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Molecular Detection Of Luxs And Rsba Genes In Proteus Mirabilis Bacteria And ALS1 Gene In Candida Albicans Isolated From Urinary Catheter Patients In Kirkuk, Iraq

Nasreen Abdulgabbar Ahmad[1], Nehan Bahaaldden Jafar[2], Sanaa H. Mohammed[3],
[1,2,3]Biology Department, Science College, University of Kirkuk, nihan@uokirkuk.edu.iq

Abstract

Background: *Catheter-associated urinary tract infection (CAUTI) is the predominant healthcare-associated infection, frequently occurring in critically ill patients with urinary obstruction and incontinence who undergo prolonged indwelling catheterization exceeding 30 days. This condition is almost universally observed in patients within 14 days of catheter insertion, leading to increased morbidity, mortality, and treatment costs.*

Aim of the study: *Isolation and identification P.mirabilis and C.albicans ,then detection both genes LuxS and RsbA in P.mirabilis and ALS1 in C.albicans.*

Materials and methods: *235 urine samples were collected from the urology, catheterization, and resuscitation ward, Al-Amal Center, and the artificial kidney unit in Kirkuk Teaching Hospital and Azadi Teaching Hospital in Kirkuk Governorate during the period from March 2024 until the end of July 2024.*

Result: *A total 235 samples were taken from patients who had urinary catheterization. 56(23%) were isolated of P. mirabilis, and 17(7.23%) isolate were for C.albicans. The results of the current study revealed the ability of the isolated P.mirabilis bacteria to form biofilms, and this was revealed morphologically by culturing the bacteria on solid Congo red (CRA) medium, as 54 isolates (96.42%) produced black colonies on solid CRA, while by using PCR analysis, 100 (56%) possess the Lux gene using PCR technology. The results showed that all bacterial isolates showed swarming (100%) on blood agar medium, and 100% possess the RsbA gene. Furthermore 17 C. albicans isolates were examined for the presence of ALS1 genes in the urine of patients who were utilizing catheters. The ALS1 gene was present in ten out of seventeen C. albicans isolates, which represents a ratio of 58.8 percent from the total.*

Conclusion: *Proteus mirabilis is the main cause of urinary tract infections in humans. According to the results of this study, P. mirabilis isolates from urinary tract infections have the ability to possess virulence factors (phenotypic and genotypic), such as biofilm and hemolysin also candida albicans have geneses responsible of virulence factors as biofilm forming isolates.*

Keywords: *Catheterization patients, P. mirabilis, LuxS and RsbA , C.albicans, ALS1.*

INTRODUCTION

Complicated urinary tract infections are associated with structural or functional abnormalities in the urinary tract such as a catheterization, calculi, immunosupression and others [1]. Devices, e.g. indwelling catheters, which are inserted in the urinary tract facilitate microbial colonization, Bacteria attach to outer and inner surfaces of a catheter and form biofilm, which is responsible for 65-85% cather-associated UT'ls. After a few days of catheterization multi-species biofilm, consisting of several organisms, is often isolated [2]. Species like: Enterococcus faecalis, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae are most frequently detected on the catheter surface [3]. The structure of multi-species bio-film is different from biofilm formed by one species of bacteria. The interactions between microorganisms which form this structure change biofilm physiology and function, facilitating the horizontal gene transfer. Consequently, in multi species biofilms resistance to antimicrobial agents and host defense is observed more frequently [4]. A prevalent problem in the management of patients requiring prolonged bladder catheterization is the repeated encrustation and obstruction of catheters. The issue originates from an infection caused by P. mirabilis [5]. Proteus species, belonging to the Enterobacteriaceae family of gram-negative bacilli, are differentiated from most other genera by their capacity to swarm across an agar surface[6]. The recurrent administration of antibiotics results in antibiotic resistance and the proliferation of antibiotic resistance genes, particularly in Gram-negative bacteria [7] A separate category of virulence genes pertains to quorum sensing (luxS and RsbA)[8]. The luxS gene in P. mirabilis encodes the LuxS enzyme, which is responsible for synthesizing the

autoinducer-2 (AI-2) signaling molecule. AI-2 participates in quorum sensing, a bacterial cell-cell communication process utilized to synchronize activities, including biofilm formation. AI-2 generated by LuxS has been associated with biofilm formation in other bacterial species [9]. The RsbA gene encodes a sensory protein that regulates swarming behavior. RsbA has been demonstrated to enhance gene expression related to extracellular polysaccharide production and biofilm formation, indicating its potential role as a protein sensor of environmental conditions. The RsbA gene encodes a histidine-containing phosphor transmitter[10]. Candiduria is rarely seen in healthy individuals but is common in hospitalized patients, especially those with risk factors such as renal abnormalities, indwelling urinary catheters, and antibiotic exposure[11]. This study sought to examine the prevalence of P. mirabilis and C. albicans, subsequently identifying the genes associated with virulence factors.

