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Antibacterial and therapeutic effects of vancomycin-resistant *Staphylococcus aureus* bacteriocin (VRSAcin) in the treatment of VRSA skin infection in mice

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ABSTRACT

Vancomycin *Staphylococcus aureus* (VRSA) is a strain of *S. aureus* that is considered the main cause of bacterial skin and soft tissue infections. It has acquired resistance to vancomycin and represents a therapeutic challenge. The current study aimed to compare the possible therapeutic effects of VRSA bacteriocin (VRSAcin) on the treatment of skin infection in mice with those of an antibiotic (linezolid). The results showed that of the fifty swabs obtained from human skin wounds. One isolate was selected for VRSAcin extraction depending on its antibiotic resistance using an antibiotic susceptibility test.An agar well diffusion test was used to determine bacteriocin's antibacterial activity, as well as its a minimum inhibitory concentration, minimum bactericidal concentration, and antibiofilm efficiency against gram-positive and gram-negative bacteria that were resistant to many medicines. The freshly developed antibacterial substance VRSAcin shows promise. Bacteriocin from VRSA was extracted and studied the optimal conditions for the Production following Purification of bacteriocin by ammonium sulfate precipitation followed by cation-exchange chromatography. The molecular weight of bacteriocin about (29 kDa) were determined by Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The typical conditions for the production of VRSAcin include a pH of 7 and a temperature of 37 °C for 48 h. In mice, VRSA-contaminated wounds revealed severe tissue distraction and inflammation that extended to the hypodermis, while VRSA-treated skin showed mild changes and localized lesions to the epidermis and upper dermis. The skin of linezolid ointment-treated mice showed moderate to severe changes. In conclusion, VRSA strain infections in human burned skin were more common than expected. In vivo studies in mice indicated that wounded skin infected with VRSA can be treated with VRSAcin as an antibacterial agent that promotes healing processes with obvious superiority to linezolid ointment. As a result, the VRSA develops bacteriocins that are appropriate for regulating AMR, Gram-positive and Gram-negative bacteria, and may be useful in wound dressings.

1. Introduction

The vancomycin-resistant *Staphylococcus aureus* (VRSA) strain is characterized by acquired resistance to vancomycin, Infections linked to Staphylococcus aureus (S. aureus) pose a serious risk to public health. aimed to identify the pattern of antibiotic resistance and characterize the genetic makeup of methicillin- and vancomycin-resistant S. aureus (VRSA) that was isolated from an alternative source. [1]. VRSA resistance is attributed to the plasmid-mediated *vanA* gene and operon [2]. When *S. aureus* invades the bloodstream or internal organs, it may cause a wide spectrum of important infections and is considered the main pathogen in humans. It causes clinical manifestations that extend from

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mild infections of the skin and soft tissue to serious and life-threatening systemic diseases, making it a great challenge for public health due to its emergence and dissemination, such as MRSA and VRSA, which have resistance mechanisms and infection characteristics [3]. One of the oldest antibiotics (lasting more than 60 years) is vancomycin, which acts by interrupting the continuity of the cell wall synthesis of susceptible bacteria. The cell wall structure coats the membrane in most bacteria to protect the bacteria from intracellular high osmotic pressure and prevent them from swelling and bursting. Recently, vancomycin has been used for treating MRSA infections and patients who are allergic to other antibiotics, such as cephalosporins or penicillin [4]. The presence of adhesive organisms and the formation of biofilms are two of the most important factors affecting the virulence of this bacterium to *Staphylococcus* bacteria, such as gram-positive bacteria, and are consistent with the adulteration patterns mentioned for negative bacteria such as *Proteus mirabilis* [5]. Resistance to vancomycin in VRSA was attributed to plasmid transposons, which increased the probability of dissemination of medically important bacteria, especially *S. aureus* [6]. The co-colonization and co-infection of MRSA and VRSA *and* resistance to vancomycin are achieved by the transformation of resistance from a donor (VRSA) to a recipient (MRSA). For these reasons, co-colonization and co-infection of both VRSE and MRSA are very common in clinical cases [7]. Since van A and its product (protein) are required to convey distinct vancomycin resistance and not van B, we conclude that van A is the source of vancomycin resistance and that resistance may be addressed by targeting it or its product. [8]. Pathogenic factors of microorganisms generated by some bacteria are small molecules named bacteriocins, which appear to be a unique strategy and strong candidates to replace or overlap the role of traditional antibiotics. Bacteriocins are peptides and proteins that suppress growth of bacteria or kill other related or unrelated microorganisms [9,10]. Bacteriocins can be used in biopreservation applications, especially for antibiotics [11]. Micrococci P1 (MP1) and garlic in KS (GarKS) are both bacteriocins with major curative potential; they are powerful against a broad range of pathogens, including many bacteria, such as *S. aureus*, *Streptococcus* spp., *Enterococcus faecium*, and *Enterococcus faecalis* [12]. However, the current study aimed to determine the possible antibacterial and therapeutic effects of VRSAcin in the treatment of skin infections and the possible antibacterial and therapeutic effects of VRSAcin in the treatment of skin infections. Because AMR bacteria, especially VRSA, are resistant to commonly used antibiotics, their global spread presents a significant public health concern. Novel antibacterial agents are therefore desperately needed. The bacterium-produced bacteriocin exhibits promise in the fight against resistant strains and gives a workable solution to this issue along with possible applications in manufacturing for higher production. It is therefore appropriate for managing AMR, specifically VRSA, and may be given topically in wound dressings in the future.

