Emergence of High Level Multi-Drug Resistance in Bacterial Isolates from UTI Cases Attending a Tertiary Care Hospital in North India

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Abstract

Introduction: Urinary tract infection (UTI) is one of the commonest causes of morbidity worldwide. Etiology and antibiotic resistance pattern of uropathogens keeps changing with time. This study was an attempt to estimate the prevalence and antimicrobial resistance pattern of various bacterial isolates in patients attending a tertiary care hospital in north India.

Materials & Methods: Total 307 urine samples were included in this study from January 2011 to December 2011 and 104 samples showed significant colony count of pathogens. Urinary isolates were identified by conventional methods and antibiotic susceptibility test of each isolate was performed by Kirby-Bauer disc-diffusion method according to CLSI guidelines.

Results: Gram-negative isolates (73.12%) pre-dominated compared to gram-positive ones (26.88%). The most prevalent bacteria were Escherichia coli (45.26%) followed by Klebsiella spp. (18.26%), Staphylococcus aureus (17.3%), Enterococcus spp., Citrobacter spp. The majority of the isolates were from females (71.15%) while the remaining was from males (28.84%). Among the gram-negative enteric bacilli, high prevalence of resistance was observed against first line antibiotics namely ampicillin, ampicillin-sulbactum, co-trimoxazole, norfloxacin, levofloxacin, nitrofurantoin, cefuroxime, cefazidime, cefoperazone-sulbactum, gentamicin and amikacin. Carbapenems, especially imipenem was most sensitive in 90% cases. Among 9 Enterococcus isolates, all were resistant to ampicillin while 4 (44.45%) to ampicillin-sulbactum, 6 (66.67%) to levofloxacin and 7 (77.78%) to gentamicin. Among 18 Staphylococcus aureus isolates 16 (88.89%) were MRSA. No Vancomycin resistance was observed in either Enterococcus or Staphylococcus isolates.

Conclusion: Multi-drug resistance in common uropathogens is emerging at an alarming rate, making urine culture and sensitivity a mandatory test for appropriate management of a very common disease like UTI.

Keywords: antimicrobial resistance, bacterial isolates, UTI, North India

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common types of bacterial infections in humans occurring both in the community and health care settings. UTI ranks the highest among the most common reasons that compel an individual to seek medical attention. Today it represents one of the most common diseases encountered in medical practice, affecting people of all ages, from the neonate to the geriatric age group.

The term urinary tract infection (UTI) denotes several distinct entities with the common feature of significant pyuria and bacteriuria. The causative pathogen profile varies from region to region, but Escherichia coli (E. coli) remains the most common causative pathogen. The sensitivity of uropathogens to different drugs varies in different areas, and changes with time. This necessitates periodic studies of the causative uropathogens and their antibiotic sensitivity pattern.

UTIs are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms. Fluoroquinolone are preferred...
as initial agents for empiric therapy of UTI in an area where resistance is likely to be of concern. This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens. The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide. Current knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy. Emerging multidrug resistant strains is of major concern to treat UTI. This study was designed to evaluate the profile of isolates causing UTI and their resistance to various antimicrobial agents in patients attending the OPD and admitted in IPD, of a tertiary care hospital in North India.

MATERIALS AND METHODS

A total of 307 urine samples from outdoor and indoor patients attending our hospital from January 2011 to December 2011 were processed in the department of microbiology. The urine samples were inoculated on MacConkey agar or Blood agar by using the calibrated loop and were incubated aerobically for 18-24 hours at 37°C. Urinary isolates from symptomatic UTI cases were identified on the basis of colony morphology, Gram’s staining, catalase test, oxidase test, coagulase test and standard biochemical tests. 104 (33.87%) samples had significant bacteriuria and were further processed for identification and antibiotic susceptibility test of the pathogen. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method. The data was analyzed using Fischer’s exact test and p value of < 0.05 was taken as statistically significant.

One specimen per patient was taken and patients with significant bacteriuria (≥105 cfu/ml) were included for the microbiological analysis. Unsterile, improperly stored or delayed specimen, were excluded from the study.