MATERIAL AND METHODS

Sample collection

235 urine samples were collected from the urology, catheterization, and resuscitation ward, Al-Amal Center, and the artificial kidney unit in Kirkuk Teaching Hospital and Azadi Teaching Hospital in Kirkuk Governorate during the period from March 2024 until the end of July 2024. Their ages were between 15-75 years, with the number of males reaching 84 and the number of females 151. A questionnaire was taken from each sample that included gender, age, condition, treatments taken, and chronic diseases.

Urine specimens

Urine specimens were collected following device removal through either the newly inserted catheter or a midstream-voided urine sample if catheter use was ceased.

Isolation and identification of Proteus mirabilis and candida albicans.

Conventional diagnosis the growing colonies of Proteus mirabilis isolates were initially diagnosed depending on [12,13,14]: Morphology on Blood agar, MacConkey agar, swarming on Blood agar, hydrogen sulfide generation on S.S agar are all morphological traits that may be observed when growing bacteria in the laboratory. microscopic examination after Gram-staining. Biochemical test: The Proteus isolates were identified using biochemical test, including the indole test and the urease test, Finally confirmed by Vitek 2 testWhile C.albicans isolation based on culture sabrourauds dextrose agar(SDA), Distinguish by chrom agar and germ tube formation as described by[15].

Molecular study

DNA extraction

The Presto™Mini g DNA Bacteria Kit from Turkey-Anatolia was utilized for genomic DNA extraction. DNA is identified by quantifying its content (ng/ml) and assessing its purity by absorbance measurements at a wavelength of 260/280 nanometers. While the virulence gene ALS1 in C.albicans detected by polymeras chain reaction technique

Primers design

specific primers were designed to identify virulence genes (LuxS and RsbA) in P. mirabilis (Table 1). These genes were amplified using a multiplex PCR technique. Primers specific for virulence genes of Candida spp. were designed specifically for this study using the NCBI GenBank Database and the Primer3 Plus primer design software. These primers were prepared by the Korean company Macrogene. The conditions of PCR for RsbA gene were as follows: initial denaturation at 94°C for five minutes, followed by denaturation at 94°C for sixty seconds, annealing at 58°C for forty-five seconds, extension at 72°C for one minute, and final extension at 72°C for seven minutes. The conditions of PCR for luxS gene were as follows: initial denaturation at 95°C for five minutes, followed by denaturation at 95°C for thirty seconds, annealing at 62°C for 30 seconds, extension at 72°C for 20 Sec, and final extension at 72°C for seven minutes.

Table(1): Primers used in this study

:r	Sequence 5'□3'	Size(bp)
:abilis		
	F: ACGTATGTCTGCACCTGCG R: CCATAGCTGCCTTCCATGCA	290
	F:TTGAAGGACGCGATCAGACC R:ACTCTGCTGTCCTGTGGGTA	467
da albicans		
	F:GACTAGTGAACCAACAAATACC AGA R: CCAGAAGAAACAGCAGGTGA	318

The conditions of PCR were as follows: initial denaturation at 94°C for five minutes, followed by denaturation at 95°C for thirty seconds, annealing at 58°C for 1 min, extension at 72°C for fifty seconds, and final extension at 72°C for five minutes.

Agarose gel electrophoresis

This method was used to separate different sized DNA molecules and electrophoresis was performed as mentioned in [16].

RESULT

A total 235 samples were taken from patients who had urinary catheterization. 56(23%) were isolated of Proteus mirabilis.

