Are We Exposing Ourselves to UTI Causing *Escherichia coli*?- Appraisal on the Virulence Factors and Antibiotic Resistant Trend Among Clinical and Washroom Water Isolates.

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of MS in Biotechnology

Department of Mathematics and Natural Sciences

BRAC University

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
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Approval

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1. This material is the authors' own original work, which has not been previously published elsewhere.
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4. The results are appropriately placed in the context of prior and existing research.
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6. All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

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Abstract

One of the most prevalent bacterial infectious diseases that cause significant morbidity and cost-effective health issues is urinary tract infection (UTI). The most frequent cause of UTIs, uropathogenic Escherichia coli (UPEC), is a type of extraintestinal pathogenic *E. coli* that exhibits extremely diverse virulence factors and capable of multi-drug resistance (MDR). This study aimed to assess the prevalence of the virulence factors and antibiotic resistant pattern among clinical and washroom water isolates of UPEC. From a tertiary diagnostic center, a total of 126 urine samples from UTI patients and total 20 washroom water samples from domestic and public washrooms were collected within a same time period in Dhaka city, Bangladesh. The samples were cultured on MacConkey agar after that DNA of suspected *E. coli* isolates were extracted for Real-Time PCR and confirmed by using ECO primer. A total of 85 isolates from clinical and a total of 38 isolates from non-clinical isolates were confirmed as *E. coli* and further the virulence genes were detected using virulence gene primers of *fim*H, *pap*C, *hly*, *cnf*, *iuc*C, and *fyu*A for both, clinical and non-clinical isolates. Antibiotic susceptibility test was performed for both, clinical and non-clinical isolates, using amoxicillin (AML) (10 μg), ciprofloxacin (CIP) (5 μg), ceftriaxone (CRO) (30 μg), azithromycin (AZM) (15 μg), cefexime (CFM) (5 μg), norfloxacin (NOR) (10 μg), nitrofurantoin (F) (300 μg), cefuroxime (CXM) (30 μg), meropenem (MEM) (10 μg), and cefepime (FEP) (30 μg). Hemolysin production was also observed for both, clinical and non-clinical isolates. According to results, among six virulence markers of pathogenicity-associated island (PAI), in both clinical and non-clinical isolates, *fim*H was found in highest percentage 80% and 52.63%, respectively. Moreover, *cnf* gene was found in least percentage, 7.06% and 2.63% for both clinical and non-clinical isolates, respectively. In case of antibiotic susceptibility pattern, the highest resistance against amoxicillin was observed 92.94% and 63.16%, in clinical and non-clinical isolates, respectively. Moreover, the lowest resistance was seen against meropenem, 57.65% in clinical isolates and 50% in non-clinical isolates. Blood Agar hemolysis pattern among clinical and non-clinical isolates showed 58.82% clinical isolates and 31.58% non-clinical isolates exhibited β-hemolytic pattern. Public water supplies in Dhaka, Bangladesh, are frequently contaminated with *E. coli* strains with virulence factors that cause UTIs and multi-drug resistance patterns. In metropolitan settings, the transmission of plasmids and resistant gene to bacteria through the supply water acts as a major risk to public health. This quick evaluation of bacterial pathogenicity may help healthcare providers treat patients with urinary tract infections more effectively.

Keywords: Urinary Tract Infection; Prevalence; Virulence factors; Uropathogenic *E. coli*; multi-drug resistance.

Dedication

To science…

Acknowledgment

First and foremost, I would like to convey my gratitude to the Almighty for keeping me healthy and giving me patience throughout my thesis work.

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List of Acronyms

ABBREVIATION	ELABORATION
AMR	Antimicrobial Resistance
AST	Antimicrobial susceptibility testing
ATP	Adenosine Triphosphate
bp	Base pairs
CLSI	Clinical & Laboratory Standards Institute
CFU	Colony Forming Unit
CNF	Cytotoxic Necrotizing Factor
DNA	Deoxyribonucleic acid
et. al	and others
HGT	Horizontal Gene Transfer
HPF	High Power Field
LB	Luria Broth
MDR	Multidrug-resistant
mm	Millimeter
ml	Milliliter
MHA	Muller-Hinton Agar
pap	Pyelonephritis Associated Pili
PAI	Pathogenicity Island
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
RT-PCR	Real-Time Polymerase chain reaction

RBC	Red Blood Cell
RTX	Repeats-in-Toxin
TE	Tris-EDTA
UTI	Urinary Tract Infection
UPEC	Uropathogenic *Escherichia coli*
VF	Virulence Factor

Chapter 1

1. Introduction and Background

1.1 Urinary Tract Infection (UTI)

Urinary tract infections (UTIs) are termed as the presence and growth of microbes in the urinary tract(McDaniel & Kaper, 1997). If the pathogen can penetrate the urinary tract system and accumulate to more than 10^5 colonies/ml in urine, a UTI results (Smelov et al., 2016). UTIs can be classified as either uncomplicated or complicated. Usually, uncomplicated UTIs affect healthy individuals where no symptoms or anatomical abnormalities are found in the genitourinary tract and are divided into lower UTIs (cystitis) and upper UTIs (pyelonephritis)(Antinori & Pezzani, 2018). Lower UTI is defined as the involvement of the urethra, bladder, prostate, epididymis, and testis (Das & Banerjee, 2015). Upper UTI is the term for the condition that affects the kidneys and ureters(Antinori & Pezzani, 2018). Bacteria most frequently enter the urinary system by the ascending transurethral avenue, but they can also enter through the bloodstream or lymphatics (Yagoob, 2009). If the lower urinary tract is affected, urinary tract infection (UTI) symptoms such as urgent urination, dysuria, frequency, strangulation, and suprapubic pain may also be present (Biswas et al, 2010). Fever and flank back pain are the symptoms of an upper (UTI). Asymptomatic urinary tract infections may be found during routine examinations (Goddard et al., 2010). When looking at the urine under a microscope for a UTI, pus cells, elevated number of epipthelial cells and/or red blood cells (RBCs) may be visible. Pyuria is defined as having six to ten or more pus cells per High Power Field (HPF) of freshly voided midstream urine that are unspun(N. G. et al., 2013). When there are two or more leucocytes in the urine, it is considered to be significant pyuria (Anígilájé & Bitto, 2013).

1.2 Global Prevalence and Associated Risk Factors

UTIs are the most common bacterial illness seen in general practice, accounting for 1-3% of consultations (Goddard et al., 2010). About 150 million individuals worldwide are diagnosed with (UTI) each year, and it affects all age groups, from neonates to the elderly(Harding & Ronald, 1994). Financially, community-acquired UTI's expensive, costing the world economy more than six billion US dollars(Prakash & Saxena, 2013). A study conducted by icddr,b found 71% culture positive UTI among 4500 patients in Dhaka city (Islam et al., 2022). Although both men and women can get infected, UTIs are typically thought of as a condition that only

affects women, who have a 50% lifetime prevalence (Foxman, 2002). Both sexes are susceptible to urinary tract infections. However, women are more likely to experience it, particularly when they are sexually active for reproduction. This is brought on by their narrow urethra, injuries sustained during sexual activity, and lack of bactericidal secretions(A. Ronald, 2003).

UTI is found to be the most recurrent bacterial infection ("13th European Congress of Clinical Microbiology and Infectious Diseases," 2003), affecting 25% of all infections in many developing countries like Bangladesh where sanitation is not adequate. It is a major source of illness and also the second most common reason for hospital visits (A. R. Ronald & Pattullo, 1991). It can affect people of all ages and populations, from newborns to elderly people (Kunin, 1994). However, women are more prone to infection (Karki et al., 2004) due to differences in anatomical structure and the greater risk of infection following bladder catheterization, which weakens natural defence mechanisms. Newborns, preschool girls, sexually active women, elderly women and men have a higher risk of developing symptomatic urinary tract infections. In 2019, a study reported that more than 404.6 million people worldwide had UTIs, resulting in nearly 236,786 deaths and contributing to 5.2 million DALYs. The age-standardized incidence rate increased from 4715.0 per 100,000 population in 1990 to 5229.3 per 100,000 population in 2019 (Zeng et al., 2022).

1.3 UTI Causing Microorganisms

It has been found that several microorganisms including *Escherichia coli, Klebsiella, Proteus, Enterobacter, Pseudomonas, Enterococcus, Staphylococcus, Candida,* and others are associated with the UTI (Patel et al., 2019). However, the most dominant causative agent for UTI is found to be *E. coli* (70-95%) and the least one is *Staphylococcus saprophyticus* in (5-10%) (Schneider & Riley, 1996a). *E. coli* strains which cause UTI are defined as uropathogenic *E. coli* (UPEC) (Mobley et al., 2009).

Further it has been reported a small percentage of UTIs are hematogenous in origin, and these infections are typically brought on by a few relatively rare bacteria (such as *Staphylococcus aureus, Candida spp.,* and *Mycobacterium tuberculosis*), which cause primary infections elsewhere in the body and then spread to the urinary tract (Wittenberg et al., 2014).

1.4 Uropathogenic *E. coli* (UPEC)

E. coli is a normal resident of the gut microbiota in humans and animals, where it helps maintain the balance and health of the intestinal microbes by interacting with the host in a beneficial way(Martinson & Walk, 2020). This bacterium does not harm healthy individuals when it stays in the gut lumen, but some strains can cause diarrhoea in some situations especially when the immune system is compromised (Evans & Evans, 1996). Moreover, some *E. coli* lineages evolved several virulence factors that allow them to infect different sites and cause diseases that are classified into three main categories: intestinal/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis (Kaper et al., 2004).

Diverse groups of *E. coli* can cause UTIs and they are classified in four main phylogroups A, B1, B2 and D (Terlizzi et al., 2017) based on the genomic variation. Indeed, these UPEC develop specialized virulence factors for attachment, colonization and damaging host cells (Hahn et al., 2002). However, certain commensal *E. coli* can be emerged as UPEC even when a single pathogenicity island (PAI) has been incorporated to their genome (Sabaté et al., 2006).

1.4.1 Character and Morphology of UPEC

The classification of *E. coli* is-

Domain: Bacteria
Kingdom: Eubacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: *E. coli.*

The bacterium *Escherichia coli* was first isolated by the German-Austrian pediatrician Dr. Theodor Escherich in 1885(Lim et al., 2010). It is a gram-negative, rod shaped, facultative anaerobic, non-spore-forming, usually motile by peritrichous flagella (Percival & Williams, 2014).

1.4.2 Virulence Factors of UPEC

Virulence factors (VFs) are defined as the contributing phenomenon of microorganism to iniate infection to a host (Sharma et al., 2017). UPEC posess several VFs to colonize, invade and cause damage to host cells.

