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The Detection of the Bacterial that cause Urinary Tract infection in women and Effect of Antimicrobial Agents on the Biofilm Production

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ABSTRACT

Of course, urinary tract infections (UTIs) are the most common infection in women. Microorganisms (M.O.) can produce potential virulence factors which are hemolysins or haemolysins. Probiotics are live harmless normal flora microorganisms, mostly represented by the strains of Lactobacillus spp., Bifidobacterium spp. and so on. However, when given in the right dosages, these microorganisms aid the health of the host. In this study, an attempt was made to isolate species of gram-negative bacteria from urinary tract infection, and test the in-vitro antibiotic and selected probiotic species against some pathogenic bacteria and search for hemolysin genes. A total of 200 urine specimens were obtained from patients with UTIs, consisting of 100 pregnant (50%) and 100 non-pregnant (50%), patients. Gram staining and microscopically analyses of bacterial colonies were done. Pathogen species were identified by biochemical tests. The Kirby-Bauer disk diffusion method was used for antibiotic testing. The well diffusion method and agar spot method were used to assess the effectiveness of probiotics against a group of isolated bacteria. Virulence genes of bacterial isolates were detected by PCR. The results revealed that 120 out of 200 urine specimens (60%) had significant bacteriuria. UPEC was present in 32/120 (26.7%) of the cultured samples, *P. aeruginosa* in 7/120 (5.8%), and *P. mirabilis* in 4/120 (3.3%). PCR diagnosis of 16S rRNA in isolates was 100% positive. *E. coli* isolates were 100% positive for both Hly-A and Hly-B genes. *P. aeruginosa* Hly-1 (plcH) and Hly-2 (exlA) were 85.7% and 71.4% positive, respectively. *P. mirabilis* Hly-A (hmpA) and Hly-B (hmpB) were 50% and 75% positive, respectively. The UPEC and *P. mirabilis* isolates showed higher resistance levels to lincomycin, rifampin, amoxicillin-clavulanate, colistin, and nalidixic acid (100%). In susceptibility testing using the well diffusion method, *L. acidophilus* was effective against 77% (P = 0.008) and *L. plantarum* against 90% (P = 0.003). In the agar spot method, *L. acidophilus* was effective against 82% (P = 0.005) and *L. plantarum* against 95% (P = 0.004). Most isolates were resistant to colistin, nalidixic acid, and azithromycin. The investigation into this mode of AR, found that Hly-A and Hly-B genes in bacterial cells enable them to be resistant to antibiotics. It has been found that Lactobacillus spp. do have antibacterial efficacy against in vitro growth of pathogenic bacteria, but junctional test indicates that whereas they can prevent the completely formation of strong biofilm in all isolates, they cannot inhibit the biofilm formation in all isolates for all period of observation.

Keywords: *Urinary tract infection (UTIs), L. acidophilus, L. plantarum, Hly-A, Hly-B, in-vitro, probiotic.*

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infections afflicting women. UTIs in women are prevalent at different stages of life. Females are much more prone to UTIs than males, mainly due to the female lower urinary tract anatomy and its proximity to the reproductive organs (Czajkowski et al., 2021). The short urethra and colonization of the peri-urethral area by pathogens from the gastrointestinal tract also contribute to this susceptibility. Pathogens from the peri-

urethral area ascend to colonize the urinary bladder or kidneys (Wing, Fassett et al., 2013; Czajkowski et al., 2021). Additionally, the incidence of UTIs is estimated at about 150 million cases per year (Al-Tulaibawi, 2019).

The microorganisms responsible for UTIs during pregnancy are the same uropathogens that are usually responsible for UTIs in non-pregnant populations. It is most frequently isolated from Escherichia coli (E. coli). Of the 90 cases of pyelonephritis in pregnant patients, E. coli was found to have been the causative agent in 82.5% of cases by an 18 year retrospective analysis. Other bacteria that may be seen include *Klebsiella pneumoniae*, *Staphylococcus*, *Streptococcus*, *Proteus*, *Pseudomonas spp.*, and *Enterococcus spp.* (Habak and Griggs, 2022).

Synthesis of Gramnegative hemolysins (cytolysins) usually leads to precursor protein formation that undergoes covalent modification to an active hemolysin and subsequent export via specific export systems that differ between various hemolysins. Lipids and proteins of hemolysins are that cause lysis of the red blood cells by forming pores and deforming the cell membrane. (Stipcevic et al., 2005). Uropathogenic *E. coli* (UPEC) and alpha-hemolysin of *Staphylococcus aureus* are the major microorganisms that produce hemolysins, which can cause cystitis, pyelonephritis, and sepsis (Kebaier et al., 2012). The expression of hemolysin correlates with the severity of infection, as up to 78% of UPEC isolates from pyelonephritis patient cases express hemolysin and harbor infectious isolates encoding up to 78% of hemolysin genes (Ristow and Welch, 2016). The organisms that cause UTIs during pregnancy are the same as those found in non-pregnant patients. *E. coli* accounts for 80-90% of infections (Balachandran et al., 2022). The most common bacteria causing UTIs in women are *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Aeromonas spp.*, *Serratia spp.*, *Neisseria spp.*, *Providencia spp.*, *Acinetobacter spp.*, *Veillonella spp.*, *Citrobacter spp.*, and *Bacteroides spp.* (Nkwelle et al., 2022).

