Prevalence of Extended Spectrum Beta Lactamase Producing Gram Negative Bacilli among Urine Isolates and their Antibiotic Susceptibility Pattern from Intensive Care Units in a Tertiary Care Hospital

M. Nishanthy*

Sree Balaji Medical College and Hospital, Chennai, India

*Corresponding author

ABSTRACT

Infection rates in ICU have been found to be high among hospital acquired infections. The most commonly used wide group of antimicrobials is beta-lactams. However, increasing trend of resistance to various beta lactams among uropathogen is mainly mediated by beta-lactamases which is of major concern. This study was done to determine the prevalence of Extended spectrum beta lactamase producing Gram negative bacilli among urine culture isolates and their trends of antibiotic susceptibility patterns which is prevalent among these isolates in our Institute from ICU patients. The study was conducted in the Department of Microbiology at Sree Balaji Medical College and Hospital from December 2017 to December 2018. Total number of urine samples received were 316 in our microbiology laboratory from ICU. Isolates were identified by Colony morphology, Gram stain and standard biochemical reactions. Antibiogram was determined by Clinical and Laboratory Standards Institute (CLSI) guidelines. Total positive culture present in the study 68 (21.51%). Among these culture positive isolates 22 were gram positive (32.35%) isolates and 46 were gram negative (67.64%). Among these 46 isolates of gram negative, 17 (36.95%) were ESBL producers. The most common isolate was E.coli, Klebsiella pneumoniae species. All the extended beta lactamase producing gram negative bacilli isolates showed 100% susceptibility to Carbapenems, Colistin and Polymyxin-B.

Introduction

Urinary tract infections are a potentially distressing, preventable infectious complication which is encountered in ICU. Prolonged hospitalization, ICU stay for a longer time, prior surgery, Foley's catheterization were all found to be associated risk factors. ESBL producing isolates poses lot of problems which are difficult to be detected and treated, thereby causing increasing mortality. In appropriate antibiotic therapy can lead to severe complications. ESBL detection among urine samples is of vital importance because it is an epidemiological marker of colonization and poses a significant threat in transfer of organisms to other patients. These urinary tract infections are due to either gram positive or gram negative organisms. Diagnosis of Urinary tract infections are usually made with urine cultures which yields a higher positive
predictive value. Early diagnosis and appropriate treatment with antibiotics can decrease the burden of urinary tract infections in critical care units. Hence this study was done to know the prevalence of extended spectrum beta lactamase producing gram negative bacilli among urine culture isolates from ICU and their trends of antibiotic susceptibility patterns which is prevalent among these isolates in our institute.

**Materials and Methods**

The study was conducted in the Department of Microbiology at Sree Balaji Medical College and hospital from December 2017 to December 2018. Total number of urine samples received were 316 in our microbiology laboratory from ICU. All these urine samples were collected from each patient under strict aseptic precautions. 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% ethyl alcohol, 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.

Urine samples were inoculated onto nutrient agar, blood agar and Mac-conkey agar plates and were incubated at 37 deg C aerobically for 24 hrs. Isolates were identified by Colony morphology, Gram stain and standard biochemical reactions [5]. The antibiotic sensitivity test was done by Kirby-Bauer disc diffusion method on Muller Hinton Agar with commercially available himedia discs ampicillin(10mcg), cefazolin (30mcg), cefuroxime (30mcg), ceftriaxone (30mcg), amoxicillin-clavulanate (20/10mcg), gentamicin (10mcg), amikacin (30mcg), ciprofloxacin (5mcg), ceftazidime (30mcg), ceftriaxone (30mcg), cefotaxime (30mcg), tobramycin (10mcg), piperacillin-tazobactam (100/10mcg), meropenem (10mcg), imipenem (10mcg), ciprofloxacin (5mcg), cotrimoxazole (1.25/23.75mcg), amikacin (30mcg), ceftazidime (30mcg), cefepime (30mcg), ciprofloxacin (5mcg), nitrofurantoin (300mcg). Results were interpreted according to CLSI guidelines [6]. These was also confirmed by automated identification and antimicrobial susceptibility system i.e. By Vitek-2 compact machine. Isolates with decreased susceptibility to cefotaxime and ceftazidime were tested for presence of extended spectrum beta lactamases by double disc synergy test and confirmation was done by phenotypic confirmatory test by cephalosporin clavulanate combination disc test and E-Test.