2. Materials and methods

2.1. Phenotypic identification

The bacterial samples were isolated from patients suffering from class 3 and 4 skin wounds in the Baghdad Medical City in Baghdad, Iraq. The bacteria was cultured on mannitol salt agar and blood agar, and then incubated for 24–48 h at 37 °C under aerobic conditions. Suspected colonies were identified morphologically and biochemically [13]. Isolates tested positive for the characteristics of MDR (multidrug-resistant bacteria). ID-gram-positive cocci (ID-GPC cards; bio Mérieux) were used to identify and confirm the positive position of every strain.

2.1.1. Antibiotic resistance test

The test was performed on plates of Mueller-Hinton agar at 37 °C for 18 h using six different antibiotic discs. After incubation, the diameter of each zone of inhibition was measured in millimeters using a ruler. The isolate was reported to be sensitive (S), intermediate (INT), or resistant (R) to a particular drug by comparison with the standard inhibition zone (Table 1) [14,15].

2.2. Extraction of bacteriocin (VRSAcin)

VRSA isolates that showed the highest zone of inhibition in the antibiotic resistance test were chosen for the production and extraction of VRSAcin. Tryptic soy broth (TSB) (2 % inoculated with 6×10^8 cells/ml VRSA) was then incubated for 24 h at 37 °C under aerobic conditions. Bacteria were harvested in PBS and centrifuged at 6000 g for 15 min [16].

2.2.1. VRSAcin activity assay

Bacteriocin-like inhibitory substance (BLIS) activity was detected by testing its inhibitory effects on indicators such as gram-positive and gram-negative bacteria obtained from the Department of Biology, College of Science, and University of Baghdad, Iraq. Crude VRSAcin antibacterial activity was tested using the agar well diffusion (AWD) method on Muller–Hinton agar [17].

2.2.2. Optimal conditions for the production of VRSAcin

To determine the conditions of the medium and culture that support maximal VRSAcin production, many optimization experiments were performed using the VRSAcin production isolate, which was selected from the screening section [18].

2.2.2.1. Effect of pH on VRSAcin. The solution of VRSAcin was mixed with 10 mM buffered potassium phosphate. The pH was adjusted from 7 to 12 (one increment in pH) using 1 N NaOH or HCl, and the mixture was incubated for 30 min at 37 °C.

2.2.2.2. Temperature effect. VRSAcin solution Samples were exposed to a range of temperatures (20, 25, 30, 37, and 40 °C) for 30 min. The protein concentrations of all the samples were measured prior to and after heat testing [19].

3. Purification of bacteriocin.

Ammonium sulfate was added gradually to the cell free supernatant with saturation ratios ranging between 40 % and 80 % and was kept for 4–6 h at 4∘Cat pH 2.5. The mixture was then mixed gently at 4 °C for 30 min. The resulting precipitate was collected by centrifugation at 10,000 rpm for 15 min at 4 °C. After centrifugation, the pellet was dissolved in 5 mL of 10 mM glycine-HCl buffer (pH 2.5) and was kept overnight in a dialysis bag for using suitable buffer for removing the excess ammonium per-sulfate [20]. Than using Tris-tricine buffer in the form of a polyacrylamide gel electrophoresis (SDS-PAGE), the molecular mass of the bacteriocin was measured. The bacteriocin was later electrophoresed at 120 V on an 18 % gel concentration using a protein ladder (10–250 kDa) (Biolabs, England) [21]. the bacteriocin solution was lyophilized at −70 °C using a freeze drier (Martin Christ/Germany), and it was then stored at 4 °C until needed.

2.2.2.3. Ion-exchange chromatography (DEAE-cellulose). A technique for separating proteins based on variations in protein charge has the name

Table (1)
Percentage of VRSA isolates susceptible to antibiotics.

Antimicrobial agent	Disc conc. µg/disk	Diameter of inhibition zone (mm)		
		Resistant (R)	Intermediate (Int)	Sensitive (S)
Ciprofloxacin Gentamicin	5	≤15	16–20	≥22
Cefoxitin Trimethoprim	10	≤12	13–14	≥15
Chloramphenicol	30	≤21	_	≥21
Linezolid	10	≤28	_	≥16
	30	≤12	13–17	≥18
	30	≤15	_	≥29

ion exchange chromatography. In the current study, DEAE Cellulose-52 was used as the filler and balanced at a flow rate of 1 mL/min utilising 0.02 mol/L pH 8.0 Tris-HCl buffer. After having freeze-dried, the desalted dialysate samples were dissolved in 0.02 mol/L of pH 8.0 Tris-HCl buffer in order to produce a solution with a 20 mg/mL concentration. After centrifuging the solution for 10 min at 10,000 rpm, the supernatant was removed for loading. Two milliliters were loaded, and the loading interval was noted. Following the passage of all the samples into the column, gradient elution was performed using a 0.02 mol/L pH 8.0 Tris-HCl buffer containing 0–0.5 mol/L NaCl, and the eluent was collected. The change in the light absorption ratio at 280 nm was then used to create the elution curve. Ultimately, every high point of the elution curve was examined for antibacterial activity, and the antibacterial activity was then utilized to determine the optimum eluent concentration [22,23].

2.3. Bacteriocin concentration

After bacteriocin extraction and purification, a concentration was prepared, and the protein concentration was measured to reach 62.50 μg/ml, which was the optimal concentration for VRSAcin activity. This concentration was measured according to the method of Lowry [24].

