Multidrug-Resistant Uropathogens, An Emerging Threat: Observational Study in a Tertiary Care Hospital of Azad Kashmir

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ABSTRACT

Objective: To detect the resistant pattern of uropathogens and find the association of different factors with the resistance in the Poonch District of Azad Kashmir.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Medicine, Shaikh Khalifa Bin Zayed Hospital, Rawalakot, from Mar to Aug 2019.

Methodology: Patients having signs and symptoms of urinary tract infection and growth of bacteria on urine culture were included in the study. The antimicrobial susceptibility of the isolates was determined by using the standard, modified Kirby Buer disc diffusion method. Zone sizes were interpreted following Clinical Laboratory Standards Institute (CLSI) 2019 guidelines.

Results: 68 culture-positive patients were included in the study, 45 (66%) patients were female. There was a previous history of urinary tract infection in 44 (64.7%) patients, and 52 (76.5%) gave a history of antibiotic use in the last three months. *E. coli* was the commonest organism isolated, followed by Klebsiella, Proteus and Pseudomonas. All patients were resistant to at least one antibiotic, 48 (72%) isolates were multi-drug resistant. Uropathogens showed the highest resistance to Ampicillin in 67 (98.5%) patients and the lowest resistance to Piperacillin/Tazobactam 2 (3%) patients only. Resistance was significantly higher in patients having a history of recurrent urinary tract infection (*p*=0.028) and the use of antibiotics in the last three months (*p*<0.001).

Conclusion: Resistance to antimicrobial agents is increasing in community-acquired urinary tract infections. Resistance is significantly higher in patients having a history of recurrent urinary tract infection and the use of antibiotics in the last three months.

Keywords: Antibiotics, Resistance, Sensitivity, Uropathogens.

INTRODUCTION

Urinary tract infection (UTI) is a prevalent infectious disease all over the world.[1] UTI is commonly caused by escherichia coli (*E. Coli*).[2] Klebsiella, staphylococcus and proteus are other common organism responsible for UTI.[2,3] It causes serious complications in old age groups and immunocompromised patients having significant morbidity, mortality and cost of treatment.[2] Like other common bacteria, antimicrobial resistance is increasing among urinary pathogens.[4]

Increasing antibiotic resistance in UTIs now leads to complications in the general population, making treatment difficult and costly also.[4] Earlier resistant pathogens were mainly isolated from co-morbid patients; resistance was also more common in patients with nosocomial infection. However, the scenario has changed; resistance is noted in many community-acquired infections in all age groups.[4] The same resistance pattern was noted among patients from nursing homes and communities in a study from Norway.[5] Although resistance strains are increasing worldwide, resistance is more common in parts of the world where antibiotics are misused and abused due to over the counter availability and prescription without indications.[6,7]

UTI is treated most of the time empirically, and it is important to have local data about the antimicrobial resistance of uropathogens to start empiric therapy.[8] Furthermore, as resistance pattern changes continuously, it is necessary to study them frequently in different areas to detect the new resistant strains and guide antibiotic selection. Unfortunately, no data on the sensitivity pattern of uropathogens in Azad Kashmir is unavailable. Therefore, we planned this study; the objective was to detect the sensitivity and resistance pattern of uropathogens and find the association of different factors with the resistance in our population.

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Received: 12 Apr 2021; revision received: 10 Aug 2021; accepted: 13 Aug 2021

METHODOLOGY

This cross-sectional study was conducted at the Department of Medicine and Department of Pathology Shaikh Khalifa Bin Zayed Teaching Hospital (CMH) Rawalakot, from March to August 2019.

Inclusion Criteria: Patients over 12 years of age, either male or female-presenting in the outpatient department (OPD) or admitted to the ward with signs and symptoms of urinary tract infection and growth of bacteria on urine culture, were included in the study.

Exclusion Criteria: Patients fulfilling the Friedman criteria for hospital-acquired urinary tract infection were excluded from the study.

Ethical approval was obtained from the Hospital Research Ethics Committee of Sheikh Khalifa Bin Zayed Hospital Rawalakot (Vide letter number 03/ SKBZ/CMH/REC/20) informed consent was taken from the patients.

