

## **Antibacterial Activity of Some Lactic Acid Bacteria Isolated From Milk Products Against *Streptococcus pyogenes* and *Staphylococcus aureus* Causing Tonsillopharyngitis**

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**Abstract.** Background: Tonsillitis and pharyngitis are the most common oral infections accompanied by risky complications, including infection recurrence and antibiotic resistance. *Streptococcus pyogenes* and *Staphylococcus aureus* are the predominant cause of such infection. Objective: This study comes to investigate the nature of bacterial interactions between probiotic lactobacilli and bacilli, isolated from milk products, with the most common pathogenic bacteria causing tonsillitis and pharyngitis in human. Methods: A 20 samples of local milk products were collected. in addition to 25 tonsil swabs were collected from patients with tonsillopharyngitis. VITEK system2 was used to confirm bacterial identification. The antibiotic susceptibility of bacterial isolates was evaluated to five tested antibiotics. Co-aggregation capability of tested bacterial species was evaluated to investigate the nature of bacterial interactions towards each other. Results: Of the 20 milk samples, the VITEK2 system confirmed that 3 (60%) of bacterial growth were identified as *Bacillus subtilis* while 8 (72.7%) were *Lactobacillus plantarum*. On the other hand, 11 (73.3%) of bacterial growth were *Streptococcus pyogenes* and 6 (100%) were *Staphylococcus aureus* isolated from tonsillopharngitis patients. The isolated *S. pyogenes* and *S. aureus* were highly resistant to the tested antibiotics, but not to vancomycin. Both bacilli and lactobacilli species showed high co-aggregation scores with the isolated pathogenic bacteria. Conclusions: The antibiotic resistance of pathogenic bacteria requires urgent, safe, and effective alternative antimicrobial agents. Beneficial bacteria capable to compete with the growth of pathogens and inhibit their growth, eventually, preventing such infections.

### **Highlights:**

1. Antibiotic-resistant *S. pyogenes* and *S. aureus* cause recurrent tonsillopharyngitis.
2. Milk-derived *L. plantarum* and *B. subtilis* co-aggregate with pathogens, inhibiting colonization.
3. Probiotics offer a natural alternative for preventing antibiotic-resistant infections.

**Keywords:** Tonsillopharyngitis, *Streptococcus pyogenes*, *Staphylococcus aureus*, bacilli and lactobacilli, auto-aggregation and co-aggregation.

## Introduction

Oral infections, sometimes manageable, however, the pain and discomfort associated with the infection are often challenging to control [1]. There is a huge microbial diversity habitat mouth and pharynx. More than 700 bacterial species were identified in the tongue, teeth, gums, inner cheek, palate, and tonsils [2]. It has been found that more than 20% of oral pathogenic bacteria were *Streptococcus* spp., the most prevalent bacterial genus, isolated from saliva [1]. Tonsillitis and pharyngitis, as commonly known as sore throat, are the most common infections in the healthcare setting [2]. The infection peak is at 50% during the childhood and adolescence of the total medical visits [2]. Pharyngitis could be auto-healed as viral infections or accompanied by risky complications, including infection recurrence and antibiotic resistance, especially when a mixture of bacterial agents, mostly *Streptococcus pyogenes* and *Staphylococcus aureus* are causing pharyngitis [3]. Acute pharyngitis may arise from non-infectious agents (such as seasonal allergies), or non-bacterial origin (such as viruses) [4]. The bacterial etiology is often group A beta-hemolytic streptococci (GABHS), causing strep throat, which is associated with acute and chronic complications [4]. In addition to group A, group C, and G streptococci are associated with pharyngitis. These strains are capable of colonizing and initiating infection, but it is difficult to determine the relative rates of these bacterial infections [5]. A single bacterial species, GABHS, may cause acute tonsillitis, but recurrent tonsillitis is produced as a result of poly-microbial infection, in which a combination of pathogenic bacteria, in addition to GABHS, causes chronic tonsillitis [4]. Recently, it has been found that beta-lactamase-producing bacteria *Staphylococcus aureus* colonize the tonsils and enhance the infection's resistance to some antibiotics, mostly penicillin. Several studies have noticed that the failure of antibiotic treatment is related to neglecting or forgetting the other microbial etiology [4], that is known to be resistant to antibiotics, such as methicillin or vancomycin-resistant *Staphylococcus aureus*. This explains the low concentration of antibiotics in tonsillar tissue, which is possibly attributed to the *Staphylococcus* production of protective enzymes, or specific antimicrobial substances, leading to antibiotic inactivation [5].

