Effect of Probiotic on "type 1 fimbrial adhesin" gene involved in Biofilm Formation of a *Klebsiella pneumoniae* isolated from Clinical samples

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ABSTRACT

Background: *Klebsiella pneumoniae* is a Gram-negative bacterium known to cause a variety of infections, especially in healthcare settings. It has the ability to form biofilms, which are organized communities of bacteria adhered to surfaces and surrounded by a self-produced extracellular matrix. Biofilms are important because they contribute to persistence and resistance to infection. *FimH* is a gene involved in biofilm formation and stress response in *K. pneumoniae*. Probiotics, live microorganisms that provide health benefits to the host, may affect the regulation of biofilm-related genes in pathogenic *K. pneumoniae*. The aim of this study was to investigate whether probiotics could influence the expression of biofilm-related genes, such as *FimH*, in *K. pneumoniae*. **Methodology**: One hundred and sixty samples, including patients with various infections such as burns, urine, sputum, wounds, lung fluid, catheters, pus, vaginal swabs, and ear swabs, were collected from hospitals in Baghdad, Iraq, between September 1, 2023, and November 30, 2023. The samples were streaked directly onto MacConkey agar, nutrient agar, blood agar, and colorimetric agar, then incubated at 37°C for 24 h. Bacterial isolates were accurately identified using the Vitek-2 system. Antibiotic susceptibility was tested using the Kirby-Bauer technique as recommended by CLSI (2023). Thirty isolates were tested for biofilm using microtiter plates. The study included molecular characterization of the biofilm gene *fimH*, while the ability of *Lactobacillus fermentum* to inhibit biofilm production was also examined. **Results**: Results showed that only 80 of the 160 samples had the same morphological and biochemical characteristics as *Klebsiella* , whereas only 50 of the 80 isolates were identified as *Klebsiella pneumoniae*. Antibiotic susceptibility showed that *K. pneumoniae* isolates exhibited varying degrees of resistance to the antibiotics used. The highest percentage of antibiotic resistance was found for Ceftriaxone (76.6%). In addition, Amikacin and Tigecycline had the lowest resistance rates, at 0% each. Molecular testing of 30 multidrug-resistant strains was performed by detecting the *fimH* gene. Results showed that all 30 *K. pneumoniae* isolates had this gene. Strong biofilm was detected in one isolate. Real-time PCR was used to detect fimH gene expression before and after probiotic and probiotic-combined antibiotic treatments.
Conclusion: The majority of *K. pneumoniae* isolates demonstrated the ability to form biofilms. All isolates have the *fimH* gene. Additionally, Combinations of *L. fermentum* and antibiotics exhibited synergistic effects against the KP1 isolate of *K. pneumoniae* compared with using the probiotic or antibiotic alone.

Keywords: *Klebsiella pneumoniae*, Lactobacillus fermentum, PCR, real-time PCR.
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INTRODUCTION

Bacterial resistance to antibiotics poses a growing threat to global public health (1). *Klebsiella* has been added to the World Health Organization's critical list of microorganisms for which new treatments are urgently needed (2). The acquisition of resistance genes in *K. pneumoniae* is a significant concern because these bacteria are able to rapidly develop resistance to antimicrobial drugs. This is facilitated by the continuous accumulation of multiple antibiotic resistance genes (ARGs) through spontaneous genomic mutations and the acquisition of conjugative plasmids (3). Plasmid conjugation plays a crucial role in the horizontal transfer of resistance genes, enabling their spread between bacterial species (4). Specific genes associated with antibiotic resistance in *K.*

pneumoniae include β-lactamases, efflux pumps, outer membrane proteins, gene replicases, protein synthesis complexes, and transcriptases (5). Mutations in these genes, as well as biofilm formation, lead to the acquisition of antibiotic resistance in *K. pneumoniae* (6). Understanding these mechanisms is essential for developing effective prevention strategies and surveillance measures against multidrug-resistant *K. pneumoniae* (7). In addition, most *K. pneumoniae* isolates can form biofilm, which can make already difficult infections even more severe. (8) Therefore, we should pay attention to the virulence characteristics of multidrug-resistant *K. pneumoniae* during biofilm formation and improve clinical awareness of this new and serious threat. Biofilm infections are 10 to 1000 times more resistant to antibiotics and other antimicrobial drugs than planktonic cells (9). There are several genes involved in biofilm formation, such as B. type 1 fimbrial adhesin (*fimH*). It is part of the type 3 pili, which are hair-like structures on the surface of the bacteria (10). These pili play a key role in the initial attachment of *K. pneumoniae* to surfaces and host tissues, which is an important step in biofilm formation. This has implications for bacterial pathogenicity and treatment resistance. *FlmH*, along with other proteins in the pili, helps bacteria adhere to surfaces and to each other, promoting biofilm formation. (11) The discovery of probiotic *lactobacilli* (beneficial bacteria) has spread worldwide as preventive and therapeutic agents to eliminate potential pathogens. There is a great need for this new therapeutics to treat diseases that are resistant to antibiotics (12). Lactic acid bacteria (genus: *Lactobacillus*) are generally considered safe and important members of the human intestinal flora. They have been reported as potential candidates for competing with harmful bacteria and potential alternatives to antibiotics (13,14). Studies have shown that the ability of probiotics to prevent disease in the population results in changes in gene regulation (15). Beneficial bacteria represent the potential for treatment, particularly in the fight against the immune system (16).
So this study aimed to investigate whether probiotics could influence the expression of biofilm-related genes, such as *FimH*, in *K. pneumoniae*.

