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The detection of biofilm formation genes in some bacterial species of catheter associated co-infections

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Abstract

Background. The biofilm formation in catheter urinary tract infections (CUTI) considered as main problem in health sector. Aim. The aim of this research was to develop a reliable and innovative procedure for the detection of multi-species biofilm in indoor patients, particularly in association with catheters and indwelling medical devices. Methods. A total of one hundred and thirty-one samples were collected from patients (urinary catheter) aged 40-70 years and ratio (1:4) male to female through period from October 2023 to March 2024, each sample was streaked on two media MacConkey and Mannitol salt agar. The 96 samples showed growth while other samples, 35, were negative. The 96 samples that showed growth were diverse between Gram positive bacteria (only six samples) and Gram-negative bacteria (ninety samples). ninety samples divided to three sections, the first included 68 samples containing single type of bacteria, second included 7 samples containing two types of bacteria (duplicate), while the last 15 samples contain three types of bacteria (triplicate). After being identified, triplicate samples tested for their ability to form biofilm. Polymerase chain reaction (PCR) was used to amplify mrpA and fimH genes. Results. The results of this study revealed that the isolated triplicate was Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli) and Proteus mirabilis (P. mirabilis). Biofilm results showed that (22/30) 73.33% isolates were biofilm producers. PCR results revealed that mrpA gene in K. pneumoniae 2/10 (20%) while fimH gene 9/10 (90%), the results of multiplex PCR in K. pneumoniae 2/10. In monoplex PCR P. mirabilis harbored mrpA only 8/10 (80%) while multiplex PCR revealed that none of the tested isolates harbored both genes. The last results of PCR of mrpA gene in E. coli 8/10 (80%) while fimH gene 5/10 (50%), the results of multiplex PCR in E. coli 3/10. Percentage of mrpA genes in (K. pneumoniae, P. mirabilis and E. coli) was 60% but percentage of fimH genes at (K. pneumoniae and E. coli) was 70%. The results of PCR in E. coli showed that isolates have more mrpA gene than fimH gene.

Keywords: Biofilm, Uropathogens, CUTI, *mrpA* gene

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Introduction

Biofilms are well-known dominant micro-ecosystems made of aggregated live microorganisms that have evolved to withstand harsh environmental conditions, they can form on biotic or abiotic moist surfaces (1). Biofilms have different features that distinguish them from free-floating bacteria (2), characterized by cells that exhibit changed phenotypes in terms of growth rate and gene transcription, and that are irreversibly attached to a substratum and embedded in a matrix of extracellular polymeric substances that they have generated (3). Biofilms are commonly found on the surface of medical equipment and body tissue, as well as in industrial settings, food processing facilities, and natural environments (4). Extracellular polymeric substance (EPS) is one of the main components of biofilm, which causes it to create a coherent three-dimensional structure, numerous methods can be used to characterize the features of the structural, chemical, and physical organizations of EPS during the biofilm growth process (5). Urinary tract infections in people whose bladders are catheterized or have been catheterized within the last 48 hours are known as catheter-associated urinary tract infections or CAUTIs (6), Risk factors for CAUTI involve age, female gender, diabetes, and lengthy catheterization (7). The length of catheterization is the primary factor that determines the development of bacteriuria (8). One of the most frequent infections seen in hospital settings are urinary tract infections (UTIs) linked to the use of an indwelling catheter (9). An extended period of catheterization has been linked to the creation of biofilm, which can act as a barrier to the penetration of antibiotics (10). Numerous known pathogens and opportunistic bacteria belonging to the Enterobacteriaceae family are linked to the development of meningitis, sepsis, urinary tract infections, and enteric diseases in humans (11). Catheter-associated urinary tract infections make of the majority of cases in complicated urinary tract infections, The most prevalent causative organisms overall after Uropathogenic Escherichia coli (UPEC) include Enterococcus spp, Klebsiella pneumoniae, Candida spp, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, and Group B Streptococcus (6). Urease is produced by uro-pathogens such P. mirabilis and K. pneumoniae, which can break down urea, release ammonia, and raise urine pH (alkaline conditions) and build crystalline biofilms on urinary catheters (12). Biofilms are well known for giving humans chronic illnesses, antibiotic resistance. Biofilms can develop on medical implants such prosthetic joints, stents, and catheters, resulting in infections that are challenging to identify and manage. The importance of research lies in uncovering hidden partners for catheter patients and determine that isolates producer biofilm.

