ORIGINAL ARTICLE

Bacterial Profile and Antimicrobial Resistance of Uropathogenic *Enterobacteriaceae*

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ABSTRACT

Aim: To determine the recent bacterial profile and antimicrobial resistance pattern of uropathogenic bacteria of *Enterobacteriaceae.*

Methods: We performed this study on a total number of 6,269 urine samples collected from various wards of the Children's Hospital Lahore. The sample processing, isolation and identification were carried out by different microbiological techniques. The antimicrobial resistance pattern was determined by using Kirby Bauer Disk Diffusion method (E-test for colistin sulphate) and reported according to CLSI guidelines. *E.coli* and *Klebsiella pneumoniae* (ATCC 25922 and 13883) used as media quality control (QC). The data was analysed through IBM SPSS v.23.

Results: A significant bacteriuria was detected in 389(6.3%) females and males patients with a proportion of 219(55.6%) and 175(44.6%), respectively. The main culprits of urinary tract infection (UTIs) belonged to the *Enterobacteriaceae* found in our study were *E. coli* 211(53.6%) and *Klebsiella* species 157(39.8%). More than 84% of the bacterial strains were resistant to all of the cephalosporin drugs, 85% co-trimoxazole, 84.0% pipemidic acid, 83.5% nalidixic acid, 80.2% norfloxacin, 77.9% ciprofloxacin and 72.0% tobramycin. A comparatively lower resistance level seen against amikacin (36.8%), fosfomycin (35.3%), nitrofurantoin (28.4%), imipenem (25.9%) and colistin sulphate (21.8%).

Conclusion: The isolation of a high number of *E. coli* and *Klebsiella* spp. shows the continued predominance of these bacterial strains as uropathogens. Multidrug resistance among the uropathogens is worrisome which left us with fewer treatment options.

Keywords: Urinary tract infection, Uropathogenic *Enterobacteriaceae,* Antimicrobial resistance pattern

INTRODUCTION

Urinary tract infections (UTIs) refers to the invasion of a structurally and functionally normal urinary tract by nonresistant infectious organisms[1]. UTI affects about 150 million people worldwide each year. History of UTI dates back to 1550 BC in Ebers Papyrus[2]. These infections usually occur between the ages of 16 to 35 years with more prevalence of females than males. The highest prevalence of the uropathogens among patients suffering from UTIs is of *E. coli and Klebsiella*[3]. The clinical manifestation of UTI is burning sensation or discomfort while passing urine. The most common symptoms include severe pain and burning above the pubic bone, frequent urination or urination urge lacking vaginal discharge, flank pain, fever, vomiting, nausea and bloody urine. In elder children loss of bladder control is also observed[4]. UTIs occurring in a healthy individual with no structural or functional abnormalities of the urinary tract is termed uncomplicated UTI that includes both upper urinary tract infection and lower urinary tract infection. All other types are considered complicated UTIs[5].

Several members of the *Enterobacteriaceae* that live in the intestine are referred to as enterobacteria or "enteric bacteria". The family *Enterobacteriaceae* includes more than 53 genera and 210 species that includes a large number of harmless symbionts and potential pathogens[6].

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The bacterial virulence factors involved in causing ascending urinary tract infection are augmented adherence and colonisation by uropathogens. These uropathogens include *E. coli*, *S. saprophyticus*, *K. pneumoniae*, *Enterobacter*, *Citrobacter*, *Morganella* and *Proteus mirabilis*[6]. Sexually active women who use birth control pills or use spermicidal agents may be at higher risk. The urethra in women is shorter that shortens the distance for bacteria to reach bladder[7]. Kidney stones or an enlarged prostate in patients who are hospitalised, paralysed or are suffering from neurological problems use a catheter to urinate, also have an increased risk of UTI[8].

The excessive usage of antimicrobials has incepted to drug-resistant germs causing selective pressure on bacterial population. The drug-resistant enzymes that *Enterobacteriaceae* produce are Amp-C mediated enzyme, extended-spectrum beta-lactamase enzyme (ESBL), and Metallo-beta-lactamase (MBL) enzyme. Mostly used treatment options against *Enterobacteriaceae* in UTIs include the use of cephalosporins, fluoroquinolones, aminoglycosides and carbapenems[9-10]. The present study intended to determine the recent bacterial profile and pattern of antimicrobial resistance of uropathogenic bacteria of *the Enterobacteriaceae* family.

