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RESEARCH ARTICLE OPEN ACCESS

Isolation and Identification of Multi-Drug Resistant (MDR) Pathogens from Patients with Urinary Tract Infection (UTI) in Dhaka City, Bangladesh

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Abstract

Urinary tract infection (UTI) is the leading cause of death among patients and majority of people visiting the hospital worldwide. In the current quantitative study, urine samples were obtained using sterile method and 100 isolates, including 60 *Escherichia coli*, 28 *Klebsiella* sp., and 12 *Proteus* sp., were investigated in a laboratory. Current study showed that *E. coli* 60/100 (60%) was the most common cause of UTI followed by *Klebsiella* sp. 28/100 (28%) and *Proteus* sp. 12/100 (12%). Cefotaxime (86.67% sensitive) was found to be the best drug for treating infections with *E. coli*, ceftazidime (100% sensitive) for infections with *Klebsiella* sp., and imipenem and levofloxacin (100% sensitive) for infections with *Proteus* sp. Overall, 51% of the isolated strains showed high multidrug resistance (MDR). Because of the alarming increase in extensively drug-resistant and MDR uropathogens, which is a concern in public health, the rational use of antimicrobial therapy should be implemented.

Keywords: UTI, MDR, Uropathogenic, *E.coli*, Dhaka

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INTRODUCTION

Increasing antimicrobial resistant (AMR) bacteria are becoming a significant threat to public health. In recent years, multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria have been explored.[1-3] MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, while XDR exhibits non-susceptibility to at least one agent in two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories).

Urinary tract infection (UTI) is becoming complex due to the above-mentioned resistant bacteria. UTI is a common infection in Bangladesh as well as in other countries of the world, caused by colonization of pathogenic and opportunistic microbes.[4,5] In Bangladesh and other developing countries, high increase in patients with UTI is found due to poor hygiene, catheterization, intercourse, pregnancy etc.[5,6] Although antibiotics are used for preventing UTIs, several studies indicated that many uropathogens are becoming resistant to a wide range of antibiotics due to underuse, abuse, and overuse.[7]

Escherichia coli is a facultative anaerobe belonging to *Enterobacteriaceae* family, and uropathogenic *E. coli* (UPEC) strains form colonies in the large intestines of warm-blooded animals, including humans. UPEC strains have been identified to cause majority of community acquired UTIs and the causal agent in 90% of all UTI cases in ambulatory individuals.[8]

Klebsiella sp. is an emerging persistent bacteria that cause UTI, posing public health problems worldwide. In recent years, there is increasing prevalence in some antibiotic resistant bacteria, especially members of *Klebsiella* genus.[9-11]

Proteus sp. also belongs to *Enterobacteriaceae* family and is distinguishable from other genera by their swarming growth across agar surface. They are widely found in the environment playing a role in decomposing organic matter. *Proteus* sp. are often associated with UTIs in individuals with structural or functional abnormalities, including patients undergoing catheterization.[12-14]

Due to increasing MDR uropathogens, this study aimed to isolate, characterize, and determine the prevalence of MDR *E. coli*, *Klebsiella* sp., and *Proteus* sp. in the urine sample of patients with UTI.

MATERIALS AND METHODS

Sample Collection

Four hundred specimens were collected in a tertiary care hospital. Using a sterile method, clean midstream urine samples were collected in glass containers. Samples were then transported in an icebox to a microbiology laboratory while maintaining the physio-chemical parameters.[15]

Demographic Data of Patients

To maintain homogeneity of variance in the study, equal number of positive samples were included in each age and gender group (Table 1). Young females and males from the age group 15–30 years old and older individuals from 30 years of age and above participated in the study. Underlying diseases were not observed in the participants.

Isolation of Uropathogens

Using calibrated sterile inoculating loops, 0.01 mL of each urine sample was plated on Mac Conkey agar, blood agar, cystine–lactose–electrolyte deficient (CLED) agar, and HIChrome agar media plates for detecting *E. coli* and *Klebsiella* sp. and on xylose lysine deoxycholate (XLD) agar for detecting *Proteus* sp. Inoculated petri plates were then incubated at 37 °C for 24–48 h. The number of colonies (CFU/mL) was counted for UTI diagnosis. Samples showing >10^5CFU/mL after incubation were positive indicator for UTI. Isolated colonies were identified based on biochemical tests.[15,16]

Table 1. Demographic data of patients

Age and Gender	Frequency
Young Female(15-30 years)	25
Older Female (>30years)	25
Young Male(15-30 years)	25
Older Male (>30years)	25

