Extended-Spectrum Beta-Lactamase (ESBL) Producing, Multidrug-Resistant (MDR) Uropathogens and Their Antimicrobial Susceptibility Profile: A Tertiary Care Hospital Experience

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ABSTRACT

Objective: To determine extended-spectrum beta-lactamase (ESBL) producing, multidrug-resistant (MDR) uropathogens and their antimicrobial susceptibility profile in various age groups and gender.

Study Design: Comparative cross sectional study.

Place and Duration of Study: Department of Pathology, Combined Military Hospital Abbottabad, from Jan to Dec 2018.

Methodology: A total of 727 urine samples were collected and inoculated on cysteine lactose electrolyte deficient (CLED) agar plates (Oxoid). Plates were incubated for 24-48 hours at 37°C ±2 aerobically. Growth was identified by standard techniques. Antimicrobial susceptibility was determined.

Results: The rate of positive culture was 19%. Uropathogens spectrum showed that there were *Escherichia coli* 82 (59.85%), *Klebsiella pneumoniae* 37 (27.01%), *Serratia spp.* 4 (2.92%), *Enterobacter spp.* 4 (2.92%), *Citrobacter spp.* 3 (2.19%), *Pseudomonas aeruginosa* 2 (1.46%), *Staphylococcus saprophyticus* 3 (2.19%) and *Enterococcus spp.* 2 (1.46%). Susceptibility was as follows: Ampicillin (2.92%), Co-amoxiclav (26.28%), Ceftriaxone (64.69%), Cefepime (58.39%), Tazobactum-piperacillin (63.50%), Imipenem (82 %), Ciprofloxacin (27.01%), Co-trimoxazole (29.93%), Amikacin (74.45%), Nitrofurantoin (81.75%) and Sulbactum-Cefoperazone (54.48%). Among 130 Gram negative bacilli (GNB) from urinary isolates 71(54.6%) were ESBL producer and 59 (44.3%) were non-ESBL producers. No isolate of *Serratia spp, Enterobacter spp.* and *Citrobacter spp*. Were producing ESBL. Out of 130, Gram negative bacilli 92 (70.8%) were MDR and 38 (29.2%) were non-MDR.

Conclusion: Extended-spectrum beta-lactamase production and MDR is significantly high in uropathogens. Empirical treatment followed by a de-escalation of antimicrobial is suggested after culture report in order to combat the problem of emerging antibiotic resistance.

Keywords: Beta-lactamases, Multidrug resistant, Urinary tract infection.

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INTRODUCTION

Urinary tract infections (UTIs) are the second most common bacterial infections after upper respiratory tract infections.[1] About 250 million cases occur annually, with the expenditure of 6 billion dollars worldwide.[2] Clinically, complicated or uncomplicated UTI is classified based on the presence or absence of functional or anatomical abnormalities.[3] As UTIs are the second most common cause of infection in human beings, resulting in a significant rise in healthcare settings workload including OPD visits, laboratory workload and hospital admissions.[4] *E.coli* followed by other members of Enterobacteriaceae and members of Gram-positive cocci are most prominent uropathogens.[5] UTIs occur more frequently in females than males (8:1) due to shortness of the female urethra.[6,7] Multidrug resistant (MDR) bacteria are increasing day by day and are difficult to treat with commonly used antibiotics.[8,9] Extended-spectrum beta-lactamases (ESBL) producing members of Enterobacteriaceae are highlighted in health care settings because of limited therapeutic options to treat these bacteria resulting in poor clinical outcomes and a prolonged hospital stay of patients.[8] ESBL producing organisms are easily acquired by hospitalized patients. The members of *Enterobacteriaceae* and *Pseudomonadaceae* produce such enzymes among them. *E.Coli* and *K. pneumoniae* are notorious for producing ESBL.[9] The knowledge of the frequency of the causative organisms and their sensitivity pattern requires reappraisal from time to time for effective treatment. The recent local data available on the subject is scarce. This study aims to determine the etiological agent of urinary tract infections, their susceptibility profile, ESBL production and MDR isolates in various age groups. The rationale of the study was to guide clinicians in

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selecting appropriate empirical treatment and in decreasing not only morbidity but also mortality, especially in hospitalized patients.

