Original Research Article

Detection of ESBLs, MBLs and AmpCs in Gram Negative Uropathogens in Tertiary Care Hospital

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A B S T R A C T

Urinary tract infections are common bacterial infections which vary from asymptomatic bacteriuria to cystitis and pyelonephritis. Infections with multidrug resistant gram negative bacilli have become a great concern as emerging β lactamases like ESBLS, AmpC and MBLs are cause of multidrug resistance in gram negative bacilli. Thus, there detection is needed to prevent UTI and to decrease the hospital stay of patients. Aim of the study is to identify uropathogens causing urinary tract infection. To detect ESBLs, MBLs, AmpC producing isolates from urine. Cross sectional study of 300 urine samples was conducted in Mediciti Institute of Medical Sciences. The samples were processed and gram negative bacilli were identified as per Mackie McCarteny 14th edition. Detection of ESBLs, MBLs and AmpCs producing organisms among the isolates was done by both screening and confirmatory test as per CLSI guidelines. Out of 300 urine samples analyzed, prevalence of gram negative bacilli was 56.6%. The predominant gram negative bacterial isolate was *Escherichia coli* 55.9%, *Klebsiella* 26.5% and *Pseudomonas* 9.4%. Among these isolates 38.8% were ESBL producers, 2.35% MBL producers and 9.4% were AmpC producers. Prevalence of gram negative pathogens in hospital area was 56.6%, predominant isolate was *Escherichia coli*. ESBLs were the predominant β lactomases produced.

Introduction

Urinary tract infections (UTI) are common bacterial infections associated with considerable morbidity and health care cost, with varied clinical spectrum of severity ranging from asymptomatic bacteriuria to cystitis and pyelonephritis to septic shock with multiorgan system failure. UTIs are specially problematic for women, 50-80% of women will suffer at least one episode of UTI in their lifetime and 20 -50% of these women will have recurrent episodes. (1)

Urinary tract infections are classified as uncomplicated UTI and complicated UTI, depending on the factors that trigger the infections. Uncomplicated UTIs are associated with bacterial infections, most often *Escherichia coli*.

The bacteria can invade and cause a urinary tract infection by two major routes, ascending or haematogenous. In females the ascending route is more common and organisms originate from faecal or vaginal source.

Several studies regarding the prevalence and antibiotic susceptibility or resistance pattern of bacterial isolates in different settings

established that community acquired urinary tract infections occurred in 85% of population while, hospital acquired infection in only 15% of cases.

Gram negative bacteria such as Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp are able to cause serious infections especially in hospitalized patients.

Treatment of infections is often complicated due to the increasing bacterial resistance against different classes of antibiotics.

Infections with multidrug resistant gram negative bacilli have become a great concern as they are associated with higher morbidity, mortality, prolonged hospital stay and raising health care costs.

As emerging betalactamases (ESBLs, Ampc and carbapenamases) are main cause of multidrug resistance in gram negative bacilli. Detection of ESBL, MBL, and Ampc is needed to prevent resistant UTIs and to decrease the hospital stay.

Materials and Methods

Cross sectional study of 300 urine samples was conducted from Nov 2011 to Aug 2013, in Mediciti Institute of Medical Sciences. The samples were processed and the gram negative bacilli were identified by microscopy, culture and standard biochemical reactions as per Mackie McCarteny 14th edition. 5-10 ml of single clean catch midstream urine was collected from symptomatic patients of urinary tract infection (Table 2).

The container was labeled with date, name, number of patient and the time of collection and immediately transported to laboratory and processed without any delay in the

microbiology laboratory at Mediciti Institute of Medical Sciences, Medchal.

All samples were processed by following technique Macroscopic appearance of urine was noted, whether it was turbid or clear.

Microscopy

Wet mount preparation: Wet mount of the urine for cytology was performed to screen for R.B.C, W.B.C bacteria and yeast cells. 5-10 ml of well mixed urine was transferred to a labeled test tube. Centrifuged at 500-1000 rpm for 5 minutes. Supernatant was discarded. The sediment was remixed by tapping the bottom of the tube. One drop of the well mixed sediment was transferred to a clean slide covered with cover slip. The preparation was examined microscopically using the 10x and 40x objective with condenser iris closed sufficiently to give good contrast.