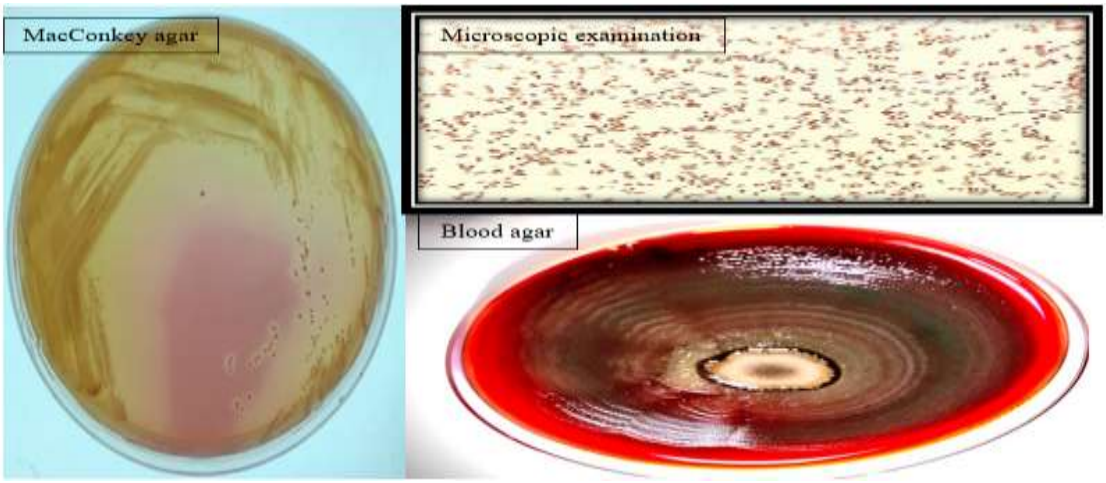
Table (2): Distribution of bacterial and fungus isolates which isolated from urinary catheterization patients

Type of isolates	No(%)
Proteus mirabilis	56(23.83)

While 50 isolates belonging to the genus Candida spp. The most isolated species belong to the C. albicans species, with 17 isolates representing 34%, followed by the C. glabrata species, with 13 isolates representing 26%, while the C. tropicalis and C. krusei species accounted for 11 and 9, representing 22% and 18%, respectively. The statistical analysis results showed significant differences between the yeast species isolated during the study.

Table (3): Percentage of fungi isolated from urine samples of patients with urinary catheters.

Candida spp	NO	%
C. albicans	17	%34
C. glabrata	13	%26
C. tropicalis	11	%22
C.krusei	9	%18
P-Value = 0.042		



Figure(1): Proteus mirabilis colonies on different media as MacConkey, Blood agar in addition microscopic examination of with 100X oil lens.

Proteus mirabilis was diagnosed based on bacteriological morphology, microscopic examination, biochemical tests, DL-20, and VITEK examination. Proteus mirabilis appeared on MacConkey Agar as single, pale, medium-sized, smooth-edged, non-lactose fermenting colonies with a fishy odor. Swarming was also observed on Blood Agar, which is a primary diagnostic feature for this bacterium. Microscopic examination of Gram-stained bacterial smears showed short, Gram-negative, motile, non-spore-forming rods

C. albicans on SDA medium showed colonies that were creamy white, shiny, smooth, oval or spherical in shape, and sticky in texture. The results of the yeast cells showed a positive reaction after staining with lactophenol blue dye that showed a spherical to oval or long, single and budding shape. The test results showed the appearance of colonies in different colors on the Chrom Agar medium, where the C.albicans colony appeared in a light green color. Germ tube formation test was performed to differentiate between Candidia spp. The results showed that all C. albicans isolates formed germ tubes.