Mid-stream urine samples were collected in a sterile container and were processed within 2 to 4 hours of collection time. Using a calibrated loop, the urine sample was inoculated on a standard culture media, Cystine-Lactose–Electrolyte-Deficient (CLED) agar. Culture plates were incubated at 35–37°C ambient air incubators for 24 to 48 hours. After the allocated time, the culture plates were visualized for the presence of bacterial colonies.

Based on the colony count method, they were reported as significant or non-significant growth. Bacterial growth was identified by their colony morphology, gram staining, cata-lase test, coagulase test, oxidase test and biochemical profile using API 20E and API 20NE.

The antimicrobial susceptibility of the isolates was determined by using the standard, modified Kirby Buer disc diffusion method. Zone sizes were interpreted following Clinical Laboratory Standards Institute (CLSI) 2018 guidelines.[9] The antimicrobial agents tested were Ampicillin (10μg), Amikacin (30μg), Amoxicillin (reported on the basis of Ampicillin), Amoxicillin /Clavulonate (20/10 μg), Chloramphenicol (30μg), Cefoperazone/Sulbactam (30μg), Ciprofloxacin (5μg), Ceftriaxone (30μg), Ceftazidime (30μg), Fosfomycin (200μg), Gentamycin (10μg), Imipenem (10μg), Meropenem (10μg), Nitrofurantoin (300μg), Piperacillin/ Tazobactam (100/10 μg), Trimethoprim/Sulfamethoxazole (1.25/23.75 μg). MDR UTI was defined as uropathogenic resistant to at least one antibiotic from three or more classes of drugs.[9,10] In addition, the previous history of UTI was defined as at least one episode of documented UTI in the last 12 months. Sociodemographic characteristics and risk factors for resistance were also collected from eligible patients.

Statistical Package for Social Sciences (SPSS) version 20.0 was used for the data analysis. Quantitative variables were summarized as mean ± SD. Frequencies and percentages were calculated for qualitative variables like gender, sensitivity pattern, resistance pattern, history of recurrent UTI, and use of antibiotics in the last three months. The chi-square test was applied to see the relationship between different factors with sensitivity pattern and resistance pattern of uropathogens, the p-value of ≤0.05 was considered significant.

RESULTS

A total of 68 culture-positive patients were included in the study. Of these 68 patients, 45 (66.2%) were female, and 23 (33.8%) were male. The mean age was 48.36 ± 18.96. There were previous history of UTI in 44 (64.7%) patients, and 52 (76.5%) gave a history of antibiotic use in the last three months. In addition, benign prostatic hyperplasia was found in 12 (17.6%), indwelling catheters in 2 (3%) and CA prostate in 1 (1.5%) patients. E. coli was the commonest organism isolated, followed by Klebsiella, Proteus and Pseudomonas; details were shown in Table-I.

Table-I: Organism isolated (n=68).

Organism	Frequency
E. Coli	41 (60.3%)
Klebsiella	7 (10.3%)
Pseudomonas	3 (4.4%)
Proteus	2 (3%)
Other	15 (22%)

All patients resisted at least one antibiotic; 48 (70.6%) isolates were multi-drug resistant. Uropathogens showed the highest resistance to ampicillin 67 (98.5%) and lowest resistance to piperacillin/ tazobactam 2 (3%), details of sensitivity and resistance pattern were shown in Table-II.

Resistance was significantly higher in patients with a previous history of UTI (p=0.028) and the use of antibiotics in the last three months (p<0.001), shown in Table-III.

Although 80% of patients having BPH were resistant to more than five antibiotics, no significant association was found between anatomical abnormalities of the urinary tract and resistance to antibiotics, shown in Table-IV).

Table-II: Resistance and sensitivity pattern of antibiotics (n=68).

Antibiotic	Sensitive	Resistant
Amoxicillin	6 (9%)	62 (91%)
Amoxicillin/Clavulonate	14 (21%)	54 (79%)
Ampicillin	1 (1.5%)	67 (98.5%)
Amikacin	59 (87%)	9 (13%)
Co-trimoxazole	14 (21)%	54 (79%)
Ciprofloxacin	20 (29%)	48 (71%)
Cefoperazone/ Sulbactam	56 (82%)	12 (18%)
Ceftazidime	37 (54%)	31 (46%)
Ceftriaxone	20 (29%)	48 (71%)
Cefixime	17 (25%)	51 (75]%)
Fosfomycin	59 (87%)	9 (13%)
Gentamycin	59 (87%)	9 (13%)
Imipenem	65 (96%)	3 (4%)
Levofloxacin	14 (21%)	54 (79%)
Meropenem	64 (94%)	4 (6%)
Nitrofurantoin	64 (94%)	4 (6%)
Piperacillin/Tazobactam	66 (97%)	2 (%)

Table-III Association of multi drug resistance with history of recurrent Urinary tract infection and antibiotic use in last 3 months.