2.4. Determination of the minimum inhibition concentration (MIC) of vancomycin

Using the broth microdilution method, the antibacterial activity of vancomycin was assessed for both gram-positive and gram-negative isolates [25]. Each vancomycin (64 μg/ml stock solution) solution was serially diluted twice in 1 ml of Mueller Hinton broth (MHB) at concentrations ranging from 4 to 64 μg/ml. Ten microplate wells containing 10 μl of a bacterial solution with a turbidity of 0.5 McFarland standard were injected with 100 μl of vancomycin dilutions. MHB medium was utilized as the negative control, and bacterial suspension in the absence of any additives was used as the positive control. MIC stands for the minimal concentration that inhibited discernible development. The minimum inhibitory concentration (MIC) of each isolate's vancomycin was determined according to the CLSI (2023) recommendations. The microplate was incubated at 37 °C for 24 h. Bacterial growth was investigated in each well after the optical density was measured at 450 nm using a microtiter plate reader. The minimum concentration that prevented noticeable growth was considered the MIC.

2.5. Experimental design

Thirty-six male albino mice were divided into 4 groups (n = 9 for each group). The mice were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). The skin area (3 × 2 cm) on the right flank of each mouse was shaved using an electrical shaver, followed by a disposable hand shaver. Liquid soap and sterile distilled water were used to clean the shaved area, and after skin drying, the area was subjected to wounding, which was induced by a sterile lancet by making three parallel superficial wound lines. The first group was considered a positive control group, and the wounds were left without any treatment, while the injured skin of the other groups was inoculated with VARS using one drop containing 1×10^8 cfu/ml [26]. Wounds in the 2nd group were left without any treatment, while wounded skin in the 3rd group was treated with a local application of one drop (0.1 ml) of VRSAcin (conc. 64 μg/ml) 2 h post infection, and the treatment was repeated every 12 h. Skin wounds in the 4th group were locally treated with linezolid ointment (ZYVOX®) (Pfizer Medical Information-US Company) 2 h post infection, and the treatment was repeated every 12 h.

2.6. Histopathological study

Three mice from each group were euthanized at 1, 3, and 5 days post injury. Skin samples (1 × 2 cm) were immediately fixed for 24 h in 10 % neutral buffer formalin; after that, the samples were routinely processed and sectioned with a microtome (4–6 μm thickness). Hematoxylin and eosin (H&E) was used to stain the slides [27].

2.6.1. Scoring

The parameters of the lesions and the healing process of the skin on each slide were subjected to semiquantitative scoring. The scores for tissue damage and inflammation were 0 for nil, 1 for mild, 2 for moderate, and 3 for severe, while the score for the healing process (epithelial regeneration) was 3 for nil, 2 for mild, 1 for moderate, and 0 for severe. The details are summarized in Table 2.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) (Tukey) and Student's *t*-test were performed to test whether the group variance was significant. Statistical significance was defined as $^{*}p < 0.05$ or $^{**}p < 0.01$. The data are expressed as the mean ± standard deviation, and statistical significance was determined using GraphPad Prism version 9 (GraphPad Software Inc., La Jolla).

3. Results

3.1. Isolation

The identification of colonies was based on colony morphology on mannitol salt agar, blood agar, Gram stain, and catalase tests. Colonies on mannitol salt agar were small (smaller than the typical yellow pinhead colonies of *Staphylococcus aureus*), white, opaque, moist, regular margins, and had a narrow zone of beta-hemolysis around the colonies on blood agar (Table 3) (Fig. 1).

The ID-Gram Positive Cocci cards (ID-GPC cards; bioMeᶜrieux) were used for identification and confirm positive results for all strains with probability (94 %–99 %) (Fig. 2).

The VRSA isolates were resistant to different antibiotics include Ciprofloxacin, gentamicin, cefoxitin, trimethoprim, chloramphenicol, and linezolid (Table 1). All strain isolates were tested for antibiotic susceptibility according to Clinical Laboratory Standards (NCCLs, 2021). A ruler was used to calculate the inhibition zone. All the *S. aureus* isolates were susceptible to 95 % chloramphenicol, 100 % linezolid and 100 % gentamicin, but all the isolates were resistant to 80 % ciprofloxacin, 60 % cefoxitin, and 50 % trimethoprim.

Table (2)
Scoring parameters and severity grades.

Scoring parameters		Severity			
		Nil	Mild	Moderate	Severe
1	Inflammation. (skin): - Dermis	0	1	2	3
2	Neutrophils	0	1	2	3
3	MNCs	0	1	2	3
4	Hemorrhage	0	1	2	3
5	Deg. and necrosis	0	1	2	3
6	Fibrin	0	1	2	3
7	Epithelial regeneration	3	2	1	0
Sum					
Mean = (sum÷8)					
Final score for each group $= \frac{\textbf{sum of means at days}}{\textbf{1 + 3 + 5 (3)}}$					

Table (3)
Prevalence of *S. aureus* (*VRSA*) and *Staphylococcus* spp. isolates.

Source of samples	No. Of Samples	No. *Staphylococcus* spp. (%)	No. of *Staphylococcus aureus* (VRSA)
Skin wounds	50	30 (60 %)	20 (40 %)
Total	50	30	20

3.2. Screening for crude VRSAcin

The bacteriocins produced by these strains showed strong antibacterial activity; their widest inhibition zone, which reached 15–18 mm on isolates among the VRSA isolates from wound infection, was chosen as a good crude bacteriocin producer among all isolates. One of the VRSA isolates was chosen among the other VRSA isolates as the preferable producer of crude VRSAcin, with a wide inhibition zone reaching 15 mm (Fig. 3).

3.2.1. Effect of the incubation temperature

Temperature plays an important role in bacterial growth and is considered an important factor influencing the lag phase of bacterial growth. Moreover, the activity of the bacteriocin strains increased constantly during the exponential phase of growth, and the highest activity was reached by the end of this phase. It was observed from the experiment that maximum production was achieved at 37 °C with an inhibition zone of 18 mm in diameter against the tested bacteria, while the bacteriocin product at 40 °C had a 13 mm inhibition zone, while other temperatures (20, 25, 30) had a minimum inhibition zone of 8, 10, and 12 mm, respectively.

