



Effect of Organic Acids and Probiotics on Methicillin and Vancomycin-Resistant *Staphylococcus aureus* Isolated from Patients in the Dhi Qar Governorate

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ABSTRACT

This study was conducted the prevalence of these strains in Dhi Qar Governorate and how to use alternative treatments based on available, inexpensive, and safe materials for human use. The goal of this study was to find out how well some organic acids and probiotics kill clinical isolates of Methicillin-resistant *Staphylococcus aureus* MRSA and Vancomycin-resistant *Staphylococcus aureus* VRSA a big health problem. From 430 specimens studied, only 314 (73%) demonstrated growth, whereas 116 (27%) indicated no growth. Out of 314 (73%), 158 (50.3%) were recognized as gram-positive bacteria, whereas 50 (31.65%) were categorized as *Staphylococcus aureus* and the growth of gram-negative bacteria 156 (49.7%). We obtained and processed 10 patient samples using traditional microbiological procedures, followed by molecular testing for validation. We treated the isolates with four types of organic acids: acetic, lactic, citric, and oxalic acids. We also used locally isolated probiotics from sheep milk, sheep whey, and cow whey, and we also used a commercially available probiotic powder formulation consisting of three probiotic bacteria. Results demonstrated that organic acids showed much more antibacterial efficacy than probiotic therapy. Acetic acid had the most efficiency among the acids, successfully suppressing bacterial growth at reduced doses. Lactic acid had the next highest efficacy, followed by citric acid with moderate activity, and oxalic acid exhibited the lowest effectiveness. The probiotic formulation decreased bacterial numbers to a degree, although its effect was far less marked than that of the organic acids. Based on these findings, organic acids, especially acetic acid, may work well as alternatives to or in addition to common antimicrobials.

Keywords: *Staphylococcus aureus*, VRSA, MRSA, antibiotics, organic acid.

INTRODUCTION

Staph. aureus has a remarkable capacity to inhabit the nasal passages of healthy individuals without eliciting any symptoms. It can cause localized infections of the skin and soft tissues. It can also affect deeper tissues or the circulation, which can lead to systemic infections like bacteremia, pneumonia, endocarditis, and osteomyelitis (Foster, 2002; Hassan, 2024; Kwiecinski and Horswill, 2020). The spread of (MRSA) and (VRSA) is a big problem in healthcare settings, especially in places like the Dhi Qar Governorate. Because they can get around standard antibiotic treatments, these antibiotic-resistant bacteria are a major threat to public health and require new ways to treat infections (Taher and Othman, 2024). Recent studies have shown that organic acids, such as lactic and acetic acid, are excellent at killing bacteria that cause these infections (Liu *et al.*, 2023). Additionally, probiotics have been shown to help prevent infections by possibly lowering the number of MRSA and VRSA infections (Sachdeva *et al.*, 2025). It has been shown that organic acids, like those made by bacteriocins, can mess up the metabolism of bacteria, which stops MRSA and VRSA from spreading, resulting in cell death. Organic acids like acetic acid, citric acid, oxalic acid, and lactic acid have been shown to stop MRSA biofilm formation, which is important for lowering antibiotic resistance. It makes vancomycin work better by breaking up biofilm structure and lowering the production of virulence factors (Alinezhad *et al.*, 2023; Gu *et al.*, 2022). Organic acids, like acetic acid, stop MRSA and VRSA by sticking to and stopping Ddl activity, which is needed for cross-linking peptidoglycans and keeping the integrity of the cell wall (Panda *et al.*, 2024). Probiotics, especially *Lactobacillus* and *Bifidobacterium* species, stop MRSA and VRSA in a number of ways (Pennypacker *et al.*, 2023). In conclusion, both organic acids and probiotics are interesting alternative ways to treat MRSA and VRSA infections. However, organic acids have been shown to directly increase the effectiveness of antibiotics. Probiotics provide a distinct benefit by influencing bacterial communication systems; hence, they diminish pathogenicity and biofilm development. More research needs to be done to fully understand how these alternative medicines work and how they might be used in therapy.

MATERIALS AND METHODS

Sample collection

430 samples were collected from patients at Imam Hussein Teaching Hospital/ Nasiriyah Teaching Hospital, and Al-Rifai Teaching Hospital in Nasiriyah from September 2024 to January 2025. These samples included 192 urine samples, 34 blood samples, 50 sputum samples, 30 wound samples, 45 burn samples, 10 spinal fluid samples, 34 abscess samples, 4 leg amputation samples, 1 breast abscess, and 30 nasal swabs. The patients were both visiting and staying at these hospitals. We took a sample from each patient, transported it to the laboratory using a carrier medium (transport media), and then transferred it to the laboratory.

