Study of Inhibitory Estimates of Probiotics and Extent of their Synergistic Effect with some Antibiotics Against Antibiotic-Resistant Bacteria that Cause Urinary Tract Infections in Children

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Abstract: The prevalence of urinary tract infections (UTIs) in children caused by antibiotic-resistant bacterial strains represents a serious public health problem. The aim of this in vitro study was to evaluate the direct antimicrobial activity of commercially available probiotic strains and to investigate their synergistic interaction with antibiotics against clinical isolates of uropathogens. From 160 urine samples, 65 antibiotic-resistant bacterial isolates (both Gram-positive and Gram-negative) were obtained. Their identification and resistance profiling were performed using the VITEK-2 system. The antimicrobial activity of probiotics and their synergism with antibiotics were assessed using the agar diffusion method. The results showed that certain probiotic strains, particularly L. rhamnosus and L. reuteri, exhibited significant inhibitory activity against uropathogens, whereas others (Bifidobacterium) were inactive. The strongest synergistic effect was observed with the combination of probiotics and tetracycline. The findings demonstrate that the antimicrobial effects and synergism with antibiotics are strictly strain-dependent and vary with the antibiotic's mechanism of action. This in vitro study highlights the potential utility of probiotics as adjunctive agents to overcome antibiotic resistance and emphasizes the need for further investigation of strain-specific interactions.

1 INTRODUCTION

One of the most prevalent forms of bacterial infections in children is urinary tract infections (UTIs). occur frequently in both females and boys. After starting in the urethra, the infection spreads to the kidney tissue. The infection's name reflects its place of origin [1]. The incidence rate is 1% for boys and 3% to 5% for girls. Studies have shown that the prevalence of urinary tract infections in children is greater than the prevalence of bacterial meningitis, pneumonia, and bacteremia. The most important risk factors for urinary tract infections (UTI) in children are gender, age, recurrence of urinary tract infections, uncircumcised children, fever, and bladder catheterization [2]. The most prevalent pathogen, *Escherichia coli*, is responsible for about 85% to 90% of UTIs. *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus species*, and *Enterococcus species* are next in line. [3].

The use of antibiotics has significantly reduced the incidence of urinary tract infections in patients, but the overuse of these antibiotics has led to the emergence of resistant strains that have different strategies to resist many drugs and the spread of super-infections caused by these organisms [4]. Finding innovative and successful therapeutic approaches is so essential. Numerous probiotic strains have been used to cure or prevent urinary tract infections by reducing the quantity of dangerous bacteria and maintaining an acidic environment [5]. Scientific studies have confirmed the ability of probiotics to inhibit pathogens, reduce inflammation, and reduce lactose tolerance. Probiotics are defined as live microorganisms that, when ingested in appropriate amounts, boost the host's health. [6].

Nowadays, therapeutic biology is used extensively in food and medicine and is of significant interest worldwide [7]. Probiotics is the Greek word that consists of (pro and bios), which means life, and it was first recognized by scientists (Stillwell, Lilly) in 1965. It was possible for these microscopic

organisms to release substances that promoted the growth of other microscopic organisms. The scientist verified that in 1989 (Roy Fuller). The success of probiotics, the possibility of using it in treatment, and their ability to benefit the health of the host. [8], Characterized by bacteria *L. plantarum*, it has great medical importance and is a bacilli gram positive. Catalase test negative result increases the pH of the medium in which it is present, and also plays a role in *L. plantarum*. It plays an important role in colonizing urinary tract epithelial cells within 24 hours of incubation and thus has antibacterial properties. [9].

2 MATERIALS AND METHODS

2.1 Collection and Isolation of Pathogens

The current study was carried out between August 8, 2024, and November 11, 2024. 65 isolates of bacteria resistant to antibiotics were isolated from 160 samples of patients under the age of 15 who visited several hospitals in Mosul, including Al-Salam Teaching Hospital, Mosul General Hospital, and Al-Khansaa Teaching Hospital. Samples of children under one year were collected using the adhesive plastic bag method. The samples were incubated for 24 hours at 37º in aerobic conditions after being cultivated on MacConkey and Blood agar. The isolates were identified by colony morphology, biochemical testing, and microscopic examination [10]. The diagnosis was confirmed using a VITEK-2 compact system utilizing ID-GNB and ID-GPB cards (BioMerieux, France) [11].

2.2 Collection and Isolation of Probiotics

In this study, some types of probiotic bacteria found in pharmacies and from reliable sources were used, in addition to types obtained from the University of Mosul, College of Agriculture. Species isolated from vaginal swabs and Activia milk were activated by growing them in MRS broth. Then incubated under anaerobic conditions for 24 hours at.37ºc After the incubation period, take 0.1It was grown on medium MRS agar. The identity of the isolates was determined through cultural characteristics and biochemical tests as stated in [12] and used it in the study.

