IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 15, Issue 6 Ver. V (June. 2016), PP 26-29
www.iosrjournals.org

Prevalence of extended spectrum □-lactamase (ESBL) producing E. coli and Klebsiella species in urinary isolates

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Abstract:
Introduction: *Bacterial resistance to β-lactam antibiotics has risen dramatically, with extended spectrum β-lactamase (ESBL) contributing to this increase. Early identification of ESBL production is becoming increasingly important.*
Methodology: *A total of 367 consecutive, urine samples from patients suspected to have UTI, were processed. All Escherichia coli and Klebsiella species isolated in significant numbers were included. The isolates were identified by standard microbiological procedures and AST was done according to CLSI guidelines. The isolates were tested for susceptibility to third generation cephalosporins and then tested for Double disc synergy test (DDST) & Phenotypic confirmatory disc diffusion test (PCDDT).*
Result: *Out of 367 urine samples processed in the study, 271 yielded various bacterial isolates. Of these, 96 were Escherichia coli and 58 were Klebsiella species. Out of these total 154 isolates, 117 showed resistance to any one of the three cephalosporins tested. ESBL production was observed in 89(57.79%) isolates by PCDDT. DDST failed to detect ESBLs in four isolates of E.coli and two isolates of Klebsiella spp.*
Conclusion: *The practice of routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases which will help in the proper treatment of the patients and also prevent further development of bacterial drug resistance.*
Keywords: *E. coli, ESBL, Klebsiella, UTI*

I. Introduction

In recent years, bacterial resistance to β-lactam antibiotics has risen dramatically, with extended spectrum β-lactamase (ESBL) contributing to this increase.[1] The ESBL producing strains are particularly feared as they are resistant to penicillin and its derivative, all cephalosporin and monobactam. Furthermore, they are often cross-resistant to trimethoprim /sulphamethaxazole and quinolones.[2]

Urinary tract infection (UTI) forms the largest single group of hospital acquired infections and account for about 40-50% of the total nosocomial infections.[3] *Escherichia coli* and *Klebsiella* species account for most of the cases of community as well as hospital acquired UTI and they have ability to produce ESBL in large quantities. The enzymes are plasmid borne and confer multiple drug resistance making urinary tract infection difficult to treat.[4] In addition, infection with ESBL producing bacteria raises mortality, and it prolongs hospital stay along with an increase in treatment cost.[2] It is therefore necessary that these strains are identified and treated accordingly at the earliest so as to prevent their dissemination into the hospital environment.[5] A knowledge about their prevalence is essential to guide the appropriate antibiotic treatment of severe infections in hospitalized patients.[6] The last two decades have seen several reports of different ESBLs produced by different mutant genes from all over the world, but the exact magnitude of problem posed by ESBLs is not known since most of the laboratories do not look for them.[5]

Early identification of ESBL production is becoming increasingly important in terms of appropriate treatment and effective infection control in hospitals. With reports on high prevalence of ESBL production in the members of *Enterobacteriaceae* family and paucity of information especially on uropathogens from our country, the present study was undertaken to find out prevalence of ESBL producers in urinary isolates of *E.coli* and *Klebsiella* species and to study their antimicrobial susceptibility pattern.

II. Material And Methods

A total of 367 consecutive, non-repetitive urine samples from patients suspected to have UTI, were processed. All *Escherichia coli* (96) and Klebsiella species (61) isolated in significant numbers ($\geq 10^5$ colony forming units/ml of urine) (referred as significant bacteriuria developed by Kass) were included in the study for detection of ESBL production. The isolates were identified based on colony morphology on blood agar, MacConkeys agar and by standard biochemical tests.[7]

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique using commercially available disc procured from Hi-media, according to CLSI guidelines. The antibiotics tested were

Co-trimoxazole (25µg), Ampicillin(10µg), Amoxycillin-clavulanic acid (20µg), Gentamycin (30µg), ciprofloxacin(5µg), lomefloxacin(10µg), Norfloxacin(10µg), Nitrofurontoin(300µg), Piperacillin-tazobactem(100/10µg), Cefazolin(30µg), Amikacin (30µg), Tetracyline (30µg) and Imipenem(10µg).

