Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

ANTIBIOGRAM PROFILING OF UROPATHOGENIC *E.COLI* FROM URINE SAMPLES OF CATHERISED PATIENTS WITH SPECIAL REFERENCE TO FIM H GENE

Parveen Anjum Sheiq[1], Khutija Sarah[2*], Lubna Iqbal[3]

Assistant Professor[1], Department of Basic Medical Sciences, Unaiza College of Medicine, Al Qassim University, KSA.

Assistant Professor[2], Department of Microbiology, Rama Medical College Hospital and Research Centre, Mandhana, Uttar Pradesh, India.

Clinical Microbiologist[3], Virinchi Hospital, Hyderabad, India.

Corresponding Author: Dr. Khutija Sarah

Email ID: dr.muzu@gmail.com

ABSTRACT

BACKGROUND : Most hospital visits globally are caused by urinary tract infections, which are also a major cause of morbidity and comorbidity in patients with underlying medical conditions. Urinary tract infections (UTIs) are a common health concern caused by a variety of bacteria, including uropathogenic *Escherichia coli* (UPEC). UPEC strains are the most common pathogens, accounting for 85% and 50% of community and hospital-acquired UTIs. UPEC strains have unique virulence characteristics, including type 1 fimbriae, which can result in worsening of UTIs.

AIM AND OBJECTIVE: To study the Antibiogram profiling of Uropathogenic *E.coli* from urine samples of catherised patients with special reference to fim H gene.

MATERIAL AND METHODS: This was a Hospital based prospective cross sectional study carried out in the Department of Microbiology. A total of 1000 freshly voided midstream urine samples were collected in a sterile wide mouth container from patients whose preliminary routine urine tests were positive for pus cells and albumin. Urine samples collected for this investigation (fever > 38ºC, urgency, frequent dysuria, or suprapubic discomfort) were processed within one hour for aerobic bacterial culture. If delayed, samples were refrigerated and processed within 4 - 6 hrs. The identification , biochemicals and the AST pattern was done according to the CLSI guidelines 2023. The DNA was extracted using the Qiagen DNA Extraction kit and the FIM H gene was detected by the conventional PCR assay.

RESULTS: In the present study total of 1000 urine samples were received in which 400 (40%) urine samples were showing significant growth for UTI. The ratio of females 254 (63.5%) were more as compared to that of the males 146 (36.5%) with the maximum age of 31-40 (43%) years of age followed by 21-30 (24.5%) being affected the most. In the age group of 0-10 years and above 71 years was the least affected with the infection. It was also observed that the maximum number of isolates were from the *E.coli* 150 (37%) followed by *Klebsiella pneumonia* 100 (25%),

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Pseudomonas aeruginosa 55 (13.7%), *Acinetobacter baumanii* 26 (6.5%) , and *Staphylococcus aureus* 30 (7.5%) for gram positive followed by *Proteus* 23 (5.75%) least for *Enterococcus* with 4%. The days of catheterization were observed to be the maximum in 4-7 followed by 8-12 days. It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates. The Molecular characterization reveals that in the current study there was FIM H gene studied. In the fim H gene there were 135 (91%) positive cases and negative were 15 (10%). It was observed that from the 5 (35.7%) negative cases that were negative for the association of biofilm formation there were 126 (92.6%) which were positive for the virulence gene Fim H gene.

CONCLUSION: The study found that more than 90% of *E. coli* isolates contained the FimH gene. FimH's high binding ability may result in increased pathogenicity of *E. coli*, making it a potential diagnostic marker and/or vaccination candidate. Regular check-ups and strict adherence to antibiotic stewardship protocols can lower the cost of UTI prophylaxis. By performing these regular examinations, the expense of UTI prevention can be decreased.

KEYWORDS: UTI, Bacteriological profiling , Antibiotic sensitivity testing, CLSI, Molecular characterization, fimH

INTRODUCTION

Urinary tract infections (UTIs) are inflammatory disorders caused by the rapid multiplication of various pathogens in the urinary apparatus, resulting in changes to the urinary tract and kidneys' proper function. UTI is particularly a large concern for females about 50-80% of the female population suffers from UTI at least once in their lives, with 20-50% of them having recrudescent occurrences [1]. Healthcare-associated infections are an important cause of prolonged hospital stay, around the globe. Urinary tract infections (UTIs) are considered as one of the most common healthcare-associated infections (HCAIs) with an estimated prevalence of 1–10% accounting for 30–40% of all HCAIs reported by hospital settings. Majority of infections of urinary tract are directly linked to the widespread use of indwelling catheters in these settings [2]. Urinary tract infections (UTIs) are the most common infections that people get. In critically sick patients, catheter-associated (CA) UTIs can induce bacteremia, and they are the major cause of morbidity and mortality in roughly 10% of hospitalised patients. Females are more susceptible to UTI as compared to males due to the short length of urethra, absence of prostatic secretion, pregnancy and easy contamination of the tract with faecal flora. The risk factors include female gender, extremes of age, diabetes mellitus, and prolonged catheterization duration [3]. The duration of catheterization is the most important factor in the development of bacteriuria, as its daily usage increases the risk of infection by 3–7% [4].

The Center for Disease Control and Prevention (CDC) provides a definition for CAUTI, which pertains to patients who have a catheter inserted and left in place for 48 hours or longer . Catheter-

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

associated urinary tract infection has been a significant factor contributing to illness and death among hospitalized patients [5,6].

CA-UTI accounts for over 80% of infections in catheterized patients admitted in intensive care units (ICUs) during their hospital stay [2]. The important predisposing factors commonly associated with CAUTIs are conditions such as diabetes, immunosuppression, renal insufficiency, and urinary incontinence commonly among neurologic and orthopedic patients [4]. Other factors for CAUTI could be patients staying on catheter for a prolonged duration of time, female and elderly patients, patients with severe illness, catheterization performed under nonsterile conditions, and catheter insertion by undertrained professionals [6].

Urinary tract is a vast reservoir of resistant microorganisms with threat of cross infection [7]. *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Coagulase-negative *Staphylococcus*, and *Enterococcus* species are the important culprits. It can cause genitourinary complications, septicemia, skeletal involvement, and over the years, bladder cancer that causes distress to the patient, prolonged hospital stay, economic loss, and mortality. It can be prevented by maintaining closed urinary drainage system and early removal of catheter. Surveillance, proper training of healthcare personnel, and implementation of bundle care approach aids in reduction of cases in ICU settings.

Escherichia coli is the most common pathogen, accounting for up to 80% of UTI cases [8]. This bacteria causes 85% of UTIs in the population and 50% in hospitals [9]. Uropathogenic *E. coli* (UPEC) strains feature unique virulence characteristics, such as pili or fimbriae, which facilitate attachment to uroepithelial and vaginal cells, resistance to human serum bactericidal action, haemolysin synthesis, and higher levels of K capsular antigen [10,11].

An essential step for beginning and development of UTI is bacterial attachment to uroepithelial cells. *E. coli* attachment is mediated by ligands of bacteria (generally small proteins placed at the tips of bacterial fimbriae) which bind to host cell wall carbohydrate residues, working as receptors . Therefore, the adherence of *E. coli* to host receptors is a function, usually mediated by adhesions of bacteria to host cell receptors [12]. The bacterial attachment permits bacteria to resist mechanical elimination by the flow of urine and bladder emptying and increasing persistence of *E. coli*. UPEC strains produce different types of adhesins, including type 1 fimbriae, which are essential for recognition and attachment to urinary tract receptors [13, 14].