METHODODOLOGY

Samples Collection

A total of 160 samples (burn, urine, sputum, wound, urine catheter, pus, viginal swab, and ear swab) were collected in sterilized containers from hospitals, including Baghdad Teaching Hospital, Ghazi Al-Hariri Hospital for Surgical Specialties and Teaching Laboratories at Medical City, AL-Almay Hospital LAB, and AL-Belsan LAB, Baghdad, Iraq, from both genders at age between (1-60) years. During the period 1/9/2023 to 30/11/2023. Samples were cultured on nutrient agar, MacConkey agar, blood agar, and Chrom agar for the isolation and identification of bacterial isolates based on standard bacteriological methods. (17)

- **Ethical approval**

The research was approved by the Research Ethics Committee of the College of Biotechnology of Al-Nahrain University (No.1705/21, dated 17/05/2026). The committee confirmed compliance with approved scientific and ethical standards.

Identification of bacterial isolates

All clinical samples were cultured on different media, as previously mentioned. Each bacterial isolate was identified using morphological, microscopic, and biochemical tests, including oxidase, catalase, indole, urease, motility, and citrate utilization. At the 98% confidence level, the Vitek-2 system (bioMérieux, France) was used to accurately identify each bacterial isolate.(17,18)

Antibiotic susceptibility test

Thirty *K. pneumoniae* isolates previously identified by morphological characteristics and the VITEK-2 system were cultured in 5 mL of broth, the supernatant was removed, and the bacterial pellet was diluted to a McFarland turbidity standard of 0.5, which contained approximately 1.5 x 10^8 CFU/mL. Antibiotic susceptibility testing was performed using the Kirby-Bauer method according to CLSI guidelines (19). The antimicrobial susceptibility test was carried out using SAM, TZP, CTX, CAZ, CZA, C/T, FEP, IPM, MEM, AK, CN, CIP, TGC, and SXT.

Detection of Biofilm by Microtiter Plates

Microtiter plates are a quantitative method for evaluating biofilms using a microplate reader. Each of the 30 bacterial isolates used in this procedure was grown on MacConkey agar at 37°C for 24 hours. After incubation, the concentration of each isolate suspension was adjusted to 0.5 McFarland. 20 microliters of the bacterial

suspension were added to 180 microliters of Mueller-Hinton broth (supplemented with 1% glucose) in each of the 96 wells. After 24 hours at 37°C, the microtiter plates were incubated. Isolates that formed biofilms on the walls of the microplate wells were stained with 150 µL of crystal violet for 15 minutes. After washing the cells in the wells twice with phosphate-buffered saline (PBS), all cells that did not form biofilm were removed, and the wells were dried at 60°C for an hour. After drying, the dye on the biofilm on the microplate walls was re-dissolved in 150 µL of 96% ethanol. This allows the microplate to be measured ELISA reader (Bio-Rad, USA) at 570 nm using a microplate reader. (20) The OD-based classification criteria were adopted as standardized interpretative parameters for evaluating the biofilm-forming capacity of the tested bacterial isolates and should not be considered direct experimental outcomes. Rather, these criteria served as a comparative framework for categorizing each isolate as none weak, moderate, or strong biofilm producer according to its optical density (OD) value relative to the calculated cut-off value (ODc). The ODc was statistically determined based on the mean optical density of the negative control supplemented with three standard deviations, thereby minimizing background interference and enhancing classification reliability. Furthermore, each isolate was analyzed in triplicate independent experiments to ensure analytical precision, reproducibility, and methodological consistency. The obtained absorbance values were subsequently expressed as Mean ± SD prior to comparison with the established ODc value for accurate determination of biofilm production intensity.

