Prevalence of the Multi-Drug Resistant Gram-Negative Bacilli in Urinary Tract Infection (UTI)

Sidra Batool1 & Dr. Amir Ali Shah2
1 Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.
2 PhD & Post-Doctorate, Assistant Professor, Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

Abstract: In the recent past years, UTI has emerged as most commonly acquired infection that may cause serious life threatening issues, if progressed to the kidneys. Therefore, appropriate knowledge regarding the resistant pathogens is required to deal with the urinary tract infection. Thus, the intended study was carried out in the microbiology department of the Pakistan institute of medical sciences (PIMS) at Islamabad, Pakistan from April – November, 2013 in order to determine the prevalence of the multidrug resistant (MDR) gram negative rods among the community-acquired urinary tract infection (CAUTI). Of total 110 samples of patient urine being tested in the study, Escherichia coli was found as the most prevalent uropathogen approaching almost 55% followed by Klebsiella spp. (23%), Enterobacter spp. (12%) and Pseudomonas (12%). Among these uropathogens, higher level of resistance was found against levofloxacin (72%) and ceftazidime (71%) and co-trimoxazole (65%). 72% of the isolates were found ESBL producers, and most of the MDR ESBL producers were isolated from the outdoor patients. Moreover, Prevalence of the MDR pathogens was found higher among young individual of 16-30 year group, with the higher prevalence rate among females as compared to the males. This prospective study suggested that the combination of antibiotic therapy is essential for the treatment of UTI.

Key words: CLSI, uropathogens, ESBL, UTI, Prevalence, multidrug resistance,

INTRODUCTION:

Inspite of the wide range of antibiotics available, their misuse has led to the emergence of bacterial resistance against antimicrobial agents (Arjunan et al., 2010). Urinary tract infection is regarded as an infectious disease acquired from community or hospital environment that can result in higher rate of sickness and loss of economy to the health centers (Hryniewicz et al., 2001; Rehman et al., 2009). Of all infectious diseases that require careful treatment, UTI is the 2nd most commonly encountered infection (Obiogbolu et al., 2009). It is estimated that on annual basis nearly 150 Million cases of UTI occurs and most of these cases relates to nosocomial origin (Drekonja et al., 2008). The risk of acquiring UTI is greater for women including a number of factors such as sexual intercourse, use of contraceptives, as well as the previous maternal history of infection (Hooton et al., 1996).

Owing to the ability to reside the urinary tract by using their ability to form the biofilm, uropathogenic Escherichia coli is responsible for almost 80% cases of UTI (Mulvey et al., 2000). Moreover, E. coli releases cytotoxin entrapped within a vauolar membrane that enhances the ir inability to envade the epithelial surface of urinary tract (Guyer et al., 2002), followed by the Klebsiella that causes 15% of the cases of UTI (Bennett et al., 1995). In patients suffering from complicated infections or using catheter tubes, Proteus spp. is the most common causing the infection Development of antibiotic resistance among members of Enterobacteriaceae is correlated with the inceasing magnitude of UTI. This increasing resistance is due to the production of specific hydrolytic enzymes by GNBs that may dissolve the antibiotic. This phenomenon is specially observed against the action of extended spectrum β-lactamases. Recent research has shown a decline in resistance in E. coli, and rapidly increasing resistance among other members of enterobacteriaeae against quinolones and cephalosporins (Hryniewicz et al., 2001).

It is recommended to use trimethoprim/ sulfamethoxazole (SXT) for the treatment of UTI, if the bacterial resistance is 20%, but in case of bacterial resistance greater than 20%, the use of Ciprofloxacin is recommended (Warren et al., 1999). Other groups of antibiotics used for combating the infection include β-lactam drugs, floroquinolones, nitrofurantoins and cephalosporins (Zervos et al., 2003).

The present study was conducted in order to determine the antimicrobial susceptibility pattern among uropathogens and to check the production
of β-lactamases by 110 isolates during six month period in the department of Microbiology of PIMS, Isb.

MATERIALS AND METHODS:

Urine sampling:
Urine sampling is an important part of study that needs to be carried out under aseptic conditions for the accurate diagnosis of the pathogens. The samples were collected from both outdoor and indoor from patients suffering from acute pain in kidney or bladder.

Procedure for Sample collection:
To prevent the contamination of urine from the normal flora of the urethra and urinary tract, it is advised to collect urine sample using the clean catch method or midstream method. This method of sample collection is applicable only for adults and cannot be done in infants owing to their nonretractable prepuce. Suprapubic aspiration method of sample collection is suitable for patients on catheter. Sterile red top containers were used for the sample collection.

Materials required:
- CLED agar with Andrade indicator
- Mackonkey agar
- Muller Hinton agar
- Triple sugar iron agar

These materials were provided by Oxoid limited, Hampshire, England.

Urine processing:
While processing urine for culture, loop calibration method was used in which nearly 1 µl of specimen was plated on CLED plate and mackonkey plate with a sterile platinum loop. After labeling, urine culture plates were placed in incubator at 37°C overnight. For further clarification of infection, Routine examination of samples was done using combor 10 strips to detect the presence of red cells, pus cells and microbial infection. The growth appeared on plates after 24-48 hours was observed visually and colonies were counted using viable colony count method taking a count of 10^9 as standard method for bacterial diagnosis.

