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Expression of bla-CTX-M and fimH genes in uropathogenic *Escherichia coli* isolated from Egyptian catheterized patients

Wesam AE. Mousa[1], Nesrene S. Omar[2], Omnia KM. Riad[1]*, Maha EA. Omran[1].

[1] Department of Microbiology and Immunology, Faculty of pharmacy, Al-Azhar university.
[2] Department of Microbiology and Immunology, Faculty of medicine, Mansoura university.

***Correspondance:** dr_omniakarem@azhar.edu.eg, Mobile number: 01006536734, Orchid ID: 0000-0001-8950-7518

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Abstract: The most common uropathogen is *Escherichia coli*, which causes more than 80% of catheter-associated urinary tract infections (CAUTI). Catheter-associated uropathogenic *E. coli* is difficult to be eradicated due to biofilm formation alongside increasing antibiotic resistance. The goal of this study is to determine the prevalence of biofilm and non-biofilm producing uropathogenic *E. coli* isolates and the antibiogram of *E. coli* isolates among catheterized patients who were admitted to Mansoura's University Hospitals. Also, the expression of bla-CTX-M and Fim H genes was determined using quantitative real-time PCR. The correlation between expression of bla-CTX-M and Fim H genes was detected. Isolated *E. coli* were highly resistant to nitrofurantoin (75%), ceftazidime (72%), ceftriaxone (71%), amikacin (71%), piperacillin-tazobactam (61%), and cefepime (53%). Biofilm formation was detected in all *E. coli* isolates. Also, *bla*-CTX-M and FimH genes were highly expressed in all *E. coli* isolates, as their expression is higher than the reference gene by 359.3 and 914.6 folds respectively. Insignificant positive correlation was detected between *bla*-CTX-M expression and FimH expression (r= 0.05, P-value= 0). These findings confirmed the serious infections caused in the catheterized patients, hence the importance of performing appropriate aseptic procedures when inserting and maintaining catheters to avoid CAUTI.

Keywords: Catheter-associated urinary tract infection; *Escherichia coli;* FimH gene; Biofilm formation; *bla*-CTX-M gene; resistance.

1. INTRODUCTION:

One of the most frequent healthcare-associated infections (HAIs) is catheter-associated urinary tract infection (CAUTI), which accounts for 30% of all HAIs[1]. The risk of CAUTI is increased by increasing the duration of catheterization. Approximately 5% of infection is increased each day at the intensive-care unit. It is strongly recommended to remove the urethral catheter whenever feasible[2]**.** When catheterization is required for a long time, it should be maintained as recommended by guideline for prevention of catheter-associated urinary tract infections, which is stated by healthcare infection control practices advisory committee (HICPAC), CDC[3,4]**.** For CAUTI prevention, CDC guidelines for prevention of CAUTI should be followed including proper aseptic catheter insertion, maintenance, and removal, as well as a minimizing catheter use[4]**.**

The most frequent uropathogen is *Escherichia coli,* which accounts for more than 80% of all cases [5]. The selection of antimicrobial therapy for uropathogenic *Escherichia coli* (UPEC) depends on the patient's tolerance, the clinical presentation, and the results of urine culture to avoid inappropriate antimicrobial use[4]**.** In many countries, trimethoprim, nitrofurantoin, β-lactams, and quinolones are the treatment of UTIs regularly. Regrettably, there is an increasing rate of resistance to these antibiotics due to the widespread and misuse of them[6]**.** The resistant uropathogen includes extended-spectrum β-lactamases (ESBLs) and carbapenemase-producing *E. coli* [5]**.** ESBL genes include CTX-M, SHV, and TEM[7].

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For their pathogenicity in the urinary system, UPEC have several virulence features such as capsule, fimbriae, flagella, toxins, iron scavenger receptors, and lipopolysaccharide[6]**.** Biofilms are difficult to eradicate with antimicrobials, as they prevent the access of antibacterial agents, white blood cells, and antibodies due to their high content of polysaccharides[8].

Uropathogenic *E. coli* forms biofilm within the bladder epithelium in urinary tract infection (UTI)[9]. Fimbrial adhesins, capsular structures, autotransporter proteins, flagella, and exopolysaccharides are just a few of the potential variables that could help *E. coli* colonize catheter surfaces and bladder epithelium[10]. P fimbriae, which is related to pyelonephritis and is expressed by pap genes[11], type 1 fimbria, which is encoded by the fim gene cluster, and S fimbrial adhesin, which is encoded by sfa genes, are the most common attachment factors in UPEC. In a Tunisian study fimbrial adhesin (FimH) was the most prevalent (68%) gene coding for the fimbrial adhesive system, followed by pap (41%), and sfa/foc (34%) [12].

In many investigations, type 1 fimbriae are important virulence factors in UTI and CAUTI[10]. Type 1 fimbriae are engaged in the early stages of biofilm formation and cause adhesion to both abiotic and biotic surfaces.[13]. Type 1 fimbriae in *E. coli* are critical during UTIs because they mediate attachment to mannose-containing receptors on the uroepithelium and promote the establishment of intracellular bacterial populations[14].

Thousands of coiled FimA domains precede the tip fibrillum domains, FimF-FimG-FimH, in each pilus of type 1 pili. To withstand mechanical forces during attachment, the domains are linked by non-covalent -strands[15]. FimA, FimF, FimG, and FimH are structural components of the fimbrial adhesin, while FimC and FimD are the assembling system for pilus[16]**.** FimB and FimE are responsible for ON/OFF orientation[17]**.** FimH is a type 1 pilus adhesin that binds D-mannose moieties on cells and secreted glycoproteins[18]**.**

This study aims to determine the prevalence of biofilm and non-biofilm producing uropathogenic *E. coli* isolates and the antibiogram of *E. coli* isolates among catheterized patients who were admitted to Mansoura's University Hospitals. Also, the expression of bla-CTX-M and Fim H genes was determined using quantitative real-time PCR. The correlation between expression of bla-CTX-M and Fim H genes was detected.