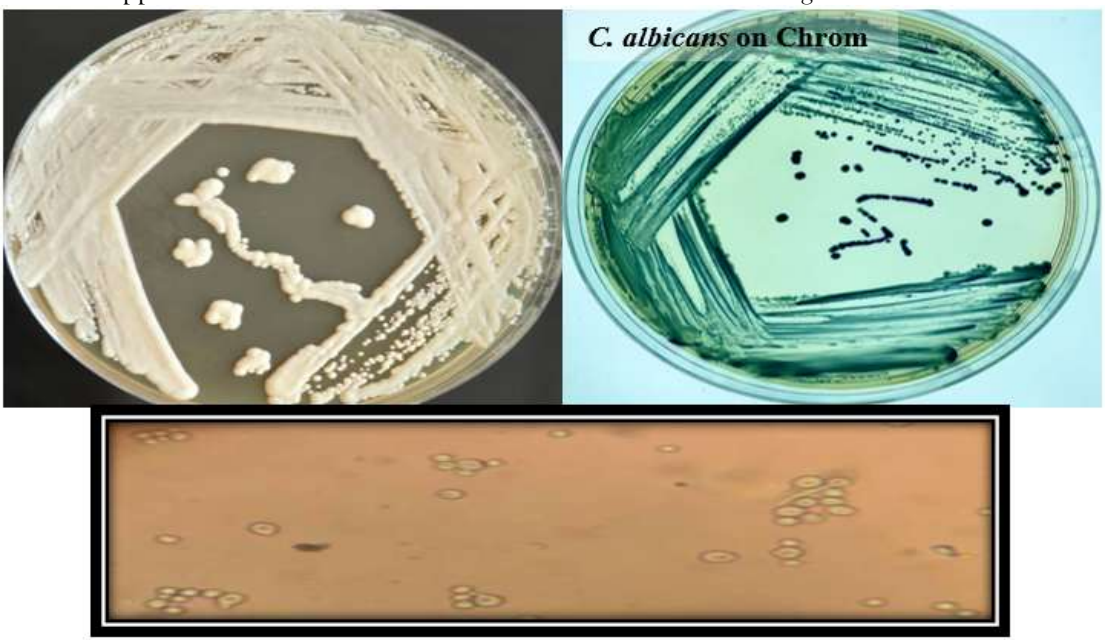


Figure (2): C. albicans colonies on SDA, chrom agar, and formation germ tube

Proteus mirabilis appeared as short, Gram-negative rods, motile and characterized by the phenomenon of cyclization, urea-degrading, non-oxidase producing due to its inability to produce the oxidase enzyme, negative for the indole test, which is used to differentiate P.mirablis from other Proteus species, as a positive result is the formation of a red ring as a result of the decomposition of the amino acid (tryptophan) and its conversion to indole. Negative for the Fox-Proscore test, positive for methyl red, consuming citrate as the sole carbon source, as the color of the medium changed from green to blue as a

result of the change in the color of bromothymol to blue due to the increase in pH, producing the enzyme catalase, indicating its ability to produce the enzyme catalase, as the positive result was the appearance of air bubbles directly after adding hydrogen peroxide H_2O_2, and it has the ability to extract the amine from phenylalanine. The diagnosed isolates exhibited a positive methyl red test and a negative Fuchs-Proskauer test, attributable to the lack of Acetyl-Methyl Carbinol production from molecular hydrolysis. As shown in Figure (3).

Figure (2): Biochemical tests for Proteus mirabilis**.**

The results of the current study revealed the ability of the isolated P.mirabilis bacteria to form biofilms, and this was revealed morphologically by culturing the bacteria on solid Congo red (CRA) medium, as 54 isolates (96.42%) produced black colonies on solid CRA medium as shown in Figure (3) and Table (2). The results of our study showed that the percentage of Proteus mirabilis bacteria possessing the Lux gene using PCR technology was (56) isolates, at a rate of 100%, as shown in Figure (4).