In wet mount >10 cells/HPF are significant for UTIs to process the specimen. In gram stain 1 cell/HPF in 7 fields corresponds to 10^4 leucocytes /ml. For suggesting pyuria at least 10^4 leucocytes must be present.

Culture

Each of the urine specimens was subjected to culture by the standard loop method and filter paper method. These specimens were inoculated on Blood agar, MacConkey agar, Cystine Lactose Electrolyte Deficient agar (CLED Himedia) agar. Culture plates were incubated aerobically at 37°C for 18-24 hours.

Semiquantitative analysis

Standard loop method

Inoculating loop of standard dimension about 4mm internal diameter and volume (0.001 ml)

of urine was taken. Loop full of well mixed uncentrifuged urine specimen was inoculated on Blood agar, MacConkey agar CLED agar. The culture plates were incubated at 37^0C for 18-24 hrs. Bacterial counts were done by counting the number of colonies and multiplying the number of colony forming units (CFU) by 1000, to determine the number of microorganisms per milliliter in the original specimen. Colony count of $>10^5$ CFU/ml was considered as significant bacteriuria.

The following biochemical tests were put up – Mackie and McCartney 14th edition (39).

For gram negative isolates, colony was subcultred into peptone water and the following tests were performed: Hanging drop (for motility), catalase test, oxidase test, Indole test, Methyl red test, Vogues Proskauer test, Simmons citrate utilization test, Urease test, Triple sugar iron test, Nitrate reduction tests, Sugar fermentation tests for the following sugars: Glucose, Lactose, Sucrose, Maltose, Mannitol and Xylose, Phenylalanine Deaminase test, Amino acid decarboxylase test: Lysine, Arginine and ornithine, Hugh Leifson's Oxidation /Fermentation test.

Testing for antibiotic susceptibility

Antibiotic testing by Kirby Bauer disc diffusion method

The test organism was subcultured into peptone water and incubated for 4-6 hrs at 37^0c. The turbidity was standardized with 0.5 Mc Farlands and swabbed over 90 mm Mueller-Hinton agar plate. Antibiotic disc were placed at 15 mm from the edge of the plate and discs were evenly placed, that they were no closer than 25 mm from centre to centre. Plate was incubated at 37^0C for 18 -24 hrs. Zones of inhibition were measured after incubation with a ruler and interpreted as per

CLSI guidelines. The commercially available antibiotics discs supplied by Himedia (Mumbai) were used.

Antibiotics tested against gram negative bacilli

Zones of clearance were measured after incubation with a ruler and interpreted as per Ampicillin (10µg), amikacin (30 µg), cefotaxime (30µg), ceftazidime (30µg), nitrofurantoin (30 µg), trimethoprim + suphamethoxazole (1.25 + 23.75 µg), cephalexin (30 µg), ciprofloxacin (5µg), ofloxacin (5 µg).

Extended spectrum β lactamase detection

Organisms resistant to third generation cephalosporins by Kirby Bauer disc diffusion method were selected for ESBL confirmatory tests. Antibiotics used were - ceftazidime (30 µg), ceftazidime + clavulanic acid (30µg + 10µg).

Confirmatory method

Disc potentiation test

In this a pair of discs containing cephalosporin with and without clavulanic acid was placed on opposite sides of the same inoculated plate. The test organism was regarded as an ESBL producer if the zone of inhibition around the combination disk was at least 5mm larger than that of the cephalosporin alone. (3)

Detection of MBLs

Testing organisms were inoculated on the Muller Hinton agar plates. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA in 100 ml of distilled water and adjusting P^H to 8.0. The EDTA solution was sterilized by autoclaving.

Two 10μg imipenem disks (one with EDTA and other without) are placed on the surface of the muller hinton agar plate.

Inhibition zones of imipenem and their EDTA impregnated discs were compared after incubation at 37°C. A zone size difference of greater than or equal to 7mm with EDTA impregnated discs is taken as indicative of metallo β lactamases production. (24).

