Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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

Rana Hussein Naser[1], Ammar Riyadh Algburi[2] , Igor Popov[3,4,5]
[1] Department of Science, College of Basic Education, University of Diyala, 32001Baqubah, Iraq ;
[2] Department of Microbiology, College of Veterinary Medicine, University of Diyala, 32001Baqubah, Iraq
[3] Bioengineering and Veterinary Medicine" and Center for Agrobiotechnology, Don State Technical University, 344000 Rostov-on-Don, Russia
[4] Division of Immunobiology and Biomedicine, Center of Genetics and Life Sciences, Sirius University of Science and Technology, 354340 Federal Territory Sirius, Russia, 5Centre for Healthy Eating & Food Innovation (HEFI), Maastricht University—Campus Venlo, 5928 SZ Venlo, The Netherlands

Email: ranaalqaysi@gmail.com, ammar.algburi@gmail.com, sc_ammaralgburi@uodiyala.edu.iq, doc.igor.popov@gmail.com

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 subtillus 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.

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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]. Tonsilitis 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].

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 ºC at 10% CO2, 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, Craponne, 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 ºC and 10% CO2. Pure culture methods were used to isolate each colony type,

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 5×105 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 ^{0}C under 10% CO2 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

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 (OD600nm) 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,

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 subtillus 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. aurues (Figure 1).

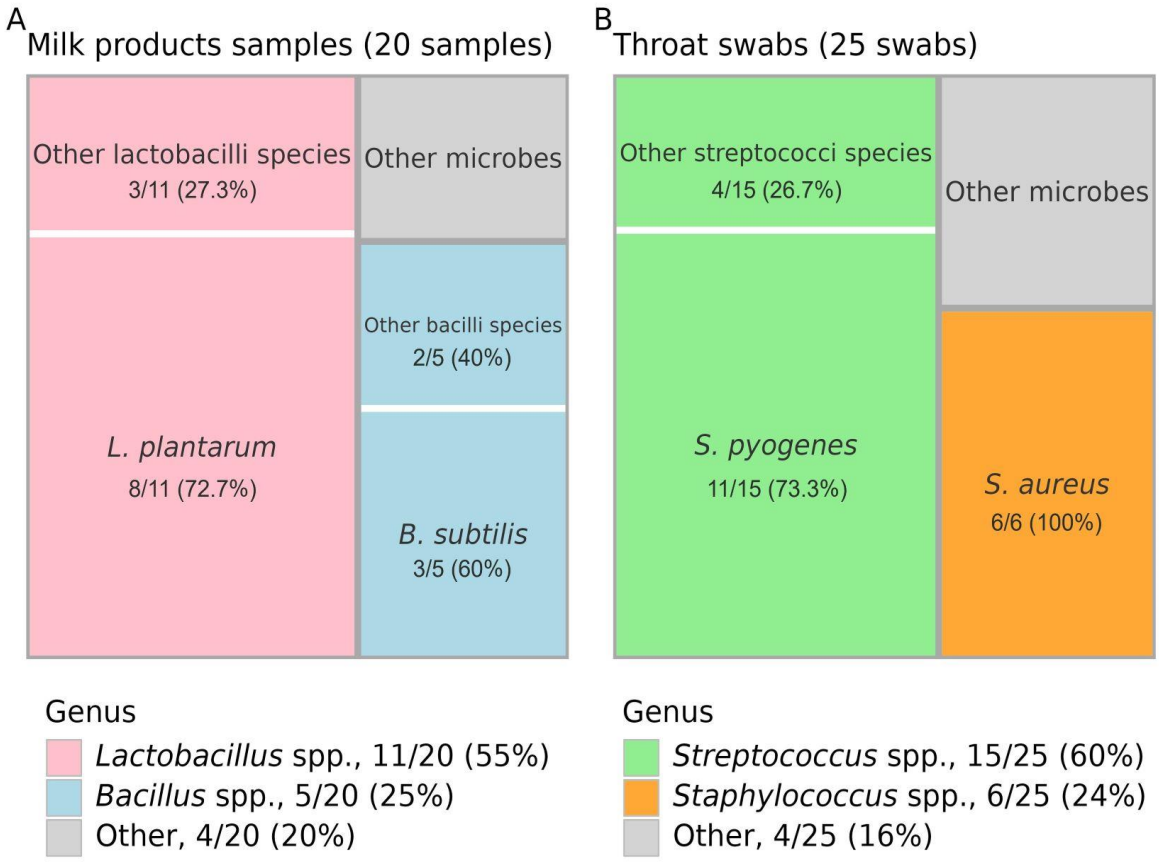

Figure 1: The number and percentages of Bacillus, Lactobacillus species isolated from milk products samples (A) and Streptococcus and Staphylococcus species isolated from tonsillopharyngitis patients (B).

