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PREVALENCE OF ESBL PRODUCERS IN FAMILY ENTEROBACTERIACEAE ISOLATED FROM D. Y. PATIL MEDICAL COLLEGE & HOSPITAL, KOLHAPUR

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ABSTRACT: Extended Spectrum Beta Lactamases (ESBL) producing Gram Negative Bacilli (GNB) is a worldwide problem. Although few studies have reported on the prevalence of ESBL producers in Indian hospitals, ESBL producing bacteria may have evolved in several hospitals all over the country. Therefore the study was carried out at Dept. of Microbiology, D. Y. Patil Hospital & Research Center, Kadamwadi, Kolhapur, to examine the incidence of ESBL producing strains & multiple drug resistance in GNB during the period of Dec. 2009 to May 2011. A total number of 197 isolates belonging to family Enterobacteriaceae were isolated from various clinical samples & studied for ESBL production by Kirby- Bauer disc diffusion test as per CLSI (Clinical Laboratory Standards Institute) standards & confirmed by using DDST (Double Disc Synergy Test) & MIC. Out of 197 isolates, 83 isolates (42.13%) were potential ESBL producers on the basis of resistance or decreased sensitivity to third generation Cephalosporins (3GC). ESBL production is detected by Jarlier DDST for these 83 isolates. Seventy (35.53%) isolates out of these 83 isolates were DDST positive. All the ESBL producers were tested for MIC and antibiotic sensitivity test (AST).

KEYWORDS: Extended Spectrum beta Lactamases, 3GC resistance, Enterobacteriaceae.

INTRODUCTION: Beta lactamase is a major defense of GNB against beta lactam antibiotics like penicillin's etc. The developments of extended spectrum antibiotics like cephalosporins are effective against GNB. 3rd generation cephalosporins (3GCs):

Cefotaxime, ceftriaxone & ceftazidime are known as extended spectrum cephalosporins (ESCs)⁽¹⁾ are used to treat GNB infections, then the bacteria gave more aggressive answer by producing ESBL enzyme that made these 3GCs ineffective due to wide use of these antibiotics, resistance to these drugs began to appear.⁽¹⁾

The present study was carried out to detect the prevalence of ESBL producing GNB in Dr. D. Y. Patil Medical College & Hospital, Kolhapur, Maharashtra.

MATERIALS & METHODS: Clinical isolates obtained from various clinical samples like pus, sputum, urine, and body fluids etc. received at the Diagnostic Microbiology Section of D. Y. Patil Medical College & Hospital Research Centre, Kadamwadi, Kolhapur, over a period of one & half year. These isolates were identified by conventional methods (Growth on Blood agar & Mac Conkey agar and by biochemical reactions)

Routine Antibiotic sensitivity testing (AST) was performed on clinical isolates by Kirby-Bauer disk diffusion method for different antibiotics, which are routinely used in the lab (Discs used–Hi Media, Mumbai):

Aztreonam (30µ), Amikacin (30µ), Gatifloxacin (5µ), Piperacillin-tazobactam (100/10µ), Amoxy-clav (20/10µ), Imipenem (10µ), Ceftazidime- clav acid (30/10µ). The results were recorded as per CLSI (Clinical Laboratory Standard Institution).⁽²⁾

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Criterion for Selection of ESBL Producing Strains: 3GCs mainly ceftazidime, cefotaxime, ceftriaxone or cefpodoxime were used. Isolates found to be resistant to or with decreased sensitivity to any one of these drugs (done in routine AST) were selected for the ESBL as per CLSI standards & taken for further study.

ESBL Detection:

Jarlier Double Disk Synergy Test (DDST):

- All the selected isolates were tested for ESBL production by this method.
- Demonstration of a synergistic reaction between 3GCs & Clavulanic acid.
- Test was done by preparing a lawn culture of test strain 0.5 McFarland standards on Muller Hinton Agar (Hi Media, Mumbai). Any one of 3GC disc (30µg) and Amoxy-clav (20/10µg) or Ceftazidime-clav acid (30/10µg) were placed 15 mm apart,⁽³⁾ and incubated overnight at 37°C.
- Enhancement of zone of inhibition towards the combination disc or increased zone of inhibition around the combination disc more than 5 mm in comparison to the 3GC disc alone was considered as ESBL producer.^(3,4)

Minimum inhibitory concentration (MIC) was observed and recorded for all the ESBL producing isolates.

Quality Control: Standard strain of *E. coli* 25922 (Sensitive to 3GC), was used as Negative control, Standard strain of *Klebsiella pneumonia* 700603 was as Positive control. Every batch of media prepared was checked for sterility by 24hrs incubation.