2. METHODS

2.1. Specimen collection:

About 147 urine specimens were collected from catheterized patients in Mansoura University Hospital, Daqahleya, Egypt under complete aseptic conditions according to Shephard (2019)[19]. The study follows the principles of the declaration of Helsinki, and informed consent was obtained from patients and. The manager of the Urology and Nephrology Center gave his written approval. Urine samples were collected only from the patient before starting antibiotic treatment to avoid false results. Following hospitals' protocol, two urine specimens were taken from each patient in capped-sterile containers: urine sample from the catheter before removal and part of urinary catheter distal to the inflating balloon. Specimens were transported with ice to Mansoura University's Faculty of Medicine's Microbiology Diagnostics and Infection Control Unit (MDICU).

The inclusion criteria for patients to be involved in the current study are presence of UTI, beside presence of state of low immunity such as diabetes or other chronic diseases. The exclusion criteria involve administration of antibiotic or infection other than UTI.

2.2. Isolation and identification of *E. coli*:

For isolation of *E. coli* [19], 0.01 ml calibrated bacteriologic loop was used to culture urine on CLED agar (Oxoid, UK) plates, with a significant count of 10^5 CFU/ml. Luria Bertani broth (Miller, USA) was inoculated with a portion of the catheter, incubated at 37°C, and then subcultured on MacConkey's and blood agar plates (Oxoid, UK). Colony morphology, Gram staining, and biochemical testing are all used to identify the isolated bacteria[20]**,** then *E. coli* isolates were selected and involved in this study after purification in cases of mixed growth.

Bacterial isolates from urine collected from catheters were included in this study. In case of finding any differences between culture results of urine collected from catheters and culture of the tip of the culture, informing infection control unit was performed with exclusion of those different isolates and notinvolved in the current study.

A standard strain of *E. coli* (ATCC25922) was kindly provided by the staff of microbiology lab, faculty of medicine, Mansoura University.

2.3. Tissue Culture Plate Method (TCP) for Biofilm Detection:

The isolate from the nutrient agar plate is inoculated into Trypticase soy broth (TSB) overnight (Oxoid, England). Then, the inoculums were put into a 96-well flat-bottom microtitre plate and inoculated in TSB with 1 percent glucose produced in various

dilutions (1:20, 1:40, 1:80, and 1:100). Then the plates were incubated for 24 hours in aerobic condition at 37ºc. After washing three times with phosphate buffer saline (PBS), methanol fixation was performed. After decantation, the wells were stained with crystal violet for 20 minutes. After washing, 33% glacial acetic acid was added to extract the stain. Using an automated ELISA plate reader, the optical density is evaluated at 490 nm. According to Nabajit Deka (2014) read, the optical density for biofilm detection was calculated[21].

2.4. Antibiotic susceptibility testing by disk diffusion test:

The antibiogram of the isolated *E. coli* was determined by disk diffusion test for chloramphenicol (C) 30 µg, cefepime (FEP) 30 µg, amikacin (AK) 30 µg, ceftriaxone (CRO) 30 µg, nitrofurantoin (F) 300 µg, trimethoprim-sulphamethoxazole (SXT) 25 mcg, piperacillin-tazobactam (TZP) 110 µg, nalidixic acid (NA) 30 µg, and ceftazidime (CAZ) 30 µg. Antibiotic discs were supplied from Oxoid, England. The test was carried out as recommended by Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI 2018 M100 28th); results were interpreted using CLSI standard breakpoints**.** The inhibition zones were evaluated using criteria from the Clinical Laboratory Standards Institute CLSI[22].

2.5. *bla*-CTX-M and Fim H expression:

2.5.1 RNA extraction:

To extract and purify bacterial RNA, the RNeasy® Mini Kit is employed (Qiagen, Hilden, Germany). Up to 1 x 10^9 bacteria were disrupted and homogenized by bead-milling bacteria in a guanidine-thiocyanate–containing lysis solution. The material is put onto RNeasy Mini spin column after it has been treated with ethanol. The RNeasy silica membrane binds the whole RNA, impurities are immediately washed away, and RNase-free water is used to elute high-quality RNA.

Following the manufacturer's instructions for the purification of the extracted RNA, Denovix Spectrophotometer (AGBL, USA) was used to ensure the purity of the extract at wavelength 260/280 nm.

2.5.2 Conversion of extracted RNA into double-stranded DNA (ds DNA):

Qiagen QuantiTect RT kit was used for the elimination of genomic DNA and reverse transcription following the manufacturer's instructions.

2.5.3 Quantitative real-time polymerase chain reaction (RT-PCR):

After cDNA synthesis by reverse transcription, in the RT-PCR mix (QIAGEN Reference Database), an aliquot of each completed reverse transcription reaction was added. 2 µL of DNA template were mixed with 10 µL of master mix, 0.5 µL for each primer (F-R) (Table 1), and 12 µL DD – RNAse – DNAse- free Water. PCR reaction conditions were 35 cycles of 3 min at 94°C for start activation, denaturation temperature was 95°C for 30 sec, annealing temperature was 66°C for 40 sec. for Fim-H & 62°C for 45 sec. for CTX-M, and the extension temperature was 73°C for 45 Sec.