The VFs of *E. coli* can be roughly classified into two major groups- one is the cell surface components including fimbriae, pili which contribute in adhesions and tissue invasion and cytokine induction (Shah et al., 2019a). Capsular lipopolysaccharide, outer membrane proteins and flagellum are also bacterial cell surface VFs. Another one is the the secreting VFs which have mostly enzymatic activities to spread and damage to cell (Emody et al., 2003). It has been found that both the cell surface and secreting VFs are associated in biofilm formation and the development of anti-microbial resistance (AMR) (Shah et al., 2019a). The most common VFs gene of UPEC are as follows-

fimH: It is the type I fimbriae coding gene(Tseng et al., 2022). Fimbriae are hair like structures and a very vital role player in the infection initiation process as they help UPEC to adhere to uroepithelial cells in genital organs. The type I fimbriae mostly produced by *fimH* gene is considered as a key attachment factors in UPEC (Bien et al., 2012a)

papC: This gene encodes Pyelonephritis associated pili (pap), another attachment factors which enable UPEC to adhere to bladder and cause pyelonephritis (Yazdanpour et al., 2020). It has been found that patients urgency in urination is higher if the infection caused by UPEC contains *papC* gene (Dadi et al., 2020a). Moreover, another study revealed that *papC* gene is highly associated with the amoxicillin resistance (Yazdanpour et al., 2020).

hly: It is the alpha hemolysin producing gene. Alpha hemolysin has been found to be toxic to a wide number of cells including red blood cell (RBC) (Wiles & Mulvey, 2013). On the other hand, study reported that this alpha hemolysin is associated with the kidney inflammation and damage during UTI caused by UPEC (Lüthje & Brauner, 2014).

cnf: This gene encodes a 110 kDa protein named cytotoxic necrotizing factor (cnf) which induces the formation of actin stress fibre (Donnenberg, 2002) and membrane ruffling

(Fiorentini et al., 1988) for the successful penetration to host cell. CNF has also be found as to induce apoptosis in bladder cell line in vitro (Mills et al., 2000).

fyuA & iucC: Iron is an essential element for bacterial growth, metabolism and interaction with host (Messenger & Barclay, 1983). However, iron is tightly bound to iron binding protein including ferittin, transferrin, lactoferrin and hemoglobin in human body (Brock, 1989). Bacteria produce certain siderophore during infection to capture iron from the human body (Page, 2019). The product of *fyuA* gene is the yersiniabactin type of siderophore which contributes to iron acquisition during infection (D'Onofrio et al., 2023). Interestingly, a study found that *fyuA* gene is highly associated with the biofilm formation by UPEC (Hancock et al., 2008). Nevertheless, the biofilm formation capability of UPEC is a serious threat in catheter mediated UTI (Trautner & Darouiche, 2004).

The product of *iucC* gene is an aerobactin, a type of siderophore used by several bacteria for iron assimilation which often makes a bacteria hypervirulent (Bailey et al., 2018). Study found that pathogenic *E. coli* always carry aerobactin synthesis gene where as avirulent strain does not (Ling et al., 2013).

1.5 Emergence of Antimicrobial Resistance (AMR) in UPEC

It has been found that half of the women and one in 10 men suffer from UTI in their lifespan (Al-Badr & Al-Shaikh, 2013). Typically, UTI is treated with antibiotics for decades but combating antibiotic resistant UTI is challenging and it is leading to more severe infection which eventually rise the hospitalization and mortality rate (Seaton, n.d.). Recent study revealed that 92 % of the UTI causing microbes are resistant to at least one commonly used antibiotic and 80% are resistant to two antibiotics (Ahmed et al., 2019).

Since UPEC accounts for 70-95% of UTI (Schneider & Riley, 1996b), the emergence of antibiotic resistant UPEC becomes a serious public health concern. A recent study found that 32% of *E. coli* causing UTI are resistant to beta-lactam antibiotics (Erdem et al., 2018). It should be noted that uncomplicated UTI is mostly treated with beta-lactam antibiotics as empirical treatment (Thakuria & Lahon, 2013). Another study conducted in Iran reported more

than 50% of UPEC were resistant to beta-lactam antibiotics (Dehbanipour et al., 2016). Therefore, the emergence of beta-lactam antibiotic resistant *E. coli* is becoming a global threat.

Not only beta-lactam, UPEC has also evolved incredible resistance against multiple antibiotics. A study reported that the highest antibiotic resistance of UPEC was found to be against tetracyclin which was 69.1% followed by 59.3% against sulphonamides and 49.4% against quinolones (Bunduki et al., 2021). Although quinolones have been used as a potent antibiotic against UTI, the study revelaed half of the *E. coli* causing UTI are resistant againt quinolones (Malekzadegan et al., 2019).

Another study revealed that 78% of UTI causing *E. coli* were found to be multi-drug resistant (MDR) and 62.5% of them were biofilm former (Katongole et al., 2020). This study also reported that 93% of *E. coli* were found to be resistant against amoxicillin and 87% against gentamycin. Indeed, the biofilm formation capabilities of UPEC is one of the main cause of spreading antimicrobial resistance through horizontal gene transfer (HGT) and AMR gene has been found to be rapidly transferred in biofilm community rather than planktonic culture (Michaelis & Grohmann, 2023).

Since UTI can lead to sepsis and renal failure (Porat et al., 2023), the early treatment with a potent antibiotic is recommended. Nevertheless, the potency of antibiotics are being diminished by the rapid emergence of antimicrobial resistant bacteria. Although the trend of antimicrobial resistance pattern differ from one geographical region to another, it is suggested to strengthen the local AMR surveillance for the better medical management in terms of infectious disease (*Strengthening AMR Surveillance*, n.d.).

1.6 Objective of the Study

This study is designed to achieve the following objectives-

i. To evaluate the antimicrobial resistant pattern of *E. coli* causing UTI
ii. To assess the virulence factors (VFs) of UPEC
iii. To compare the VFs and antimicrobial resistant pattern among UPEC and *E. coli* isolated from washroom water

1.7 Novelty of the Study

Although several studies on UPEC and antimicrobial resistant pattern have been conducted in Bangladesh but the degree of virulence factors are not well studied in this region. Our study focus on both the antimicrobial resistance and VFs.

Washing genital organ after urination is a very common practice by Muslims and Bangladeshi people. Still, we do not have enough data on the quality of the washroom water and if there is any potential UTI causing microorganism. This is the first time in Bangladesh we have compared the VFs among Clinical UPEC and *E. coli* isolated from different washroom water.

Chapter 2

2. Methodology

The experiments were carried out in collaboration with a tertiary diagnostic center of Dhaka city and the life sciences laboratory of Brac University to study the antibiotic susceptibility pattern and virulence factors of *E. coli* isolated from urinary tract infection and several washroom water.

All the suspected *E. coli* isolates were firstly subjected to molecular identification by Real-Time PCR (RT-PCR). Secondly, PCR confirmed isolates were further studied for the presence of virulence factors. Finally, antibiotic sensitivity test was done for both the clinical and washroom water isolates and were compared.

2.1 Sample Collection

All the uropathogenic *E. coli* were collected from the routine urine test of a tertiary care diagnostic center from December 2022 to February 2023. Mid-stream urine from the urinary tract infection (UTI) suspected patients was collected and without delay, one loopfull of urine (1 µl) was separately inoculated on Nutrient Agar & MacConkey agar and after 24 hours of incubation growth of more than 10 colonies on Nutrient Agar (resembles 10^4 to 10^5 CFU/ml was interpreted that particular patient was considered as UTI positive (*Guideline for Urine Culture and Biochemical Identification of Bacterial Urinary Pathogens in Low-Resource Settings - PMC*, n.d.). Based on colony morphology on MacConkey agar, 106 suspected *Escherichia coli* were selected and further 85 *E. coli* isolates were confirmed by Real Time PCR (RT-PCR) using ECO primer.

To collect non-clinical isolates, water samples were collected from different washrooms in the Dhaka city, for example household washrooms (n=5), public washrooms (n=5), hospital washrooms (n=5) and washrooms of some universities (n=5). The samples were collected aseptically in a sterile container in an amount of 100 ml and taken in the laboratory within two hours. Then the water samples were serially diluted up to 10^{-4} and 100µl of water was spreaded

on the MacConkey agar. Based on colony morphology, 58 suspected *E. coli* isolates were taken and 38 of them were confirmed by RT-PCR using ECO primer.

2.2 Media preparation:

MacConkey Agar was used for the identification and enumeration of Coliforms in the isolate. Media was prepared according to the manufacturer's instruction (HiMedia). 51.53g of anhydrous media was dissolved in 1000 ml of distilled water, it was heated with gentle swirling for a few minutes. Then the media was autoclaved at 121°C for 15 minutes having about 15 pounds of pressure and cooled down to 45-50°C while pouring into plate.

The Luria Broth culture medium is non-selective rich medium which is widely used to culture Enterobacteriaceae as well as facultative organisms. The media was prepared by adding Tryptone 10g, Yeast Extract 5g, and Sodium Chloride 10g into a conical and dissolved in 1000 ml of distilled water. The mixture was heated for some times to avoid clumps and then autoclaved at 121°C for 15 minutes having about 15 pounds of pressure. The autoclaved media was cooled down to 45-50°C before pouring it into sterile test tubes.

Muller-Hinton Agar **(**MHA) medium was mainly used to observe the antimicrobial activities of the antibiotics. The required amount of agar was first measured in electronic balance and mixed with distilled water in two conical flasks and then were heated and dissolved by heating until the agar melted. The mouth of the conical flask was covered with aluminum foil paper. After sealing the mouth, it was then placed into an autoclave machine at 121^0 C and 15 psi for 15 minutes. After autoclaving the mixture was then poured into the sterile large sized petri dishes. When the agar solidified the petri dishes were then labeled and were stored at 4^0 C inside the refrigerator for further use.

2.3 Preparation of Physiological Saline

Physiological saline was made to prepare bacterial suspension and it was matched with McFarland standard 1 solution. At first 0. 9 g NaCl was dissolved in 80 ml deionized or distilled

water in clean conical flask. Then the water was added to bring total solution volume to 100 ml. After mixing saline was transferred to 15 ml test tube and autoclaved.

2.4 DNA Extraction:

DNA extraction was performed using the boiling method. Before extraction, the isolates were grown in LB (Luria Broth) at 37 °C for 24 hours. Microcentrifuge tubes were filled with 700 µl of culture broth and centrifuged at 3000 x g for 10 minutes, the supernatant was discarded and pellet was washed with PBS (Phosphate buffered saline). Washing process was repeated twice and the solution was centrifuged at 14000 x g for 5 minutes. Then the supernatant was discarded and 200 µl of TE (Tris-EDTA) buffer was added to the pellet. Then the solution was placed in the heating block to boil at 100◦C for 15 mins and immediately was placed in ice for cold shock for 10minutes. Then contents of microcentrifuge tubes were then centrifuged at 1400x g for 5minutes. As cell debris was precipitated, supernatant was collected and stored in the freezer at -20°C for further analysis.

2.5 Primers for the Target Gene Identification

Among surface virulent factors: attachment factors, type 1 fimbriae encoding *fim*H gene and P fimbriae encoding *pap*C was detected using specific primers (table 1). Among secreted virulence factors: α-hemolysin producing *hly* gene, cytotoxic necrotizing factor encoding *cnf* gene, siderophore producing systems such as *iuc*C genes encoding aerobactin system and fyuA encoding yersiniabactin system were also detected using specific primers (table 1) in both sample, clinical and non-clinical.

The following primers were used in this study to identify *E. coli* isolates and also their virulence factors.

