Study Antagonistic effects of *Lactobacillus acidophilus* on *Staphylococcus aureus* Methicillin resistance

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ABSTRACT

Recent studies point to probiotics as a promising option for fighting biofilms. Probiotics are live microorganisms which, when administered in sufficient quantities, are of health benefit to the host. *Lactobacillus* is the dominant group of bacteria with proven probiotic effect, where *Lactobacillus* is more effective. The current study aimed to study effects of *Lactobacillus acidophilus* on *Staphylococcus aureus*.It was observed in this study the *Lactobacillus acidophilus* broth culture appeared inhibitory activity against isolates of *Staphylococcus*. While the supernatant did not give inhibitory activity except for the isolate of Staph1.Also,it was observed that the biofilm of all methicillin-resistant *S. aureus* isolates using *Lactobacillus acidophilus* broth showed significant differences compared to control. In addition, the scanning electron microscope results showed a decrease in the thickness of the biofilm after treatment with *L. acidophilus*

Keywords: *Lactobacillus acidophilus, Staphylococcus aureus,* Methicillin resistance

Introduction

Recent studies point to probiotics as a promising option for fighting biofilms. Probiotics are live microorganisms which, when administered in sufficient quantities, are of health benefit to the host. *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus* and *Leuconostoc* are the dominant group of bacteria with proven probiotic effect, where *Lactobacillus* is more effective. This group of bacteria can grow in different habitats using diverse sources of carbon. From glucose metabolism, lactic acid bacteria are classified as fermenters, producing exclusively lactic acid, and producing many other metabolites besides lactic acid, such as ethanol and acetic acid (Carvalho et al.,2021).

Staphylococcus aureus is a diffuse, and highly adaptable pathogen that colonizes the skin and mucous membranes of the anterior nose, gastrointestinal tract, peritoneum, urogenital tract, and pharynx (den Heijer et al., 2013), and is the causative agent of a variety of human and animal diseases. Which has a significant impact on public health (Luzzago et al., 2014; Bitrus et al., 2018).It demonstrates the opportunistic pathogenic behaviour of Staphylococcus aureus in both humans and animals, and can cause several disorders such as suppurative dermatitis or abscesses, endocarditis, sepsis, and urinary tract infections, mastitis, meningitis, osteomyelitis, food poisoning, biofilm-related infections and septicemia (Singh, 2017; Scudiero et al., 2020). It represents of the most common bacterial causes of skin infection. Staphylococcus aureus is responsible for folliculitis, furunculosis, impetigo, syndrome of toxic shock, etc (Saha et al., 2019). This study aimed to study effects of

Lactobacillus acidophilus on *Staphylococcus aureus*.

Methods

Sample collection

Samples were collected from different sources (burns, wounds, and urine) of *S. aureus* isolates from patients admitted to Hawija Hospital / Kirkuk city. It was 250 samples

Isolation and identification of *S. aureus*

The isolates of S. aureus were diagnosed microscopically. Also , it was used Vitek 2 compact system to diagnosis this bacteria. Moreover , *S. aureus* methicillin resistance detected by using methicillin resistance on Muller Hinton agar

Antagonistic effect of *Lactobacillus* on *S. aureus*

Lactobacillus acidophilus (L.a) was obtain from Selçuk University/Turkey .It was used to inhibition of *S. aureus* methicillin resistance. Where it was applied suspension and supernatant of *Lactobacillus plantarum* to detect using agar diffusion to detect activity against four isolates of *S. aureus*.

Detection of biofilm formation

It was detected biofilm by using the plate consisting of micro-calibration tubes (Mathur et al.,2006)

Results and discussion

Detection of *Staphylococcus aureus*

The number of isolates obtained was S. aureus 130 samples out of 250 samples .The microscopic examination of the isolates showed that they are Gram-positive in pairs, and often in clusters, as they appeared in the form of short chains, and they do not contain a capsule and do not contain spores (Versalovic, 2011).The diagnosis of *Staphylococcus aureus* was confirmed using Vitek 2Compact. Also, it was tested 30 isolates for their resistance to methicillin using Methicillin and all of them were resistant to Methicillin (100%) as shown in Figure 1.