Criteria for selection of ESBL producing strains:

The isolates were tested for susceptibility to third generation cephalosporins (Ceftazidime, cefotaxime & Ceftriaxone) by using standard disc diffusion method. If the zone diameter of ≤22mm for Ceftazidime, ≤ 27mm for cefotaxime and ≤ 25mm for Ceftriaxone were recorded, the strain was considered to be suspicious for ESBL production.

Tests for ESBL productions

1. **Double disc synergy test (DDST)**
   A lawn culture of colony suspension was made on Muller Hinton agar after adjusting turbidity to McFarland 0.5 standard. Antibiotic discs of amoxicillin/clavulanic acid (20/10µg) and cefotaxime(30µg) were placed at a distance of 15 mm apart and incubated. Organisms that showed a clear extension of cefotaxime inhibition zone towards the disc containing clavalunate were considered as an ESBL producer.

2. **Phenotypic confirmatory disc diffusion test (PCDDT)**
   While performing antibiotic testing, ceftazidime(30µg) and Ceftazidime plus clavulanic acid (30/10µg) were placed on MHA and incubated. Organism was considered as an ESBL producer if there was a ≥5mm increase in zone diameter of Ceftazidime/ clavulanate disc and that of Ceftazidime disc alone. Positive results were also confirmed by using cefotaxime and cefotaxime- clavulanic acid combination disc.

Quality control: every batch of the media prepared was checked for sterility for 24 hours. E.coli ATCC 25922 strain was used for quality control.

III. Result

Out of 367 urine samples processed in the study, 271 yielded various bacterial isolates. Of these, 96 were *Escherichia coli* and 58 were Klebsiella species. (Table 1)

TABLE: **1** Bacteriological profile of urinary tract infection

Name of the isolate	Total number (n=367)	Percentage
E. coli	96	26.16
Klebsiella spp.	58	15.80
S. aureus	40	10.90
Pseudomonas aeruginosa	37	10.08
Citrobacter spp.	15	4.09
Proteus spp.	10	2.72
Acinetobacter spp.	9	2.45
Enterococcus spp.	6	1.63
No growth /non significant growth	96	26.16

Out of total 154 isolates 117(75.97%) (70 *E.coli*, 47 Klebsiella species) showed resistance or decreased susceptibility to any one of the three cephalosporins tested. These were considered as potential ESBL producers and were further studied for ESBL production by double disc synergy test and phenotypic confirmatory test. ESBL production was observed in 89(57.79%) isolates by PCDDT, of which 40(68.96%) were Klebsiella spp. & 49(51.04%) were *E. coli*. DDST failed to detect ESBLs in four isolates of *E.coli* and two isolates of Klebsiella spp. (Table 2) The ESBL positive strains were isolated from almost all the wards of the hospital, specifically from the surgical wards.

Table: 2 ESBL productions by two different methods

Method	PCDDT	DDST
E. coli (n=96)	49 (51.04%)	45 (46.87%)
Klebsiella spp. (n=58)	40(68.96%)	38 (65.51%)
Total (n=154)	89 (57.79%)	83 (53.90%)

The antibiogram of ESBL producers and non- producers is shown in table 3. Significant proportions of ESBL producing strains were found to be resistant to Ampicillin (89.89%), Norfloxacin (83.15%), Co-trimoxazole (82.02%), Amoxycillin/clavulanic acid (80.90%), Ciprofloxacin (79.78%) and Nitrofurantoin (77.53%). Amikacin, Lomefloxacin and Gentamicin constitute the reasonable option for treatment of UTI, as 64.04%, 53.93% and 46.07% isolates were sensitive to these antibiotics respectively. Imipenem was found to be the most effective antibiotic for ESBL producers as well as non- producers as 100% isolates were sensitive to it.

ESBL producing isolates were resistant to most of antimicrobial agents than ESBL non-producers and the difference was statistically significant except for Lomefloxacin and Nitrofurantoin where resistance was more among ESBL producers than non-producers but difference was not significant.