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,

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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**	
	S. pyogenes **Resistance (%)**	***S. aureus*** **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. subtillus + 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.

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

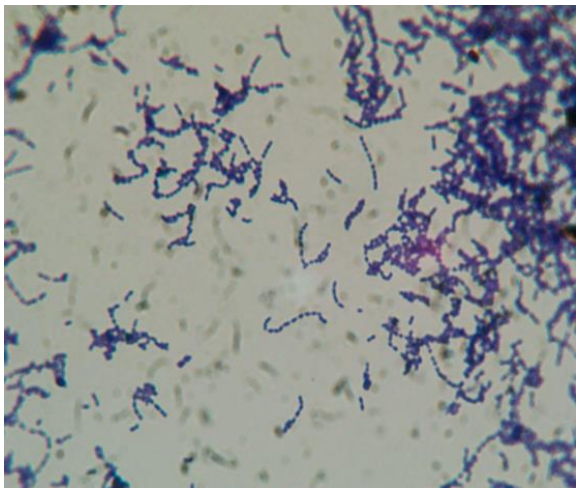

Figure : Auto-aggregation of S. pyogens.

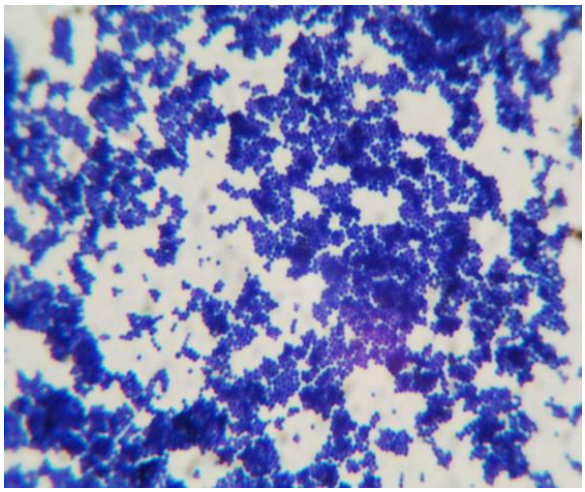

Figure 3: Auto-aggregation of S. aureus.

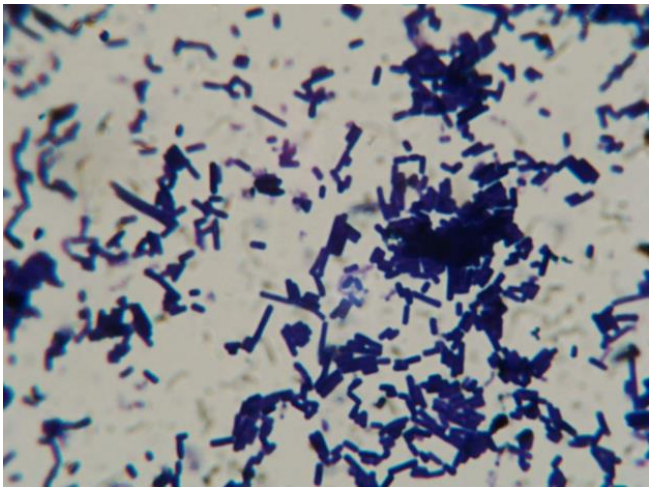

Figure 4: Auto-aggregation of Bacillus species

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

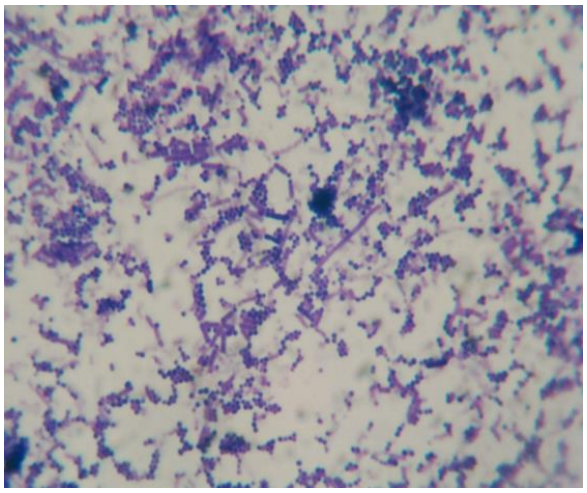

Figure 5: Co-aggregation of B. subtilis with S. aureus (+4).