Table 1 Primer sequences of Identification and virulent genes

Targeted gene(s)	Primer Name	Primer Sequence (5′- 3′)	Size of amplicon (bp)	References
fimH	fimH-f	5′-AACAGCGATGATTTCCAGTTTGTGTG-3′	465	Dadi et al., 2020
	fimH-r	5′-ATTGCGTACCAGCATTAGCAATGTCC-3′		
papC	pap1	5′-GACGGCTGTACTGCAGGGTGTGGCG-3’	328	Dadi et al., 2020
	pap2	5′-ATATCCTTTCTGCAGGGATGCAATA-3’		
hly region	hly s	5′-AGATTCTTGGGCATGTATCCT-3’	556	Dadi et al., 2020
	hly as	5′-TTGCTTTGCAGACTGTAGTGT-3’		
cnf	cnf s	5′-TTATATAGTCGTCAAGATGGA-3’	693	Dadi et al., 2020
	cnf as	5′-CACTAAGCTTTACAATATTGA-3’		
iucC	aer s	5′-AAACCTGGCTTACGCAACTGT-3’	269	Dadi et al., 2020
	aer as	5′-ACCCGTCTGCAAATCATGGAT-3’		
fyuA	F	5’-TGATTAACCCCGCGACGGGAA-3’	787	Dadi et al., 2020;
	R	(5’-CGCAGTAGGCACGATGTTGTA-3’		(Tarchouna et al., 2013)
16S rRNA	ECO-f	GAC CTC GGT TTA GTT CAC AGA	585	Moawad et al., 2017
	ECO-r	CAC ACG CTG ACG CTG ACC A		

2.6 PCR Amplification:

All the PCR was carried out in CFX Opus 96 Real-time PCR System and the reaction volume was 13 µl which had 2 µl of primers (1 µl of each forward and reverse primer), 2 µl of template DNA and 6 µl of Luna® Universal qPCR Master Mix. Conditions consist of an initial denaturation at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 2 min, annealing at a specific temperature for 30 s (table 1), and extension at 72 °C for 1 min.

2.7 Antimicrobial Susceptibility Test:

In-vitro antimicrobial susceptibility testing of the bacterial isolates was performed by Kirby-Bauer disc diffusion method. A 24 hours old culture of the bacterial isolate (10^6 CFU/ml of 0.5 McFarland Standard) was used to make lawn on sterile Mueller-Hinton Agar and antibiotics disks were placed on the lawned media. Antibiotics discs were placed on solidified agar plates at equal distance apart. The plates were kept standby for 10 min. Then the media plates were incubated for 24 hours at 37 °C. After 24 hours, diameters of zones of inhibition were using measuring scale and result was interpreted based on the standard set CLSI. In this study, *E. coli* ATCC 25922 was taken as a reference strain. The following antimicrobial discs were used with their respective concentration: amoxicillin (AML) (10 µg), ciprofloxacin (CIP) (5 µg), ceftriaxone (CRO) (30 µg), azithromycin (AZM) (15 µg), cefexime (CFM) (5 µg), norfloxacin (NOR) (10 µg), nitrofurantoin (F) (300 µg), cefuroxime (CXM) (30 µg), meropenem (MEM) (10 µg), and cefepime (FEP) (30 µg).

2.8 Hemolysin Production Test:

The blood agar hemolysis test was done for the detection of α-hemolysis produced by the *E. coli*. The bacteria were inoculated into 5% sheep blood agar and incubated overnight at 37°C. Hemolysin production was detected by the presence of a zone of complete lysis of the erythrocytes around the colony and clearing of the medium (Ranjan et al., 2010).

Chapter 3

3. Results:

A total of 126 mid-stream urine samples from UTI suspected patients and non-clinical water samples from household washrooms (n=5), public washrooms (n=5), hospital washrooms (n=5) also from washrooms of randomly chosen universities (n=5). The isolates were examined in order to observe whether there was any of potential pathogen of interest. One hundred six isolates which resulted from clinical samples were screened by RT-PCR using ECO primer and 85 isolates were found positive for uropathogenic *E. coli*. Among 58 isolates which resulted from non-clinical samples, 38 isolates were revealed as UPEC by RT-PCR.

3.1 Prevalence of virulence genes in different samples:

Total 85 isolates were confirmed as *E. coli* by pcr from clinical samples. Six different virulence genes were detected in UPEC strain of urine samples (clinical isolates). The most frequent virulent gene found was *fim*H in 68 (80%) isolates, followed by *hly* in 42 (49.41%), *pap*C in 38 (44.7%), *iuc*C in 26 (30.59%), *fyu*A in 23 (27.05%) and *cnf* in 6 (7.06%) isolates. (Figure 1)

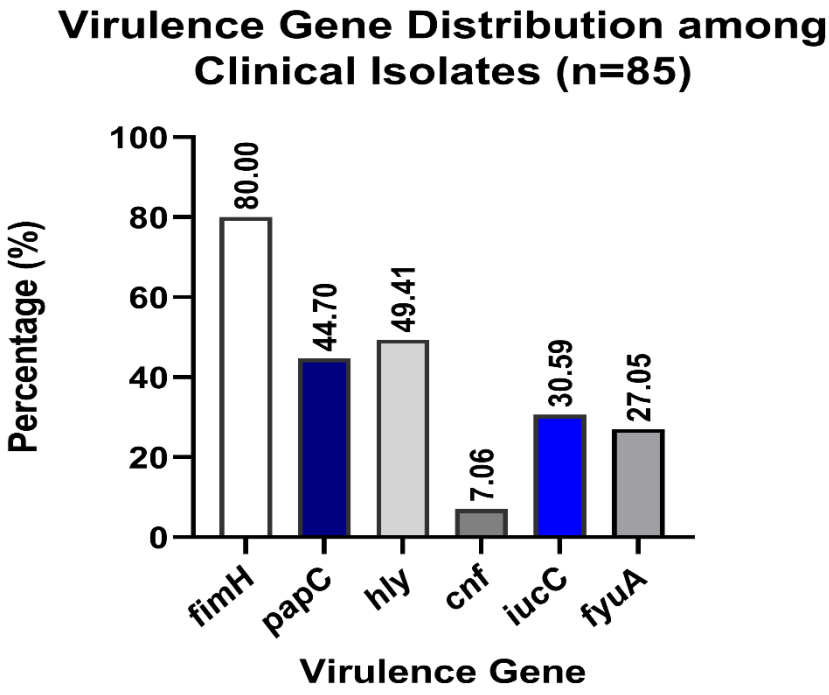

Figure 1: Virulence gene distribution among clinical isolates showed most frequent appearance of *fim*H gene which appeared in highest percentage (80%), followed by the least appeared gene *cnf* (7.06%)

In case of non-clinical samples, some virulence genes were detected which were also found in clinical samples. Two among 6 virulence genes were detected from household washrooms (n=5) isolates. A total of 4 isolates were selected from household washroom samples. The most frequent virulence factor identified *fim*H in 3 (75%) isolates, followed by *hly* in 1 (25%) isolate. (Figure 2)

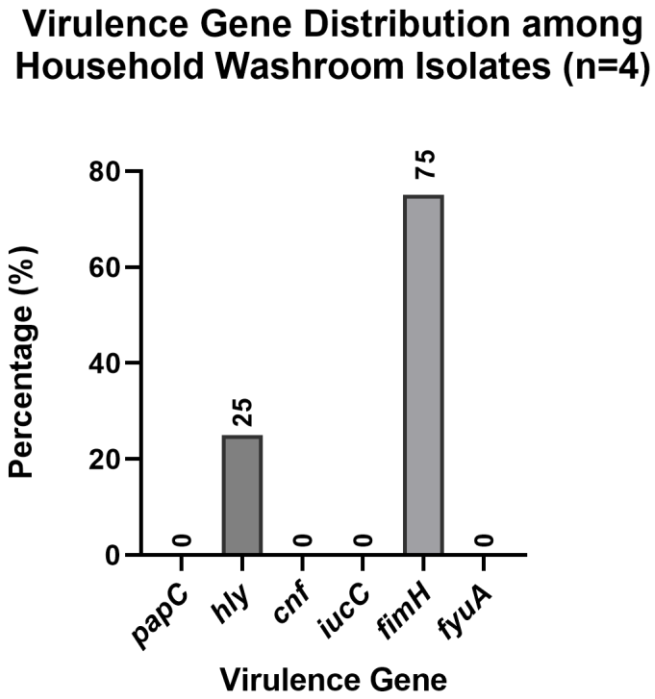

Figure 2: Virulence gene distribution among household washroom isolates showed most frequent appearance of *fim*H (75%) gene, followed by least appeared gene, *hly* (25%).

From the water samples of public washrooms (n=5), a total of 11 isolates were confirmed as *E. coli* and 5 virulence genes were found by RT-PCR. The most frequent virulence genes were *pap* C found in 6 (54.54%) isolates and *hly* in 6 (54.54%) isolates, followed by *fim*H in 5 (45.45%) isolates, *iuc*C in 3 (27.27%) isolates, *fyu*A in 3 (27.27%) isolates. (Figure 3)

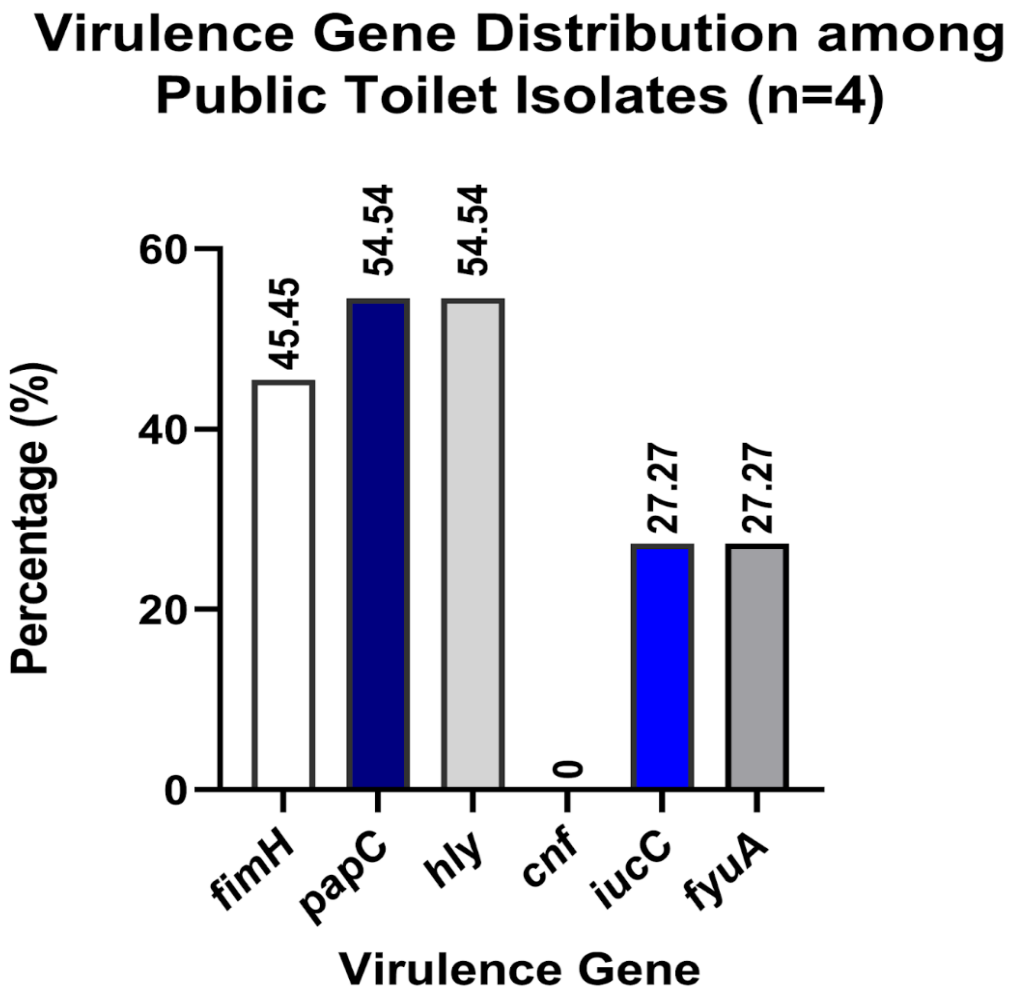

Figure 3: Virulence gene distribution among public washroom isolates show most frequent appearance of *pap*C (54.54%) gene, *hly* (54.54%) gene, followed by least appeared *iuc*C (27.27%) gene and *fyu*A (27.27%)gene.