Escherichia coli (*E. coli*) is a A Gram negative, facultative anaerobic, rod shaped coliform bacterium of the genus Escherichia. In addition, the UPEC strains have a great genetic diversity, which is associated with colonization and maintenance in the urinary tract, even in immunocompetent patients. Despite the numerous virulence factor VFs related to the occurrence of UTIs, including Alpha-hemolysin (*HlyA*), encoded by the *hlyA* gene in a pathogenicity island, causes the lysis of erythrocytes, endothelial cells, and urinary tract cells, enabling bacteria to capture iron and escape from phagocytes (Nascimento *et al*., 2021).

Proteus mirabilis (*P. mirabilis*) is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. It shows swarming motility and urease activity. In addition, *P. mirabilis* causes 90% of all Proteus infections in humans(Majeed and Aljanaby, 2019). In addition, *P. mirabilis* has many factors of virulence, Proteus mirabilis hemolysin genes are a two-part secretion (*hpmA* and *hpmB*). (Etxaniz *et al*., 2020).

Pseudomonas aeruginosa (*P. aeruginosa*) is a common encapsulated, Gram-negative, aerobic–facultatively anaerobic, rod-shaped bacterium (Nascimento *et al*., 2021). In addition, probiotics were live M.O that are intended to have health benefits when consumed or applied to the body. An October 2001 report by the World Health Organization (WHO) defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host (National Center for Complementary and Integrative Health, 2019).

2. MATERIALS AND METHODS

Patients and Study design

One hundred pregnant individuals (50%) and 100 non-pregnant individuals (50%) of the age group of 13 to 44 years who were suffering from urinary tract infection (UTI) were identified and their urine specimens were collected during the period from 1st August 2022 to 25 December 2022 from Al-Kut Maternity and Child Hospital, and Al Zahraa Teaching Hospital as well as private clinics in Wasit governorate in Iraq. The sample was conducted with sterile disposable cotton swabs and transport swabs. The organisms were cultivated on Blood Agar Base, Brain Heart Infusion Agar, Brain Heart Infusion Broth, Muller-Hinton Agar, and Nutrient Agar plates prior to aerobic and anaerobic incubation with CO2 at 37 ºC for 24 to 48 hours. Based on colony shape, Gram staining microscopy, blood hemolysis capacity, standard biochemical assays, the Vitek 2 system, and 16S rRNA analysis, the researchers conducted the following binary categorization assessments.

Antimicrobial susceptibility test

According to The Clinical and Laboratory Standards Institute (CLSI, 2022) standards, the antibiotic sensitivity of isolates was determined on Mueller Hinton agar using the Kirby-Bauer disk diffusion method (Sharma and Srivastava, 2016).

Molecular detection

In the current study, the designed primers of Polymerase chain reactions (PCR) were achieved using NCBI Genbank sequence database design, online software and synthesized by Scientific Researcher Co.Ltd, Iraq (Table2).

Table 1: Sequences of all primers were used in the present study

Primer	Sequence (5'-3')		Product Size	Genbank
16S *rRNA* gene *Escherichia coli*	F	TCCGGAGCTAACGCGTTAAG	459bp	LC682250.1
	R	AGTTGCAGACTCCAATCCGG		
16S rRNA gene *Proteus mirabilis*	F	AGTTGCAGACTCCAATCCGG	363bp	FN650811.1
	R	TTCGATGCAACGCGAAGAAC		
16S rRNA gene *Pseudomonas aeruginosa*	F	ATGCCTAGGAATCTGCCTGG	700bp	LN874213.1
	R	TCGTTTACGGCGTGGACTAC		
Primer	**Sequence (5'-3')**		**Product Size**	**Ref or Genbank**
Hla gene *E. coli*	F	AGGGGATGCTTTACTCGCAG	513bp	KM596784.1
	R	AACTCCTTCGGTTGAGCCTC		
Hlb gene *E. coli*	F	GGAGTTAGTGCAGCCTCCAG	419bp	U12572.1
	R	AACTCCTTCGGTTGAGCCTC		
hmpA gene *P. mirabilis*	F	GTTGAGGGGCGTTATCAAGAGTC	709bp	Ghaima *et al*., 2019
	R	GATAACTGTTTTGCCCTTTTGTGC		
hmpB gene *P. mirabilis*	F	CAGTGGATTAAGCGCAAATG	422bp	Ghaima *et al*., 2019
	R	CCTTCAATACGTTCAACAAACC		
Hemolysin 1gene *P. aeruginosa*	F	CTGGTACCTGTACGTCGACG	328bp	FN650811.1
	R	GAGGATGAACACGGTCCTGG		
Hemolysin 2gene *P. aeruginosa*	F	GACTACATCCTGGCCAACCC	512bp	LN874213.1
	R	TTCAGGCTGTTGTCGGGAAG		

PCR thermo cycler program

Polymerase chain reaction thermo cycler conditions for *S. aureus* and *Str. sanguinis* amplification reactions were done using convential PCR thermo cycler system is same for each gene except for annealing temperature as following the Tables 3.