OD value	Biofilm formation activity
OD ≤ ODc	NO biofilm producer
ODc < OD ≤ 2 ODc	Weak biofilm producer
2 ODc < OD≤ 4 ODc	Moderate biofilm producer
4ODc < OD	Strong biofilm producer

The cut-off value (ODc) is defined as three standard deviations (SD) above the mean OD of the negative control: ODc = average OD of negative control + (3× SD of negative control)

DNA extraction and PCR reaction

The genomic DNA of 30(Promega, USA) clinical isolates of *K. pneumoniae* exhibited the strongest biofilm formation and the greatest antibiotic resistance was extracted using a Wizard Genomic DNA Purification Kit (Promega, USA, Madison), and the purity and concentration of the DNA were determined using a spectrophotometer (Nanodrop) by measuring the optical density (O.D). The extracted DNA was then kept at -20°C for later use.

The primers were used in both conventional PCR and RT-PCR in this study, as shown in Table 1.

Table (1): Primers and their sequences

Primer	Sequences (5`----3`)	bp	GC%	Product size (bp)	Promega Tm
*[1]*FimH*	F: GGTATTACCTCTCCGGCACC	20	60	236	64
	R: GATGATCGACTGCACGTTGC	20	55		65
RpoB	F: TTGACTCCGGTGTTACTGCC	20	55	102	
	R: CCGGGTACATCTCGTCTTCG	20	60		
Reference		Designed in this study using Primer3Plus (Whitehead Institute; Cambridge, Massachusetts; United Kingdom) *[1]targeting the region 4397811-4398719 of the *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286 chromosome (NC_016845.1) *[2]targeting NC_016845.1:227354-231382 *Klebsiella pneumoniae* subsp. *pneumoniae* HS11286 chromosome, complete genome			

The primer pairs presented in Table (1) were designed and applied for dual purposes, including molecular detection of the *FimH* by conventional PCR and quantitative evaluation of its expression by RT-qPCR. The use of identical primer sequences in both assays enhanced amplification specificity and methodological consistency. Meanwhile, *RpoB* was employed solely as an internal reference (housekeeping) gene to normalize relative gene expression levels and ensure the reliability of RT-qPCR quantification.

PCR reaction mixture

The primers, nuclease-free water, and master mix were all contained in the 0.2 mL Eppendorf tube. The total volume of the PCR mixture was 20 µl, as shown in Table 2.

Table (2): Reaction mixture of PCR

Components	Reaction volume (µl)	Final concentration
Master Mix (2X)	10	1X
Forward primer (10 µM)	1	0.4 µM
Reverse primer (10 µM)	1	0.4 µM
DNA template	2	< 250 ng
Nuclease-free water	6	------

The PCR tubes were placed in the thermal cycler to begin the amplification reaction according to the optimized program for each primer pair.

Preparation of probiotic filtrates

The unconcentrated cell-free filtrate of the *Lactobacillus fermentum* isolate was prepared by inoculating 1 mL of an overnight culture into 9 mL of MRS broth for 24 hours at 37°C. Next, 2% (v/v) of the actively growing culture was transferred into 100 milliliters of MRS broth and incubated for 48 hours at 37°C in a candle jar. Subsequently, the culture was centrifuged for 15 minutes at 6000 rpm at 4°C, and the suspension was filtered through Millipore 0.22 µm filters (21).

Determination of the *L. fermentum supernatant* minimal inhibitory concentration (MIC)

The inhibitory activity of probiotic filtrate against biofilm formation by *K. pneumoniae* strains was determined using the well polystyrene plate method. Each *K. pneumoniae* strain was cultured on MacConkey agar at 37°C for 24 hours. After culture, a portion of the grown colonies was suspended in physiological solution, and the concentrations of all isolate suspensions were balanced to 0.5 McFarland. A portion of Mueller-Hinton broth (180 µL) (enriched with 1% glucose) was added to each of the 96 wells, followed by 20 µL of the bacterial suspension. The microtiter plate was incubated at 37°C for 24 hours. Subsequently, the supernatant was carefully removed, and each well was washed twice with 200 µL of phosphate-buffered saline (PBS).

Next, 100 µL of pure Mueller-Hinton broth was added to each well of the polystyrene microtiter plate. To treat biofilms with the probiotic *L. fermentum*, 100 µL of broth containing the probiotic was added vertically to the first well of the plate, mixed, and then 100 µL was transferred to the second well, and this process was repeated to the last well. After mixing and draining, a positive control was prepared by adding only broth to each sample, and a negative control was prepared by adding only broth. Each well was then stained with 20 µL of resazurin. This will be chemically reduced by aerobic respiration caused by cell proliferation, changing color from blue and nonfluorescent to pink and highly fluorescent, indicating cell viability. The plates were incubated at 37°C for 24 hours. The stained biofilms were measured at 570 nm using a microtiter reader. Each strain was examined in triplicate, and the average was taken (19,20).

The minimum inhibitory concentrations (MICs) of the antibiotic meropenem and the probiotics combination with meropenem were also determined using the same method.