Detection of AMP C

Double Disc Synergy Tests using 500 μg was put up for all non-ESBLs producing strains using inhibitor of AmpC β lactamases. Using Mueller Hinton plate for each test organism, a lawn culture was made, the disc of the inhibitor was placed in the centre and the distance between this disc and cefotaxime (30μg) and ceftazidime (30μg), one on either side from centre to centre, was kept as 15 mm.

After overnight incubation at 37ºC, expansion of inhibitory zone of either one or both, ceftazidime and cefotaxime, towards the inhibitory discs was interpreted as positive results for production of AmpC β lactamases by the isolates (37).

Results and Discussion

Our study was undertaken to know the distribution and the antibiotic susceptibility pattern of uropathogens, isolated from patients in tertiary care hospital.

In our study female to male ratio was 2:1, 60% were female and 30% were male patients (female to male ratio was 2:1) (Table 4).

The present study correlated with study conducted by Marie-Vic-o *et al.,* (4) in 1998, reported that majority of patients were female, the ratio between female and male was 4.6:1. In this study, urinary tract infections were most commonly found in the age group between 31-35 years. The present study was similar to Dimtrov *et al.,* in 2003 who reported significant bacteriuria among young and middle age patients (20-40) years (Table 3).

In our study most of the isolated organisms were found to be sensitive to amikacin (85.7%) and nitrofurantoin (71.4%) and resistant to ampicillin (28.57%) cotrimoxazole (57.1%) Asad Khan *et al.,* (41) in 2006 found that 90% of isolates were resistant to ampicillin followed by chloramphenicol (60%) (Tables 6, 7 and 8).

In the present study, among the gram negative isolates, the maximum percentage of ESBLs were detected in *Escherichia coli* (42.85%) followed by *Klebsiella pnuemoniae* (30%). This data correlated well with the study conducted by Mohammed Akram *et al.,* (40) in 2007 in JNMC Hospital, Aligarh, who detected 34.42% of *E. coli*, 27.3% *Klebsiella pneumoniae* to be the most prevalent ESBL producers in community acquired urinary tract infections.

Similarly, Nachimuth Ramesh *et al.,* (32) in 2008 observed 71.5% of isolated were ESBL producers in hospital isolates. While Shukla *et al.,* (42) in 2004 reported only 30.18% ESBL producers.

In our study we have observed 2.35 % of Pseudomonas among gram negative isolates as MBL producers by imepenem, imepenem EDTA method (Figs. 1–3).

Gupta *et al.,* (24) in 2006 conducted a study at Government medical college, Chandigarh and found 7.5 % of pseudomonads and *Acinetobacter* were shown to be MBL producers with imepenem and imepenem EDTA method in concordance with present study (Tables 9 and 10).

Table.1 Distribution of patients

Patient distribution	No. of patients
Inpatient	180 (60%)
Outpatient	120 (40%)
Total	300

A total of 300 urine samples were screened for pathogenic organisms. Out of which 40 % of samples were from out patient and 60 % of samples are from inpatient.

Table.2 Symptom analysis of UTI

Symptoms	M=120	F=180
Burning micturation	50	45
Frequency	25	35
Fever	20	40
Lower abdomen pain	10	35

In all patients burning micturition is most common presenting symptom.

Table.3 Age wise distribution of clinically suspected cases of UTI

Age (Yrs)	Number of patients
10-19 Years	40 (13.33%)
20-29 Years	98 (32.6%)
30-39 Years	120 (40%)
40-49 Years	30 (10 %)
50-59 Years	12 (4%)

Out of 300 patients with suspected UTI maximum number (40 %) of patients are from age group of 30 – 39 years.

Table.4 Sex wise distribution of cultural results of UTI

	Culture +ve	Culture –ve	Total=300
Male	60(35.29%)	60(54.5%)	120 (40 %)
Female	110 (64.7%)	70(63.6%)	180 (60 %)

Out of 300 patients selected for study 40 % are males, in which 35.29 % are culture positives, 60% are females, in which 64.7 % are culture positives, indicating maximum incidence of UTI in females.