Table 2: PCR thermocycler system of 16SrRNA, *Hly-A gene* and *Hly-B* gene.

PCR step.	Temp.	Time	No. cycles
Initial denaturation	95	5mnts	1
Denaturation	95	30 sec	35
Annealing	58[1]	30 sec	
Extension	72	30 sec	
Final-extension	72	5mnts	1
Hold	4	∞	12

All PCR products were detected by 2% agarose gel (100volts for 45minut) and visualized by staining with 1µl of Ethidium bromide stain then documentation was performed by the gel documentation saving picture (vision, UK).

Methods for detection anti-bacterial activity of Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus acidophilus and plantarum together

Lactobacillus acidophilus, Lactobacillus plantarum, and their combination were sourced from a commercial capsule provided by Wasit University. The two bacteria were subsequently grown in 10 ml of MRS broth at 37°C under anaerobic conditions for 24 to 48 hours.

Well diffusion method

Lactobacillus acidophilus, Lactobacillus plantarum, and a combination of both were cultivated in MRS broth at 37°C with 5% CO2 for 24 hours, serving as the broth culture bacteria (BCB). The isolates were cultured in broth for 24 hours at 37°C. Additionally, the cell-free supernatant (CFS) of Lactobacillus spp. was obtained by centrifuging the culture at 10,000 rpm for 15 minutes, followed by clarification using a 0.22 µm pore size filter paper.

The suspension had a turbidity of 0.5 McFarland. Then, 100µl of pathogenic bacteria were swabbed onto the nutrient agar plates as an inoculum. The plate then departed for an hour. On the cultured plates, three wells (6 mm in diameter) were formed. After that, add 50µl of Lactobacillus spp., each BCB and CFS were independently poured into wells (El-Mokhtar *et al.*, 2020). Following a 24-hour CO2 and 37°C incubation period. Zone diameter of inhibitions (ZDIs) values were measured in mm and classified as less active when ZDIs were less than 10, moderately active when ZDIs were between 11 and 14, and extremely active when ZDIs were greater than 15. (Halder *et al.*, 2017).

Agar spot method

The antibacterial efficacy of all strains against the pathogen isolates was evaluated. To isolate Lactobacillus spp. from the surface of MRS agar, an overnight culture in MRS broth was performed, followed by a 24-hour incubation at 37°C. Following a 24-hour culture, the pathogenic bacteria were transferred to a semi-solid nutritional medium (1.3g nutrient broth and 0.75g nutrient agar in 100ml distilled water) and subsequently applied to MRS agar. The samples were incubated at 37°C for 24 hours, after which an inhibitory zone was examined.

. Inhibition areas were categorized as no visible inhibition (...), inhibition of between 0.5 and 6mm (+), between 7 and 12mm (++) and more than 12mm (+++) (Abe Sato *et al*., 2021).

Statistical analysis

All data is analyzed statistically using the Statistical Package for the Social Sciences, version 25.0 for Windows. The Chi Square test was employed to examine all findings at a significance level of $P \leq 0.05$.

The results

Patient age groups

The results were distributed according to the patient age between 13-44 years old. The lowest incidence was among 35-44 Y age group (17.0%), while the highest incidence was among 25-34 Y age group (43.5%).

Isolation and identification of pathogen

Test the cultural characteristics of urine isolates about different media such as (Blood agar, MacConkey agar, Cetrimide agar, Eosin Methylene Blue (EMB), and Mannitol salt agar), gram stain, biochemical tests, vitek-2 system result and finally by 16sRNA Identification showed that upon confirmation the total number of patients with significant bacteriuria was 120/200 (60%) on positive culture and the negative (no growth) 80/200 (40%) may be due to fungal or virus infections. The number of patients with significant bacteriuria was higher in pregnant, n= 66/120(55%).

In the currant the most prevalence pathogen in UTI that cause heamolysis on blood agar was G+ve bacteria 25 (62.5%), *S.aureus* and *S.haemolyticus* with 15(37.5%) and 10(25%) respectivelly, and the G-ve bacteria that cause heamolysis on blood agar was 15 (37.5%), *P.aeuroginosa* n=7(17.5%), *Proteus* n=4(10%) and *E.coli* n=4(10%). As showen in Table 1.

Table 3: show the most prevalence pathogenic bacteria in pregnant and non-pregnant with age group.

Bacteria	No.	%	Patient's with Bacteria				
			Pregnancy		Age		
			Pregnant	Non Pregnant	13-24 Y	25-34 Y	35-44 Y
S.haemolyticus	10	25%	5	5	5	3	2
S.aureus	15	37.5%	8	7	6	8	1