Expression of biofilm-associated genes

Total RNA Extraction

Total RNA was extracted from *K. pneumoniae* isolates exhibiting the strongest biofilm formation using the TransZol plus RNA isolation kit protocol. RNA samples were quantified using the NanoDrop Spectrophotometer by measuring absorbance at wavelengths of 260 and 280 (A260 and A280, respectively) nm. The absorbance quotient (A260/A280) was used as an indicator of RNA purity, with a value of 2

considered indicative of well-purified RNA. The extracted RNA was subsequently stored at -80°C for future use.

Synthesis of cDNA

RNA was transcribed into cDNA using GoScript™ Reverse Transcriptase following the manufacturer's protocol.

After that, the mixture was placed in a thermal cycler, and the following thermal cycling conditions were applied as in Table 3.

Table (3): Thermal cycling conditions for Reverse Transcription

Stage	Temperature (°C)	Time (min)	Number of Cycle
Primer annealing	25	5	1
cDNA synthesis (Reverse transcription)	42	60	1
Reverse transcriptase inactivation	70	15	1

Real-time PCR: (qRT-PCR)

The *K. pneumoniae* (K.P1) isolate was chosen to investigate the expression of the *fimH* biofilm gene. With a housekeeping gene (*rpoB*) by (Thermo Fisher Scientific, USA) (22) after treating with the probiotic, meropenem, and probiotic-antibiotic combinations.

Table (4): Components of quantitative real-time PCR used for *fimH* and the housekeeping *rpoB* genes expression experiment

Component	Volume (µl)
cDNA Template	2
Forward Primer	0.4
Reverse Primer	0.4
Nuclease-Free Water	7.2
qPCR Master Mix, (SYBR)	10
Total volume	20

The cycling parameters were set as in Table 5:

Table (5): qRT-PCR Cycling Program

Steps	Temperature (°C)	Time (min)	Number of cycles
Initial denaturation	94	2	1
Denaturation	94	0.5	40
Annealing	60	0.5	40
Extension	72	0.5	40
Melting curve	65-95	0.5	1

Analysis of Gene Expression Data

Schmittgen and Livak (23) introduced the delta Ct (ΔΔCt) technique, which involves comparing the Ct values of the target gene with a reference gene (often a housekeeping gene) to determine qRT-PCR results. This technique allows for the assessment of gene expression levels (fold change) by analyzing the ΔΔCt values.

RESULTS

Out of the 160 collected clinical samples, only 80 isolates were preliminarily identified as *K. pneumoniae* based on their morphological and biochemical characteristics, while the remaining isolates belonged to other Gram-negative bacteria, including *Burkholderia cepacia*, *Escherichia coli*, *Aeromonas salmonicida*, and *Serratia fonticola*. All samples were initially inoculated into nutrient broth and incubated at 37°C as a pre-enrichment step to enhance bacterial recovery and increase growth density. Subsequently, the isolates were subcultured onto nutrient agar, MacConkey agar, and blood agar plates, followed by incubation at 37°C for 24 hrs. On MacConkey agar, presumptive *K. pneumoniae* isolates exhibited lactose-fermenting, large mucoid pink colonies, whereas on blood agar they appeared as mucoid, grey-white, non-hemolytic colonies. Colonies demonstrating typical Klebsiella-like morphology were further subcultured onto fresh MacConkey agar and CHROM agar plates to obtain pure single colonies for subsequent identification procedures. After incubation, the presumptive *Klebsiella* isolates produced characteristic metallic blue colonies on CHROM agar, as illustrated in Figure 1. These observations represented preliminary phenotypic identification only and were subsequently confirmed using the VITEK-2 system to ensure accurate species-level identification.

A **B** **C**

Figure (1): Growth of *K. pneumoniae during* a 24-hour incubation period at 37°C (A): on MacConkey agar, appearing pink due to lactose fermentation (B): on blood agar, presenting as mucoid colonies, non-hemolytic grey-white (C): on Chrom agar, displaying as metallic blue colonies.

To determine the profile of antibiotic susceptibility, the Kirby-Bauer technique was employed on 30 isolates of *K. pneumoniae*. The results indicated that the *K. pneumoniae* exhibited varying levels of susceptibility and resistance to these antibiotics. Table 6 presents the susceptibility of *K. pneumoniae* isolates to 14 different antibiotics.

Table (6): Susceptibility test of *K. pneumoniae* isolates to 14 different antibiotics.