From the clinically suspected patients of UTI, midstream clean catch specimen of urine from both male and female patients was collected in a sterile, screw-capped, wide mouthed universal container. The bacterial count in the urine sample was determined by Semi-quantitative culture method using 3.26 mm internal diameter standard loop (Hi- Media laboratories Ltd.).

Antimicrobial sensitivity testing of all significant isolates was performed by Kirby-Bauer disk diffusion method, according to CLSI 2011 guidelines. Commercially available antibiotic disc of 6mm (Hi-Media lab) were used. Antibiotic disc were selected according to bacterial isolates. In the present study the antibiotic disc tested were: Amoxicillin (10μg), Amoxicillin/sulbactum (10/10μg), Cotrimoxazole (25 μg), Gentamicin (10μg), Nitrofurantoin (300 μg), Nalidixic acid (30μg), NorFloxacin (10μg), Ciprofloxacin (5μg), Amikacin (30μg), Levofloxacin (5μg), Cefotaxime (30μg), Cefotaxime/clavulanic acid (30/10μg), Ceftriaxone (30μg), Ceftriaxone/sulbactam (30/15μg), Ceftazidime (30μg), Ceftazidime/clavulanic acid (30/10μg), Imipenem/cilastin (10/10μg), Meropenem (10μg), Polymyxin B and Tigecycline (15μg).

RESULTS

Total 307 samples were included in the present study to estimate the prevalence of antimicrobial resistance in patients suffering from Urinary tract infection attending the OPD and IPD of the hospital. Total 104 (33.87%) samples showed significant colony count (106 cfu/ml) of pathogens, 153 (49.8%) samples were sterile, 30 (9.8%) samples showed multiple isolates (samples grossly contaminated during collection) and 20 (6.5%) samples showed insignificant colony count. Out of 104 culture positive samples 58 (55.76%) were from IP and 46 (44.24%) were from OP patients.

All patients were between the ages of 1 to 85 years. Majority of patients were females 74 (71.16%) compared to males 30 (28.84%). Females of reproductive age group (between 21 and 40 years) constituted 52.89% of the total patients. However, males of >40 years, had a higher prevalence of 12.51% compared to females’ 6.73% of the same age group. (Table 1 and Figure 1)

Out of total 104 isolates, majority were gram negative 77 (74.03%) while 27 (25.96%) were gram positive. Escherichia coli (E. coli) was the most common organism isolated from 47 (45.19%) cases, followed by Klebsiella spp. 19 (18.26%), S. aureus 18 (17.30%) and Enterococcus spp. 9 (8.65%). Uncommon bacterial isolates obtained in the study were Citrobacter spp., Pseudomonas spp.,

<p>| Table 1: Age and sex distribution of urinary tract infection cases |
|--------------------------------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>07 (6.73)</td>
<td>6 (5.77)</td>
</tr>
<tr>
<td>11-20</td>
<td>11 (10.57)</td>
<td>5 (4.80)</td>
</tr>
<tr>
<td>21-30</td>
<td>40 (38.46)</td>
<td>35 (33.66)</td>
</tr>
<tr>
<td>31-40</td>
<td>27 (25.96)</td>
<td>20 (19.23)</td>
</tr>
<tr>
<td>41-50</td>
<td>12 (11.54)</td>
<td>6 (5.77)</td>
</tr>
<tr>
<td>51-60</td>
<td>04 (3.86)</td>
<td>1 (0.96)</td>
</tr>
<tr>
<td>61-70</td>
<td>01 (0.96)</td>
<td>0</td>
</tr>
<tr>
<td>71-80</td>
<td>01 (0.96)</td>
<td>0</td>
</tr>
<tr>
<td>81-90</td>
<td>01 (0.96)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>104 (100)</td>
<td>74 (71.16)</td>
</tr>
</tbody>
</table>

*P value significant, < 0.05
Acinetobacter spp., Proteus spp., Enterobacter spp. and CoNS (Table 2 and Figure 2).

