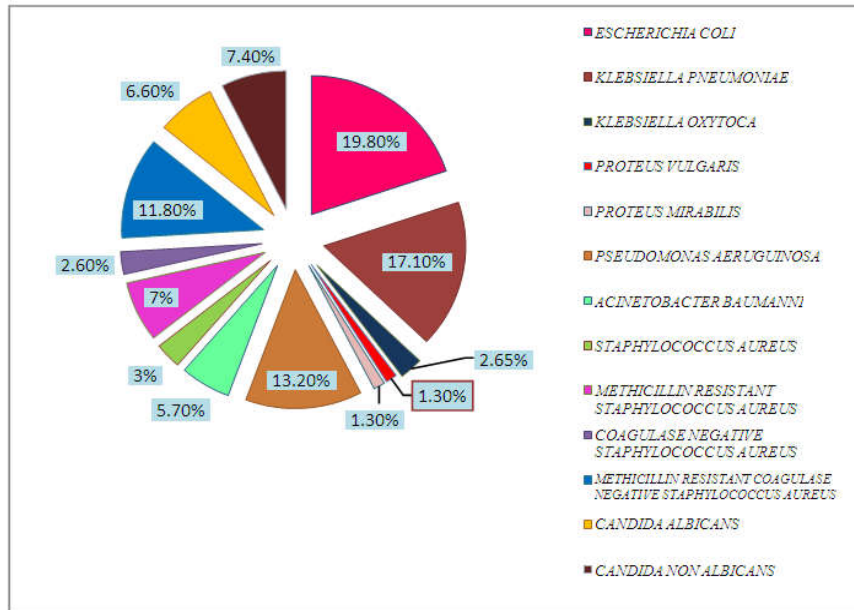


Fig. 2. Distribution of various organisms isolated in ICU at the Tertiary Care Hospital

From 139 gram negative bacilli, 27 (19.42%) were ESBL producers. The results shows 44 urinary isolates, 16 (36.36%) were ESBL producers. From Blood total number of isolates were 50, in that 8 (16.00%) were ESBL producers. Out of 10 sputum isolates, 1 (10.00%) were ESBL producers. Total number of isolates from wound swab were 22, in that which 2 (9.09%) were ESBL producers. Drainage tube tip isolates were 13 but they were not ESBL producers.

From the 50 isolates of gram negative bacilli, 8 (16.00%) were ESBL producers. From table shows 15 Escherichia coli isolates, 7 (46.66%) were ESBL producers. Out of 11 Klebsiella pneumoniae isolates, 1 (9.09%) were ESBL producer. Klebsiella oxytoca isolate was 1, Proteus vulgaris isolate was 1, Pseudomonas aeruginosa isolates were 14, and Acinetobacter baumannii isolates were 8, but they were not ESBL producers.

Out of 10 isolates of gram negative bacilli, 1 (10.00%) were ESBL producers. Out 8 Klebsiella

pneumoniae isolates, 1 (12.50%) were ESBL producer. Pseudomonas aeruginosa isolates were 2 but they were not ESBL producers.

From 27 ESBL producers, 9 (33.33%) sensitive to Cotrimoxazole, 6 (22.22%) were sensitive to Ciprofloxacin, 11(40.7%) sensitive to gentamicin, 13(48.14%) sensitive to Amikacin, 100.00% sensitivity was with Meropenem and 26(96.29%) sensitivity with Imipenem. Piperacillintazobactam showed 26 (96.29%) sensitivity.

4. DISCUSSION

ESBL which are plasmid encoded emerged due to the extensive use of extended spectrum cephalosporins, since 1980s a significant evolution of antimicrobial resistance developed. Globally ESBL have become a problem for hospitalized patients. Prevalence of ESBL varies widely among different geographic areas and keeps changing overtime. There are only few studies conducted in Indian hospitals to find the prevalence of ESBL producers, but ESBL

producing gram negative bacilli have evolved in various hospitals all over the country.[8] Since the prevalence of ESBL from ICU was found to be high from various studies, this study was conducted to find out the prevalence of ESBL producing organisms during the period of December 2017 - December 2018 in the Intensive care unit of Sree Balaji Medical College and Hospital from Urine, Blood, Sputum, Wound swab, Drainage tube tip.

Screening of ESBL producing gram negative bacilli in this study was done by double disc synergy testing which is a simple and cost effective procedure. 227 cases of both male and female between 20 - >80 yrs of age were included in this study which gives a ratio of 1:0.7 [9-11].

Among 337 samples found 227 gram negative bacilli. Among 139 gram negative bacilli, 67 were resistant to third generation cephalosporins (48.20%) and 27 out of them were positive by DDST for ESBL(19.42%) correlating with study of

Jerestin Hansotia et al. (25.7%), Bithika Duttaroy et al. 73(28.3%), Sumeeta et al. 74(30.2%), Ashwin et al. 75 (33.3%). In this study ESBL from urine was 36.36%, which is consistent in relation with the study done by Baby padmini et al. (40.3%). 52%(25/44) of Escherichia coli isolated from urine were ESBL producers which correlates with study by Bithika Duttaroy et al. (46.5%) [12-15].