Antibiotic resistance and infection recurrence are the most challenging issues related to chronic pharyngitis and tonsillitis. Several studies mentioned that antibiotic resistance to the infection is associated with multi-microbial colonization, especially when methicillin-resistant *Staphylococcus aureus* (MRSA) is included [6]. The current antibiotic treatment is used only to reduce the severity and duration of symptoms, in addition to limiting the spread of infection [7]. Recent studies showed that the new generation of antibiotics, such as azithromycin for 3-6 days, gave better treatment results compared to conventional antibiotics [8]. The activity of antibiotics may be limited but failing to eliminate the infection completely, sometimes they are useless. The random prescription of antibiotics may contribute to the development and spread of bacterial resistance to antibiotics. Therefore, effective alternative therapeutic agents are urgently required to avoid antibiotic resistance.

The microbial content of the milk and its products, especially, lactobacilli, are generally recognized as safe (GRAS) for animal and human health care applications [9]. Lactobacilli which are abundantly present in cow milk have several medical importance as efficient biocontrol agents and significant broad-spectrum antibacterial against multidrug-resistant infections of plants, animals and human [9]. Consuming milk supplemented with lactobacilli reduces the symptoms of respiratory infections in infants attending municipal daycare centers [10]. In addition, studies have also indicated that taking lactobacilli orally reduces the symptoms of the common cold [11]. Moreover, using of beneficial bacteria, as over-the-counter (OTC) supplements, has been extensively studied as human health-promoting. Several studies referred to the improvement of the digestive system after the oral consumption of lactic acid probiotics [12]. Moreover, the probiotics' effect on the human microbiota has been previously observed in the oral cavity, to prevent the formation of dental caries *Streptococcus salivarius* K12 strain is used orally to control or treat pharyngeal infections in children and adults [13]. Yogurt is one of the most famous fermented products, its ingredients are known to humans. Regular consumption of yogurt made from pure cow's milk stimulates the growth and activity of beneficial microbes in the intestines [14]. In addition to Yogurt from cow's milk, goat's milk can also be an alternative for making yogurt. Compared to cow's milk, goat's milk is a highly nutritional product composed of proteins, lipids, sugars, and vitamins, so it is an ideal growth medium for various microorganisms [15]. Probiotics in

yogurt milk are capable of producing antimicrobial compounds including; organic acids, diacetyl, and bacteriocins. These compounds possibly play a major role in preventing the growth of pathogenic bacteria and controlling bacterial infections, including tonsillitis and pharyngitis [16].

This study comes to evaluate antibiotic susceptibility of *S. pyogenes* and *S. aureus* isolated from tonsillitis and pharyngitis, In addition, to investigate in vitro the nature of interactions between the probiotic, lactobacilli and bacilli, isolated milk products with pathogenic bacterial isolates. Bacterial interaction (co-aggregation) indicates probiotics' capability to compete with pathogenic bacteria on the site and nutritional sources, and eventually, prevent colonization and infection establishment

## Methods

### **Samples collection**

In this study, 20 samples of milk products were collected from various local stores in order to isolate the lactic acid bacteria growing on these products; cream, yogurt, and cheese. 1 mL of each sample was prepared in sterile phosphate-buffered saline (PBS) and inoculated into 9 mL of de Man, Rogosa, and Sharpe (MRS) broth (Becton Dickinson and Company, Sparks, MD, USA) for LAB growth and maintenance. In addition, 25 throat swabs were collected from patients with tonsillitis and pharyngitis. These swabs were inoculated onto enrichment culture media, including brain-heart infusion (BHI) medium (HiMedia, Mumbai, India) and blood agar (HiMedia, Mumbai, India) supplemented with 5% sheep blood.

### **Bacterial growth and identification**

Regarding milk product samples, they were inoculated into MRS broth and incubated for 48 hours at 37 °C at 10% CO<sub>2</sub>, using an anaerobic jar. Pure culture methods were used to isolate each colony type, lactobacilli, and bacilli, separately. The Initial diagnostic investigations were performed using Gram staining techniques, catalase, urease, oxidase, citrate, esculin hydrolysis, arginine hydrolysis, IMViC, and TSI. Then, the VITEK 2 compact system (BioMerieux, Craaponne, France) was used to confirm lactobacilli and bacilli species identification. Throat swabs were inoculated into BHI agar, sheep blood agar, and chocolate agar. Then, the agar plates were incubated for 48 hrs at 37 °C and 10% CO<sub>2</sub>. Pure culture methods were used to isolate each colony type,

staphylococci and streptococci, separately based on their colony morphology. Streptococcus species were isolated on sheep blood agar while Staphylococcus species were isolated onto mannitol salt agar, as a differential and selective culture medium. Gram staining, catalase, and Streptex™ Latex Agglutination Test (Thermo Scientific™, Waltham, MA, USA) were applied to initially identify the pathogenic bacterial species. Then, the VITEK 2 system was used to confirm bacterial species causing tonsillitis and pharyngitis.