From the water samples of hospital washrooms (n=5), total 15 isolates were confirmed as *E. coli*. The most frequently found virulence genes were *fim*H 9 (60%), followed by *hly* 8 (53.33%), *fyu*A 7 (46.67%), *pap* C 4 (26.67%), *iuc*C 4 (26.67%) and *cnf* 1 (6.67%) (Figure 4)

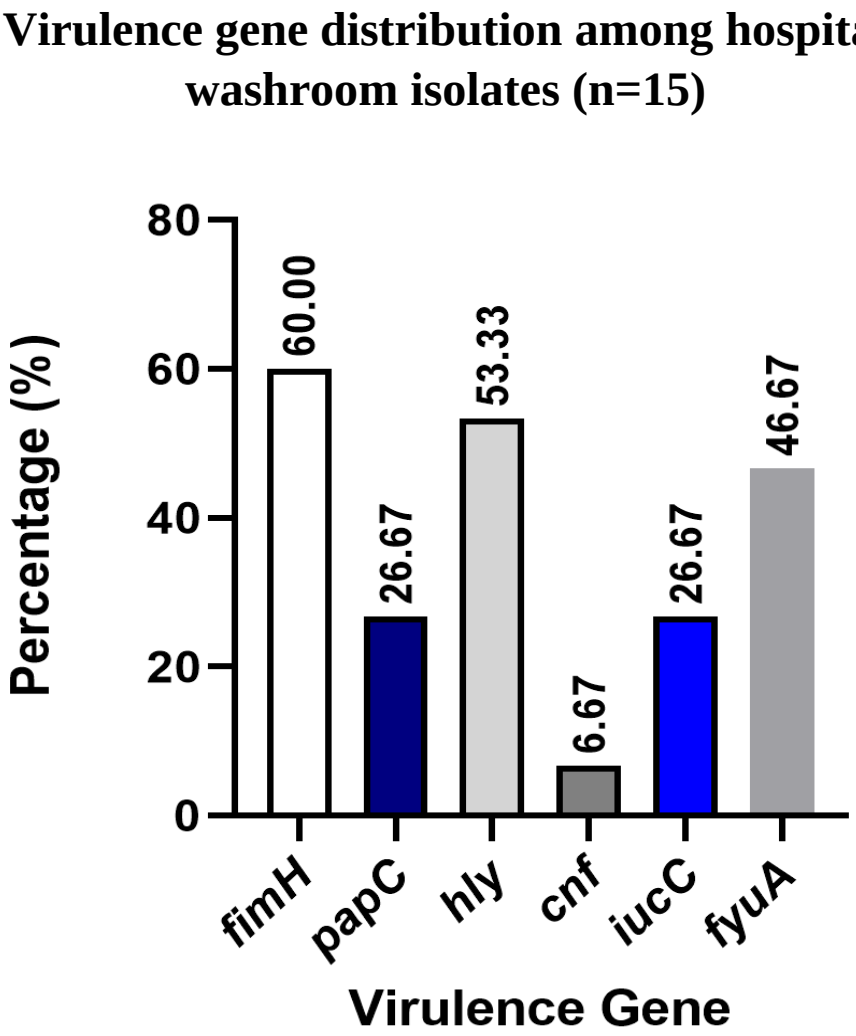

Figure 4: Virulence gene distribution among hospital washroom isolates show most frequent appearance of *fim*H (60%) gene, followed by least appeared gene, *cnf* (6.67%).

Among 5 water samples from university washrooms, 8 isolates were confirmed as *E. coli*. The most frequently found genes were *fim*H 3 (37.5%), *pap* C 3 (37.5%), followed by *hly* 2 (25%) and *fyu*A 1 (12.5%) (Figure 5)

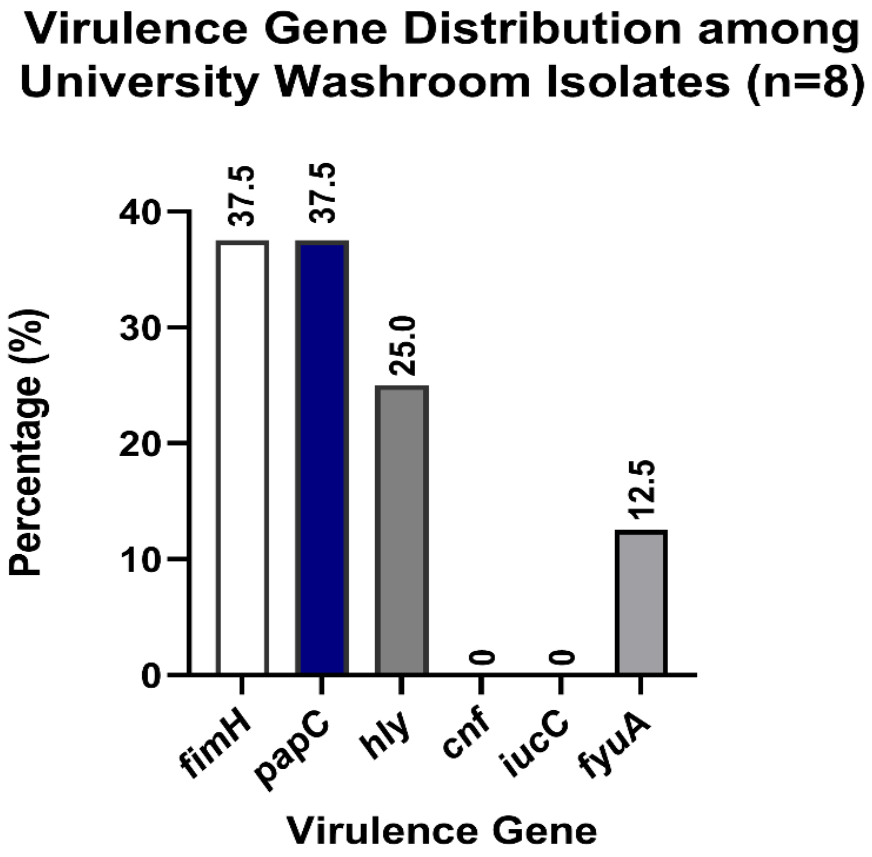

Figure 5: Virulence gene distribution among university washroom isolates show most frequent appearance of *fim*H (37.5%) and *pap*C (37.5%) gene, followed by least appeared *fyu*A (12.5%)gene.

3.2 Comparison between prevalence of virulence genes among clinical and non-clinical isolates:

A total 85 isolates were confirmed as *E. coli* among 106 isolates of clinical samples and 38 isolates were confirmed as *E. coli* among 58 isolates of non-clinical samples (washroom water samples). The presence of same 6 virulent genes were observed in both type of samples. The most prevalent virulence gene found was *fim*H which had the frequency of 68 (80%) in clinical isolates and 20 (52.63%) in washroom isolates. Among the adhesion virulence genes, *pap*C was found in 38 (44.7%) clinical isolates where as it was found in 13 (34.21%) washroom isolates. The virulence factor for hemolysin, *hly* gene was found in 42 (49.41%) clinical isolates and in 17 (44.73%) isolates of non-clinical samples. The cytotoxic necrotizing factor, *cnf* gene was found in 6 (7.06%) clinical isolates where as it was found in only 1 (2.63%) isolate identified from non-clinical samples. The *iucC* gene which codes for aerobactin virulence, was found in 26 (30.59%) isolates of clinical sample and in 7(18.42%) isolates of non-clinical samples. The *fyu*A gene, encoding the yersiniabactin receptor, was found in 23 (27.05%) isolates of clinical sample and in 11 (28.94%) isolates of non-clinical samples. (Figure 6)

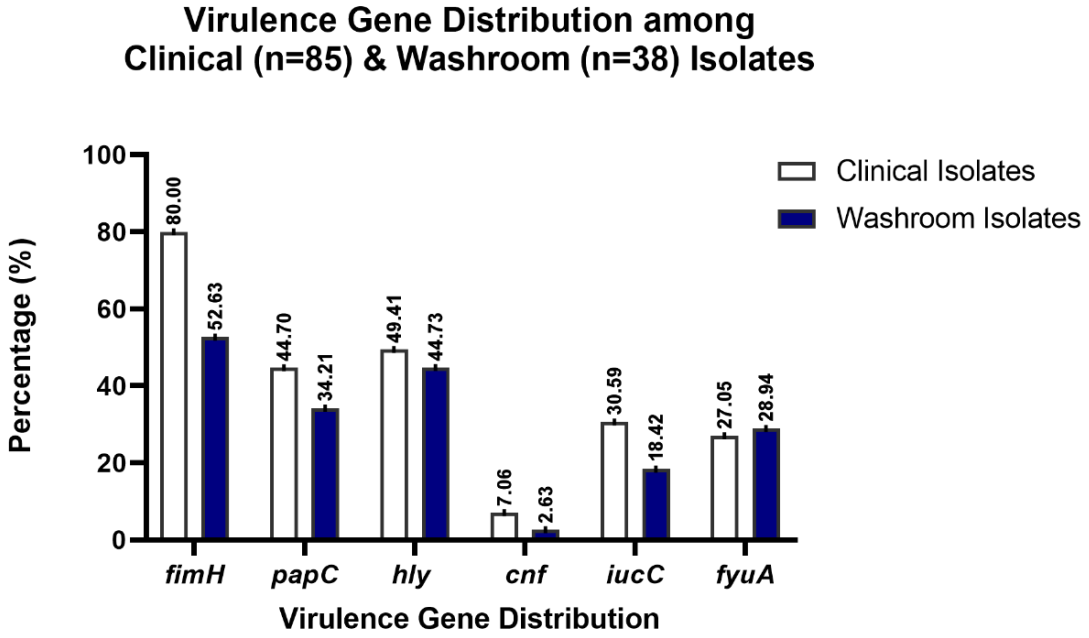

Figure 6: Distribution of virulence gene among clinical and non-clinical samples. Each gene was found in prominent number in clinical isolates compared to non-clinical isolates. In clinical and non-clinical isolates *fim*H was found in highest percentage 80% and 52.63%, respectively. Moreover, *cnf* gene was found in least percentage, 7.06% and 2.63% for both clinical and non-clinical isolates, respectively.