3.2.2. Effect of medium pH

The optimal pH for VRSAcin growth was investigated to cover all the potential possibilities since the normal pH of the stratum corneum is 4.1–7. In addition, physiological gaps, which include the axillae, groin, toe, and anus, exhibit pH values between 6.1 and 7.4; minimum pH

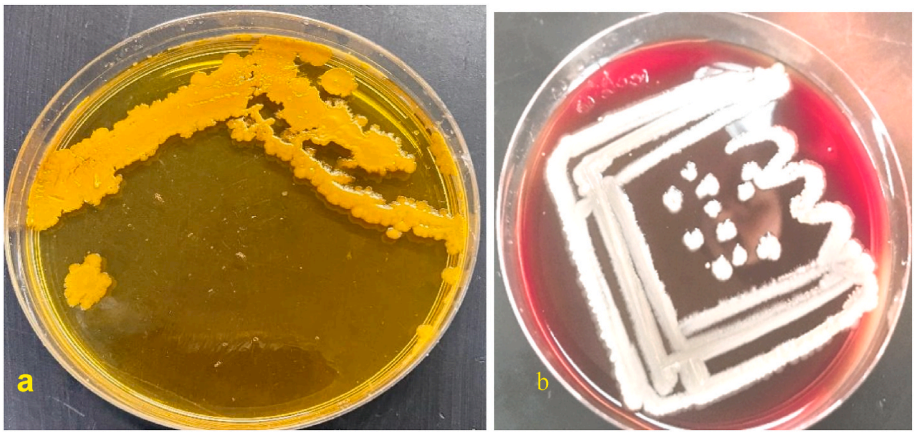

Figure (1). VRSA on (A) MSA (mannitol fermenter isolate) and (B) blood agar at 37 °C for 24 h.

bioMérieux Customer: Microbiology Chart Report Printed October 13, 2024 3:23:18 PM CST

Patient Name: Patient ID:
Location: Physician:
Lab ID: Perynaz 18 Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus (VRSA)

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:** 4.97 hours	**Status:** Final
Selected Organism	96% Probability	**Staphylococcus aureus (VRSA)**
	Bionumber:	050402032763229
ID Analysis Messages		

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	(+)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	+	37	dGAL	-
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Fig. 2. The Vitek 2 System results show different Staphylococcus aureus (VRSA).

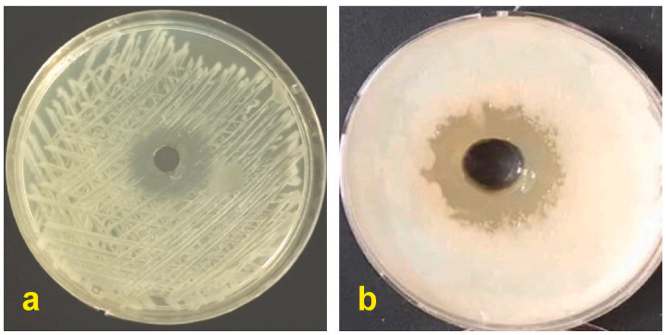

Fig. 3. Screening of crude VRSAcin from VRSA against A) *E. coli* B) *Staphylococcus aureus* on MHA at 37 °C for 48 h.

values between 6.1 and 7.4; and a minimum pH of 7.

3.3. Purification of bacteriocin

The concentrated bacteriocin was successively subjected to ion exchange chromatography (IEC) on a DEAE-cellulose column. The elution profile (Fig. 4) showed one peak between activity and absorption at 280 nm. The maximum bacteriocin activity was detected in fractions 16, 41, and 57. The active fractions were pooled and used for further study. Ion exchange chromatography with DEAE cellulose increased the protein concentration of the bacteriocin to 0.88 mg/ml in the 25.2 mm zone of inhibition (Table 4).

Tricine-SDS-PAGE was used to evaluate the purified Bacteriocin sample as was obtained from various purification stages. The appearance of a single target band (Fig. 5) indicates that the resulting bacteriocin is a single active peptide. The molecular weight of S. haemolyticus was roughly 29 kDa after the gel length and migration distance of the sample had been determined and compared with a protein standard (Biolabs, England; broad range 10–250 kDa).

3.4. Minimum inhibitory concentration of vancomycin

To combat vancomycin-resistant gram-positive infections, researchers are investigating nontraditional antimicrobial agents, such as antimicrobial peptides, bacteriophages, and nanoparticles. To address the growing problem of antibiotic resistance and offer long-term solutions for treating gram-positive infections, these substitutes and cutting-edge tactics have been developed.

Vancomycin is used to treat a variety of diseases caused by gram-positive bacteria because it is particularly effective against these bacteria. Among the most prevalent gram-positive infections in which vancomycin is used to treat skin infection.

A comparative analysis of the inhibition zone diameter of VRSA

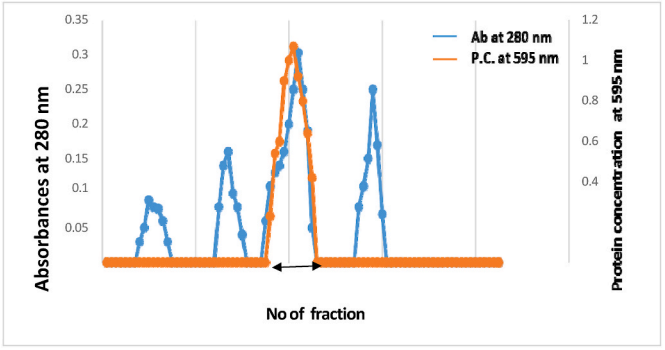

Figure (4). Ion exchange chromatography of VRSAcin using a DEAE-cellulose column (2 × 40 cm).

