International Journal of Community Medicine and Public Health
Farjana NE et al. Int J Community Med Public Health. 2021 May;8(5):2144-2149
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20211730

Original Research Article

Bacterial association in urinary tract infection and their drug resistance among patients in Rajshahi, Bangladesh

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Received: 15 February 2021
Accepted: 16 March 2021

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ABSTRACT

Background: Urinary tract infection (UTI) is a very common infection worldwide and drug resistance makes the treatment more difficult which may lead to complications along with extended hospital stay. Therefore, this study was designed to find out the prevalence of UTI, and antimicrobial susceptibility pattern of isolates collected from patients of UTI.
Methods: This study was conducted among 1,760 suspected UTI patients from January 2019 to November 2020. Urine samples from mid-stream were transferred to microbiology laboratory following microbiological standard protocol. Microbial identification was performed by microscopy, colony morphology, and biochemical characteristics. Antibiogram was pursued following the standard Kirby-Bauer disk diffusion method.
Results: Our study showed almost 30% of samples were positive and *E. coli* was found as the highest predominating organism (50.09%) followed by *Klebsiella* spp., *Pseudomonas* spp., *Enterococcus* spp., and *Citrobacter* spp. The percentage of infection in females was 65.28% which is higher than males. The highest prevalence of UTI has been observed in females aged 21-30 years and males aged 31-40 years. However, *E. coli* and *Pseudomonas* spp. showed the highest resistance against Amoxiclav whereas, *Klebsiella* spp. showed it against co-trimoxazole. *Citrobacter* spp. showed 100% resistance towards Amikacin, cefixime, nalidixic acid, co-trimoxazole, amoxiclav, ceftazidime, and *Enterococcus* spp. showed against nalidixic acid and amoxiclav. Moreover, 95.8% isolates collected were resistant towards 3 or more than 3 antibiotics which is very alarming.
Conclusions: Changing trend in antibiotic sensitivity profile of the isolates needs to be monitored as there is limited availability of newer drugs.

Keywords: Urinary tract infection, Antibiotics, Susceptibility, Kirby-Bauer disk diffusion, Bangladesh

INTRODUCTION

Urinary tract infection (UTI) is an infection in any part of the urinary systems such as kidney, ureters, bladder, and urethra. It can be classified as the upper tract infection or the infection of the kidneys (pyelonephritis) and lower tract infection or the infection of the bladder (cystitis), but the infection of the urethra and prostate are also considered as the lower UTIs. This infection can be asymptomatic, acute, chronic, and complicated, or uncomplicated according to how they occur along the urinary tract.[1]

Over time, UTI has become a severe public health concern worldwide. UTI that is caused by antibiotic-resistant bacteria can make the treatment difficult and can lead to complications.[2,3] According to the most recent global epidemiological data of UTIs, around 150 million people globally suffer from UTI every year. Women in the age range 16-64 are more likely to get UTIs and one in every five women globally suffers from UTI at least one time in

their life. Women are more affected by UTI than men because of their physical structure though men over the age of 60 years with prostatic hypertrophy are at a greater risk.[4] Some factors such as gender, age, race, circumcision, human immunodeficiency virus (HIV), diabetes, urinary catheter, genitourinary tract abnormalities, and pregnancy are the major risk factors for UTIs.[1,5-13]

UTIs can be community-acquired or nosocomial. According to recent data, the prevalence of community-associated UTI (CAUTI) is 17% with major risk factors are age, history of UTI, sexual activity, and diabetes. However, the occurrence of hospital-associated UTI (HAUTI) is 12.9, 19.6, and 24% in the United States, Europe, and developing countries, respectively.[14] Although *E. coli* is the most predominant microorganism in UTI, some other pathogens include *Klebsiella*, *Enterococcus*, *Staphylococcus*, *Pseudomonas*, *Proteus*, *Streptococcus*, *Acinetobacter*, *Citrobacter* and *Candida*.[15-17]

Bangladesh is a densely populated country and most of the people in this country living in a condition where people get affected by various bacterial infections due to lack of knowledge, and unhygienic lifestyle. According to a recent study in Bangladesh, pathogens responsible for UTI acquired resistance to antibiotics. Scientists warn that there could be a higher risk of drug resistance if antibiotics are prescribed without sensitivity or laboratory culture tests. Taking the recent scenario regarding drug resistance into consideration, scientists anticipated that death will rise from 7, 000, 00 (current death) to 10 million people per year by the year 2050 due to antibiotic-resistant bacterial infections.[18] Therefore, our study was designed to detect the prevalence, causative agents, and their antimicrobial susceptibility pattern among UTI patients over a period of 23 months. As drug resistance pattern among antibiotics varies with their selective use in treatment within different localities, here goes the aspiration of our effort to take a depiction of bacterial antibiotic sensitivity in our region.