METHODS

The current cross-sectional study was carried out at the Microbiology Department of The Children's Hospital,

Lahore for six months (July to December 2017). A total of 6,269 urine samples of pediatric patients were collected through convenient sampling technique from inpatient and outpatient departments. The cultures exhibiting mixed, fungal growth and bacterial count of $<10^5$ CFU/ml were excluded from the study. We used CLED (Cystine Lactose Electrolyte Deficient) agar plates to inoculate urine specimens, and calibrated wire loop of 2 µl size was used to inoculate the agar plate. The CLED agar plates were incubated overnight at 37°C for 24 hours.

Bacterial identification was performed through the morphology of bacterial colonies, their shape and size, colour, surface elevation, margins and translucency. The cultural characteristics were further analysed for the fermentation of lactose. The final identification was made based on API 10S (bioMerieux). Antimicrobial resitance analysis was done by means of 0.5 McFarland turbidity scale through the modified Kirby-Bauer disk diffusion method and E-test method used to assess minimum inhibitory concentration (MIC) of colistin sulphate.

The suspension was inoculated onto Muller Hinton plate and spread over the plate using a sterile swab. The available antibiotic disks were applied on the media with the help of multi-disk dispenser and incubated at 37ºC for 24 hours. After 18-22 hours of incubation, the zones of inhibition of antibiotics was measured in millimetres to report the organism as resistant, intermediate sensitive or sensitive using CLSI guidelines 2017[11]. *E.coli* and *Klebsiella pneumoniae* (ATCC 25922 and 13883) were used as QC for the CLED agar. The statistical data analysis was performed using IBM corporation SPSS statistics V. 23.

RESULTS

Out of the 6,269 urine samples, 394 (6.3%) came out as positive cultures. Significant bacteriuria was detected in 389 (6.3%) cases of females and males with a proportion of 219 (55.6%) and 175 (44.6%), respectively. The isolation rate of uropathogenic bacteria from different wards primarily showed 97 (24.6%) bacterial strains from Urology, 94 (23.9%) Medical, 52 (13.2%) Outpatient Department (OPD) and 38 (9.6%) Nephrology. A less number of uropathogenic isolates were obtained from the remaining wards (Table- I).

The major bacteria found in the study were *E. coli* 211/389 (53.6%) followed by *Klebsiella* spp. 157/389 (39.8%). The other *Enterobacteriaceae* found in the study were *Serratia* spp. 6/389 (1.5%), *Citrobacter* spp. 6/389 (1.5%), *Proteus* spp. 6/389 (1.5%), *Enterobacter* spp. 5/389 (1.3%) and *Morganella* spp. 3/389 (Table II).

The antibiotic resistance profile of uropathogenic bacteria showed very high resistance against the majority of the antibiotics. Overall > 84% of the bacterial strains were resistant to all of the cephalosporin drugs. The uropathogenic strains exhibited high resistance against co-trimoxazole (335; 85.0%), pipemidic acid (330; 84.0%), nalidixic acid (329; 83.5%), norfloxacin (316; 80.2%), ciprofloxacin (307; 77.9%) and tobramycin (284; 72.0%). A comparatively lower resistance level seen against amikacin (145; 36.8%), fosfomycin (78; 35.3%), nitrofurantoin (112; 28.4%), imipenem (102; 25.9%) and colistin sulphate (86; 21.8%). The resistance profile of uropathogens against the other antibiotics has shown in Table III.

Table-I: Demographic distribution of patients with positive cultures

Gender	**Frequency (n)**	**%age**
Female	219	55.6
Male	175	44.4
Wards		
Urology	97	24.6
Medical Ward	94	23.9
Outpatient Department	52	13.2
Nephrology	38	9.6
Private	19	4.8
Neonatal Nursery	15	3.8
Gastroenterology	15	3.8
Medical Emergency	13	3.3
Intensive Care Unit	12	3
Developmental	10	2.5
Oncology	10	2.5
Ear Nose Throat	6	1.5
Cardiology Ward	6	1.5
Neurology	5	1.3
Surgery	2	0.5

Table-II: Bacterial profile of uropathogens (n=394)

Organisms Isolated	**Frequency (n)**	**%age**
Escherichia coli	211	53.6
Klebsiella spp.	157	39.8
Serratia spp.	6	1.5
Citrobacter spp.	6	1.5
Proteus spp.	6	1.5
Enterobacter spp.	5	1.3
Morganella spp.	3	0.8