Antimicrobial Susceptibility

Antibacterial susceptibility testing was performed using Kirby–Bauer disk diffusion method against different antibiotics, including amoxycillin (AMC), amikacin (AMK), azithromycin (AZM), cefixime (CFX), ciprofloxacin (CIP), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (CEFE), cefotaxime (CEFO), gentamycin (GEN), nitrofurantoin (NIT), levofloxacin (LEVO), imipenem (IMI), and sulfamethoxazole (SXT).[14-17]

RESULTS AND DISCUSSION

The misuse and abuse of antibiotics are the major cause of drug resistance, while some pathogens develop innate resistance by environmental adaptation. Resistance develops also due to selective pressure of antibiotics.[18,19]

This study aimed to investigate the prevalence of AMR uropathogens isolated from patients with UTI.

A total of 60 *E. coli*, 28 *Klebsiella* sp., and 12 *Proteus* sp. were identified to observe the antibiotic susceptibility having significant growth (Table 2). Previous studies demonstrated high abundance of *E. coli* ranging from 53.7%[20] to 61.45%[21,22] among uropathogenic isolates.

We observed that 13.33% and 60% of the *E. coli* strains were resistant to CEFO and NIT, respectively (Figure 1), *Klebsiella* sp. strains showed no resistance to CAZ, while 64.52% were resistant to AMK (Figure 1); *Proteus* sp. strains showed susceptibility to IMI and LEVO, whereas 100% resistance to AZM (Figure 1 and Table 3).

CEFO (86.67% sensitive) was found to be the most effective drug for treating infections with

Table 2. Prevalence of organism in each patient group

Organism	Young Female	Elder Female	Young male	Elder Male	Total
E. coli	15/25(60%)	15/25(60%)	15/25(60%)	15/25(60%)	60/100(60%)
Klebsiella sp.	7/25(28%)	7/25(28%)	7/25(28%)	7/25(28%)	28/100(28%)
Proteus sp.	3/25(12%)	3/25(12%)	3/25(12%)	3/25(12%)	12/100(12%)

Table 3. Antibiotic resistance of bacterial Isolates

Organism	AMC	AMK	AZM	CFX	CIP	CAZ	CRO	CEFE	CEFO	GEN	NIT	LEVO	IMI	SXT
E. coli(%)	50	35	28.33	40	38.33	28.33	16.66	48.33	13.33	43.33	60	40	18.33	50
Klebsiella(%)	23.57	64.52	46.43	25.00	42.86	0	23.33	37.62	57.14	25.00	23.33	20.48	28.57	32.14
Proteus(%)	16.66	83.33	100	75	83.33	75	50	50	41.66	66.67	16.67	0	0	33.33

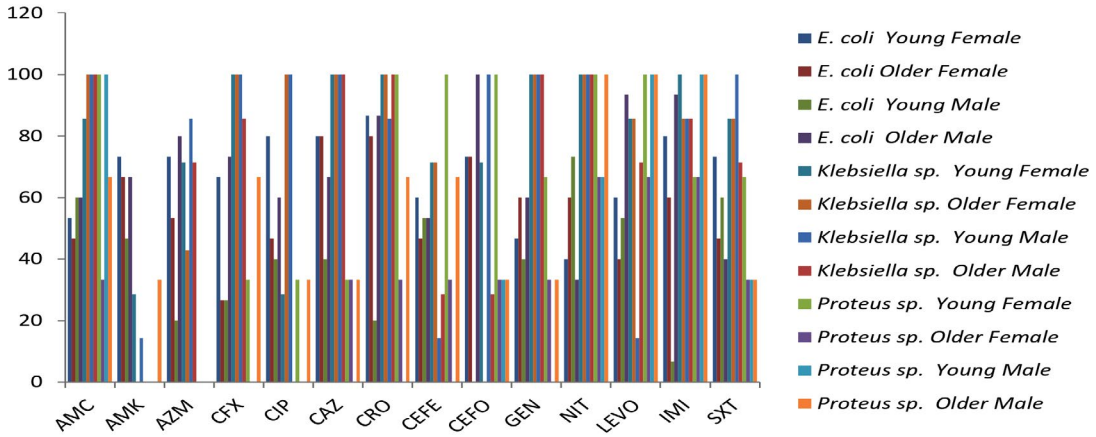

Figure 1. Antibiotic Sensitivity of *E. coli, Proteus* sp. & *Klebsiella* sp. in different age and sex groups.