METHODS

Study area, period, and population

This study was carried out in the microbiology laboratory of Jazeera diagnostic center which is located in Kajihata, Lakhmipur, Rajshahi city, Bangladesh. About 1,760 UTI suspected patients were included to conduct this study and the duration of this study was from January 2019 to November 2020 (a total of 23 months).

Collection of samples

In brief, midstream urine samples were collected in a wide-necked screw-top sterile container, and then, all the containers were labelled with the date, time, and name of the patients. Sample processing was performed within 2 hours of sample collection and in certain cases, they were kept at 4°C for less than 24 hours.

Process of culturing urine samples

To culture, 0.1 ml urine sample was spread on the surface of sterile nutrient agar (NA), and MacConkey agar (MAC) plates (60×15 mm) following standard bacteriological technique. After inoculation, all the plates were incubated aerobically at 37°C for 18-24 hours and following incubation, all the plates were examined in terms of the number of colonies and the colony morphology. The samples were considered positive when the colony number was $\geq 10^5$ colony forming unit/ml. Colony characteristics of those isolates were marked and recorded.

Characterization of isolates

To obtain pure culture, colonies were picked up from agar media and sub-cultured. Gram staining and biochemical tests were also performed to identify the isolates as per the standard protocol followed in the microbiology laboratory.[19] Antibiotic susceptibility tests were performed following the identification of the pure culture isolates.

Antibiotic susceptibility test

The modified Kirby-Bauer disc diffusion method was followed to observe the sensitivity pattern of the isolates. In brief, isolated colonies were spread over Muller-Hinton Agar media, and then, paper discs of antibiotics were placed on top of it. Following incubation at 37°C for 24 hours, the zone of inhibitions (ZOIs) were measured around the colonies to detect their susceptibility or resistance by comparing with the clinical and laboratory standard institute (CLSI) guideline.[20] However, antibiograms were performed against a total of 14 antibiotics namely amikacin (30 mcg), cefixime (5 mcg), imipenem (10 mcg), meropenem (10 mcg), nalidixic acid (50 mcg), nitrofurantoin (300 mcg), gentamicin (10 mcg), co-trimoxazole (25 mcg), amoxiclav (25 mcg), cefuroxime (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), ciprofloxacin (5 mcg), and levofloxacin (5 mcg).

RESULTS

Distribution of positive and negative samples

Figure 1A shows the number of positive and negative samples from January 2019 to November 2020. Among 846 suspected patients, a total of 242 infected and 604 uninfected patients were found in the year 2019. Moreover, the number of infected and uninfected patients in the year 2020 remained 285 and 629 out of 914 total assumed patients, respectively.

However, there was no significant consistency observed in the increase or decrease of infected patients' number month-wise among 23 months study period. The highest number of infected patients was found in August 2019 (30) and in July 2020 (45). Conversely, the lowest number of infected patients was received in April 2019 (13) and January 2020 (18).

In our study, it was observed that almost 70% of samples were found as negative (1233 samples) and 30% of samples were positive (527 samples) throughout the study period (Figure 1B).

Age- and gender-wise distribution of positive samples

The highest infection was observed in the age group 31-40 and 21-30 for males and females, respectively (Figure 2A). However, the incidence of infection slowly declined in both males and females following the age range. Our data demonstrated that among all the positive samples, 65.28% (344) were female and 34.72% (183) were male patients (Figure 2B).

Distribution of microorganisms among the positive samples

Our data revealed that *E. coli* was responsible for most of the urinary tract infections. As the most predominant organism, *E. coli* was approximately 50.09% followed by *Klebsiella* spp. (30.55%), *Pseudomonas* spp. (18.02%), *Enterococcus* spp. (0.379%), and *Citrobacter* spp. (0.948%) (Table 1).

During our study period, we did not observe any multiple positive urine cultures from any patient.

Antibiotic sensitivity test

A degree of sensitivity/resistance was observed against *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. The lowest sensitivity was observed against Amoxiclav by both *E. coli* (15.91%) and *Pseudomonas* spp. (16.84%), where *Klebsiella* spp. showed the lowest 25.47% sensitivity against co-trimoxazole. However, 0% sensitivity was observed against nalidixic acid and amoxiclav by *Enterococcus* spp.; and against amikacin, cefixime, nalidixic acid, co-trimoxazole, amoxiclav, and ceftazidime by *Citrobacter* spp. Except for *Pseudomonas* spp. that showed the highest sensitivity against meropenem, all the other isolates showed the highest sensitivity against nitrofurantoin. However, *Enterococcus* spp. and *Citrobacter* spp. showed 100% sensitivity against the three antibiotics namely imipenem, meropenem, nitrofurantoin. Overall, the highest resistance was observed against amoxiclav and the highest sensitivity was detected against nitrofurantoin (Table 2). Nevertheless, our data revealed a scary scenario where only 5 bacterial isolates showed resistance to only one antibiotic, 17 isolates showed resistance to two antibiotics, 305 isolates showed resistance to 3 antibiotics and 200 bacterial isolates showed resistance to 4 or more than 4 antibiotics (Figure 3).