### **Ethical consideration**

The throat swab samples were collected from patients based on the instructions of the Institutional Ethical Clearance Committee No.12367 on 22 September 2020. These samples were taken, processed, and stored following the Guiding Principles for Ethical Research issued by the University of Diyala, Baqubah, Iraq.

### **Antibiogram of the pathogenic bacteria isolated from tonsillopharyngitis patients**

Based on the Clinical and Laboratory Standards Institute (CLSI) [17], the Kirby-Bauer method was used to evaluate antibiotic susceptibility to the five tested antibiotics discs; penicillin G 10 IU, amoxicillin 30mg, cefotaxime 30mg, cefotazidim 30mg, and vancomycin 30mg based on the recommendation of local physicians. The bacterial growth in BHI broth was diluted into Muller-Hinton (MH) broth and justified to McFarland No. 0.5. Then, 100 µl of bacterial dilution (approximately  $5 \times 10^5$  CFU/ml) was streaked and overloaded on the surface of MH agar. Then, the selected antibiotic discs were applied onto the surface of the MH agar, previously inoculated with the bacterial cells. After 24 hrs incubation at 37 °C under 10% CO<sub>2</sub> condition, the zones of bacterial growth inhibition (around the discs) were measured by millimeter. The susceptibility/resistance to the tested antibiotics was expressed based on clinical and laboratory standards institute guidelines [17].

### **Auto-aggregation and co-aggregation**

This assay was performed following the method described by Al-Dulaimi et al. [18] with minor modifications in order to evaluate the co-aggregation capability of milk product isolates; Bacillus subtilis and Lactobacillus plantarum with the most bacterial etiology of tonsillitis and pharyngitis. Both bacterial isolates (beneficial and pathogens) were grown into BHI broth for 24-36 hrs at 37°C. Then, bacterial cells were precipitated

by centrifugation using 4480g for 15 minutes at 23°C. The precipitated cells were washed with PBS, Thrice. The washed-harvested cells were re-suspended in PBS and their optical density (OD<sub>600nm</sub>) was adjusted to 0.2 using a microplate reader (Molecular Diagnostics, Sunnyvale, CA, USA). For the co-aggregation assay, 100 µl of each tested pathogenic bacteria (*S. pyogenes* or *S. aureus*, separately) was mixed with 100 µl of each species of *B. subtilis* or *L. palntarum* into a 96-wells microplate. For auto-aggregation, 200 µl of the single bacterial species suspension was added, individually, to a 96-wells microplate. The microplate was incubated in the incubator at 37°C, and a drop (5 µl) was taken from each well, during different periods of incubation (0, 2, 24 hours). The drop was stained following the Gram staining procedure to examine the strength of the aggregation that occurs between *Bacillus*/*Lactobacillus* species and the isolated pathogenic bacteria using a light microscope.

#### **Light microscope for co-aggregation identification**

Bacterial co-aggregation on slides was monitored after 0, 2, and 24 hours, using an optical microscope, after the slides were stained with Gram stain. Images were pictured using a NIS- Elements D3.0 software microscope-mounted camera (Kopacam, China) under 100 x (oil immersion lens). The quantity of aggregation was analyzed using a light microscope (Roth GmbH & Co. KG, Karlsruhe, Germany), and recorded using a scoring system: 0 being no classification of aggregation and 4 being an abundance of aggregation. The results analysis was based on Al-Dulaimi et al. [18] method.

#### **Statistical analysis**

In this study, Microsoft Excel and the «ggplot2» R package were used, performing the student T-test to analyze the effect of antibiotics on bacterial growth and bacterial co-aggregation. P-values <0.05 and ≤ 0.001 were considered statistically significant

## **Result and Discussion**

In this study, 20 samples of milk products were collected in order to isolate the LAB, lactobacilli, and bacilli species. In addition, 25 throat swabs were taken in order to isolate the most common pathogenic bacteria causing tonsillitis and pharyngitis. It has been found that only 4 (20%) samples show no bacterial growth. While 16 (80%) samples showed growth of both; bacilli (5 (25%)) and lactobacilli species (11(55%)), based on the initial tests: Gram staining, catalase, urease, oxidase, citrate utilization,

esculin hydrolysis, arginine hydrolysis, IMViC and TSI (Figure 1A). Regarding pathogenic bacteria causing sore throat infection, 15 (60%) were streptococci, and 6 (24%) were staphylococci, while 4 (18%) isolates were other microbial growth, based on the phenotypic features and the initial biochemical tests (Figure 1B). The VITEK 2 compact system confirmed that 3 (60%) of bacilli isolates were identified as *Bacillus subtilis* while 8 (72.7%) of lactobacilli isolates were *Lactobacillus plantarum*. On the other hand, 11 (73.3%) of isolated streptococci were *S. pyogenes* and 6 (100%) of staphylococci were *S. aureus* (Figure 1).

