Khwaja Yunus Ali University Journal

Publisher homepage: www.kyau.edu.bd

OPEN ACCESS

ISSN: 2791-3759 (Online), 2521-3121 (Print)

Journal homepage:www.journal.kyau.edu.bd

Research Article

Bacteremic Urinary Tract Infection and *in vitro* Antimicrobial Responsiveness Patterns at AMZ Hospital Ltd., Dhaka.

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ABSTRACT

The objective of the cross-sectional study was to culture urine samples from patients with UTIs and The cross-sectional study aimed to identify the most common bacterial pathogens that cause urinary tract infections and the drug susceptibility patterns of those isolates. A 671 of fresh morning midstream urine samples were examined microscopically and cultured on Chromogenic UTI agar, Blood agar and MacConkey agar to isolate the pathogens. Isolated pathogens were identified through colony morphology, microscopic studies, and biochemical indications. Finally, antimicrobial susceptibility patterns were determined by Kirby-Bauer Disc Diffusion method according to the Clinical and Laboratory Standard Institute (CLSI, 2018). Ninety-seven urine samples out of 671 samples yielded a positive culture. Most of the bacteria among all isolates were Escherichia coli and Klebsiella pneumoniae, which accounted for 62.9% and 25.7% respectively and others 11.4% of urinary pathogens namely Pseudomonas spp. (5.2%), Enterococcus spp. (4.1%), and Proteus spp. (2.1%). Female patients were more likely to be isolated (72.16%) than male patients (27.84%). The fourth generation Carbapenems antibiotics of Imipenem and Meropenem were more effective to in all bacterial pathogens. The β-lactam antibiotic of Amoxycillin and cephalosporin antibiotic of Cefixime were showed highly resistant to both of Gram positive and Gram negative uropathogens. Complicated urinary tract infections and antimicrobial resistance pose a significant threat to treating this infection. Therefore, using antimicrobial agents properly and developing public awareness can reduce the issue of antimicrobial resistance.

Keywords: Antimicrobial susceptibility, Urinary tract infection, Bacterial Pathogens Antimicrobial agents.

1. Introduction

Urinary tract infection (UTI) means microbial invasion and inflammation of the urinary tract. After statistical analysis, according to estimates, about 150 million people worldwide suffer from UTIs each year (Sharmin

et al.,2022). In developed countries such as the USA and developing nations like Bangladesh, UTIs continue to be one of the leading causes of mortality and morbidity (Abedin *et al*.,2020). *Escherichia coli* are the most common bacteriological cause of urinary tract infection among urinary tract pathogens (Nakhjavani *et al*.,2007). *K.*
pneumoniae, *Pseudomonas* spp., *Proteus* spp., and other Gram-negative pathogens that cause urinary tract infections. However, *Enterococcus* spp. and coagulase-negative Staphylococci are the most common gram-positive bacteria that cause UTIs (Goering *et al*.,2008).

Anaerobic organisms are rarely present in the urinary tract. 35% of healthy women suffer from signs and symptoms of a urinary tract infection at some point. UTIs can be asymptomatic in many cases, while up to 90% of the patients with UTIs have urinary tract symptoms such as burning while urinating, fever, itching, the formation of blisters and ulcers in the genital area, lower abdominal pain (LAP), genital and suprapubic pain, and so on. Early diagnosis and adequate treatment are vitally important to avoid chronic illness and long-term renal damage. Most UTIs are preceded by the association of sexual activity with colonization and vaginal acquisition, pregnancy, and obstruction, which are responsible for increasing the frequency of UTIs in females. Though anyone is susceptible to UTIs, the prevalence of infection typically varies with age, gender, and certain predisposing factors such as diabetes, pregnancy, and impaired bladder voiding (Abedin *et al*.,2020).

The purpose of this study was to provide information on bacteria isolated from the urine of suspected UTI patients in Bangladesh, as well as their sensitivity and resistance patterns to locally available antibacterial agents commonly prescribed by doctors, in order to identify appropriate antimicrobial agents to treat UTI.

2. Materials and Methods

Ethical concern:
The Research Ethics Board of AMZ Hospital Ltd., Dhaka, Bangladesh, has approved the protocol.

Population and study area

The present study was conducted at AMZ Hospital Ltd. in Dhaka, Bangladesh, from September 2022 to December 2022. The 671 consecutively obtained urine samples from midstream and catheter came from individuals of all ages and sexes who were clinically highly suspected of having UTIs. The patients' age, gender, and other criteria have been taken from the authorities of the study's participants.

Sample collection and processing

Following the CLSI (2018), clean, sterile, 50-ml screw-capped containers were used to catch urine samples. Moreover, they were transported to the laboratory immediately in ice-pack bags for analysis. To avoid false-positive cultures or bacterial overgrowth if sample processing was postponed for longer than two hours, buffered boric acid (2000 mg) was included in the samples (Sharmin *et al*.,2022).