Identification of bacterial isolates

Following standard methods to separate *S. aureus*, all samples were grown on differentiation and enrichment mediums such as blood agar (Accumix/India), mannitol salt agar (Bio mark/India), after being in an aerobic environment at 37°C for 18-24 hours (Al-Shaar and Altai, 2025; Yasir, 2024). The morphological properties of bacterial colonies cultivated on the aforementioned medium, including their shape, texture, color, and margin, together with other traits, such as pigment production, were used for the preliminary diagnosis of the colonies. A single pure colony was moved to a microscope slide using a clean loop so that it could be studied. The slide was then heated to dryness, and stained with Gram stain, these tests include the catalase and coagulase assays. These tests help tell *Staph. aureus* apart from other bacteria by looking at its Gram-positive traits and its ability to make enzymes. And checked using the API Staph System (BioMérieux/France) (Abdulbaqi and Ibrahim, 2023; Prihandani *et al.*, 2024).

Culture Characteristics: You can cultivate *S. aureus* on selective media, such as mannitol salt agar and blood agar, where it exhibits distinct growth patterns. For example, *S. aureus* colonies on

mannitol salt agar may metabolize mannitol, resulting in a color change in the medium (Khalid *et al.*, 2022; Sadeq and Lafta, 2024).

Antibiotic susceptibility test: The antibiotic susceptibility test was conducted. The disk diffusion methodology is documented in Bauer (1966), and subsequently revised by Vandepitte (2003) (Al-Jubouri and Abod, 2024; Salih *et al.*, 2021). Antibiotics from 12 types were used in the concentrations specified in (Table 1).

Table (1): Utilized antibiotics and their concentrations.

NO.	Antibiotic disc	Symbol	Concentration/ μ g	Company/ origin
1	Cefoxitin	FOX	30 μ g	Liofilchem/ Italy
2	Augmentin	AMC	30 μ g	Bioanalys / Turkey
3	Ciprofloxacin	CIP	5 μ g	Liofilchem/ Italy
4	Erythromycin	E	15 μ g	Liofilchem/ Italy
5	Gentamycin	GEN	10 μ g	Liofilchem/ Italy
6	Norfloxacin	NOR	10 μ g	Liofilchem/ Italy
7	Oxacillin	OX	1 μ g	Bioanalys / Turkey
8	Tetracycline	TE	30 μ g	Bioanalys / Turkey
9	Trimethoprim-Sulfamethoxazole	SXT	1.25/23.75 μ g	Liofilchem/ Italy
10	Methicillin	ME	10 μ g	Liofilchem/ Italy
11	Clindamycin	DA	2 μ g	Bioanalys / Turkey
12	Vancomycin	VA	30 μ g	Liofilchem/ Italy

Identification and susceptibility testing by the VITEK-2 system

The VITEK-2 system is a valuable tool for identifying MRSA and VRSA, the VITEK-2 system is proficient in assessing oxacillin susceptibility, essential for the identification of MRSA. Nonetheless, its precision may fluctuate. A study that compared VITEK-2 and agar dilution found that they agreed on oxacillin susceptibility by 0.88%, which suggests that the data is not the same (Dhar *et al.*, 2023; Wang *et al.*, 2024).

The VITEK-2 system and the broth microdilution techniques all gave similar results for vancomycin susceptibility in most isolates. However, some isolates showed differences, which shows the need to cross-check data using more than one method (Bushra *et al.*, 2022).

Organic acids such as citric acid, acetic acid, lactic acid, and oxalic acid, along with probiotics, effect on *staph. aureus*:

Organic acids Probiotics and have been shown to help fight *Staphylococcus aureus* (*Staph. aureus*), even methicillin-resistant strains (MRSA) and vancomycin-resistant strains (VRSA).

Organic acids, including citric, acetic, lactic, and oxalic acids, have been investigated for their antibacterial efficacy against *Staph. aureus*. Probiotics, especially those that generate organic acids, may augment the effectiveness of traditional antibiotics against *Staph. aureus* (Panda *et al.*, 2024; Rodríguez-Sánchez *et al.*, 2022).