Table 1: The primers that were used in this study:

Gene	Sequence	Melting temperature (T_m)
FimH	**Forward:** AAAACGAGGCGGTATTGGTG **Reverse:** GCTGTGATGTTTCTGCTCGT	62, 59
***bla*-CTX-M**	**Forward:** CGCTTTGCGATGTGCAG **Reverse:** ACCGCGATATCGTTGGT	61, 57
16-S (Reference gene)	**Forward:** AGAGTTTGATCMTGGCTCAG **Reverse:** ACGAGCTGACGACARCCATG	67, 60

2.6. Statistics and data analysis

Statistics were done using GraphPad prism 6. Number and percent were used to describe qualitative data. Mean and standard deviation (SD) were used to describe parametric data. The association between CTX-M and FimH gene expression was determined using a correlation test. Significance was considered at (0.05).

3. RESULTS:

3.1. Patients' characteristics:

Urine specimens were collected from 147 catheterized patients who were admitted to intensive care units in Mansoura's hospital (Mansoura university, Daqahleya, Egypt).

The age of patients included in the study ranged between 3 and 82 (mean age ± SD= 58.9±17.62) years old. Out of the 147 studied patients, 103

(70.06%) were males and 44 (29.9%) were females (Figure 1).

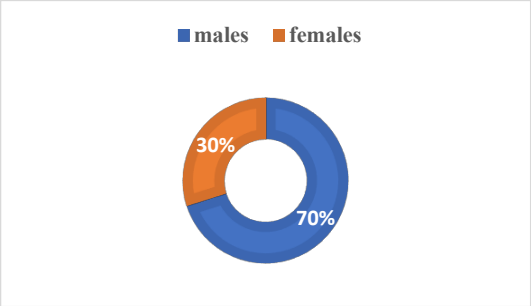

Figure 1: Frequency of studied patients according to their gender.

The urine culture of the 147 catheterized patients were positive in 78 (53%) patients. Urine culture of 69 (46.9%) catheterized patients were free from microorganisms (Figure 2).

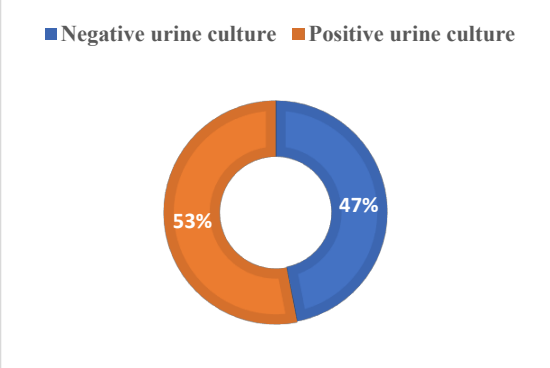

Figure 2: Frequency of studied patients according to their urine culture results.

3.2. *E. coli* isolation and identification:

In the current study, mono-microbial colonization represented 79.5% (62/78) in catheterized patients for short-term catheterization less than 10 days and poly microbial colonization constituted about 20.5 % (16/78) of patients with long-term catheterization in which catheters were inserted for one month (Figure 3).

The urine culture of the 147 catheterized patients was positive in 78 (53%) patients. Urine cultures of 69 (46.9%) catheterized patients were free from microorganisms.

About 151 bacterial isolates were retrieved. The most commonly isolated bacteria were *E. coli* (n=100, 66%) followed by *Candida spp.* (n=20, 13%), *Staphylococcus aureus* (n=16, 11%), and *Klebsiella spp.* (n=12, 8%) (Figure 4).

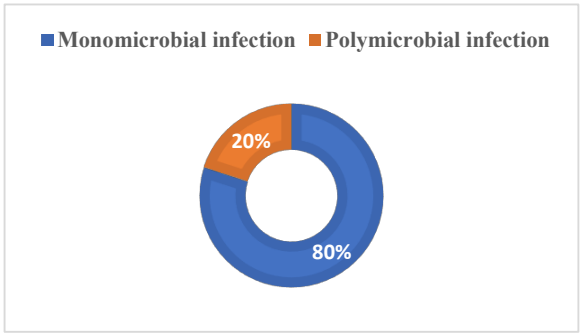

Figure 3: Frequency of monomicrobial and polymicrobial infection among catheterized patients.

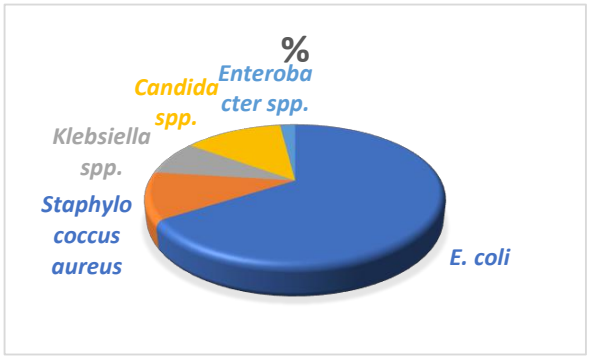

Figure 4: Distribution of microorganisms retrieved from urine culture of catheterized patients.

3.3. Antibiogram of the isolated *E. coli*:

Isolated *E. coli* were resistant to nitrofurantoin (75%), ceftazidime (72%), ceftriaxone (71%), amikacin (71%), piperacillin-tazobactam (61%), cefepime (53%), and nalidixic acid (44%). Isolated *E. coli* were least resistant to chloramphenicol (18%), and trimethoprim-sulphamethoxazole (30%) (Figure 5).

About 81% of tested *E. coli* were multi-drug resistant.