Furthermore, virulence factors of UPEC strains play an important role in the development of UTIs. UPEC-dependent virulence factors include adhesions (type 1 fimbriae, p fimbriae, curli fimbriae, a fibrillar adhesion, and flagellum), aerobactins, hemolysins, and cytotoxic necrotizing factor . The virulence factors play key roles in UPEC colonisation, extraintestinal survival, and the generation of cytopathic consequences. Furthermore, the expression of UPEC-specific virulence factors can contribute to uropathogenicity and worsen UTIs [11].

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Currently UTI is mostly managed empirically without urine culture or susceptibility testing this may lead to the frequent misuse of antibiotics. The antimicrobial susceptibility data of UTI-causing microorganisms is variable it changes from time to time and from place to place. Most commonly UTIs are treated empirically; in that case the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogen and its expected resistance pattern in that geographic area [7]. Among UPEC adhesions, the sticky subunit of type 1 fimbriae, FimH, is a crucial factor, with high tropism for urinary tract receptors; consequently, FimH adhesion is important in colonising diverse niches of *E. coli*. As a result, regular monitoring of the etiologic agents of UTI and their resistance patterns in the population is crucial. Therefore, the current study was conducted to examine the antibiogram in catheterized patients with urinary tract infections with special reference to FIMH gene.

MATERIAL AND METHODS

This was a Cross sectional study carried out in the Department of Microbiology for a period of 12 months i.e, between March 2023 to March 2024. A total of 1000 freshly voided midstream urine samples from the people whose initial routine urine tests were positive for pus cells and albumin were collected in a sterile wide mouth container. Within an hour of being collected, all urine samples were processed for aerobic bacterial culture. If samples were delayed, they were refrigerated and processed in 4 to 6 hours.

INCLUSION CRITERIA:

Patients of all the age groups and both sex with indwelling urinary catheters for at least 2 days, who were suffering from the symptoms of UTIs (fever > 38ºC, urgency, frequency dysuria or suprapubic tenderness) were included in this study .

EXCLUSION CRITERIA:

The non-catheterized patients with urinary tract infections was excluded from the study.

Patients who were undergoing treatment for UTI when the catheter was inserted were excluded.

Patient with symptoms of UTI prior to the catheterization were also excluded.

Microscopic Study

One of the diagnosis criteria of UTI was based on microscopic findings of more than 10 pus cells/ high power field (40×) in urine were included in the study.

Collection and process of urine samples

Midstream urine samples were collected in a sterile container and processed within two hours of collection. Urine samples were also centrifuged, and the resulting sediment was examined under a microscope for red blood cells (RBCs), leukocytes, epithelial cells, casts, crystals, and parasites. In normal urine sediment, a few RBCs, pus cells (0-5/high power field), and epithelial cells may

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

be present. The number of epithelial cells was reported as "few," "moderate," or "many" per low-power field.

Isolation and Identification of Uropathogens

Using a calibrated (1 L) loop, a urine sample was inoculated onto a standard culture media called Cystine-Lactose- Electrolyte-Deficient (CLED) agar.
For 18 hours, culture plates were incubated in an ambient air incubator at 35–37°C. The culture plates were examined for the presence of bacterial colonies after the allotted time was over. Using the colony count method, their growth was classified as significant or not. By growing isolated colonies on various media, such as MacConkeys agar and blood agar, they were further described based on cultural traits
In cases where culture (growth) was unsuccessful, the plates were incubated at 37°C for an additional 24 and 48 hours. The identification, biochemicals, and AST pattern were completed in accordance with CLSI guidelines for 2023 [15]. All chemicals and reagents required for culture media were purchased from HiMedia Laboratories Pvt Ltd., Mumbai.
Urine culture using the calibrated loop/surface streak method.

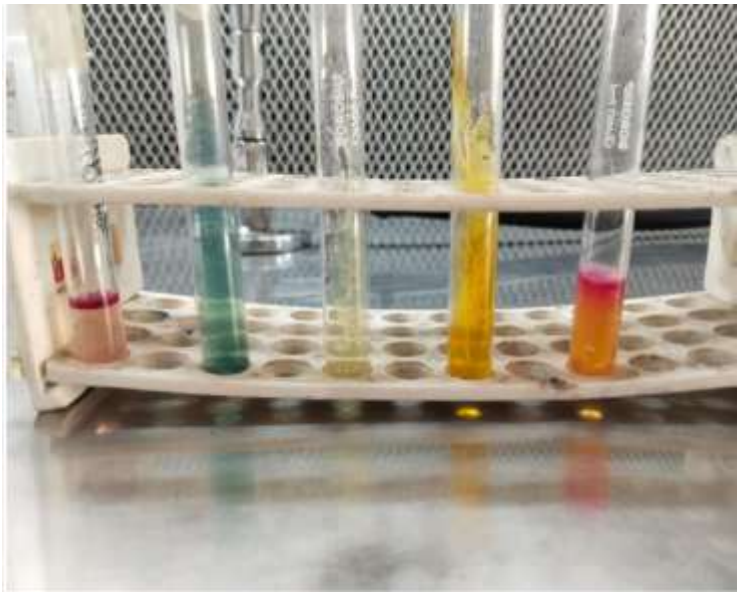


Figure 1: Biochemicals test for (a.) *E.coli* (b.) *S.aureus* ; (c.) *Klebsiella pneumonia* ; (d.) *Pseudomonas aeruginosa*

Genotypic Detection Method

Detection of Resistance Gene fim H from uropathogenic *E.coli* was performed using PCR assay [10].

The Bacterial DNA was extracted. The primers were purchased from "**Saha gene'** and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

The Molecular Characterization of the Gene by phenotypic method

The DNA was isolated using the Qiamp DNA Blood Mini Kit (QIAGEN, Germany) as per the manufactures guidelines. The extracted DNA and the gene was confirmed by the PCR to detect the presence of the fim H gene.

The DNA was eluted in 60 µl elution buffer and preserve at -20 °C till PCR analysis.

Figure No. 2: The fimH primer

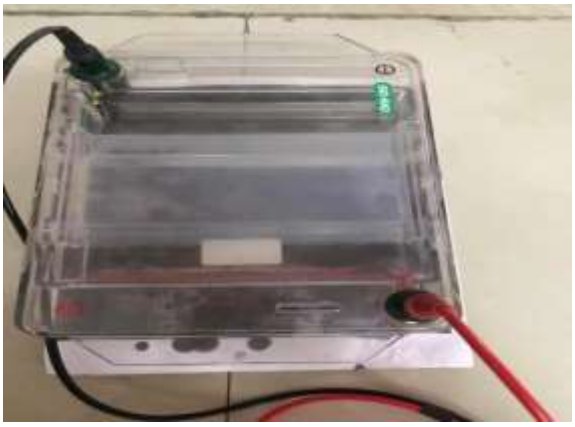


Figure No.3: Gel Electrophoresis for the DNA Extraction

The Primers

Gene	Primer sequence	Length (bp)	Reference

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

fimH	Forward- 5-GAGAAGAGGTTTGATTTAACTTATTG-3	559bp	269 [16]
	Reverse 5-AGAGCCGCTGTAGAACTGAGG- -3		

Table No. 1: Primers for FIM H Gene Polymorphism

- The primer for FimH for the detection of gene of interest was designed and confirmed by NCBI.

