

Using Lactic Acid Bacteria as Probiotic Against Staphylococcus aureus Isolated from Different Sources in Erbil City

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Abstract. A total of 50 different samples were collected randomly from November 2021 to the end of February 2022 in Erbil city, In order to isolate different types of Staphylococcus which are isolated from different sources like (milk, cream, bakery products, poultry, hand worker). Number of testing that were used to identify the bacteria: mannitol fermentation, Gram staining, coagulase, hemolysis, catalase, Test results confirmed with the Vitik 2 compact system. Using probiotic Lactic Acid Bacteria agents staphylococcus aureus that the results have shown the LAB effect on S. aureus which is inhibition zone 5.3 cm , 5.2cm, 5.2cm, 5.1cm, 5.1cm, 4.9cm, 4.8cm,4.8cm, 4.2cm, 4.0cm, 3.9cm,3.8cm,3.5cm,3.3cm,2.5cm.

Keywords. Staphylococcus aureus, Gram staining, Mannitol fermentation, Probiotic, LAB.

1. Introduction

The gram-positive pathogen is named because its tendency to grow as clusters of grapes and generate golden colonies on agar. When compared to other staphylococcus species it is easily distinguishable [1]. Thirty percent of people have S. aureus in their nasal passages without any ill effects, but this opportunistic organism may cause a wide range of disorders, from skin and soft tissue infections to more serious conditions including toxic shock syndrome, necrotizing pneumonia and endocarditis. Food poisoning from S. aureus is also a possibility, especially if the food is handled carelessly [2].

Virulence factors produced by S. aureus include several matrix-binding proteins that let it to cling to host surfaces, as well as a diversified collection of secreted toxin and hydrolytic enzymes that allow it to be very invasive. It is also well-known that this pathogen is capable of producing biofilms, which aid in adhesion to medical devices, antibiotic resistance, immunological evasion, and genetic variation [3].

Human infections caused by S. aureus include bacteremia, infective endocarditis, skin and soft tissue infections (e.g. impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g. pneumonia and empyema), gastroenteritis, and meningitis, to name [4]. Toxin-mediated illnesses and invasive infections are possible with these bacteria, depending on the strains involved and the location of infection. Different strains of Staphylococcus aureus have different pathogenesis. [5].

One of S. aureus' several virulence factors facilitates tissue adhesion and invasion, as well as avoiding the host immune response. Since S. aureus has the capacity to develop resistance to a wide range of

medicines, it is a particularly difficult organism to eradicate. There has been a significant rise in the number of people who have died as a consequence of methicillin-resistant *S. aureus* (MRSA) strains emerging and spreading around the world [6]. "Live microorganisms that when supplied in suitable proportions offer a health advantage to the host" has been defined by the Food Agricultural Organization/World Health Organization (FAO/WHO) [7]. Probiotics are typically found in fermented foods, such as yogurt and fermented milk beverages, as well as in nutritional supplements, such as yoghurt. Probiotics are live microorganisms, either mono or mixed cultures, that improve the balance of the host's indigenous microflora and stimulate the host's immune system when taken in sufficient amounts as part of the diet FAO/WHO (2001) [8]. Lactic acid bacteria were used as a probiotic in this research to combat staphylococcus aureus obtained from Erbil city.

2. Material and Method

2.1. Sample Collection

A total of 50 different samples were collected randomly at the end of 2021 to 2022 in Erbil province, from (milk, cream, bakery products, poultry, hand worker) of which 10 were milk, 10 samples of cream, 10 samples of bakery products, 10 from poultry, 10 samples from hand worker. Samples were transported in a cooler box with ice packs (four to eight degrees) to the lab for examination in disposable sterile screw-cap containers, and some poultry samples were taken by disposable swab. samples were dispersed over Mannitol Salt agar and incubated at 37 degrees Celsius for 24 hours.

2.2. Isolation and Identification of *S. aureus*

Each sample was incubated at 37°C for 24 hours on mannitol salt agar medium. There were a number of testing that were used to identify the bacteria: mannitol fermentation, Gram staining, coagulase, hemolysis, catalase, Test results can be confirmed with the Vitik 2 compact system [9].

2.3. Effect of the Probiotic LAB Against *Staphylococcus aureus*

LAB cultures were grown in MRSB and incubated aerobically for 24 h at 37°C; also *S. aureus*, was grown in MSA for 24 h at 37 °C. Then for probiotic test prepare Muller hinton agar and for each petri dish create two halls and covered by *Staphylococcus aureus* then one of halls add 200 microliter distilled water as a control, Another one add 200 microliter of LAB with MRSBroth, The plates were then incubated at 37°C for 24 h, and the diameter of the growth inhibition zone was measured [10,11].

3. Result and Discussion

Out of a total of 50 different samples were collected, The results shown in Table 1 showed that 38 samples (76%) produced bacterial growth, while 12 samples (24 %) did not produce growth of bacteria, and no growth of bacteria, which could have been due to the use of antibiotics, the pathogen not being germs and researched, or the pathogen being harder to identify in usual ways. Cream and Hand worker samples (18%) were the most common source of *Staphylococcus aureus* Bakery product samples were the second most common source (16 %), with Milk coming in third, after that Poultry swab (10%).

Table 1. Numbers and percentages of isolated samples.

Type of samples	Total samples		Positive samples		Negative samples	
	No.	%	No.	%	No.	%
Milk	10	20%	7	14%	3	6%
Cream	10	20%	9	18%	1	2%
Bakery products	10	20%	8	16%	2	4%
Poultry	10	20%	5	10%	5	10%
Hand worker	10	20%	9	18%	1	2%
Total	50	100%	38	76%	12	24%

For *Staphylococcus aureus* the most important biochemical test results are listed in Table 2, which is (Mannitol fermentation, Gram stain, Catalase, Hemolysin, Coagulase) were positive with the diagnostic step using Vitik 2 serving as a complement and confirmed diagnostic step [12].

Table 2. The biochemical tests of *Staphylococcus aureus*.

Test	Result
Mannitol fermentation	Posative
Gram stain	Posative
Catalase	Posative
Hemolysin	Posative
Coagulase	Posative

LAB was used against *S. aureus*, and It was clearly that the used probiotics had aweak inhibitory effect on *Staphylococci aureus*, and the zone of inhibition ranged from 3.5 – 5.3 cm. As illustrated in Table (3), The inhibition zones were measured in cm. The obtained results revealed that the best antibacterial effect was obtained by mixture of probiotics dairy products. these results are in agreement with the results reported by several investigators who reported that LABs can inhibit the growth of *S. aureus* [10-13].

Table 3. The antimicrobial activity of probiotics against *S.aureus*.

Type of Samples	Measurement of inhibition zone (cm)
Milk	3.5 , 2.5, 3,9
Cream	5.2, 4.0, 4.8
Bakery products	4.8, 4.2, 3.3
Poultry	5.3, 5.1, 4.9
Hand worker	5.2, 5.1, 3.8



Figure 1. LAB was used against *S. aureus*.

Conclusions

In terms of isolation, *Staphylococcus aureus* appears to be more prevalent in various sources, with the hand worker and cream ranking first, followed by bakery products. Then comes milk and poultry. the culture of probiotic strains could increase the protective effects against *S. aureus* infection.

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