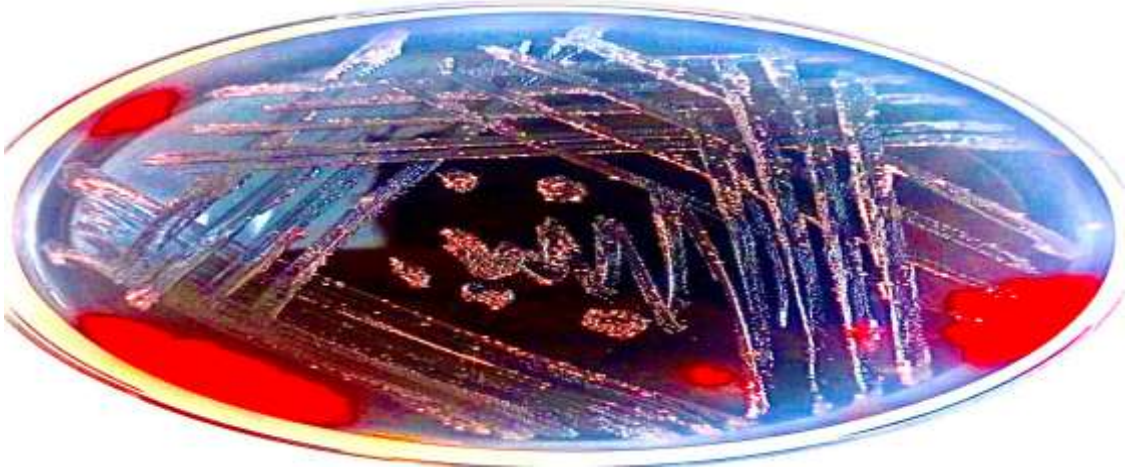


Figure (3): Biofilm production in Proteus mirabilis on Congo red agar medium.

Table (4): Numbers and percentages of biofilm production by Proteus mirabilis**, both phenotypically and genetically.**

	Lux		Biofilm formation by P.mirabilis			
Total	Numbers and percentages of genetically		Number and Percentage by CRA		Number and Percentage by MTP	
	-ve	+ ve	-ve	+ ve	-ve	+ ve
56(100%)	0	56 (100%)	2(3.6%)	54(96.4)	1(%1.8)	55(98.2%)

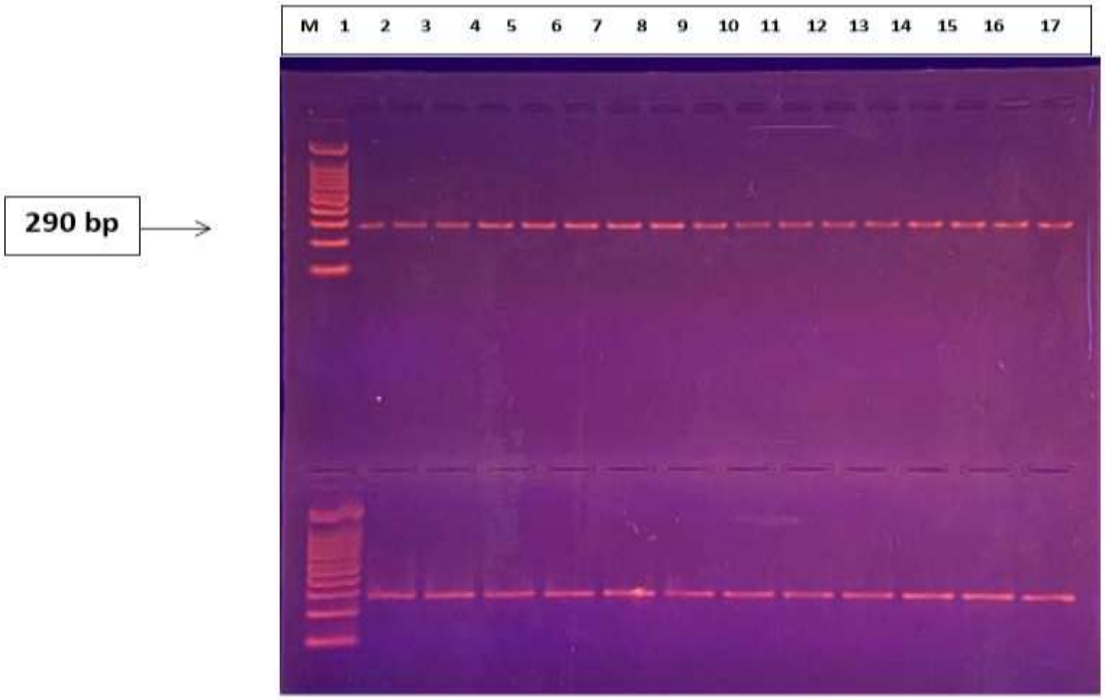


Figure (4) shows the result of the PCR reaction with a size of 290 bp for genetic detection of the Lux gene of the P.mirabilis, which was transferred in agarose gel at a concentration of (1)% with a potential difference of (70) volts for (60) minutes. All isolates appear from 1 to 21 isolates.

The results showed that all bacterial isolates isolated from the urinary catheter showed swarming (100%) on blood agar medium, and they were in the form of concentric waves, known as the bull's-eye pattern. The results of our study showed that the percentage of Proteus mirabilis bacteria possessing the RsbA gene using PCR technology was (56) isolates, at a rate of 100%, as shown in Table (3) and Figure (5).