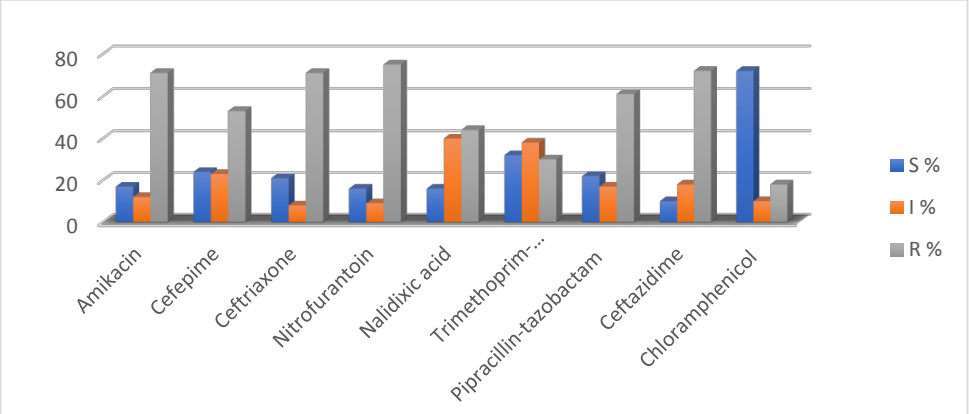

Figure 5: Antibiogram of *E. coli* isolates.

3.4. Biofilm formation:

Biofilm formation was confirmed in 94% *E. coli* isolates, which had been isolated from the urine culture of catheterized patients as shown in figure 6.

3.5. *bla*-CTX-M and Fim H expression:

bla-CTX-M and FimH genes were expressed in all *E. coli* isolates (n=100). Upon comparing the expressed results to the negative control, the mean CTX-M was highly expressed by 359.3 folds. FimH was highly expressed by 914.6 folds (Table 2, Figure 7, 8).

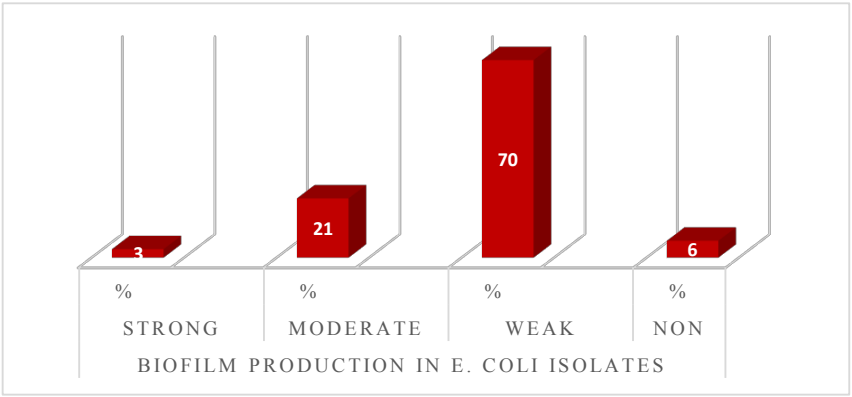

Figure 6: Classification of *E. coli* isolates according to the degree of biofilm formation.

Table 2: Expression of *bla*-CTX-M and Fim H genes:

Gene	Ct		ΔCt		ΔΔCt		Fold Change	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CTX-M	22.24	0.6173	3.479	0.6828	-8.336	0.6872	359.3	177.7
Fim H	20.66	0.512	2.802	0.5849	-9.728	0.5849	914.6	342.4

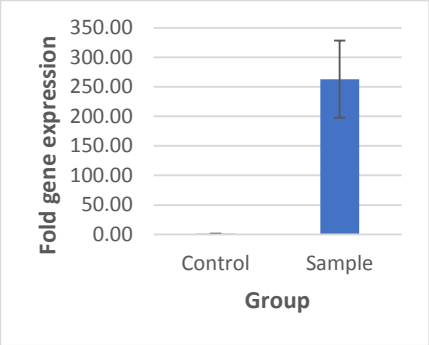

Figure 7: *bla*-CTX gene expression in comparison to reference gene (16-S).

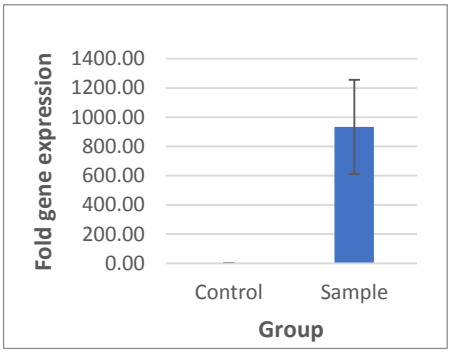

Figure 8: FimH gene expression in comparison to reference gene (16-S).

3.6. Correlation between expression of *bla*-CTX-M and FimH:

A positive correlation was detected between *bla*-CTX-M expression and FimH expression (r= 0.05), but this correlation is insignificant correlation as P-value= 0.6 (p-value > 0.05) (Table 3, Figure 9).

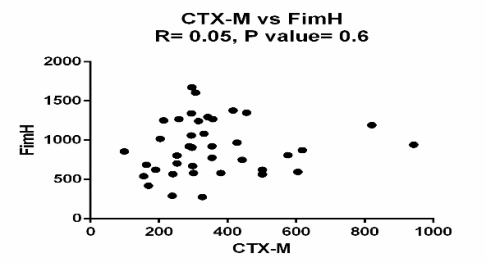

Figure 9: Correlation between *bla*-CTX-M and FimH genes.

4. DISCUSSION

Seeking good catheter care is indispensable to prevent risks of CAUTIs. CAUTIs lead to decreased life quality, increased mortality and increased risk of hospitalization [23]. 70% to 80% of complicated UTIs are caused by catheterization. Infection with UPEC is the most common cause of UTIs [24].