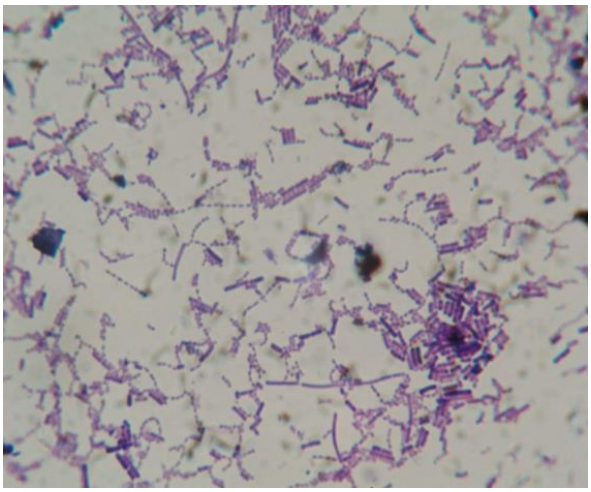

Figure 6: Co-aggregation of L. plantarum with S. pyogenes (+4).

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

Bacterial species	Co-aggregation score after 2 hours	Co-aggregation score after 24 hours
L. plantarum + *S.pyogenes*	+1	+4
L. plantarum + *S.aureus*	0	+3
B. subtillus + *S. pyogens*	+1	+3
B. subtillus + *S. aureus*	+1	+4
P value = 0.0008		

Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): April
ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 habitat 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 subtillus 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-foaming 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-γ-glutamic acid (γ-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

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 %

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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. pyogens 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 tonsilitis 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-

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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. subtillus + 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 athors 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

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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 enrichement 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 flash 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.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

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

References

[1] J. L. Evans, "Think Outside the Gut: Probiotics for Oral Health," Agro Food Industry Hi Tec, vol. 27, no. 3, pp. 48-53, 2016.

[2] S. M. Schappert and E. A. Rechsteiner, "Ambulatory Medical Care Utilization Estimates for 2006," National Health Statistics Reports, no. 8, pp. 1-32, 2008.

[3] S. T. Shulman, A. L. Bisno, H. W. Clegg, M. A. Gerber, E. L. Kaplan, G. Lee, et al., "Clinical Practice Guideline for the Diagnosis and Management of Group A Streptococcal Pharyngitis: 2012 Update by the Infectious Diseases Society of America," Clinical Infectious Diseases, vol. 55, no. 10, pp. e86-e102, 2012.

[4] L. K. Kociolek and S. T. Shulman, "In the Clinic: Pharyngitis," Annals of Internal Medicine, vol. 157, no. 5, pp. ITC3-1-ITC3-16, 2012.

[5] P. Varosy and T. Newman, "Acute Pharyngitis," The New England Journal of Medicine, vol. 344, no. 19, p. 1479, 2001.

[6] A. E. Zautner, M. Krause, G. Stropahl, S. Holtfreter, H. Frickmann, C. Maletzki, et al., "Intracellular Persisting Staphylococcus Aureus is the Major Pathogen in Recurrent Tonsillitis," PLOS One, vol. 5, no. 3, p. e9452, 2010.

[7] V. P. Cavalcanti, L. A. Camargo, F. S. Moura, E. J. D. Melo Fernandes, J. Lamaro-Cardoso, C. Braga, et al., "Staphylococcus Aureus in Tonsils of Patients with Recurrent Tonsillitis: Prevalence, Susceptibility Profile, and Genotypic Characterization," Brazilian Journal of Infectious Diseases, vol. 23, pp. 8-14, 2019.

[8] L. Thomer, C. Emolo, V. Thammavongsa, H. K. Kim, M. E. McAdow, W. Yu, et al., "Antibodies Against a Secreted Product of Staphylococcus Aureus Trigger

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

Phagocytic Killing," Journal of Experimental Medicine, vol. 213, no. 3, pp. 293-301, 2016.