The antibiotic sensitivity pattern of gram negative isolates is shown in Table 3. In first line antibiotics, all gram negative isolates were resistant to ampicillin while cotrimoxazole was found to be sensitive only in 6 (12.8%) E. coli and 2 (10.5%) Klebsiella spp. isolates, nitrofurantoin in 10 (21.3%) E. coli and 4 (21%) Klebsiella spp. isolates, norfloxacin in 6 (12.8%) E. coli, 4 (21%) Klebsiella spp., 1 (25%) Citrobacter spp. and 1 (33.3%) Pseudomonas spp. isolates, gentamicin in 9 (9.1%) E. coli, 5 (26.3%) Klebsiella spp., one isolate each of Citrobacter spp. (25%), Pseudomonas spp. (33.3%) and Acinetobacter spp. (50%). In second line antibiotics, amikacin was found to be sensitive only in 7 (14.5%) E. coli, 2 (10.5%) Klebsiella spp., 1 (25%) Citrobacter spp. and 1 (100%) Enterobacter spp. isolate; ceftazidime in only 1(2.1%) E. coli isolate, cefuroxime in 1 (2.1%) E. coli and 2 (10.5%) Klebsiella spp. isolates, cefoperazone/sulbactum in 2 (4.2%) E. coli, 1 (5.25%) Klebsiella spp., 1 (25%) Citrobacter spp. and 1 (33.3%) Pseudomonas spp. isolates, gentamicin in 9 (9.1%) E. coli, 5 (26.3%) Klebsiella spp., one isolate each of Citrobacter spp. (25%), Pseudomonas spp. (33.3%) and Acinetobacter spp. (50%). In reserved antibiotics category, majority of isolates of E. coli (72.3%) and rest of all other isolates were sensitive to imipenem, followed by meropenem in 29 (61.7%) E. coli, 13 (68.4%) Klebsiella spp., 3 (75%) Citrobacter spp., 2 (66.6%) Pseudomonas spp., 1 (50%) Acinetobacter spp., all isolates of Enterobacter spp. and Proteus spp. Most sensitive drugs were found to be polymixin B and tigecycline, with sensitivity in 44 (93.6%) and 42 (89.4%) isolates of E. coli, 16 (84.2%) and only 4 (21%) Klebsiella spp. isolates, 3 (75%) and 1 (25%) Citrobacter spp. isolates and all Pseudomonas spp., Acinetobacter spp., Enterobacter spp. and Proteus spp. isolates respectively (Table 3).

In gram positive isolates, only 2 (11.11%) Staphylococcus spp. were sensitive to ampicillin, followed by 4 (22.2%) to norfloxacin, 6 (33.3%) to cotrimoxazole and levofloxacin each, 7 (38.9%) to ampicillin/sublactum, 10 (55.5%) to gentamicin, 14 (77.8%) to clindamycin. Only 2 (11.1%) Staphylococcus spp. isolates were cefoxitin sensitive. In Enterococcus spp. no isolate was sensitive to ampicillin, 2 (22.2%) to gentamicin, 3 (33.3%) to levofloxacin and 5 (55.5%) to ampicillin/sublactum, All Staphylococcus spp.
and Enterococcus spp. isolates were sensitive to vancomycin (Table 4).

DISCUSSION

Urinary tract infections are one of the most common types of bacterial infections occurring in humans. This study was undertaken to identify pathogenic bacteria responsible for urinary tract infection and to determine their antimicrobial resistance pattern.

Out of the total 307 urine samples, 104 (33.9%) samples yielded significant colony count, 153 (49.8%) samples were sterile, 30 (9.8%) samples showed multiple isolates (samples grossly contaminated during collection) and 20 (6.5%) samples showed insignificant colony count. Our results are similar to a previous study done by Mandal et al., who reported 62% samples sterile, 26.01% showed significant growth, 2.3% showed insignificant growth and 9.6% were found contaminated. It shows that culture positivity rate varies from one laboratory to other.

Higher prevalence of UTI in females than in males, is a well recognized fact; and our findings in accordance with culture positivity in 74 (71.2%) females compared to only 30 (28.8%) in males. However, a reversal of prevalence was seen in >50 years age groups, where higher prevalence of 5.8% was found in males compared to only 0.96% in females. This might be due to decrease in prostatic secretions in males and decreased sexual activity in females, as reported previously by Astal et al. and Khalifa et al.