In this study, *E. coli* was the most predominant isolate (66%) followed by *Candida spp.* (13%), *Staphylococci* (11%), and *Klebsiella spp.* (8%). This is following several studies like that of Tamadonfar and his colleagues (2019) who stated that UPEC is responsible for 65% of CAUTI[24]. Also, *Enterococci* (11%) and Staphylococci are involved in CAUTIs[24, 25]. Another study that was conducted in Taiwan confirmed that *Candida spp.* (25.8%) is the most common isolate causing CAUTI followed by *E. coli* (15.2%)[26].

Catheterization creates the proper environment for biofilm formation. Biofilm may be crystalline and cause blockage problems if caused by urease-producing bacteria[27]. Biofilms promote the establishment of multidrug-resistant organisms by allowing for poor antibiotic penetration and horizontal virulence gene transfer[28]. In the current study, biofilm formation was confirmed in 94% of *E. coli* isolates. This is following some studies which reveal that 84% of UPEC have a high tendency to make biofilm[29]. Other studies showed lower values such as 61%[30] and 62.5% [28].

High percent of weak biofilm formation that were detected in this study may be attributed to the use of silicon-catheters which cause less irritation to the mucosa in opposite to latex-catheters which showed higher rate of biofilm production than silicon catheter, as latex causes more irritation to the mucosa and had rough surface which enhances biofilm formation in comparison to smooth surface of silicon[31].

Antibiotic resistance has been linked to CAUTIs all around the world; as resistance to most of the available antibiotics is increasing. This increased resistance to antibiotics is due to biofilm formation which prevents access of antibiotics to kill the pathogenic bacteria, and to the increased antibiotic administration for a reason other than UTIs [32]. Continuous emergence of antibiotic-resistant bacteria needs more investigations and studies to combat this increasing problem by applying different strategies or new treatment methods[33].

In the current study, the isolated *E. coli* were most resistant to nitrofurantoin (75%), ceftazidime (72%), ceftriaxone (71%), amikacin (71%), piperacillin-tazobactam (61%), cefepime (53%), and nalidixic acid (44%). They were least resistant to chloramphenicol (18%), and trimethoprim-sulphamethoxazole (30%). The antibiotic resistance in the current study is following several studies[34, 35], but the resistance pattern to different antibiotics differs among different countries or different hospitals in the same country according to the used protocol.

In a study, the antibiogram of UPEC showed increased resistance to ampicillin, ceftriaxone, ciprofloxacin, TMP/SMX, and gentamicin (100%, 96%, 89%, 53%, and 57% respectively). UPEC were least resistant to carbapenems, nitrofurantoin, and fosfomycin (0%, 1.3%, and 6.6%) [35]**.** In another study, uropathogenic bacteria were extremely vulnerable to erythromycin, penicillin G, cefazolin, cefuroxime, cefotaxime, and aztreonam (90.52%, 74.01%, 73.41%, 72.52%, 71.41%, and 70.11%). They were least susceptible to vancomycin, amikacin, linezolid, and imipenem (0.92%, 3.17%, 3.47%, and 5.74%)[34]**.**

Carbapenem-resistant Enterobacteriaceae (CRE) and extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae are two prevalent multi-resistant gram-negative bacteria discovered in chronic indwelling catheters[32].

In the current study, 81% of the isolated *E. coli* were multi-drug resistant (MDR), which is more or less in agreement with many studies that reported that multidrug-resistant uropathogenic *E. coli* was 63% [36]**,** 62.5% [28]**,** 77.8% [37]**.**

In addition to biofilm formation especially in presence of catheters, *fim*H has the main role in the binding of *E. coli* with host cell receptors. Mutations disrupt the expression of *fim*H results in the decreased virulence of UPEC [38]**.** In this study *fim*H were expressed in all (100%) *E. coli* isolates.

In other studies, *fim*H was found in more than 71% of multi-drug resistant *E. coli* isolates [36] and in more than 50% in urosepsis isolates[37]**.**

*fim*H gene was detected in 100% of *E. coli* isolated from bovine endometritis, in which they were positive phenotypically for biofilm formation[39]. In an Egyptian study, it was detected in 75% of E. coli isolated from cheese[40]**.**

E. coli isolates from catheterized patients in the present study are not only characterized by high rates of resistance and biofilm formation but there is also a high rate of expression of *bla*-CTX-M (100%). This is in agreement with some studies as that of the Egyptian study who detected the bla-CTX-M gene in 98% of isolates[41]**.** Also, an Indonesian study reported CTX-M-15 gene in more than 89% of isolates [42]**.**

Worldwide spread of the CTX-M-type ESBL-producing Enterobacteriaceae (CTX-M Ent) are predominant, *E. coli* harboring CTX-M-15 (CTXM-1 group) being the most commonly reported in both adult and pediatric studies. However, several other CTX-M types continue to circulate[43]. Also, in another study, CTX-M-1 and CTX-M-15 presence were reported in ESBL positive patients as 100%, 95.5%, respectively[44].

In another study, CTX-M-1 and CTX-M-15 were detected in 73%, 37% in UTIs, respectively. They also clarified that Presence of CTX-M-1 genes among *E. coli* strains isolated from inpatients were found statistically higher than outpatients[45].

According to authors' knowledge, there is no available study which measure the expression of bla-CTX-M from the same point of view of the current study to compare with it.

In the current study, there is a positive insignificant correlation between *bla*-CTX-M expression and FimH expression (r= 0.05, P-value= 0.6). There is no similar study to compare our findings with other studies. But some studies mentioned that biofilm production has been linked to antibiotic resistance and virulence factors[29]**.** Another study discovered a significant prevalence of biofilm-forming UPEC strains, which have been associated with the MDR phenotype[28].