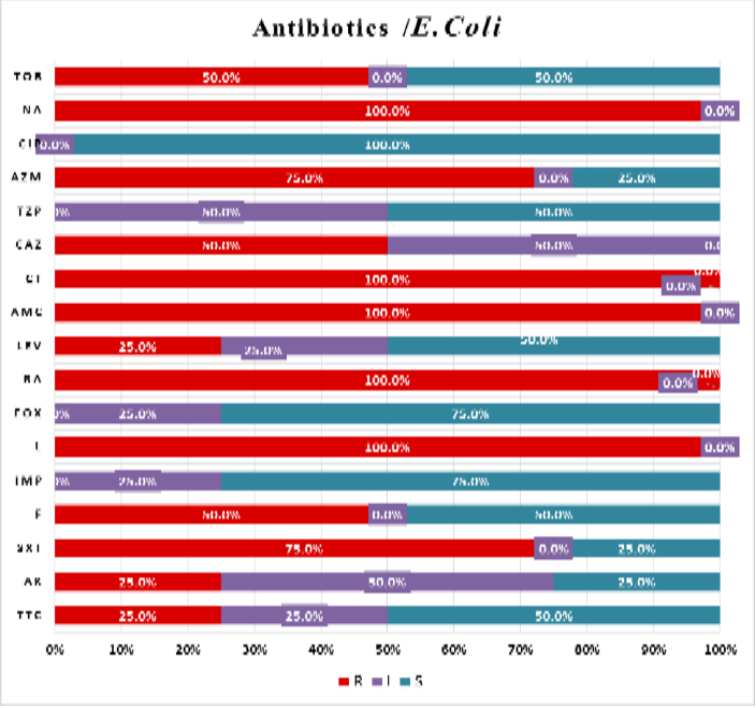
Proteus	4	10%	4	0	1	2	1
E.coli	4	10%	2	2	1	3	0
P.aeuroginosa	7	17.5%	4	3	2	5	0
***P*-Value**			0.495		0.656		

The *UPEC* was presented 32/120 (26.7%) of samples culture, *K. pneumoniae* 16/120 (13.3%), *P. aeruginosa* 7/120 (5.8%) and *P. mirabilis* 4/120 (3.3%).

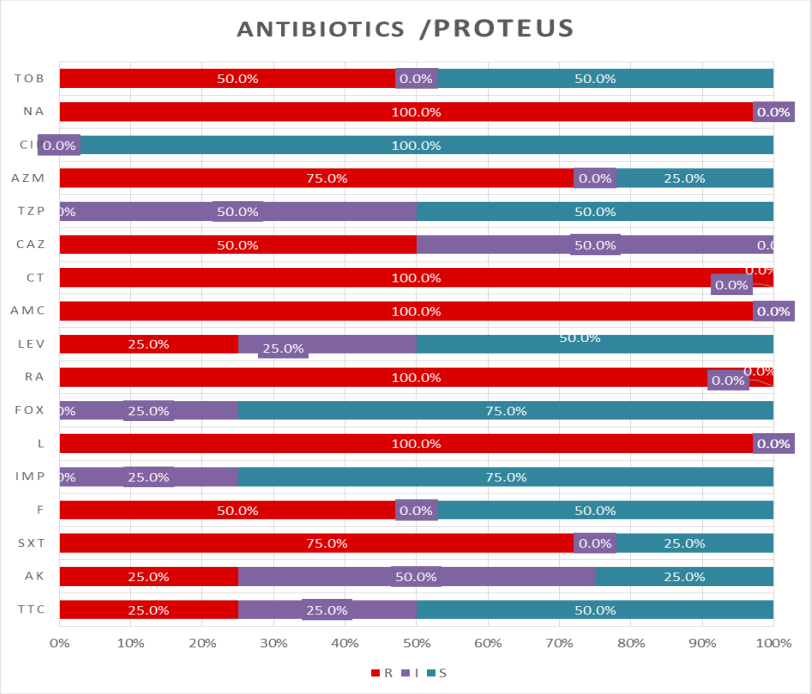
Antibiotic susceptibility

According to CLSI 2022, guidelines and by Kirby-Bauer disk diffusion method, on Muller-Hinton agar a total of 15 of *S. aureus* isolates, 10 *S. haemolyticus*, 4 *E. Coli*, 4 *P. mirabilis*, and 7 *P.aeuroginosa*, were exposed to susceptibility testing using different antibiotics as mentioned above.

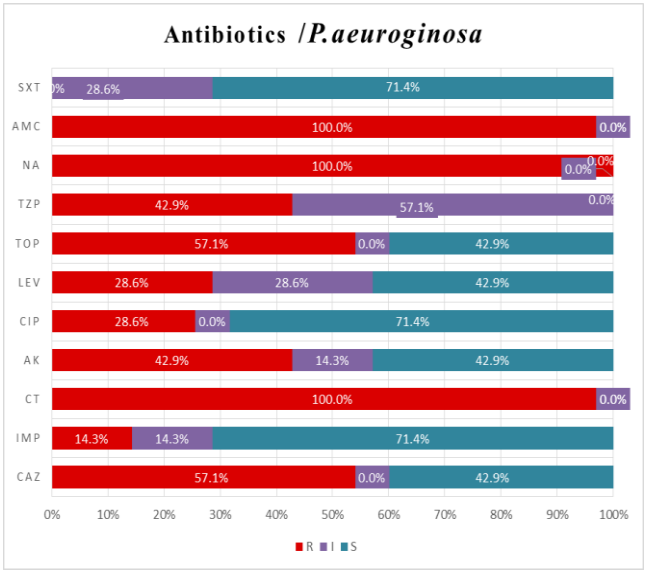
The Antibiotic susceptibility test of UPEC isolates Showed higher resistance level of the present study for lincomycin, Rifampin, Amoxicillin-clavulanate, colistin and Nalidixic acid were 100%, and Trimethoprim-sulfamethoxazole and Azithromycin were 75%, while the lowest resistancy were to Imipenem, Cefoxitin, Pipracacilin – tazobactam and Ciprofloxacin were 0.0%, with high significant P-Value 0.001. as figure 1