Table (3): Numbers and percentages of biofilm production, both phenotypically and molecularly.

Total number	RsbA		Swarming	
	Numbers and percentages of genetically		Numbers and percentages of phenotipically	
	-ve	+ ve	-ve	+ ve
(%100)56	0	56(100%)	0	56(100%)

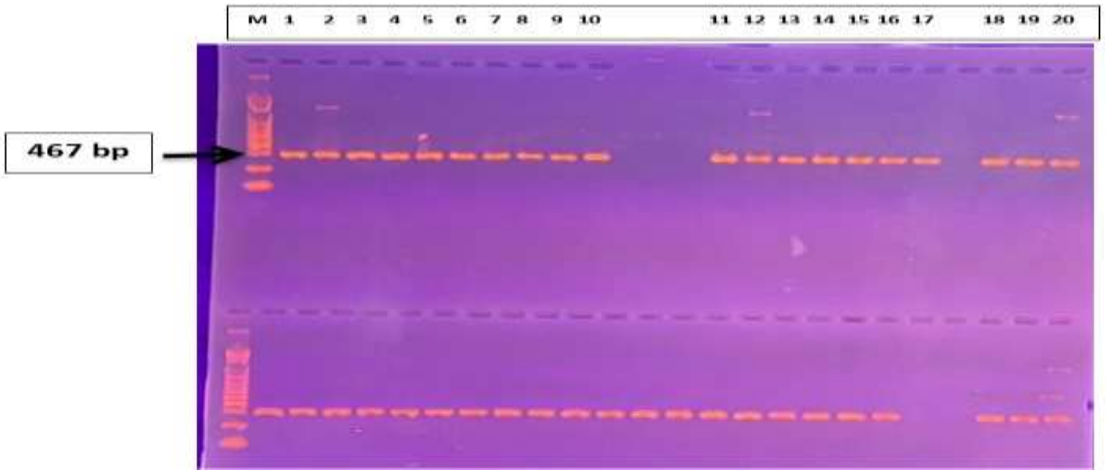


Figure (5) shows the result of the PCR reaction with a size of 467 bp for genetic detection of the rsbA gene of P.mirabilis bacteria, which was transferred in agarose gel at a concentration of (1)% with a potential difference of (70) volts for (60) minutes. All isolates appear from 1 to 24 isolates.

Using PCR analysis, 17 C. albicans isolates were examined for the presence of ALS1 genes in the urine of patients who were utilizing catheters. 10(58.8%) were have gene ALS1.

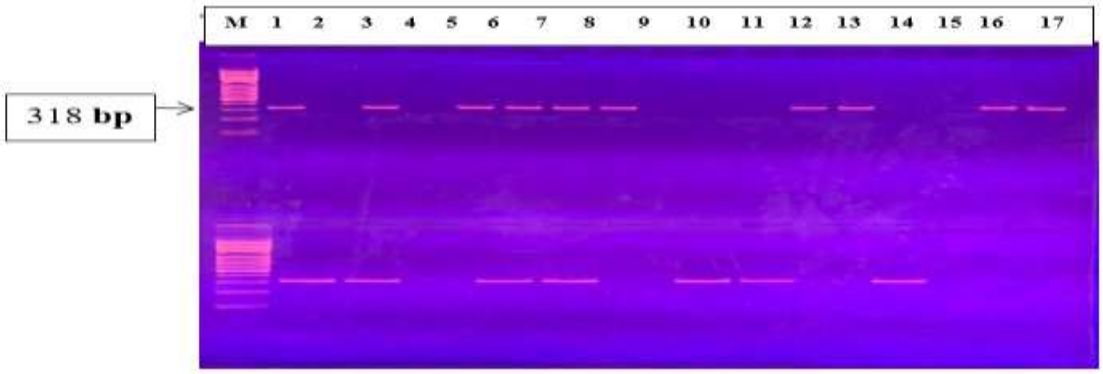


Figure (6) shows the result of the PCR reaction with a size of 318 bp for genetic detection of the ALS1 gene of C.albicans, which was transferred in agarose gel at a concentration of (1)% with a potential difference of (70) volts for (60) minutes. All isolates appear from 1 to 17 isolates.