3.3 Antibiotic Susceptibility Pattern:

Among the antibiotics tested against total 85 clinical isolates, every isolate was found multidrug resistant (MDR) (≥ 3 classes of antiobiotics). The isolates showed highest resistance to amoxicillin (92.94%), followed by nitrofurantoin (88.24%), cefixime (78.82%), and among 2nd generation cephalosporin, cefuroxime (78.82%). Among macrolides, isolates showed resistance against azithromycin (77.65%) and among 2nd generation fluoroquinolones, resistance pattern was observed against norfloxacin (72.94%) and ciprofloxacin (71.76%). The isolates also showed resistance to 3rd generation cephalosporin, ceftriaxone (68.24%) and among 4th generation cephalosporin, isolates showed resistance to cefepime (58.82%). Moreover, resistance pattern against meropenem (57.65%) was observed. Though there was specific pattern of sensitivity but interestingly, no intermediate zone was observed. (Figure 7)

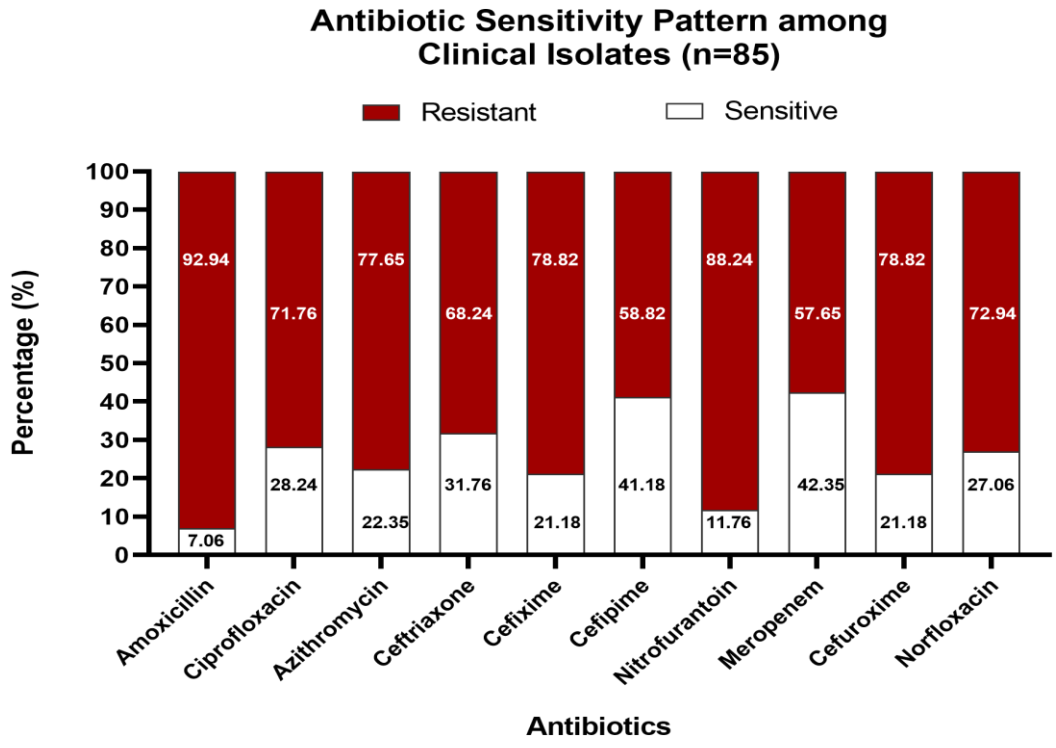

Figure 7: Antibiotic susceptibility pattern among clinical isolates which showed highest resistance against amoxicillin (92.94%) and least resistance to meropenem (57.65%).

Among non-clinical samples, isolates from household washroom water sample showed highest resistance against ceftriaxone (75%), cefepime (75%), and cefuroxime (75%), followed by amoxicillin (50%), ciprofloxacin (50%), and nitrofurantoin (50%). The isolates also showed resistance against azithromycin (25%), cefixime (25%), meropenem (25%), and norfloxacin (25%). No intermediate zone was observed here as well. (Figure 8)

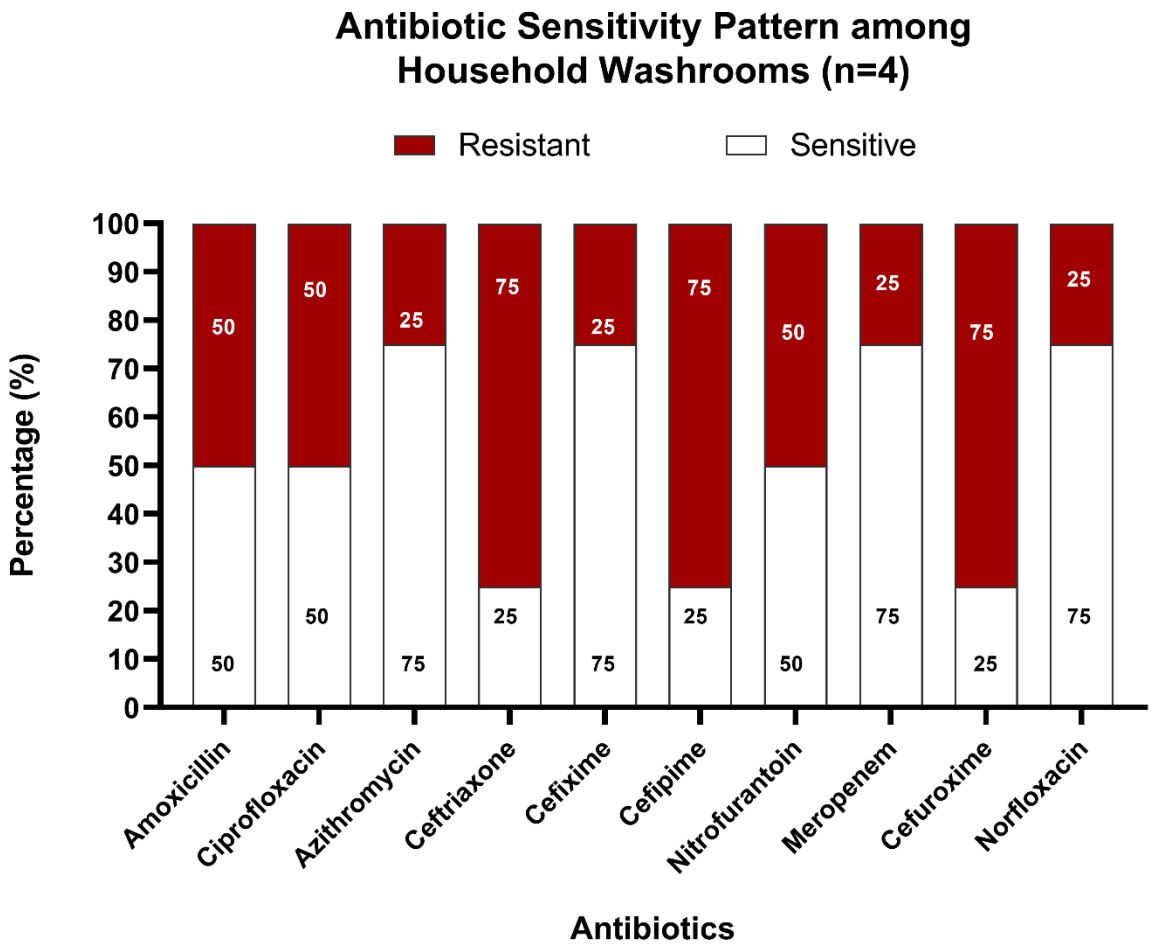

Figure 8: Antibiotic susceptibility pattern was observed among isolates of household washroom water samples which showed highest resistance against ceftriaxone (75%), cefepime (75%), and cefuroxime (75%) and least resistance against azithromycin (25%), cefixime (25%), meropenem (25%), and norfloxacin (25%).

In case of the public washroom water sample, isolates exhibited highest resistance to norfloxacin (72.73%) and cefuroxime (72.73%), followed by amoxicillin (63.64%), ciprofloxacin (63.64%), azithromycin (63.64%), and cefixime (63.64%). The isolates also showed resistance to ceftriaxone (54.55%), cefepime (54.55%), nitrofurantoin (54.55%), and meropenem (45.45%). No intermediate zone was observed here in this case. (Figure 9)

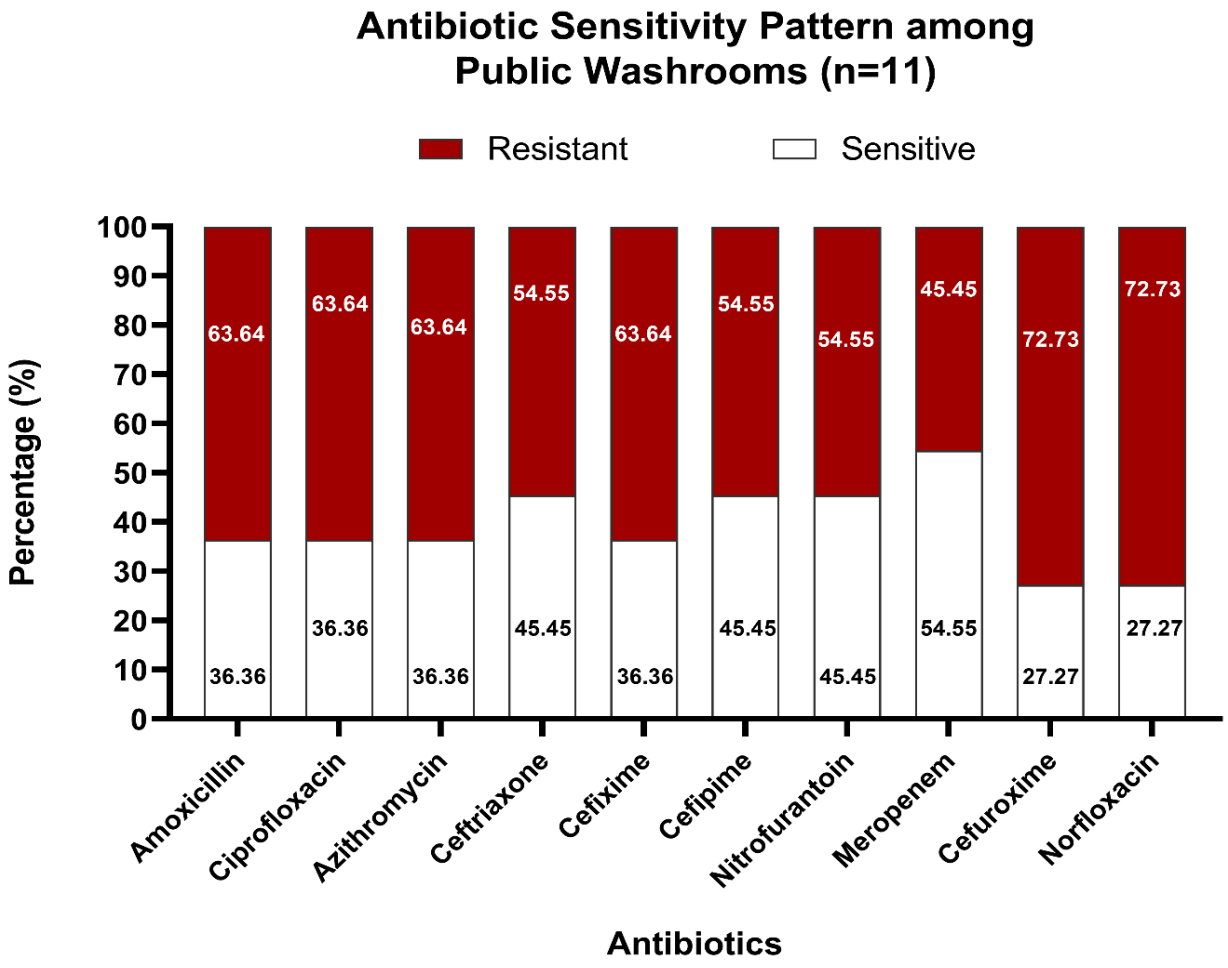

Figure 9: Antibiotic susceptibility pattern was observed among isolates of public washroom water samples which showed highest resistance to norfloxacin (72.73%), cefuroxime (72.73%) and least resistance to meropenem (45.45%).