Antibiotic	S Isolate No. (%)	I Isolate No. (%)	R Isolate No. (%)
Ampicillin / Sulbactam	11 (36.67%)	2 (6.67%)	17 (56.67%)
Piperacillin / Tazobactam	22 (73.33%)	2 (6.67%)	6 (20%)
Cefotaxime	8 (25.81%)	0 (0%)	23 (74.19%)
Ceftazidime	13 (43.33%)	1 (3.33%)	16 (53.33%)
Ceftazidime / Avibactam	27 (90%)	0 (0%)	3 (10%)
Ceftolozane / Tazobactam	25 (83.33%)	0 (0%)	5 (16.67%)
Cefepime	18 (60%)	0 (0%)	12 (40%)
Imipenem	26 (86.67%)	1 (3.33%)	3 (10%)
Meropenem	26 (86.67%)	2 (6.67%)	2 (6.67%)
Amikacin	27 (90%)	3 (10%)	0 (0%)
Gentamicin	21 (70%)	1 (3.33%)	8 (26.67%)
Ciprofloxacin	27 (90%)	1 (3.33%)	2 (6.67%)
Tigecycline	29 (96.67%)	1 (3.33%)	0 (0%)
Trimethoprim / Sulfamethoxazole	11 (36.67%)	2 (6.67%)	17 (56.67%)

S: Sensitive, I: Intermediate, R: Resistance

According to the results of the antibiotic susceptibility test, as shown in Table 6, the cephalosporin antibiotic Cefotaxime exhibited the highest percentage of antibiotic resistance (76.6%), followed by Trimethoprim-Sulfamethoxazole and Ampicillin/Sulbactam (56.6%), Ceftazidime (53.3%), Cefepime (40%), Gentamicin (26.6%), Piperacillin/Tazobactam (20%), Ceftolozane/Tazobactam (16.6%), Imipenem and Ceftazidime/Avibactam (10%), and Ceftazidime/Avibactam and Ciprofloxacin (6.6%). Additionally, the lowest resistance percentage was observed for Amikacin and Tigecycline (0%). The results indicated a notably high resistance rate (76.6%) to cefotaxime among *K. pneumoniae* isolates.

Based on the results, 30 clinical *K. pneumoniae* isolates were tested for biofilm formation using OD readings. The findings showed that 24 isolates (80%) were biofilm producers, while 6 isolates (20%) were non-producers. Most of the positive isolates exhibited weak biofilm production, whereas only one isolate showed moderate production and one isolate showed strong production. The highest OD value was recorded in isolate KP1, with a mean OD of 0.305, indicating strong biofilm-forming ability. Since most isolates were obtained from urine samples, these results suggest that biofilm formation may play an important role in *K. pneumoniae* infections, particularly those associated with UTI.

Molecular study

The polymerase chain reaction (PCR) technique was applied to the isolated DNA in order to identify the genes. After the concentrations were determined, Nanodrop estimated the purity range of 1.8–2 and the concentrations of all the isolates to be between 50 and 530 ng/µl. In this investigation, PCR amplification was used to screen one gene, and it was detected in all 30 isolates (100%) of *K. pneumoniae* identified by previous identification methods. The detection of the fimH gene was performed using specific primers *fimH-F* and *fimH-R*, resulting in an amplified fragment size of 263 bp, as shown in Figure 2.

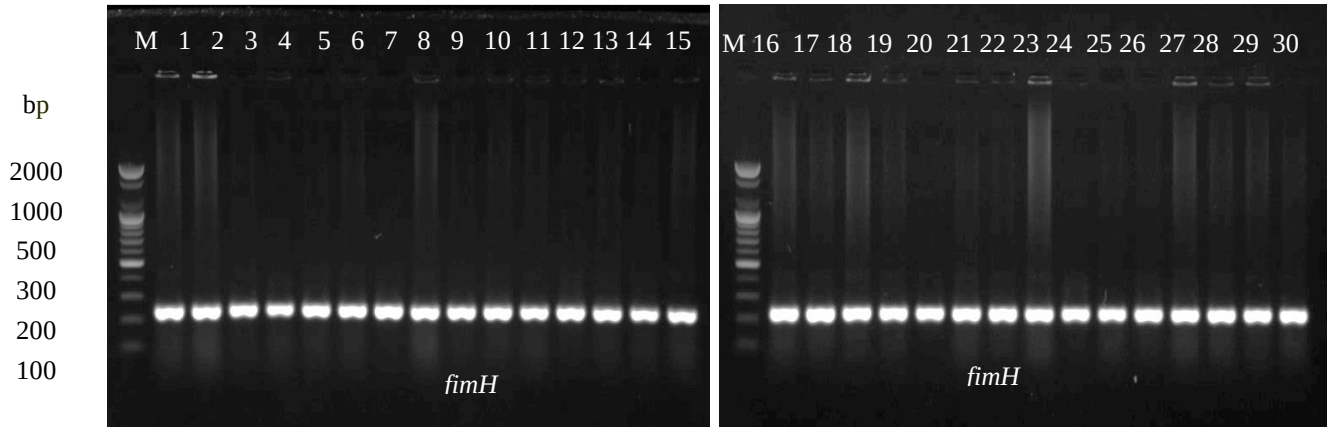

Figure (2): Gel electrophoresis of amplified PCR product of *fimH* gene (263 bp) of *K. pneumoniae*. Electrophoresis was performed on a 1% agarose gel stained with Ethidium Bromide (0.5 µg/mL), and run at 5 V/cm for 1 h in TBE buffer (1X). Lane M:100 bp DNA marker, Lanes 1-30: *fimH* bands.