The outcome of the antibiotic susceptibility assessment of P. mirabilis isolates The current study demonstrated that the isolates exhibited complete resistance (100%) to lincomycin, rifampin, amoxicillin-clavulanate, colistin, and nalidixic acid. Resistance to cefoxitin, piperacillin-tazobactam, and azithromycin was observed at 75%, while intermediate resistance was noted for ticarcillin-clavulanic acid. Conversely, the majority of isolates were fully sensitive (100%) to ciprofloxacin, with sensitivity to amikacin, imipenem, and levofloxacin at 75%. P-value: 0.0003 (Figure2).



Antibiotic susceptibility test of *P. aeruginosa* isolates, The current study showed that the isolates were most resistant to colistin, Nalidixic acid and Amoxicillin-clavulanate were 100%, and Trimethoprim-sulfamethoxazole was 0%, and the higher sensitivity was to Imipenem, Ciprofloxacin and Trimethoprim-sulfamethoxazole with 71.4%. With high significant *P*-Value 0.0001. Figure 3.



Molecular results

Extraction of DNA in different isolates of pathogenic bacteria

The DNA for *P. aeruginosa*, *P. mirabilis* and *E. coli* were extracted. Additionally, NanoDrop was used to confirm the nucleic acid purity and concentration.

Molecular detection of 16S rRNA of isolates

Diagnosis using PCR was regarded the golden and confirmatory diagnosis which takes a short period compared to other methods this specific detection of most conserved region of target bacteria recorded *E. coli* 4(100%), *P. aeruginosa* 7(100%), *P. mirabilis* were 4(100%), isolates, as showed in the Figure4, 5, and 6.

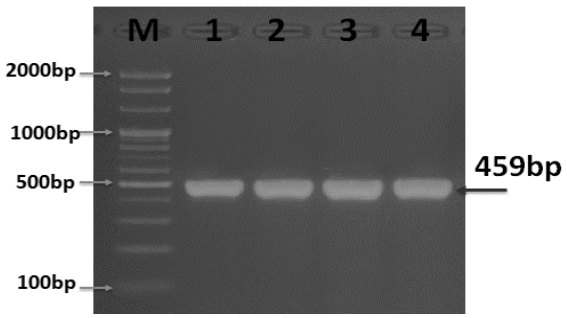


Figure (4): Analysis of PCR products for the 16S ribosomal RNA gene to detect Escherichia coli isolates was conducted via agarose gel electrophoresis imaging. M (Marker ladder 2000-100 bp). Several positive Escherichia coli isolates exhibited the 16S ribosomal RNA gene with a product size of 459 bp in lanes 1-4.

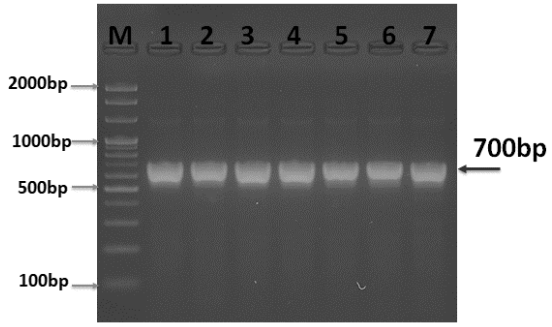


Figure (5): Analysis of PCR products of the 16S ribosomal RNA gene for the detection of Pseudomonas aeruginosa isolates via agarose gel electrophoresis picture. M (Marker ladder 2000-100 bp). Positive Pseudomonas aeruginosa isolates exhibited a 700 bp 16S ribosomal RNA gene product in lanes 1 to 7.

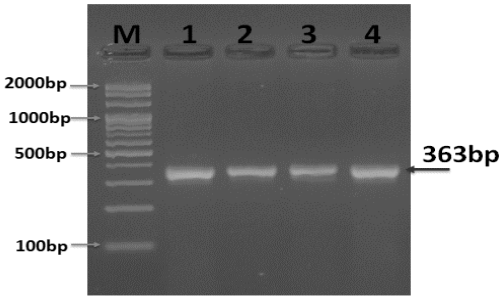


Figure (6): The analysis of PCR products for the 16S ribosomal RNA gene to detect Proteus mirabilis isolates was conducted using agarose gel electrophoresis images. M (Marker ladder 2000-100 bp). Once more, Lane (1-4) exhibited several positive Proteus mirabilis isolates with a 363 bp product size of the 16S ribosomal RNA gene.

Detection of some virulence genes of bacterial isolates

The polymerase chain reaction was performed to detect the presence of the genes (*Hly -A* and *Hly-B*) for *E. coli*, *P. aeruginosa*, and *P. mirabilis.* As showen in the table 2.

In current study the Hemolysin gene of the *E. coli* isolates was 4(100%) for both *Hly-A* and *Hly-B* gene. Figure 7.