Table III: Antimicrobial resistance pattern of uropathogenic isolates

Antibiotic	**Frequency (n)**	**%age**
Cefuroxime	363	92.1
Cefixime	357	90.6
Ceftazidime	342	86.8
Co-trimoxazole	335	85.0
Ceftriaxone	333	84.5
Cefotaxime	331	84.0
Pipemidic Acid	330	84.0
Nalidixic Acid	329	83.5
Norfloxacin	316	80.2
Ciprofloxacin	307	77.9
Tobramycin	284	72.0
Co-amoxiclav	275	69.8
Gentamicin	206	52.3
Meropenem	166	42.2
Piperacillin-tazobactam	154	39.1
Cefoperazone-sulbactam	148	37.6
Chloramphenicol	146	37.1
Amikacin	145	36.8
Fosfomycin (n=221)	78	35.3
Nitrofurantoin	112	28.4
Imipenem	102	25.9
Colistin sulphate	86	21.8

DISCUSSION

Literature from different studies suggested and proved that *E. coli* remained the major etiological uropathogen, comprising of 77% proportion of all the UTI cases irrespective of the hospital setting, community or country[12-14]. UTI cases are treated empirically in the developing

communities where often patients cannot have enough money to pay a physician, and the urine samples for diagnostic tests do not respond to treatment due to the overrepresentation of microorganisms[15]. UTI is among most frequent infections in paediatric patients and a notable cause of morbidity, occurring as febrile children (2%) febrile infants (5%) and of <5 years of age when particularly associated with structural abnormalities of urinary tract[16]. The most common uropathogen isolated in the present work was *E. coli* 53.5%. This finding is similar to the different studies which showed 42.3% and 81.7% *E. coli* as a prominent pathogen[13,14]. Most of *E. coli* were isolated from females 55.6% and this observation is comparable to the study reported by Balkhi *et al.* and Ghanbari *et al.* which reported most bacterial strains from female patients[17,18].

Klebsiella is the second most frequent organism isolated in the present study accounting for UTI. The finding is similar to the observations in other studies where *Klebsiella* was isolated in 16.7% to 25%, although, rare isolation of *Klebsiella* (5.5%) has also been reported by Haque *et al.* and Kaur *et al.* in Bangladesh[19,20]. The results of the sensitivity pattern to different antibiotics showed that all organisms isolated had reduced sensitivity to the common antibiotics used in the community, i.e., ampicillin and co-trimoxazole. Increased antimicrobial resistance among urinary tract isolates to co-trimoxazole, ampicillin and the first-generation cephalosporin has earlier been reported by many researchers[21]. The present study showed similar sensitivity results for nitrofurantoin with the reports of other studies which reported 87.8% and 80% sensitivity results respectively[18,22]. Because of the broad-spectrum activity of carbapenems, the imipenem was the most active antimicrobial drug reported 70% activity. However, the results are contradictory to the findings of Majumder *et al.* from Bangladesh who reported 98% sensitivity of uropathogens to meropenem and 93% sensitivity to imipenem against the bacterial isolates[23].

Cefixime is a third-generation oral cephalosporin which has good palatability and tolerance in children due to its taste and convenient dosage regimen, *E. coli* Showed 90% resistance to cefixime, and 92% resistance pattern for cefuroxime highlighted the of extensive drug resistance development. These findings are comparable with the study of Dreshaj *et al.* which showed 88% effectiveness of cefixime in uncomplicated UTI[24]. The cephalosporins, ceftazidime had 86% resistance among all isolates, the findings are contradictory to the report of from South Western Nigeria which observed least resistance[25]. Among fluoroquinolone class, ciprofloxacin showed 78% resistance, and the findings are similar to a study which reported 82% resistance. However the contradictory results 48% were found in the study of Ghanbari *et al.* in Iran[18]. The effectiveness of piperacillin-tazobactam, imipenem and nitrofurantoin against the multidrug-resistant uropathogens has already been reported[26,27,28,29]. The data for the local antibiotic susceptibilities should be used to determine the changes in the resistance profile of the bacteria due to the unjustified use of antibiotics[30,31,32,33]. The existence of resistant strains in hospital settings could also be a potential source of emerging infection among hospitalised patients. The exogenous nosocomial infections can be reduced with good decontamination practices[34].

CONCLUSION

The isolation of a high number of *E. coli* and *Klebsiella* spp. shows the continued predominance of these bacterial strains as uropathogens. The multidrug resistance among the uropathogens is worrisome which left us with few treatment options. We found comparatively lower resistance against amikacin, fosfomycin, nitrofurantoin, imipenem and colistin sulphate which can be used as empirical drugs against the multidrug-resistant uropathogens. Improved hygiene and infection control measures could help to reduce infection rates by the uropathogens. The injudicious use of antibiotics should be minimised.

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