RESULTS AND DISCUSSION

Isolates of the bacterium were identified based on various cultural characteristics specific to Gram-positive bacteria, including colony morphology, hemolytic type, and pigmentation on blood agar. *Staphylococcus aureus* is typically surrounded by distinct areas of β -hemolysis. It possesses a large, round shape with a smooth, lustrous surface and exhibits a golden yellow pigmentation on the mannitol salt agar. From the 430 specimens studied, only 314 (73%) demonstrated growth, whereas 116 (27%) indicated no growth. Out of 314 (73%), 158 (50.3%) were recognized as gram-positive bacteria, whereas 50 (31.65%) were categorized as *Staphylococcus aureus* and the growth of gram-negative bacteria 156 (49.7%). Shown in Fig. (1).

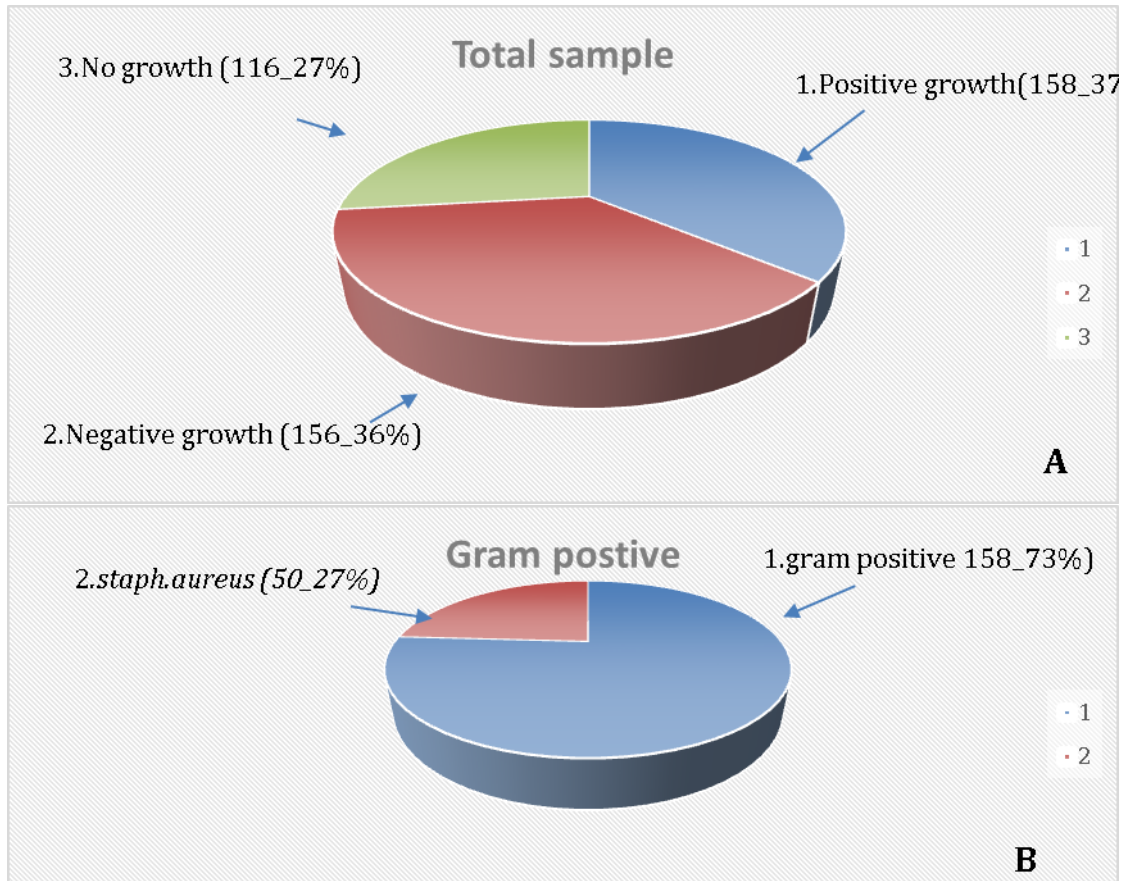


Fig. 1: Culture results of *Staph. aureus*. (A) positive results and percentage, (B) *Staph. aureus* isolation percentage.

Colony morphology

Staphylococcus aureus colonies on blood agar are round, convex, and measure 1-4 mm in diameter, with a prominent border and typically displaying a light to golden yellow pigment. In contrast, colonies of *S. epidermidis* and *S. saprophyticus* are either bright yellow or white. *Staphylococcus aureus* generally displays distinct β -hemolytic zones on blood agar (Spies *et al.*, 2024; Yasir, 2024). Shown in Fig. (2).