Isolation and identification of urinary isolates:
Further identification of urine isolates was done using three basic techniques including the colony morphology, Gram staining and biochemical tests (Myers and Koshi, 1982). Initial identification was done on the basis of colony size on plates, their pigmentation and elevations at margins of colony. For differentiation b/w gram positive and gram negative microbes, gram staining technique developed by Hans Christian Gram was used. The bacterial isolates were further identified by biochemical tests using the standard methods of Bergey’s manual of Determinative microbiology. Four basic tests used for identification were SIM, TSI, Citrate utilization test, Urease test. The antimicrobial susceptibility testing of the urine isolates was carried out by using the Modified Disk Diffusion Method.

The Antimicrobial susceptibility test:
The antimicrobial sensitivity of urinary isolates was performed in order to determine their antibiogram using the standard method of disk diffusion method developed by CLSI (clinical lab standard institute). The sensitivity method was done on the Muller-hinton agar plates provided by Oxoid, ltd. In order to determine the resistance pattern of MDR gram negative pathogens, a total of eight groups of antimicrobials containing 16 antibiotics were used, and the interpretation was done on the basis of criteria given by M100 CLSI, 100. The antibiotic disks used in study were provided by Oxoid, including a number of third generation antibiotics such as Cephalosporins (30µg), Floroquinolones (05µg), trimethoprim-sulfamethoxazole (05µg), tetracyclines (30µg), Carbapenems (10µg), Aminoglycosides (10µg) and β-lactams (100µg).

Investigation for the ESBL production by GNBs:
The screening for the ESBL production was done using the standards provided by CLSI. Nutrient broth was prepared and refrigerated after placing in auto-claved screw-caped 5ml test tubes. After 24 hours, inoculum was taken in tube containing broth and spread over Muller-Hinton plate to form an evenly distributed lawn using a sterile cotton swab. After swabbing, the amoxicillin+calavulanic acid disk was placed in center with cefuroxime and ceftazidime disk on either side. After incubation, the formation of band between AMC and other antibiotics was reported.

RESULTS
Appropriate statistical data analysis was applied to the results of urine culture by using SPSS 20.0 provided by the Statistical Package of Social Sciences. A total of 110 gram negative urine isolates were selected for study on the basis of positive results of routine examination, positive culture results and biochemical results. It was observed that the percentage of gram negative pathogen was greater as compared to other pathogens, with E. coli as the most prevalent pathogen (55%) followed by klebsiella (23%), Enterobacter (12%) and Pseudomonas (12%); and is summarized in Table.1. Moreover, the prevalence of the infection was greater from OPD as compared to IPD, and most of the patients were from the emergency. Higher prevalence of urinary
tract infection was found among females (52%) as compared to the males (48%). Prevalence of the pathogens was found different in different age groups with higher percentage in 16 yr to 30 yr group among females and 61 yr to 75 yr group among males.

### Table 1. Bacterial isolates causing the UTI

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>No. of isolates and their percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>26 (50.9)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>09 (17.6)</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>02 (3.92)</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>01 (1.96)</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>01 (1.96)</td>
</tr>
<tr>
<td>Providencia spp.</td>
<td>01 (1.96)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51 (100)</td>
</tr>
</tbody>
</table>

### Table 2. Common antibacterial resistance pattern in MDR gram negative bacilli.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Percent of isolates resistant to antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli (n=60)</td>
</tr>
<tr>
<td>AMC</td>
<td>61.6</td>
</tr>
<tr>
<td>CAZ</td>
<td>73</td>
</tr>
<tr>
<td>CRO</td>
<td>77</td>
</tr>
<tr>
<td>TEP</td>
<td>30</td>
</tr>
<tr>
<td>SXT</td>
<td>68</td>
</tr>
<tr>
<td>IPM</td>
<td>15</td>
</tr>
<tr>
<td>LEV</td>
<td>73</td>
</tr>
<tr>
<td>CN</td>
<td>63</td>
</tr>
<tr>
<td>F</td>
<td>21</td>
</tr>
<tr>
<td>FOS</td>
<td>05</td>
</tr>
<tr>
<td>SCF</td>
<td>48</td>
</tr>
<tr>
<td>CIP</td>
<td>63</td>
</tr>
<tr>
<td>MEM</td>
<td>20</td>
</tr>
<tr>
<td>TOB</td>
<td>61</td>
</tr>
<tr>
<td>DO</td>
<td>78</td>
</tr>
<tr>
<td>AK</td>
<td>18</td>
</tr>
</tbody>
</table>

Note: The Panel of Antibiotics for Pseudomonas is different as compared to other pathogens.

