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Frequency and Distribution of Multidrug Resistance Enterobacteriaceae Isolated from Hospital and Community Acquired Urinary Tract Infection Patient Attended at a Tertiary Level Care Hospital in Dhaka City

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Abstract

Background: Urinary tract infection is caused by different bacteria of family enterobacteriaceae in the hospital and community acquired infection. **Objective:** The purpose of the present study was to find out the bacteria causing urinary tract infection, frequency as well as their antibiotic resistance pattern. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology at National Institute of Neurosciences and Hospital, Dhaka, Bangladesh from June 2017 to November 2017 for a period of six months. Different urine samples of patient who were presented with infection in the urinary tract were included as study population. Using sterile container urine samples were taken from both IPD and OPD patients of National Institute of Neurosciences and Hospital, Dhaka, Bangladesh. The urine specimen was inoculated on blood and MacConkey agar plates and incubated at 37°C for 24 hours. Identification was based on standard microbiological methods The antibiogram testing was done by using modified Kirby-Bauer method. **Results:** A total of 200 urine samples were included for study of which 118(59%) cases showed positive and 82(41.0%) cases showed negative growth. Among 118 samples *Escherichia coli* were in 56(47.5%) isolates followed by *Klebsiella pneumoniae* were in 22(18.6%); *Citrobacter freundii* were in 20(16.9%); *Pseudomonas* species were 12(10.2%) and *Proteus mirabilis* were 8(6.8%). *Escherichia coli* was most predominant urinary tract infection causing species (47.5%) and found most resistant against cefixime (96.4%), azithromycin (92.9%), ciprofloxacin (71.4%); *Klebsiella pneumonia* was most resistant to amikacin, azithromycin (81.82%), ciprofloxacin, tazobactum/piperacillin (72.7%) cefixime, meropenem (63.7%). *Citrobacter freundii* was resistance to ciprofloxacin, cefixime (80.0%). **Conclusion:** *Escherichia coli* infections are more common in urinary tract infection patients and predominant resistant against cefixime followed by *Klebsiella pneumonia*. [*Bangladesh Journal of Infectious Diseases, December 2022;9(2):47-52*]

Keywords: UTI; azithromycin; ciprofloxacin; cefixime; resistance

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Introduction

Enterobacteriaceae fall within the domain bacteria, phylum Proteobacteria, class Gamma proteobacteria, order Enterobacteriales. They are Gram-negative, rod shaped, non -spore- forming, facultative anaerobes that ferment glucose and other sugars, are motile by virtue of peritrichous flagella or non-motile, reduce nitrate to nitrite, and produce catalase but do not produce oxidase[1]. In 2011, WHO declared combat drug resistance: no action today, no cure tomorrow[2]. In current years, strains of multidrug resistant organisms have become quadrupled worldwide[3]. Presently, anti-microbial resistance (AMR) poses a major threat to patient's treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the patient and nation[4]. The clinical isolates such as *Pseudomonas aeruginosa*, methicillin resistant *Staphylococcus aureus* (MRSA), *Enterococci* especially vancomycin resistant *Enterococci* (VRE), and members of family enterobacteriaceae, for example, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus* species, rapidly develop antibiotic resistance and spread in the hospital environment. Actually, the health care planners have declared "Health for All by the year 2000." In the last two decades, there were so much increase of infectious diseases that the standard of public health in many parts of the world is equivalent to pre antibiotic era[5].

As per standardized international terminology created by European Centre for Disease Control (ECDC) and Centre for Disease Control and Prevention (CDC), Atlanta, the multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria have been well defined[6]. Urinary tract infections (UTI) are among the most common bacterial infections for which patients seek medical attention[7]. It is estimated that global incidence of urinary tract infections is 250 million per year. Symptomatic urinary tract infections result in approximately 7 million visits to outpatient clinics (OPDs), one million visits to emergency departments, and 100,000 hospitalizations each year[8]. UTIs account for a large number of antibacterial drug consumption, costing about six billion US dollars a year[9].