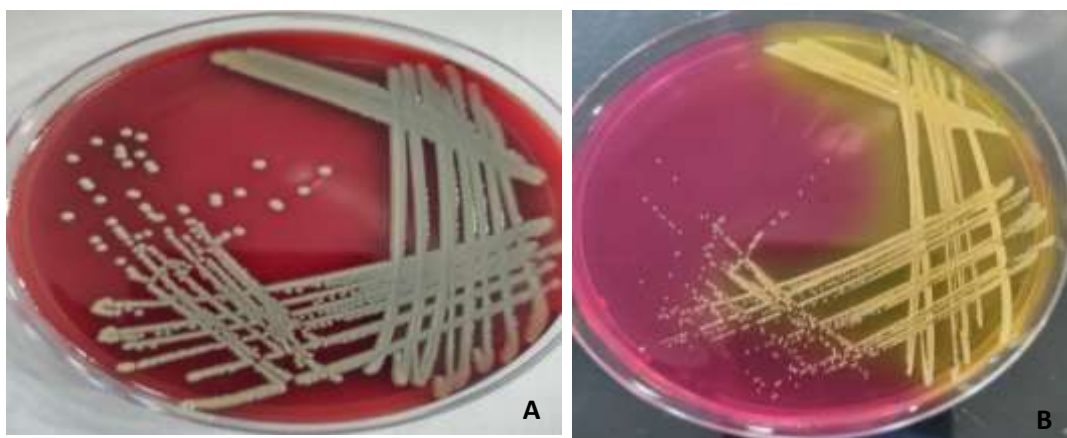


Fig. 2: This image shows *Staph. aureus* (A) on the blood agar (B) on the mannitol salt agar.

Microscopic examination

We conducted a microscopic examination following the Gram staining of 50 out of 158 isolates to assess their response to the stain. As illustrated in the referenced figure, the cells manifested as gram-positive cocci, predominantly aggregated in irregular clusters reminiscent of grapes. *Staph. aureus* is a gram-positive bacterium because it has a strong, highly cross-linked peptidoglycan layer (20 to 80 nm) that traps the main stain-mordant complex inside the cell wall (Wang *et al.*, 2015).

Identification by biochemical tests and VITEK-2 system

Catalase and coagulase assays are crucial to the biochemical characterization of *Staph. aureus*. The coagulase test tells the difference between *Staph. aureus* and other staphylococci, while the catalase test, which shows bubbles, makes sure that the catalase enzyme is present (AL-Wasmee, 2024; Hamed and Alnazzal, 2023). Shown in Fig. (3).

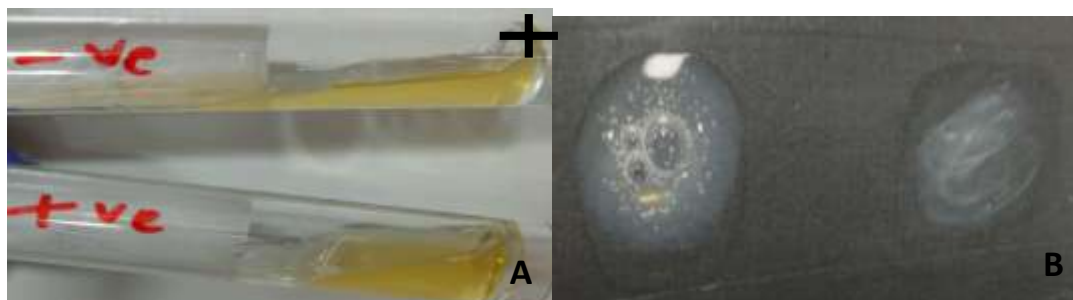


Fig. 3: This image shows: (A) Coagulase test. (B) Catalase test for *Staph. aureus*.

We used the VITEK-2 system to precisely identify the isolates and complete the remaining tests and experiments on those isolates. Based on the current findings, we identified 50 (27 %) of the 158 samples as *Staphylococcus aureus*, a type of gram-positive bacteria. Due to its high sensitivity and specificity for fully identifying *S. aureus* isolates, the Vitek-2 technique is used to confirm the diagnosis of standard biochemical tests. The results showed that manual identification had previously recognized all 50 isolates as *Staph. aureus* (Duraye *et al.*, 2024).