Media and chemicals

To isolate and identify the microorganisms causing urinary tract infections, blood agar, MacConkey agar, and chromogenic UTI agar media were used. Muller-Hinton agar (MHA) was then used to test the selected urine cultures for antibiotic susceptibility. H_2O_2 (for the catalase test), tetramethyl-p-phenylenediamine (for oxidase), Kovac's reagents (for the indole test), Triple Sugar Iron (TSI), and Simmons Citrate Agar were used for biochemical analysis.

Preparation of media

All agar media were purchased from Oxoid in the UK as a dehydrated powder. Following the guidelines and recommendations of the manufacturer, culture Petriplate were made on –site.

Isolation and identification of uropathogens
Bacterial identification was accomplished through phenotypic examination of culture media-specific uropathogenic organisms. One loop of urine samples was used to inoculate chromogenic UTI agar, MacConkey agar, and blood agar media, which were then incubated aerobically for 24 hours at 37o C. The number of bacterial colonies was counted following incubation.

Criteria for significant bacterial numbers (Cheeseborough, 2007**).**

Colony-forming units (CFU), or the number of bacteria per milliliter of urine, were calculated. Reports on the bacterial counts are as follows;

- Insignificant: $<10^4$/mL
- Possibly significant: 10^4-10^5/mL
- Significant bacteria: 10^5/ml

Antimicrobial susceptibility test

Antimicrobial susceptibility testing (AST) is performed using Muller-Hinton agar following the Kirby-Bauer disc diffusion technique (Bauer,. 1966 & CLSI,2018) using a panel of 14 antibiotics (Oxoid, UK), including Amikacin (30 mcg), Amoxycillin (30 mcg), Cefixime (30 mcg), Ceftriaxone (30 mcg), Co-trimoxazole (25 mcg), Gentamicin (10 mcg), Imipenem (10mcg), Meropenem (10 mcg), Netilmicin (30 mcg), Nitrofurantoin (300 mcg), Polymixin-B (300iu), and Tazobactum (110 mcg). Depending on the size of the zone of inhibition, susceptibility was classified as being sensitive, resistant, or intermediate (CLSI, 2018).

3. Data analysis

Excel 2016 and SPSS version 20 were used to analyze the data. Descriptive statistics and chi-square tests were done to check the statistical evaluation. "< 0.5" was the significant value of the p-value considered in this study.

4. Results

In this study, a total of 671 patients from different age and sex groups were included. Among all cases, 97 (14.5%) urine samples yielded significant positive bacterial isolates, while the remaining 574 (85.5%) urine samples yielded no growth of any pathogenic isolates. The bacterial isolates were both Gram positive and Gram-negative and isolated by conventional microbiological techniques as tabulated 1.

Table 1: Distribution of bacterial isolates from suspected patients

SN.	Bacterial isolates	Total Number	Frequency (%)	Gram reaction
01.	*Escherichia coli*	61	62.9	Gram negative bacilli (95.9%)
02.	*Klebsiella pneumoniae*	25	25.7	
03.	*Pseudomonas* spp.	5	5.2	
04	*Proteus* spp.	2	2.1	
05.	*Enterococcus* spp.	4	4.1	Gram positive cocci (4.1%)
	Total	97	100	

Table 2: Gender breakdown of urine culture positive cases

Gender	Number	Frequency (%)
Male	27	27.8%
Female	70	72.2%
Total	97	100%

Table 3: Age based distribution of cases of urinary tract infections.

Age (Years)	No.	Frequency (%)
0-12	8	8.25
13-24	3	3.09
25-36	16	16.49
37-48	13	13.40
>49	57	58.77
	97	**100.00**

The antimicrobial sensitivity for fourteen selected antimicrobial agents against the five most frequent UTI pathogens is listed in **Table 4**.

Table 4: The antimicrobial sensitivity patterns of five most frequent UTI pathogens

Antimicrobial agents	*E. coli*	*Klebsiella pneumoniae*	*Pseudomonas* spp.	*Enterococcus* spp.	*Proteus* spp.
Amikacin	85.2%	92%	80%	100%	100%
Amoxycillin	13.1%	0%	0%	0%	0%
Cefixime	36.1%	60%	60%	100%	100%
Ceftrixone	50.8%	48%	80%	75%	100%
Ciprofloxacin	67.2%	60%	100%	100%	0%
Co-trimoxazole	62.3%	64%	60%	75%	100%
Gentamycin	73.8%	80%	60%	100%	50%
Imipenem	91.8%	100%	100%	100%	100%
Levofloxacin	60.7%	64%	60%	75%	0%
Meropenem	83.6%	96%	100%	75%	100%
Netilmicin	83.6%	92%	80%	100%	100%
Nitrofurantoin	72.1%	84%	80%	75%	100%
Polymixin-B	47.5%	48%	20%	0%	50%
Tazobactam	85.2%	92%	60%	100%	100%

5. Discussion

Due to the current emergency of multi-drug-resistant uropathogens, urinary tract infection is among the most prevalent public infection rates worldwide including Bangladesh and only some effective alternative antibiotics are available, treating UTIs has become complicated and unpredictable. Doctors and microbiologists must work closely to combat the multi-drug-resistant bug because local variation is also seen among uropathogens (Abedin *et al*.,2020). This study also included an analysis of the uropathogens that cause uncomplicated UTIs and their antimicrobial sensitivity patterns.