E. coli, CAZ (100% sensitive) against *Klebsiella* sp., and IMI and LEVO (100% sensitive) against *Proteus* sp. Previous studies found 91.3% sensitivity to meropenem (Figure 2).[22] Akter et al. showed that carbapenems, aminoglycoside, and piperacillin-tazobactam were the most effective drugs against the *E. coli* strains; however, progression of drug resistance in these isolates was observed within 4 years.[6]

Fifteen (60%) MDR bacteria were found in the samples of older males, 13 (52%) in the samples of young males, eight (32%) in the samples of young females, and 15 (60%) in the samples of older females.

Among the *E. coli* isolates from older female samples, four isolates were resistant to five drugs, including AMC, AZM, CFX, CIP, and NIT; eight isolates were resistant to at least six drugs, including AMC, AZM, CRO, CEFO, GEN, IMI, CEFE, AMK, NIT, CFX, CAZ, LEVO, and SXT; ten isolates were resistant to seven drugs, including CAZ, CEFE, AMK, AZM, GEN, LEVO, SXT, AMC, CFX, CIP, CEFO, IMI, CRO, and NIT; and eleven isolates were resistant to eight or more drugs, including AMK, CAZ, SXT, AMC, CEFE, GEN, NIT, CIP, CRO, CEFO, LEVO, and IMI.

Among *E. coli* isolates from young female samples, four isolates were resistant to five drugs, including AMC, AZM, CFX, CIP, CRO, CEFO, NIT, and LEVO; at least six isolates showed resistance to six drugs, including AMC, AMK, AZM, CIP, CAZ, CRO, CEFE, CEFO, NIT, LEVO, IMI, and SXT; four isolates

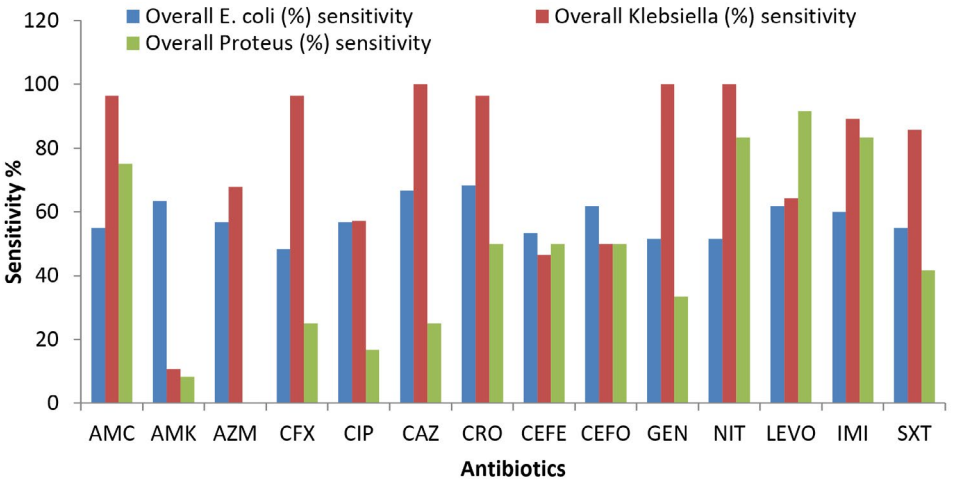

Figure 2. Overall Sensitivity (%) of organisms to each antibiotics.

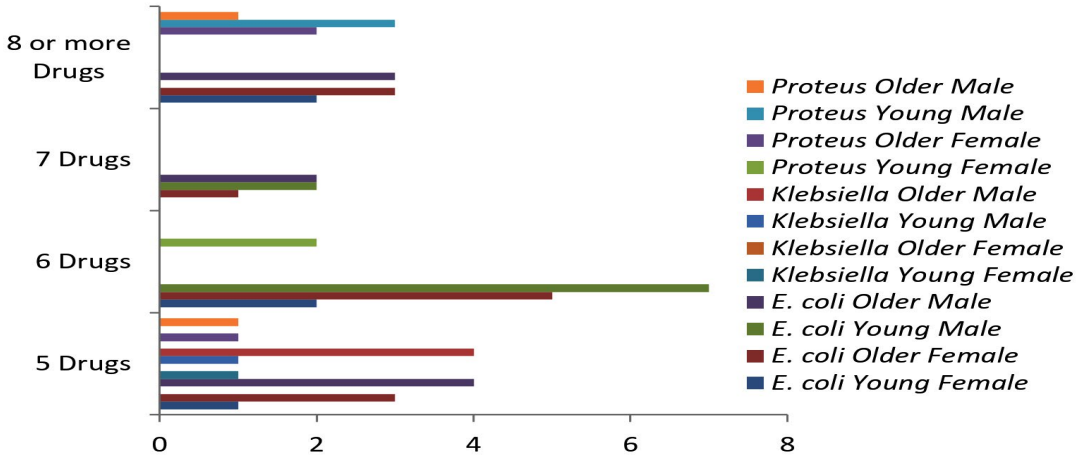

Figure 3. Multi-drug resistance pattern of *E.coli, Klebsiella* sp. & *Proteus* sp.