Table (4)
Purification steps of VRSAcin from VRSA.

Purification step	Volume (ml)	Zones of inhibition (mm)	Protein concentration (mg/ml)
Culture filtrate	400	16	0.32
$^{(NH}4)2^{SO}4$ precipitate	70	17.5	0.65
DEAE-cellulose	30	25.2	0.88

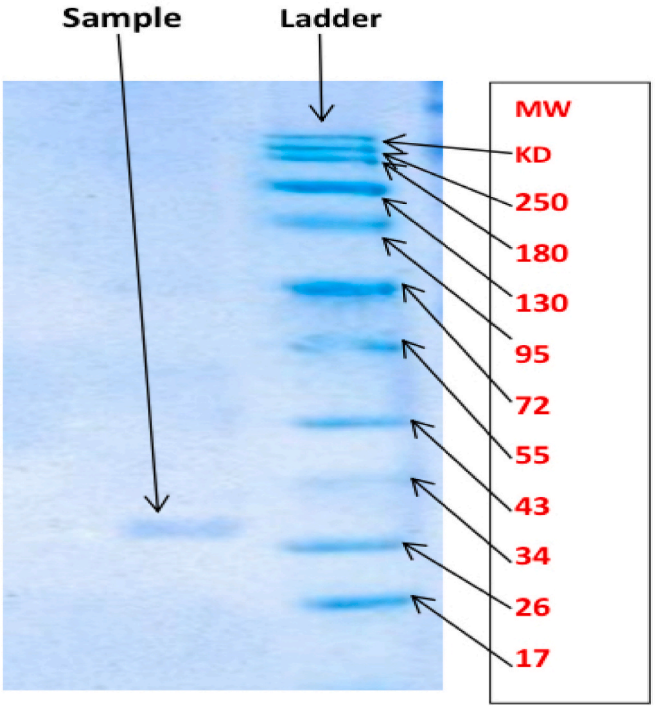

Figure (5). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) of the purified bacteriocin with molecular weight 29 KDa from *S.haemolyticus* (S23) by stain Commassie Brilliant Blue G-250.

following treatment with varying vancomycin concentrations revealed that the lowest concentration (4 μg/ml) and the lowest concentration of *S. aureus* bacteria strongly inhibited each other. Using the well diffusion assay (WDA) method, the inhibitory zone size of 15 mm was widened with increasing concentrations of 32 μg/ml.

3.5. Antimicrobial activity of VRSAcin and vancomycin

The activity of the crude VRSAcin alone or in combination with WDA against different pathogenic bacteria (gram-positive and gram-negative) was recorded. The highest inhibition zone recorded against gram-positive bacteria *(S. aureus)* when tested with bacteriocin reached 25 mm, causing bacteriocin to be more active against a cross-reactive strain than vancomycin is against *Pseudomonas. aeruginosa* reached an inhibition zone of 12 mm, whereas vancomycin reached inhibition zones of 25 and 15 mm, respectively. The statistical analysis revealed a significant difference ($p < 0.05$) in the mean zone of bacterial inhibition after treatment with 32 μg/mL vancomycin compared to that after treatment with VRSAcin. The differences between gram-negative bacteria and *S. aureus* were less significant. (Table 5).

3.6. Histopathology

The lowest score was observed in the positive control group (0.79), followed by the treated group (1.1) and the antibiotic linezolid-treated

Table (5)
Antimicrobial activity of VRSAcin and vancomycin against different bacterial strains.

Bacterial Isolate	Zone of bacterial inhibition in mm (Mean ± SD)		*p* Value
	Vancomycin (32 g/mL)	VRSAcin	
S.epidermidis	18.3 ± 0.4	19.23 ± 0.5	**0.0037** **
S. aureus	15.4 ± 0.4	25.5 ± 0.4	**<0.0001** **
P. aeruginosa	12.2 ± 0.2	15. 3 ± 0.3	**<0.0001** **
Salmonella	15.5 ± 0.6	22.3 ± 0.34	**<0.0001** **
Klebsiella Sp.	17.16 ± 0.21	21.6 ± 0.25	**<0.0001** **
P. mirabilis	12.3 ± 0.3	19.13 ± 0.15	**<0.0001** **
E. coli	19.3 ± 0.31	21.61 ± 0.25	**<0.0001** **

*$p < 0.05$ between groups in a row.

group (1.47), while the VRSA-infected group had the highest score (2.37). The scores of all the groups are summarized in Table 6, while Table 7 showed the comparison between difference groups in Scores of skin response.