Table 1: Distribution of microorganisms isolated from urine samples.

Bacterial isolates	Number	%
Gram-negative bacteria		
E. coli	264	50.09
Klebsiella spp.	161	30.55
Pseudomonas spp.	95	18.02
Citrobacter spp.	2	0.948
Gram-positive bacteria		
Enterococcus spp.	5	0.379
Total	527	100

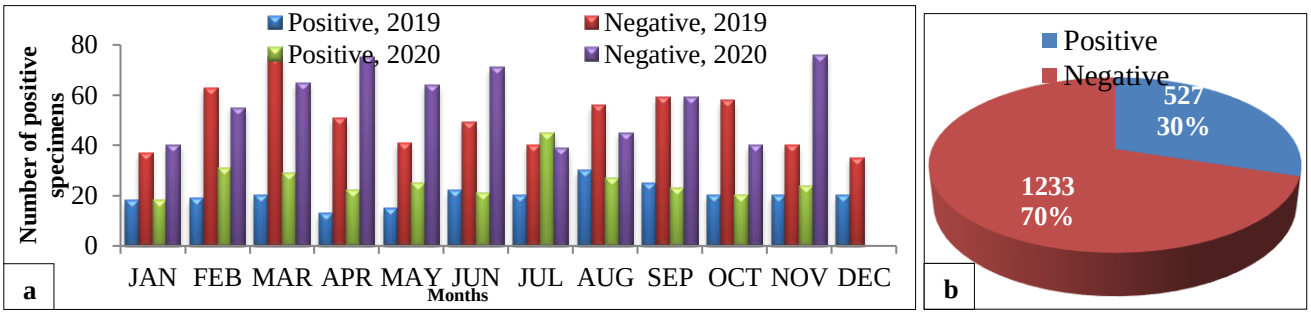

Figure 1: (a) Frequency distribution of positive and negative samples in every month of the study period, and (b) distribution of the total positive and negative urine samples.

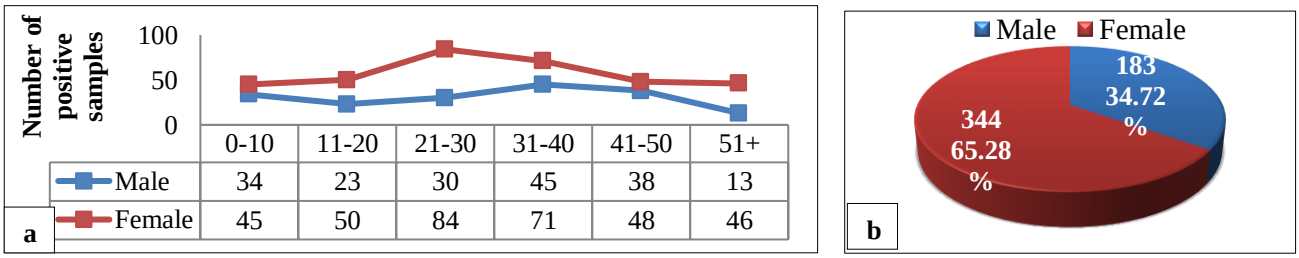

Figure 2: (a): Age-wise distribution of male and female patients in the study period, and (b) percentage of total male and female patients throughout the study period.

Table 2: Antibiotic sensitivity of bacterial isolates against a pool of antibiotics.

Isolates	No. of isolat-es	Amikacin	Cefixime	Imipenem	Meropenem	Nalidixic acid	Nitrofurantion	Gentamicin	Co-trimoxazole	Amoxiclav	Cefuroxime	Ceftriaxone	Ceftazidime	Ciprofloxacin	Levofloxacin
E. coli	264	55.68	39.02	86.74	81.44	26.89	95.45	25.38	28.41	15.91	59.09	73.11	35.98	25.76	51.52
***Klebsiella* spp.**	161	33.54	33.54	65.43	91.30	52.80	95.03	35.40	25.47	27.95	42.24	67.70	34.16	38.51	54.66
***Pseudomo-nas* spp.**	95	22.11	34.74	97.89	98.95	26.32	89.47	44.21	28.42	16.84	30.53	62.11	24.21	28.42	45.26
***Enterococcus* spp.**	5	40	60	100	100	0	100	40	20	0	60	80	20	40	60
***Citrobacte-r* spp.**	2	0	0	100	100	0	100	50	0	0	50	66.67	0	50	50