The *K. pneumoniae* isolate (K.P1) used in the previous steps, which gave a resistant result for more than 10 antibiotics in the antibiotic susceptibility test and strong biofilm formation, was further subjected to gene expression analysis to assess the impact of probiotic (*L. fermentum*), antibiotic (meropenem), and probiotic antibiotic combinations on genes associated with biofilm formation. *FimH* was selected due to its prevalence in clinical *K. pneumoniae* isolates. The results indicate that *fimH* expression in clinical isolates was up-regulated upon treatment with *L. fermentum*. Conversely, treatment with meropenem, probiotics + meropenem resulted in downregulation, as shown in Table 7.

Table (7): The effect of Lactobacillus supernatant, meropenem, and probiotics + meropenem, on gene expression of *fimH* gene. Red colors indicate up-regulation, blue colors indicate down-regulation of the gene expression.

Treatment	*rpo*B	*fimH*	ΔCT	ΔΔCT	folding
Control	21.05	21.94	0.89	0	1
Probiotic	18.94	19.61	0.67	-0.22	1.16
Meropenem	15.75	16.42	0.67	-0.22	0.95
Probiotic + Meropenem	22.03	23.74	1.71	0.82	0.68

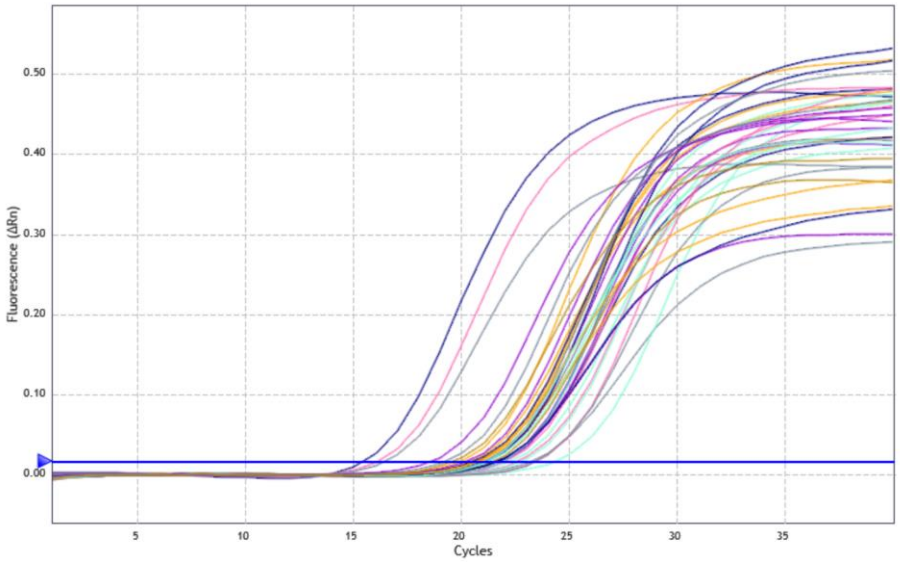

Figure (3): Real-time amplification dynamics of fimh gene expression in *Klebsiella pneumoniae*

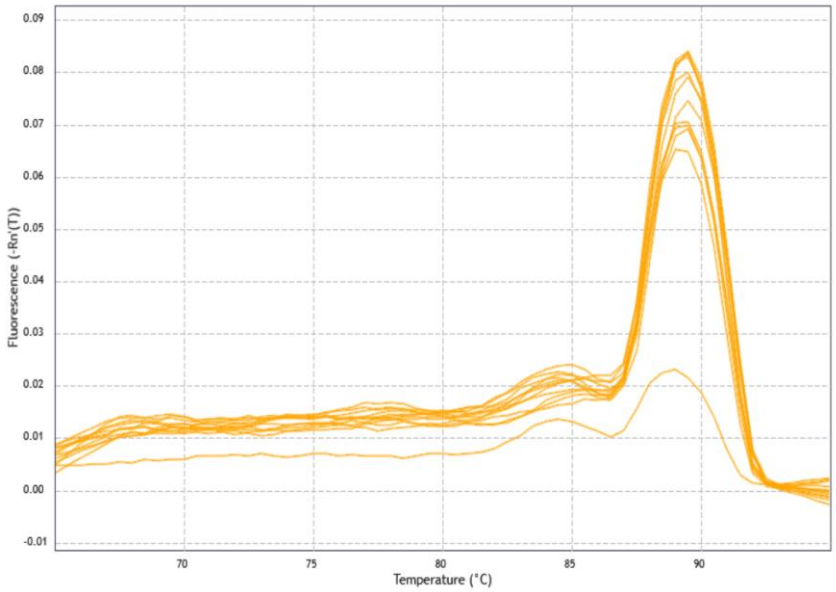

Figure (4): Melting curve analysis confirming the specificity of fimH gene amplification in *Klebsiella pneumoniae*