2.3 Determination Of AST and Minimal Inhibitory Concentration of Antibiotic Using the Vitek2 Compact System

Antibiotic susceptibility tests and minimum inhibitory concentrations (MICs) for all isolates under study were carried out to identify the prevalence of antibiotic resistance among isolates by using the VITEK-2 compact system [13]. In this study, we used two types of sensitivity cards: AST card for Gram-positive bacteria (AST-580) and the AST card for Gram-negative bacteria (AST-N222).

2.4 Testing the Inhibitory Activity of Isolates Probiotics Invitro

2.4.1 Estimation of Inhibitory Activity of Precipitate Organisms Probiotic

Carried out as described in [14-16]. After minor adjustments, 0.1 ml of bacterial suspension was added, with a concentration of $1.5x10^8$ bacterial cells/ml. Using a cotton swab, they were applied in three different directions to the Muller-Hinton agar medium while the plate was rotated at a 60° angle. The plates were then left to dry at room temperature for ten to fifteen minutes. A sterile corkborer was used to create wells of five millimeters in the middle of the Muller-Hinton agar on the pathogenic bacteria that had grown. For control and comparison, 0.1 ml of the bacterial isolate's suspension Probiotics was added to each well, and one well was filled with MRS broth free of bacterial growth and incubated for 24–48–72 hours at 37ºC surrounding the holes were measured following the incubation time, and the results were recorded and compared with the control coefficient that contained MRS broth without vaccination [17].

2.4.2 Estimation of Inhibitory Activity of Filtrate Probiotics

The Well diffusion experiment was utilized to determine the inhibitory activity of therapeutic filtrate probiotics, with a few modifications. The impact of liquid culture filtrate on the growth of isolates of therapeutic organisms Probiotics (0.5) MacFarlane Within test tubes filled with MRS broth after adjusting the pH to 5.7, the tubes were incubated at 37ºC for 24 hours under anaerobic conditions. Following incubation, the tubes were centrifuged at 4000 rpm for 10 minutes to extract the bacterial-free supernatant. The liquid was then

filtered through filters having a 0.45 micrometer diameter. In the process, the turbidity constant was compared with the suspension of harmful bacteria. The suspension was then applied to Mueller Hinton agar medium using a sterilized cotton swab. 50 µL of liquid culture filtrate of isolates probiotics was transferred to the solid medium after holes measuring 5 mm in diameter were created with a corkborer. and incubated for 24-48-72 hours at 37ºC. The inhibition zones surrounding the holes were measured following the incubation time, and the results were recorded and compared with the control coefficient that contained MRS broth without vaccination [17].

2.5 Combined Effects of Probiotics and Antibiotics Against Urinary Tract Infection Pathogens

The diffusion test was used with some modifications to measure the effectiveness of synergism in inhibiting pathogenic strains (*E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*). A bacterial suspension of pathogenic bacteria and turbidity comparison was prepared (0.5) with McFarland scale and brushed with a cotton swab on Mueller Hinton agar medium and 100 microliter of the probiotic that caused the highest inhibition was mixed with (100) µL (100µg/ml) of antibiotics (Tetracycline, Amikacin, Gentamycin, Amoxicillin, Ciprofloxacin, Cefuroxime). Then transfer (50) µL from the mixture to the holes and incubated for 24 hours at a temperature 37 ºC. After the incubation period, the inhibition zones around the holes were calculated, and the results were recorded and compared with the control coefficient containing MRS broth without vaccine [18].

3 RESULTS AND DISCUSSION

3.1 Isolation and Identification of Pathogens

Between August 8, 2024, and November 11, 2024, 160 urine samples were collected from children under 15 who had UTIs and visited hospitals in Mosul City. According to the findings, 65 samples were positive culture and 95 were negative culture, as indicated in Figure 1. The isolates were identified using the Gram stain, cell morphology, and culture characteristics in the blood agar and MacConkey agar. The diagnosis was confirmed by Vitek2 compact system. The rates of recurrent urinary tract infections are still high and lead to increased relapses and deaths around the world. The excessive and inappropriate use of antibiotics has led to a decrease in the effectiveness of antibiotics and an increase in bacterial resistance, not only at the individual level but also at the community level [19].

3.2 Percentage of Gram-Negative Bacterial Species

Figure 3 shows that Gram-negative bacterial isolates were represented by Escherichia coli 22(55%), Pseudomonas aeruginosa 6 (15%), Klebsiella pneumonia 5 (12.5%), Enterobacter cloacae 4(10%), Proteus mirabilis 2(5%) and Serratia marcescens 1(2.5%). The percentage of Gram-negative bacterial species is shown in Figure 3.