ESBL among Klebsiella pneumoniae from urine was 30% in the present study which correlates with study by Baby padmini et al. 76(41%). In this study ESBL producing Klebsiella oxytoca was isolated from one wound swab. Study done by Christopher et al. 77 from Virginia in 1997 was 4.4%. This present study showed that there were 16.00% (8/50) ESBL producing organisms in blood which almost correlates with study done by Bithika et al. 73 which is around 11.32% (6/53). Increased percentage of ESBL producing organisms in present study may be due to the increased duration of patient stay in intensive care unit in our study [16].

Table 4. Esbl producing gram negative organisms among blood isolates (n = 50)

Isolates	Total	ESBL producers	Percentage
<i>Escherichia coli</i>	15	7	46.66%
<i>Klebsiella pneumoniae</i>	11	1	9.09%
<i>Klebsiella oxytoca</i>	1	0	0.00%
<i>Proteus vulgaris</i>	1	0	0.00%
<i>Pseudomonas aeruginosa</i>	14	0	0.00%
<i>Acinetobacter baumannii</i>	8	0	0.00%
Total	50	8	16.00%

Table 5. Esbl producing gram negative organisms among sputum isolates (n = 10)

Isolates	Total	ESBL producers	Percentage
<i>Klebsiella pneumoniae</i>	8	1	12.50%
<i>Pseudomonasaeruginosa</i>	2	0	0.00%
Total	10	1	10.00%

Table 6. Ntibiotic susceptibility of esbl producing and non esbl producing gram negative organisms to various non-β lactam antibiotics

Antibiotics	Resistant strains(n = 67)		Sensitive strains	
	Esblproducers (N = 27)		Non-esbl producers (n = 72)	
			(n = 40)	
Cotrimoxazole	9(33.33%)	9(22.50%)	24(33.33%)	
Ciprofloxacin	6(22.22%)	10(25.00%)	27(37.50%)	
Gentamicin	11(40.7%)	6(15.00%)	37(51.38%)	
Amikacin	13(48.14%)	18(45.00%)	41(56.9%)	
Meropenem	27(100.00%)	40(100.00%)	72(100.00%)	
Imipenem	26(96.29%)	40(100.00%)	72(100.00%)	
Piperacillin Tazobactam	26(96.29%)	31(77.50%)	72(100.00%)	

From blood, ESBL producing *Escherichia coli* was 46.66% (7/15). But in study done by Bithika et al. it was 18.18% (6/33)%. sputum, ESBL producing *Klebsiella pneumoniae* was 12.5% (1/8) in the present study which is consistent in relation with the study done by Patel SC et al. 79 in 2019 it is 27.7%. This difference may be due to low number of isolates in our study. This difference may be due to the decreased number of isolates in Bithika et al. study from Baroda medical college. ESBL producing *Klebsiella pneumoniae* in blood in the present study was 9.09 [17] Minimum inhibitory concentration was reduced to 0.19µg/ml-0.38µg/ml of Ceftazidime in presence of clavulanic acid by Ezy MIC test strip (E-test) method for 20 isolates of ESBL producing *Escherichia coli*, 6 isolates of ESBL producing *Klebsiella pneumoniae*. Minimum inhibitory concentration was reduced to 0.25µg/ml for single isolate of *Klebsiella oxytoca*.

In the present study out of the 27 ESBL producing organisms, 9 (33.33%) were sensitive to Cotrimoxazole, 6 (22.22%) were sensitive to Ciprofloxacin, 11(40.7%) were sensitive to Gentamicin, 13(48.14%) were sensitive to Amikacin which is almost consistent in relation with study done in Coimbatore by Baby padmini76 et al. in which the sensitivity was 26% for Cotrimoxazole, 89% for Amikacin, 9% for Ciprofloxacin. All the ESBL producing gram negative bacilli showed 100% sensitivity with Meropenem which is consistent in relation with the study done by Shukla [7] et al. from Haryana [18].

Extended Spectrum Beta Lactamase producing gram negative bacilli isolated from the Intensive care unit of Sree Balaji Medical College and Hospital during the period from December 2017 to December 2018 was 19.42% by double disc synergy test and phenotypic confirmatory cephalosporin - clavulanate combination disc test. Prevalence of ESBL in our intensive care unit is less when compared with other studies from India. ESBL producing gram negative bacilli organisms that were isolated was found to be more in urine samples (36.36%), followed by blood samples (16.0%), sputum (10.0%) and wound swab (9.09%).

In Intensive care units extensive use of third generation cephalosporins, prolonged intubations and prolonged stay poses an important factor for the development of Extended Spectrum Beta Lactamase mediated resistance. Hence

appropriate antibiotics and proper infection control practices can curtail spread of ESBL production. Being a simpler and cheaper method DDST should be included as a routine test in all Microbiology laboratories for early detection of ESBL producing organisms particularly when samples received from critically ill patients.

Although Meropenem is 100% sensitive to all ESBL producing gram negative bacilli, but still sensitivity also observed with some cheaper drugs like Cotrimoxazole (33.33%), Amikacin (48.14%), Gentamicin (40.7%), Ciprofloxacin (22.22%). Hence by vigilant screening we can reduce the cost of treatment for patients infected with Extended Spectrum Beta Lactamase producing organisms.

5. CONCLUSION

The alarming frequency of beta-lactamase producing infections among patients in the ICU is obtained. These beta-lactamase producing isolates are extremely MDR and revealed a high burden of resistance to antibiotic drugs and anti-metabolite classes of antibiotics. Thus, screening for these and taking appropriate measures for control of their spread are of major importance.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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