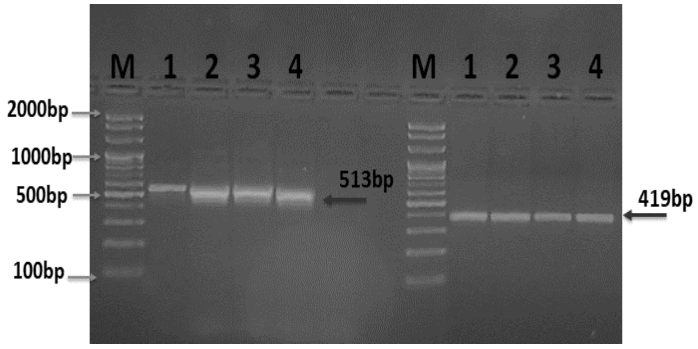


Figure (7): PCR product analysis of hla and hlb genes on bacterial Eschericial isolates examined by agarose gel electrophoresis image. M (Marker ladder 2000-100bp). The results of the hla assay of Lane (1-4) positive with 513bp product size, while the positive with 419bp product size of the hlb assay of Lane (1-4) .

Diagnosis for hemolysin gene using PCR recorded that the *P. aeruginosa Hly*-1(*plcH*) and *Hly*-2(exlA) were 6(85.7%) and 5(71.4%) respectivelly figure 8.

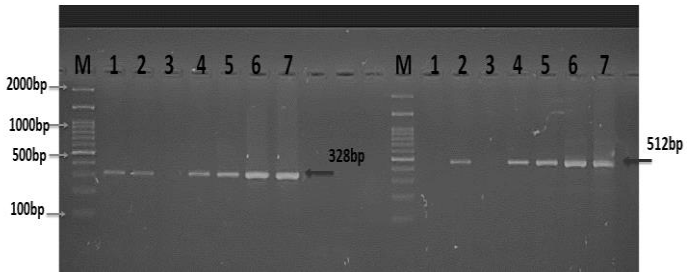


Figure (8): The agarose gel electrophoresis image showing the analysis of the PCR product of Hemolysin 1 and Hemolysin 2 genes in Pseudomonas aeruginosa isolates. M (Marker ladder 2000-100bp). The results indicate that Lane (1-7) showed some positive Hemolysin 1 gene Pseudomonas aeruginosa isolates at 328bp product size and Lane (1-7) showed some positive Hemolysin 2 gene Pseudomonas aeruginosa isolates at 512bp product size .

The results in the present study revealed that the *P. mirabilis Hly-A*(*hmpA*) and *Hly-B* (*hmpB*) 2(50%) 3(75%) respectivelly, As showen in figure 9.

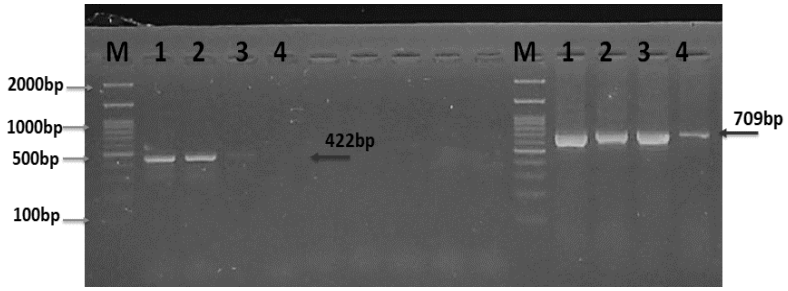


Figure (9): PCR product analysis of hmpA and hmpB genes in Proteus mirabilis isolates was done on agarose gel electrophoresis image. M (Marker ladder 2000-100bp). Proteus mirabilis isolates showing positive hmpA gene had product size of 709bp and some positive hmpB gene Proteus mirabilis isolates had product size of 422bp (Lane 1 – 4).

Effect of some probiotic on bacteria isolated from UTIs

An evaluation of the antibacterial efficacy of Lactobacillus acidophilus, Lactobacillus plantarum, and their combination use efficacy against specific bacteria isolated from urinary tract infections by the means of well diffusion and agar spot techniques. The metric was the inhibitory zone (mm). The current findings concerning the use of probiotic bacteria against the various bacterial isolated from urinary tract infection illustrated the range of their inhibition abilities, with the BCB caused 11 - 24 mm inhibition zones and the CFS 9 - 15 mm when subjected the well diffusion method and 12 - 26 mm when carried out the agar spot method. (Table 4).

Table 4. Antimicrobial effect (%) of Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus acidophilus and plantarum together against some bacteria isolate using well diffusion method.

Bacteria	*L.Acido*		*L.Plantarium*		*L.Acido & L.Plantarum*		P-Value
	No.	%	No.	%	No.	%	
Proteus	3	75%	4	100%	4	100%	0.008
e.coli	4	100%	4	100%	4	100%	1.000
P.aeuroginosa	4	57%	5	71.4%	6	85.7%	0.013

Inhibition of the growth of BCB by Lactobacillus plantarum was 24 mm and by L. acidophilus was 19 mm, and Lactobacillus acidophilus and plantarum combined was 26–29 mm inhibition zone using well diffusion technique. Further, Lactobacillus plantarum exhibited the highest inhibition zone of 15 mm and 11 mm in Lactobacillus acidophilus and combined in Lactobacillus acidophilus and Lactobacillus plantarum was 24–28-mm

Table 5: Antimicrobial effect (%) of Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus acidophilus and plantarum together against some bacteria isolate using CFS well diffusion method.