Antibiotic test results: The (Table 2) presents the resistance (R), intermediate susceptibility (IN), and susceptibility (S) of Vancomycin-resistant *Staphylococcus aureus* (VRSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) strains to various antibiotics. Below is an interpretation of the results: 1. Antibiotics with total resistance (100%): FOX (Cefoxitin), AMC (Amoxicillin/Clavulanic Acid), OX (Oxacillin), and ME (Methicillin) demonstrated 100% resistance, so proving that the isolates are MRSA. DA (Clindamycin) exhibited 80% resistance, signifying that the majority of isolates are unresponsive to this antibiotic. 2. Antibiotics exhibiting moderate resistance levels: CIP (Ciprofloxacin): 16% resistance, 84% susceptibility, indicating its continued efficacy against the majority of isolates. E (Erythromycin): 68% resistance, 4% intermediate susceptibility, and 28% susceptibility, demonstrating a significant prevalence of resistance. GEN (Gentamicin): 14% resistance indicates it may remain a viable treatment option. NOR (Norfloxacin): 18% resistance, 78% susceptibility, demonstrating relative efficacy. Tetracycline (TE) has 56% resistance, 28% intermediate susceptibility, and 16% susceptibility, indicating it is not the optimal selection. SXT (Trimethoprim/Sulfamethoxazole): 22% resistance and 74% susceptibility indicate its potential as a viable treatment option. Vancomycin resistance (VA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) Concern: 26% of isolates exhibited resistance to vancomycin, 6% demonstrated intermediate susceptibility, and 68% were susceptible (Gülbay and Doğan, 2024; Khalid *et al.*, 2024; Özbek *et al.*, 2021). This indicates that while the majority of isolates are still susceptible to vancomycin, a subset has acquired resistance, prompting worries over the onset of VRSA. Shown in Fig. (4) Clinical Implications: The analyzed strain is confirmed as MRSA due to its resistance to oxacillin and methicillin. The rise of vancomycin resistance is especially alarming, as it restricts treatment alternatives. Antibiotics that may be effective include ciprofloxacin, gentamicin,

norfloxacin, and trimethoprim/sulfamethoxazole; however, treatment choices should be informed by specific susceptibility testing for each patient. Shown in (Table 2).

Table (2): Shows the number of resistant, intermediately resistant and sensitive isolates and the percentages for each, noting that the total number of isolates is 50.

Antibiotics	R (100%)	IN (100%)	S (100%)
Cefoxitin	50 (100%)	0	0
Augmentin	50 (100%)	0	0
Ciprofloxacin	8 (16%)	0	42 (84%)
Erythromycin	34 (68%)	2 (4%)	14 (28%)
Gentamycin	7 (14%)	1 (2 %)	42 (84%)
Norfloxacin	9 (18%)	2 (4%)	39 (78%)
Oxacillin	50 (100%)	0	0
Tetracycline	28 (56%)	14 (28 %)	8 (16 %)
Trimethoprim-Sulfamethoxazole	11 (22%)	2 (4%)	37 (74 %)
Methicillin	50 (100%)	0	0
Clindamycin	40 (80%)	0	10 (20%)
Vancomycin	13 (26%)	3 (6%)	34 (68%)



Fig. 4: This image shows the antimicrobial susceptibility test.

Result effect of organic acids and probiotics

Below is a compilation of data on the inhibitory effects of several organic acids and probiotics against *Staphylococcus aureus*, listed in order of decreasing inhibition:

Acetic acid has the most pronounced inhibitory impact on *S. aureus*. Its relatively low and strong antibacterial properties allow it to damage the bacterial cell membrane and metabolic pathways, which significantly slows down the growth of bacteria.

Lactic acid has effectiveness subsequent to acetic acid. It not only reduces ambient pH but also disrupts bacterial enzyme function. Although effective, its inhibitory action is rather less apparent than that of acetic acid.

Citric acid has mild antibacterial activity. It can bind to important metal ions and change how cells let things in and out, which makes it an inhibitor, but it is not as strong as acetic or lactic acid.

Oxalic acid has the least inhibitory efficacy among the organic acids analyzed. The reduced acidity and distinct interaction with bacterial cells explain the more restricted antibacterial efficacy (Liu *et al.*, 2024; Panda *et al.*, 2024).

Probiotics often have a much-reduced direct inhibitory impact on *Staph. aureus* compared to organic acids. They usually kill bacteria indirectly, mostly by keeping other bacteria from growing, controlling the immune system, and making small amounts of organic acids or bacteriocins. Shown

in Fig. (5). Nonetheless, these effects are less quick and powerful than the direct action of the previously described acids (Díaz *et al.*, 2024). Shown in (Table 3).

Table (3): The effect of organic acid and the probiotic on the *Staph. aureus* MRSA and VRSA using well defusing method millimeter.

