ETIOLOGY AND ANTIBIOGRAM OF COMMUNITY ACQUIRED URINARY TRACT INFECTION IN HYDERABAD

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ABSTRACT

Introduction- Urinary tract infection (UTI) is the second most common infectious presentation in community practice. The most common pathogenic organisms of UTI are Escherichia coli, Staphylococcus saprophyticus. Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. The aim of the study was to determine the prevalence of organisms that causes urinary tract infections and the antibiotic sensitivity and resistance pattern of pathogenic organisms isolated.

Materials and Methods- A total of 100 urine samples were screened for pathogenic organisms. 5-10 ml of single clean catch midstream urine was collected from symptomatic patients of urinary tract infection. The samples were inoculated on Blood agar, MacConkey agar, Cystine Lactose Electrolyte Deficient agar (CLED), Urichrome (Himedia) agar. Culture plates were incubated aerobically at 37°C for 18-24 hours. The isolated organism was identified by standard biochemical tests and antibiogram was done.

Results – Out of 100 samples, females were of 76% and males 34%. Urinary tract infections were most commonly found in the age group between 31-40 years in females and 36-40 years in males. The most common isolate was Escherichia coli (58.3%).

Conclusion - A high prevalence of ESBL (Extended Spectrum Beta Lactamases) and AmpC beta lactamase in community acquired infection is a matter of concern. It is quite alarming to note that, multidrug resistant isolates including ESBL and AmpC beta lactamase producing bacteria circulating in community. Henceforth it is important to formulate a strict antibiotic policy.

KEY WORDS – UTI, ESBL, E.coli.
INTRODUCTION
Urinary tract infection (UTI) is the second most common infectious presentation in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion dollars.\(^1\) It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually.\(^2\)

The most common pathogenic organisms of UTI are Escherichia coli, Staphylococcus saprophyticus and less common organisms are Proteus sp., Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococci and Candida albicans.\(^3\) UTI is the presence of bacteria in the urine (bacteriuria) and defined as the growth of a single pathogen of >10\(^5\) colony-forming units/mL from properly collected mid-stream urine specimens.\(^4\) Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens.\(^2\)

As the result of extensive uses of antimicrobial agents, nosocomial pathogens have shifted away from easily treatable bacteria towards more resistant bacteria. This change is important problem for nosocomial infection control and prevention.\(^5\) The aim of this study was to determine the prevalence of organisms that causes urinary tract infections among the patients attending out patients departments of Medicine, Obstetrics & Gynaecology and Urology and the antibiotic sensitivity and resistance pattern of pathogenic organisms isolated.

MATERIALS AND METHODS
The study was conducted from October 2009 to November 2010 at Gandhi hospital.
The study includes 100 adult patients attending out patient departments of Medicine, Obstetrics & Gynaecology and Urology at Gandhi Hospital with the complaints of urinary tract infections.

Inclusion criteria
Adults of both sexes
Age group between 18-50 years with one or more symptoms of urinary tract infection such as burning micturition, frequency, urgency of urine, lower abdomen pain, fever with chills and without fever.
Exclusion criteria
Asymptomatic patients
Hospitalized patients
Prior antibiotic therapy

Sample collection
A sterile, dry, wide necked and leak proof container was given to the patients.
Following instructions were given to the patients before collection of urine
Men: Wash hands before collection of urine.
Pull back the fore skin and hold back the fold of skin and then collect mid stream urine in a container.
Women: Wash hands before collection of urine
Spread the labia and clean the vulva, labia and then collect the mid stream urine in a container.
5-10 ml of single clean catch midstream urine was collected from symptomatic patients of urinary tract infectionsymptomatic patients of urinary tract infection. The container was labelled with the date, name, and number of patient and the time of the collection and immediately transported to the laboratory and processed without any delay in the Microbiology laboratory at Gandhi Hospital.

Methodology
All samples were processed by the following technique
Macroscopic appearance of urine was noted. Whether it was turbid or clear

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 labelled test tube. Centrifuged at 500-1000rpm 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 and covered with cover slip. The preparation was examined microscopically using the 10x and 40x objective with condenser iris closed sufficiently to give good contrast.

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

**Semiquantitative analysis**

Two methods
a. Standard loop method
b. Filter paper method

Standard loop method: Inoculating loop of standard dimension about 4mm internal diameter and volume (0.001ml) of urine was taken. Loop full of well mixed uncentrifuged urine specimen was inoculated on Blood agar, Mac Conkey agar, CLED agar and Urichrome (Himedia) agar. The culture plates were incubated at 37°C 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 millilitre in the original specimen. Colony count > 10^5 CFU/ml was considered as significant bacteriuria.