Females of the reproductive age group (between 21 and 50 years) constituted 58.66% of total cases, compared to only 16.6% male patients. However, males (50 years or more) had a higher incidence of UTI (5.8%) compared to only 0.96% females of the same age group. Approximately same findings were reported by Akram et al., who reported that, women of reproductive age group formed the main group of adult patients with UTI (42.34% of all UTI detected in women of age 21-50 years). UTIs were reported in 62.42% of females and in 37.67% of males. It has been extensively reported that adult women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors.

This study showed that E. coli was the commonest pathogen causing UTI in 47 (45.19%) cases and it was similar to the findings of Mandal et al., Sharma and Paul.

The antimicrobial sensitivity pattern of the E. coli isolates in our study presents a disturbing pattern of antibiotic resistance, compared to previous studies done in India. The comparison of resistance patterns of uropathogenic E. coli in various studies is shown in Table 5.

Alarmingly, high level of drug resistance to first line of antibiotics was observed in our study, as all gram negative
isolates were resistant to ampicillin, while ampicillin/sulbactum was found to be sensitive in only 5 (10.6%) E. coli and 2 (10.5%) Klebsiella spp. isolates. E. coli and Klebsiella spp. isolates were also less sensitive to cotrimoxazole (12.8% and 10.5% respectively), nitrofurantoin (21.3% and 21% respectively), norfloxacin (12.8% and 21% respectively) and gentamicin (19.1% and 26.3% respectively). While for second-line antibiotics, E. coli was more resistant (69.5%) than Klebsiella spp. (46.1%) in this region. Indian isolates showed higher resistance against ampicillin and co-trimoxazole than the isolates from USA (39.1% and 18.6%)21, 22.

Nitrofurantoin has been used for more than five decades for the treatment of uncomplicated cystitis and it was found to remain active against common uropathogens. Recent data suggests that nitrofurantoin has retained a good amount of sensitivity (90.98%), both against ESBL producers and non-ESBL producers23. In our study, nitrofurantoin was found to be sensitive in only E. coli and Klebsiella spp. isolates, at frequency of 21.3% and 21% respectively and we are first to report such high level of resistance to nitrofurantoin from India.

Carbapenems have a broad spectrum of antibacterial activity, and these are resistant to hydrolysis by most β-lactamases including extended spectrum β-lactamases (ESBL) and Amp C β-lactamases. Overall imipenem resistance was less common compared to meropenem, as only 27.7% isolates of E. coli and 50% of Acinetobacter spp. were resistant to imipenem compared to 50% of Acinetobacter spp., 33.4% of Pseudomonas spp., 32.3% of E. coli, 31.6% of Klebsiella spp. and 25% of Citrobacter spp. isolates were resistant to meropenem.

Least resistance was observed against reserved category of drugs i.e. polymyxin B and tigecycline; nevertheless, resistance to these drugs was observed in 6.4% and 10.6% of E. coli and 15.8% and 79% of Klebsiella spp. isolates respectively. This data is worrisome, as it hints towards emergence of pan-drug resistant common uropathogens.

Staphylococcus spp. was the third most common isolate. High level of resistance to ampicillin and methicillin was documented in 88.89% of isolates, followed by norfloxacin in 77.8%, cotrimoxazole and levofloxacin in 66.67%, ampicillin/sulbactum in 62.12%, gentamicin in 44.45% and clindamycin in 22.23% isolates. However, all isolates were sensitive to vancomycin, but a watchful vigilance is required for emergence of vancomycin resistance in view of recent reports of reduced susceptibility of S. aureus to vancomycin24.

Enterococcus spp. was the fourth most common isolate. No isolate was sensitive to ampicillin but 55.55% isolates were sensitive to ampicillin/sulbactum followed by, 33.33% to levofloxacin and only 22.22% to gentamicin. All isolates were sensitive to vancomycin.

**CONCLUSION**

The susceptibility patterns seen in our study seem to suggest that slowly but steadily multi-drug resistance in common uropathogens is emerging at an alarming rate, making urine culture and sensitivity absolutely necessary, before initiation of antibiotic therapy in cases of suspected UTIs.

**REFERENCES**


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