MATERIALS AND METHODS

Collection of samples

The 131 samples that were gathered from urinary catheterized patients that aged 40-70 years and ratio (1:4) male to female in Baghdad hospitals (Al Yarmuk Teaching Hospital and Kadhimiya Teaching Hospital) through period from October 2023 to March 2024. Samples were taken based on age and removal catheter from personals after period at least 2 days. All samples were transported from hospitals to laboratory by sterilized Plane tubes that contain nutrient broth.

Isolation and Identification of bacteria

The collected samples were cultured immediately in nutrient broth and incubated for 24 hours at 37°C, then the positive growth subcultured on two culture media Mannitol salt Agar and MacConkey agar. The identification of the isolates included morphological characteristics and biochemical tests which carried out depending on Bergy's Manual of Systematic Bacteriology, 2nd edition (13,14). The confirmation tests were done by using Vitek 2 compact system.

Detection of biofilm formation by Congo red agar

This approach has been described by Freeman et al. (15). The Congo red agar medium inoculated by tested bacterial isolates by streaking method then incubates at 37 °C for 24h. The black colonies indicate that isolates are producers of biofilm while red colonies indicate the tested isolates non producers of biofilm.

Molecular detection of biofilm formation genes

DNA extracted according to the methodology of Gram-negative bacteria provided by Genomic DNA mini-Kit (Transgene, China). The primers used to detect adhesions coding genes mrpA and fimH were designated for this study. Forward and reversed primers of MrpA gene: MrpA-F is (TTGGTTCAATCATTGATGCTC) and MrpA-R (GGGTGCCTAATTTGATTTGTT) for the amplification of internal gene with PCR product 294 bp. FimH-F (AAAATAATCCCCCTGTTCACC) and FimH-R (GAAGGAGTCGCTATTGTAGTT) for the amplification of internal gene with PCR product 486 bp. Using a 25 μL reaction mixture that includes 5 μL of DNA and 3.5 μL of nuclease-free water, 1 μL of each forward primer and 1 μL of each reverse primer were added to 12.5 μL of Green Master Mix in order to amplify the MrpA and FimH genes. The PCR reaction was occurred in Thermal Block, with one round of initial denaturation for five minutes at 94°C preceding gene amplification. 32 cycles of denaturation at 94°C for 30 seconds, annealing for 45 seconds at 46°C for the (MrpA and FimH) genes, extension for 45 seconds at 72°C, and final extension for 7 minutes at 72°C were then applied to the reaction mixture. An agarose gel was used to electrophorese the PCR products that had been amplified.

Amplify of PCR Product in agarose gel

The detection of PCR products was tested using agarose gel electrophoresis. A 2% concentration of agarose gel was made 1.5g of agarose powder is dissolved in 1X TBE buffer, then boiled, cooled to 50°C, and added red safe dye, and wells were formed by a special comb. Each well was loaded with 10 μL of each sample, while the first well was loaded with 10 μL of 100 bp DNA ladder, and the gel was closed and exposed to 80 volts of electric current. The red safe stained bands in gel were seen using the gel documenting system.

Statistical Analysis

Statistical packages of Social Sciences-SPSS program were used to detect the effect of difference factors in study parameters. Chi-square test was significant compared to the percentage (0.05 and 0.01 probability) in this study.

Ethical Approval

We confirm that all tables and figures in the manuscript present the results of the current study. The authors have adhered to ethical considerations, with approval granted by the Research Ethics Committee and the Scientific Committee designated by the Biology Department, College of Science, University of Baghdad, under reference number CSEC/1023/0097.

Results

From all these 131 samples of catheter, aged 40-70 years and ratio (1:4) male to female most of them 96/131(73.28%) samples showed growth while 35/131(26.71%) didn't show any growth. The Gram-negative bacteria were most prevalent bacteria 90/96 (93.75%) while G+ve bacteria occupied 6/90 (6.25%) only of the positive growth showed in (table 1).

Table (1): Frequency of G+ve and G-ve bacteria isolated from this study.

	G-ve	G+ve
No. of samples	90	6
Percentage of samples	93.75%	6.25%
Chi-Square: χ^2 (P-value)	73.500 ** (0.0001)	
** ($P \leq 0.01$).		