**Filter paper method**

A standard 6mm wide strip of absorbent fluff less blotting or filter paper was bent into an “L” shaped with a 12mm long foot (area 12x6mm) and sterilised at 160°C for 1hr. The whole of the angulated end and foot was dipped into the mixed uncentrifused sample of urine, it was withdrawn and a few seconds were waited to allow it into the paper. The foot was pressed onto the surface of a marked section of a well dried plate agar culture medium.

The strip was removed and the plate was incubated at 37°C for 18-24 hours. After incubation, the colonies were counted which were grown on the impression area. Up to 50 colonies might be countable and heavier growth was noted as being semi-confluent (+) or (++). The number of viable bacteria per ml of urine was estimated from the count of colonies on the impression area or the pattern of semi-confluent or confluent growth. The value of 10^5 bacteria per ml corresponds to a count of 25 colonies of bacilli. Organisms were identified after incubation by the standard scheme of biochemical reactions.

**Antibiotic testing by Kirby Bauer disc diffusion method**

The test organism was subcultured into peptone water and incubated for 4-6hrs at 37°C. The turbidity was standardised with 0.5MC Farlands and swabbed over 90mm Mueller-Hinton agar plate. Antibiotic disk were placed at 15mm from the edge of the plate and disks were evenly placed, that they were no closer than 25mm from centre to centre. Plate was incubated
at 37°C 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 were used, supplied by Himedia (Mumbai).

**Antibiotics tested against Gram negative bacilli**

Ampicillin (10μg), amikacine (30μg), cefotaxime (30μg), ceftazidine (30μg), nitrofurantion (30μg), trimethprine+ sulphomethoxazole (1.25μg + 23.75μg), cephalexin (30μg), ciprofloxacine (5μg), oflaxacin (5μg )

**Antibiotics tested against Gram positive cocci**

Ampicillin (10μg), ciprofloxaclin (5μg), cephataxime (30μg), tetracycline (30μg), trimethoprine+ sulphamethoxazole discs (1.25μg+23.75μg), oxacillin (1μg), cephalaxine (30μg), vancomycin (30μg), erythomycin (15μg), ciprofloxacine (5μg).

**Extended spectrum β lactamase detection:**

Organisms resistant to third generation cephalosporins by Kirby Bauer disc diffusion method were selected for ESBL confirmatory tests and later confirmed by various methods using standard strains. A non ESBL producing organism (Escherichia coli ATCC25922) was used as a negative control and an ESBL-producing organism (Klebsiella pneumonia ATCC 700603) was used as positive control. Antibiotic used were : - cefotaxime (30μg), ceftazidine (30μg), amoxycillin+clavulanlic acid (20+10μg), ceftazidine+clavulanic acid (30+10μg).

Confirmatory methods for ESBL producers: As per clinical and laboratory standards institute (CLSI) guidelines.

**Double disk diffusion (Double disk approximation method or Modified double disc synergy test)**

Inoculum was standardized with 0.5MC Farlands and swabbed on to a 90mm Mueller-Hinton agar plate. A susceptibility disk, containing Amoxycillin/clavulanate was placed in the centre of the plate and discs of ceftazidine, cefotaxime were placed 20mm (centre to centre) from the amoxicillin / clavulanate disc. The presence of distinctive enhancement of the inhibition zone towards the amoxicillin / clavulanate disc was, considered as positive for ESBL production.
Disc potentiation test
In this test 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.

Disc- on - disc test
Inoculum was standardized with 0.5MC Farlands and swabbed onto a 90mm Mueller-Hinton agar plate. cefotaxime and ceftazidime disks were tested against test organism both alone and in combination with a co-amoxiclav disk, which was placed on top of the cephalosporin disk. If the zone diameter around the combination disk was >5mm more than the cephalosporin disk. The organism was considered as ESBL producer.

Disc replacement method
In this method, two amoxyclav discs were applied to a 90mm Mueller-Hinton agar plate inoculated with the test organism which was standardized to 0.5 McFarlands standards. After 1 hour at room temperature, these antibiotic discs were removed and replaced on the same spot by discs containing cefotaxime and ceftazidime. Control discs of these three antibiotics were simultaneously placed at 30mm from these locations. A positive test was indicated by a zone increase of ≥ 5mm around the discs, which had replaced the amoxyclav discs compared to the control discs.

Detection of AmpC ß lactamase production
AmpC disk method
The test is based on use of Tris-EDTA to permabilize a bacterial cell and release beta lactamases into the external environment. AmpC disc was prepared in house by applying 20μl of a 1:1 mixture of saline and 100x Tris-EDTA to sterile filter paper discs, allowed discs to dry and storing at 2-8°C. Surface of Mueller-Hinton agar plate was inoculated with a lawn of cefoxitin susceptible E.coli ATCC 25922 according to standard disc diffusion method. Immediately prior to use, AmpC discs were rehydrated with 20μl of saline and several colonies of each test organism were applied to a disc. A 30μg cefoxitine disc was applied on the inoculated surface of the Muellar-Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disc with the inoculated disc face in contact with the agar surface. The plate was then inverted and incubated overnight at 35C in ambient air. After incubation, plates were examined for either an indentation or a flattening of the zone of
inhibition indicating enzymatic inactivation of cefoxitin, or the absence of a distortion, indicating no significant inactivation of cefoxitin.

