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Research Article

Detection of extended spectrum β-lactamase among gram negative clinical isolates from a tertiary care hospital in South India

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ABSTRACT

Background: The β lactamase enzymes produced by the organisms break down the structural beta-lactam ring of β lactam antibiotics. Many genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β lactamase and also some are plasmid mediated β lactamases. The objective of the study was to detect extended spectrum β lactamases among gram negative clinical isolates.
Methods: 200 clinical were subjected to routine disc diffusion technique and zone diameter of ≤27mm for Cefotaxime and ≤22mm for Ceftazidime or ≤25mm for Ceftriaxone were included in this study. The strains are subjected to double disc synergy test.
Results: Of 200 samples, 174 yielded organisms belonging to *enterobacteriaceae* and 26 yielded growth of nonfermenters. Out of 174 members of *enterobacteriaceae* family, 122 were *E. coli*, 36 *Klebsiella spp*, 8 *Proteus spp*, 5 *Enterobacter spp* and 3 *Citrobacter spp*. Out of 26 nonfermenters, 18 were *Pseudomonas spp* and 8 were *Acinetobacter*.
Conclusions: In the present study prevalence of ESBL was 23.3%, the high prevalence may be due to irrational use of third generation cephalosporins in both the hospital and community.

Keywords: Extended spectrum β lactamases, Cefotaxime, Ceftazidime, Double disc synergy test

INTRODUCTION

Penicillin was discovered by Alexander Fleming used to treat Gram positive bacterial infections, however Flory and Chain demonstrated therapeutic activity of penicillin. Simultaneously bacteria too have developed various mechanism to overcome inhibitory action of such antibiotics, of which elaboration of enzymes is one of important mechanism of inactivating antibiotics.[1] β lactamases are structurally related to PBP's and coevolved with β lactam antibiotics. The β lactamase enzymes produced by the organisms break down the structural beta-lactam ring of β lactam antibiotics. Many genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β lactamase and also some are plasmid mediated β lactamases. This development was likely to the selective pressure exerted by β lactam producing soil organisms found in the environment. In early 1960s TEM-1 was the 1st plasmid mediated β lactamase described in gram negative organisms. Another common plasmid mediated β lactamase is SHV-1.[2] In recent years there has been an increased incidence and prevalence of extended spectrum β lactamases [ESBLs], enzymes that hydrolyse and cause resistance to oxyimino cephalosporins and Aztreonam.[3] They represent a major group of β lactamases belonging to Ambler class A penicillinases currently being identified worldwide in large numbers and are now found in a significant percentage of *E. coli* and *K. pneumoniae* strains. They have also been found in other *Enterobacteriaceae* strains like *Enterobacter, Citrobacter, Proteus, Morganella morganii, Serratia marsescens, Shigella dysenteriae, Pseudomonas aeruginosa*.[3] Today over 150 different ESBLS has been

described ESBLS were first observed in 1983 in isolates of Klebsiella ozaenae in Germany.[2] Thereafter, within one year there was wide spread dissemination of ESBL producing organisms in hospitals of Europe. Now major outbreaks' involving these resistant organisms has been reported all over the world in many members of the *Enterobacteriaceae* and *Pseudomonas* species, resulting in limitation of therapeutic options. ESBL producing strains are probably more prevalent than currently recognized because they are often undetected by routine susceptibility testing methods.[4] ESBL strains have been associated with resistance to other non beta lactam antibiotics like the Aminoglycosides and Chloramphenicol. Another property of these ESBL strains is that they might show a false sensitive zone of inhibition in the Kirby- Bauer disk diffusion method.[5] The present study was conducted with an objective to find out the presence of ESBL producing Enterobacteriaceae and formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.

METHODS

The present study was under taken to look for extended spectrum β-lactamases (ESBL) production in gram negative bacterial isolates, from inpatients who were admitted to different wards and ICU at our hospital. 200 isolates were included in the study.

These strains were subjected to routine disc diffusion technique and zone diameter of ≤27mm for Cefotaxime and ≤22mm for Ceftazidime or ≤25mm for Ceftriaxone were included in this study. All the strains were stored at 4˚C in stock vials containing semisolid agar medium for the study. At the time of testing, the stock vials were sub cultured onto blood agar to check for purity and viability and inoculated onto peptone water and incubated at 35°C in the incubator for 4 hours. The resulting growth was matched to 0.5 McFarland turbidity and inoculated onto Muller Hinton agar (MHA) plate using a sterile cotton swab.