Table: 3 Antibiogram of ESBL producers and non- producers

Antibiotics	**ESBL producers (n=89)**	**ESBL non-producers (n=65)**	P value
Ampicillin	80(89.89%)	45(69.23%)	≤ 0.01
Amoxycillin/ clavulanic acid	72(80.90%)	36(55.38%)	≤ 0.01
Cefazolin	61(68.54%)	28(43.08%)	≤ 0.01
Piperacillin+tazobactam	57(64.04%)	23(35.38%)	≤ 0.01
Co-trimoxazole	73(82.02%)	25(38.46%)	≤ 0.01
Tetracycline	66(74.16%)	30(46.15%)	≤ 0.01
Ciprofloxacin	71(79.78%)	31(47.69%)	≤ 0.01
Norfloxacin	74(83.15%)	28(43.08%)	≤ 0.01
Lomefloxacin	41(46.07%)	17(26.15%)	**0.01**
Nitrofurantoin	69(77.53%)	43(66.15%)	**0.01**
Gentamycin	48(53.93%)	21(32.31%)	≤ 0.01
Amikacin	32(35.96%)	11(16.92%)	≤ 0.01
Imipenem	0(0%)	0(0%)	

IV. Discussion

The emergence of plasmid encoded ESBLs due to extensive use of expanded spectrum cephalosporins since 1980s is a significant evaluation in antimicrobial resistance.[9] Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures.[10] Incidence of these organisms is being continuously increasing throughout the world with limited therapeutic alternatives.[11]

In India, there have been several reports on the prevalence of ESBLs in recent years. ESBL production has been observed in large percentage of urinary isolates and majority of ESBL producing strains worldwide are of Klebsiella spp. & *E. coli*.[18] In our study, ESBL production was observed in 57.79% of the urinary isolates. Somewhat higher percentage (66.9%) was given by Gaurav Dalela *et al* while Dechen C Tsering *et al* noted a lower (34.03%) prevalence. In our study, we found that the ESBL production was more among Klebsiella spp.(68.96%) followed by *E. coli* (51.04%) as also reported by others,[1,10,13,14,15,17] while Gaurav Dalela et al reported that ESBL production was more common among *E.coli* (73.5%) than *Klebsiella pneumoniae*. ESBL production in Klebsiella spp. has been variably reported as 76.5%[6], 62%[10], 59.1%[20], 57.14%[13], 25.6%[15] & 6.19%[4]. ESBL positivity in *E.coli* (51.04%) in our study fairly correlates with other reports.[3, 10, and 19]

ESBL production co-existed with resistance to several other antibiotics. ESBLs are encoded by plasmids which also carry resistant genes for other antibiotics.[3] We found such associated resistance with Tetracycline, Co-trimoxazole, Norfloxacin, ciprofloxacin, Gentamycin & Amikacin as also reported by Dechen C Tsering & Duttaroy et al. Most of the ESBL producing strains were significantly resistant to various antimicrobials as compared to non-ESBL producers. Similar observations were shown in other studies.[3,1] A point of interest noted in the present study that all the strains were at least resistant to 3-5 antibiotics indicating multidrug resistance pattern as also reported by others.[3,13] All the isolates in the present study were uniformly sensitive to Imipenem. The carbapenems still remain adequately active for almost all Klebsiella spp. & *E. coli*, however they are expensive and require prolonged intravenous administration. The circumstances have changed & growing carbapenem resistance is a major clinical concern.

Of the two tests used in the present study, we found PCDDT as a more superior procedure for detection of ESBL than DDST as ESBL detection was missed in some isolates by DDST, but the difference was not significant. Other researchers found this method to be reproducible, easy to perform and cost effective for use in a busy diagnostic laboratory where large number of clinical isolates are to be screened.[14,18] The continuing worldwide transmission of ESBLs is partly as a result of the fact that clinical laboratories are not able to detect these enzymes as effectively or as rapidly as needed. The laboratories which do not perform tests for detection of ESBLs and do not report ESBL producers as resistant to penicillin and all cephalosporins, risk poor outcome.[9] All the isolates positive by confirmatory tests should be reported as resistant to pencillin, all cephalosporins and aztreonam even if the organism is susceptible by routine disc diffusion methods.

In the present study, a high percentage of *E.coli* and Klebsiella spp. causing UTI were found to be ESBL producers. Patients with UTI due to these organisms are unable to respond to penicillin, cephalosporins and aztreonam. Since, the routine antimicrobial susceptibility testing method may not detect these organisms; the microbiological laboratories should look for ESBL production routinely and explicitly report their presence so that appropriate therapy can be instituted.

V. Conclusion

The present study reports a high percentage of *E.coli* and Klebsiella spp. causing UTI to be ESBL producers and there is increase in resistance by these strains to a number of commonly used antibiotics to an alarming level. In view of this emerging drug resistance the practice of routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases which will help in the proper treatment of the patients and also prevent further development of bacterial drug resistance.

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