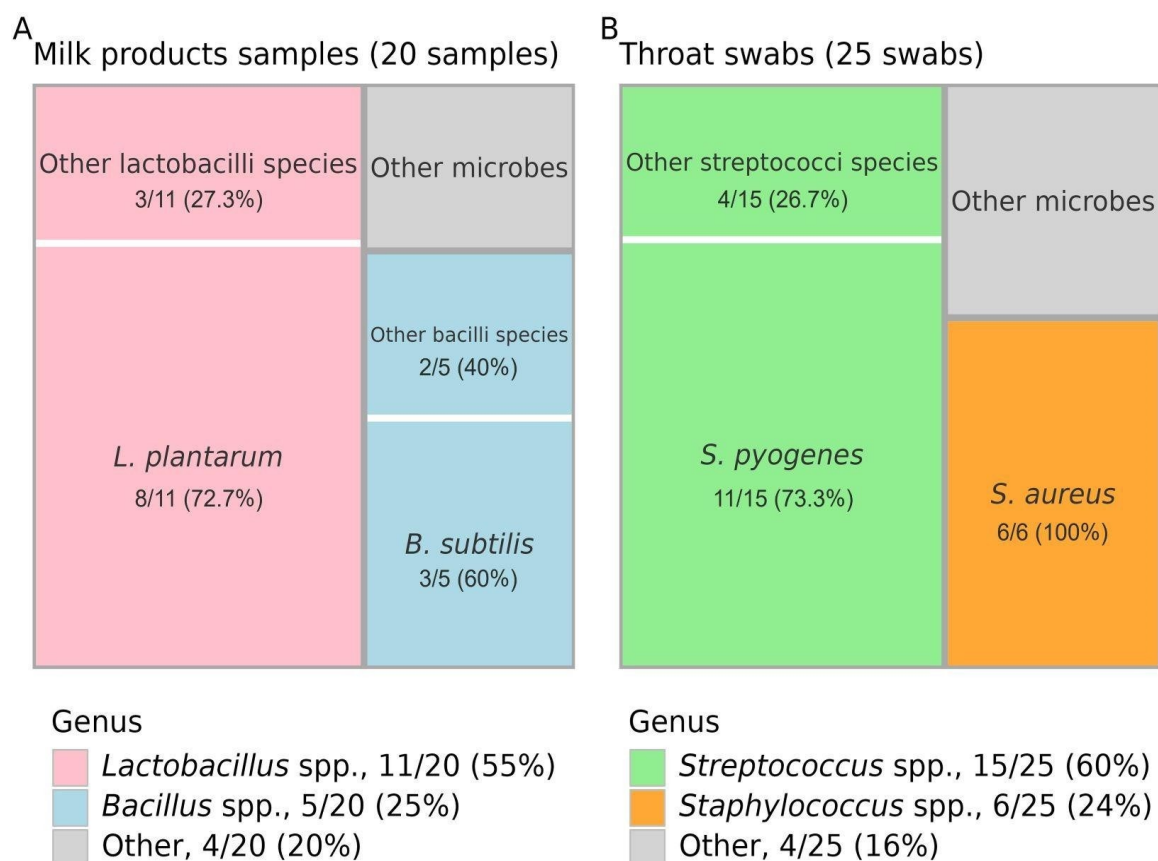


Figure 1: The number and percentages of *Bacillus*, *Lactobacillus* species isolated from milk products samples (A) and *Streptococcus* and *Staphylococcus* species isolated from tonsillopharyngitis patients (B).

In this study, five types of antibiotics were applied. The data of this study noticed that 81.8% of *S. pyogenes* were resistant to both; pencillin G 10IU and amoxicillin 30mg,



while 63.3% were resistant to cefotaxime 30mg and ceftazidime 30mg. Streptococcal resistance to vancomycin was very low, 9.1% compared to the other tested antibiotics. In regard to *S. aureus*, 83.3% of bacterial species were resistant to amoxicillin 30mg and penicillin G10 IU. In addition, 50% of the tested *S. aureus* were resistant to ceftazidime 30mg, while only 16.6% were resistant to vancomycin 30 mg (Table 1). No significant difference was identified in the bacterial resistance to antibiotics between the two isolated species, P value=0.25.