[9] A. R. Costa, D. W. Batistão, R. M. Ribas, A. M. Sousa, M. O. Pereira, and C. M. Botelho, "Staphylococcus Aureus Virulence Factors and Disease," in Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education, Formatex Research Center, 2013, pp. 702-710.

[10] M. N. Asha, M. S. Chowdhury, H. Hossain, M. A. Rahman, A. A. Emon, F. Y. Tanni, et al., "Antibacterial Potential of Lactic Acid Bacteria Isolated from Raw Cow Milk in Sylhet District, Bangladesh: A Molecular Approach," Veterinary Medicine and Science, vol. 10, no. 3, p. e1463, 2024.

[11] G. Humphreys and A. McBain, "Antagonistic Effects of Streptococcus and Lactobacillus Probiotics in Pharyngeal Biofilms," Letters in Applied Microbiology, vol. 68, no. 4, pp. 303-312, 2019.

[12] A. Berggren, I. Lazou Ahrén, N. Larsson, and G. Önning, "Randomised, Double-Blind and Placebo-Controlled Study Using New Probiotic Lactobacilli for Strengthening the Body Immune Defence Against Viral Infections," European Journal of Nutrition, vol. 50, pp. 203-210, 2011.

[13] M. G. Cagetti, S. Mastroberardino, E. Milia, F. Cocco, P. Lingström, and G. Campus, "The Use of Probiotic Strains in Caries Prevention: A Systematic Review," Nutrients, vol. 5, no. 7, pp. 2530-2550, 2013.

[14] E. M. Fakri, S. Lim, N. Musa, M. H. Hasan, A. Adam, and K. Ramasamy, "Lactobacillus Fermentum LAB 9-Fermented Soymilk with Enriched Isoflavones and Antioxidants Improved Memory in Vivo," Journal of Soybean Medicine, vol. 45, no. 9, pp. 1289-1297, 2016.

[15] G. Haenlein, "Goat Milk in Human Nutrition," Small Ruminant Research, vol. 51, no. 2, pp. 155-163, 2004. doi:10.1016/j.smallrumres.2003.08.010.

[16] B. González-Alzaga, M. Lacasaña, C. Aguilar-Garduño, M. Rodríguez-Barranco, F. Ballester, M. Rebagliato, et al., "A Systematic Review of Neurodevelopmental Effects of Prenatal and Postnatal Organophosphate Pesticide Exposure," Toxicology Letters, vol. 230, no. 2, pp. 104-121, 2014.

[17] Clinical and Laboratory Standards Institute (CLSI), "M100 Performance Standards for Antimicrobial Susceptibility Testing," 31st ed., CLSI, 2021, p. 352.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

[18] M. Al-Dulaimi, A. Algburi, A. Abdelhameed, M. S. Mazanko, D. V. Rudoy, A. M. Ermakov, et al., "Antimicrobial and Anti-Biofilm Activity of Polymyxin E Alone and in Combination with Probiotic Strains of Bacillus Subtilis Katmira1933 and Bacillus Amyloliquefaciens B-1895 Against Clinical Isolates of Selected Acinetobacter spp.: A Preliminary Study," Pathogens, vol. 10, no. 12, p. 1574, 2021.

[19] M. Aspri, P. Papademas, and D. Tsaltas, "Review on Non-Dairy Probiotics and Their Use in Non-Dairy Based Products," Fermentation, vol. 6, no. 1, p. 30, 2020.

[20] Y. Widyastuti and A. Febrisiantosa, "The Role of Lactic Acid Bacteria in Milk Fermentation," Food and Nutrition Sciences, vol. 5, no. 4, pp. 8-12, 2014.

[21] S. Karami, M. Roayaei, H. Hamzavi, M. Bahmani, H. Hassanzad-Azar, L. M., et al., "Isolation and Identification of Probiotic Lactobacillus from Local Dairy and Evaluating Their Antagonistic Effect on Pathogens," International Journal of Pharmaceutical Investigation, vol. 7, no. 3, pp. 137-141, 2017.

[22] A. A. Aroutcheva, J. A. Simoes, and S. Faro, "Antimicrobial Protein Produced by Vaginal Lactobacillus acidophilus That Inhibits Gardnerella vaginalis," Infectious Diseases in Obstetrics and Gynecology, vol. 9, pp. 33-39, 2001.