The culture of samples found that 68/90 of samples contain single isolate, 7/90 of samples contain duplicate isolates and the last 15/90 were triplicate samples. findings of this investigation demonstrated that triplicates were *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae*, this was the most prevalent (66.66%) while the other triplicates were less frequent.

Congo red agar method

The results of this study showed that 22/30 (73.33%) of the co-isolates were biofilm produces figure (1).

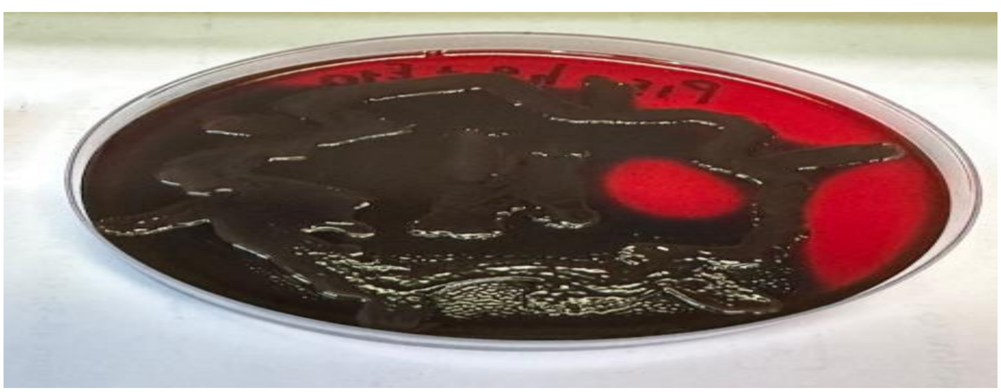

Figure (1): Congo red agar cultured with positive results of biofilm production for multispecies infections: *P. mirabilis*, *E. coli* and *K. pneumoniae*.

Multiplex PCR for biofilm gene detection

The results of this study referred to presence of the *fimH* gene in *Klebsiella pneumoniae* 9/10 (90%) but results of *mrpA* gene only 2/10 (20%). There is no previous research indicating the presence of a gene *mrpA* in *K. pneumoniae*, as this study indicated the presence of this gene. In monoplex PCR *Proteus mirabilis* harbored *mrpA* only 8/10 (80%). The presence of *fimH* gene in *Escherichia coli* 5/10 (50%), but the results of *mrpA* gene was 8/10 (80%). There has been no prior research on the presence of the *mrpA* gene in *E. coli*, and the high percentage of the gene in *E. coli* is due to horizontal gene transfer. The results of multiplex PCR were 2/10 of *K. pneumoniae* isolates, regarding *P. mirabilis* the genetic results of multiplex PCR revealed that none of the tested isolates harbored both genes while result of *E. coli* 3/10 (figure 2,3,4).

Figure (2): Agarose gel electrophoresis for multiplex PCR for *MrpA* and *FimH* genes (2% agarose gel, TAE buffer, 80 min). *MrpA* gene (size 294 bp) and *FimH* gene (486 bp) ,80 volts, L: DNA Ladder (100bp).