Table 1: Antibiotic susceptibility of bacterial species isolated from tonsillopharyngitis patients.

Antibiotics	Bacterial Species	
	<i>S. pyogenes</i> Resistance (%)	<i>S. aureus</i> Resistance (%)
Pencillen G	81.8%	83.4%
Amoxicillin	81.8%	83.4%
Cefotaxime	63.3%	50%
Cefotazidim	63.3%	50%
Vancomycin	9.1%	16.6%
P value=0.25		

The bacterial aggregation was conducted in order to investigate the nature of bacterial interactions of beneficial bacteria with pathogenic bacterial isolates. This study found that bacterial isolates were highly auto-aggregated among the same species (Figures 2, 3, and 4). Moreover, the highest co-aggregation scores were observed when the following bacterial interactions were made; *L. plantarum* + *S. pyogenes* and *B. subtilis* + *S. aureus*, the co-aggregation score was +4 (Figures 5 and 6, respectively), compared to the other bacterial combinations (Table 2). A significant difference was observed in bacterial co-aggregation between 2 and 24-hour incubation, P value = 0.0008.



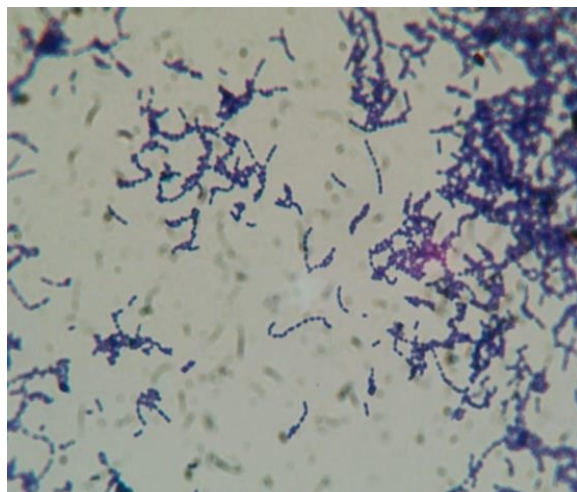


Figure : Auto-aggregation of *S. pyogenes*.

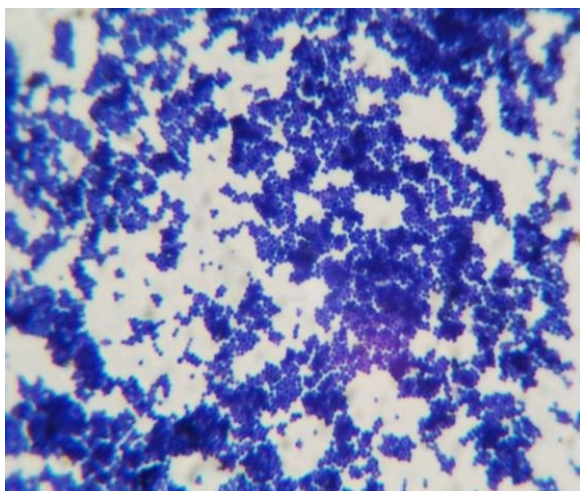


Figure 3: Auto-aggregation of *S. aureus*.