Histopathological changes in the positive control group one day post injury revealed mild infiltration of neutrophils in the dermis under necrotic epithelium (Fig. 6a). On day 5, there was slight regeneration of the epidermal epithelium from the edges of the incision site; the new epithelium showed vacuolar degeneration (Fig. 6b); and on day 7, there was an intact new epithelium in the incision site with regular collagen fibers (Fig. 6c). The VRSA group revealed the following results: at 1 day post infection, there was severe necrosis in the dermal layer, which extended to the dermis with loose collagen fibers (Fig. 7a), while on day 5, the necrosis extended to the deep dermis and hypodermis with hemorrhage, neutrophils, MNC infiltration, and multiple abscesses (Fig. 7b). At 7 days post infection, the lesion became more severe, and a large abscess was observed in the dermis and hypodermis (Fig. 7c). In the treated group, on day 1, there was severe necrosis in the epidermis and dermis layers, necrotic tissue sloughed from the skin (Fig. 8a), and mild infiltration of neutrophils in the epidermal layer. On the 5th day of treatment, there was mild regeneration of the epidermis and multifocal aggregation of MNCs, especially around necrotic hair follicles (Fig. 8b), while on day 7, there was complete regeneration of the skin epithelia, which separated the necrotic tissue from the skin, and the dermis showed irregular, dense collagen fibers (Fig. 8C). On day 1 post treatment, the epidermis and upper dermis were necrotic in the linezolid-treated group (Fig. 9a); on day 5, the lesion extended to the hypodermis as multiple abscesses (Fig. 9b); and at 7 days, the lesion was characterized by the presence of a large abscess extending to the dermis and hypodermis (Fig. 9c).

Table 7
Comparison between difference groups in Scores of skin response.

Group	Sum of score	Mean ± SE
Positive control	2.37	0.790 ± 0.16 c
VRSA-infected	7.10	2.37 ± 0.25 a
VRSAcin-treated	3.30	1.10 ± 0.04 bc
Linezolid-treated	4.42	1.47 ± 0.11 b
L.S.D. (P-value)	–	0.439 ** (0.0062)

Means having with the different letters in column differed significantly.
** ($P \leq 0.01$).

4. Discussion

The colony morphology of *S. aureus* in this study was typical of most references, but some strains showed yellow fermented colonies, while others showed whitish pink fermented colonies [28]. The results of the antibiotic susceptibility test coincided with those of [29], who reported that *S. aureus* was resistant to ciprofloxacin (54.2 %) and gentamicin (12.5 %). According to Ref. [30], 37 % and 29 % of strains are resistant to ciprofloxacin and gentamicin, respectively, while according to Refs. [31,32], VRE clones, or vancomycin-resistant enterococci, are becoming a global public health concern. At this time, the only treatment available for Enterococcus-related infections that are resistant to ampicillin and aminoglycoside antibiotics is glycopeptide antibiotics [33]. MRSA is resistant to ciprofloxacin (51 %) and gentamicin (72 %). On the other hand, *S. aureus* and MRSA strains isolated from nonclinical specimens, such as those from cosmetic tools, showed resistance to most antibiotics, such as vancomycin and ciprofloxacin [34]. The results of screening for crude VRSAcin agreed with those of [35], who showed that the antimicrobial activity of VRSAcin disappeared under different laboratory conditions, which indicates that the presence of the indicator strains in culture may induce bacteriocin production. On the other hand, the effects of S-pyocin bacteriocin on acne and fungi have been estimated [36]. Other results showed that *Staphylococcus* spp. isolates were resistant to many antibiotics: 99.99 % of the isolates were resistant to CLR, P, and AMP, while 92.30 % of the isolates were resistant [37]. The effects of incubation temperature and medium pH in other studies revealed that extracellular protease production occurred at optimum temperatures ranging from 25 °C to 55 °C [38], and the optimum temperature for *S. epidermidis* Y73 growth was 37 °C [39]. The optimal pH for VRSAcin growth was investigated to cover all the potential possibilities since the

Table 6
Scores of skin response.

Scoring system	Groups (n = 9 each)[a]											
	Positive control			VRSA-infected			VRSAcin-treated			Linezolid-treated		
	1 d (n = 3)	5 d (n = 3)	7d (n = 3)	1 d (n = 3)	5 d (n = 3)	7d (n = 3)	1 d (n = 3)	5 d (n = 3)	7d (n = 3)	1 d (n = 3)	5 d (n = 3)	7d (n = 3)
Inflam. (skin):												
- **Dermis**	0.7	1.7	1.3	2.7	3	3	1.7	2.3	2	2.3	2.7	**2.3**
- **S/C**	0	0	0	2	2.3	2.6	0	0.6	0.3	0	0.6	**0.6**
Neutrophils	2.2	1.3	1	2.7	3	3	2	1.7	1.3	2.3	2.7	**2.0.3**
MNCs	0.3	1.7	1.4	0.7	2	2.4	0.7	2	1.7	0.7	2	**2**
Hemorrhage	0	0	0	2	2.7	2.2	0.3	0	0	1.3	0.3	**0**
Deg. And Necrosis	2	1	0.3	3	3	3	2.7	1.7	1	2.7	2	**1.3**
Fibrin	0	0	0	1.7	1.2	1.2	0	0	0	1.2	0	**0**
Epithelial regeneration	2.7	1.3	0	3	2.3	2	2.7	1	0.7	2.7	2	**1.3**
Total Sum	7.9	7	4	17.8	19.5	19.4	10.1	9.3	7	13.2	12.3	**9.8**
(sum÷ 8)	0.99	0.88	0.5	2.23	2.44	2.43	1.26	1.16	0.88	1.65	1.54	**1.23**
Mean	2.37			7.1								
							3.3			**4.42**		
Score (Mean ÷ 3)	**0.79**			**2.37**			**1.1**			**1.47**		

[a] G Power >0.999.