The Polymerase Chain Reaction

For the PCR amplification, 2 µl of template DNA was added to 18 µl reaction containing 10 µl of Qiagen master mix, 2 µl of primer mix (1 µl each of the respective forward and reverse primers) and 6 µl of molecular-grade water.

The cyclic conditions for FIMH gene, initial denaturation at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s and 72 °C for 1 min 30 s were followed by extension of 72 °C for 10 min.

The PCR cycling conditions

Step	**Program** **FIM H GENE** **Time Temperature**	**Cycles**
Initial denaturation **Denaturation** **Annealing** **Extension**	15 min 95 ºC 30 s 94 ºC 1 min30 s 59 ºC 1 min 30 s 72º C	30
Final extension	10 min 72º C	

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Table No. 2 : The PCR cycling conditions to amplify FIMH gene fragments.

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System

The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments. The resulting PCR product was subjected to 1% agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA). A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample [16].

Statistical analysis

Data recorded on the report form and structured proforma were subsequently entered into a spreadsheet. Data management and analysis were performed using Microsoft Excel.

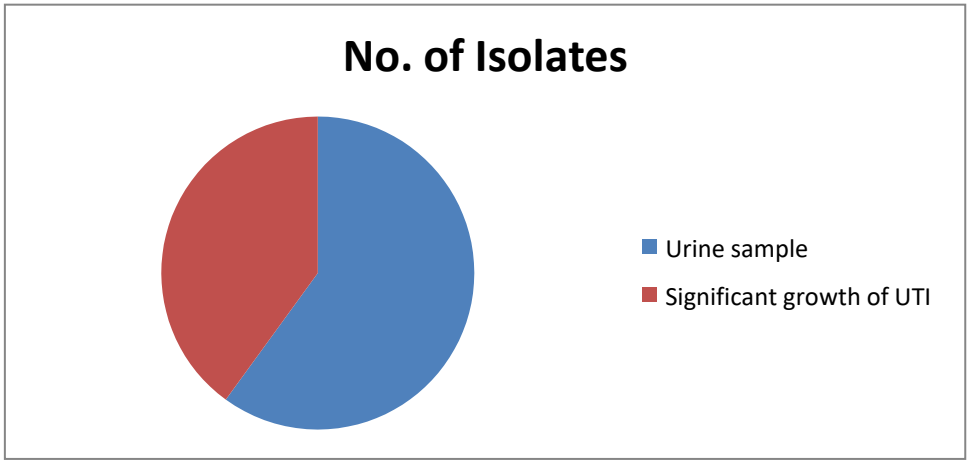
RESULTS

In the present study out of the 1000 urine samples received in the Microbiology Laboratory 400 urine samples shows significant growth for UTI. Therefore, the prevalence rate of UTI was found to be 40%.

S.No.	Type of Isolates	Total No. of samples (n=1000)	Percentage
1.	UTI	400	40%
2.	Other Isolates	600	60%

Table No. 3 : Samplewise distribution of the clinical isolates

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024



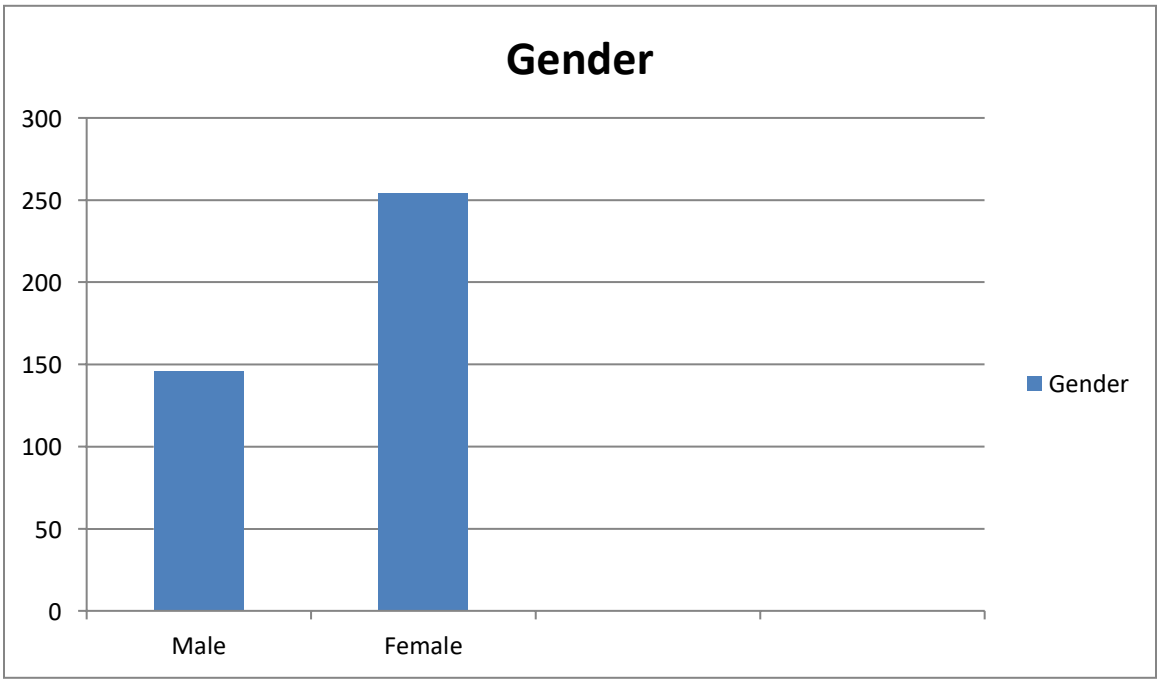
Graph No. 1: Graphical Representation of Samplewise distribution of the clinical isolates

S.NO.	GENDER	TOTAL NO. OF ISOLATES (N=400)	PERCENTAGE
1.	Male	146	36.5%
2.	Female	254	63.5%

Table No. 4: Genderwise distribution of the Isolates

The ratio of females 254 (63.5%) were more as compared to that of the males 146 (36.5%) [Table no. 4] .