The antibacterial sensitivity pattern of the 16 selected antibiotics against the most prevalent GNBs was checked using the disk diffusion method with higher resistance pattern against levofloxacin (73%) and ceftazidime (73%) followed by ceftriaxone (71%). The resistance pattern of 16 selected drugs by urinary pathogens is presented in figure 1. The best drug action was shown by nitrofurantoin, fosfomycin, amikacin and carbapenems. The multidrug resistance of GNBs was also checked among both IPD and OPD and the resistance pattern was found higher for specimen received from OPD. Of total 110 isolates, nearly 72% isolates were found ESBL producers and remaining 28% were non producers of ESBL. Of total 60 isolates of E.coli and 25 isolates of Klebsiella, nearly 47% isolates of E.coli were ESBL producers followed by 40% isolates of Klebsiella, as summarized in Table 3.
DISCUSSION:
This study described the overall prevalence of the urinary tract infection caused by the gram negative bacilli resistant to multiple range of drugs along with their susceptibility patterns and distribution of ESBL producers among 110 bacterial pathogens isolated from urine cultures, from April to November, 2013 in the Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan. In this study, *Escherichia coli* was found most prevalent cause of infection causing nearly 54% cases of infection, followed by *Klebsiella* (22%) and *Pseudomonas spp.* (12%), which correlates with the previous findings by Farshad et al., (2010), Khadri et al., (2009). The highest prevalence of *E. coli* is associated with the ability of biofilm formation as well as the presence of certain types of virulence factors that might evade the host immune system. In 2013, study conducted on the β-lactamase production in bangladesh also demonstrated the higher prevalence of of *E. coli* among all urinary pathogens causing the infection (Khan et al., 2013). *E. coli* is responsible for the formation of quiescent intercellular reservoir (QIR) in the bladder’s epithelial cells therefore able to invade the urinary tract more easily as compared to other pathogens. The formation of glycoseamineglycan (GAG) in human’s bladder act as a barrier for the entry of pathogen’s entry in the urinary tract. In menstruated females, a change in the GAG layer renders females more prone to infection (Mysorekar et al., 2006).

In this investigative study, it was shown that the infection caused by the MDR gram negative bacilli showed greater prevalence among adult patient age groups of 16yr to 30yr, which was found consistent
with the reported study by Linhares et al., 2013. The higher occurrence of infection in this age group is associated with the higher frequency of sexual activity. The study conducted by Linhares in 1989 also showed that uropathogens are more likely to be transmitted by sexual intercourse. The second most common group of age susceptible to infection include 61yr to 75yr which is associated with the use of catheters and prostrate enlargement. The given data demonstrated the higher prevalence of infection among females (51%) as compared to the males (45%) and the paeds (4%). This study is consistent with other reported study in different part of the world (Gradwohl et al., 2006; De Backer et al., 2008; Omoregie et al., 2008; Linhares et al., 2013).

In this study, uropathogens resistant to three or more antimicrobial agents were considered multidrug resistant, which is consistent to the study by Magiorakes et al., 2011. The highest pattern of resistance was observed against the cephalosporins (78%), tetracyclines (77%), trimethoprim (72%) and floroquinolones (72%). In this study, a large number of urinary isolates are found resistant to cephalosporins due to the production of β-lactamases by the isolates. This study on cephalosporins is parallel to the study done by Faridah et al., 1997 showing that the increasing resistance in isolates is attributed to the production of β-lactamase enzymes. Tetracyclines have long been found effective for the treatment of bacterial infection, but the increasing resistance against tetracycline in this study has shown evolution of resistance mechanism by pathogens including the efflux pumps or change in ribosomes (Yamaguchi, 1997). In floroquinolones group, the higher pattern of resistance is found in ciprofloxacin (62%) and levofloxacin (72%) which is new finding as compared to previously reviewed data showing resistance nearly 3% to 7%. (Mathai et al., 2001). In 2001, study conducted on the prevalence of urinary isolates showed the increasing resistance against the SXT which shows that SXT is least effective for the treatment of UTI. In combating infection, fosfomycin is found most effective in killing the E. coli. In 2003, Marchese et al., has shown nearly 95% effectiveness for the treatment of UTI, due to the longer stay of fos in the urine that is effective for killing the pathogen. Discussing the resistance pattern against Carbapenems, 3.9% resistance was found during the study conducted in 2011 (Eshwarappa et al., 2011) while, present study has shown nearly 28% level of resistance.

In the present study, the production of ESBL by the resistant isolates was found nearly 49%. Among these isolates, nearly 47% of E. coli isolates are ESBL producers while only 40% of Klebsiella isolates are ESBL producers. The study conducted by Khan et al., 2013 has shown the production of ESBL by isolates is nearly 53%, showing higher prevalence of Klebsiella for the production of β-lactamases.

CONCLUSION:

Prevalence rate of E. coli and Klebsiella is found higher in the community, while the prevalence of the Pseudomonas is higher in the Hospital environment. Mostly the GNBs causing the UTI are isolated from the patients in the OPD of urology, Emergency and Nephrology. According to the susceptibility profiles, nearly 70% to 80% isolates are found resistant to the 3rd generation cephaporphins and the resistance to the β-lactam drugs is due to the production of ESBL by the urinary isolates.

Acknowledgements:

This work was supported in part by a grant from Mr. Taqee Razza, Mr. Hussain Bux Chandio & Mr. Ali Razza. Members of Microbiology dept. of PIMS are also gratefully acknowledged for their great contribution in the whole study.

References:


