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Molecular Detection of Biofilm-Related Genes (*mrpA*, *fimH*) in Urinary Tract Co-Infections

Mohammed Abdulwahab Abdulshafi[1], Marwa Hameed Alkhafaji[2]
[1]*Department of medical laboratory Techniques, Al-Farahidi University, Baghdad, 10021, Iraq*
[2]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq*.

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Abstract

Urinary tract co-infections are particularly significant due to their potential to facilitate the transfer of biofilm formation genes between pathogens. The research aims to uncover hidden partners for catheter patients and determine, that isolates producer biofilm. 131 samples were collected from urinary catheter patients, during the period from October 2023 to March 2024. Each sample was cultured on mannitol salt agar and MacConkey agar plates to differentiate between G+ve and -ve bacteria. Then, identification was completed by using chemical tests and the Vitek 2 compact system. Bacterial DNA was extracted, and specific primers were designed to amplify *mrpA* and *fimH* genes using multiplex PCR. 96/131 (73.28%) of samples showed positive growth, while 35/131 (26.71%) showed negative growth. On MacConkey agar, the results showed a growth percent of 93.75% (90 samples), while on mannitol salt agar, the growth percent was as low as 6.25% (6) samples. The results of MacConkey agar showed single species 68/90 (75.55%), double 7/90 (7.77%), and triple 15/90 (16.66%). Triplicates were *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli,* which were the most prevalent (66.66%). Biofilm detection was done using three methods, congo red agar method, microtiter plate method, and electron microscopic examination. In conclusion*, P. mirabilis* was found to reduce the biofilm formation potential of *K. pneumoniae* and *E. coli*. The *mrpA* gene was detected in 60% of *K. pneumoniae*, *P. mirabilis*, and *E. coli* isolates, while the *fimH* gene was present in 70% of *K. pneumoniae* and *E. coli* isolates. Notably, *E. coli* isolates had a higher percentage of the *mrpA* gene than *fimH*, likely due to horizontal gene transfer.

Keywords: Biofilm, *fimH, mrpA*, Triplicate co-infections, Urinary tract infection.

الكشف الجزيئي عن الجينات المرتبطة بالأغشية الحيوية في الالتهابات المشتركة للمسالك البولية

محمد عبدالوهاب عبدالشافي[1] ، مروى حميد مطشر[2]
[1]قسم تقنيات المختبرات الطبية، جامعة الفراهيدي، بغداد، 10021، العراق
[2]قسم علوم الحياة, كليه العلوم, جامعه بغداد, بغداد, العراق

الخلاصه:

تعد عدوى المسالك البولية المصاحبة ذات أهمية خاصة نظرًا لقدرتها على تسهيل نقل جينات تكوين الأغشية الحيوية بين مسببات الأمراض، لذا فإن هدف البحث يكمن في الكشف عن الشركاء المخفيين لمرضى القسطرة وتحديد العزلات المنتجة للأغشية الحيوية .تم جمع 131 عينة من مرضى القسطرة البولية، خلال الفترة

*Email: Mohammed.Abdulwahab1602a@sc.uobaghdad.edu.iq

من تشرين الاول 2023 إلى اذار 2024. تمت زراعة كل عينة على أجار ملح المانيتول وأجار ماكونكي للتمييز بين البكتيريا G+ve و .ve–ثم تم استكمال التعريف باستخدام الاختبارات الكيميائية ونظام Vitek 2 compact. تم استخراج الحمض النووي للبكتيريا، وتم تصميم بادئات محددة لتضخيم جينات *mrpA* و*fimH* باستخدام تفاعل البوليميراز المتسلسل المتعدد .كانت 131/96 (73.28٪) من العينات ذات نمو إيجابي، بينما كانت 131/35 (26.71٪) سلبية .أظهرت النتائج على أجار ماكونكي أن نسبة النمو بلغت 93.75% (90 عينة)، بينما كانت نسبة النمو على أجار ملح المانيتول منخفضة حيث بلغت 6.25% (6) عينات .أظهرت نتائج أجار ماكونكي أن النوع الواحد 90/68 (75.55%)، والنوع المزدوج 90/7 (7.77%) والنوع الثلاثي 90/15 (16.66%). وكانت الأنواع الثلاثية هي *P. mirabilis* ، *K. pneumoniae* ، *E. coli* وكانت الأكثر انتشارًا(66.66%) .تم الكشف عن الأغشية الحيوية بثلاث طرق، طريقة أجار الكونغو الأحمر، طريقة لوحة الميكروتيتر والفحص المجهري الإلكتروني .وفي الختام، وجد أن بروتيوس ميرابيليس يقلل من إمكانية تكوين الأغشية الحيوية لـ *K. pneumoniae* و *E. coli*. تم اكتشاف جين *mrpA* في 60% من عزلات *K. pneumoniae* و*P. mirabilis* و*E. coli*، بينما كان جين *fimH* موجودًا في 70% من عزلات *K. pneumoniae* و E. coli. ومن الجدير بالذكر أن عزلات *E. coli* تحتوي على نسبة أعلى من جين *mrpA* مقارنة بـ*fimH* ، ويرجع ذلك على الأرجح إلى النقل الجيني الأفقي.

Introduction

Due to its connection to bacterial virulence and other clinical environmental issues, biofilm development is one of the largest issues facing scientists [1]. Biofilms are well-known dominant micro-ecosystems made of aggregated live microorganisms that have evolved to withstand harsh environmental conditions [2]. Biofilm poses a serious threat to global health concerns because it can show signs of growing resistance to traditional antibiotics and cause sickness through infections linked to devices and unrelated to devices (tissue). For this reason, treating and inhibiting biofilm-associated illnesses requires early detection as well as searching for novel and alternative treatments [3]. Multiple microbial species form the majority of biofilms found in nature, and these mixed species biofilms reflect the true lives of bacteria, fungi, viruses (phages), and/or protozoa [4]. Multi-species biofilms are commonplace in natural and artificial settings and are effective forms of life on Earth. Multispecies biofilms are endowed with emergent properties—like improved resource uptake, stress tolerance, and enhanced metabolic activity—that are not predictable from studies of individual planktonic cells. The majority of elemental biogeochemical cycling activities in soil and water are also caused by microbes found in multispecies biofilms [5]. 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 [3]. Biofilms have different features that distinguish them from free-floating bacteria [6]; it was characterized by cells that show changed phenotypes in terms of growth rate and gene transcription; and that are irreversibly connected to a substratum and embedded in a matrix of extracellular polymeric substances that they have produced [7]. One of the most frequent infections seen in hospital settings is urinary tract infections (UTIs) linked to the use of an indwelling catheter [8]. An extended period of catheterization has been linked to the creation of biofilm, which can act as a barrier to the penetration of antibiotics [9]. These indwelling catheters and the biofilms that are connected to them serve as a significant reservoir for Gram-negative bacteria, such as *Klebsiella pneumoniae* and *Escherichia coli* [10]. One of the primary constituents of biofilm is extracellular polymeric substances (EPS), prompting biofilm to form a cohesive three-dimensional framework [11]. *E. coli* is one of the most studied microorganisms due to its widespread use across medical, biological, and commercial fields [12]. UPEC are *E. coli* strains that have migrated from their commensal role as intestinal flora to cause urinary tract infections (UTIs) via several different virulence mechanisms. UPEC refers to *E. coli* strains that have been repeatedly linked to uropathogenicity [13]. Uropathogenic *E. coli* strains have important virulence factors, such as type 1 fimbriae, which

are responsible for bacterial pathogenicity and biofilm production. Type 1 fimbriae are expressed by more than 95% of all *E. coli* strains [14]. The genus *Klebsiella* is facultative, anaerobic, non-motile, Gram-negative rods with a conspicuous polysaccharide capsule [15]. *K. pneumoniae* is one of the multidrug-resistant organisms that has been recognized as a serious hazard to human health, infections from it are especially problematic in the hospital context for newborns, the elderly, and immunocompromised patients [16]. Fimbrial adhesions aid in adhesion to both biotic and abiotic surfaces. *K. pneumoniae* expresses type 1 and type 3 fimbriae of two fimbrial adhesions [17]. All members of the Enterobacteriaceae family have type 1 fimbriae, which are necessary for *K. pneumonae* to produce urinary tract infections (UTI). The *Fim H* adhesion, which is found on the tip of the fimbriae and is encoded by operon fim, helps them to exert their sticky qualities [18]. *Proteus mirabilis* is a Gram-negative bacterium that is a frequent cause of catheter-associated urinary tract infections (CAUTIs). Its ability to cause such infections is mostly related to the formation of biofilms on catheter surfaces. In order to form biofilms, *P. mirabilis* expresses a number of virulence factors. Such factors may include adhesion proteins, quorum-sensing molecules, lipopolysaccharides, efflux pumps, and urease enzyme [19]. The MR/P pili gene is important because it provides many virulence traits, such as colony formation, formation of biofilms, and bacterial attachment [20]. The importance of research lies in uncovering hidden partners for catheter patients and determining that isolates producer biofilm.

Materials and methods

Collection of samples

One hundred and thirty-one samples were collected from two hospitals in Baghdad city (Al Yarmuk Teaching Hospital and Kadhimiya Teaching Hospital). All these samples were transported from hospitals to the laboratory in the department by using sterilized plane tubes that contained nutrient broth and incubated at 37°C for one day.

Isolation of bacteria and Identification of isolates

The positive growth of samples was cultured on two culture media: mannitol salt agar and MacConkey agar. The identification of the isolates included morphological characteristics and biochemical tests, which were carried out depending on Bergy's Manual of Systematic Bacteriology, 2nd edition [21]. The confirmation tests were done using the Vitek 2 compact system.

Criteria for taking samples were the age of patients from 40-70 years, gender of patients (ratio of male to female 1:4), catheterized patients, and co-infection after culture samples on media, while exclusions criteria of samples were young patients and single infections after culture samples on media. Taken Ethical approval for collected samples.

Identification by VITEK-2 compact system

The VITEK-2 compact system is an identification system, that depends on the biochemical reactions between the bacterial isolates suspended in their solution, and the media in the VITEK-2 compact system identification cards, to identify the isolates.

The bacterial isolates were inoculated on-to MacConkey agar plates and then incubated over-night at 37 ºC. A single colony was then taken and suspended in to solution. The turbidity of the bacterial suspension was adjusted with the VITEK-2 compact system (Bio Merienx) to match the MacFarland 0.5 standard in 0.45% sodium chloride. Then, the VITEK-2 compact system ID.GN (Gram-negative) card and the bacterial suspension tubes were manually loaded into the VITEK-2 compact system. Following steps on the software were done according to the manufacturer's instructions (Bio Merieux, France).

Detection of biofilm formation

A-*Congo red agar method*

This method, described by Freeman *et al.*, [22], is considered a qualitative method and depends on color change. Consists of brain heart infusion broth (37 g), sucrose (50 g), agar-agar (15 g) dissolving in 900Ml of distilled water and then autoclaved. After that, prepare Congo red solution (melting Congo red dye 0.8g in one hundred milliliters of distilled water and then autoclaved). Cooled mixing to fifty-five and sixty degrees Celsius and merged with Congo red solution. Thereafter, poured into sterile Petri dishes. The purpose of using this medium is to determine the ability of bacteria to form biofilm. The black colonies indicate that isolates producers of biofilm, while red colonies indicate non-producers.

B-*Microtiter plate assay*

Considered quantitative method and described by Christensen *et al.,* [23], bacterial suspension cultures overnight were inoculated in brain heart infusion broth (BHIB). The Eppendorf tube was filled with 980 µL of BHI broth that had been supplemented with 1% glucose following comparison with a 0.5 McFarland tube and 20 µL of the bacterial suspension was added to the Eppendorf tube. By using a microtiter plate, the first three wells were used as a negative control; 200 µL of BHI broth with glucose was placed in these wells. At least three repetitions of each isolate were performed using the 200 µL of bacterial suspension from Eppendorf that was transferred to other wells and incubated at 37°C for 24 hours. Unattached bacteria were eliminated by washing the wells three times with Phosphate buffer saline (PBS) and letting them air dry at room temperature. Poured 200 µL of crystal violet into all wells, permitted to sit a quarter of an hour. After that, wells were washed with PBS three times to remove unbound dye before being allowed to dry at room temperature. Crystal violet solution was then removed, and the dye that bound to the adherent cells was resolubilized with 200 µL glacial acetic acid 33%. Using an ELISA reader, each well's optical density (OD) was calculated at 630 nm. These OD values were used to measure the number of bacteria that adhered to surfaces and formed biofilms according to the following equation in Table (1).

Table 1**:** Equation for biofilm formation in a microtiter plate

Mean OD	OD value Biofilm classification
$OD \leq ODc$	Negative
$ODc < OD \leq 2*ODc$	Positive (weak)
$2*ODc < OD \leq 4*ODc$	Positive (moderate)
$4*ODc < OD$	Positive (strong)

Average of the three repetitions of control was equation for cut-off-value (ODc) calculation.

C. *Electron microscopic examination for biofilm formation on abiotic surfaces*

The smear for scanning electron microscope (SEM) has been prepared by culture of two different types of isolates in the same glass tube of nutrient broth containing sucrose. The former white ring on the glass wall of the tube (that referred to biofilm formation) was removed and placed on a slide for examination.

DNA Extraction

Klebsiella pneumoniae, *P. mirabilis*, and *E. coli* isolates were grown in nutrient broth for 24 hours at 37°C. Bacterial DNA was extracted using (EasyPure® Genomic DNA Kit, Transgene® China).

Primers

All the utilized primers were designated for this study and supplied from Macrogen (Korea). Primer sequences and size were used in this study to identify *mrpA* and *fimH* genes, as shown in Table 2.

Table 2**:** Primers used in this study

Primer Name	sequence	Product size (bp)
mrpA-F	5`- TTGGTTCAATCATTGATGCTC-3`	294
mrpA-R	5`- GGGTGCCTAATTTGATTTGTT-3	
fimH-F	5`-AAAATAATCCCCCTGTTCACC-3`	486
fimH-R	5`-GAAGGAGTCGC TATTGTAGTT-3`	

Molecular Amplification of multiplex (fimH and mrpA) genes

This step was carried out by adding (12.5µL) master mix (2X), and 1µL (10 pmol) of each of the following: forward primer1, reverse primer1, forward primer 2, and reverse primer 2;- (3.5µL) nuclease-free water, and 5Ml of DNA template. Optimal PCR conditions for genes are shown in Table 3**.**

Table 3**:** PCR multiplex conditions for (*mrpA*, *fimH*) genes

Cycle No	Stage	Temperature	Time
1	Initial Denaturation	94 ºC	5 min
30x	Denaturation Annealing Extension	94 ºC 48 ºC 72 ºC	30 sec 45sec 45sec
1	Final Extension	72 ºC	7min

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of different factors on study parameters. The chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study.

Results

Bacterial isolation and identification

Out of 131 catheter samples, 73.28% (96/131) were positive for bacterial growth, while 26.71% (35/131) were negative. On MacConkey agar, the results showed the growth of lactose fermenters (L.F) and non-lactose fermenters (N.L.F) was 93.75% (90/96), while on mannitol salt agar, the growth percent was as low as 6.25% (6/90).

Gram-negative bacteria were the prevalent group in this study (93.75%). These bacterial groups that caused UTI were classified depending on their type of existence in the cultured samples, and the results of this study showed that some of them were single species 75.55% (68/90), others were double 7.77% (7/90) and triple 16.66% (15/90), as shown in-Table 4.

Table 4**:** Distribution of sample study according to type in total number of samples

Total number	Single	Double	Triple
90	68/90	7/90	15/90
Percentage 100%	75.555%	7.777%	16.666%
Chi-Square: χ^2 (*P*-value)	74.015 ** (0.0001)		
** (P≤0.01).			

Triplicate co-infections were characterized initially on MacConkey agar as two groups: lactose fermenters and non-lactose fermenters. Using biochemical tests such as urease, motility, hemolysis, and H_2S production, the triplicate co-infections were diagnosed. Results of this study showed that triplicates were *P. mirabilis*, *K. pneumoniae*, and *E. coli*-, this was the most prevalent 10/15 (66.66%) while the other triplicates were less frequent (Figure 1).

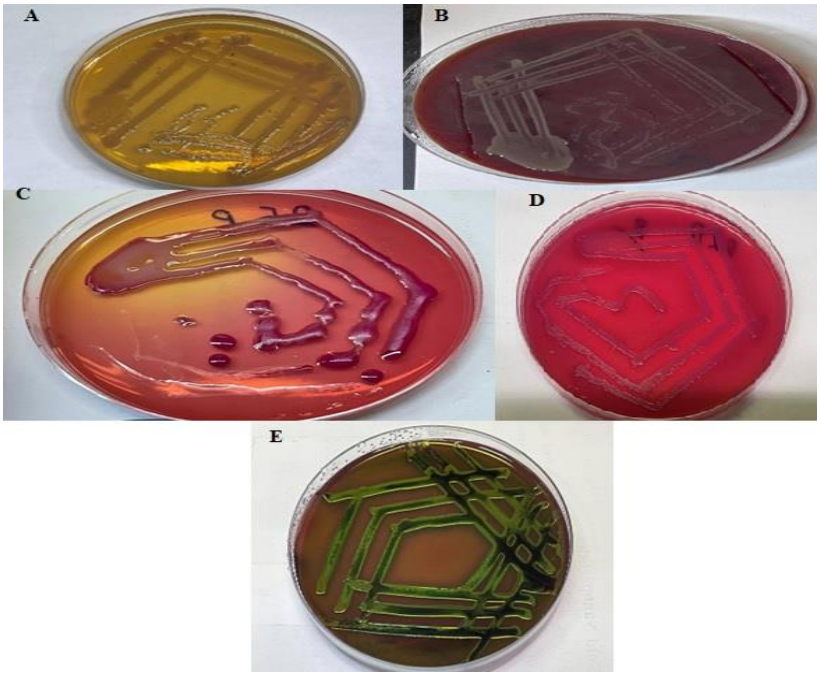

Figure 1: Different isolates of triplicate samples on different media whereas A: *Proteus mirabilis* on MacConkey agar, B: *Proteus mirabilis* on blood agar, C: *Klebsiella pneumoniae* on MacConkey agar, D: *E. coli* on MacConkey agar, and E: Green metallic sheen of *E. coli* on EMB agar.

Identification of isolates by theVITEK-2 compact system

VITEK 2 report to confirm the identification of isolates in Figures 2,3, and 4.

bioMérieux Customer: Microbiology Chart Report Printed November 18, 2023 9:04:17 AM AST

Patient Name: 15PP J, . Patient ID: DFHDFHNDF
Location: Physician:
Lab ID: 185 Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus mirabilis

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.30 hours	**Status:**	Final
Selected Organism	99% Probability	**Proteus mirabilis**		
	Bionumber:	0013000240040210		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure 2: Identification results by VITEK system confirming *Proteus mirabilis*

bioMérieux Customer: Microbiology Chart Report Printed November 18, 2023 9:04:15 AM AST

Patient Name: 21B, . Patient ID: SDGD
Location: Physician:
Lab ID: 183 Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.07 hours	**Status:**	Final
Selected Organism	99% Probability	**Klebsiella pneumoniae ssp pneumoniae**		
	Bionumber:	6607734673564010		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure 3: Identification results by VITEK system confirming *Klebsiella pneumoniae*

bioMérieux Customer: Microbiology Chart Report Printed November 13, 2023 3:11:15 AM CST
Patient Name: D
Location:
Lab ID: 7
Patient ID: 7
Physician: .
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: Urine **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.80 hours	**Status:**	Final
Selected Organism	89% Probability	**Escherichia coli**		
	Bionumber:	0405611750527353		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	(-)	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	+	47	ODC	+	48	LDC	+	53	lHISa	+	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	+	64	lLATa	+			

Figure 4: Identification results by VITEK system confirming *Escherichia coli* isolates

Biofilm formation

The results of biofilm formation by Congo red showed that out of all the 30 triplets, 22 (73.33%) of the co-isolates were biofilm produced while 8/30(26.67%) were negative. *P*-value (0.0019) showed highly significant differences between isolates that form biofilm and those that do not form.

After examining the ability of the isolates to form biofilm separately, all isolates were cultured in the same tube using a nutrient broth. All of these tubes were incubated overnight and then streaked on Congo red agar. Figure 5 clarifies the positive result which appears as black color.

Figure 5: Congo red agar cultured with positive results of multispecies infections: *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae*

Table 5: Percentage of biofilm formation by microtiter plate assay

Type of infections	**Total number**	**Positive No. (%)**	**Negative No. (%)**	***P*-value**
Single infections	30	24 (80.00%)	6 (20.00%)	0.0010 **
Double infections	24	13 (54.17%)	11 (45.83%)	0.683 NS
Triple infections	8	4 (50.00%)	4 (50.00%)	1.00 NS
P-value	---	0.0006 **	0.0492 *	---
* (P≤0.05), ** (P≤0.01).				

*Significant (P≤0.05)
**Highly Significant (P≤0.01)

Mixed species biofilm was examined under scanning electron microscope (Figure 6).

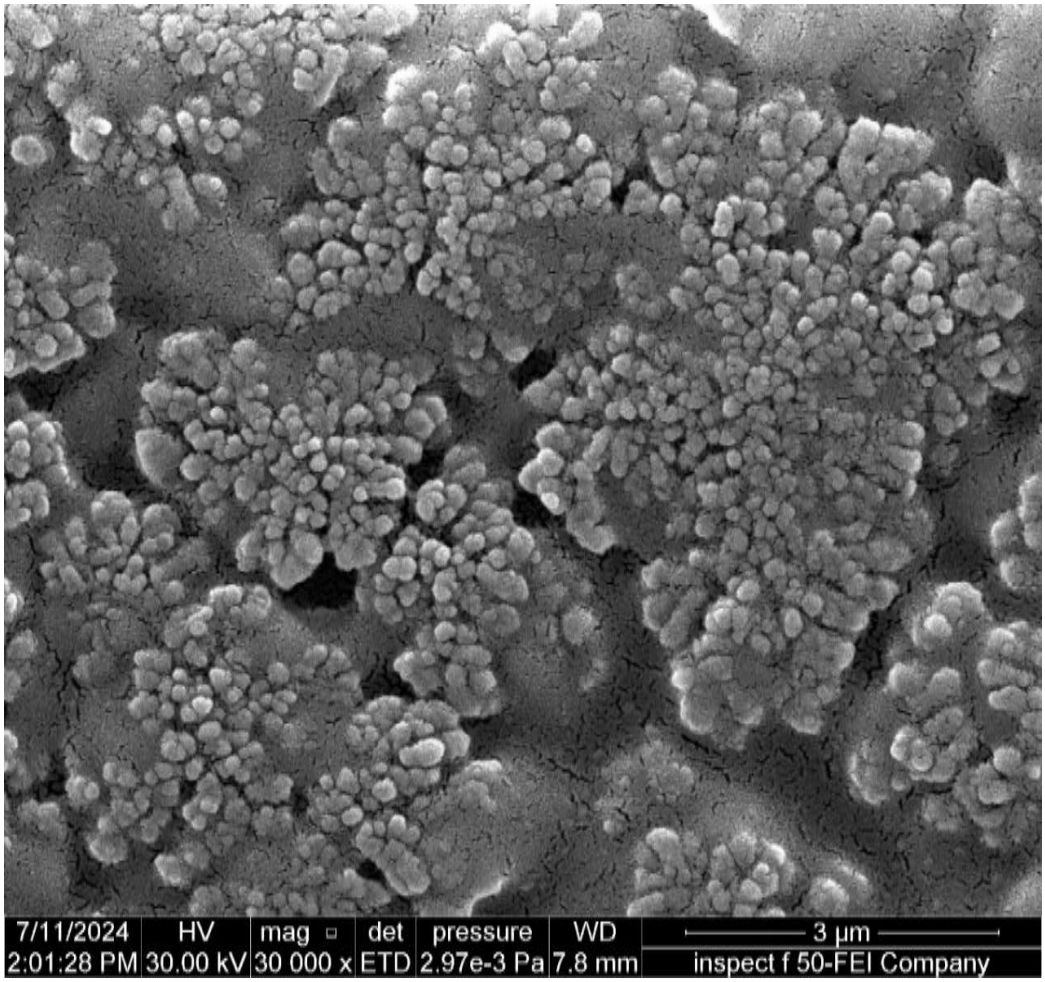

Figure 6: Scanning electron microscope images of the biofilms formed on a biotic surface between two species of bacteria (*Escherichia coli* and *Klebsiella pneumoniae*)