3.3 Percentage of Gram-Positive and Gram-Negative Bacterial Species

Distribution of bacterial isolates was as follows: 25/65 (38.5%) Gram-positive and 40/65 (61.5%) Gram-negative bacteria (Figure 1). Gram-positive isolates comprised: Staphylococcus haemolyticus 11 (44%), S. aureus 10 (40%), Staphylococcus hominis 2 (8%), and Enterococcus faecalis 2 (8%) (Figure 2).

3.4 Sensitivity of Isolates and Minimum Inhibitory Concentration to Antibiotics

All 65 isolates under study were subjected to antibiotic susceptibility testing and minimum inhibitory concentration (MIC) to determine the prevalence of antibiotic resistance. The MIC was determined by Vitek2 compact system which is based on a series of dilutions of antibiotics prepared in a card AST for Gram-positive and Gram-negative bacterial isolates, the antibiotic was considered sensitive if the value was MIC is at the lowest stopping point according to (CLSI-2024) and the results were MIC for isolation as follows:

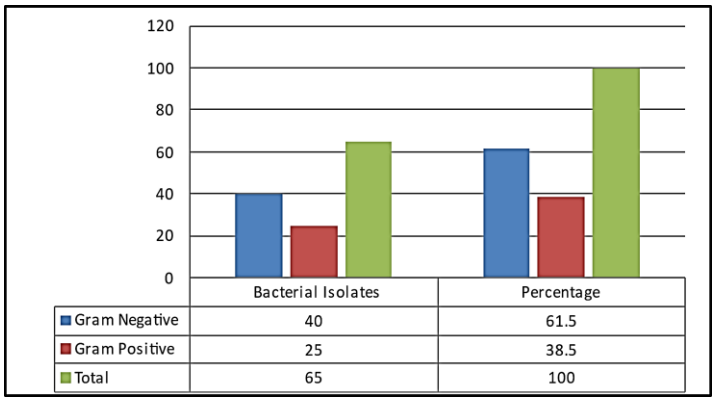

Figure 1: The demographic characteristics of the study.

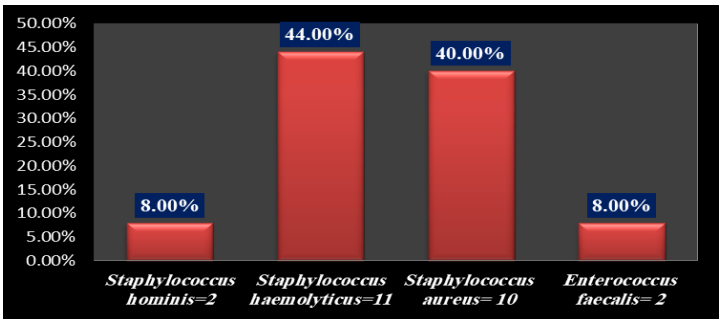

Figure 2: Percentage of Gram-positive bacterial species.

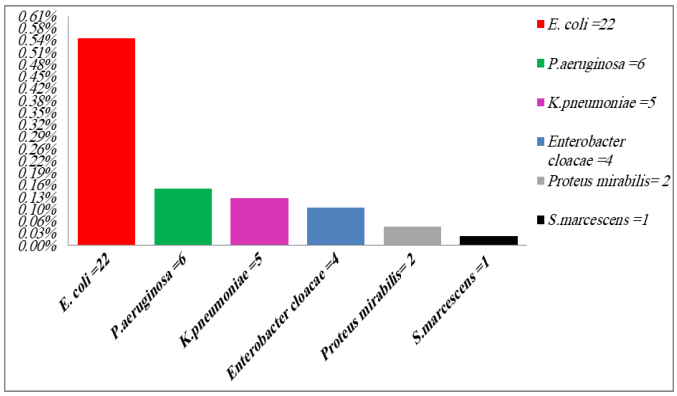

Figure 3**:** Percentage of Gram-negative bacterial species.

3.5 AST and MIC Profile for Gram Positive and Gram-Negative Bacteria

According to Table 1, all Staphylococcus aureus isolates were 100% sensitive to antibiotics (Moxifloxacin, Gentamycin, Teicoplanin, Linezolid, Vancomycin, Trimethoprim/Sulfamethoxazole) respectively. The minimum inhibition concentration was (⩾0.5µg) for each of (Vancomycin, Teicoplanin, Gentamycin) respectively, while the MIC was (⩽0.5µg, ⩽2µg, 10µg) for each of (Moxifloxacin, Linezolid, Trimethoprim/sulfamethoxazole) respectively. The highest resistance was recorded 70% by (Benzylpenicillin, Oxacillin) and the MIC was (⩾4µg, ⩾0.5µg) respectively, while the lowest resistance was recorded 10% by (Tetracycline, Rifampicin) and the MIC recorded was (2µg,1µg)