Bacteria			*Proteus*		*e.coli*		*P.aeuroginosa*	
			No.	%	No.	%	No.	%
L.Acido	**Active**	**≥15 mm**						
	Moderate	**11-14 mm**	**1**	**25%**	**1**	**25%**	**2**	**28.6%**
	Less active	**≤10 mm**	**2**	**50%**	**3**	**75%**	**2**	**28.6%**
L.Plantarium	**Active**	**≥15 mm**						
	Moderate	**11-14 mm**	**3**	**75%**	**2**	**50%**	**2**	**28.6%**
	Less active	**≤10 mm**	**1**	**25%**	**2**	**50%**	**3**	**42.9%**
L.Acido & L.Plantarum	**Active**	**≥15 mm**						
	Moderate	**11-14 mm**	**4**	**100%**	**4**	**100%**	**3**	**42.9%**
	Less active	**≤10 mm**	**0**	**0%**		**0%**	**3**	**42.9%**

Table 6: Antimicrobial effect (%) of Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus acidophilus and plantarum together against some bacteria isolate using BCB well diffusion method.

Bacteria			*Proteus*		*e.coli*		*P.aeuroginosa*	
			No.	**%**	**No.**	**%**	**No.**	**%**
L.Acido	**Active**	**≥15 mm**	2	50%	2	50%	3	42.9%
	Moderate	**11-14 mm**	1	25%	2	50%	1	14.3%
	Less active	**≤10 mm**						
L.Plantarium	**Active**	**≥15 mm**	4	100%	3	75%	3	42.9%
	Moderate	**11-14 mm**	0	0%	1	2%	2	29%
	Less active	**≤10 mm**						
L.Acido & L.Plantarum	**Active**	**≥15 mm**	4	100%	4	100%	6	85.7%
	Moderate	**11-14 mm**						
	Less active	**≤10 mm**						

Agar spot method is another method to detect the antimicrobial effect of Lactobacillus plantarum and Lactobacillus acidophilus and Lactobacillus plantarum together against some bacteria isolate, as showed in the Table 3.15.

Table 7: Antimicrobial efficacy (%) of Lactobacillus acidophilus, Lactobacillus plantarum, and the combination of Lactobacillus acidophilus and Lactobacillus plantarum against various bacterial isolates utilizing the agar spot method.

Bacteria	***L.Acido***		***L.Plantarium***		***L.Acido & L.Plantarum***		**P-Value**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Proteus	3	75%	4	100%	4	100%	**0.008**
E.coli	4	100%	4	100%	4	100%	**1.000**
P.aeuroginosa	5	71%	6	85.7%	7	100.0%	**0.009**

Antibiotics vs. Probiotics: Impact on Biofilm Formation by Pathogenic Skin Wound Bacteria

The effect of Lactobacillus acidophilus, Lactobacillus plantarum and mix of Lactobacillus acidophilus and plantarum on biofilm production and prevention was studied for bacteria statically in this current study therefore well diffusion method and agar spot method with P-Value=0.00712, stress, means all Lactobacilli had significant effect on biofilm formation by bacteria. Besides, statically analysis revealed that each of Lactobacillus acidophilus and plantarum alone has a relationship with inhibition the biofilm formation of the pathogen bacteria using well diffusion method (P = 0.007), while statically analysis (P = 0.0089) indicated that a combination of Lactobacillus acidophilus and plantarum also had a relationship with the inhibition of the biofilm formation of the pathogen bacteria using agar spot method. Statistical study indicates a

connection between probiotics and the suppression of robust biofilm development by both Lactobacillus spp. bacteria.

Table 8: Antimicrobial effect (%) of Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus acidophilus and plantarum together against some bacteria isolate using well diffusion method.

Antibacterial	***Proteus***		***E.coli***		***P.aeuroginosa***	
	No.	**%**	**No.**	**%**	**No.**	**%**
Strong Biofilm	2	50%	3	75%	7	100%
L.Acido	3	75%	4	100%	4	57%
L.Plantarum	4	100%	4	100%	5	71%
L.Acido & L.Plantarum	4	100%	4	100%	6	86%
TOP	S(50%)		S(50%)		R(57.1)	
NA	R(100%)		R(100%)		R(100%)	
CIP	R(100%)		S(100%)		S(71.4%)	
AZTH	R(75%)		R(75%)			
TZP	S(50%)		S(50%)		I(57.1%)	
CAZ	R(50%)		R(50%)		R(57.1)	
CT	R(100%)		R(100%)		R(100%)	
AMC	R(100%)		R(100%)		R(100%)	
LEV	S(50%)		S(50%)		S(42.9%)	
RA	R(100%)		R(100%)			
FOX	S(75%)		S(75%)			
L	R(100%)		R(100%)			
IMP	S(75%)		S(75%)		S(71.4%)	
F	S(50%)		R(50%)			
SXT	R(75%)		R(75%)		S(71.4%)	
AK	I(50%)		I(50%)		R(42.9%)	
TTC	S(50%)		S(50%)			

Chi-Sqaure=13.584 , P-Value=0.00712

3. DISCUSSION

The pregnant women appeared to be significantly infected with UTIs and uropathogenic bacteria than the non pregnant women which can be associated with an adverse outcome for both the mother and the fetus (Habak et al., 2019). According to Ali et al. (2022), the sample percentage of G-negative bacteria was 97%, in which 49 (71%) were G-negative bacilli. The Ab sensitivity values of UBEC were similar to the values reported by Rahman et al., 2022. The findings of Mohammed et al., 2021 in Iraq.