The results of molecular detection revealed the presence of the *fimH* gene in *E. coli* was 50% (5/10), and the result of the *mrpA* gene was 80% (8/10). The high percentage of the *mrpA* gene in *E. coli* could be caused by horizontal gene transfer, and there is no published data about the founding of the *mrpA* gene in these bacteria. Monoplex PCR of *P. mirabilis* showed that the bacteria harbored only *mrpA*-8/10 (80%). The presence of the *fimH* gene in *K. pneumoniae* was 9/10 (90%), but the result of the *mrpA* gene was only 2/10 (20%). There is no published data indicating the presence of the gene *mrpA* in *K. pneumoniae* bacteria, as this study indicated the presence of this gene. The results of multiplex PCR for both genes were 2/10 of *K. pneumoniae* isolates and *E. coli* 3/10, while in *P. mirabilis*, the molecular results of multiplex PCR revealed that none of the tested isolates harbored both *mrpA*, and *fimH* genes, as shown in Figures 7,8, and 9 in addition to table 6.

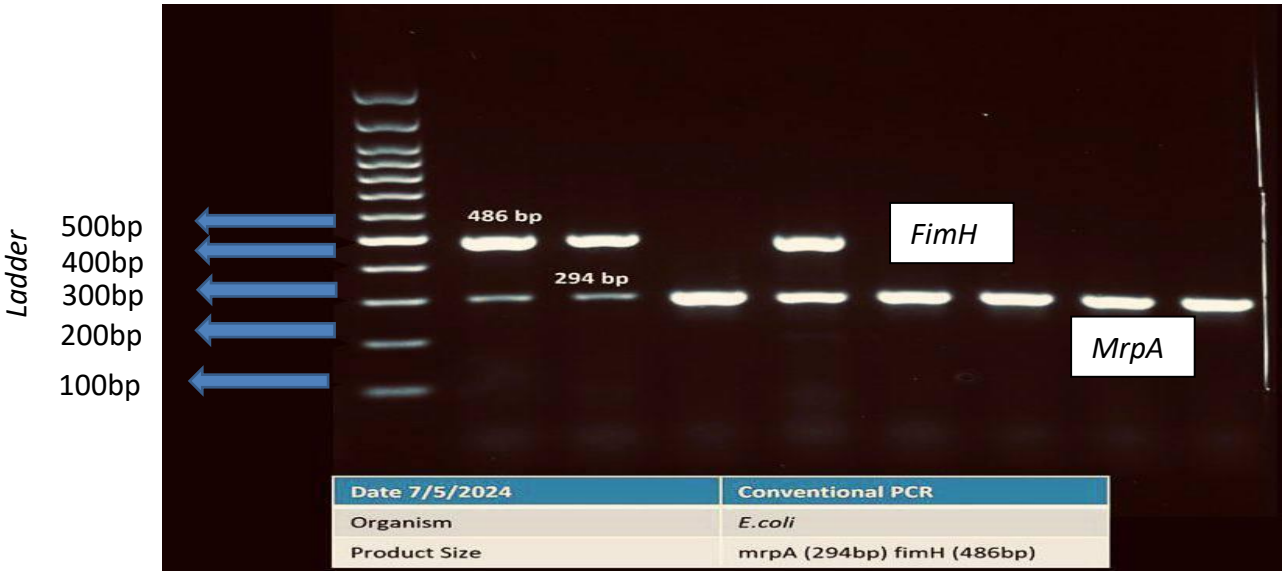

Figure 7: Multiplex PCR for *mrpA* and *fimH* genes in *Escherichia coli* (2% agarose gel, TAE buffer, 80 min). *MrpA* gene (size 294 bp) and *FimH* gene (486) ,80 volts, L: Ladder (100bp).

