# Community-acquired urinary tract infections by extended-spectrum beta-lactamase-producing Gram negative bacteria

Y.V.S.Annapurna, V.V.Lakshmi

**Abstract**— Antibiotic resistance among uropathogens causing community acquired urinary tract infection (CAUTI) is increasing worldwide. Severity and endemicity persists despite of wide spread availability of drugs. Knowledge of the aetiology and antimicrobial susceptibility patterns of uropathogens is important in order to determine the best empiric treatment option. Extended-spectrum beta-lactamase (ESBL) producing strains of Enterobacteriaceae and other Gram negative bacteria are a cause of increasing concern. Delay in the detection and reporting of ESBL production by gram-negative bacteria is associated with prolonged hospital stay, increased morbidity, mortality and health-care cost. This study was aimed to determine the aetiology and antimicrobial susceptibility of uropathogens in culture-positive, CAUTIs. The incidence, MAR index and virulence characters of ESBL-producing strains were also analyzed. Double-disk synergy test (DDST) and E test was performed according to CLSI recommendation in order to detect the ESBL producers. In addition to antibiotic resistance, virulence characters like mucoid and hydrophobic nature of isolates,Protease and Biofilm production were studied. The commonest organism isolated was *Escherichia coli* and *Klebsiella* spp. Among the antibiotics tested,isolates were most susceptible to Amikacin ,Netilimicin and Gentamicin. Maximum resistance was observed with cephalosporins.ESBL production was predominant in *Escherichia coli* and *Klebsiella* spp. Most commonest virulence feature in the present study was found to be exopolysaccharide production

**Index Terms**— Antimicrobial susceptibility, community-acquired urinary tract infection, ESBL-producers, *E. coli*, *K. pneumonia*, Uropathogens

## 1 Introduction

Extended spectrum β-lactamase (ESBL) isolates were first detected in Western Europe in the mid-1980s. Since then, their incidence has been increasing steadily. A large number of outbreaks of infections due to ESBL producing organisms have been described on every continent of the globe. This alarming increase may further lead to increased patient mortality when antibiotics inactive against ESBL producers are used. Therefore, control of the initial outbreak of ESBL producing organisms is of critical importance[1]. Among Gram-negative pathogens, beta-lactamases remain the most important contributing factor to beta-lactam resistance[2].Special efforts have been undertaken by clinical microbiology laboratories as recommended by the Clinical and Laboratory Standards Institute (CLSI) for ESBL detection. Additional use of ESBL detection methods has originated because some ESBL producing organisms appeared susceptible to cephalosporins, using conventional breakpoints. It has been recommended that physicians should avoid all penicillins, aztreonam, and cephalosporins if an ESBL producing organism is present[1] Amp C lactamases, in contrast to ESBLs, hydrolyse broad and extended-spectrum cephalosporins but are not inhibited by clavulanic acid or other lactamase inhibitors[3]

Urinary tract infections (UTIs) due to ESBL producing organisms constitute a common clinical problem not only in adult population but also in pediatric hospitals[4].Detection of ESBL producing organism from samples such as urine may be important because this represents an epidemiologic marker of colonization, and therefore there is potential for transfer of such organisms to other patients. Hence, the present study was designed to detect ESBL production among uropathogens.

## 2 Material and Methods

### 2.1 Sample collection

Male and female patients attending the Central Railway Hospital, Mettuguda, Hyderabad, India as out- patients during the period January-December 2013, presenting symptoms of Urinary tract infection were included in the study.

### 2.2 Isolation, identification and confirmation of isolates

Samples with predominantly one bacterial isolate were subjected to standard identification procedures of colony morphology, Gram staining reaction, motility, sugar fermentation and IMViC tests [5],[6]. The enzyme tests like catalase, oxidase, and urease test were also tested. The samples were further processed to determine antibiogram.

### 2.3 Antibiotic Susceptibility Testing:

Susceptibility of isolates to antibiotics were tested using the disk diffusion Bauer-Kirby Method, against the following commonly used antibiotics using discs obtained from Himedia, Mumbai. The spectrum of antibiotics tested include β lactam group- Cefoperazone (CFP:75µg),cefaclor

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(CEC:30µg),Cefuroxime (CXM:30µg), Ceftriaxone (CRO:30µg), Cefadroxil (CFR:30µg), Aminoglycoside group like Amikacin(AMK:30µg), Netilmicin(NET:30µg), Gentamicin (GEN:10µg) and Norfloxacin (NOR:10µg), Ciprofloxacin (CIP:5µg), Ofloxacin (OFX:5µg) that belong to Quinolone group. Discs were consistently tested for efficacy against standard strains recommended by Clinical Laboratory Standards Institute. Inhibition zones sizes were interpreted in accordance to Performance Standards for Antimicrobial Disk Susceptibility Tests, CLSI [7].