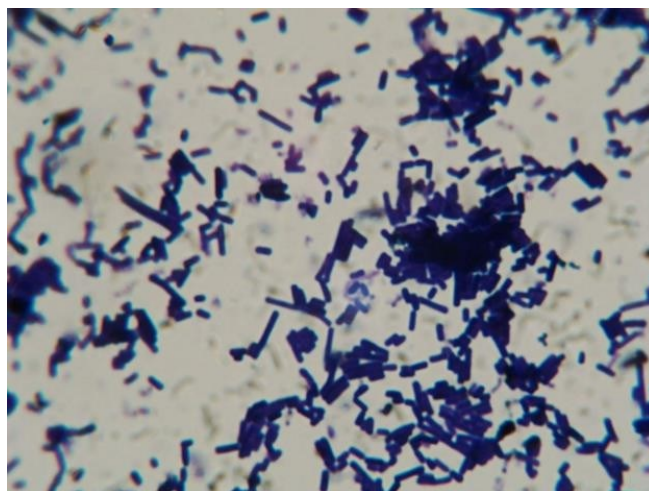


Figure 4: Auto-aggregation of *Bacillus* species

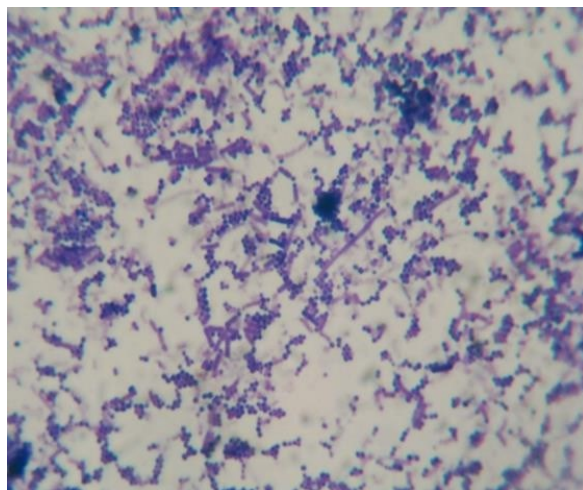
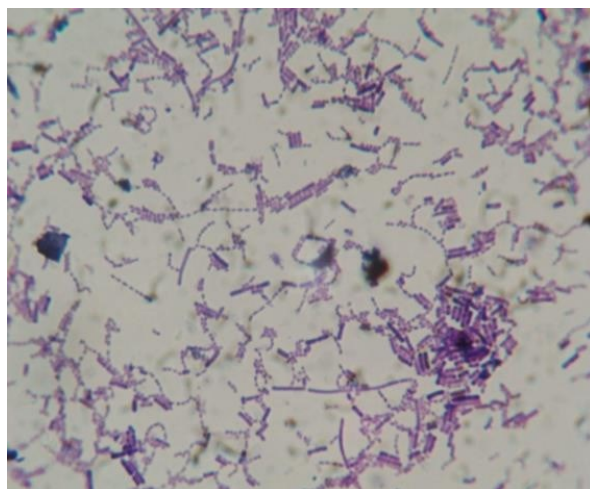
Figure 5: Co-aggregation of *B. subtilis* with *S. aureus* (+4).Figure 6: Co-aggregation of *L. plantarum* with *S. pyogenes* (+4).

Table 2: Co-aggregation score of the tested bacterial species

Bacterial species	Co-aggregation score after 2 hours	Co-aggregation score after 24 hours
<i>L. plantarum</i> + <i>S. pyogenes</i>	+1	+4
<i>L. plantarum</i> + <i>S. aureus</i>	0	+3
<i>B. subtilis</i> + <i>S. pyogenes</i>	+1	+3
<i>B. subtilis</i> + <i>S. aureus</i>	+1	+4
P value = 0.0008		

## Discussion

Probiotics that were commonly prescribed from dairy products were consumed without harmful side effects, for a long time [19]. They are generally recognized as safe (GRAS) status microbes that contribute to human health [20]. Probiotics, as normal flora that inhabit different parts of the human body, could prevent infections, stimulating the immune system, reducing cholesterol and the risk of cancer [21]. Probiotic bacteria are known to produce lactic acid and organic acids, lower the pH environment, and produce antimicrobial compounds such as bacteriocins [22].

Out of 20 samples of milk products collected in this study, 3 (60%) isolates of *Bacillus subtilis* and 8 (72.7%) isolates of *Lactobacillus plantarum* were identified using VITECK system 2. In regards to percentages of probiotics isolation from the milk products. Fguiri et al. [23] noticed that *L. plantarum* is predominantly isolated from the test milk products, compared to the other lactobacilli isolates, using initial biochemical tests and API galleries. Some studies referred to the isolation of *L. plantarum* in Sudanese fermented camel milk [24]. In addition, Sun et al. [25] reported the presence of *L. plantarum* in the traditional fermented milk in Mongolia.

*Bacillus* isolates, including *B. subtilis*, is a spore-forming bacteria used as a probiotic in fermented foods, including milk products [26]. Our findings are in agreement with Lee et al. [27] who confirmed *B. subtilis* and *L. plantarum* isolation from the milk products. The authors noticed that viable count of *B. subtilis* was 6.80 log CFU/mL, and increased after 1 day to 8.74 log CFU/mL. Moreover, an increase of the viable cell counts of *L. plantarum* was noticed; it increased from 7.61 log CFU/mL to 9.06 log CFU/mL. It is worth mentioning that *Bacillus* strains are potential probiotics due to their thermostable and acid-tolerant features [28], *Bacillus* species are valuable bacteria in the fermented food producing legume-based fermented foods and poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA), hydrolytic enzymes, and peptides [29]. *L. plantarum* in some fermented milk products, such as yogurt, kefir, sauerkraut, and kimchi show glutamate decarboxylase activity, that required for the production of gamma-aminobutyric acid [30].