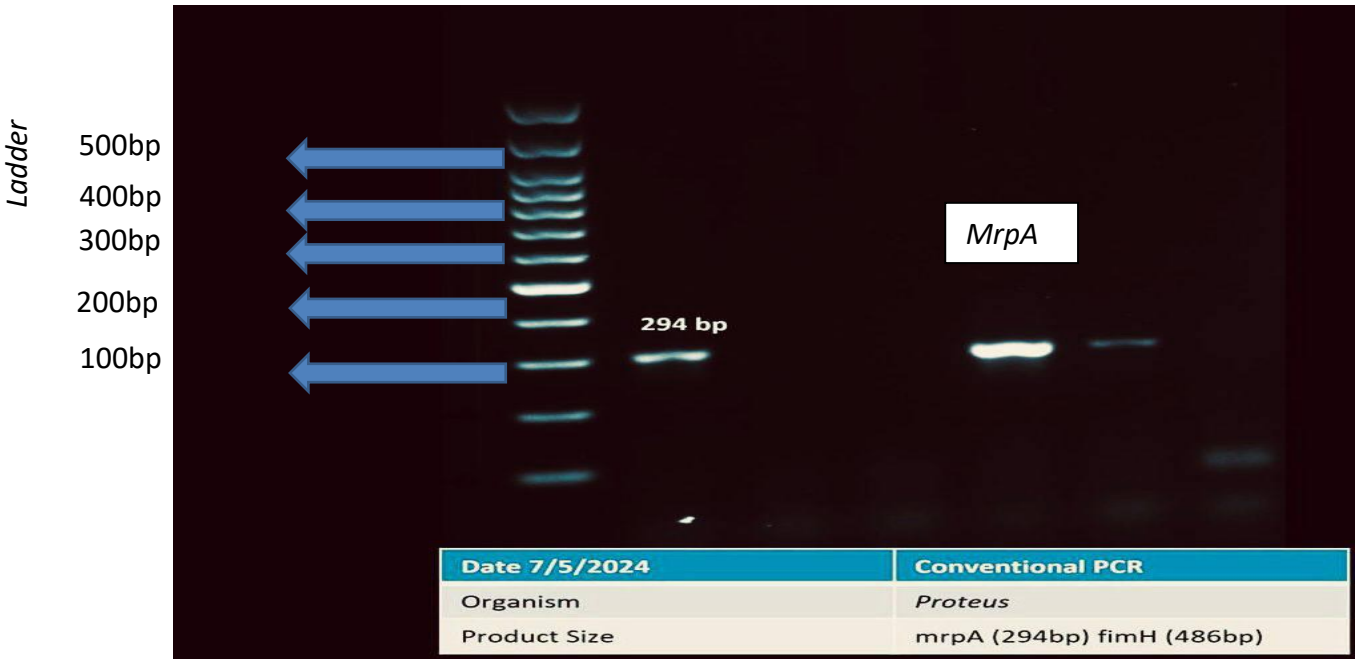

Figure 8: Multiplex PCR for *mrpA* and *fimH* genes in *Proteus mirabilis* (2% agarose gel, TAE buffer, 80 min). *MrpA* gene (size 294 bp) and *FimH* gene (486) ,80 volts, L: Ladder (100bp).