Hence, to counteract biofilm growth, new tactics must be developed during catheterization and increase awareness about hygienic practices and self-management in patients living with urinary catheters to improve their life quality and prevent CAUTI[46]**.**

5. CONCLUSIONS

Catheterization causes several problems especially CAUTIs. UPEC is the most common pathogen causing CAUTIs. Biofilm formation and increased resistance to antibiotics are major complications in catheterized patients. High expression of resistant gene bla-CTX-M and fimH gene (the main gene incorporated in fimbriae type1 composition) are detected in all catheterized patients in this study. So, following strict precautions including aseptic insertion, maintenance, and appropriate use of urinary catheters as in approved guidelines is highly recommended to prevent CAUTIs and their complications especially biofilm formation and increased antibiotic resistance.

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Acknowledgments: Not available
Conflict of interest: The authors declare that they have no conflict of interests regarding this study.
Ethical Statement: Although the study is carried out on the isolated bacteria isolated from urine samples that were routinely sent to lab, informed consent was

taken from patients and the study follows the principles of declaration of Helsinki. The manager of the Urology and Nephrology Center gave his written approval.

Authors' contributions: Wesam Mousa carried out the laboratory analysis. Writing the original draft was performed by Wesam Mousa & Omnia Riad. Maha Omran & Nesrene Omar were responsible for conceptualization of the study. Maha Omran, Nesrene Omar & Omnia Riad was responsible for revising and editing the original draft, visualizing, validating and supervising the laboratory work in this study.

Authors consent of publication: All authors agree to publish this study.

List of Abbreviations: AK: Amikacin; C: Chloramphenicol; CAUTI: Catheter-Associated Urinary Tract Infections; CAZ: Ceftazidime; CLSI: Clinical Laboratory Standards Institute; CRE: Carbapenem-Resistant; Enterobacteriaceae; CRO: Ceftriaxone; Ct: Cycle threshold; ds DNA: double-stranded DNA; ESBLs: Extended-Spectrum B-Lactamases; F: Nitrofurantoin; FEP: Cefepime; Hais: Healthcare-Associated Infections ; MDR: Multi-Drug Resistant; NA: Nalidixic Acid ; PBS: Phosphate Buffer Saline ; RT- PCR: Real-Time Polymerase Chain Reaction; SD: Standard Deviation; SXT: Trimethoprim-Sulphamethoxazole; TCP: Tissue Culture Plate Method; TSB: Trypticase Soy Broth; TZP: Piperacillin-Tazobactam; UPEC: Uropathogenic *Escherichia Coli*; UPEC: Uropathogenic *Escherichia Coli*; UTI: Urinary Tract Infection

REFERENCES

1. Davis C. Catheter-associated urinary tract infection: signs, diagnosis, prevention. Br J Nurs. 2019 Jan 24;28(2):96-100. doi: 10.12968/bjon.2019.28.2.96. PMID: 30673326.

2. Fukuoka K, Furuichi M, Ito K, Morikawa Y, Watanabe I, Shimizu N, et al. Duration of Urinary Catheterization Increases Catheter-Associated Urinary Tract Infection in PICU. Pediatr Crit Care Med. 2018 Oct;19(10):e547-e550. doi: 10.1097/PCC.0000000000001628. PMID: 29905633.

3. Nicolle LE. Urinary Tract Infections in the Older Adult. *Clin Geriatr Med*. 2016;32(3):523-538. doi:10.1016/j.cger.2016.03.002.

4. Gould CV, Umscheid CA, Agarwal RK, Kuntz G, Pegues DA, and the Healthcare Infection Control Practices Advisory Committee (HICPAC) Guideline for prevention of catheter-associated urinary tract infections. Healthcare infection control practices advisory committee (HICPAC) CDC. (2009), last update: 6 June 2019, Available at: https://www.cdc.gov/infectioncontrol/guidelines/cauti/

5. de Cueto M, Aliaga L, Alós JI, Canut A, Los-Arcos I, Martínez JA, et al. Executive summary of the diagnosis and treatment of urinary tract infection: Guidelines of the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC). Enferm Infecc Microbiol Clin. 2017 May;35(5):314-320. English, Spanish. doi: 10.1016/j.eimc.2016.11.005. Epub 2016 Dec 23. PMID: 28017477.

6. Asadi Karam MR, Habibi M, Bouzari S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli. Mol Immunol. 2019 Apr;108:56-67. doi: 10.1016/j.molimm.2019.02.007. Epub 2019 Feb 18. PMID: 30784763.

7. Hassuna NA, Khairalla AS, Farahat EM, Hammad AM, Abdel-Fattah M. Molecular characterization of Extended-spectrum β lactamase- producing E. coli recovered from community-acquired urinary tract infections in Upper Egypt. *Sci Rep*. 2020;10(1):2772. Published 2020 Feb 17. doi:10.1038/s41598-020-59772-z

8. Spoering AM, Lewis K. Biofilm and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. J Bacteriol 2001; 183:6746-6751.

9. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm like pods in urinary tract infections. Science 2003; 301:105-107.

10. Guiton PS, Cusumano CK, Kline KA, Dodson KW, Han Z, Janetka JW,Henderson JP, Caparon MG, Hultgren SJ. Combinatorial small-molecule therapy prevents uropathogenic *Escherichia coli* catheter-associated urinary tract infections in mice. Antimicrob.Agents Chemother 2012; 56:4738–4745.

11. Snyder JA, LIoyd AL, Lockatell CV, Johnson DE, Mobley HL. Role of phase variation of type 1 fimbriae in a uropathogenic *Escherichia coli* cystitis isolate during urinary tract infection.

Infect Immun. 2006; 74(2):1387-93. 10.1128/iai.72.2.1387-1393.2006.

12. Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in Escherichia coli isolated from patients with urinary tract infection. *Int J Infect Dis*. 2013;17(6):e450-e453. doi:10.1016/j.ijid.2013.01.025

13. Schembri MA, Hjerrild L, Gjermansen M, Klemm P. Differential Expression of the *Escherichia coli* Autoaggregation Factor Antigen 43. J Bacteriol 2003; 185: 2236–2242.

14. Wright KJ, Seed PC, Hultgren SJ. Development of intracellular bacterial communities of uropathogenic *Escherichia coli* depends on type 1 pili. Cell Microbiol **2007**; 9: 2230–2241.

15. Alonso-Caballero A, Schönfelder J, Poly S, Corsetti F, De Sancho D, Artacho E, Perez-Jimenez R. Mechanical architecture and folding of E. coli type 1 pilus domains. Nat Commun. 2018 Jul 16;9(1):2758. doi: 10.1038/s41467-018-05107-6. PMID: 30013059; PMCID: PMC6048123.

16. Orndorff PE, Falkow S. Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. J Bacteriol 1984;159: 736–744.

17. Klemm P. Two regulatory fim genes, fimB and fimE, control the phase variation of type 1 fimbriae in Escherichia coli. EMBO J. 1986 Jun;5(6):1389-93. PMID: 2874022; PMCID: PMC1166953.

18. Connell I, Agace W, Klemm P, Schembri M, Mărild S, Svanborg C. Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. Proc Natl Acad Sci U S A. 1996 Sep 3;93(18):9827-32. doi: 10.1073/pnas.93.18.9827. PMID: 8790416; PMCID: PMC38514.

19. Shephard E. Specimen collection 1: general principles and procedure for obtaining a midstream urine specimen. Nursing Times. 2017; 113:7, 45-47.

20. Koneman E, Allen S, Janda W, Schreckenberger P, Winn, W. Color Atlas and text book of diagnostic microbiology. 5th edn. J.B. Lippincott Company, Philadelphia, 1997.

21. Nabajit Deka. Comparison of Tissue Culture plate method, Tube Method and Congo Red Agar Method for the detection of biofilm formation by Coagulase Negative Staphylococcus isolated from Non-clinical Isolates. Int J Curr Microbiol App Sci. 2014; 3(10) 810-815.

22. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty third information supplements. CLSI document M100- S28. Clinical and Laboratory Standards Institute, Wayne, PA, 2018.

23. Waskiewicz A, Alexis O, Cross D. Supporting patients with long-term catheterisation to reduce risk of catheter-associated urinary tract infection. Br J Nurs. 2019 May 9;28(9):S4-S17. doi: 10.12968/bjon.2019.28.9.S4. PMID: 31070971.

24. Tamadonfar KO, Omattage NS, Spaulding CN, Hultgren SJ. Reaching the End of the Line: Urinary Tract Infections. Microbiol Spectr. 2019 May;7(3). doi: 10.1128/microbiolspec.BAI-0014-2019. PMID: 31172909.

25. Conover MS, Flores-Mireles AL, Hibbing ME, Dodson K, Hultgren SJ. Establishment and Characterization of UTI and CAUTI in a Mouse Model. J Vis Exp. 2015 Jun 23;(100):e52892. doi: 10.3791/52892. PMID: 26132341; PMCID: PMC4544389.

26. Lai CC, Lee CM, Chiang HT, Hung CT, Chen YC, Su LH, *et al.* Infection Control Society of Taiwan. Implementation of a national bundle care program to reduce catheter-associated urinary tract infection in high-risk units of hospitals in Taiwan. J Microbiol Immunol Infect. 2017 Aug;50(4):464-470. doi: 10.1016/j.jmii.2017.01.006. Epub 2017 Jun 26. PMID: 28711430. Ramírez-Castillo FY, Moreno-Flores AC, Avelar-González FJ, Márquez-Díaz F, Harel J, Guerrero-Barrera AL. An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. Ann Clin Microbiol Antimicrob. 2018 Jul 24;17(1):34. doi: 10.1186/s12941-018-0286-5. PMID: 30041652; PMCID: PMC6057003.

27. Pelling H, Nzakizwanayo J, Milo S, Denham EL, MacFarlane WM, Bock LJ, Sutton JM, Jones BV. Bacterial biofilm formation on indwelling urethral catheters. Lett Appl

Microbiol. 2019 Apr;68(4):277-293. doi: 10.1111/lam.13144. PMID: 30811615.

28. Katongole P, Nalubega F, Florence NC, Asiimwe B, Andia I. Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic Escherichia coli isolated from clinical isolates in Uganda. BMC Infect Dis. 2020 Jun 29;20(1):453. doi: 10.1186/s12879-020-05186-1. PMID: 32600258; PMCID: PMC7325280.

29. Zhao F, Yang H, Bi D, Khaledi A, Qiao M. A systematic review and meta-analysis of antibiotic resistance patterns, and the correlation between biofilm formation with virulence factors in uropathogenic E. coli isolated from urinary tract infections. Microb Pathog. 2020 Jul;144:104196. doi: 10.1016/j.micpath.2020.104196. Epub 2020 Apr 10. PMID: 32283258.

30. Hagos DG, Mezgebo TA, Berhane S, Medhanyie AA. Biofilm and hemagglutinin formation: a Hallmark for drug resistant uropathogenic Escherichia coli. BMC Res Notes. 2019 Jun 24;12(1):358. doi: 10.1186/s13104-019-4382-1. PMID: 31234908; PMCID: PMC6591996.

31. Ramadan R, Omar N, Dawaba M, Moemen D. Bacterial biofilm dependent catheter associated urinary tract infections: Characterization, antibiotic resistance pattern and risk factors. Egyptian J Basic Appl Sci, 2021; 8:1, 64-74, DOI: 10.1080/2314808X.2021.1905464.