Basic Characteristics	Study Groups		p-value
	Non Multidrug Resistant Uropathogens (n=20)	Multidrug resistant Uropathogens (n=48)	
History of Recurrent Urinary Tract Infection			
Yes (n=44)	9 (45%)	35 (73%)	0.028
No (n=24)	11 (55%)	13 (27%)	
Antibiotic use in Last 3 Months			
Yes (n=52)	10 (50%)	42 (87.5%)	<0.001
No (n=16)	10 (50%)	6 (12.5%)	

Table-IV: Association of multi drug resistance with abnormalities of urinary tract.

Abnormalities of Urinary Tract	Study Groups		p-value
	Non Multidrug Resistant Uropathogens (n=20)	Multidrug Resistant Uropathogens (n=48)	
No (n=53)	16 (80%)	37 (77%)	
Benign Prostate Hyperplasia (n=12)	4 (20%)	8 (17%)	1.35
Carcinoma Prostate (n=1)	-	1 (2%)	
Indwelling catheter (n=2)	-	2 (4%)	

DISCUSSION

We found that resistance to antimicrobial agents is increasing in community-acquired urinary tract infections. All patients were resistant to at least one antibiotic; multi-drug resistant pathogens were isolated in 71% of patients. Nitrofurantoin and Fos fomycin were the only oral agents with more than 85% sensitivity. Increasing resistance was significantly associated with the use of antibiotics in the last three months and previous history of UTIs.

The commonest organism isolated in our study was E. coli (60%), followed by Klebsiella, proteus and pseudomonas. This is in accordance with the previous studies. Previous literature also shows that E. coli is the most common cause of complicated and uncomplicated UT.[12] E. coli was responsible for community-acquired UTI in 66.6% of the patients in a study conducted by Erdem *et al*, followed by Klebsiella, enterobacter and pseudomona,[11] almost comparable with our findings. A study from the United States also shows that E. Coli (65.1%) was the most common organism isolated from UTI patients; supporting our findings, Enterococcus spp was the second most common organism, and Klebsiella was third in the list.[12] This slight difference may be due to a different study population and environment. E. coli commonly colonizes the periurethral region due to colon contamination and later reaches the bladder. Uropathogenic species of E. coli have many characteristics that help them to colonize the urinary tract. Fimbriae and pili increase the attachment of E. coli to host cells, whereas biofilm increases the adherence to the uroepithelium,[13] making it the most common organism in UTI.

Studies from Pakistan have also shown that E. coli is the common isolate from the patients with UTIs, followed by klebsiella,[14,15] supporting our findings.

We found that 100% of isolates were resistant to at least one antibiotic, which is very alarming. We could not find such findings in previous studies, although numerous studies from different parts of the world have demonstrated the rising trend of resistance among uropathogens.[4] About 71% of uropathogens were multidrug-resistant in our study. Multi-drug resistance among pathogens responsible for UTIs is a common finding in many studies, although the percentage is lower than ours. A study by Shabbir *et al*, showed that 58.9% of isolates were multi-drug resistant.[16] Almost 54% of strains were MDR in a study from Serbia.[17] These findings indicate that multi-drug resistance is increasing with time, which is alarming.

Resistance against commonly used antibiotics in UTIs is on the rise around the globe. A study from New York State showed that resistance to Co-trimoxazole and Quinolones was quite high,[12] although

it was not as high as we observed in our study. Resistance to Ampicillin, Co-trimoxazole, Co-amoxiclav, Cefixime and Quinolones were also very high in different studies from Pakistan,[14] these results are comparable with our findings as resistance to these antibiotics was also very high in our study. This increasing resistance may be due to misuse and overuse of antibiotics as it is more common in the world where antibiotics are available over the counter without prescription,[17,18] another reason is the unregulated use of antibiotics in livestock.[6] Resistance to many second-line injectable antibiotics Ceftriaxone (71%), ceftazidime (46%), cefoperazone/sulbactam (18%) and gentamycin (13%) was also very high in our study. This is very threatening as this limits the choice of IV antibiotics for complicated UTIs. Resistance to Ceftriaxone, Ceftazidime, and Gentamycin was 78%, 45.8% and 31%, respectively, in a study published two years back in Pakistan, almost comparable with our results, but resistance to cefoperazone/sulbactam was only 6%.[19] This contrast is possibly due to changing resistant patterns over time.