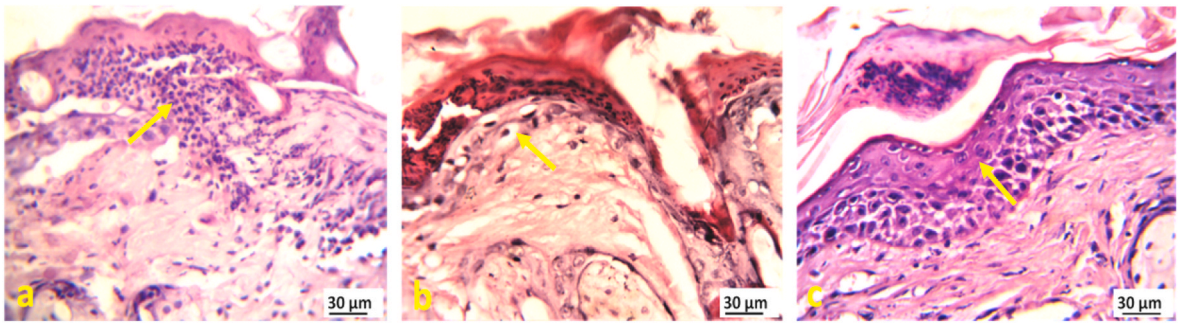

Fig. 6. Skin sections of mice in the positive control group (H&E Stain).
a- Day 1 post injury: mild infiltration of neutrophils in the dermis under the necrotic epithelial layer (arrow).
b- Day 5 post injury, the epidermal epithelium regenerated from the edge of the incision under the necrotic tissue, and the new epithelia showed vacuolar degeneration (arrow).
c-Day 7 post injury: complete regeneration of the dermal epithelium (arrow) under necrotic tissue with mild subepidermal MNC infiltration.

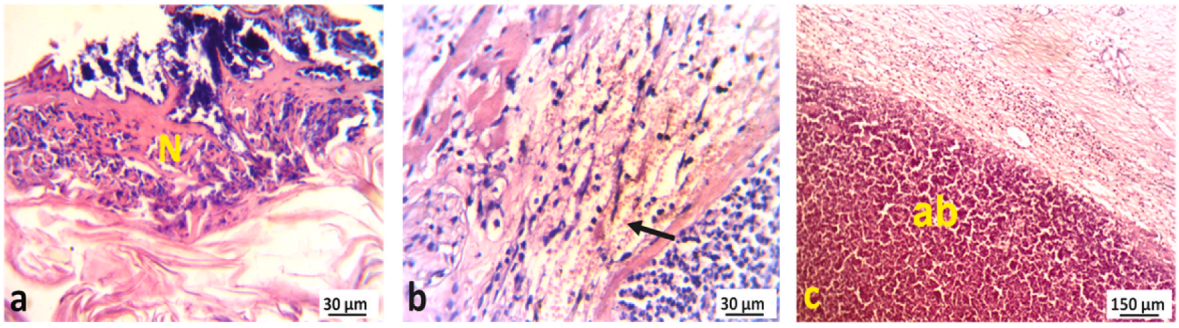

Fig. 7. Skin sections of mice in the VRSA-infected group (H&E stain).
a- Day 1 post infection, severe necrosis (N) of the epidermal layer extended to the dermal layer with loose collagen in the dermis.
b- Day5 post infection: hemorrhage (arrow) in the dermal layer with abscess formation.
c- Day 7 post infection, a large abscess (ab) in the dermis extended to the subcutaneous tissue.

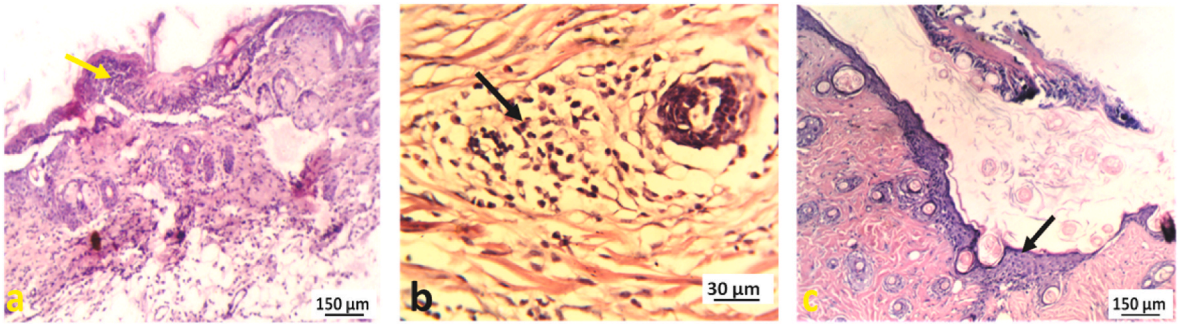

Fig. 8. Skin sections of mice in the VRSAcin-treated group (H&E Stain).
a- Day 1 post treatment, severe necrosis in the epidermis and dermis layers was observed, and the necrotic tissue sloughed from the skin (arrow).
b- Day 5 post treatment: focal aggregation of MNCs around necrotic hair follicles (arrow).
c- Day 7 post treatment, the healed ulcer was characterized by complete regeneration of the epidermal epithelium (arrow) and separation of the necrotic tissue from the skin.

normal pH of the stratum corneum is 4.1–7. In addition, the pH values of physiological gaps, which include the axillae, groin, toe, and anus, range between 6.1 and 7.4. Thus, using such a range is justified by the natural variety of human pH values [40]. Ion exchange (DEAE-Cellulose) features are used in this step due to the synergistic effects of the high-resolution Nisin A bacteriocin from *Lactobacillus* spp. with silver nanoparticles for antibacterial isolation from local food markets [41,42]. The results showed that there was a decrease in the volume of purified salvaricin after treatment with DEAE-cellulose. The maximum activity of MAR-pyocin in the fractions was 14, and the specific activity of these fractions was 1200 AU/mg protein with a 1.2-fold purification and 17 % yield. [43]. According to the histopathological study, the positive control group revealed heavy neutrophils and moderate infiltration of MNCs, indicating a sequence of events that normally proceeded to wound healing. The peak neutrophils count began to decrease 5 days post injury, and these results agreed with previous findings [44].