DISCUSSION

Previous investigations on the bacterial biomass associated with nosocomial infections have indicated that gram-negative bacilli are prominent in urinary tract infections[17]. Moreover, additional results suggest that the identified strains of Proteus mirabilis rank among the most commonly isolated nonfermentative bacterial species from clinical specimens of hospitalized patients[18]. The toxicity of these bacterial species is minimal, and their virulence manifests solely in individuals with compromised immune systems and those utilizing invasive equipment.The severity of biofilm formation depends on the day of catheterization. The current results showed that all isolates formed biofilms, ranging from strong to moderate to weak. Many bacterial pathogens form cell aggregates known as biofilms, which significantly limit the success of antibiotic treatment and human immune defense. Biofilm formation is an important virulence factor for bacteria, allowing them to survive longer at the site of infection [19]. Proteobacteria isolated from urinary catheters have a greater ability to form biofilms than bacteria isolated from urine, specifically from the middle bladder. This is because the surfaces of urinary catheters facilitate bacterial adhesion without filtration, aided by the absence of the bladder's immune defense system. A study done by [20] demonstrated that all P. mirabilis isolates obtained from urinary catheters formed biofilms. Another study by [21] reported that P. mirabilis isolates obtained from urinary catheters had a higher biofilm-forming capacity than those isolated from feces. A similar study by [22] found that P. mirabilis isolates obtained from patients who had undergone urinary catheterization had a higher percentage (43.3%) of biofilm-forming capacity than those obtained from non-catheter samples (30%). Conversely, [23] reported no difference in biofilm-forming capacity between catheterized and non-catheterized urine isolates.Swarming, a phenomenon produced by Proteus mirabilis, facilitates the movement and migration of bacteria from the peri-urethral area and along the surface of the urinary catheter to the bladder, causing urinary tract infections in catheterized patients [24]. The results of the previous study showed that 28 (80%) of the samples possessed the rsbA gene. This is roughly consistent with the findings of [25], who found that 70% of Proteus mirabilis isolates possessed the rsbA gene. In another study, [26] Proteus mirabilis possessed the rsbA gene at a rate of 53%.The gene responsible for biofilm production in P. mirabilis isolated from catheter-associated urinary tract infections was genetically identified using PCR. The results showed that all 56 P. mirabilis isolates carried the gene responsible for biofilm production (100%). This differs from what was shown by phenotypic detection. This is due to the accuracy and speed of PCR, or it may be due to the two isolates possessing a latent gene, which can only be detected phenotypically molecularly. A study done by [27] indicated a correlation between the genes responsible for biofilm formation and the phenotypic appearance of the cell membrane, which revealed the presence of 100% of the genes responsible for membrane formation, while 96% gave a positive result for its phenotypic appearance. A study done by [28] demonstrated the luxS gene in 100% of their isolates. On the other hand, [26] reported that only 47% of their isolates carried the luxS gene, while 35% carried the mrpA gene. The results of the study conducted by [29] showed that all isolates had the ability to form

biofilms, with the percentage ranging from strong (30%) to moderate (50%) to weak (20%). This study documents the prevalence of urinary candiduria among patients with indwelling urinary catheters and highlights the high prevalence of the ALS1 gene among Candida isolates isolated from catheter-associated candiduria. The results of this study are consistent with a study conducted in Turkey by [30] in which the ALS1 gene was found in 53.9%. The ALS1 gene was detected in 46.4% of the isolates. A study conducted by [31] showed a higher prevalence of the ALS1 gene (65%) in C. albicans isolates isolated from hospital-acquired infections. A previous study conducted in Iraq by [32] also showed higher prevalence of the ALS1 gene (100%) among C. albicans isolates. The discrepancy in the prevalence of virulence genes between different studies may be attributed to several factors, including the number of isolates studied and the different locations of C. albicans strains' isolation.Adhesion is the initial step in biofilm formation. Genes in the ALS family encode cell surface proteins linked to glycosylphosphatidyl inositol (GPI) molecules, which act as adhesins and bind to glycoproteins, facilitating the attachment of Candida spp. strains to mucosal surfaces[33].

CONCLUSION

Proteus mirabilis is the main cause of urinary tract infections in humans. According to the results of this study, P. mirabilis isolates from urinary tract infections have the ability to possess virulence factors (phenotypic and genotypic), such as biofilm and hemolysin also candida albicans have geneses responsible of virulence factors as biofilm forming.

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