Among all oral antibiotics tested for sensitivity in our study, only Nitrofurantoin and Fosfomycin showed more than 85% sensitivity against all uropathogens. This is in accordance with the results from previous studies. A study in Turkey also showed that more than 85% of isolates from community-acquired urinary tract infections were sensitive to Nitrofurantoin and Fosfomycin.[11] Nitrofurantoin sensi-tivity was more than 90% in another study conducted by Rank *et al*, almost the same findings as in our study; however, Fosfomycin was not tested in this study.[12] Studies from Pakistan also show the same results supporting our findings in this regard.[15]

Sensitivity to Imipenem, Meropenem and Piperacillin/Tazobactam was more than 95%. It has also been shown that Carbapenems have very good activity against uropathogens.[18] A study by Biswas *et al*, showed that 98 % of patients were sensitive to Imipenem and meropenem,[20] comparable with our findings. As shown by previous studies, Piperacillin/Tazobactam is also very effective in UTIs. In a study conducted in Pakistan, almost 93% of isolates were sensitive to Piperacillin/Tazobactam supporting our findings.[16] Sensitivity to Amikacin was 87% in our study; other studies conducted in Pakistan earlier have shown almost identical results.[16] A study from Bangladesh showed that 86.4% of isolates were sensitive to amikacin, comparable to our findings.[20]

There was a history of previous UTI (one episode in the last 12 months) in 64.7% of patients in our study. Recurrent UTI was a common finding in different studies, but the percentage in our study was very high. For example, it was 14% in a Canadian study.[21] This difference may be due to increased resistance against commonly used antibiotics and partial treatment of UTIs in our population. Many patients stop antibiotics when they feel asymptomatic without completing the course of antibiotics and do not use the proper dosage advised leading to recurrent infections.[22] Our study found a previous history of UTI as an important risk factor for multi-drug resistance (*p*=0.028). This is in accordance with previous studies. A systematic analysis shows that in more than 60% of studies in which a previous UTI was studied as a risk factor, a significant association was found between multi-drug resistance and previous history of UTI.[23]

A history of antibiotic use in the last three months was found in 76% of participants in our study. This very high percentage indicates the unnecessary use of antibiotics in our population. This is not surprising as over-prescription by doctors and self medications by patients are very common in Pakistan. Furthermore, antibiotics are available over the counter without prescription, so misuse is routine.[24,25] In addition, we found a strong association between multi-drug resistance and the use of antibiotics in the last three months. Many previous studies have the same results, a meta-analysis shows that this was the most commonly identified risk factor for multi-drug resistance in UTIs, the use of antibiotics in the last three months was associated with multidrug resistance in 75 % of studies in that meta-analysis.[23]

We did not find any significant association between age, gender, urinary tract abnormalities and the patient's socioeconomic status and multi-drug resistance in UTIs. Old age was found as a risk factor in some studies,[23] this contrasts with our results. Similarly, the association between gender and drug resistance has conflicting results in different studies. Maximum studies showed no association between the female gender and drug resistance. However, more than 60% of studies showed a significant association between the male gender and drug resistance.[21,15] Many studies found an indwelling catheter as a risk factor for multi-drug resistance. However, we did not find any such association with a catheter or any other abnormality in the urinary tract.[24,25] This contrast may be due to our study design. We did not consider

indwelling catheters as a separate risk factor. Instead, we put it in the group of urinary tract abnormalities.

CONCLUSION

Resistance to antimicrobial agents is increasing in community-acquired urinary tract infections. In addition, resistance is significantly higher in patients having a history of recurrent urinary tract infections and the use of antibiotics in the last three months.

Conflict of Interest: None.

Authors' Contribution

MN: Conception, design, analysis, data collection, drafting, MAZ: Conception, design, data collection, revision, THT: Conception, design, data collection, final approval, SS:, JZ: Conception, design, drafting, MAM: Design, analysis, data collection, AA: design analysis, drafting.

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