Urine is normally sterile. An infection occurs when bacteria, usually from gastrointestinal tract, gain access to urethra. Theodor Escherich, a German pediatrician, cultured "bacterium coli" in 1885 from the faeces of healthy individuals. It could be found universally in the colon, hence the name coli. It was renamed as *Escherichia coli* in 1919. Initially it was considered a harmless normal commensal forming a beneficial symbiotic relationship with its host[10]. Soon it was found that some strains of *Escherichia coli* were pathogenic and could cause serious disease both within the intestinal tract and elsewhere in the body.

These pathogenic strains were broadly categorized as diarrheagenic and extra intestinal pathogenic *E coli* (EXPEC)[11]. Among EXPEC, strains of uropathogenic *Escherichia coli* (UPEC) were most commonly associated with human disease. Various diseases caused by EXPEC include urinary tract infection, neonatal meningitis, sepsis, pneumonia, surgical site infections, and infections in other extra intestinal locations[12]. *Escherichia coli* are the most common cause of urinary tract infection, responsible for 80.0% to 90.0% of community acquired and 30.0% to 50.0% of nosocomial urinary tract infection[13]. Recurrent or relapsing Urinary tract infection is especially problematic. *E coli* causing urinary tract infection in a particular individual usually come from his own digestive tract as they match the person's rectal isolates. However, a single clonal group of uropathogenic *Escherichia coli* may spread within the community via contaminated foods or other consumables[14]. These organisms can also be transmitted sexually and uropathogenic *Escherichia coli* strains in sexually active patients often match faecal isolates of their partners[15]. The purpose of the present study was to find out the bacteria causing urinary tract infection, frequency as well as their antibiotic resistance pattern.

Methodology

Study Settings and Population: This cross-sectional study was conducted in the Department of Microbiology at National Institute of Neurosciences and Hospital, Dhaka, Bangladesh from August, 2017 to November, 2017 for a period of six months. Different urine samples of patient who were presented with infection in the urinary tract were included as study population. Using sterile container urine samples were taken from both IPD and OPD patients of National Institute of Neurosciences and Hospital, Dhaka, Bangladesh.

Study Procedure: Urine samples were collected using sterile container with screw capped it must be bearing the patients name, age, sex, indoor, outdoor etc.

Isolation and Identification of Bacteria: The urine specimen was inoculated on blood and

MacConkey agar plates. The streaked plates were incubated at 37^0 C for 24 hr. Bacterial colonies on blood agar plates were later Gram stained. Characterization of bacterial isolates was based on standard microbiological methods. Identification of isolates were done based on colony morphology, motility, catalase test, oxidase test, coagulase test and biochemical tests like Kligler's iron agar, hydrogen sulfide, motility, indole, urease and citrate utilization test.

Antibiogram Susceptibility Testing: The antibiogram susceptibility testing was done as per as CLSI guidelines using modified Kirby-Bauer method. Few colonies from the culture plate was inoculated into 2ml of peptone water and incubated at 37^0 C for 2 hr. Turbidity was compared to that of 0.5 McFarland standards. A cotton swab was immersed and rotated in this inoculums, the swab was then pressed to the side of the tube so as to remove excess inoculums. It was then used for carpet streaking on Muller Hinton agar plate. Then appropriate antibiotic disc was placed on plate and was incubated at 37^0 C for 24 hours. The zone of inhibition (in mm) was measured and recorded as resistance, sensitive and intermediate sensitive as per CLSI guidelines.

Statistical Analysis: The statistical analysis was carried out using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Qualitative variables like gender, classification, blood group, clinical features were expressed as frequency and percentage; quantitative variables were expressed as mean ±standard deviation.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee.

Results

A total of 200 urine samples were included for study of which 118(59.0%) cases showed positive and 82(41.0%) cases showed negative growth (Figure I & II). Among 118 samples, 46(38.98%) cases were male patients and 72(61.0%) cases were female and 66(55.9%) cases were indoor and rest 52(44.1%) cases were outdoor patients (Table 1).