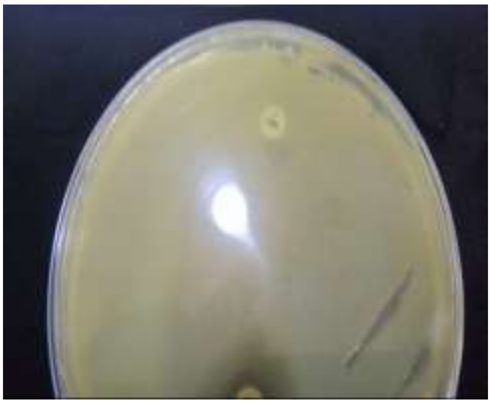

Figure1. *Staphylococcus aureus* (A)methicillin resistance on Muller Hinton agar

It agreed with several studies to detect the resistance of bacteria to methicillin, one of study showed the resistance of *Staphylococcus aureus* isolates to Oxacillin 100% (Jahan et al., 2015). So the percentage of resistant isolates was 37.5% out of 165 samples tested (Pillai et al., 2012). Another used the Oxacillin tablet method, where out of 116 isolates of *S. aureus*, 33 (28.44%) were methicillin-resistant MRSA isolates by Oxacillin tablet diffusion test, the sensitivity, specificity, and accuracy of the Oxacillin disc diffusion test were 100%, 94.31%, and 95.68%, respectively (Chowdhury et al., 2014).

Antagonistic activity test of *Lactobacillus acidophilus* on methicillin-resistant *Staphylococcus aureus* (MARSA)

Lactobacillus plantarum (L.p) was tested to detect antibacterial activity against methicillin-resistant Staphylococcus aureus, and Lactobacillus supernatant and broth culture were used by agar diffusion method. The results showed through the diameters of inhibition (Table 1 and 2) that *Lactobacillus* *plantarum* gave inhibitory activity against isolates of *Staphylococcus*. While the supernatant did not give inhibitory activity except for the isolate of Staph1.

Table 1. Inhibition diameters of bacterial culture of *Lactobacillus acidophilus* towards methicillin-resistant *Staphylococcus aureus* isolates.

MARSA	L.a1	L.a2
Staph1	12	15
Staph2	10	15
Staph3	11	14
Staph4	13	15

Table 2. Inhibition diameters of bacterial supernatant of *Lactobacillus acidophilus* towards methicillin-resistant *Staphylococcus aureus* isolates.

MARSA	L.a1	L.a2
Staph1	7	5
Staph2	4	3
Staph3	0	0
Staph4	8	0

During several studies, including Kubba (2006) noted that the inhibitory effect of Lactobacillus spp. It was higher in MRS liquid medium as it has a stimulating effect on Lactobacillus bacteria which inhibits Gram-negative and Gram-positive bacteria. It was also noted that the three isolates of Lactobacillus bacteria, L. acidophilus, L. plantarum L. casei, Where they had an inhibitory effect on bacteria with inhibition diameters of 13.6 mm, 10.3 and 10.6, as well as a mixture of these types was used and they had a strong inhibitory effect when mixed with them with inhibition diameters The highest ranges between 14.3-20.3 mm (Bhola and Bhadekar, 2019).

Also Xu et al.(2020) showed that *L. casei* inhibits the growth of *Bacillus cereus*, *Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli*. In another study, the activity of L. acidophilus, *L.casei* bacteria against methicillin-resistant *S. aureus* was observed when grown on solid and liquid media of a mixture of *Lactobacillus* bacteria of the species used with methicillin-resistant *S. aureus*, where as a result of the antagonistic reaction between them, the methicillin-resistant bacteria were eliminated with 99% after an incubation period of 24 hours at 37°C (Karska-Wysocki et al., 2010).

Antagonistic activity test of Lactobacillus on biofilm formation for methicillin-resistant *Staphylococcus aureus* (MARSA)

The results showed (Table 3 and 4) the antibacterial effect of Lactobacillus towards the biofilm of methicillin-resistant S. aureus isolates, as it was observed that the biofilm of all methicillin-resistant S. aureus isolates using *Lactobacillus acidophilus* broth showed significant differences compared to isolates that were not treated with Lactobacillus bacteria. The results of using *Lactobacillus* supernatant also showed a decrease in the biofilm formation of *S. aureus* when treated with the supernatant. In addition, the scanning electron microscope results showed a decrease in the thickness of the biofilm after treatment with *L. acidophilus* compared to the control (without treatment) as shown in Figure 2