respectively. Staphylococcus haemolyticus isolates showed 100% resistance to (Oxacillin, Benzylpenicillin) and recorded MIC (⩾2µg, ⩾0.25µg, ⩾0.5µg) respectively and were 100% sensitive to antibiotics (Linezolid, Vancomycin, Tigecycline, Rifampicin) and the MIC was (⩾0.5µg,0,25µg) respectively and were 90% sensitive to antibiotics (Moxifloxacin, Clindamycin, Teicoplanin). Staphylococcus hominis showed 100% resistance to antibiotics (Oxacillin, Fusidic Acid, Erythromycin) and the MIC was (0.5µg, ⩾8µg, 4µg) respectively and were sensitive to many antibiotics 100% (Gentamycin, Clindamycin, Teicoplanin, Linezolid, Vancomycin, Tigecycline, Rifampicin) MIC was between (⩾0.25µg, 1µg). Enterococcus faecalis was 100% resistant to the antibiotic (Gentamycin) while 100% sensitive to (Teicoplanin, Linezolid) MIC (⩾0.5µg, 1µg) respectively. According to Table 2, the isolates of E. coli were 100% sensitive to each of the antibiotics (Imipenem, Meropenem, Amikacin, Colistin) and the MIC was (0.25µg⩾ µg2), while 100% resistant to the antibiotics (Ticarcillin) MIC is (⩾128µg). Klebsiella Pneumonia isolates were 100% resistant to each of the antibiotics (Aztreonam, Ticarcillin) and the MIC was (32µg, ⩾128µg) respectively, while the isolates recorded high sensitivity of 100% to the antibiotics (Imipenem, Meropenem, Amikacin, Colistin) recorded MIC (0.5µg, 2µg, ⩽0.25µg). Proteus mirabilis isolates were 100% resistant to each of the antibiotics (Piperacillin, Imipenem, Tobramycin, Colistin, Ticarcillin, Trimethoprim/ Sulfamethoxazole) and the MIC was (2µg, ⩾128µg), while the isolates were 100% sensitive to the antibiotics (Cefepime, Meropenem, Amikacin) and the MIC was (2µg, 16µg) respectively. The isolates of Pseudomonas aurogenosa were 83% resistant to each of the antibiotics (Ticarcillin/Clavulanic acid, Piperacillin, Imipenem, Meropenem) and recorded MIC (0.5µg, ⩽0.25µg, 32µg,) respectively while the highest sensitivity to antibiotics was recorded by Colistin was 66%, while the MIC was (⩽o.5µg). Enterobacter cloacae isolates were 100% resistant to the antibiotic Colistin and the recorded MIC (⩾0.5µg), while they were 100% sensitive to the antibiotics (Cefepime,

Meropenem, Amikacin, Imipenem) and the MIC was between (⩾0.25µg, 2µg). Serratia marcescen recorded 100% resistance to the antibiotic Colistin and the MIC (2µg). 100% sensitive to all antibiotics used in the study, MIC was between (⩾0.12µg, 4µg). Gram-positive bacterial isolates According to Table 1 showed high sensitivity to antibiotics (Vancomycin, Linezolid Teicoplanin, Tigecycline). The reason is attributed to their lack of mechanisms that help them resist drugs, such as the lack of sufficient defense mechanisms or the lack of resistance genes. Glycopeptides antibiotics (Teicoplanin, Vancomycin) act on the cell wall, which It represents the basic part of the bacterial cell, and (Tigecycline, Linezolid) work from the oxazolidinones, Tetracyclines class that inhibit protein synthesis, which leads to stopping protein synthesis. Staphylococcus aureus, Staphylococcus haemolyticus have a high level of resistance to(Oxacillin, Benzylpenicillin) This study agreed with a study conducted with Diaullah Mirza and a study in Rajshahi [20], [21] while it did not agree with a study in Egypt (Mahfouz et al., 2023) where the resistance rate was medium while it gave a resistance rate to each of Fusidic acid, Erythromycin, Clindamycin)) reached 20% and a study in Iraq and Jordan (AL Husain et al., 2020; [22] showed high resistance due to the presence of genes resistance. But gram-negative bacteria were between resistant and sensitive to the antibiotics used in the study, and the cause of resistance is attributed to the possession of ß-lactamase enzymes that break the ß-Lactams ring in antibiotics, and changes in the cell membrane in addition to the acquisition of mutated genes through plasmid transfer processes, but the cause of sensitivities The lack of these factors makes them more susceptible to drugs [23] in this study, according to Table 2 the most gram-negative bacterial isolates were highly sensitive to (Colistin, Imipenem, meropenem, Amikacin) and the results were consistent with studies in Mosul and Iran [24], [25] and the difference of the results with the study of performances in Erbil [26] showed the result Different proportions of 40% resistance against (Amikacin) When isolates showed moderate resistance to (Ciprofloxacin, Aztreonam), except for Klebsiella Pneumonia isolates, it was 100% resistant, but Pseudomonas aeruginosa isolates showed 66% high sensitivity to(Colistin) it was different from a previous study in Diyala (Mohammed, 2021) where isolates showed Multiple drug resistance. Antibiotic failure if the bacteria show moderate resistance and if not diagnosed in time. According to the study [27], with the increase of resistant bacteria, it led to the lack of therapeutic options in the treatment of bacterial infections, and we now face a big challenge in employing active treatment.