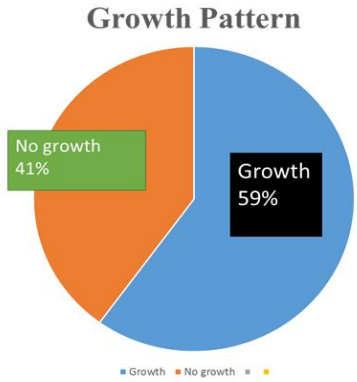

Figure I: Growth pattern of urine samples.

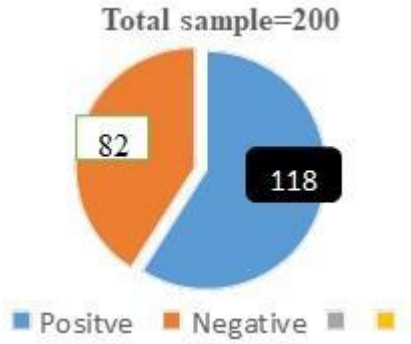

Figure II: Distributions by positive & negative growth (n=200)

Table 1: Distribution of Indoor and Outdoor Patients (n=118)

Patient type	Frequency	Percent
IPD	66	55.9
OPD	52	44.1
Total	**118**	**100.0**

Among 118 samples *Escherichia coli* were 56(47.46%) followed by *Klebsiella pneumonia* were 22 (18.64%), *Citrobacter freundii* were20(16.9%), *Pseudomonas* species were 12(10.2%) and *Proteus mirabilis* were in the rate of 8(6.78%) which was very high among the all isolates (Table 2).

Table 2: Frequency and percentage of species (n=118)

Name of bacteria	Frequency	Percent
Escherichia coli	56	47.46
Klebsiella pneumonia	22	18.64
Citrobacter freundii	20	16.95
Pseudomonas spp	12	10.17
Proteus mirabilis	08	06.78
Total	**118**	**100%**

Table 3: Resistance Pattern of Different MDR Enterobacteriaceae

Antibiotic	***Escherichia coli***	***Klebsiella pneumoniae***	***Citrobacter freundii***	***Pseudomonas* spp.**	***Proteus mirabilis***
Amikacin	42.9%	81.8%	10.0%	50.0%	25.0%
Azithromycin	92.9%	81.8%	40.0%	33.3%	37.5%
Ciprofloxacin	71.4%	72.7%	80.0%	50.0%	62.5%
Cefixime	96.4%	63.7%	80.0%	83.3%	25.0%
Colistin	46.4%	18.2%	10.0%	16.7%	25.0%
Meropenem	14.3%	63.7%	40.0%	33.3%	12.5
Nitrofurantoin	14.3%	18.2%	30.0%	33.3%	25.0%
Tazobactum/ Piperacillin	57.1%	72.7%	50.0%	16.7%	37.5%

Escherichia coli was most predominant urinary tract infection causing species (47.46%) and found most resistant against cefixime (96.43%), azithromycin (92.86%), ciprofloxacin (71.42%), *Klebsiella pneumonia* was most resistant to amikacin, azithromycin (81.82%), ciprofloxacin, Tazobactum/piperacillin (72.7%) cefixime, meropenem (63.7%). *Citrobacter freundii* was resistance to ciprofloxacin, cefixime (80.0%), *Pseudomonas* species was resistance to cefixime (83.3%), *Proteus. mirabilis* was resistance to ciprofloxacin (62.5%) (Table 3).

Discussion

The member of family enterobacteriaceae such as *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Pseudomonas* sp. are the common causative agents of urinary tract infections. The emerging resistant genes in such bacteria by various mechanisms are a matter of concern. After the end of the study it showed that, the most common bacteria those causes urinary tract infections are *Escherichia coli, Klebsiella pneumonia, Citrobacter freundii*, *Pseudomonas* species and *Proteus mirabilis.* Out of this species the *Escherichia coli* (47.46%) was the most predominant member of family enterobacteriaceae that causes urinary tract infections.