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024



Graph No. 2: Graphical Representation of the Genderwise distribution

S.NO.	Age	No. of Isolates (n=400)	Percentage	
1.	0-10	3	0.75%	
2.	11-20	12	3%	
3.	**21-30**	**98**	**24.5%**	
4.	**31-40**	**172**	**43%**	

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

5.	41-50	61	15.2%	
6.	51-60	25	6.25%	
7.	61-70	16	4%	
8.	≤ 71	13	3.25 %	

Table No. 5: Agewise distribution of the Isolates

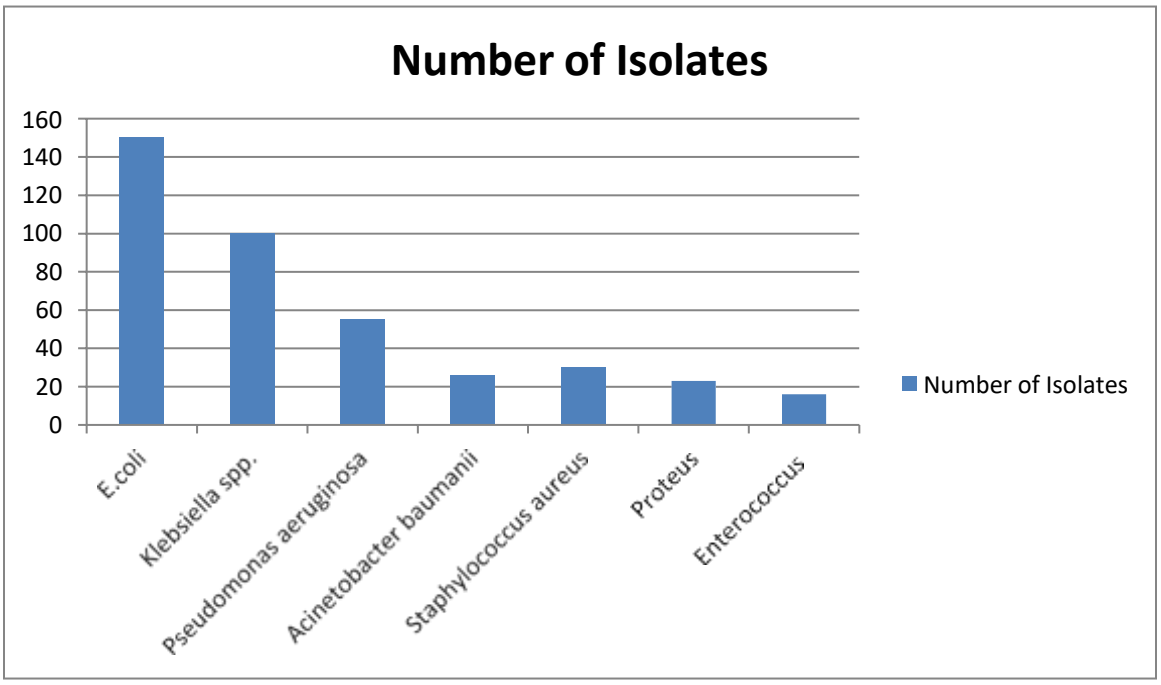
From the present study it was also noted that the age group of 31-40 (43%) years of age followed by 21-30 (24.5%) was affected the most. In the age group of 0-10 years and above 71 years was the least affected with the infection.

To study the different Phenotypic Tests For the detection and Identification of:. identified by studying colony characteristics, production of pyocyanin pigments, grapelike odour, growth at 42^{o}C, motility test, Gram staining, and biochemicals was performed according to the CLSI guidelines [15].

Type	No. of Isolates	Percentage
E.coli	150	37%
Klebsiella spp.	100	25%
Pseudomonas aeruginosa	55	13.7%
Acinetobacter baumanii	26	6.5%
Staphylococcus aureus	30	7.5%
Proteus	23	5.75%
Enterococcus	16	4%
Total	400	

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Table 6 : Types and Number of isolation of patients studied



Graph No. 3: Graphical Representation of the Types and Number of isolates of patients studied

From the Table No. 6 it was observed that the maximum number of isolates were from the *E.coli* 150 (37%) followed by *Klebsiella pneumonia* 100 (25%), *Pseudomonas aeruginosa* 55 (13.7%), *Acinetobacter baumanii* 26 (6.5%) , and *Staphylococcus aureus* 30 (7.5%) for gram positive followed by *Proteus* 23 (5.75%) least for *Enterococcus* with 4%.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

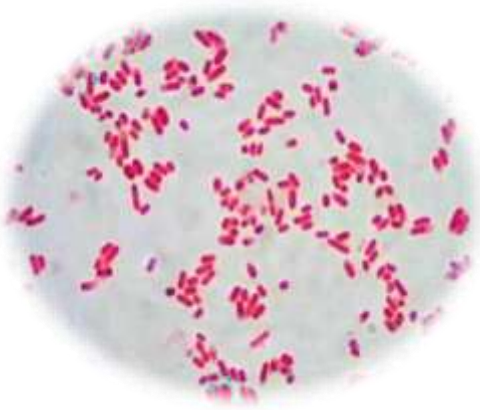


Figure No.4: Microscopic examination of *E.coli*

Days of catheterization	Gender		Total
	Female	Male	
1-3	15 (5.9%)	5 (3.4%)	20(5%)
4-7	**155 (61%)**	**72(49.3%)**	**227 (56.7%)**
8-12	**81(31.8%)**	**65 (43.8%)**	**146 (36.5%)**
13-14	3 (1.1%)	4 (2.7%)	7(1.75%)
Total	254	146	400(100%)

Table No. 7 : Days of catheterization- Frequency distribution of patients studied

The days of catheterization were observed to be the maximum in 4-7 followed by 8-12 days [Table No. 7].

Variables	Gender	Total

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

	Female	Male	
DIABETICS			
• No	225 (88.5%)	121 (82.8%)	346(86.5%)
• Yes	29 (11%)	25 (17.1%)	54 (13.5%)
HYPERTENSION			
• No	195 (76.7%)	90 (61%)	285 (71.25%)
• Yes	59(23.2%)	56 (38%)	115 (28%)
KIDNEY DISEASE			
• No	200 (78.7%)	118 (80.8%)	318 (79.5%)
• Yes	54 (21.2%)	28 (19.1%)	82 (20.5%)
Total	254(100%)	146(100%)	400 (100%)

Table No. 8 :COMORBID CONDITIONS- Frequency distribution of patients studied

From the Table 8 it was clear that out of the total samples the comorbidity with diabetes was found to be low (13.5%). It was also observed that patients with hypertension and the kidney disease were observed to be low as compared to the healthy individuals.

Days since Fever	Gender		Total
	Female	Male	
1-3	108(42.3)	59 (40%)	167(41%)
4-7	140(55%)	81(55%)	221(44%)
8-12	6 (2.36%)	6(4%)	12 (3%)
Total	254 (100%)	146(100%)	400(100%)

Table No. 9: Days since Fever (DAYS)- Frequency distribution of patients studied

The maximum number of days with fever was observed to be maximum in 4-7 days, least for 8-12 days [Table No. 9].

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Variables	Gender		Total
	Female	Male	
DYSURIA			
• No	54(21%)	19 (13%)	73 (18.5%)
• Yes	200(78%)	127 (86.9%)	327 (81.75%)
ABDOMINAL PAINS			
• No	66 (25%)	35 (23%)	101 (25%)
• Yes	188 (74%)	111 (76%)	299(74%)
CHILLS			
• No	74 (29%)	22 (15%)	96 (24%)
• Yes	180 (70.8%)	124 (84.9%)	304 (76%)
Total	254 (100%)	146(100%)	400(100%)

Table No. 10 :SIGNS AND SYMPTOMS

From the Table 10 it was observed that dysuria observed in female was 200 (78%) in male 127 (86.9%) , the abdominal pain in female 188 (74%) followed by the chills with the same ratio of female 180 (70.8%).

The Identification of Drug Resistance Pattern : Antibiotic susceptibility testing was performed by Kirby bauer Disk diffusion method as per the CLSI guidelines [15].