Table 4. MDR prevalence among different groups of patients

Organism	Patient groups	5 Drugs	6 Drugs	7 Drugs	8 or more drugs
E. coli	Young Female	1	2	0	2
	Older Female	3	5	1	3
	Young Male	0	7	2	0
	Older Male	4	0	2	3
Klebsiella sp.	Young Female	1	0	0	0
	Older Female	0	0	0	0
	Young Male	1	0	0	0
	Older Male	4	0	0	0
Proteus sp.	Young Female	0	2	0	0
	Older Female	1	0	0	2
	Young Male	0	0	0	3
	Older Male	1	0	0	1

were resistant to seven drugs in all antibiotics except CFX and GEN; and thirteen isolates were resistant to eight or more antibiotics.

Among *E. coli* isolates from young male samples, two isolates were resistant to five drugs, including AMC, AMK, AZM, CFX, CRO, NIT, LEVO, and SXT, 10 isolates were resistant to at least six drugs except CAZ; eight isolates were resistant to seven drugs, including AMC, AMK, CIP, CEFE, CEFO, NIT, IMI, and SXT; and two isolates were resistant to eight or more drugs.

Among *E. coli* isolates in older male samples, one isolate was resistant to five drugs, including CFX, CAZ, CEFE, NIT, and LEVO; five isolates were resistant to at least six drugs in all antibiotics except GEN; twelve isolates were resistant to seven drugs in all tested drugs, and eleven isolates were resistant to eight or more drugs.

Among *Klebsiella* sp. isolates from older female samples, one isolate was resistant to five drugs, three isolates were resistant to at least six drugs in all antibiotics except GEN, two isolates were resistant to seven drugs, and eight isolates were resistant to eight or more drugs. Among *Klebsiella* sp. isolates from the samples of young females, two isolates were resistant to five drugs, one isolate was resistant to at least six drugs, no isolate was resistant to seven drugs, and eight isolates were resistant to eight or more drugs in all drugs. Among *Klebsiella* isolates from young male samples, one isolate was resistant to five drugs, six isolates were resistant to at least six drugs in all drugs, two isolates were resistant to seven drugs, and three isolates were resistant to eight or more drugs. Among *Klebsiella* isolates from older male samples, four isolates were resistant to five drugs, five isolates were resistant to at least six drugs in all drugs, no isolate was resistant to seven drugs, and six isolates were resistant to eight or more drugs in all drugs.

Among *Proteus* sp. isolates from older female samples, two isolates were resistant to five drugs, no isolate was resistant to at least six drugs, three isolates were resistant to seven drugs, and two isolates were resistant to eight or more drugs in all drugs. Among *Proteus* sp. isolates from young female samples, one isolate was resistant to five drugs no isolate was resistant to at least six drugs, one isolate was resistant to seven drugs, and six isolates were resistant to eight or more drugs in all drugs. Among *Proteus* sp. isolates from young male samples, no isolate was resistant to five drugs, two isolates were resistant to at least six drugs, one isolate was resistant to seven drugs, and five isolates were resistant to eight or more drugs in all drugs. Among *Proteus* isolates in older male samples, one isolate was resistant to five, six, and seven drugs, and four isolates were resistant to eight or more drugs in all drugs (Figure 3, Table 4). MDR cases were reported to be 54.2% by Asaduzzaman et al.,[23] while 70.67% by Begum et al.[15] In a previous study, *E. coli* was the most predominant isolate (72.4%),[18] and similar results were observed in several other studies.[6] Nevertheless, in the current study, 68.62% *E. coli* isolates showed MDR (Table 4).

CONCLUSION

The current study revealed that MDR cases are increasing continuously. Within the last couple of years, highly effective antibiotics were almost useless against newly identified uropathogenic strains due to drug resistance. By controlling the abuse of antibiotics and reducing selective pressure from available antibiotics, it may be possible to reduce the rise in resistant bacteria. Health care professionals need to be aware of the current situation of antibiotic resistance and focus on prescribing antibiotics after obtaining culture and sensitivity reports.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethical Approval Committee (IEAC), Primeasia University, India with reference number PAU/IEAC/22/108.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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