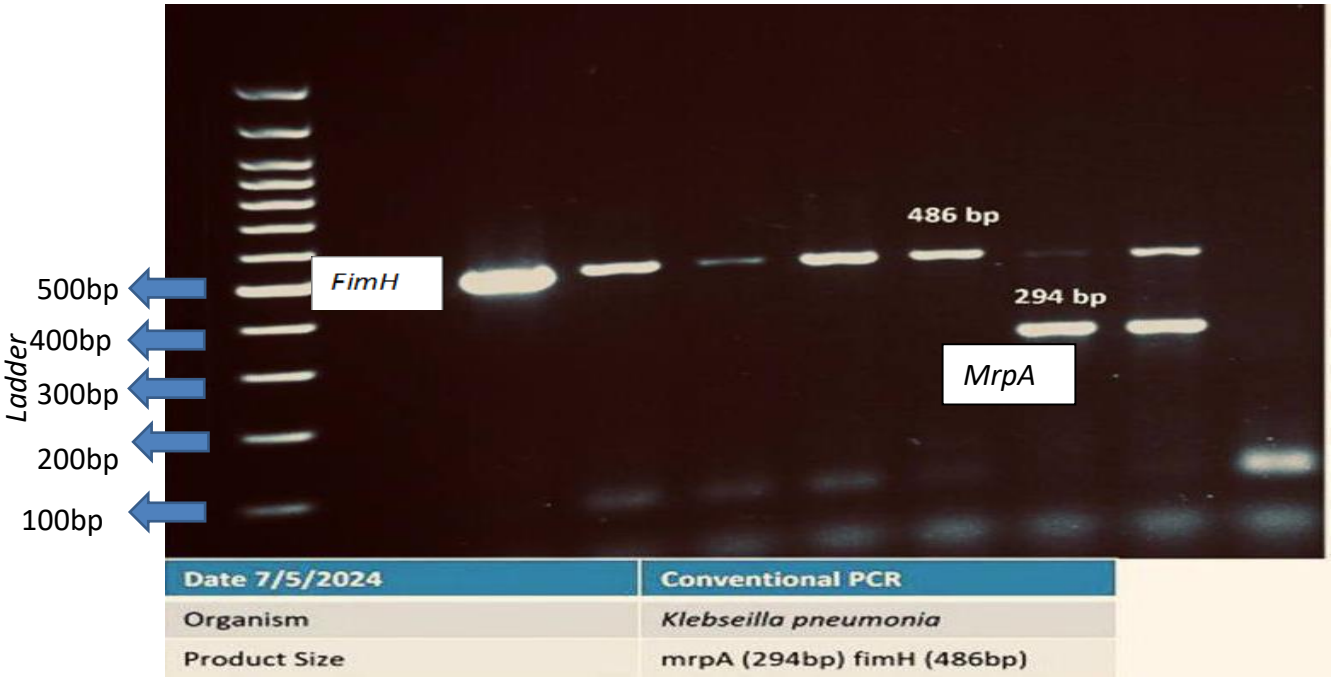

Figure 9: Multiplex PCR for *mrpA* and *fimH* genes in *Klebsiella pneumoniae* (2% agarose gel, TAE buffer, 80 min). *MrpA* gene (size 294 bp) and *FimH* gene (486) ,80 volts, L: Ladder (100bp).

Table 6: 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

Several studies showed co-infection and multi-species catheter- associated infection. In a previous study, urine samples from removed catheters yielded 47 bacterial isolates. The predominant species was *E. coli* (31.92%), followed by S. *aureus* (23.40%), *P. mirabilis* and *P. vulgaris* (10.64% each), *P. aeruginosa* and *S. saprophyticus* (8.51% each), and *K. pneumoniae* (6.38%) [24]. Saleem *et al*., investigated 150 catheterized patients, and their results showed that 36 cases (24%) developed catheter-associated urinary tract infections (CAUTI). *E. coli* was the most prevalent pathogen, accounting for 53% (19 isolates), followed by *K. pneumoniae*, *P. aeruginosa*, and *Enterococcus faecalis* [25]. Other results highlight the dominance of Gram-negative bacteria in CAUTI cases, in a study analyzed 310 urine samples from CAUTI cases at Sohag University Hospitals reported that *E. coli* was the most prevalent bacteria (41.7%; 75/180), followed by *K. pneumoniae* (21.7%; 39/180) and *E. cloacae* (13.3%; 24/180) [26]. Shen *et al*., reinforced the vital role of Gram-negative bacteria in CAUTI etiology, while highlighting the significant contributions of Gram-positive bacteria and fungi to these infections. Their study included 7,295 patients, and the results showed that CAUTI was observed in 182 cases, with 276 pathogenic strains isolated from them. Gram-negative bacteria were the predominant pathogens (47.83%), followed by Gram-positive bacteria (32.97%) and fungi (19.20%) [27].

In this study, several bacterial species were identified in urine samples, with triplicate co-infections causing CAUTI cases. Following the identification of these bacterial isolates, and testing their virulence factors, attention shifted to examining the genetic determinants that contribute to their pathogenicity. One such gene, *fimH*, which encodes an adhesin crucial for bacterial adhesion to urinary tract epithelium, was found to be highly prevalent in *E. coli* strains in a current study.

Electrophoresis analysis revealed that 82.53% of *E. coli* isolates carried the *fimH* gene, highlighting its significant role in uropathogenesis [28]. A recent study in Baghdad included 150 urine samples collected from patients with confirmed UTIs. Among the isolates, 52/150 (35%) were identified as *E. coli*, and 100% of these isolates harbored the *fimH* gene [29]. Similarly, a study by Musafer *et al*., identified 50 clinical *E. coli* isolates from UTI patients using PCR, with 86% of these isolates carrying the *fimH* gene [30].

Al-Hamdani and Al-Hashimy [31] conducted a study on 50 urine samples from patients, identifying *P. mirabilis* through routine tests and the VITEK 2 system, and detected the *mrpA* gene in 80% (8/10) of the samples. In a related study, Hussein *et al*., [32] found that 92.1% of *P. mirabilis* isolates from UTI patients harbored the *mrpA* gene.

In a study conducted with 250 clinical samples collected from several hospitals in Baghdad, 68 *K. pneumoniae* isolates (27.2%) were identified. 24 strains of them, were multi-drug resistance (MDR) and biofilm producers. The *fimH* gene was detected in 100% of these isolates [33]. Similarly, Muhsin *et al* studied 60 clinical *K. pneumoniae* isolates from urine samples collected from three hospitals in Baghdad, and found the *fimH1* gene was in 78.3% of the isolates [34].

Conclusion

P. mirabilis was found to reduce the biofilm formation potential of *K. pneumoniae* and *E. coli*. The *mrpA* gene was detected in 60% of *K. pneumoniae*, *P. mirabilis*, and *E. coli* isolates, while the *fimH* gene was present in 70% of *K. pneumoniae* and *E. coli* isolates. Notably, *E. coli* isolates had a higher percentage of the *mrpA* gene than *fimH*, likely due to horizontal gene transfer.

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.

Conflict of Interest

The authors declare that they have no conflict of interest.

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