Antibiotic susceptibility testing: The antibiotic disks (HiMedia) used were ampicillin (10 μg), piperacillin/tazobactam (100/10 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), amikacin (30 μg), gentamicin (10 μg), cotrimoxazole (1.25/23.75 μg), cefoperazone + sulbactam (75/30 μg), imipenem (10 μg), meropenem (MRP; 10 μg) and Nitrofuratoin(30 μg) . Antibiotic susceptibility will be determined by using standard Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines 2023 [15]

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Antibiotic	RESISTANCE N=400	SENSITIVITY N=400
AMP	371 (92%)	29 (7.25%)
PTZ	170 (42%)	240 (60%)
CTR	350 (87%)	50(12%)
CTX	350 (87%)	50(12%)
CIP	371 (92%)	29 (7.25%)
NOR	348 (87%)	52 (13%)
AMK	90 (22%)	310 (77.5%)
GEN	340 (85%)	60 (15%)
COT	348 (87%)	52 (13%)
CFS	60 (15%)	340 (85%)
IMP	30 (7%)	370 (93%)
MERO	75 (18%)	325 (81.5%)
NIT	30 (7%)	370 (93%)
Total	400(100%)	400 (100%)

Table 11: Antibiotic resistance/Sensitivity pattern of patients studied

In the present study the resistant rate for Ampicillin was observed to be 92% and Imipenem and Nitrofurantoin were sensitive with (93%).
It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

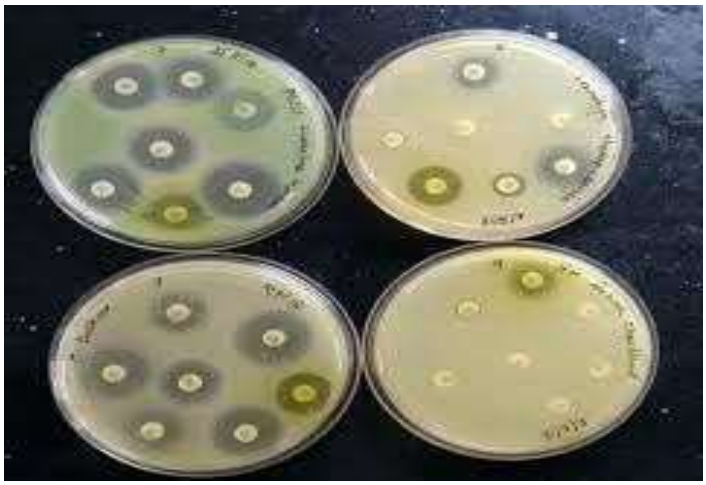


Figure No.5: AST plate for *E.coli*

against Antibiotics ; zones of growth inhibition for Meropenem referred > or = 14 (susceptible), 12-13 (intermediate) and < or = 11 (resistant) mm.

Variables	No. of Patients	%
FIM H		
• Negative	15	10
• Positive	135	90
Total	150	100.0

Table 12: Detection of fim H Gene

In the current study there was fim H gene studied. In the fim H gene there were 135 (91%) positive cases and negative were 15 (10%).

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Variables	Biofilm Result		Total
	Negative	Positive	
fim H			
• Negative	5(35.7%)	10(7.3%)	15(10%)
• Positive	9(64.2%)	126(92.6%)	135(90%)

Table 13:FIM H GENE-Frequency distribution in relation to Biofilm results

In the current study it was clear that from the 5 (35.7%) negative cases that were negative for the association of biofilm formation there were 126 (92.6%) which were positive for the virulence gene Fim H gene Table.13

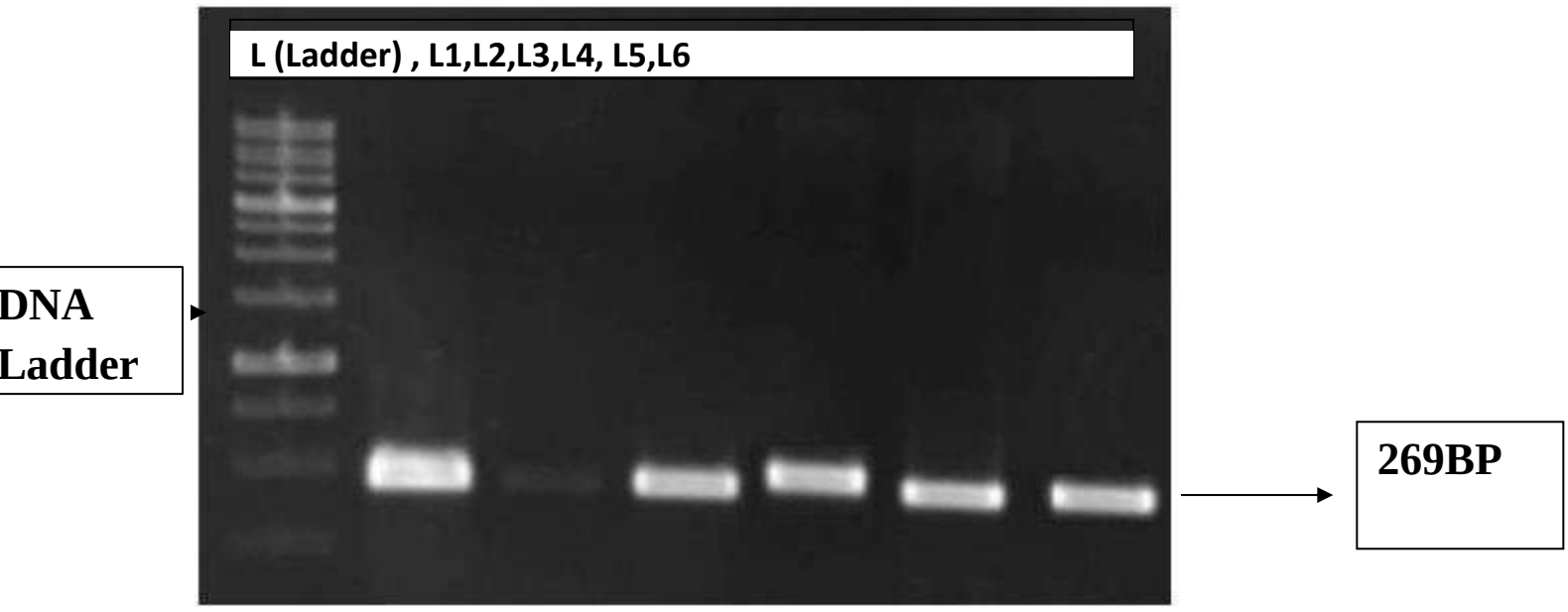


Figure No.6: The Gene Extraction FIMH gene:

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

L corresponds to the DNA Ladder; L1 corresponds to the sample positive for FIMH gene;L2 is the Negative Control for FIMH gene; L3-L6 are the sample positive for FIMH gen

DISCUSSION

Urinary Tract Infection (UTI) is one of the most common infections encountered in clinical practice. Empirical treatment for both complicated and uncomplicated UTI has been practiced throughout the world because a failure in timely treatment might lead to increased morbidity and mortality [1,3]. Urinary tract infection (UTI) is one of the most common infectious diseases worldwide. It is more prevalent among females with an incidence rate 50-fold higher among the 20–50 years age group.