Samples taken from hospital washrooms; 15 isolates showed highest resistance pattern against amoxicillin (80%), cefixime (80%), nitrofurantoin (80%), and cefuroxime (80%). Resistance pattern was also observed against ceftriaxone (73.33%), cefepime (73.33%), ciprofloxacin (66.67%), azithromycin (66.67%), meropenem (66.67%), norfloxacin (66.67%). Though some sensitivity patterns were shown but no intermediate zone was observed. (Figure 10)

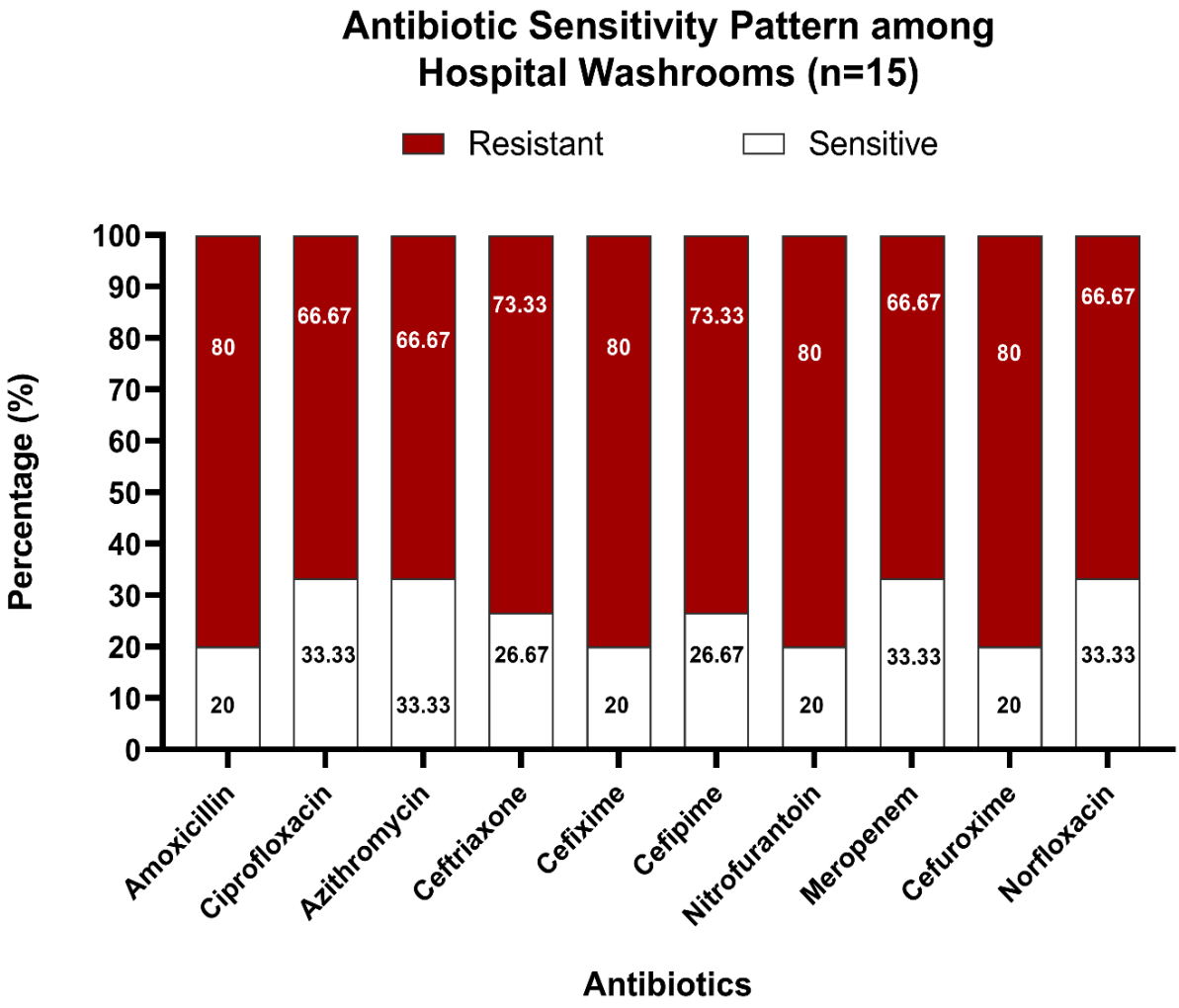

Figure 10: Antibiotic susceptibility pattern was observed among isolates of hospital washroom water samples which showed highest resistance pattern against amoxicillin (80%), cefixime (80%), nitrofurantoin (80%), cefuroxime (80%) and least resistance to ciprofloxacin (66.67%), azithromycin (66.67%), meropenem (66.67%), norfloxacin (66.67%).

Eight isolates were selected as *E. coli* from university washroom water samples. These isolates were also found as multidrug resistance (MDR). All isolates showed resistance to ciprofloxacin (50%), azithromycin (50%), followed by amoxicillin (37.5%), ceftriaxone (37.5%), meropenem (37.5%), cefuroxime (37.5%) and norfloxacin (37.5%). The isolates were also resistance against cefixime (25%), cefepime (25%) and nitrofurantoin (25%). Though there was specific pattern of sensitivity but interestingly, no intermediate zone was observed. (Figure 11)

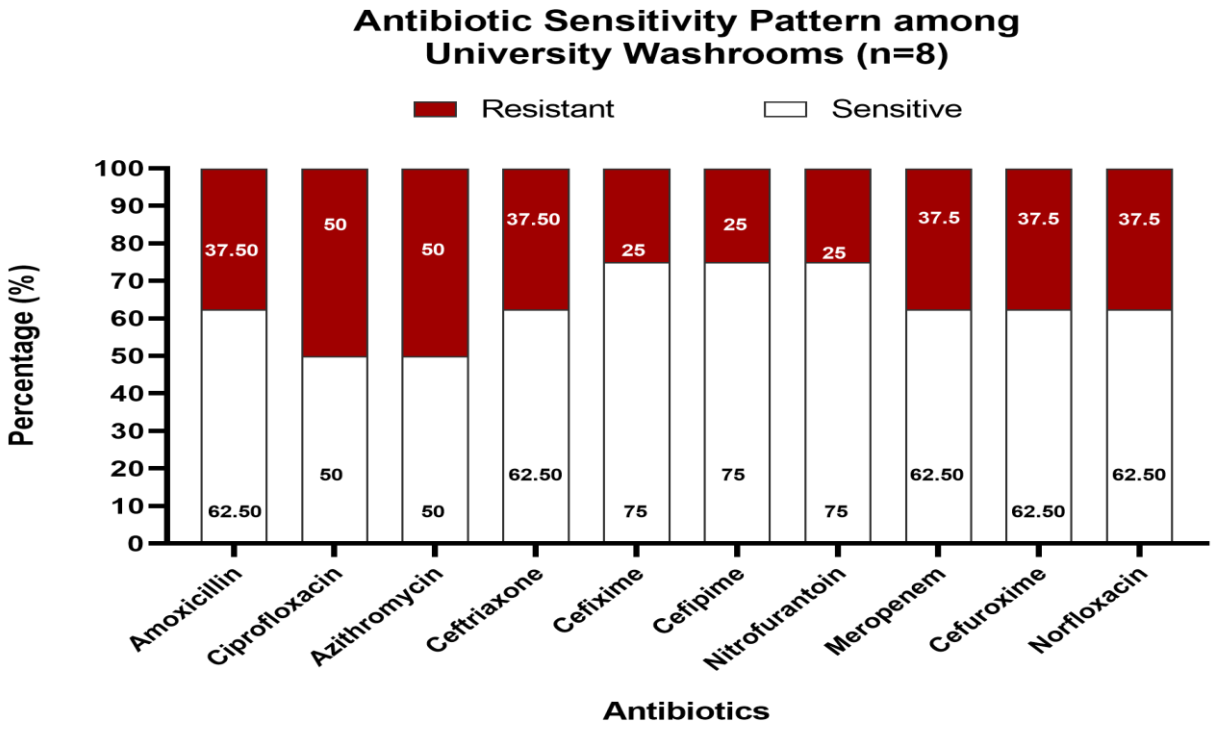

Figure 11: Antibiotic susceptibility pattern was observed among isolates of university washroom water samples which showed resistance to ciprofloxacin (50%), azithromycin (50%) and least resistance to cefixime (25%), cefepime (25%) and nitrofurantoin (25%).

3.4 Comparison between antibiotic susceptibility pattern of clinical and non-clinical sample:

A total 85 isolates among clinical samples and 38 isolates among non-clinical samples were confirmed as *E. coli.* All these isolates showed resistance pattern against the antibiotics used for this study and all these isolates were found as multidrug resistant (MDR). Clinical isolates showed highest resistance to amoxicillin which was 92.94% whereas 63.16% of non-clinical isolates showed resistance to amoxicillin.

Isolates from clinical samples showed resistance to nitrofurantoin which was 88.24% whereas it was 57.89% in case of non-clinical isolates. Cefixime resistance was noted in 78.82% of clinical isolates but it was resistant to 57.89% of non-clinical isolates. An antibiotic among 2nd generation cephalosporin, cefuroxime, was found resistant to 78.82% of clinical isolates and 68.42% of non-clinical isolates. Resistance against azithromycin was found in 77.65% of clinical isolates and in 57.89% of non-clinical isolates. Norfloxacin resistance was found in 72.94% of clinical isolates and 57.89% of non-clinical isolates. Clinical isolates showed resistance to ciprofloxacin which was 71.76% whilst 60.52% of non-clinical isolates were resistant to it. Isolates from clinical samples exhibited resistance against ceftriaxone which was 68.24% whereas 60.53% of non-clinical isolates were resistant to it. Cefepime resistance was noted in 58.82% of clinical isolates and in 57.89% of non-clinical isolates. A resistance pattern was observed against meropenem as well, where 57.65% of clinical and 50% of non-clinical isolates were found resistant to it. (Figure 12)

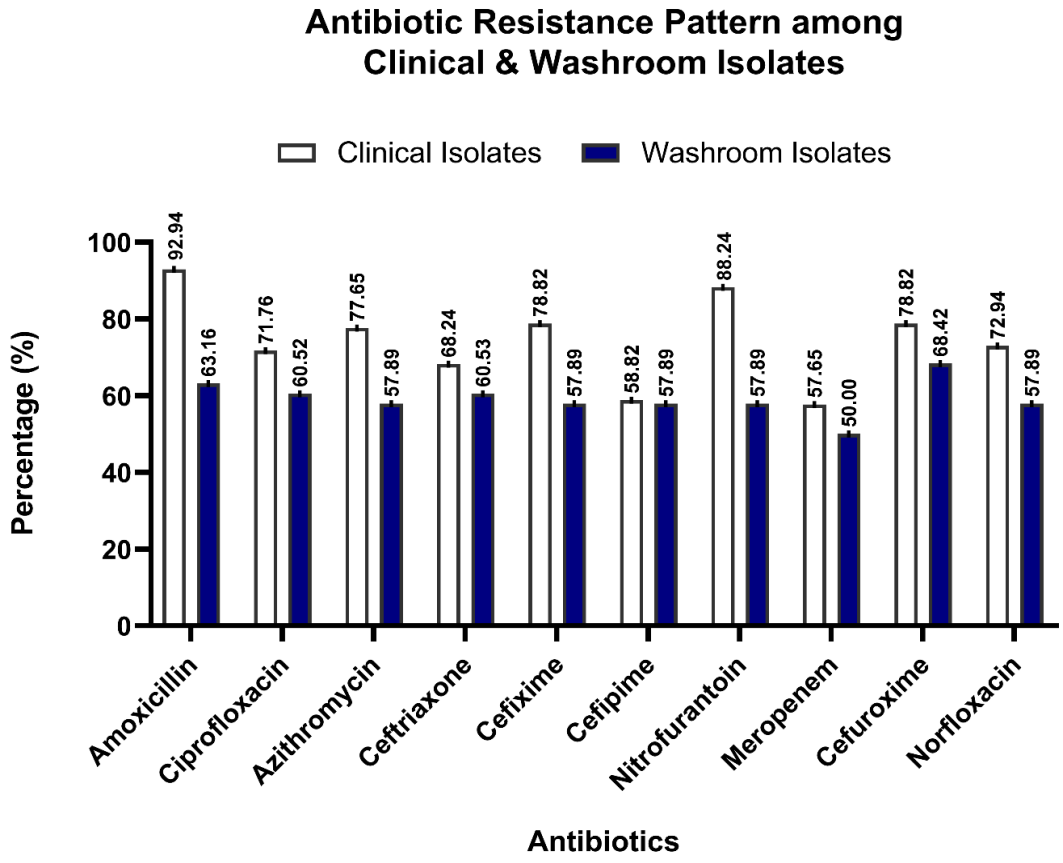

Figure 12: Comparison of antibiotic resistance pattern among clinical and non-clinical samples where highest resistance against amoxicillin was observed 92.94% and 63.16%, in clinical and non-clinical isolates, respectively. Moreover, the lowest resistance was seen against meropenem, 57.65% in clinical isolates and 50% in non-clinical isolates.