ATCC E.coli 25922 was used as negative control.

RESULTS

A total of 100 urine samples were included in the study out of which 76 were of females and 24 males. Male to female ratio was 3.15:1. High incidence of UTI was seen in the age group 36-40 years in both males and females.(75% and 62.5%) Burning micturition was the most common symptom (32.89%) in females and fever was the most common symptom (29.17%) in males (Table 1)

Table 1: Symptomatic analysis of UTI

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. of females(n=76)</th>
<th>No. of males(n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture positive(n=27)</td>
<td>Culture negative(n=49)</td>
</tr>
<tr>
<td>Burning micturition</td>
<td>25(32.89%)</td>
<td>51(67.1%)</td>
</tr>
<tr>
<td>Urgency of urine</td>
<td>22(28.95%)</td>
<td>54(71.05%)</td>
</tr>
<tr>
<td>Frequency of urine</td>
<td>24(31.57%)</td>
<td>52(68.43%)</td>
</tr>
<tr>
<td>fever</td>
<td>18(23.68%)</td>
<td>58(76.32%)</td>
</tr>
<tr>
<td>Lower abdomen pain</td>
<td>20(26.32%)</td>
<td>56(73.68%)</td>
</tr>
</tbody>
</table>

The sensitivity of wet mount was 75% and specificity 53.12%. P value is <0.005, indicates that the test was significant. Positive predictive value of wet mount - 47.37%

Most common isolate was E.coli (58.33%) followed by K.pneumoniae (27.77%)(table 2)

Table 2: Organisms isolated (n=36)

<table>
<thead>
<tr>
<th>Organism isolated</th>
<th>Total No</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>21</td>
<td>58.33%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10</td>
<td>27.77%</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>01</td>
<td>2.75%</td>
</tr>
<tr>
<td>Acinetobacter baumanii</td>
<td>01</td>
<td>2.75%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>02</td>
<td>5.55%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>01</td>
<td>2.75%</td>
</tr>
</tbody>
</table>

Gram negative bacilli showed higher colony count (78.78%), > 10^5 CFU/ml whereas lower colonies count (10^3-10^4 CFU/ml) was seen in S.aureus and C.albicans.

Most of the isolates were sensitive to amikacin (86.11%), nitrofurantoin (83.33%) and resistant to cephalexin (91.67%), ampicillin (88.89%), cotrimoxazole (80.56%). (Table 3)
Table 3: Antibiotic susceptibility pattern of isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of isolates tested</th>
<th>% of sensitivity</th>
<th>% of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>36</td>
<td>11.11%</td>
<td>88.89%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>36</td>
<td>86.11%</td>
<td>13.89%</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>36</td>
<td>83.33%</td>
<td>16.67%</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>36</td>
<td>19.44%</td>
<td>80.56%</td>
</tr>
<tr>
<td>Cephalexine</td>
<td>36</td>
<td>8.33%</td>
<td>91.67%</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>36</td>
<td>52.77%</td>
<td>47.23%</td>
</tr>
<tr>
<td>Cephatoxime</td>
<td>36</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>2</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>2</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>36</td>
<td>11 (30.56%)</td>
<td>25 (69.44%)</td>
</tr>
</tbody>
</table>

16 isolates were tested for ESBL producers. Out of this, 12 isolates were ESBL producers. Disc potentiation method was found to be more sensitive in comparison to other phenotypic methods of ESBL detection. E.coli showed highest percentage (42.85%) of ESBLs and AmpC β-lactamase (14.28%) production.

DISCUSSION

Urinary tract infection constitutes a major problem for clinicians in diagnosis, management and treatment. The methods available for assessing the presence of UTI are clinical symptoms such as fever, dysuria and the presence of burning micturition, microscopic examination such as wet mount, Gram stain, chemical methods and isolation of pathogenic organisms in culture. The diagnosis of urinary tract infection (UTI) is currently based on concept of quantitative bacteriuria.