UTI is a public health problem accounting for more than 15% of all the antibiotics prescriptions among outpatients [4]. Infections forming biofilm are associated with AMR and recurrent UTIs [5,6] both of which are currently increasing globally [7]. One of the most typical infections, particularly among women, is UTI. According to the National Ambulatory Medical Care Survey, UTI alone accounts for up to one million visits to hospital emergency rooms and roughly seven million outpatient department (OPD) visits, leading to approximately 100,000 inpatient stays [12].

In the present study the prevalence of UTI was found to be 40%. This finding was similar to the study performed by the other authors Ahmad S et al and Suhail A. et al., where the prevalence was found to be 20.54% and 32% respectively [17,18].
In the current study the maximum number of isolates were from the Females where the ratio of females 254 (63.5%) were more as compared to that of the males 146 (36.5%) . This study was similar to the study by Suhail A. et al, and Martin Odoki et al., in 2019 where the ratio of females was more as compared to the males [18, 19].
Higher prevalence of UTI among females is due to various factors that predispose women to UTI [20]. From the present study it was also noted that the age group of 31-40 (43%) years of age followed by 21-30 (24.5%) was affected the most. In the age group of 0-10 years and above 71 years was the least affected with the infection. This study was parallel to the study performed by author [19].
It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates. It was observed that the maximum number of isolates were from the *E.coli* 150 (37%) followed by *Klebsiella pneumonia* 100 (25%), *Pseudomonas aeruginosa* 55 (13.7%), *Acinetobacter baumanii* 26 (6.5%) , and *Staphylococcus aureus* 30 (7.5%) for gram positive followed by *Proteus* 23 (5.75%) least for *Enterococcus* with 4%. Similar study was performed by the other research workers where among 206 bacterial isolates obtained from 417 urine samples, majority of the isolates (99%) were Gram negative bacteria which included *Escherichia coli* (56.79%), *Klebsiella sps* (19.9%), *Pseudomonas sps* (6.3%), *Pro- teus sps* (5.8%), *Enterobacter sps* (3.8%), *Citrobacter sps* (1.4%), *Enterococcus sps* (0.9%), and other *NFGNB* (4.8%) [21].

This finding is similar to other reports which suggest that gram negative bacteria, particularly *E. coli* was the commonest pathogens isolated from patients with UTI [22-24]. The incidence of *E.*

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

coli in our study was higher when compared with the Nigerian studies reporting 42.10% [25] and 51% [26]. Most of the studies conducted in Africa and Arab countries showed less than 50% isolation of *E coli* from the UTI patients but re- ported a higher percentage (29%) of *S aureus* as second most frequently isolated bacteria from UTI cases.
In the present study it was found that antimicrobial resistance was seen both in Gram-positive and Gram-negative bacteria.

In the present study the resistant rate for Ampicillin was observed to be 92% and Imipenem and Nitrofurantoin were sensitive with (93%). It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates. The high resistance in trimethoprim/sulfamethoxazole susceptibility pattern may be due to non-judicious use and over-the-counter selling of this antibiotic [27].
The antibiotic susceptibility of uropathogenic bacteria is known to change with time and is inconsistent in different regions . Here, we have described the impact of the best antimicrobials with low resistance rate (overall resistance %) against the uropathogens in this study. The best antimicrobials for Gram-negative organisms was meropenem, amikacin , gentamicin , tobramycin , and cefepime and moderate resistance rate were ciprofloxacin , cefotaxime , cefoxitin , norfloxacin , ceftazidime , cefpodoxime , piperacillin/tazobactam ,
and cefuroxime. It was noteworthy that high resistance rate was found to be against cefuroxime ,trimethoprim/sulfamethoxazole , nitrofurantoin , amoxicillin/clavulanic acid piperacillin and ampicillin .
In contrast, the antimicrobial sensitivity pattern of antimicrobials for Gram-positive organisms linezolid , teicoplanin , vancomycin , cefalotin screen , moxifloxacin , nitrofurantoin , and levofloxacin ; however, the high resistance rate was found to be against erythromycin , trimethoprim/sulfamethoxazole , gentamicin , tobramycin , fosfomycin , clindamycin , oxacillin (tetracycline and benzylpenicillin (100%) resistance rate. Our finding was in supportr with study by the other authors [28,29]. In the current study the maximum number of days since fever was observed in 7-12 days. This study was in comparison with the study performed by the other author Eshwarappa M etal.[30] where maximum number of days since fever was observed in 7-12 days. Similar study by Wagenlehner FME et al [31] was observed where maximum number of days since were lasting >7 days and BM Lary recorded maximum number of days since fever was observed in 7-12 days [32].

In the present study dysuria was the most common in case of UTI observed in female with 200 (78%) and in male 127 (86.9%) , the abdominal pain in female 188 (74%) followed by the chills with the ratio of female 74 (28%). There were other studies which were similar to the present study where the common signs and symptoms include fever, dysuria, rigors, lower back pain,suprapubic pain/tenderness [32]. Another study by Wasson M Bono and JMwas also in supported to the present study where dysuria was most commonly observed [33,34].

Table No. 14: Comparison of Organisms distribution of the cases with the other studies

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

S.No.	Study	Place	Year	Results
1.	Odoki M et al.,[35]	Uganda	2017	*E. coli* being common bacterial uropathogen with 36/86 (41.9%).
2.	Mishra D et al., [36]	Port Blair	2019	The commonest causative uropathogen in the study was found to be *Escherichia coli* (n=26)
3.	Folliero V et al., [37]	Italy	2020	Among the Gram-negative bacteria, *E. coli* (53.5%) was the most detected one.
4.	Muhammad Khan I [38]	China	2020	Collectively the commonest single isolate was *E. coli* (69.7%)
5.	Birhman N et al. [39]	Noida, UP	2020	*Escherichia coli* remains the common bacterial isolates for patients who develop symptoms of UTI in a short course catheterization, although it comprises fewer than one-third of isolates. In this study, the total no. of *Escherichia coli* isolates was 39 (51.3%) out of 69
6.	**In the present study**	**Tertiary Care**	**2024**	**Maximum number of** the *E.coli* 150 (37%) was observed to be the commonest .

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

In the present study the resistant rate for Ampicillin was observed to be 92% and Imipenem and Nitrofurantoin were sensitive with (93%). It was noted that the maximum number of isolates were from the gram negative isolates as compared to the gram positive isolates. There was another study by Akter T., *et al.*,[40]where susceptibility of *Escherichia coli* was 89.19%, Azithromycin(89.19%), Ciprofloxacin (83.78%), which was higher than our findings and another study conducted by Bhuwan Khatri., *et al.* where found 52.4% susceptibility to Ciprofloxacin [41]. Study by Patel et al., was also similar to the present study where Imipenem 91,69%, Meropenem 91.89%, Nitrofurantoin 72.33%, Piperacillin+Tazobactam51.77% was observed in *E. coli* [42].

In the present study it was observed that between the resistance gene the percentage Resistance by fim H-gene was with 90% . This study was in accordance to the study performed by the other author where fimH gene was observed to be more prelevant [43,44].