The susceptibility results of P. mirabilis to antibiotics contradicted those reported by Mirzaei et al. (2019), which indicated resistance rates of 15.5% to ciprofloxacin, 13.6% to norfloxacin, 12.7% to tobramycin, 11.8% to imipenem, 4.5% to meropenem, and 2.7% to amoxicillin-clavulanic acid. Similar study Hussein et al., 2020/ Iraq, revealed that the susceptibility rate ceftriaxone 96.8%, then norfloxacin 82.5%, ciprofloxacin 69.8%, Nalidixic acid 42.9%, sulfamethoxazole 39.7%,

Nitrofurantoin 39.7%. This class of antibiotics was extensively misused over the years and consequently resulted in high degree of resistance to such antibiotics among P. mirabilis isolates (Mirzaei et al., 2019). He et al., 2022 perform a similar investigation of susceptibility for P. aeruginosa results, and found 54% resistance for ciprofloxacin. Piperacillin/tazobactam showed 26% POS (25% RO), Imipenem showed POS of 14% (RO 14%). However, as other Bekele et al., 2015; and Tet et al., 2023 conducted shows that Ciprofloxacin as the most effective antibiotic in UTIs treatment against P. aeruginosa had 100% percent.

The results in the present study of Hly gene of *E. coli* agreed with study achieved by Noori and jassim Mohamad, 2023 in Kirkok/ Iraq, showed that *E. coli* isolates possessed the *hlyA* gene isolated from urine was (75%). While, the result in the present study disagreed with another study achieved by Audrey and Procter, 2015, who showed that *hly-A* gene expressed in the *E. coli* was 26 carried *hlyA* (21.7%). The result conducted by Moeinizadeh and Shaheli, 2021, Shiraz city/Iran, revealed that the frequencies of *hlyA* and *hlyB* genes were calculated as 50% and 43%, respectively and that disagree with the results in current study. The result in current study for *Hly* of *P. aeruginosa* agreed with Ghanem et al., 2023 who showed that the PCR amplification results presence of *plcH* (*Hly-A*) was 75.2% genes. The result in current study agreed with Andhale et al., 2021 who showed that the PCR amplification results presence of *plcH* (*Hly-A*) genes in 22 (73.33%). The results in the present study agreed with another study achieved by Faraji et al., 2016, who showed that plcH gene in Pseudomonas aeruginosa were 79%.

Our investigation confirmed that, consistent with the findings of Kais et al. (2019), hemolysin genes (hpmA and hpmB) were identified in P. mirabilis; specifically, both hpmA and hpmB were present in 96 out of 110 P. mirabilis isolates, or 87.3% of the samples. Cestari et al. (2013) corroborated the current findings, indicating that the hpmA and hpmB genes were detected by PCR in 97.15% of the 211 isolates. There is evidence that hemolysin increases the virulence of infections by P. mirabilis because production of this protein has been correlated with cytotoxicity in *Vetro* cells, also it was found that observed that *P. mirabilis* that produced hemolysin associated to the cell produced a lethal dose 50 % greater than the non-hemolytic isolates when injected transurethrally in mice (Kais et al., 2019). These results of Antimicrobial effect of Lactobacillus spp. come in accordant to another study conducted by Al-Azzawi et al. (2020), who showed that the inhibition zone by BCB higher than CFS.

The results in the current study come in accordant to another study achieved by Dallal et al. (2017), who revealed that the probiotic *Lactobacillus plantarum* enhancement of inhibitory zone diameters in well diffusion method higher than others against *P. aeruginosa* with 100% effect. In addition, these results in the current study agreed with another study achieved by Al-Asady *et al*. (2020), who showed that the effect of *L. acidophilus* (13mm) with 100%. Moreover , Soltani et al. (2022), who found that L acidophilus had ability to inhibit of *E. coli* isolated from UTIs was16 mm with 100%. Tigu et al. 2016, The Lactobacillus isolates have also been shown to inhibit the growth of E. coli with inhibition zones of 10 to 14 mm in diameters. The results of the present study showed that agar spot method is a good but slightly more effective method in comparison with well diffusion method. The agar spot method in general was highly effective compared to the well diffusion method. The results of this study matched those of another study which continues to cultivate the liquid culture with those conducted by Al-Azzawi et al (2020).

4. CONCLUSION

This study show that the majority of isolates possess the hemolysin gene. Most isolates exhibit resistance to lincomycin, rifampin, amoxicillin-clavulanate, colistin, and nalidixic acid. Lactobacillus spp. has antibacterial efficacy and immunological properties against pathogenic microorganisms responsible for urinary tract infections (UTIs).

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