**2.4 Identification of MDR and determining MAR index:**

A multiple drug resistance (MDR) phenotype was defined as resistance to 3 or more antimicrobial agents. The multidrug resistance character of the isolates was identified by observing the resistance pattern of the isolates to the antibiotics [2]. Multiple antibiotic resistance (MAR) index, referred to as the number of antibiotics to which test isolate displayed resistance divided by total number of antibiotic to which the test organism was calculated. The MAR index of profile is a good indicator that helps to evaluate the heath risk of the environments for each test isolates by method of Krumperman[8].

**2.5 Detection of virulence factors**

The virulence features like mucoid nature, cell surface hydrophobicity, gelatinase, and biofilm production of the MDR isolates were determined.

**2.5.1 Mucoid nature**-A bacterial loop was touched to a suspect colony on a agar plate and withdrawn slowly. Bacteria forming a 'string' of at least 5 mm were determined to be positive[9].

**2.5.2 Cell surface hydrophobicity** The cell surface hydrophobicity of isolates was determined by using Salt Aggregation Test(SAT)[6]. Ten microliter of isolate suspension made in phosphate buffer was mixed with equal amounts of ammonium sulphate solution of different molar concentrations (0.2, 0.4, a bacterial loop was touched to a suspect colony on a 5% sheep blood agar plate and withdrawn slowly. Bacteria forming a 'string' of at least 5 mm were determined to be positive1, 1.4, 2M) on a glass slide. Visible clumping or aggregation of the organism was observed for one minute while rotating . UPEC strains that had SAT value less than or equal to 1.4M were considered hydrophobic. Strains showing aggregation in 0.002M phosphate buffer (pH 6.8) alone were taken as auto agglutination.

**2.5.3 Gelatinase test:** Gelatinase production was tested using gelatin agar [6]. The plate is inoculated with test organism and inoculated at 37°C for24h.The plate is then flooded with mercuric chloride solution . Development of opacity in the medium and zone of clearing around colonies were considered positive for gelatinase.

**Biofilm production:** Congo red agar (CRA) medium was prepared with brain heart infusion broth , sucrose, agar and congo red indicator[10],[11],[12]. CRA plates were inoculated with test organism and incubated at 37°C for 24 hrs. Black colonies with dry consistency indicate strong biofilm formation.Brownish or reddish growth was considered as negative biofilm formation.

**2.5.4 Phenotypic confirmatory test for ESBL production** The phenotypic confirmatory test for ESBL production was performed as per CLSI guidelines [7]. For this purpose, following four antibiotic discs were used: Cefotaxime (30 µg), ceftazidime (30 µg), cefotaxime-clavulanic acid (30/10 µg) and ceftazidime-clavulanic acid (30/10 µg) (HiMedia Laboratories Pvt, Ltd., Mumbai, India). Discs were placed 25 mm apart on a MHA plate inoculated with 0.5 McFarland suspension of the test isolate. Plates were incubated at 35°C for 18 h at ambient atmosphere. After incubation the zone diameters around each of the disc were measured. A difference of ≥5 mm between the zone diameters of either of the cephalosporin discs and their respective cephalosporin/clavulanic acid disc was considered as positive phenotypic confirmatory test for ESBL production.

**2.5.5 E test for ESBL :**The strains were phenotypically screened by E-test MIC using ceftazidime, +/- clavulanic acid, cefotaxime +/- clavulanic acid ( HiMedia Laboratories Pvt, Ltd., Mumbai, India ). A reduction of MIC by ≥3 twofold dilutions of the cephalosporin in the presence of clavulanic acid, i. e. a MIC ratio of ≥ 8,was considered indicative of ESBL phenotype [2].

## 3 Results and Discussion

Among the total 500 patients with urinary tract infection, 470 samples showed significant growth. Of 470 isolates,*E.coli* was seen in 229 (48.72%),*Klebsiella sp* in 185(39.36%),and 56(11.91%) belonged to non- lactose fermenters. Preliminary identification of *E.coli* and *Klebsiella* sp were done based on their Gram negative character, formation of lactose fermenting colonies on Macconkey media. *E.coli* showed colonies with metallic sheen on Eosin methylene blue media. Further confirmation of the isolates was done by the results obtained from biochemical reactions[5],[6].

Out of 229 *E.coli* strains studied 136 ( 59.38%) and from 185 *Klebsiella* isolates *95(51.35%)* were multidrug resistant. The presence of multi drug resistance may be related to the dissemination of antibiotic resistance among hospital isolates. Multi-drug resistance (MDR) is a major problem in the management of uropathogens This MDR may be due to plasmids harboring several resistance genes which are transferred from one bacterium to another and have linked such resistance pattern to the presence of integrons[11].