According to the results of the PCR test for the identification of surveyed virulence genes, the highest frequency belonged to the FimHgene, which was detected in 93.8% (135 isolates) of the isolates [43]. Similar study was performed by Adnan Malooh Jaber et al.,[45] stated that 57 out of 60 isolates(95%) have fimHgene. The present results agreed with Merza, (2017) [46], who detected that 94.5% of the isolates, were positive for the fimH gene.Other studies submitted by Hojati*et al.,* (2015) [47] ; Al-Taai*et al.,* (2018) [48] ; Salih *et al*., (2015) [49] demonstrated that 92.2%, 100%, 91% respectively of the isolates were positive for fimHgene.

A similar study was conducted in Ethiopia by Dadi BR et al, 2020 [50**]** in which genetic determinants were studied including those coding for type 1 fimbriae [fimH], pili associated with pyelonephritis[pap], S and F1C fimbriae [sfa and foc], afimbrialadhesins [afa], hemolysin [hly], cytotoxic necrotizingfactor [cnf], and aerobactin [aer]. Virulence genes in E. coli isolates. The most frequent E.coli virulence gene was *fimH*164 (82%), followed by *aer*109 (54.5%), hly 103 (51.5%), pap 59 (29.5%), cnf 58(29%), sfa 50 (25%) and afa 24 (12%). This finding was also in accordance with our findings. This is also in agreement with studies conducted in Romania, 86%;Mongolia, 89.9%, Iran, 86.17% and China, 87.4%.

The results of the present indicate a strong relationship between the virulence genes fimH gene and the biofilm. In the current study it was observed that biofilms pose a serious threat in the hospital settings due to its relation with high antibiotic resistance. Biofilms are considered as one cause of antibiotic resistance but other mechanisms like the production of ESBL (Extended Spectrum of Beta Lactamases) and MBL (Metallo Beta Lactamases) were also observed as the reason behind antibiotic resistance. Furthermore, biofilm acts as a barrier for antibiotics due to which many antimicrobial agents are restricted to enter the cell which severely limits therapeutic options for treating infections and thus cause some of the devastating infections

CONCLUSION

The current study observed that beta-lactam antibiotics had limited efficacy in treating UTI in patients, perhaps due to the high prevalence of antibiotic resistance among *E. coli* in this region.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

The right measures may help to lower the risk of UTI infection because of these related factors, such as resistance, which may lead to an inaccurate antibiotic prescription, which may then select for new resistance genes.
It is noteworthy that multidrug resistance (MDR) is increasingly spreading globally. This is alarming, since it indicates that we are quickly running out of options for treating simple bacterial infections. Educating practitioners on the high probability of multidrug resistance should be a priority. Hence, the emergence of MDR that our study revealed poses a major danger to the management of CAUTI patients.

It is therefore recommended that routine microbiological analysis, antibiotic sensitivity test of mid-stream urine samples and biofilm detection of patients with symptoms of UTI and other asymptomatic patients be carried out so as enhance in the administration of drugs for the treatment and management of UTIs. Also safe usage techniques of indwelling catheters are suggested. So, targeting *fim-H*as a potential vaccine candidate is important for the prevention of UTI in patient with underlying conditions and currently is under investigation.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

REFERENCES

1. O. Amali, M. D. Indinyero, E. U. Umeh, and N. O. Awodi, “Urinary tract infections among female students of the university of agriculture, Makurdi, Benue State, Nigeria,” *Internet Journal of Microbiology* 2009; 7(1) :1–5.

2. Agarwal J, Srivastava S, Singh M. Pathogenomics of uropathogenic Escherichia coli. *Indian J Med Microbiol.* 2012; 30(2):141–9.

3. Chenoweth CE, Gould CV, Saint S. Diagnosis, management, and prevention of catheter-associated urinary tract infections. *Infect Dis Clin.* 2014; 28(1):105–119.

4. Bereket W, Hemalatha K, Getenet B, Wondwossen T, Solomon A, Zeynudin A, et al. Update on bacterial nosocomial infections. *Eur Rev Med Pharmacol Sci.* 2012; 16(8):1039–1044.

5. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control.* 2008; 36(5):309–332.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

6. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The diagnosis of urinary tract infection: a systematic review. *Dtsch Arztebl Int.* 2010; 107:361–367.

7. Verma S, Naik SA, Deepak TS. Etiology and risk factors of catheter associated urinary tract infections in ICU patients. *IP Int J Med Microbiol Trop Dis.* 2017; 3(2):65–70.

8. Su C. Female lower urinary tract infection. *JTUA.* 2008; 19:12–20.

9. Emody L, Kerenyi M, Nagy G. Virulence factors of uropathogenic Escherichia coli. *Int J Antimicrob Agents.* 2003; 22 Suppl 2:29–33.

10. Soutourina OA, Bertin PN. Regulation cascade of flagellar expression in Gram-negative bacteria. *FEMS Microbiol Rev.* 2003; 27(4):505–23.

11. Mladin C, Usein CR, Chifiriuc M, Palade A, Slavu CL, Negut M, et al. Genetic analysis of virulence and pathogenicity features of uropathogenic Escherichia coli isolated from patients with neurogenic bladder. *Rom Biotech Lett.* 2009; 14(6):4906–11.

12. Le Bouguenec C. Adhesins and invasins of pathogenic Escherichia coli. *Int J Med Microbiol.* 2005; 295(6-7):471–8.

13. Oliveira FA, Paludo KS, Arend LN, Farah SM, Pedrosa FO, Souza EM, et al. Virulence characteristics and antimicrobial susceptibility of uropathogenic Escherichia coli strains. *Genet Mol Res.* 2011; 10(4):4114–25.

14. Hooton TM, Bradley SF, Cardenas DD, Colgan R, Geerlings SE, Rice JC, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. *Clin Infect Dis.* 2010; 50(5):625–663.

15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 28th ed. M100. Wayne: Clinical and Laboratory Standards Institute (CLSI); 2023 p. 38.

16. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010; 7:653-60.

17. Ahmad S, Ahmad F. Urinary tract infection at a specialist hospital in Saudi Arabia. Bangladesh Med Res Counc Bull .1995; 21:95-8.

18. Syed Suhail Ahmed, Ali Shariq, Abdulaziz Ajlan Alsalloom, Ibrahim H. Babikir, Badr N. Alhomoud. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. International Journal of Health Sciences. 2019; Vol. 13, Issue 2 .

19. Martin Odoki , Adamu Almustapha Aliero , Julius Tibyangye , Josephat Nyabayo Maniga, Eddie Wampande, Charles Drago Kato, Ezera Agwu, and Joel Bazira . Prevalence of Bacterial

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda International Journal of Microbiology .2019, Article ID 4246780, 8 pages

20. August SL, De Rosa MJ. Evaluation of the prevalence of urinary tract infection in rural panamanian women. PLoS One 2012; 7:e47752

21. Manjula N. G., Girish C. Math., Shripad A. Patil, Subhashchandra M. Gaddad, Channappa T. Shivannavar. Incidence of Urinary Tract Infections and Its Aetiological Agents among Pregnant Women in Karnataka Region. Advances in Microbiology. 2013; 3, 473-478.

22. A. K. Onifade, F. O. Omoya and D. V. Adegunloye, "In- cidence and Control of Urinary Tract Infections among Pregnant Women Attending Antennal Clinics in Gov-ernment Hospitals in Ondo State, Nigeria," *Journal of Food Agriculture and Environment*. 2005; 3(1): pp. 37-38.