[23] I. Fguiri, M. Ziadi, M. Atigui, N. Ayeb, S. Arroum, M. Assadi, et al., "Isolation and Characterisation of Lactic Acid Bacteria Strains from Raw Camel Milk for Potential Use in the Production of Fermented Tunisian Dairy Products," International Journal of Dairy Technology, vol. 69, no. 1, pp. 103-113, 2016.

[24] A. Ashmaig, A. Hasan, and E. El Gaali, "Identification of Lactic Acid Bacteria Isolated from Traditional Sudanese Fermented Camel's Milk (Gariss)," African Journal of Microbiology Research, vol. 3, no. 8, pp. 451-457, 2009.

[25] Z. Sun, W. Liu, J. Zhang, J. Yu, W. Gao, J. Miri, et al., "Identification and Characterization of the Dominant Lactic Acid Bacteria Isolated from Traditional Fermented Milk in Mongolia," Folia Microbiologica, vol. 55, pp. 270-276, 2010.

[26] S. Sudan, R. Flick, L. Nong, and J. Li, "Potential Probiotic Bacillus subtilis Isolated from a Novel Niche Exhibits Broad-Range Antibacterial Activity and Causes Virulence and Metabolic Dysregulation in Enterotoxic E. coli," Microorganisms, vol. 9, no. 7, p. 1483, 2021.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

[27] S.-P. Lee, W.-K. Yoon, J. Lee, and C. V. Garcia, "Fermented Milk Product Enriched with γ-PGA, Peptides, and GABA by Novel Co-Fermentation with Bacillus subtilis and Lactiplantibacillus plantarum," Fermentation, vol. 8, no. 8, p. 404, 2022.

[28] D. Ramesh, S. Souissi, and T. S. Ahamed, "Effects of the Potential Probiotics Bacillus aerophilus KADR3 in Inducing Immunity and Disease Resistance in Labeo rohita," Fish & Shellfish Immunology, vol. 70, pp. 408-415, 2017.

[29] B. Halmschlag, S. P. Putri, E. Fukusaki, and L. M. Blank, "Identification of Key Metabolites in Poly-γ-Glutamic Acid Production by Tuning γ-PGA Synthetase Expression," Frontiers in Bioengineering and Biotechnology, vol. 8, p. 38, 2020.

[30] D. Das and A. Goyal, "Antioxidant Activity and γ-Aminobutyric Acid (GABA) Producing Ability of Probiotic Lactobacillus plantarum DM5 Isolated from Marcha of Sikkim," LWT - Food Science and Technology, vol. 61, no. 1, pp. 263-268, 2015.

[31] M. R. Wessels, "Pharyngitis and Scarlet Fever," in Streptococcus pyogenes: Basic Biology to Clinical Manifestations, University of Oklahoma Health Sciences Center, Oklahoma City, OK, 2016.

[32] D. Kebede, A. Admas, and D. Mekonnen, "Prevalence and Antibiotic Susceptibility Profiles of Streptococcus pyogenes Among Pediatric Patients with Acute Pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia," BMC Microbiology, vol. 21, no. 1, p. 135, 2021.

[33] H. M. Fahad, "Types of Aerobic Bacteria Isolated from Iraqi Patients with Acute Tonsillitis and Their Susceptibility to Different Antibiotics," Journal of Pure and Applied Microbiology, vol. 12, no. 4, pp. 1855-1859, 2018.

[34] C. Timon, V. McAllister, M. Walsh, and M. Cafferkey, "Changes in Tonsillar Bacteriology of Recurrent Acute Tonsillitis: 1980 vs. 1989," Respiratory Medicine, vol. 84, no. 5, pp. 395-400, 1990.

[35] H. H. Darod, A. Melese, M. Kibret, and W. Mulu, "Bacterial Tonsillitis and Antimicrobial Resistance Profiles Among Children within Five Years of Age at Hargeisa Group of Hospital, Somaliland: A Cross-Sectional Study," 2021.

[36] Z. Heussien, N. Yousef, H. Temark, and A. Ebnawaled, "Isolation, Identification and Antibiogram Study of Pathogenic Bacteria Mediated Children Chronic Tonsillitis at Qena University Hospital, Egypt," SVU International Journal of Medical Sciences, vol. 3, no. 2, pp. 1-8, 2020.