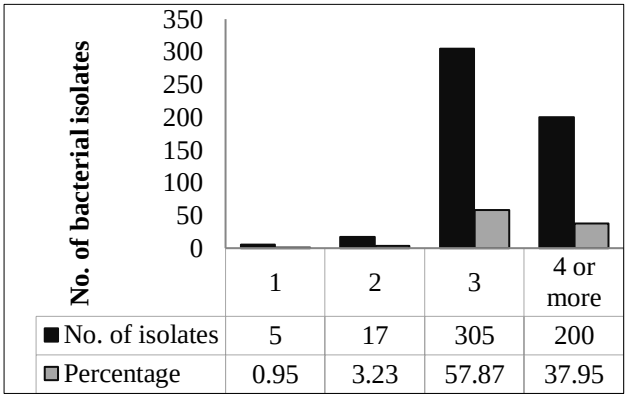

Figure 3: Prevalence of antibiotics resistant bacterial infection in UTI.

DISCUSSION

Urinary tract infection has reached a critical stage where people in the USA had to expense about 3.5 billion dollars per year on treatment but the scenario is even worse in developing countries like Bangladesh. In the USA, about 2.8 million bacterial infections have acquired antibiotic resistance per year.[21] Drug resistance has made this infection more complicated. According to some recent research, most of the etiological agents of UTI are becoming resistant to several antibiotics such as Ampicillin which was largely used against UTI.[22] Our study aimed to evaluate the predominating microorganisms present in UTI infection and antibiotic susceptibility pattern of the isolated bacteria. To conduct this study, a total of 1760 suspected patients' samples were tested where 70% of samples did not show any incidence of UTI.

Gram-negative organisms that in general reside in the gut are more responsible for UTI and in most cases, *E. coli* is accountable for 80-90% of primary infection.[23] Our study highlighted *E. coli* as the highest predominant organism with 50.09% prevalence which is comparable to other studies where 40-46% of UTI infections were caused by *E. coli*.[24-27] In our study, *Klebsiella* spp. was found as the second highest predominating organism (30.55%) followed by *Pseudomonas* spp. (18.02%), *Enterococcus* spp. (0.379%), and *Citrobacter* spp. (0.948%).

Out of suspected patients of UTI, 30% were found infected where 70% did not show any significant infection which very closely correlates with a previous study where 31% samples were found positive for UTI.[28] Nonetheless, the highest rate of infection was observed among the females (65.28%) aged 21-40. A higher incidence of UTI infection in females than males was very commonly observed in the earlier investigations and the prevalence is due to their physical structure where the anus is close to the vagina in females in association with the vaginal colonization with uropathogens, sexual activity, pregnancy and obstruction.[28,29-34] Conversely, in men, the reasons behind UTI may include insertive anal intercourse or lack of circumcision or having sexual partner with vaginal colonization with uropathogenic microorganisms or lack of immunity.[35, 36]

In our study, we observed a wide extent of resistance of uropathogens against several antibiotics which is a matter of great concern. The most predominating *E. coli* showed 95.45% sensitivity to Nitrofurantoin and 84.09% resistance to Amoxiclav. Moreover, most of the uropathogens of this study including *E. coli*, *Pseudomonas* spp., *Enterococcus* spp., and *Citrobacter* spp. showed the highest resistance to amoxiclav which is being used as a first-line treatment for lower UTIs. Our result also revealed that 95.8% isolates were resistant to 3 or more than 3 antibiotics which we may call multi-drug resistant (MDR) which is comparable with an earlier report.[37] A recent research in 2019 claimed that Bangladesh, along with some of its neighboring countries, is responsibly harboring drug-resistant microbes that might play a role in disseminating them throughout the world by humans, animals,

agricultural products, and water. This research addresses significant gaps in surveillance, susceptibility testing methods, their interpretation that is creating doubt in methodologies and therefore, making it difficult to compare with other studies.[38]

CONCLUSION

Antibiotic resistance can be reduced by conducting such antibiotic surveillance studies, creating awareness in public and by concerted efforts between doctors, scientists, students, pharmaceutical industries, and policymakers. Changing trends in the antibiotic sensitivity profiles of the isolates need to be monitored as there is limited availability of newer drugs and the emergence of resistant bacteria far exceeds the rate of new drug development.

ACKNOWLEDGEMENTS

The authors hereby express their deep gratitude to the Jazeera diagnostic center, Kajihata, Lakhmipur, Rajshahi, Bangladesh for the technical supports provided throughout the study period.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Farjana NE, Islam MA, Zerin T, Begum MA. Bacterial association in urinary tract infection and their drug resistance among patients in Rajshahi, Bangladesh. Int J Community Med Public Health 2021;8:2144-9.