3.5 Hemolysis Pattern:

E. coli encodes for a gene (*hly*) that can produce hemolysin toxin which is a member of repeats-in-toxin (RTX) family. Among 85 of clinical isolates, 21 (24.79%) isolates caused α-hemolysis, 50 (58.83%) isolates caused β-hemolysis and 14 (16.47%) isolates showed γ- hemolysis pattern. (Figure 13)

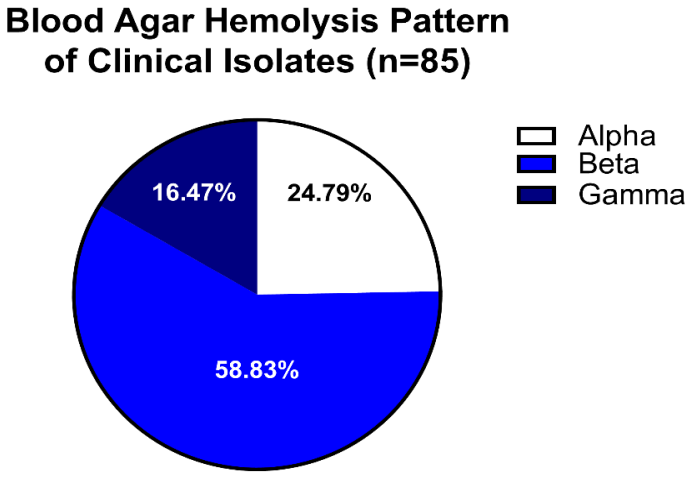

Figure 13: Hemolysis pattern of clinical isolates on blood agar where 58.83% isolates exhibited β-hemolytic pattern

Among 38 isolates of non-clinical samples, 4 isolates were confirmed as *E. coli* from household washroom water samples. Among which 1 (25%) isolate showed α-hemolysis and 3 (75%) isolates showed γ- hemolysis. (Figure 14)

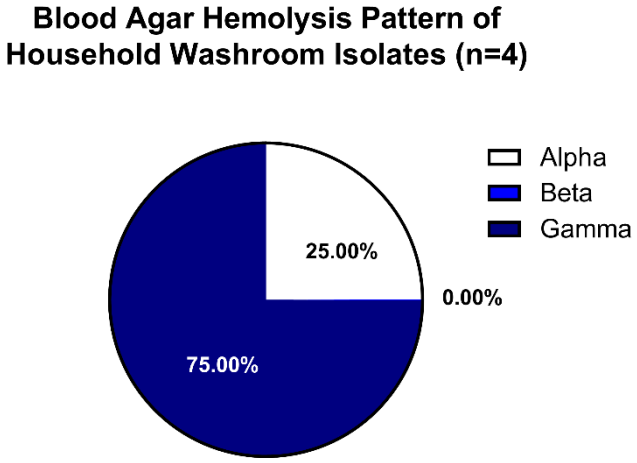

Figure 14: Blood Agar hemolysis pattern of isolates from household washroom water sample where 75% isolates showed β-hemolytic pattern.

A total of 11 isolates were selected from public washroom water samples as *E. coli*. Among which 4 (36.36%) isolates showed α-hemolysis, 4 (36.36%) isolates showed β-hemolysis and 3 (27.27%) isolates showed γ- hemolysis pattern. (Figure 15)

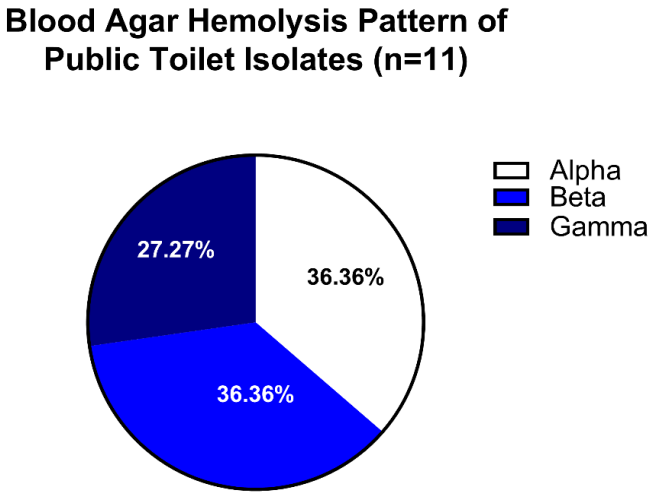

Figure 15: Blood Agar hemolysis pattern of isolates from public washroom water sample where 36.36% isolates showed β-hemolytic pattern

A total of 15 isolates were selected from hospital washroom water samples as *E. coli*. Among which 5 (33.33%) isolates showed α-hemolysis, 8 (53.34%) isolates showed β-hemolysis and 2 (13.33%) isolates showed γ- hemolysis. (Figure 16)

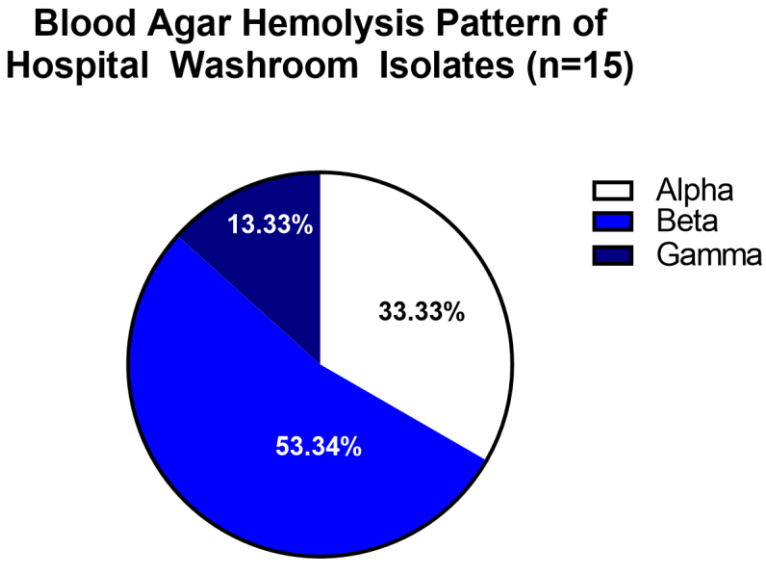

Figure 16: Blood Agar hemolysis pattern of isolates from hospital washroom water sample where 53.34% isolates showed β-hemolytic pattern

Eight isolates were selected from university washroom water samples as *E. coli*. Among which 5 (62.44%) isolates showed α-hemolysis and 3 (37.46%) isolates showed γ- hemolysis pattern. (Figure 17)

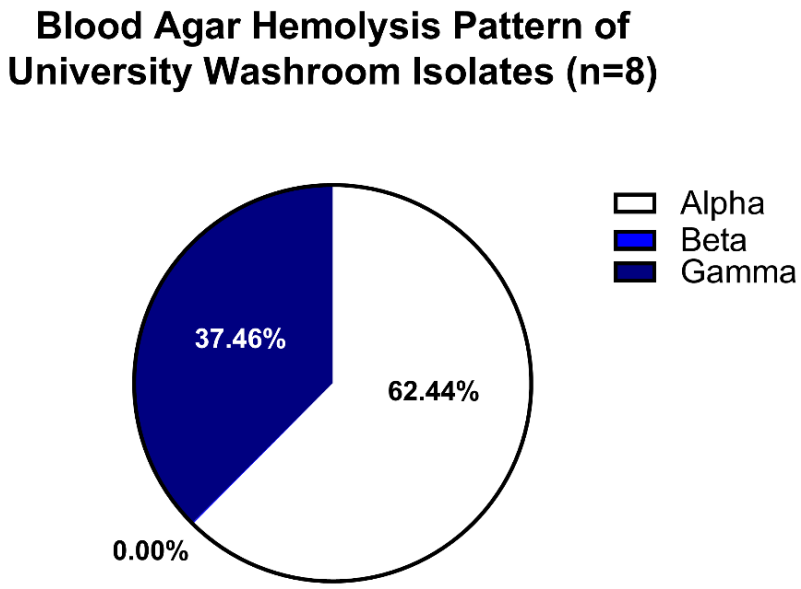

Figure 17: Blood Agar hemolysis pattern of isolates from university washroom water sample where none of the isolates had shown β-hemolytic pattern

3.6 Comparison of hemolysis pattern among clinical and non-clinical isolates:

Twenty-one (24.70%) among 85 isolates from clinical sample showed α-hemolysis whereas 15 (39.47%) isolates among 38 isolates of non-clinical sample showed α-hemolysis. In case of clinical samples, 50 (58.52%) isolates showed β-hemolysis but only 12 (31.58%) isolates showed β-hemolysis from non-clinical samples. A total of 14 (16.47%) isolates showed γ-hemolysis pattern from clinical samples whereas 11 (28.95%) isolates from non-clinical sample showed γ- hemolysis. (Figure 18)

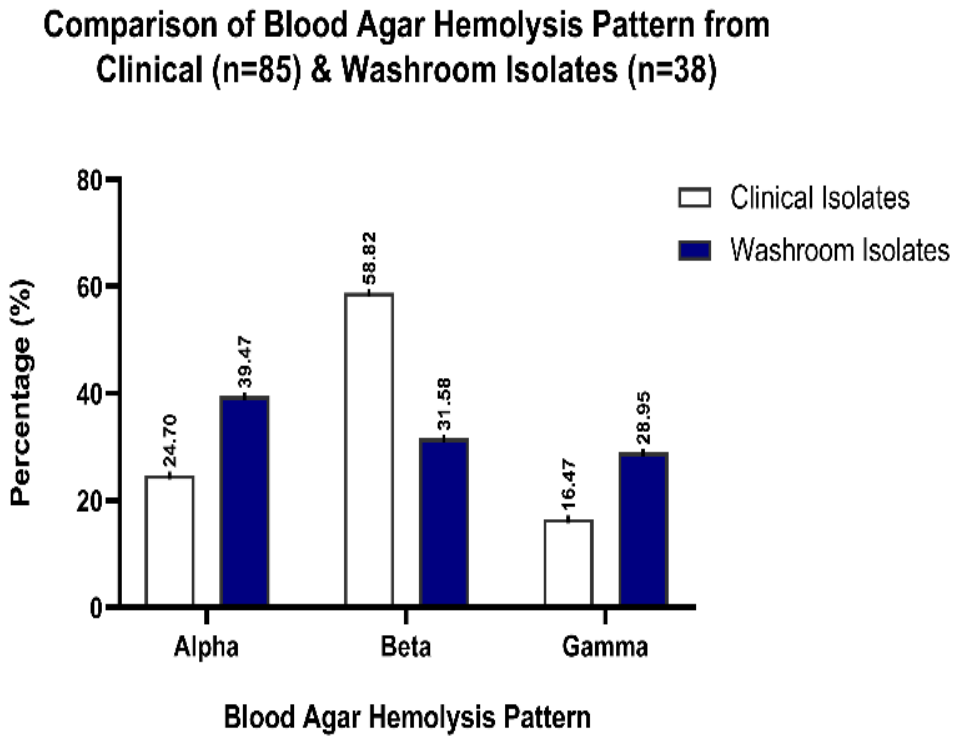

Figure 18: Comparison of Blood Agar hemolysis pattern among clinical and non-clinical isolates which showed 58.82% clinical isolates and 31.58% non-clinical isolates exhibited β-hemolytic pattern.