Double disc synergy test[6]

The test inoculums (0.5 McFarland tube) was spread as a lawn onto Muller Hinton agar plate using a sterile cotton swab. A disc of Ceftazidime (30 µg) + Clavulanic acid (10 µg) were placed on the surface of MHA, then the disc of Ceftazidime (30 µg) were placed at the distance at the distance of 15 mm from Ceftazidime + Clavulanic acid disc by edge to edge. The inoculated plates were incubated at 35°C in the incubator for 18-24 hours. The zone of inhibition between the Ceftazidime and Ceftazidime + Clavulanic acid was compared. Difference in the zone diameter of ≥5 mm was interpreted as positive for ESBL production.

RESULTS

Of these 200 samples, 174 yielded organisms belonging to *enterobacteriaceae* and 26 yielded growth of nonfermenters. Out of 174 members of *enterobacteriaceae* family, 122 were *E. coli*, 36 *klebsiella spp*, 8 *proteus spp*, 5 *enterobacter spp* and 3 *citrobacter spp*. Out of 26 nonfermenters, 18 were *pseudomonas spp* and 8 were *acinetobacter spp*. The breakup of the specimens and organisms is given in the following table 1 and table 2.

Table 1: Distribution of sample from various clinical specimens.

Specimen	Number of samples	Percentage (%)
Urine	119	59.5
Pus	32	16
Wound swab	28	14
Sputum	10	5
Vaginal discharge	04	2
Stool	02	1
Renal abscess	03	1.5
Blood	02	1

Table 2: Detection rate of different isolates in the study population.

Organism	Number of samples	Percentage (%)
E. coli	122	61
Klebsiella spp	36	18
Proteus	08	4
Enterobacter	05	2.5
Citrobacter	03	1.5
Pseudomonas	18	9
Acinetobacter	08	4

Table 3: Distribution of various ESBL producing strains.

Organism	Total	ESBL positive (%)
E. coli	122	24 (19.6%)
Klebsiella spp	36	15 (41.6%)
Proteus spp	08	03 (37.5%)
Enterobacter	05	01 (20%)
Citrobacter	03	01 (33.3%)
Pseudomonas	18	02 (11.1%)

Overall, 46 (23%) isolates, (24 *E. coli*, 15 *Klebsiella spp*, 3 *Proteus spp*, 3 *Enterobacter*, 1 *Citrobacter*, 2 *Pseudomonas*) were characterized as ESBL producers. The distribution of ESBL producer strain was shown in table 3.

DISCUSSION

ESBL producing strains are a major problem in many hospitals leading to increased morbidity, mortality and health care costs. Cephalosporins are first line drugs in treatment of infections caused by *Enterobacteriaceae* family, however because of extensive use of third generation cephalosporins has resulted in increased prevalence of ESBL and plasmid mediated AmpC among organism.

In the present study, a total of 200 Gram negative strains were isolated from various clinical specimens of which majority are from urine 59.5% (119/200) followed by pus 16% (32/200), wound swab pus 14% (28/200) (Table 1). Among the isolated organisms *E. coli* was the most predominant 61% (122/200), followed by *klebsiella spp* 18% (36/200), *Pseudomonas spp* 9% (18/200), *Proteus spp* 4% (08/200), *Enterobacter* 2.5% (5/200) and *Citrobacter* 1.5% (3/200).

In the present study 19.6% and 41.6% isolates of *E. coli* and *Klebsiella spp* are ESBL producers. Similar to our study Kumar et al in 2004[7], observed ESBL productions in *E. coli* is 43.2% and *Klebsiella spp* 39.5%. In the same year study by Babypadmini S et al, 57.89% of ESBL production by *Klebsiella spp* followed by *Proteus spp* 50.0%, *E. coli* 47.83% and *pseudomonas spp* 31.35%.[8]

In the present study prevalence of ESBL was 23.3%, which was very similar to two Indian studies by Shukla et al[9] and Shivaprakasha et al[10] were prevalence of ESBL to be 27.3% and 25.8%. In contrast to our study, studies by Arunkumar et al[11] and Hansotia et al[12] observed ESBL production is 70% and 53.4%. The high prevalence may be due to irrational use of third generation cephalosporins in both the hospital and community and is believed to be the major cause of mutations in these enzymes that has led to the emergence of the ESBLs.

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