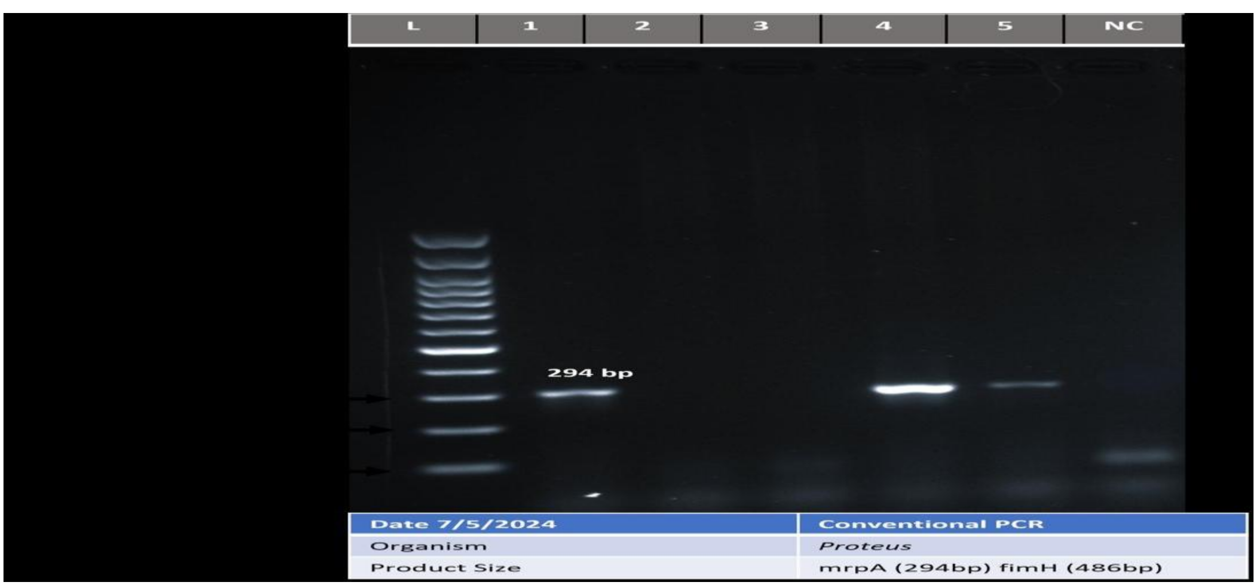

Figure (3): Agarose gel electrophoresis for multiplex PCR for *MrpA* and *FimH* genes (2% agarose gel, TAE buffer, 80 min). *MrpA* gene (size 294 bp) and *FimH* gene (486) ,80 volts, L: DNA Ladder (100bp).

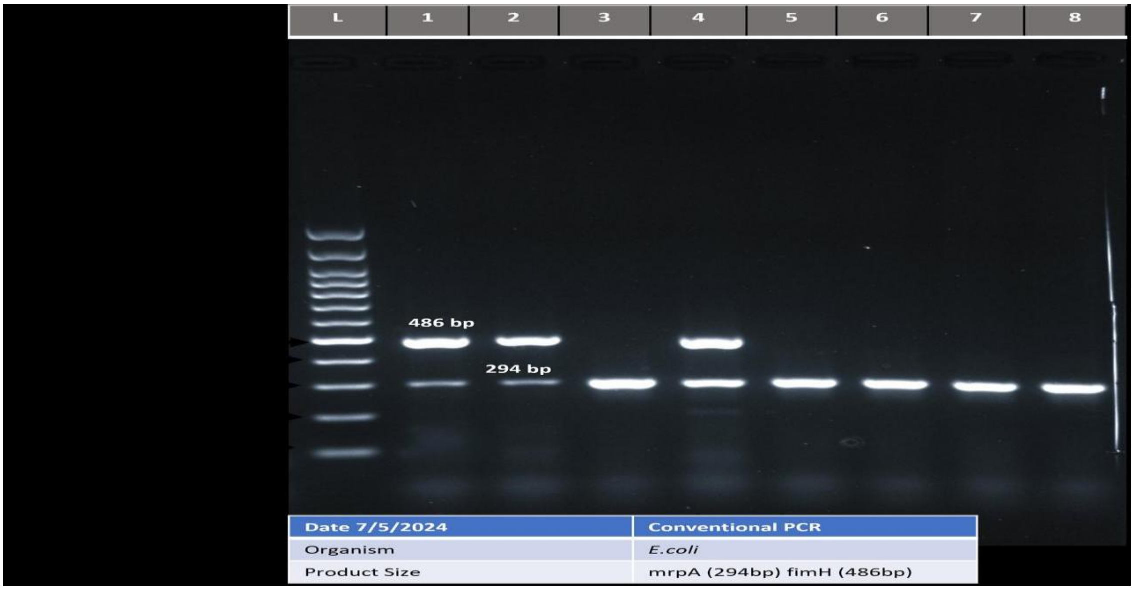

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Table (2): Result PCR Amplification of *MrpA* gene by PCR

Isolates	Total	Positive *MrpA*	Percentage (%)	Negative *MrpA*	Percentage (%)	P-value
K. pneumonia	10	2	20%	8	80%	0.0498 *
P. mirabilis	10	8	80%	2	20%	0.0498 *
E. coli	10	8	80%	2	20%	0.0498 *
P-value	---	---	0.0437 *	---	0.0483 *	---
* (P≤0.05).						

Table (3): Result PCR Amplification of *FimH* gene by PCR

Isolates	Total	Positive *FimH*	Percentage (%)	Negative *FimH*	Percentage (%)	P-value
K. pneumonia	10	9	90%	1	1%	0.0001 **
P. mirabilis	10	0	0%	10	100%	0.0001 **
E. coli	10	5	50%	5	50%	1.00 NS
P-value	---	---	0.0001 **	---	0.0001 **	---
** (P≤0.01).						