**Results and Discussion**

A total number of 316 urine samples from patient suspected of urinary tract infections, admitted in intensive care unit of our hospital were routinely processed for urine culture in the Department of Microbiology from December 2017 to December 2018. Out of these samples males were 198 (62.65%), females were 118(37.34%).

Total positive culture present in the study 68 (21.51%). Among these culture positive isolates 22 were gram positive(32.35%) and 46 were gram negative (67.64%).

The most common isolate was *E.coli*, *Klebsiella pneumoniae* species. All the extended spectrum beta lactamase producing gram negative isolates showed 100% susceptibility to Carbapenems, Colistin and Polymyxin-B.

Among 46 gram negative isolates, 17isolates (36.95%) were ESBL producers, out of them 9isolates were *E.coli* (52.94%), 8isolates were
Klebsiella pneumoniae (47.05%) species. All these gram negative isolates showed weak activity against third generation cephalosporins but showed 100% susceptibility to Carbapenems, Colistin and Polymyxin-B.

Prevalence of extended spectrum beta lactamase in India has been reported since 1990s [1]. In a study done by Aruna et al., 2012 indicates a higher prevalence of extended spectrum beta lactamase producing E. coli are found to be the highest in India (60 %), followed by Hong Kong (48 %) and Singapore (33 %) [7]. Most common encountered drug resistant uropathogen is E. coli [8] Klebsiella pneumoniae is the second most common encountered uropathogen which is frequently an ESBL producer [9].

Table 1 Antibiotic Sensitivity Pattern of Extended Spectrum Beta Lactamase Producing Gram Negative Bacilli among Urine Culture Isolates from Intensive Care Unit

| Isolates (n=46) | MRP | IMP | PIT | CIP | COT | AK | AMC | GEN |
|----------------|-----|-----|-----|-----|-----|----|-----|-----|
| E.coli (n=9)   | 9 (100%) | 9 (100%) | 9 (100%) | 3 (33%) | 2 (22%) | 8 (89%) | 4 (44%) | 4 (44%) |
| Klebsiella pneumoniae (n=8) | 8 (100%) | 7 (88%) | 7 (88%) | 3 (38%) | 2 (25%) | 5 (63%) | 3 (38%) | 3 (38%) |

Fig.1

SEX DISTRIBUTION

- Male 63%
- Female 37%

Fig.2

CULTURE POSITIVE ORGANISMS

- Gram Positive 32%
- Gram Negative 68%
The present study provides information regarding prevalence of extended spectrum beta lactamase producing gram negative bacilli among urine isolates and antibiotic susceptibility pattern from intensive care units in a tertiary care hospital. The urine culture positivity rate present in the study 68 (21.51%). Among these culture positive isolates 22 were gram positive (32.35%) isolates and 46 were gram negative (67.64%). The most common isolate was *E.coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* species. All the extended beta lactamase producing gram negative bacilli isolates showed 100% susceptibility to Carbapenems, Colistin and Polymyxin-B.

Irrational use and the over counter availability of antibiotics have led to higher prevalence of resistance among micro-organisms. All the gram negative organisms showed poor susceptibility to beta-lactam antibiotics because of the fact that beta-lactam antibiotics are the most commonly prescribed drugs for both inpatients and outpatients could be the reason for such high level of resistance. The members of the family enterobacteriaceae showed good susceptibility to carbapenems, piperacillin tazobactam and amikacin.

Minimum inhibitory concentration of ceftazidime by E-test strip method for 17 Esbl producing organisms in this study was between 16µg/ml to 32 µg/ml. Minimum inhibitory concentration of ceftazidime for 9 isolates of ESBL producing *Escherichia coli* was between 16µg/ml to 32µg/ml. Minimum inhibitory concentration of ceftazidime by E-test strip method for 8 isolates of Esbl producing *Klebsiella pneumoniae* was between 16µg/ml to 32µg/ml. Major threat is the infection caused by these organisms poses a greater risk and makes very limited antibiotics available for treatment. With limited options available for treatment and increasing resistance, clinicians are left with so called drugs like Polymyxin –B and Colistin which could soon lead to most dreaded condition like pan drug resistance.

In conclusion the present study showed prevalence of extended spectrum beta lactamase producing gram negative bacilli among urine culture isolates from intensive care units is around 36.95%. This implies urine culture must always be done in suspected cases of urinary tract infection, once antibiotic susceptibility profile of organisms is known de-escalation of higher end antibiotics should be done to reduce antimicrobial pressure. However, effective stringent hospital infection control policy and good antibiotic policy with routine surveillance for baseline resistance will go a long way in combatting drug resistance.

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