In regards to the isolation of *S. pyogenes* and *S. aureus* from patients with tonsillitis and pharyngitis, several published studies reported that multiple bacterial species could participate in initiation tonsillitis and pharyngitis, predominately, *S. pyogenes* and *S. aureus* [31]. Our findings were close to Kebede et al. [32] who found

that the prevalence of *S. pyogenes* isolation was 95% of the total throat swabs (154 swabs). In addition, the isolation of *S. pyogenes* in this study was comparable with the studies conducted in Ethiopia, India, Japan, Indonesia and Nepal [32]. Moreover, Fahad found that the isolation percentage of *S. pyogenes* (88.6 %) was more than *S. aureus* (9.6 %) [33]. Results of Timon, et al. [34] study was, also, in agreement with our findings that *S. aureus*, is predominant and the second to *S. pyogenes* causing tonsils and pharynx infection. Darod et al. [35] found that *S. pyogenes* was the most frequently bacterial isolate 55% while *S. aureus* was 29% the second causative agent of bacterial tonsillitis. However, this study disagrees with an Egyptian study by Heusseina et al. [36] who found that the isolation percentages were as follows: *S. aureus* (40%) and *S. pyogenes* (35%), out of 35 throat swab samples. In addition, Babaiwa et al. from Nigeria [37] reported that the most common bacterial species isolated from tonsillitis was *S. aureus*, followed by *S. pyogenes*. An Iraqi study of Bakir and Ali [38] found that *S. aureus* isolated from tonsillitis at the rate of (30.5%). It has been found that *S. pyogenes* causes sore throat cases in > 37% of all diagnosed, and in children more than in adults [39]. Agrawal et al. [40] noticed that *S. pyogenes* isolated from patients with chronic tonsillitis represent 2.14%, compared to the other bacterial species. The difference in bacterial species prevalence data is related to the difference in the size of samples, variation in the experimental designs and conditions, in addition to the techniques used in these studies for bacterial identification.

The data published by the Centers for Disease Control and Prevention (CDC) showed that antibiotic resistance is associated with 2 million infections, more than 20 thousand deaths, and costs 55 billion dollars, every year in the USA [41]. In this study, tonsillitis/pharyngitis-associated bacteria were resistant to three or more tested antibiotics. We found that most of the isolated *S. pyogenes* and *S. aureus* were resistant to penicillin G10 and amoxicillin 30mg. Also, Most of the isolates showed a high resistance to cephalosporin groups (cefotaxime and ceftazidime), but, low resistance % were reported to vancomycin. Several published works were in line with our findings. Babaiwa et al. [37] in their data referred to the sensitivity percentages of *S. pyogenes* and *S. aureus* to some tested antibiotics, including amoxicillin. They found that 100% of both bacterial isolates were resistant to amoxicillin. In addition, the data from Fahad's study was close to our findings, the author found that 86.6% of *S. aureus*, but 25.6 %



of *S. pyogenes*, isolated from tonsillitis were resistant to penicillin [33]. A study of Pichichero [42], explained that penicillin therapy was not effective in the treatment of streptococcal pharyngitis compared to using the cephalosporin group, which was more effective. However, some studies disagree with our results, in regards to the sensitivity of *S. pyogenes* to penicillin. For example, Skoog Ståhlgren et al. (43) noticed that when penicillin V was given 4 times a day for 5 days, it was considered an effective alternative antibiotic for patients with pharyngotonsillitis caused by group A streptococci. Some publications recommended penicillin as the best choice for controlling streptococcal tonsillitis because of its low cost and the rare cases of bacterial resistance [44]. However, it is important to mention that in patients with an IgE-mediated penicillin allergy, beta-lactam antibiotics should be taken with caution to avoid the risk of anaphylaxis [45].

This study was in agreement also with the report of Heussien et al. [36] who found that 85.71% and 81.25% of *S. aureus* and *S. pyogenes*, respectively isolated from tonsillitis were resistant to amoxicillin. Moreover, the authors reported that 50-62.5% of *S. aureus*, but 70-85.71% of *S. pyogenes* were resistant to the selected cephalosporin group [36]. Darod et al. [35] reported that the tested bacteria revealed an 83.3–100 % rate of resistance to ampicillin, in addition, the overall percentages of multidrug resistance were 50.4% and 52.6% of *S. aureus* and *S. pyogenes*, respectively. Regarding cephalosporin resistance, our findings disagreed with Fahad [33] who found that all the tonsillitis isolates were sensitive to cephalosporin. In addition, Fahad mentioned that cephalosporin was more effective compared to other tested antibiotics. In the same regard, Berwal et al. [46] found that out of 48 *S. pyogenes* isolates isolated from oral infection, 46 (95.8%) were sensitive to cefotaxime.