DISSCUSION

The colonization of *K. pneumoniae* across a range of medical settings generates questions about the organism's ability to form biofilms, and this ability is intimately linked to specific virulence traits. (24). Given the increasing frequency of resistant *K. pneumoniae* in environmental and clinical specimens, the high mortality rate caused by this pathogen, and its propensity to develop resistance to antibiotics, especially beta-lactamase antibiotics like carbapenem, which are considered the last line of defense against Grame- negative bacteria resistant to beta-lactamases, underscore the necessity for developing alternative treatments and practices to curb the spread of these microbes beyond antibiotic use.(25) Numerous studies have shown that probiotics' ability to prevent disease in the population results in changes in gene regulation (15). Several previous studies have identified biofilm-associated genes in *K. pneumoniae* strains that cause pneumonia, urinary tract infections, and nosocomial gastrointestinal infections (26, 27,28). In the present study, we identified a gene involved in the biosynthesis of surface molecules required for biofilm formation, such as the B. pneumoniae fimH gene. To investigate this gene, PCR amplification was performed using specific primers, which demonstrated that 100% multidrug-resistant *K. pneumoniae* tested isolates harbor this gene. This result is consistent with the findings of Alcantar-Curiel *et al*. (29), whose aim was to determine the prevalence of the fimA and mrkA fimbrial genes of type 1 and type 3 pili, respectively, in 69 clinical and environmental *K. pneumoniae* strains by PCR-based assays and to establish a correlation with pili production during cell adhesion and biofilm formation. The fim operon is present in 100% of the strains and is sufficient to promote bacterial interactions leading to the efficient development of biofilms and/or other previously unidentified adhesins. Critical to biofilm formation is their ability to produce an adhesive capsule and a variety of adhesives (fimbriae and non-fimbriae).

Real-time PCR differs from other gene expression methods in its accuracy, sensitivity, and rapid response time. This technique has become the best in the field of gene expression analysis. Also known as comparative CT technique or relative quantification, it allows for comparative analysis of target gene expression in different samples or conditions. In relative PCR, the expression level of the target gene is compared to a reference gene, also known as a housekeeping gene. The housekeeping gene used in the study was RpoB (102 bp). Many classify the RpoB gene as a housekeeping gene (22). It is important for DNA repair and recombination processes that are necessary to maintain the integrity of the genome in every cell. It is believed to be consistently expressed in all samples. Relative changes in gene expression in all samples must be calculated, rather than a standard curve or known concentrations. Harshitha and Arunraj (30) reported their results as the difference in expression between the reference and target genes. After treating the isolates with probiotics and probiotics combined with antibiotics (meropenem), the results showed that the combination of probiotics and meropenem had a useful potential inhibitory effect on the growth of *K. pneumoniae* isolates. These results are consistent with several studies that have shown that antimicrobial activity is increased when antibiotics and probiotics are used in combination rather than antibiotics alone or probiotics alone (31,32). Combination therapy can help produce synergistic effects, prevent drug resistance, and improve effectiveness against polymicrobial infections. (33). According to Amrutha et al (34), lactic acid and other organic acids primarily have potent inhibitory effects on bacteria. According to Hossain *et al*. (35), organic acid (lactic acid) penetrates pathogens' outer barrier, allowing other probiotic molecules to enter the bacterium and ultimately causing the pathogen to die of a lowered internal pH. The most popular method of eliminating or preventing the development of susceptible bacteria was pore creation, which created ion channels in the plasma membrane and caused membrane depolarization (36). The hole's development causes phosphate and occasionally K+ to be effluxed, which depletes cytoplasmic ATP and may decrease cells' adherence to uroepithelial cells, affect their ability to form biofilms, or obstruct the invasion process. The extensive action of hydrogen peroxide on pathogenic bacteria is another mechanism (33, 36).

CONCLUSION

The majority of *K. pneumoniae* isolates were demonstrated to produce biofilm. Each isolate tested positive for the PCR detection of *fimH* gene. Additionally, Combinations of *L. fermentum* and antibiotic exhibited synergistic effects against the KP1 isolate of *K. pneumoniae* compared to the use of the probiotic or antibiotic alone.