23 E. E. A. Okonofua and B. N. Okonofua, "Incidence and Pattern of Asymptomatic Bacteriuria of Pregnancy in Ni- gerian Women," *The Nigerian Medical Practitioner*. 1989; 17: 354-358.

24 I. O. Okonko, L. A. Ijandipe, O. A. Ilusanya, *et al*., "In- cidence of Urinary Tract Infection (UTI) among Pregnant Women in Ibadan, South-Western Nigeria," *African Journal of Biotechnology*. 2009; 8(2) 3: 6649- 6657.

25. P. I. Nwanze, L. M. Nwaru, S. Oranusi, U. Dimkpa, M. U. Okwu, B. B. Babatunde, A. Anake, W. Jatto and C. E. Asagwara, "Urinary Tract Infection in Okada Village: Prevalence and Antimicrobial Susceptibility Pattern," *Scientific Research and Essays*. 2007; 2(4): . 112-116.

26 M. Akram, M. Shahid and A. U. Khan, "Aetiology and Antibiotic Resistance Patterns of Community Acquired Urinary Tract Infections in JNMC Hospital Aligarh, In- dia," *Annals of Clinical Microbiology and Antimicrobials*. 2007; 6 :4-10.

27. Syed Suhail Ahmed, Ali Shariq, Abdulaziz Ajlan Alsalloom, Ibrahim H. Babikir, Badr N. Alhomoud. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. International Journal of Health Sciences. 2019; 13(2) .

28. August SL, De Rosa MJ. Evaluation of the prevalence of urinary tract infection in rural panamanian women. PLoS One 2012; 7:e47752

29. Ibrahim ME, Bilal NE, Hamid ME. Increased multi-drug resistant *Escherichia coli* from hospitals in Khartoum state, Sudan. Afr Health Sci .2012; 12:368-75.

30. P. Kempegowda. Clinico-microbiological profile of urinary tract infection in south India. Indian J Nephrol. 2011; 21(1): 30–36.

31. Wagenlehner FME, Bjerklund Johansen TE, Cai T, Koves B, Kranz J, Pilatz A, Tandogdu Z. Epidemiology, definition and treatment of complicated urinary tract infections. Nat Rev Urol. 2020 ;17(10):586-600.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

32. Larry M. Bush Fever in adults. MSD MANUALS. Reviewed/Revised Aug 2022 | Modified Sep 2022

33. Nirmala Poddar, Kumudini Panigrahil, Basanti Pathi, DiptiPattnaik, Ashok Praharaj, Jagdananda Jena. Microbiological Profile Of Catheter Associated Urinary Tract Infection In ICU'S Of A Tertiary Care Hospital Bhubaneswar,Odisha, India. International Joirnal of Medical Microbiology and Tropical Diseases. 2020; 6(2):107-112.

34.Michael J. Bono; Stephen W. Leslie; Wanda C. Reygaert..Urinary Tract Infection. National Libraray of Medicine. 2022.

35. Martin Odoki , Adamu AlmustaphaAliero , Julius Tibyangye , Josephat Nyabayo Maniga , Eddie Wampande , Charles Drago Kato , Ezera Agwu , Joel Bazira . Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. 2019; 4246780.

36.. Debadutta Mishra, Kodukula Bhaskara Rao. Catheter associated urinary tract infection in an acute care setting of a tertiary care centre in South India. International Journal of Research in Medical Sciences *Mishra D et al. Int J Res Med Sci.* 2019 ; 7(6):2182-2186.

37. Veronica Folliero , Pina Caputo , Maria Teresa Della Rocca , Annalisa Chianese Marilena Galdiero , Maria R. Iovene , Cameron Hay , Gianluigi Franci , and Massimiliano Galdiero. Prevalence and Antimicrobial Susceptibility Patterns of Bacterial Pathogens in Urinary Tract Infections inUniversity Hospital of Campania "Luigi Vanvitelli"between 2017 and 2018. 2020; 28; 9(5):215.

38. Muhammad Bilal D, Yi Hua AndFenfen Lia. Assessment Of Multidrug Resistance In Bacterial Isolates From Urinary Tract-Infected Patients. Journal Of Radiation Research And Applied Sciences 2020; 13(1): 267–275.

39. Nikita Birhman, Sneha Mohan, Tarana Sarwat, Mariyah Yousufand Dalip K Kakru**.** Bacteriological Profile of Catheter Associated Urinary Tract Infection. Act a Scientific Microbiology (ISSN: 2581-3226). 2020; 3.5 : 77-80.

40. M Kibret and B Abera. Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. Afr Health Sci. 2011 ; 11(Suppl 1): S40–S45.

41. Khatri B., *et al*. Etiology and antimicrobial susceptibility pattern of bacterial pathogens from urinary tract infection. *Nepal Medical College Journal* 2012; 14(2): 129-132.

42. Patel, H., Soni, S., Bhagyalaxmi, A., & Patel, N. Causative agents of urinary tract infections and theirantimicrobial susceptibility patterns at a referral center in Western India: An audit to help clinicians preventantibiotic misuse. Journal of Family Medicine and Primary Care. 2019; 8, 154.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

43. H Hyun M, Lee JY, Kim HA. Differences of virulence factors, and antimicrobial susceptibility according to phylogenetic group inuropathogenic Escherichia coli strains isolated from Korean patients. *Research Square*. 2021: 10; 20(1):77.

44. Mostafa Boroumand , Asghar Sharifi , Mohammad Amin Ghatei and Mohsen Sadrinasab. Evaluation of Biofilm Formation and Virulence Genes and Association with Antibiotic Resistance Patterns of Uropathogenic*Escherichia coli* Strains in Southwestern Iran. Jundishapur J Microbiol. 2021 September; 14(9):e117785.

45. Adnan Malooh Jaber and Hasan A. Aal Owaif. Detection of genes involved in biofilms formation by *Escherichia coli* isolated from patients suffering of urinary tract infections. *plant archives*. 2020; 20 (2) :5987-5992.

46. Merza, N.S. Prevalence and Molecular Characterizationof *Fim H* Gene in *Escherichia Coli* Isolates RecoveredFrom Patients With Utis. *Medical Journal of Babylon*. 2017; 14(3): 470–477.

47. Hojati, Z., B. Zamanzad, M. Hashemzadeh, R. Molaie and A.Gholipour . Detection of fimh gene in uropathogenicescherichia coli strains isolated from patients with urinary tract infection. *Jundishapur Journal of Microbiology*. 2015; 8(2): 12-15.

48. Al-Taai, H.R.R., Z.A.S. Al-Jebouri, B.H. Khalaf and Y.Q.Mohammed . Antibiotic resistance patterns andadhesion ability of uropathogenic Escherichia coli in children. *Iraqi Journal of Biotechnology*. 2018; 17(1): 18-26.

49. Salih, E.G.M., M.I. Nader and M.N. Rasheed .*RapidDetection of Uropathogenic Escherichia Coli virulencefactors in Iraqi patients by multiplex polymerase chain Reaction. 2015*

50. Belayneh Regasa Dadi, Tamrat Abebe, Lixin Zhang, Adane Mihret, Workeabeba Abebe and WondwossenAmogne, Distribution of virulence genes and phylogenetics of uropathogenic Escherichia coli among urinary tract infection patients in Addis Ababa, Ethiopia; BMC Infectious Diseases. 2020; 20:108.