32. Köves B, Magyar A, Tenke P. Spectrum and antibiotic resistance of catheter-associated urinary tract infections. GMS Infect Dis. 2017 Nov 22;5:Doc06. doi: 10.3205/id000032. PMID: 30671328; PMCID: PMC6301742.

33. Mahmoud ER, Ahmed HA, Abo-senna AS, Riad OK, Abo- Shadi MM. Isolation and characterization of six gamma-irradiated bacteriophages specific for MRSA and VRSA isolated from skin infections. J Radiat Res Appl Sci. 2020; DOI:10.1080/16878507.2020.1795564

34. Wang J, He L, Sha J, Zhu H, Huang L, Zhu X, *et al.* Etiology and antimicrobial resistance patterns in pediatric urinary tract infection. Pediatr Int. 2018 May;60(5):418-422. doi: 10.1111/ped.13526. Epub 2018 Mar 30. PMID: 29394522.

35. Zavala-Cerna MG, Segura-Cobos M, Gonzalez R, Zavala-Trujillo IG, Navarro-Perez SF, *et al.* The Clinical Significance of High Antimicrobial Resistance in Community-Acquired Urinary Tract Infections. Can J Infect Dis Med Microbiol. 2020 Jun 4;2020:2967260. doi: 10.1155/2020/2967260. PMID: 32566057; PMCID: PMC7293720.

36. Ramírez-Castillo FY, Moreno-Flores AC, Avelar-González FJ, Márquez-Díaz F, Harel J, Guerrero-Barrera AL. An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. Ann Clin Microbiol Antimicrob. 2018 Jul 24;17(1):34. doi: 10.1186/s12941-018-0286-5. PMID: 30041652; PMCID: PMC6057003.

37. Malekzadegan Y, Khashei R, Sedigh Ebrahim-Saraie H, Jahanabadi Z. Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic Escherichia coli isolates from Iranian patients. BMC Infect Dis. 2018 Nov 15;18(1):572. doi: 10.1186/s12879-018-3467-0. PMID: 30442101; PMCID: PMC6238375.

38. McLellan LK, McAllaster MR, Kim AS, Tóthová Ľ, Olson PD, Pinkner JS, *et al.* A host receptor enables type 1 pilus-mediated pathogenesis of Escherichia coli pyelonephritis. PLoS Pathog. 2021 Jan 29;17(1):e1009314. doi: 10.1371/journal.ppat.1009314. PMID: 33513212; PMCID: PMC7875428.

39. Raheel IAER, Hassan WH, Salem SSR, Salam HSH. Biofilm forming potentiality of *Escherichia coli* isolated from bovine endometritis and their antibiotic resistance profiles. J Adv Vet Anim Res. 2020 Aug 5;7(3):442-451. doi: 10.5455/javar.2020.g440. PMID: 33005670; PMCID: PMC7521812.

40. Hussien H, Elbehiry A, Saad M, Hadad G, Moussa I, Dawoud T, Mubarak A, Marzouk E. Molecular characterization of *Escherichia coli* isolated from cheese and biocontrol of Shiga toxigenic *E. coli* with essential oils. Ital J Food Saf. 2019 Oct 1;8(3):8291. doi: 10.4081/ijfs.2019.8291. PMID: 31632932; PMCID: PMC6784595.

41. Shash RY, Elshimy AA, Soliman MY, Mosharafa AA. Molecular Characterization of Extended-Spectrum β-Lactamase *Enterobacteriaceae* Isolated from

Egyptian Patients with Community- and Hospital-Acquired Urinary Tract Infection. Am J Trop Med Hyg. 2019 Mar;100(3):522-528. doi: 10.4269/ajtmh.18-0396. PMID: 30594263; PMCID: PMC6402938.

42. Yamasaki S, Shigemura K, Osawa K, Kitagawa K, Ishii A, Kuntaman K, *et al.* Genetic analysis of ESBL-producing Klebsiella pneumoniae isolated from UTI patients in Indonesia. J Infect Chemother. 2021 Jan;27(1):55-61. doi: 10.1016/j.jiac.2020.08.007. Epub 2020 Sep 2. PMID: 32888833.

43. Logan LK, Medernach RL, Domitrovic TN, Rispens JR, Hujer AM, Qureshi NK, et al. The Clinical and Molecular Epidemiology of CTX-M-9 Group Producing Enterobacteriaceae Infections in Children. Infect Dis Ther. 2019 Jun;8(2):243-254. doi: 10.1007/s40121-019-0237-2. Epub 2019 Feb 16. PMID: 30772921; PMCID: PMC6522575.

44. Namaei MH, Yousefi M, Ziaee M, Salehabadi A, Ghannadkafi M, Amini E, et al. First report of prevalence of CTX-M-15-producing Escherichia coli O25b/ST131from Iran. Microb Drug Resist 2017;23:879–84, http://dx.doi.org/10.1089/mdr.2016.0272.

45. Demirci M, Ünlü Ö, İstanbullu Tosun A. Detection of O25b-ST131 clone, CTX-M-1 and CTX-M-15 genes via real-time PCR in Escherichia coli strains in patients with UTIs obtained from a university hospital in Istanbul. J Infect Public Health. 2019 Sep-Oct;12(5):640-644. doi: 10.1016/j.jiph.2019.02.017. Epub 2019 Feb 27. PMID: 30826300.

46. Alex J, Salamonson Y, Ramjan LM, Montayre J, Fitzsimons J, Ferguson C. The impact of educational interventions for patients living with indwelling urinary catheters: A scoping review. Contemp Nurse. 2020 Aug;56(4):309-330. doi: 10.1080/10376178.2020.1835509. Epub 2020 Oct 29. PMID: 33043843.