A total of 200 urine samples were included for study of which 118(59.0%) showed positive and 82(41.0%) showed negative growth. Among 118 samples, 66(55.9%) were indoor and the rest 52 (44.07%) were outdoor patients. Among 118 samples *Escherichia coli* were 56(47.5%) followed by *Klebsiella pneumoniae* were 22(18.6%), *Citrobacter freundii* were 20(16.9%), *Pseudomonas* species were 12(10.2%) and *P. mirabilis* were 8(6.78%) that compare to the study where highest prevalence of *Escherichia coli* (55.5%) followed by *Klebsiella pneumoniae* (23.2%), *Proteus mirabilis* (4.5%), *Pseudomonas species* (3.2%), *Enterobacter species* (2.6%), *Enterococcus faecalis* (2.6%) and others species (8.4%). In this study the *E. coli* and *Klebsiella pneumoniae was* found 75.10% and *Escherichia coli* and *Klebsiella pneumoniae* represent 78.7% of all isolated bacteria.

Total enterobacteriaceae was found 106(89.8%) that compare to the another study[5]. Antimicrobial resistance in Escherichia coli strains from urinary tract infections[16] where showed that more than 60.0% were *Enterobacteriaceae*. *Escherichia coli* was most predominant urinary tract infection causing species (47.5%) that compare to the another study[11]. Frequency of *Escherichia coli* in patients with community acquired urinary tract infection and their resistance pattern against some commonly used antibacterials[17] where *E. coli* were the most common cause of UTI patients (40.0%). *Escherichia coli* are the most common cause of urinary tract infection, responsible for 80.0% to 90.0% of community acquired and 30.0% to 50.0% of nosocomial UTI that was found in a study[11]. Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*[13]. *Escherichia coli* were found most resistant against azithromycin (92.86%), ciprofloxacin (71.4%) where 15.0% resistant showed in the another study[15]. Antimicrobial resistance of urinary *Escherichia coli* isolates[18] and 80.0% resistant showed in the another study[11]. Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections[16]. In this study *Escherichia coli* show sensitive to amikacin (57.14%) and nitrofurantoin (85.7%) where sensitive to amikacin (96.25%) and nitrofurantoin (92.5%) were showed in another study[12]. Antimicrobial resistance pattern of *Escherichia coli* isolated from urine samples in patients visiting tertiary health care centre in Eastern Nepal[19]. Cefixime (96.43%), *Klebsiella*

pneumoniae was most resistant to amikacin, azithromycin (81.82%), ciprofloxacin, tazobactum/piperacillin (72.73%) cefixime, meropenem (63.67%). *Citrobacter freundii* was resistance to ciprofloxacin, cefixime (80%), *Pseudomonas* species was resistance to cefixime (83.33%), *Proteus mirabilis* was resistance to ciprofloxacin (62.50%).

Conclusion

The ratio of *Escherichia coli* infection in urinary tract infection patient is greater as compared to non-operated. *Escherichia coli* infections are more common in urinary tract infection patients and predominant resistant against cefixime followed by *Klebsiella pneumonia* was second highest infections in urinary tract infection patients with resistant against amikacin and azithromycin, *Citrobacter freundii* was resistant to ciprofloxacin and cefixime, *Pseudomonas* species was resistance to cefixime, *Proteus.mirabilis* was resistance to Ciprofloxacin. The most common remarkable resistant antibiotic against *Escherichia coli, Klebsiella pneumonia, Citrobacter freundii, Pseudomonas* species was Cefixime.

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None

Conflict of Interest
We declare that we have no conflict of interest.

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Contribution to authors:
Islam MS, Yusuf MA: Conception and design, or design of the research; or the acquisition, analysis, or interpretation of data; conceptualized and designed the overall study. Debnath AC, Shil R is involved in data collection; Drafting the manuscript or revising it critically for important intellectual content. Islam MS: involved in data input and data cleaning. Islam MS, Yusuf MA: conducted data analysis. Siddiqui UR drafted the manuscript. All authors reviewed and approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporti ng data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a retrospective study, not every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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