In regards to vancomycin, the data of many recently published studies were close to our findings. The study of Darod et al. [35] found that only 12.8 % of *S. pyogenes* and 33.3% of *S. aureus* were resistant to vancomycin. A low resistance rate was reported for vancomycin, 13.8 and 13.3% of both *S. pyogenes* and *S. aureus*, respectively [45]. Also, Kebede et al. [32] reported that 35.5% of *S. pyogenes* were resistant to vancomycin, furthermore, Anja et al. [47] noticed that 25.7% of *S. pyogenes* isolates from tonsillitis were resistant to vancomycin.

The bacterial isolates resistance to the mentioned antibiotics is attributed to the various mechanisms of actions related to bacterial enzymatic inactivation, such as beta-

lactamase production and those mediated by aminoglycoside-modifying enzymes (AMEs), in addition to the mutations of quinolones resistance-determining region (QRDR) [35]. Despite their extraordinary value and their continued effectiveness, bacterial resistance to antibiotics was described in several publications. The failure of antibiotic treatment could be attributed to a lack of compliance, in addition to the misuse and overuse of antimicrobial agents. It is important to mention that the more antibiotics are taken, the more bacterial resistance mechanisms will develop to evade them [47].

Probiotic lactobacilli and bacilli exhibit ecological and metabolic adaptability. They are isolated from various environmental niches, such as fermented foods, meats, plants, and the gastrointestinal tract [28]. The advantages of probiotics include; (i) natural restoration of health making them a suitable goal in medicinal science, (ii) encounter of antibiotic resistance, and (iii) competition with the pathogenic bacteria on the site of colonization, preventing several bacterial infections [28,29]. This study comes to explore their activity, through their co-aggregation capability with the most oral-pharyngeal pathogens, *S. pyogenes* and *S. aureus* and to initially investigate the mechanisms behind their antimicrobial activity.

This study observed that bacterial isolates were highly auto-aggregated (among the same species) (Figures 2, 3, and 4). Our data found that the highest co-aggregation scores were observed (+4) when *L. plantarum* + *S. pyogenes* and *B. subtilis* + *S. aureus* were mixed (Figure 5 and 6, respectively, and Table 2). Our findings were close to Algburi et al. [48] who mentioned that the percentages of co-aggregation of probiotic bacilli with the tested pathogenic bacteria, after 24 hours of incubation were higher in comparison to 4 hours. The authors reported high levels of co-aggregation of *P. mirabilis* isolates from urinary tract infections, with *B. amyloliquefaciens* B-1895 and *B. subtilis* KATMIRA1933 after 24 hours of incubation.

Co-aggregation is a desirable feature of probiotic microbes when two or more genetically different bacteria are aggregated and competed on the attached surfaces, and nutritional sources [49]. Co-aggregation capability of beneficial organisms may also help the bacterium adhesion to different surfaces, which eventually creates a barrier that effectively inhibits colonization and biofilm formation of pathogenic bacteria [50]. The auto-aggregation of beneficial strains plays an essential role in their adhesion to the epithelial cells, keeping them from being flushed outside our body [50]. Further and

deep research works are required to investigate the complex aspects of auto-aggregation and co-aggregation, therefore, not all mechanisms of action have been demonstrated.

## Conclusion

the present study revealed that there is a considerable rate of tonsillitis/pharyngitis caused by multidrug-resistant bacteria, predominately *S. pyogenes* followed by *S. aureus*. These bacterial etiology are resistant to commonly used antibiotics and could have a potential risk of rheumatic fever and rheumatic heart disease. Antibiotic resistance and infection recurrence should attract researchers to investigate safe, alternative, and broad-spectrum antibacterial agents to effectively encounter antibiotic resistance and rational use of antimicrobials. In this study, the authors shed lights on the importance of milk products as enrichment source of probiotic lactobacilli and how to engaging these beneficial bacteria as a suggested bio-strategy in the precaution and controlling bacterial tonsillopharyngitis. Competition of beneficial bacteria, through co-aggregation, on the site of attachment may inhibit the pathogenic bacterial colonization on the tonsillar tissue, and eventually, prevent infection. Furthermore, bacterial co-aggregation may increase the opportunity to inhibit pathogenic bacteria and flush them out of the host immune system. Further investigation in vitro and in vivo is required to demonstrate the antibacterial activity of the postbiotics; enzymes, organic acids, and bacteriocins produced by probiotics, in terms of therapeutics, against tonsillopharyngitis-associated bacteria

## Conflict Of Interests

No conflict of interests was declared by the authors.

## Funding Source

The authors did not receive any source of fund.

## Data Sharing

statement Supplementary data can be shared with the corresponding author upon reasonable request.



## Acknowledgements

The authors would like to thank the medical staff and laboratory of Baqubah Teching Hospital for their support and helps in collecting throat swabs and bacterial isolation/ identification.

## Conflict Of Interest

There are no conflicts of interest

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