METHODOLOGY

This comparative cross-sectional study was conducted at the Department of Pathology, Combined Military Hospital Abbottabad Pakistan, from January to December 2018.

Inclusion Criteria: All the urine specimens collected from indoor and outdoor patients, clinically diagnosed with UTI were included in the study.

Exclusion Criteria: None.

Samples were collected from the patient in a sterile container. Consecutive, non-duplicate urine sampling was carried out. Sample size of 137 was calculated using WHO sample size-calculator. Samples were processed as early as possible. 0.2µl of urine was inoculated semi-quantitatively using filter paper on Cysteine Lactose Electrolyte Deficient (CLED) agar plates. Culture plates were incubated for 24-48 hours at 37°C ± 2 aerobically. UTI was diagnosed when 105 colonies were cultured in sampling with the clean-catch method, more than 104 in catheter sampling and any colony from suprapubic sampling. The growth of two or more, two types of colonies was taken as mixed growth and considered contamination. Urinary isolates were identified by colony morphology followed by Gram stain and biochemical reactions by using analytical profile index 20E (API20E). Gram-positive isolates were identified by catalase, coagulase and biochemical laboratory tests.

Antimicrobial sensitivity testing was performed using the modified Kirby Bauer disc diffusion method as per CLSI guidelines.[10] The bacterial suspension was prepared by comparing 0.5 MacFarland standard and inoculated on the Muller-Hinton agar. Antibiotic discs Ampicillin, Amikacin, Co-amoxiclav, Co-trimoxazole, Cefepime, Ceftriaxone, Ciprofloxacin, Imipenem, Piperacillin-tazobactam, Cefoperazone-sulbactam and Nitrofurantoin were placed on the Muller-Hinton agar. After the 18-24 hours incubation at 37°C ± 2 aerobically zone diameter of inhibition was measured. According to Clinical and Laboratory Standards Institute (CLSI) guidelines, susceptibility break points were elucidated. *E.coli* ATCC 25922 was used as a quality control strain.[11] Phenotypic confirmation of ESBL producing isolates was carried out by double-disk diffusion method using Ceftazidime (30µg) and Cefotaxime (30µg) antimicrobial disks with and without Clavulanic acid (30µg). A zone difference of >5 mm between disks was considered confirmed positive for ESBL producing isolates.[11] The ESBL screening was carried out. *K. pneumoniae* ATCC700603 was used as a quality control strain. According to the Centre for Disease Control and Prevention (CDC), bacterial isolate resistant to at least one agent in three or more antimicrobial categories is referred to as MDR.[12]

Statistical Package for Social Sciences (SPSS) version 21 was used for data analysis. Data was catagorozed according to age, gender, ESBL production and multidrug resistance. Chi-square test was applied with the *p*-value of ≤0.05 considered as significant.

RESULTS

Out of 727 cultured samples, 137 (18.8%) samples yielded pure bacterial growth of pathogens. The mean age of the patients was 42 ± 18.95 years. The majority 77 (56.2%), of the isolate were from females while the remaining were from the males 60 (43.80%), as shown in Table-I.

Table-I: Positive samples according to gender (n=727).

	Gender	
	Male, n (%)	**Female, n (%)**
Positive	77 (56.2%)	60 (43.7%)
Negative	354 (60%)	236 (40%)

In female patients, the common age group was 31-40 years, while male patients mainly were >60 years of age. The most prevalent isolated etiological agents of UTI were *E. coli* 82 (59.85%) followed by *K. pneumoniae* 37 (27.01%), *Serratia spp.* 4 (2.92%), *Enterobacter spp.* 4 (2.92%), *Citrobacter spp.* 3 (2.19%), *P. aeruginosa* 2 (1.46%), *Staphylococcus saprophyticus* 3 (2.19%) and *Enterococcus spp.* 2 (1.46%) as shown in Table-II.

Table-II: Pattern of uropathogens in positive samples according to gender (n=137).