Chapter 4

Discussion:

UTI caused by UPEC is one of the most prevalent infectious diseases leading to renal failure (Bien et al., 2012b). The degree of pathogenicity of UPEC strains is dependent on the presence of virulence factors.

In our study, we found prevalence of virulence genes in clinical samples was higher than the non-clinical samples. This study showed that *fim*H adhesion gene was the most common and present in 68 (80%) uropathogenic *E. coli* isolates which is in agreement with studies conducted in Ethiopia, 82% (Dadi et al., 2020b). *Fim*H is the type 1 fimbriae that have shown to enhance bacterial survival, to stimulate mucosal inflammation, and to promote invasion and growth as a biofilm. (Bien et al., 2012b) . Pyelonephritis associated pili (*pap*) gene was found in 38 (44.7%) *E. coli* isolates in this study but the frequency was lower in study conducted in Iran, 16.6% (Neamati et al., 2015). P fimbriae are the second common virulence factor of UPEC, which plays an important role in the pathogenesis of ascending UTIs and pyelonephritis in humans. (Bien et al., 2012b). The variation in frequency of pap gene among different studies can be due to the fact that UPEC strains use a diverse group of adhesins to bind to the urinary epithelial cells and initiate the infection (Neamati et al., 2015). Uropathogenic *E. coli* secretes toxins like α-haemolysin (*hly*) and cytotoxic necrotizing factor (*cnf*). *Hly* Alpha promotes bladder cell exfoliation and cell lysis, which facilitates iron and nutrient acquisition by the bacteria. *Cnf* involved in bladder cell exfoliation and increased levels of bacterial internalization (Dadi et al., 2020b). In this study 42 (49.41%) uropathogenic *E. coli* isolates carried hemolysin (*hly*) gene which is comparable to studies conducted in Ethiopia, 51.5% (Dadi et al., 2020b). This indicates that hemolysin may be responsible for clinical manifestation in UTI patients. Alpha-hemolysin encoded by *hly* is an extracellular cytolytic protein toxin that is produced by up to 50% of UPEC isolates. Alpha-hemolysin has been associated with clinical severity in UTI patients (Dadi et al., 2020b). In our study we found *cnf* in 6 (7.06%) isolates of *E. coli* but the frequency was higher in study conducted in Ethiopia, 29% (Dadi et al., 2020b). Furthermore, siderophores (iron chelating molecule) encoding genes were also detected which allow *E. coli* to obtain iron from the host, as well as in its colonization and survival; at the same time. Among four distinct siderophore systems found in *E. coli*, *iuc*C genes which encodes for aerobactin system and *fyu*A encodes which yersiniabactin system were detected from our study samples. We found *iuc*C in 26 (30.59%) isolates and *fyu*A in 23 (27.05%) isolates.

From clinical point of view UTI is classified as upper (proximal) urinary tract infection and lower (distal) urinary tract infection. Upper urinary tract infection includes pyelonephritis while lower urinary tract infection includes cystitis and urethritis. The common clinical symptom of upper urinary includes flank pain, fever, chills and costovertebral angle tenderness. The common clinical symptom of lower urinary includes dysuria and frequency. The above-mentioned clinical symptoms of UTI that is correlated with virulence genes should be interpreted in this aspect. The difference in virulence genes prevalence between our study and different studies abroad may be due to sample size difference and methodology difference (Dadi et al., 2020b).

The emergence of drug resistant microorganism among UPEC strains increase the serious threat to global health. Therefore, knowledge regarding local prevalence of UPEC and antimicrobial resistance is essential for optimal management of UTI (Shah et al., 2019b). Thus, this study was designed to find resistance pattern among clinical and non-clinical samples. In clinical samples, we found resistance against norfloxacin in 72.94% isolates where as the resistance was found in 52% of isolates in a study conducted in Nepal (Shah et al., 2019b). In our study resistance to meropenem and nitrofurantoin were 57.65% and 72.94% of isolates, respectively but in a study conducted in Nepal, resistance to meropenem and nitrofurantoin were found in 26% and 38% of isolates, respectively (Shah et al., 2019b). The resistance to ceftriaxone was found in 68.24% of isolates in the current study which was higher than 49% which was found in the study of Nepal (Shah et al., 2019b). In our study, we found resistance to ciprofloxacin was 71.76% whereas it was 47.2% in the city of Bamenda (Fonseca-Martínez et al., 2023). We found resistance to cefepime in 58.82% of isolates but resistance was found in lower percentage in a study of China, 11.6% (Wang et al., 2014). In this study, the highest resistance was found against amoxicillin, 92.94% but it was found in a lower percentage in a study of Russia, 69.8% (Sarra et al., 2021). Moreover, this study revealed that 78.82% of *E. coli* isolates were resistance to cefixime but resistance was in lower percentage in a study in Pakistan, 55% (Ehsan et al., 2023). In our study, we also found that 77.65% of clinical isolates were resistance against azithromycin.

Escherichia coli strains that cause extraintestinal infections possess numerous virulence factors, including hemolysin production. *E. coli* α-hemolysin (*hly*) produces large, clear zones

of hemolysis around colonies on blood agar. The hemolysin is present in cell-free filtrates and is the best characterized member of the RTX (repeats in toxin) toxin family. *Hly* lyses cells by the creation of pores in the target cell membrane and affects erythrocytes, leukocytes, and renal tubular cells. Its activity on polymorphonuclear granulocytes liberates leukotrienes, histamine, and ATP and is neutralized by specific antiserum (Kerényi et al., 2005). All *hly* positive *E. coli* can exhibit highly hemoytic (β-hemolysis), moderately hemolytic (α-hemolysis) and poorly hemolytic (γ-hemolysis) (Bashir et al., 2012). In our study, we found 50 (58.52%) isolates showed β-hemolysis, 21 (24.70%) isolates showed α-hemolysis and 14 (16.47%) isolates showed γ- hemolysis.

Household water supply provided by the municipal authority is an important shared resource for millions of people living in Dhaka metropolitan area. *E. coli* is commonly isolated from water sources, including the municipal water supply of Dhaka city (Talukdar et al., 2013).

Unsafe water supplies continue to raise public health concerns, especially in urban areas in low resource countries (Talukdar et al., 2013). To understand the extent of public health risk attributed to supply water in Dhaka city, Bangladesh, *Escherichia coli* isolated from tap water samples collected from different households, hospital washrooms, university washrooms and public washrooms. The samples were characterized for their antibiotic resistance and pathogenic properties.

Antimicrobial resistance among enteropathogens, including *E. coli* has been reported to be increasing in recent years (Pitout & Laupland, 2008), sometimes leading to point-break situations where no antibiotic treatment options remain (Lynch et al., 2013). These situations are of serious concern in developing countries where enteropathogens are frequently encountered and cause life-threatening infections(Talukdar et al., 2013).

The antibiotic resistance factors is carried by mobile genetic elements such as plasmids or transposons (Talukdar et al., 2013). Horizontal gene transfer (HGT) is one of the most common mechanisms by which antibiotic resistance traits are transferred from one organism to another. In *Enterobacteriaceae,* plasmids are the major vectors for HGT. In vivo transfer of resistance traits between *Enterobacteriaceae* in natural ecosystem has been reported (Martinez, 2009).

Generic *E. coli* are frequently used as indicator bacteria to monitor the trends in antimicrobial resistance because they are the prevalent commensal enteric bacteria in humans and animals, can be cultured easily and inexpensively (Talukdar et al., 2013), and they can acquire and preserve resistance genes from other organisms in the environment and in animal populations. *E. coli* is also considered as a good indicator of the selective pressure imposed by antimicrobial use in food animals (Talukdar et al., 2013)

The presence of *E. coli* in the water sample indicates the presence of microorganisms that might be potentially hazardous for human health and presence of virulence factors which is also present in *E. coli* isolated from UTI patient indicates supply water can cause community acquired UTI.

It becomes a serious threat when these *E. coli* exhibit resistance to multiple antibiotics and pathogenic properties that cause diseases like UTI in people who use this contaminated water. Studies conducted in other countries demonstrated the presence of MDR pathogenic bacteria in water sources including rivers, ponds and lakes (Talukdar et al., 2013)

In our study, we found UTI causing virulence genes in the sample supply waters. The Type 1 fimbriae, *fim*H, was found in the highest number (52.63%) of *E. coli* isolates. The other genes such as *pap*C, *hly*, *iuc*C, *fyu*A and *cnf* were also present in a significant number. All the *E. coli* isolates collected from water samples, were resistant to at least three classes of antibiotics tested which defines them as MDR.

Due to Bangladeshi culture and religious believe, people in Bangladesh use the supplied water for maintaining hygiene and sanitization. The household water supply is normally used without any pre-treatment. Hence, the presence of multi-drug resistant uropathogenic *E. coli* in household water supply in Dhaka has important implications for health of the urban population. The MDR *E. coli* may represent an important reservoir of genetic determinants of antimicrobial resistance that can easily be transferred to other microorganisms in the environment through HGT, including the potential human pathogens.

4.1 Limitations:

One of the limitations of the study is that we could not collect water samples from the entire areas of the city. Therefore, the level of contamination of water might not representative of other areas of the city. However, most Dhaka residents rely on the municipal water, which is mainly abstracted from underground sources and circulated to households following the same system. As such, the risk of exposure to MDR pathogenic organisms via water supply system in Dhaka residents would not be significantly different from one area to the other. Moreover, Whole Genome Sequencing was not done which could help us to find whether there were similar strain of UPEC present in clinical and non-clinical samples.

Chapter 5

Conclusion

Escherichia coli is an important cause of UTIs, enteric infections, and systemic infections in humans. In our study, we found similar virulence factors in both clinical and non-clinical isolates. So far, this is the first molecular study of *E. coli* strains isolated from UTI patient and non-clinical (water) samples in Dhaka city of Bangladesh. This comparison can be a step towards improving the knowledge regarding their virulence genetic determinants and antibiotic resistance pattern. Like many pathogens, UPEC employs multiple virulence mechanism to evade and manipulate host barrier defense and innate immune responses. Our increased understanding of these host-pathogen interactions has unfolded novel therapeutic strategies that could be used to combat UPEC mediated UTI. In future more detailed study of virulent genes and their association with antibiotics sensitivity studies will be steppingstone towards epidemiological research on UTI in Bangladesh. Since the presence to several putative virulence genes has been positively linked with the pathogenicity of UPEC. These data can be used as a baseline for further investigation of association with UTI causing *E. coli* found in various group of patients and supply water from all over the country. As an emerging issue, continuous surveillance is needed to understand antibiotic resistant pattern among UPEC strain in this region.

Poor sanitation and hygiene, overcrowded situation and lack of knowledge are the some of the precipitating factors for massive amount of UTI patients in Bangladesh. Effective prevention strategies are needed all over the country to limit the widespread circulation of these bacteria in the community and to contain the threat of emerging drug resistance among various enteric bacterial pathogens.

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