Our study was undertaken to know the distribution and their antibiotic susceptibility pattern of uropathogens, isolated from patients with community acquired urinary tract infections. In our study 76% were female patients and 24% were male patients. Male and female ratio was 3.15:1. Majority of the patients were females. The present study correlated well with the studies conducted by other authors Marie-Vic-o et al reported that ratio between Female and Male was 4.6: 1.8 Majority of patients were females. 8 In our study, urinary tract infections were most commonly found in the age group between 31-40 years in females and 36-40 years in males. The present study was similar to Dimitrov et al who reported significant bacteriuria among young and middle age patients (20-40) yrs. 9 Similarly Mohammed Akram et al conducted a study on newborn to 80 years of age and found that most cases of UTIs were recorded among young and middle age patients (20-49yrs). 1 In our study, burning micturition was the most common symptom of urinary tract infections and colony count > 10^5CFU/ml
was more significant. This observation was similar to other studies, J.I Alos et al studied community acquired urinary tract infections and described that, most common symptoms of UTIs were, dysuria, frequency, urgency and suprapubic tenderness.\textsuperscript{10} In our study Escherichia coli (58.33\%) was the most common organism isolated which was similar to studies conducted by Marie-vic-Raco, et al. Dimitrov, et al., and Jose Anastacio Dias Neto who reported 49.37\%, 48.65\% and 58\% respectively.\textsuperscript{8,9,11} In our study we have observed that colony count of all isolates were similar on three media, Bood agar, Mac Conkey agar and Urichrome agar whereas on CLED agar less number of isolates were grown. This observation correlated well with that of Lakshmi et al who observed that the colony count of all isolates on four culture media, Blood agar, Mac conkey agar, CLED agar and urichrome agar was similar.\textsuperscript{12} C. Chaux et al, compared the performance of three chromogenic agar plates, CPS ID2, Chromogenic UTI and USA and their performance rates were found to be 99.1\%, 97.1\% and 96.6\% respectively and reported that it was easy to identify different bacterial colonies on three chromogenic media.\textsuperscript{13} Most of the isolated organisms were found to be sensitive to amikacin (86.11\%) and nitrofurantoin (83.333\%) and resistant to ampicillin (88.89\%), cotrimoxazole (80.56\%) and cephalaxin (91.67\%). This observation correlated well with that of Dimitrov et al who reported high prevalence of resistance of gram negative bacilli to ampicillin followed by amoxicillin/clavulanic acid while imipenem had the widest coverage, followed by the 3\textsuperscript{rd} generation cephalosporins.\textsuperscript{9} In the present study, the maximum percentage of ESBLs were detected in Escherichia coli (42.85\%) followed by Klebsiella pneumoniae (30\%). This data correlated well with the study conducted by Mohammed Akram et al in JNMC Hospital, Aligarh, who detected 34.42\% of E.coli, 27.3\% of Klebsiella pneumonia to be the most prevalent ESBL producers in community acquired urinary tract infections.\textsuperscript{1}

In the present study, it has been found that among the phenotypic confirmatory test for ESBLs, Disc potentiation test showed the highest percentage of 43.75\% in confirming ESBLs. This correlated well with the findings of Hanan et al conducted a study on multi drug resistant isolates of members of Enterobacteriaceae and found the majority of Escherichia coli (37\%) and Klebsiella pneumonia (34\%) of ESBLs were confirmed by Disc potentiation method.\textsuperscript{14}In our study we have observed 24.28\% of the organisms isolated from community acquired UTIs to be AmpC \beta lactamase producers by AmpC disc test with Tris-EDTA whereas Vikas Manchand et al conducted a study at Guru TeghBahadur Hospital in Delhi, India. They obtained samples from NICU, ICU, inpatients and outpatient units and found that 20.7\% of all patients harboured AmpC \beta lactamase producers.\textsuperscript{15} Jennifer et al
in 2005 reported 31% of positive AmpC beta lactamases by AmpC disc test with Tris-EDTA.16

CONCLUSION
The prevalence of uropathogens in community acquired urinary tract infections was 36%. Semiquantitative culture method was found to be the gold standard for determination of significant bacteriuria. Incidence of community acquired urinary tract infections was found to be predominant in women in their reproductive age group i.e. 20-40 years. Escherichia coli was the most common uropathogen 58.33%. Most of the organisms were sensitive to amikacin and nitrofurantoin and resistant to ampicillin, cotrimaxazole and cephalexin. Escherichia coli harboured the highest percentage of ESBLs 56.25%. Disk potentiation test had the highest percentage of sensitivity among the diffusion tests. Highest percentage of (24.28%) AmpC beta lactamase production was detected. ESBL and AmpC disc tests were easy, convenient and economical. A high prevalence of ESBL and AmpC beta lactamase in community acquired infection is a matter of concern. It is quite alarming to note that, multidrug resistant isolates including ESBL and AmpC beta lactamase producing bacteria circulating in community. Henceforth, it is important to formulate a strict antibiotic policy. 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 and AmpC beta lactamases also varied in different places. It ranges from 0-100% in different reports. Another contributing factor affecting the prevalence is the increased usage of 3rd generation cephalosporin antibiotics in clinical practice. A continuous surveillance is therefore essential along with prudent use of the implicated antibiotics.

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