Table 3. Inhibitory effect of *Lactobacillus acidophilus* broth on the biofilm formation by *S. aureus*(mean±SE)

Isolates of *S. aureus*	*L. acidophilus*	O.D630nm Control	O.D 630nm Treatment
Staph1	L.a1	0.9±0.06a	0.33±0.2b
	L.a2	1.1±0.15a	0.3±0.1b
Staph2	L.a1	1±0.6a	0.45±0.3b
	L.a2	1.2±0.62a	0.505±0.5b
Staph3	L.a1	1.1±0.3a	0.335±0.8b
	L.a2	1.2±0.4a	0.45±0.7b
Staph4	L.a1	0.95±0.05a	0.35±0.4b
	L.a2	0.85±0.15a	0.46±0.2b

Different letter in rows indicated significant differences(P<0.05)

Table4. Inhibitory effect of *Lactobacillus acidophilus* supernatant on the biofilm formation by *S. aureus*(mean±SE)

Isolates of *S. aureus*	*L. acidophilus*	O.D630nm Control	O.D 630nm Treatment
Staph1	L.a1	0.65±0.09a	0.35±0.05b
	L.a2	0.7±0.3a	0.33±0.6b
Staph2	L.a1	0.74±0.53a	0.37±0.08b
	L.a2	0.65±0.7a	0.35±0.06a
Staph3	L.a1	0.74±0.08a	0.60±0.5a
	L.a2	0.7±0.06a	0.63±0.3a
Staph4	L.a1	1.0±0.15a	0.73±0.08a
	L.a2	0.6±0.8a	0.53±0.5a

Different letter in rows indicated significant differences (P<0.05)

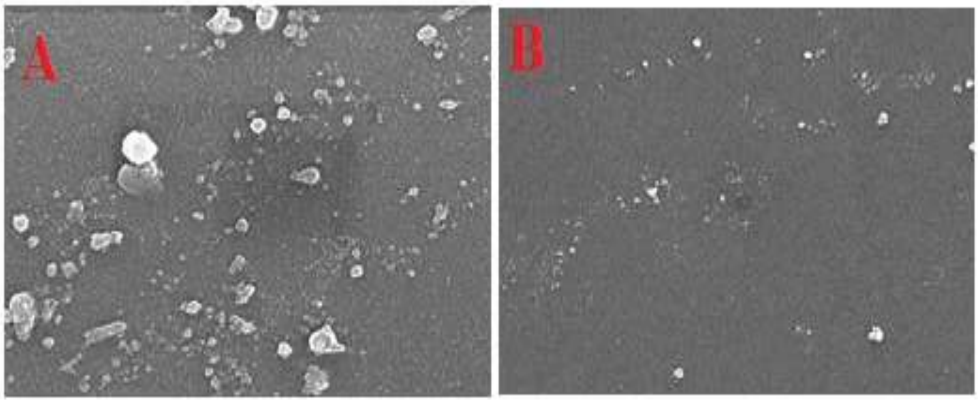

Figure2. Biofilm in scanning electron microscope of *S. aureus* (A) Control (B) Treated with *Lactobacillus*

Several studies on the susceptibility of *Lactobacillus* bacteria to inhibit biofilm and antagonistic of S. aureus showed that the surfactants produced by *Lactobacillus acidophilus* had an inhibitory effect on microbes (*S. aureus, E.coli, Pseudomonas aeruginosa* and *Micrococcus luteus*). Also, the antibacterial effect of surfactants against S. aureus depends on their concentrations (Nataraj et al.,2021). *Lactobacillus* bacteria contain surfactants that are produced by a variety of microorganisms with different biological functions. Whereas lactic acid bacteria were examined for their emulsifying properties. The ability of Lactobacillus plantarum to produce surface-active peptides was verified. Biosurfactant derived from L. plantarum has been shown to have the ability to reduce surface tension (Al-Seraih et al., 2022).

Conclusions

It was observed in this study the *Lactobacillus acidophilus,*broth culture appeared inhibitory activity against isolates of *Staphylococcus*. While the supernatant did not give inhibitory activity except for the isolate of Staph1.Also,it was observed that the biofilm of all methicillin-resistant *S. aureus* isolates using *Lactobacillus acidophilus* broth culture showed significant differences compared to control. In addition, the scanning electron microscope results showed a decrease in the thickness of the biofilm after treatment with *L. plantarum*

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