Table (4): Result Multiplex PCR Amplification of *MrpA and FimH* genes

Isolates	Total	Positive	Percentage (%)	Negative	Percentage (%)	P-value
K. pneumoniae	10	2	20%	8	80%	0.0086 **
P. mirabilis	10	0	0%	10	100%	0.0001 **
E. coli	10	3	30%	7	70%	0.0319 *
P-value	---	---	0.071 NS	---	0.0748 NS	---
* (P≤0.05), ** (P≤0.01).						

Discussion

In a prior study in Kerman, southeast Iran, 146 *K. pneumoniae* isolates were isolated from various clinical specimens, it was found the percentage of *fimH* was 48.5% in all isolates (16). Muhsin *et al*. (17) studied 60 clinical isolates of *K. pneumoniae* that were collected from three sources including urine samples (20) from 3 hospitals in Baghdad, the gene *fim-H1* was detected in (78.3%) of all clinical isolates.

Al-Hamdani and Al-Hashimy (18) fifty urine samples were taken from UTI patients in total from different sites in Baghdad, the urine of these 10 patients was identified by routine tests, biochemical tests and also VITEK 2 system used for more identification, they detected four important virulence genes and one of these genes was *mrpA* 8/10 (80%). Hussein *et al*. (19) investigated eleven virulence genes of *P. mirabilis* isolated from individuals suffering from urinary tract infections, Genes that cause virulence (11) were detected in more than 90% of the isolates (*mrpA* was 92.1%). The 100 *P. mirabilis* isolates that Tabatabaei *et al*. (20) obtained from patients who visited a tertiary hospital in Iran were split into 35 (35%) strains that were isolated from catheterized patients' urine and 65 (65%) strains that were isolated from non-catheterized patients' urine. They also looked into nine virulent genes, one of which was *mrpA* (90/100), and the percentage of this gene was 90%. De Oliveira *et al*. (21) eight genes were examined in the collection of 183 strains recovered from the urine of CA-UTI patients in Londrina, Paraná State, Brazil; *mrpA* was one of these genes, and all strains tested positive for *mrpA*.

Tarchouna *et al*. (22) PCR was used to check for the presence of seven virulence genes in 90 uropathogenic *E. coli* strains (one of them *fimH*), this gene was 68%. Another study, Abdul-Ghaffar and Abu-Risha (23) 129 clinical urine specimens were obtained from patients with UTIs of different ages and genders who were taken to Baghdad's AL-Yarmouk Teaching Hospital; 92 of these specimens came from hospitalized patients who had urinary catheters, and 37 were not, their study carried out to 43 *E. coli* isolates (33.3%), hospital urinary catheterized patients accounted for 21 (22.8%) of the isolates, whereas non-catheterized patients accounted for 22 (59.4%), the presence of *fimH* in 38 isolates (88.37%), the percent of this gene in catheterized patients (20 isolates,95.23%) higher than non-catheterized patients (18 isolates,81.81%). According to Al-Taai *et al*. (24), 250 *E. coli* isolates obtained from children in camp saad of displaced /Diyala; the *fimH* gene was 100 % (in 15 isolates) frequent. In other study, Musafer *et al*. (25) used PCR for four genes to identify 50 *E. Coli* clinical isolates from UTI patients; the results showed *fimH* (86%). Ghafer *et al*. (26) 54 isolates of UPEC were collected from patients with UTIs attending different hospitals in Baghdad/Iraq, these isolates identified by culturing on differential media and using Vitek 2 automated system for confirmed the identification, by using PCR assay with specific primers screened the prevalence of *fimH* gene among 45 UPEC isolates, the result of this gene (95.6%). 119 clinical samples were collected from patients at several hospitals in Baghdad, determined 76 of samples as *E. coli* by Vitek 2 assay, the DNA of 30 isolates was successfully extracted then 21/30 (70%) of isolates have *fimH* gene (27).

Conclusion: *P. mirabilis* reduces the ability of the other two species to form biofilms. Percentage of *mrpA* gene in (*K. pneumoniae*, *P. mirabilis* and *E. coli*) was 60% but the percentage of *fimH* gene in (*K. pneumoniae* and *E. coli*) was 70%. The results of PCR in *E. coli* showed that isolates have more *MrpA* gene than *FimH* gene may be because horizontal gene transfer.

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