A total of 671 samples of various ages and sexes were collected from suspected UTI patients and only 97 (14.5%) showed a positive result for UTIs. All bacterial isolates were single pure growths, and no polymicrobial growths showed partial similarity with some reports from Pakistan, Bangladesh, and India (Rani *et al*., 2012). Among the positive cases, 27 (27.8%) were male, and 70 (72.2%) were female. It is understandable why women are more likely to develop UTIs than men, and our analysis's findings concur with those of a recent study by Deshpande *et al*., (2011). Our evaluation agrees with a few prior reports with Keah *et al*.,(2007) regarding the prevalence of uropathogenic.

According to Tables 3, there were 8 (5.25%) cases in people aged 0–12 years old, 3 (3.09%) cases in people aged 13–24 years old, 16 (16.49%) cases in people aged 25–36 years old, 13 (13.40%) cases in people aged 37–48 years old, and 57 (57.77%) cases in people aged > 49

years old. Another UTI study conducted in Bangladesh discovered that the age groups of 21–30 years old (44% of cases) and 41–50 years old (37% of cases) accounted for the majority of UTI cases (Rani *et al.*, 2012).

In this study, the Gram-negative bacilli contributed to 95.9% of the total isolates, while the Gram-positive cocci constituted 4.1%. In our study, *E.coli* was the most commonly detected species. There were 61 (62.9%) out of 61 positive cases of *E. coli*. The second-most isolates of *Klebsiella pneumonia*e (25.8%) were followed by *Pseudomonas* sp. (5.2%), *Enterococcus sp.* (4.1%), and *Proteus* sp. (2.1%). This result is consistent with reports from other studies by Abedin *et al*. (2020M, 2021, and Sanjee *et al.*, 2017), and it was the most frequent pathogen causing UTI in all these studies.

Antimicrobial sensitivity of uropathogens is expected to differ among countries and over time. Imipenem was found to have the highest sensitivity to *E. coli* (91.8%), followed by Amikacin and Tazobactum, which were (85.2%) and 85.2%, respectively; Netilmicin and Imipenem, which were 83.6% and 83.6%; and Gentamycin and Nitrofurantoin, which were 73.8%.Imipenem (100%) was the most effective antibiotic against *Klebsiella pneumoniae*, followed by Meropenem (96%), Amikacin (92%), Netilmicin (92%), and Tazobactum (92%) (Table 4).

Ciprofloxacin (100%), Meropenem (100%), Imipenem (100%), Amikacin (80%), Netilmicin (80%), and Nitrofurantoin (80%) showed the highest susceptibilities against *Pseudomonas* spp. and *Enterococcus* spp., followed by Amikacin (100%), Cefixime (100%), Ciprofloxacin (100%), Gentamycin (100%), Imipenem (100%), and Netilmicin (100% and 100%). Regarding *Proteus* spp., they were sensitive to Amikacin (100%), Cefixime (100%), Ceftriaxone (100%), Co-trimoxazole (100%), Imipenem (100%), Meropenem (100%), Netilmicin (100%), Nitrofurantoin (100%), and Tazobactum (100%) (Table 4). These highest sensitivities are similar with Abedin *et al*., (2020,2021), Parveen *et al*., (2018) & Khondakar *et al*. (2016).

In this investigation, it was discovered that all of these antibiotics had a medium level satisfactory spectrum of antimicrobial activity against uropathogenic bacteria, which is concerning in terms of choosing an appropriate medication for treating UTIs.

7. Conclusion

E.coli was found to be the most common isolate. The commonest isolated among Gram negative bacilli (GNB) were *K. pneumoniae*, *Pseudomonas sp.*, *Enterococcus sp.*, and *Proteus* sp and found to be 100% resistant. Imipenem and Meropenem were the highly susceptible antibiotics to treat the UTIs. The nature of microbial UTI infections should be taken into consideration in empirical antimicrobial therapy of UTI patients. Indiscriminate and overuse of broad-spectrum antibiotics predisposes hospitalized due to the multi drug resistant virulent micro flora. In conclusion, present observations seem to be helpful in providing useful guidelines for choosing effective therapy against isolates from UTI patients.

8. Acknowledgement

We, authors and co-authors, are very grateful to all the patients of AMZ Hospital Ltd. from whom the clinical isolates were obtained.

9. Authors Contributions

Research concept and Research design - Mohammad Zakerin Abedin, Materials & Data collection –Md. Nazmul Huda, Data analysis and Interpretation- Mohammad Zakerin Abedin & Md. Nazmul Huda , Literature search & Writing article- Md. Nazmul Huda, Critical review & Article editing-Mohammad Zakerin Abedin, Final approval- All authors .

10. Funding

This study did not receive any specific grants from public, commercial, or non-profit funding bodies.

11. Disclosure

The authors declare that they have no conflicts of interest in this study.

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Citation: Abedin, M.Z., & Huda, M.N.(2023). Bacteremic Urinary Tract Infection and *in vitro* Antimicrobial Responsiveness Patterns at AMZ Hospital Ltd., Dhaka. *Khwaja Yunus Ali Uni.J*, 5(2),1-6