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تأثير البروبيوتيك على جين اللاكتين النوع الأول للشعرات المتورط في تكوين الأغشية الحيوية لجرثومة *Klebsiella pneumoniae* المعزولة من العينات السريرية

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الخلاصة

خلفية البحث ***Klebsiella pneumoniae*** :هي بكتيريا سالبة الجرام معروفة بتسببها في مجموعة متنوعة من العدوى، خصوصًا في البيئات الصحية. لديها القدرة على تكوين الأغشية الحيوية، وهي مجتمعات منظمة من البكتيريا متصلة بالأسطح ومحيطة بمصفوفة خارج الخلية تنتجها البكتيريا نفسها. تُعتبر الأغشية الحيوية ذات أهمية لأنها تسهم في استمرارية ومقاومة العدوى. يُعتبر جين FimH من الجينات المتورطة في تكوين الأغشية الحيوية واستجابة الإجهاد في *K. pneumoniae*. يمكن أن تؤثر البروبيوتيك، وهي كائنات دقيقة حية توفر فوائد صحية للمضيف، على تنظيم الجينات المرتبطة بالأغشية الحيوية في *K. pneumoniae* الممرضة. تهدف هذه الدراسة إلى فحص ما إذا كانت البروبيوتيك يمكن أن تؤثر على تعبير الجينات المرتبطة بالأغشية الحيوية مثل fimH في*K. pneumoniae*.

المواد وطرق العمل: تم جمع 160 عينة من مرضى يعانون من عدوى متنوعة، مثل الحروق، البول، البلغم، الجروح، سائل الرئة، القسطرة البولية، الصديد، المسحات المهبلية، ومسحات الأذن، من مستشفيات في بغداد، العراق، بين 1 سبتمبر 2023 و30 نوفمبر 2023. تم زراعة العينات مباشرة على أغار MacConkey وأغار المغذيات وأغار الدم وأغار الكروم، ومن ثم حضنها لمدة 24 ساعة عند 37 درجة مئوية. تم تحديد العزلات البكتيرية بدقة باستخدام نظام .Vitek-2 تم استخدام تقنية Kirby-Bauer لاختبار الحساسية للمضادات الحيوية وفقًا لتوصيات .CLSI (2023) تم الكشف عن الأغشية الحيوية لثلاثين عينة باستخدام صفيحة الميكروتيتر. تضمنت الدراسة التعرف الجزيئي على جين الأغشية الحيويةfimH ، والتي نظرت أيضًا في قدرة Lactobacillus fermentum على تثبيط إنتاج الأغشية الحيوية. **النتائج:** من بين العينات الكلية البالغ عددها 160 عينة، أظهرت النتائج أن 80 عزلة فقط تشترك في نفس الخصائص المورفولوجية والكيميائية الحيوية مثل .Klebsiella spp ومن بين هذه العزلات، أعطت 50 عزلة فقط .*K. pneumoniae* أظهرت حساسية المضادات الحيوية أن عزلات *K. pneumoniae* لديها مستويات متفاوتة من المقاومة للمضادات الحيوية المستخدمة. أظهر السيفوتاكسيم أعلى نسبة مقاومة للمضادات الحيوية (76.6%)، بالإضافة إلى ذلك، لاحظنا أن الأميكاسين والتيجي سايكلين كانت لديهما أدنى نسبة مقاومة، حيث كانت 0% لكل منهما. تم إخضاع ثلاثين عزلة مقاومة للمضادات الحيوية للدراسة الجزيئية عن طريق الكشف عن جين .fimH أظهرت النتائج أن جميع العزلات الثلاثين (100%) من *K. pneumoniae* تم التعرف عليها من خلال طرق التعرف السابقة، وتم الكشف عن غشاء حيوي قوي في عزلة واحدة. اكتشفت تقنية تفاعل البوليميراز المتسلسل في الوقت الحقيقي تعبير جين fimH قبل وبعد العلاج بالبروبيوتيك والبروبيوتيك بالتزامن مع المضادات الحيوية. **الاستنتاجات:** أظهرت الغالبية العظمى من عزلات *Klebsiella pneumoniae* القدرة على تكوين الأغشية الحيوية. تم اختبار إيجابية كل عزلة للكشف عن جين fimH باستخدام تفاعل البوليميراز المتسلسل. بالإضافة إلى ذلك، أظهرت التركيبات من .L fermentum والمضاد الحيوي تأثيرات تآزرية ضد عزلة KP1من *K. pneumoniae* مقارنةً باستخدام البروبيوتيك أو المضاد الحيوي بمفرده.

الكلمات المفتاحية: الكلبسيلة الرئوية، اللاكتوباسيلس فيرمينتوم، تفاعل البوليميراز المتسلسل، تفاعل البوليميراز المتسلسل في الوقت الحقيقي.