Bacterial Isolates	Male, n (%)	Female, n (%)
E.coli	35 (58.33)	47 (61.04)
K. pneumoniae	18 (31.67)	19 (23.38)
Serratiaspp	-	4 (100)
Enterobacter spp	1(25)	3 (75)
Citrobacterspp	1 (33)	2 (67)
P. aeruginosa	1 (50)	1 (50)
S.saprophyticus	2 (67)	1 (33)
Enterococcus spp	1 (50)	1 (50)

E.coli showed a higher sensitivity pattern to Imipenem while *K .pneumoniae* was more susceptible to Nitrofurantoin. A high resistance was noted in Gram-negative bacilli against Ampicillin, Co-amoxiclav and

Co-trimoxazole. Gram-positive isolates *S. saprophyticus* and *Enterococcus spp*. displayed a 100% sensitive profile against Vancomycin (Table-III).

Table III: Pattern of susceptibility against various antimicrobials.

Anti-Bacterial Drugs	*Escherichia coli*	*K. pneumoniae*	*Enterobacter spp*	*Citrobacter spp*	*Serratiaspp*	*Pseudomonas Aeruginosa*	*S. Saprophyticus*	*Enterococcus spp*
	n=82 (%)	n=37 (%)	n=4 (%)	n=3 (%)	n=4 (%)	n=2, (%)	n=3 (%)	n=2 (%)
Ampicillin	2 (2.4)	-	-	-	-	-	2 (67)	2 (100)
Co-amoxiclav	33 (40.2)	-	3(75)	-	-	-	-	-
Amikacin	65 (79.2)	23 (62.1)	4(100)	2 (66.6)	2 (50)	1 (50)	3 (67)	2 (100)
Cefepime	51 (62)	21(56.7)	3(75)	-	3 (75)	2 (100)	-	-
Ciprofloxacin	23 (28)	8 (21.6)	-	2 (66.6)	2 (50)	2 (100)	2 (67)	2 (100)
Ceftriaxone	60 (73)	18 (48.6)	2(50)	3 (100)	2 (50)	2 (100)	-	-
Co-trimoxazole	21 (25.6)	15 (40.5)	1(25)	-	-	1 (50)	2 (67)	1 (50)
Tazobactum-Piperacillin	65 (79.2)	12 (32.4)	2(50)	3 (100)	3 (75)	2 (100)	-	-
Imipenem	75 (91.4)	22 (59)	3(75)	3 (100)	4 (100)	2 (100)	-	1 (50)
Cefoperazone-Sulbactum	38 (46.3)	22 (59)	4(100)	2 (66.6)	4 (100)	2 (100)	-	1 (50)
Nitrofuratoin	67 (81.7)	28 (75.6)	4(100)	2 (66.6)	4 (100)	2 (100)	3 (100)	2 (100)
Rifampicin	-	-	-	-	-	-	2 (67)	2 (100)
Vancomycin	-	-	-	-	-	-	3 (100)	2 (100)
Tetracycline	-	-	-	-	-	-		2 (100)

Out of 137 uropathogens, 132 were Gram-negative bacilli (GNB). Among 130 *Enterobacteriaceae*, 71 (54.6%) were Extended-Spectrum Beta-Lactamase (ESBL) producers and 59 (44.3%) were non-ESBL producers. Out of 82 *E.coliisolates*, 51 (62%) were ESBL producers and 31 (38%) were non-ESBL producers. Out of 37, *K. pneumonia* 20 (54%) were ESBL producers and 17 (46%) were non-producers. No isolate of *Serratiaspp, Enterobacterspp* and *Citrobacterspp* was ESBL producer. Among 130 GNB uropathogens, 92 (70.8%) were MDR and 38 (29.2%) were non-MDR. Out of 92 MDR isolates *E.coli* was more common (56.6%) followed by *K.pneumoniae* 25 (25.5%). Association of ESBL production with age groups, gender and multidrug resistance were shown in Table-IV.

Table-IV: Association of extended-spectrum beta-lacta-mase production with age groups, gender and multidrug resistance.