3.6 Probiotics Development and Isolation

Probiotic isolates were obtained as where it was activated on MRS broth. for 24 hours at 37°C under anaerobic conditions, then purified on MRS agar medium and incubated anaerobically for 48 hours at 37°C. The colonies were small to medium sized flat circular colonies. The colony color was white to creamy, shiny, sticky, usually smooth, with edges and slightly convex, while the cells appeared under the microscope as long or short rods, sometimes oval, in pairs or Single and Gram positive [28]. It is non-spore-forming and negative for catalase, oxidase, indole and urease tests and positive for carbohydrate fermentation.

3.7 Study of the Inhibitory Activity of Probiotic Isolates

The inhibition activity of the samples obtained was tested using MRS agar medium. against pathogenic bacterial isolates and selection of the most resistant to antibiotics. As shown in Figure 4 several methods were used to study the inhibitory activity of probiotics, as the rate of inhibition diameters for the isolate (L. rhamnosus, L. reuteri) against pathogenic bacteria ranged between 17-24mm, while the rate of inhibition diameters for the bacterial isolate (L. plantarium) ranged between 15-20mm. As for the bacteria isolated from the vagina, an inhibition rate was recorded between 6-10mm, while no inhibition was recorded by the milk isolate (Bifidobactrium).

Figure 4: The diameters of inhibition of probiotics against pathogens.

An inhibiion rate was recorded by the commercial isolate consisting of several types of probiotics, and it was between 14-20mm. The highest inhibition rate was recorded by the types used in this study against the pathogenic bacteria (Proteus mirabilis), as the inhibition rate ranged between 19-24mm, while the inhibition rate varied against other pathogenic types, as shown in Table 3. It was also found in this study that the ideal incubation period at which the diameters of inhibition reached their highest measurements was 24 hours, which is consistent with what was stated by [29] While no inhibition was recorded by the probiotic filtrate or it was of little effect for all pathogenic bacterial isolates.

3.8 The Combined Effect of Probiotics and Antibiotics

According to the results shown, the most efficient isolates were selected from probiotics were used in the study of the synergistic effect with the antibiotic. Three pathogenic isolates that were most resistant to antibiotics were selected. The results of the synergistic effect between the antibiotic and probiotics were as shown in Table 4, where the synergism between the antibiotic Tetracycline with the probiotic isolates used recorded the highest inhibition and was between (21-33mm). The highest inhibition was on *Staphylococcus aureus* bacteria. No inhibitory effect was recorded when the Probiotic isolates were synergized with the antibiotics (Amoxicillin, Cefuroxime) against pathogenic isolates (*E. coli, Klebsiella Pneumonia*), while it was an inhibitor on *Staphylococcus aureus* isolates. Synergism was not recorded between the antibiotic Gentamycin with *L. plantarium* and the product containing different types of probiotics against the *Klebsiella pneumonia* isolate. The use of commercially obtained combinations of Probiotics against bacterial isolates causing urinary tract infections and resistant to multiple antibiotics was suggested. The selection of antibiotics was based on studying the synergistic effects for their use in clinical prescriptions in the hospital to treat infections. Most antibiotics had a synergistic effect with Probiotics. Using the diffusion method in the holes, it was observed that the diameter of the inhibition zone against pathogens when mixing (Tetracycline + Probiotics) was higher than the inhibition zone when mixing (Gentamycin, Amikacin) with Probiotics [30]. In this study, it was recorded that the effect of inhibiting the growth of pathogens by antibiotics alone was higher than the synergistic effect with Probiotics, which is consistent

with what was stated in the study [31], where synergy had negative effects and reduced the zone of inhibition of pathogen growth, contrary to what was stated in a study conducted by [18]. The synergy between antibiotics and Probiotics had higher inhibitory effects on the growth of pathogens than when used alone, so it seems that the type of antibiotic and the type of probiotic are important in creating synergistic effects. This depends on the mechanism of action of the antibiotic. For example, the antibiotic Tetracycline acts on the 30S subunit and inhibits protein synthesis. Therefore, when used separately, the antibiotic has a better effect on pathogens than when mixed with probiotics. Through our evaluation of the ability Probiotics inhibit the growth of pathogens and found their effect on both Gram-positive and Gram-negative bacteria, this is consistent with a study conducted in Iraq by [32]. While it differed from a study in Turkey conducted by [33], in which probiotics were found to be effective on Gram-positive bacteria only. The variation in inhibition may be related to the type of pathogenic bacteria, the type and quantity of the inhibitor, and its ability to inhibit.