In the VRSA-infected group, severe damage to tissue can be attributed to virulence factors, which include toxins, enzymes, cell surface proteins, adhesion proteins, and other virulence factors, in addition to the ability of VRSA to evade the immune system [45]. Strains of *S. aureus* produce the PVL protein, which may be responsible for severe tissue necrosis [46]. In addition, α-hemolysin (α-toxin) secreted by strains of

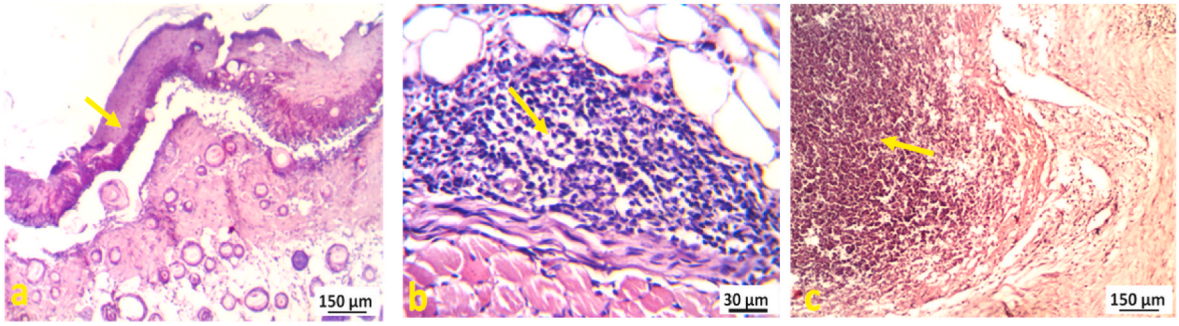

Fig. 9. Skin sections of mice in the linezolid-treated group (H&E stain).
a- Day 1 post treatment: necrotic skin with inflammatory cells sloughed from host tissue (arrow).
b- Day 5 post treatment: abscess in the hypodermis (arrow).
c- Day 7 post treatment: large abscess in the dermis (arrow).

S. aureus may cause superficial dermonecrosis when the lesion extends from subcutaneous tissue [47]. Additionally, abscess formation in the VRSA (induced large abscess), VRSAcin, and linezolid groups revealed that the infection extended to the dermis on day 3 and to the hypodermis on day 7 post wound infection, which may indicate that the recruitment of neutrophils to the infected site may be required to induce an effective immune response. However, other research indicated the same results of abscess formation after inoculation *of wounded skin with S. aureus* and MRSA [48,49]. The localization of skin lesions in the VRSAcin-infected group may be attributed to lysostaphin, which is an *S. aureus* bacteriocin that cleaves the cell wall pentaglycine bridge and kills staphylococcal bacteria [50]. The results of the linezolid-treated group showed moderate to severe damage to the skin, and the effectiveness of this antibiotic was lower than that of VRSAcin. This may suggest several differences between them, including the mechanisms of action and other properties; for example, bacteriocins are more temperature-stable than antibiotics and tolerate extreme pH. [51]. The results from additional research supported the conclusions of a substantial amount of earlier research on the use of bacteriocins as a potent substitute for antibiotics. L. crispatus IS30 cells found in a cream recipe work well against a few common vaginal infections. [52]. The use of active bacteriocin as a probiotic in ointments or emulsion gels may provide a defense against infections of the outer ear. However, medical professionals must be aware of the advantageous characteristics of the bacterial microflora that is naturally present in the human body, such as the outer ear, and their treatment plans should prioritize control over eradication of this microflora [53].

5. Conclusion

There is serious concern about antibiotic resistance in harmful bacteria. Therefore, it is essential to create new therapies for illnesses brought on by bacteria that the antibiotics that are currently on the market cannot eradicate. The fight against germs that are resistant to drugs has benefited from the discovery of bacteriocins. Bacteriocins have been introduced as a possible substitute for these conventional antimicrobial medications due to the sharp increase in antibiotic resistance and the negative consequences that go along with it. VRSA strain infections in burned human skin were found to be more common than expected. An in vivo study in mice indicated that wounded skin infected with VRSA can be treated with VRSAcin as an antibacterial agent that promotes the healing process with obvious superiority to linezolid ointment.

CRediT authorship contribution statement

Ahmed Qassim Al-Awadi: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Mais Emad Ahmed:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Mohammad Y. Alfaifi:** Writing – original draft, Validation, Resources, Investigation, Formal analysis, Data curation. **Ali A. Shati:** Writing – original draft, Validation, Software, Methodology, Investigation, Data curation. **Serag Eldin I. Elbehairi:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mohammed Aufy:** Writing – review & editing, Writing – original draft, Validation, Software, Investigation, Data curation. **Ahmed M. Hussein:** Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Investigation, Data curation, Conceptualization.

Ethics approval

The human part of the current research was approved by the ethics committee of Baghdad Medical City Hospital in Baghdad City and the Collage of Science Research Ethics Committee-University of Baghdad (Approval number: CSEC/0923/0095; Sept. 28, 2023). We confirm that our study compatible with all local national and international regulations governing research involving human subjects. Furthermore, all necessary steps to protect the Patient confidentiality were taken.

The animal part of the current research was approved by the College of Veterinary Medicine Ethics Committee- University of Baghdad (Approval number COVM/710 at April 4, 2024).

Data availability

Data will be made available on request.

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Declaration of competing interest

The authors of this manuscript hereby declare that they have no financial, professional, or personal conflicts of interest that could have appeared to influence the work reported in this paper.

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