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

[37] U. Babaiwa, N. Onyeagwara, and J. Akerele, "Bacterial Tonsillar Microbiota and Antibiogram in Recurrent Tonsillitis," Biomedical Research, vol. 24, no. 3, pp. 298-302, 2013.

[38] S. Bakir and F. Ali, "Evaluation of Multi-Drug Resistance and β-Lactamase Production in Throat Infected by Gram-Positive Bacteria," European Journal of Pharmaceutical Sciences, vol. 3, no. 2, pp. 68-76, 2016.

[39] N. M. Wijesundara and H. V. Rupasinghe, "Herbal Tea for the Management of Pharyngitis: Inhibition of Streptococcus pyogenes Growth and Biofilm Formation by Herbal Infusions," Biomedicines, vol. 7, no. 3, p. 63, 2019.

[40] A. Agrawal, D. Kumar, A. Goyal, R. Gupta, and S. Bhooshan, "Bacteriological Evaluation and Their Antibiotic Sensitivity Pattern in Tonsillitis," IOSR Journal of Dental and Medical Sciences, vol. 13, no. 3, pp. 51-55, 2014.

[41] A. Reta, A. B. Kifilie, and A. Mengist, "Bacterial Infections and Their Antibiotic Resistance Pattern in Ethiopia: A Systematic Review," Preventive Medicine, vol. 2019, p. 4380309, 2019.

[42] M. E. Pichichero, "Explanations and Therapies for Penicillin Failure in Streptococcal Pharyngitis," Clinical Pediatrics, vol. 31, pp. 642-649, 1992.

[43] G. S. Ståhlgren et al., "Penicillin V Four Times Daily for Five Days Versus Three Times Daily for 10 Days in Patients with Pharyngotonsillitis Caused by Group A Streptococci: Randomised Controlled, Open Label, Non-Inferiority Study," BMJ, vol. 367, p. l5337, 2019.

[44] K. Hedin, S. Thorning, and M. L. van Driel, "Different Antibiotic Treatments for Group A Streptococcal Pharyngitis," Cochrane Database of Systematic Reviews, vol. 3, no. 3, p. CD004406, 2021.

[45] R. S. Gruchalla and M. Pirmohamed, "Clinical Practice: Antibiotic Allergy," New England Journal of Medicine, vol. 354, no. 6, pp. 601-609, 2006.

[46] A. Berwal, K. Chawla, S. Shetty, and A. Gupta, "Trend of Antibiotic Susceptibility of Streptococcus Pyogenes Isolated from Respiratory Tract Infections in a Tertiary Care Hospital in South Karnataka," Iranian Journal of Medical Microbiology, vol. 11, no. 1, pp. 13-18, 2019.

[47] A. Anja, G. Beyene, Z. S/Mariam, and D. Daka, "Asymptomatic Pharyngeal Carriage Rate of Streptococcus Pyogenes, Its Associated Factors, and Antibiotic

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i2.97

Susceptibility Pattern Among School Children in Hawassa Town, Southern Ethiopia," BMC Research Notes, vol. 12, p. 564, 2019.

[48] A. Algburi, S. A. Alazzawi, A. I. A. Al-Ezzy, R. Weeks, V. Chistyakov, and M. L. Chikindas, "Potential Probiotics Bacillus Subtilis KATMIRA1933 and Bacillus Amyloliquefaciens B-1895 Co-Aggregate with Clinical Isolates of Proteus Mirabilis and Prevent Biofilm Formation," Probiotics and Antimicrobial Proteins, vol. 12, no. 4, pp. 1471-1483, 2020.

[49] R. G. Ledder, A. S. Timperley, M. K. Friswell, S. Macfarlane, and A. J. McBain, "Coaggregation Between and Among Human Intestinal and Oral Bacteria," FEMS Microbiology Ecology, vol. 66, no. 3, pp. 630-636, 2008.

[50] B. Kos, J. Šušković, S. Vuković, M. Šimpraga, J. Frece, and S. Matošić, "Adhesion and Aggregation Ability of Probiotic Strain Lactobacillus Acidophilus M92," Journal of Applied Microbiology, vol. 94, no. 6, pp. 981-987, 2003.