RESULTS AND DISCUSSION: Out of 197 GNB isolated from various clinical samples in the present study 83 isolates were potential ESBL producers detected by screening test (resistance to any one of 3GC mainly Ceftazidime, Cefotaxime, Ceftriaxone) as per CLSI standards,⁽²⁾ & most of these isolates were multidrug resistant. The most common GNB isolated from various clinical samples were *E. coli* (39), *Klebsiella pneumonia* (19), *Klebsiella oxytoca* (4), *Proteus vulgaris* (7), *Proteus mirabilis* (6), *Citrobacter* spp (8) as shown in figure no 1 in the present study.

Fig. 1

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Photograph No. 1: DDST- showing zone of combination disc (Ceftazidime-clav acid) is more than the individual discs (Ceftazidime & ceftriaxone).

Photograph 1

Fig. 2

These 83 isolates were tested for ESBL production by Jarlier DDST. Out of these 83 isolates 70 (35.53%) isolates were positive by DDST as shown in photograph no 1. Remaining 13 isolates were positive by screening test but negative by DDST. Out of these 13 isolates 8 isolates had shown sensitivity to 3GC and 5 isolates were resistant to all antibiotics except Imipenem (they could be AmpC β -lactamase producers). All these 13 strains were excluded from the study. The 70 isolates positive for DDST, were then tested for MIC by agar dilution method and there was 100% correlation between results of MIC and CLSI confirmatory test.

DISCUSSION: Prevalence of ESBL producing GNB in the present study was 35.53% and ESBL production was highest in *E. coli* 33(39.75%) followed by *Klebsiella pneumoniae* 16(37.20%) from various clinical samples as shown in figure no 2. Similar results were reported by Babypadmini et al.⁽⁵⁾ and Nath et al.⁽³⁾

Previous studies from India have showed prevalence of ESBL producers to be 6.6% to 87%.^(5,6,7) ESBL production was reported among GNB by Anantan et al, University of Madras, Taramani, Chennai in 2002,⁽⁸⁾ Babypadmini et al PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, in 2004, reported 31.6% were ESBL producers, where *E. coli* contributed

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41% and *Klebsiella pneumoniae* 40(5)Nath et al, Assam Medical College and Hoaspital, Dibrugarh, Assam in 2006 had reported 37.89% GNB were ESBL producers.(3)

Duttaroy et al, Medical College Baroda, Gujrat,⁽¹⁾ in 2005, had reported 53% GNB were ESBL producers.

Arora et al reported 56.5% of *E.coli* & 26.1% of *Klebsiella pneumoniae* were ESBL producers,⁽⁹⁾ Tankhiwale et al reported 48.3% of urinary isolates tested were ESBL producers,⁽¹⁰⁾ Manchanda et al reported 87% of *Klebsiella pneumoniae* were ESBL producers.⁽⁶⁾

E.coli (19; 57.5%) was most common organism isolated followed by *Klebsiella pneumoniae* (06,18.18%) in the present study form urine sample. Similar results have been reported by Tankhiwale et al.,⁽⁹⁾ and also by Agrawal et al.,⁽¹⁰⁾ 30% of *E.coli* and 16% of *Klebsiella* spp were found to be ESBL producers. In the present study ESBL producers which were isolated from urine samples (33) were tested for Nitrofurantoin, Nalidixic acid and Norfloxacin. 66.6% ESBL producers showed susceptibility to Nitrofurantoin, 90.9% showed resistance to Nalidixic acid and 87.8% were resistant to Norfloxacin. Various organisms which have been reported to be isolated from UTI were mostly *E.coli*. From pus samples also the most common organism isolated was *E.coli* (47.6%) followed by *Klebsiella pneumoniae* (14.28%). Similar results were noted by Agarwal et al ⁽¹⁰⁾ *E.coli* (52%) was the most common, followed by *Klebsiella pneumoniae* (28%).

CONCLUSION: The prevalence of ESBL producing GNB of family Enterobacteriaceae in the present study was 35.53%. ESBL production was highest in *E.coli* isolates 39.75% followed by *Klebsiella pneumoniae* 37.5%. *E.coli* was the most common ESBL producing organism isolated from various samples like urine 57.5%, pus 47.6%, and, blood 33.3%, followed by *Klebsiella pneumoniae* isolated from various clinical samples like urine 18.18%, pus 14.28%, sputum 100%, and, blood 66.6% etc.

Regular monitoring of prevalence and incidence of ESBL producers in GNB from various clinical areas including critical care units like ICU, NICU of the hospital is very important. To prevent spread of ESBL producing organisms and failure of antibiotic therapy, hospital must have:

- i. A hospital drug policy.
- ii. A functioning hospital infection control committee.

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