Sample number	Acetic acid	Oxalic acid	Citric acid	Lactic acid	Sheep milk	Sheep whey	Cow whey	Mix powder
19A	40	6	37	32	6	6	6	16
79A	42	9	35	35	6	8	6	20
15A	41	24	28	32	6	6	6	21
55A	43	18	26	29	6	6	6	22
11A	46	15	32	35	6	6	6	24
26A	40	6	31	30	6	6	6	17
200A	40	18	20	34	6	12	6	25
14A	42	19	25	40	6	6	6	15
59A	36	25	30	34	6	6	6	26



Fig. 5: The effect of organic acids and probiotics on *Staph. aureus*.

CONCLUSIONS

Overall, this research represents an important step toward finding innovative solutions for dealing with antibiotic resistance in MRSA and VRSA. The findings indicate that organic acids, notably acetic acid, can play a crucial role in inhibiting the growth of pathogenic bacteria, while probiotics serve a supportive role by modulating bacterial communication and biofilm formation.

Expanding the scope of this study could lead to improved treatment protocols and offer safer and more effective therapeutic options to confront future challenges in combating resistant bacterial infections.

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تأثير الأحماض العضوية والبروبيوتيك على العزلات السريرية لمكورات العنقودية الذهبية المقاومة للميثيسيلين وللغانكوميسين من المرضى في محافظة ذي قار

رياض مكطوف جاسم الزالمي

دائرة صحة ذي قار/ ذي قار/ العراق

هيام عادل الطائي

قسم علوم الحياة/ كلية العلوم/ جامعة الموصل/ الموصل/ العراق

ايكور ايفانوفسكي

قسم علوم الأحياء/ جامعة سانت جوزيف/ حرم لونغ آيلاند/ نيويورك/ الولايات المتحدة الأمريكية

الملخص

تمت في هذه الدراسة على انتشار هذه السلالات في محافظة ذي قار، وكيفية استخدام علاجات بديلة تعتمد على مواد متوفرة ورخيصة وآمنة للاستخدام البشري. هدف هذه الدراسة هو معرفة مدى فاعلية بعض الأحماض العضوية والبروبيوتيك في القضاء على العزلات السريرية لمكورات العنقودية الذهبية المقاومة للميثيسيلين والمقاومة للغانكوميسين، والتي تُعد مشكلة صحية كبيرة. من بين 430 عينة تمت دراستها، أظهرت 314 عينة فقط (73%) نمواً، بينما أشارت 116 عينة (27%) إلى عدم وجود نمو. من بين 314 عينة (73%)، تم التعرف على 158 عينة (50.3%) على أنها بكتيريا موجبة الجرام، بينما تم تصنيف 50 عينة (31.65%) على أنها بكتيريا المكورات العنقودية الذهبية ونمو البكتيريا سالبة الجرام 156 عينة (49.7%).

تم اختيار عشر عينات من المرضى وتشخيصها باستخدام الطرق الميكروبيولوجية التقليدية، تلتها الاختبارات الجزيئية لتأكيد النتائج. تم معالجة العزلات بأربعة أنواع من الأحماض العضوية: حمض الأسيتيك، حمض اللاكتيك، حمض الستريك، وحمض الأوكساليك. كما استُخدم بروبيوتيك معزول محلياً من حليب الغنم، ومصل الغنم، ومصل الأبقار، بالإضافة إلى تركيبة تجارية لبروبيوتيك تحتوي على ثلاثة أنواع من بكتيريا البروبايتك أظهرت النتائج أن الأحماض العضوية أظهرت فاعلية مضادة للبكتيريا أكبر بكثير مقارنة بالعلاج بالبروبيوتيك. حيث كان حمض الأسيتيك الأعلى فاعلية بين الأحماض، إذ نجح في تثبيط نمو البكتيريا بجرعات منخفضة، تلاه حمض اللاكتيك من حيث الفاعلية، ثم حمض الستريك بفاعلية متوسطة، بينما كان حمض الأوكساليك الأقل فاعلية. أما تركيبة البروبيوتيك، فقد ساعدت في تقليل أعداد البكتيريا إلى حد ما، لكن تأثيرها كان أقل بكثير من تأثير الأحماض العضوية. وبناءً على هذه النتائج، قد تُستخدم الأحماض العضوية، خاصة حمض الأسيتيك، كبديل أو مكملات للمضادات الحيوية التقليدية.

الكلمات الدالة: المكورات العنقودية الذهبية، VRSA، MRSA، المضادات الحيوية، الأحماض العضوية.