Table 1**:** Antibiotics susceptibility tests in AST card for Gram-Positive bacteria based on CLSI,2024.

No o	AB	Gram-Positive bacterial isolates											
		Staphylococcus aureus (10)			*Staphylococcus haemolyticus (11)*			*Staphylococcus Hominis* (2)			*Enterococcus faecalis* (2)		
		R%	S%	MIC	R%	S%	MIC	R%	S%	MIC		S%	MIC
1	P	7 (70)	3 (30)	⩾0.03µg	11 (100)	0 (0)	⩾0.5µg	1 (50)	1 (50)	⩾0.03µg	-	-	-
2	OXI	7 (70)	3 (30)	⩾0.25µg	11 (100)	0 (0)	⩾4µg	2 100	0(0)	0.5µg	-	-	-
3	FA	2 (20)	8 (80)	⩾0.5µg	9 (82)	2 (18)	⩾0.5µg	2 100	0(0)	4µg	-	-	-
4	GM	0 (0)	10 (100)	⩾0.5µg	6 (55)	5 (45)	⩾0.5µg	0(0)	2 100	⩾0.5µg	2 100	0(0)	SYN
5	E	2 (20)	8 (80)	⩾0.25µg	9 (82)	2 (18)	⩾0.25µg	2 (100)	0(0)	⩾8µg	-	-	-
6	MO	0 (0)	10 (100)	⩾0.25µg	2 (18)	9 (82)	⩾0.25µg	1 (50)	1 (50)	⩾0.25µg	-	-	-
7	CM	2 (20)	8 (80)	⩾0.25µg	2 (18)	9 (82)	⩾0.25µg	0(0)	2 100	⩾0.25µg	-	-	-
8	TEC	0 (0)	10 (100)	⩾0.5µg	1 (9)	10 (91)	8µg	0(0)	2 100	1µg	0(0)	2 100	⩾0.5µg
9	LNZ	0 (0)	10 (100)	2µg	0 (0)	11 (100)	2µg	0(0)	2 100	2µg	0(0)	2 100	1µg
10	VA	0 (0)	10 (100)	⩾0.5µg	0 (0)	11 (100)	⩾0.5µg	0(0)	2 100	⩾0.5µg	1 (50)	1 (50)	4µg
11	TE	1 (10)	9 (90)	⩾1µg	9 (82)	2 (18)	⩾1µg	1 (50)	1 (50)	⩾1µg	1 (50)	1 (50)	⩾1µg
12	TGC	0 (0)	10 (100)	⩾0.12µg	0 (0)	11 (100)	0.25µg	0(0)	2 100	⩾1µg	0(0)	2 100	⩾0.12µg
13	RA	1 (10)	9 (90)	⩾0.5µg	0 (0)	11 (100)	⩾0.5µg	0(0)	2 (100)	⩾0.5µg	-	-	-
14	SXT	0 (0)	10 (100)	⩾10µg	5 (45)	6 (55)	⩾10µg	1 (50)	1 (50)	⩾10µg	-	-	-

Table 2: Antibiotics susceptibility tests in AST card for Gram-Negative bacteria based on CLSI,2024.