Characteristics	ESBL, n (%)	Non-ESBL, n (%)	*p*-value
Age Groups			
0-9 years	4 (3)	1 (0.7)	
10-19 years	2 (1.5)	6 (4.6)	
20-29 years	20 (15)	8 (6)	0.001
30-39 years	8 (6)	8 (6)	
40-49 years	6 (4.6)	19 (14.6)	
50-59 years	11 (8.4)	3 (2.3)	
>60 years	20 (15.4)	14 (10.7)	
Gender			
Female	41 (31.5)	33 (25.3)	0.815
Male	30 (23)	26 (20)	
Multidrug Resistance			
MDR	61 (47)	31 (23.8)	0.001
Non-MDR	10 (7.7)	28 (21.5)	

DISCUSSION

ESBL production and resistance to multiple anti-microbials classes was found significantly high. The emergence of ESBL and MDR urinary isolates are alarming as it results in high morbidity mortality and increased health care cost. Empirical therapy followed by culture directed de-escalation is suggested. Implementation Infection control practices and antimicrobial stewardship programs are need of the hour to curtail antimicrobial resistance.

UTIs are common community-acquired and hospital-acquired bacterial infections.[12] The present study has highlighted the bacterial profile in urinary tract infections and their antimicrobial susceptibility pattern in outdoor and indoor patients visiting Combined Military Hospital, Abbottabad. Out of a total of 727 samples, 137 (19%) showed significant bacteriuria, which was lower than similar studies, which may be due to the patient taking the antibiotics before submitting the samples.[5] UTI was more prominent in female subjects than male subjects, which was in agreement with several earlier studies.[13] Present study showed that the spectrum of organisms causing UTI was comparable to other studies across Pakistan. The second most common cause of UTI followed by *E. Coli* was *K. pneumoniae* which was similar to another study in which *E. coli* was found to be the predominant organism 37.41%, followed by *Klebsiella spp* 32.79%.[14]

Antimicrobial resistance among urinary tract infections is a major threat to public health.[15] Emerging antimicrobial resistance among UTI needs regular monitoring of susceptibility profile of etiological agents.[16]

Our study revealed that urinary isolates were least sensitive to Ampicillin which was in agreement with a study done in Libya in which susceptibility to Ampicillin was around 10%.[17] *E.coli* was the most frequent uropathogen. It showed a high pattern of (91.46%). A previous study in Western India showed sensitivity against Imipenem as 91.69%.[18] The present study revealed the sensitivity of Nitrofurantoin (81.71%) against *E. coli* and *K. pneumoniae* was 82% and 75% which was comparable to earlier studies in Pakistan.[13] Unfortunately, both isolates are sources of hospital-acquired infections.[19] They are the leading cause of septicemia and UTI in health care settings.[20] This is substantial evidence to support the idea of increased morbidity and mortality ESBL outbreaks, particularly in nosocomial environments. The third-generation Cephalosporins are resistant to ESBL producing isolates which is a more significant threat because of limited treatment options.[17]

MDR are micro-organisms resistant to at least one drug in 3 or more classes of antibiotics.[11] In our study, it is revealed that out of 130 GNB, 92 (71%) were MDR and 38 (29%) were non-MDR, which was significantly high number. A study conducted in India reported a comparatively low (38%) burden of MDR.[19] In our investigation, the Vancomycin sensitivity rate against Gram-positive isolates was 100%, which strongly agreed with earlier findings.[21]

It is recommended that before the start of empirical therapy, urine samples should be collected for culture and sensitivity. The treatment may be modified after the culture and sensitivity report. Based on the study result, it is suggested that Nitrofurantoin is a suitable antibiotic for empiric therapy of uncomplicated cystitis over other antibiotics like Co-trimoxazole and Ciprofloxacin. Carbapenems and Aminoglycosides should be preferred for upper urinary tract infections like complicated and uncomplicated pyelonephritis over Cephalosporins and Fluoroquinolones. It is time to formulate an antibiogram that helps clinicians select appropriate antimicrobials for therapy and prevent antibiotic resistance.

CONCLUSION

Extended-spectrum beta-lactamase production and MDR is significantly high in uropathogens. Empirical treatment followed by a de-escalation of antimicrobial is suggested after culture report in order to combat the problem of emerging antibiotic resistance.

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Conflict of Interest: None.

Authors' Contribution

MF: Literature review, data analysis and over all supervision, AG: Data collection & literature review, AM: Literature review, MA: Literature review, TSM: Data collection, AA: Data collection.

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