**Table 1 Antibiotic resistance pattern among *E.coli and Klebsiella sp***

| Antibiotics | R% of *E.coli* | R% of *Klebsiella sp* |
|---|---|---|
| Amikacin | 15,28 | 14.05 |
| Netilmicin | 11.35 | 11.89 |
| Gentamicin | 37.55 | 38.92 |
| Ofloxacin | 44.10 | 41.62 |
| Norfloxacin | 62.88 | 58.38 |
| Ciprofloxacin | 58.95 | 43.78 |
| Cefaclor | 61.57 | 56.22 |
| Cefuroxime | 65.50 | 56.76 |
| Cefoperazone | 59.39 | 55.14 |
| Ceftriaxone | 56.77 | 51.35 |
| Cefadroxil | 66.38 | 65.41 |

As shown in Table 1 Maximum resistance was observed with Cefadroxil in both *E.coli* and *Klebsiella sp* among the antibiotics tested. Apart from showing higher resistance to Cephalosporins group of antibiotics, significant resistance was also observed with Norfloxacin. Higher Sensitivity was observed with Aminoglycosides - Amikacin and Netilimicin followed by Gentamicin where the resistance percent of isolates was between 11.35% to 38.92%.

As indicated in Table 2, highest MAR index was 0.91 seen in 13 isolates, whereas 7isolates showed a Mar index of 0.75 and majority of the isolates have MAR above 0.2.

**Table 2 MAR index among the isolates**

| Mar Index | ***E.coli*** (n=229) | ***Klebsiella sp*** (n=185) |
|---|---|---|
| 0 | 39 | 39 |
| 0.09 | 6 | 4 |
| 0.18 | 5 | 8 |
| 0.27 | 14 | 6 |
| 0.36 | 13 | 14 |
| 0.45 | 16 | 19 |
| 0.55 | 25 | 19 |
| 0.64 | 44 | 28 |
| 0.73 | 33 | 26 |
| 0.82 | 21 | 15 |
| 0.91 | 13 | 7 |

A MAR index greater that 0.2 implies that the strain of such bacteria originate from an environment where several antibiotics have been used (10). The present results suggest that a very large proportion of the *E.coli* isolates and *Klebsiella sp* isolates have been exposed to antibiotics resulting in a alarming trend of rise of multiple antibiotic resistance and accumulation of MAR. MAR is considered as a good tool for risk assessment.This also gives an idea of the number of bacteria showing antibiotic resistance in the risk zone in the study's routine susceptibility testing. This MAR index also recommended that all isolates, somehow, originated from the environment where antibiotics were over used[13],[14].

Fifty isolates, all having Mar index above 0.7 were further tested for virulence phenotypes. . Colonies which have mucoid and ropy phenotype were identified.Table 3 depicts the virulence features of uropathogens tested. Mucoid nature is often related to the presence of exopolysaccharides. Capsule confers serum and phagocyte resistance and this could be attributed to sialic acid residues that subvert the ability of bacterial surface to activate complement by alternative pathway thereby augmenting the virulence potential of such pathogens[12].In the present study all the mucoid isolates showed biofilm production when tested on congo red plates.

**Table 3 Virulence phenotypes expressed among uropathogens**

| **Isolates Total 50** | **Mucoid (6)** | **Biofilm production(22)** | **Hydrophobicity (16)** | **Gelatinase (06)** | **ESBL (12)** |
|---|---|---|---|---|---|
| *E.coli* ( n=13) | 01 (7.6%) | 08 (61%) | 04 (30.7%) | 03 (23%) | 05 (38%) |
| *Klebsiella sp* (n=20) | 05 (25%) | 14 (70%) | 12 (60%) | 03 (15%) | 07 (35%) |

The results showed that a large proportion of the isolates,had the capacity to form biofilms which is an important factor determining virulence.These microorganisms survive in hospital environments despite unfavorable conditions such as desiccation, nutritional starvation, and antimicrobial treatment. These exopolysaccharide producers may also increase the incidence of UTI due to their increased ability to bind to catheters.

Out of 12 Strains detected ESBL positive with Double disc synergy,10 isolates were ESBLpositive when retested with E test.Reproducibility of both the tests-DDST and E-test are good though both have their own limitations. Strains that produce enzymes that are not inhibited by clavulanic acid will not be distinguished and false positive results may occur. Infec-

tion by ESBL producing *Enterobacteriaceae* has become a serious problem in India. Various authors have reported the prevalence of ESBLs to be in the range 6-88 per cent in various hospitals, especially among *Klebsiella pneumoniae* and *Escherichia coli*[15]

## 4 Conclusions

The knowledge of trends in virulence features such as hemolysin production, cell surface hydrophobicity,and biofilm production are pertinent in evaluating the pathogenicity of isolate. This knowledge, in addition to the antibiotic sensitivity profiles can greatly enhance the treatment strategy adopted for UTI causing organisms. The use of these tests may contribute to a wider recognition and more careful monitoring of this emerging resistance problem among some *Enterobacteriaceae* (*E. coli* and *Klebsiella* spp.). Furthermore, these tests are useful for selecting strains for more detailed molecular analysis. Clinical isolates of ESBL-producing members of *Escherichia coli* and *Klebsiella* species should be characterized at molecular level.

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