No	AB*	Gram-Negative bacterial isolates																		
		Escherichia Coli (22)			*Klebsiella pneumonia (5)*			*Pseudomonas (6) aeruginosa*			*Proteus mirabilis* (2)			*Enterobacter cloacae (4)*			*Serratia marcescen* (1)		
		R%	S%	MIC	R%	S%	MIC	R%	S%	MIC	R%	S%	MIC	R%	S%	MIC	R%	S%	MIC
1	P	10 (42.8)	12 (57.2)	8 µg	3 60%	2 40 %	⩽8	5 (83)	1 (17)	32µ g	1 (5 0)	1 (5 0)	64µ g	1 (2 5)	3 (7 5)	⩽8µg	0 0	1 1 0 0	⩽8µg
2	PE	22 (100)	0 (0)	⩾128 µg	4 80%	1 20 %	⩾12 8 µg	5 (83)	1 (17)	32µ g	2 1 0 0	0(0)	16µ g	2 (5 0)	2 (5 0)	⩾4µg	0 0	1 1 0 0	⩽4µg
3	C AZ	9 (38)	13 (62)	⩽1 µg	4 80%	1 20 %	16 µg	4 (66)	2 (34)	⩽1µ g	1 (5 0)	1 (5 0)	⩽1µ g	2 (5 0)	2 (5 0)	⩾1µg	0 0	1 100	⩽1µ g
4	G M	9 (38)	13 (62)	⩽1 µg	2 40%	3 60 %	⩾16 µg	3 (50)	3 (50)	⩽1µ g	1 (5 0)	1 (5 0)	8µg	2 (5 0)	2 (5 0)	⩾1µg	0 0	1 1 0 0	⩽1µg
5	FE D	5 (2 4)	17 (76)	⩽1 µg	3 60%	2 40 %	⩽1 µg	4 (66)	2 (34)	⩽1µ g	0(0)	2 1 0 0	4 µg	0 (0)	4 (1 00)	⩾1µg	0 0	1 1 0 0	⩽1µg
6	IP M	0 (0)	22 (10 0)	⩽0.25 µg	0 0%	5 100 %	⩽0.2 5 µg	5 (83)	1 (17)	⩽0. 25µ g	2 1 0 0	0(0)	2µg	0 (0)	4 (1 00)	⩽0.5µ g	-	-	-
7	M E M	0 (0)	22 (10 0)	⩽0.25 µg	0 0%	5 100 %	⩽0.2 5 µg	5 (83)	1 (17)	0.5µ g	0(0)	2 1 0 0	⩽0. 25 µg	0 (0)	4 (1 00)	⩽0.25 µg	0 0	1 100	⩽0.2 5µg
8	A K	0 (0)	22 (100)	⩽2 µg	0 0%	5 100 %	⩽2 µg	3 (50)	3 (50)	32µ g	0(0)	2 1 0 0	⩽2 µg	0 (0)	4 (1 00)	⩽2µg	1 1 0 0	0 0	⩽2µ g
9	TO b	8 (38)	14 (62)	⩽1 µg	2 40%	3 60 %	⩽1 µg	4 (66)	2 (34)	⩽1µ g	2 1 0 0	0(0)	⩾16 µg	2 (5 0)	2 (5 0)	⩾1µg	1 1 0 0	0 0	⩽1µ g
10	CS	0 (0)	22 (100)	⩽0.5 µg	0 0%	5 100 %	⩽0.5 µg	2 (34)	4 (66)	⩽0. 5µg	2 1 0 0	0(0)	⩾16 µg	4 (1 00)	0 (0)	⩽0.5 µg	1 1 0 0	0 0	2µg
11	CI P	12 (5 7)	10 (43)	⩽0.2 5 µg	3 60%	2 40%	0.5⩽ µg	3 (50)	3 (50)	⩽0. 06µ g	1 (5 0)	1 (5 0)	⩽0. 25µ g	1 (2 5)	3 (7 5)	⩾0.25 µg	0 0	1 1 0 0	⩾0.25µg
12	A Z	12 (53)	10 (47)	⩽1 µg	5 100%	0 0%	32 µg	-	-	-	1 (5 0)	1 (5 0)	⩽1 µg	2 (5 0)	2 (5 0)	⩾1µg	0 0	1 1 0 0	⩽1µg
13	Ti c	22 (1 00)	0 (0)	⩾12 8 µg	5 100%	0 0%	⩾12 8 µg	-	-	-	2 1 0 0	0(0)	⩾64 µg	2 (5 0)	2 (5 0)	⩾16µg	0 0	1 100	⩽8µ g
14	S	17 (8 1)	5 (19)	⩽20 µg	3 60%	2 40%	⩽20 µg	-	-	32µ g	2 1 0 0	0(0)	⩾32 0µg	2 (5 0)	2 (5 0)	⩾320 µg	0 0	1 100	⩽20 µg

Table 3: The inhibitory effect of probiotic strains against bacterial pathogens.

Pathogen bacteria / Probiotics	*S. auras*	*E. coli*	*Pseudomonas. aurogenosa*	*Klebsiella pneumonia*	*Proteus mirabilis*
L.rhamnosus, L. reuteri	Inhibition zone in diameter				
	17 mm	18 mm	17 mm	17 mm	24 mm
L.plantarium	16 mm	15 mm	20 mm	14 mm	20 mm
L.acidophilus	15 mm	15 mm	14 mm	6 mm	20 mm
Bifidobacterium	-	-	-	-	-
L.casei, L. paracasei, L gasseri. L. Salivarius, L. bulgaricus . fermentum, L	20 mm	15 mm	14 mm	16 mm	19 mm

Table 4: The antibacterial activity of probiotics and antibiotics alone and in combination against pathogen isolates based on the inhibitory zone's diameter.