Table.5 Bacteriological profile of UTI

Gram negative isolate	No. of isolates
Escherichia coli	95 (55.88%)
Klebsiella	45 (26.47%)
Pseudomonas	16 (9.4%)
Proteus	8 (4.7%)
Citrobacter	6 (3.5%)
Total	170 (100 %)

Out of 300 samples studied for UTI, predominant isolate was *E. coli* (55.88%), followed by *Klebsiella* (26.47%).

Table.6 Antibiotic susceptibility pattern of test isolates

Antibiotics	Sensitivity
Ampicillin	35.7 %
Amikacin	85.7 %
Nitrofurantoin	71.4 %
Cotrimoxazole	57.1 %
Cephalexine	76.1 %
Ceftazidime	60 %
Cephotaxime	50 %
Gentamicin	85.7 %
Cepodoxime	70.4 %

Antibiogram of isolates showed maximum sensitivity to amikacin & Gentamicin (85.7%), followed by cephalexin (76.15%).

Table.7 Antibiotic susceptibility pattern of *Escherichia coli*

Ampicillin	30 %
Amikacin	87%
Nitrofurantoin	80%
Cotrimoxazole	60 %
Cephlexine	51.4 %
Ceftazidime	47.1 %
Cephotaxime	50 %
Gentamicin	85.7 %
Cepodoxime	70.4 %
Ciprofloxacin	45%
Imepenem	37%

In antibiogram of *Escherichia coli* which is predominant isolate in study maximum sensitivity was noted for Amikacin (87%) followed by Gentamicin and nitrofurantoin (85.7 %, 80 %) least with ampicillin (30%).

Table.8 Antibiotic susceptibility pattern of *Klebsiella* species

Ampicillin	20 %
Amikacin	87 %
Nitrofurantoin	76 %
Cotrimoxazole	60%
Cephlexine	51 %
Ceftazidime	60 %
Cephotaxime	58 %
Gentamicin	86 %
Cepodoxime	70.4 %
Ciprofloxacin	46 %
Imepenem	35 %

In antibiogram of Klebsiella which is second predominant isolate in study maximum sensitivity was noted for Amikacin (87 %) followed by Gentamicin and nitrofurantoin (80 %, 76 %) least with ampicillin (20%.)

Table.9 Prevalence of ESBL, MBL and Amp C: No. Tested =170 / No. Detected=86

Resistance pattern	Prevalence of resistance n=170
ESBL	66 (38.82%)
MBL	4 (2.35%)
AmpC	16 (9.41%)
Total	86 (50.58%)

Among the isolates in present study 38.82 % are ESBL producers, 2.35% are MBL producers and 9.41% are Amp C producers.

Table.10 Organism wise prevalence of ESBL, MBL and AmpC in different isolates

Organism	ESBL	MBL	AmpC
Escherichia coli N=95	40 (42.1 %)	-	12 (12.63 %)
Klebsiella N=45	20 (44.44 %)	-	3 (6.66 %)
Pseudomonas N=16	2 (12.5 %)	4 (25 %)	-
Proteus N=8	2 (25 %)	-	-
Citrobacter	2(33.3%)	-	1
Total	66	4	16

Among the ESBL producers predominant isolate is *Klebsiella*, and Ampc predominant isolate is *Escherichia coli*.

Fig.1 Detection of ESBL

Fig.2 Detection of MBL

Fig.3 Detection of Amp C

In our study 9.4% AmpC producers were isolated, by double disc synergy method, with cefotaxime, cefatazidime and cloxacillin.

Sasirekha Bakthavatchalu *et al.,* (38) in 2011 conducted a study at centre of postgraduate studies in Jain University and found 5.4% Amp c producers out of 259 isolates by CLSI double disc diffusion method and it is in concordance with present study. To summarize, the prevalence of urinary tract infections and antibiotic resistance pattern varied from country to country, place to place and time to time. The prevalence of ESBL, MBL and AmpC β

lactamases also varied in different places. It ranges from 0-100% in different reports. Other contributing factor affecting the prevalence is increased usage of 3rd generation cephalosporin antibiotics in clinical practice. A continuous surveillance is therefore essential along with prudent use of implicated antibiotics.

everybody associated to make it a grand success.

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