Antibiotics	Zone of inhibition in mm of pathogenic bacteria and antibiotics											
	E. coli				Klebsiella pneumonia				Staphylococcus aureus			
	antib	1+ Antibio	2+ Antibio	5+ Antibio	antib	1+ Antibio	2+ Antibio	5+ Antibio	antib	1+ Antibio	2+ Antibio	5+ Antibio
GM	17mm	13mm	12mm	18mm	19mm	11mm	0	0	22mm	17mm	18mm	18mm
AK	23mm	21mm	20mm	22mm	20mm	16mm	15mm	10mm	19mm	15mm	15mm	14mm
CIP	5mm	0	0	0	23mm	18mm	21mm	16mm	12mm	0	0	0
AM	0	0	0	0	0	0	0	0	16mm	14mm	12mm	14mm
TE	33mm	30mm	23mm	21mm	25mm	22mm	23mm	23mm	38mm	30mm	30mm	33mm
CX	0	0	0	0	0	0	0	0	22mm	20mm	20mm	20mm

Abbreviations: GM= Gentamycin, AK =Amikacin, CIP= ciprofloxacin, AM =Amoxicillin, TET= Tetracycline, CX= Cefuroxime, Antib= Antibiotics, 2=L.plantarium,5= MIX of probiotics. 1=Lactobacillus rhamnosus, L. reuteri

Table 5: Comparison of the percentage of survival of different probiotic strains at different pH values.

Probiotics	pH at 3 hours				pH at 6 hours			
	pH 2	pH 3.5	pH5	pH 6.5	pH 2	pH 3.5	pH5	pH 6.5
L. rhamnosus, L. reuteri	54.16%	60%	68.7%	93.3%	28%	41.17%	87.5%	97.2%
L.plantarium	40%	38%	48%	96.5%	33.3%	35.3%	45%	1oo.o8%
L.casei, L. paracasei L gasseri. L. Salivarius L. bulgaricus L . fermentum,	33.3%	33.3%	98%	99.3%	48%	37.5%	100%	100.5%

3.9 Acidity Tolerance pH of Probiotic Isolates

Acidity tolerance was carried out according to the method described [34] with some modifications, incubation of Probiotics isolates anaerobically overnight in 5 ml of MRS broth at a temperature of 37°C, after incubation, the culture was diluted with 5 ml of MRS broth to obtain a bacterial suspension (10^7 CFU) and then were washed twice with Phosphate buffer solution(pbs) with a pH of 7.2, and resuspended in 5 mL of sterile MRS broth which was adjusted to pH use of 2, 3.5, 5, and 6.5 which has been modified by using hydrochloric acid (HCL)and incubated for 3 and 6 hours in the aerobical at 37ºc. With a temperature of 37ºc and anaerobic, the percent of survivors of the acid challenge was calculated as the ratio of bacterial concentrations of colonies counted in MRS after acid challenge (N1) divided by to the initial bacterial number concentration at time zero (N0):

$$Survivors\ (\%) = cfu/ml(N0)/cfu/ml(N1) \times 100.$$

According to Table 5 The difference in the tolerance of probiotics isolates to different degrees of pH to simulate the environment of the human stomach showed that all the isolates used in the study showed a general level of tolerance at pH (5, 6.5). These results agreed with the results reported by [35]. According to the reported study [36], the survival rate of isolates with pH was low.

4 CONCLUSIONS

This in vitro study was designed to evaluate the antimicrobial activity of different probiotics and their ability to act synergistically with antibiotics against clinical isolates of bacteria causing UTIs in children. The main conclusions are as follows:

- The antimicrobial activity of probiotics was shown to be strain-dependent. Lactobacillus rhamnosus and Lactobacillus reuteri demonstrated the highest in vitro efficacy.
- The synergistic effect between probiotics and antibiotics is not universal. It varies according to the specific probiotic strain–antibiotic combination. The strongest synergistic effect in our experiment was observed with tetracycline.
- In certain cases, the combination of a probiotic and an antibiotic may result in an antagonistic effect or show no difference compared to the antibiotic alone, underscoring the complexity and selectivity of these interactions.
- The obtained in vitro data highlight the potential utility of specific probiotic strains as adjunctive agents for enhancing the effectiveness of antibiotics and overcoming uropathogen resistance.

Thus, the results of the study indicate that selected probiotic strains may exhibit antimicrobial activity and enhance the action of certain antibiotics under in vitro conditions. Further, more comprehensive research is required to determine the mechanisms underlying these interactions and their potential clinical significance.

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