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

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Abstract Vancomycin *Staphylococcus aureus* (VRSA) is a strain belonging to *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 compared with an antibiotic (linezolid). The results showed that from fifty swabs obtained from human skin wounds, only 30 samples were identified as *Staphylococcus spp.*, and 20 samples of them were identified as VRSA strains. One isolate was selected for VRSAcin extraction depending on its antibiotic resistance using an antibiotic susceptibility test. The typical conditions for the production of VRSAcin include pH 7 and a temperature of 37°C for 48 hours. 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. Linezolid-ointment-treated skin shows moderate to severe changes. In conclusion, VRSA strain infections in human burned skin were found to be 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.

Keywords: VRSA, Bacteriocin, linezolid, skin, inflammation.

Introduction

Vancomycin-resistant *Staphylococcusaureus* (VRSA) strains belong to *Staphylococcus aureus*, which is characterized by acquired resistance to vancomycin, which is a glycopeptide antibiotic [1]. VRSA resistance is attributed to the plasmid-mediated *vanA* gene and operon [2]. When *S. aureus*

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invades the bloodstream or the internal organs, it may cause a wide spectrum of important infections and is considered a main pathogen for humans. It causes clinical manifestations that extend from 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 (more than 60 years) is vancomycin, which acts by interrupting the continuity of susceptible bacteria cell wall synthesis. The cell wall structure coats the membrane in most bacteria to protect the bacteria from intracellular high osmotic pressure and prevent it from swelling and bursting. Recently, vancomycin has been used for MRSA infections and to treat patients who were allergic to other antibiotics such as cephalosporins or penicillin [4]. The adhesive organism and formation of biofilm were two of the most important factors in its virulence to *Staphylococcus* bacteria, such as Gram-positive bacteria, and they are consistent with the adulteration patterns mentioned therein for negative bacteria like *Proteus mirabilis* [5]. Resistance to vancomycin in VRSA was attributed to the plasmid transposons, which raised the probability of dissemination of medically important bacteria, especially *S. aureus* [6].*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 the growth of bacteria or kill other related or unrelated microorganisms [9, 10]. Bacteriocins can be used in biopreservative applications, especially in an antibiotic role [11]. Micrococci P1 (MP1) and garlic in KS (GarKS) both are 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 aims to determine

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the possible antibacterial and therapeutic effects of VRSAcin on the treatment of skin infections.possible antibacterial and therapeutic effects of VRSAcin on the treatment of skin infections.

Materials and Methods

Phenotypic Identification

Fifty wound samples were collected from Baghdad Medical City in Baghdad, Iraq, and cultured on Mannitol salt agar and blood agar, then incubated for 24-48 hours at 37 °C under aerobic conditions. Suspected colonies were identified morphologically and biochemically [13].

Confirmation of VRSA Isolates

Antibiotic Resistance Test

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

Table (1): Percentage of antibiotic susceptibility for VRSA isolates.

NO.	Antimicrobial agent	Disc conc. µg/disk	Diameter of inhibition zone (mm)		
			Resistant (R)	Intermediate (Int)	Sensitive (S)
1	Ciprofloxacin	5	≤15	16-20	≥22
2	Gentamicin	10	≤12	13-14	≥15
3	Cefoxitin	30	≤21	_	≥21
4	Trimethoprim	10	≤28	_	≥16
5	Chloramphenicol	30	≤12	13-17	≥18
6	Linezolid	30	≤15	_	≥29

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 Soay broth (TSB) (2)% inoculated with 6×10^{8} cells/ml of VRSA then incubated for 24 hrs. at 37 °C under aerobic conditions. Bacteria was harvested in PBS solution and centrifuged at 6000 rpm for 15 minutes [16].

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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, University of Baghdad, Iraq. Crude VRSAcin antibacterial activity was tested using the Agar Well Diffusion (AWD) method on Muller-Hinton agar. [17]

Optimal Conditions for the Production of VRSAcin

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

Effect of pH on VRSAcin

The solution of VRSAcin was mixed with 10 mM of buffered potassium phosphate. The pH value was adjusted from 7 to 12 (one increment pH unit) using 1N NaOH or HCl and incubated for thirty minutes at 37°C.

Temperature Effect:

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

Ion-exchange Chromatography (DEAE-Cellulose)

This method was prepared using DEAE-Cellulose powder, which was suspended in DW. This step was repeated many times until the supernatant became clear and left to settle. After the supernatant was discarded, 0.25N HCl was used for 30 minutes to activate the DEAE-Cellulose, which was then filtrated by a Buchner funnel containing Whatman No. 1 filter paper and packaged in a column with a dimension of 2×40 cm. The absorbance for each fraction was measured at 280 nm, measuring protein concentration. [20] [21] .

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 detected to be the best point for VRSAcin activity. This concentration was measured according to the method of Lowry [22].

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Determination of minimum Inhibition Concentration (MIC) of vancomycin

Using the broth microdilution method, the antibacterial activity of vancomycin was assessed for both gram positive and negative isolates [23].Each Vancomycin (64 µg/ml stock solution) was serially diluted twice in 1 ml of Mueller Hinson 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 additions as the positive control. MIC stands for minimal concentration that inhibited discernible development. According to CLSI (2023) recommendations, each isolate's vancomycin minimum inhibitory concentration (MIC) was determined. The microplate was incubated at 37°C for 24 hr. Bacterial growth was investigated in each well after the optical density was measured at 450 nm using microtiter plate reader. The minimum concentration that prevented noticeable growth was considered as MIC.

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 mouse right flank 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 then, 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 [24]. Wounds in the 2nd group left without any treatment, while wounded skin in the 3rd group was treated with local application of one drop (0.1 ml) of VRSAcin (conc. 64 µg\ ml) two hours post-infection and the treatment was repeated every 12 hours. Skin wounds in the 4th group were locally treated using linezolid ointment (ZYVOX®) (Pfizer Medical Information-US company) after 2 hours post-infection and the treatment was repeated every 12 hours.

Histopathological Study

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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 hours in 10% neutral buffer formalin; after that, samples were routinely processed and sectioned by microtome (4-6 µm thickness). Hematoxyline and eosin stain were used to stain the slides. [25].

Scoring:

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

Table (2): Scoring parameters and grades of severity.

Scoring parameters		Severity			
		Nil	Mild	Moderat	Severe
1	Inflammation. (skin): - Dermis - S/C	0 0	1 1	2 2	3 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 = $(\frac{\text{sum of means at days 1+3+5}}{3})$					

Statistical Analysis

one-way analysis of variance ANOVA (Tukey) and the Student's *t*-test were performed to test whether the group variance was significant or not. Statistical significance was defined as * $p<0.05$ or ** $p<0.01$. Data were expressed as mean±standard deviation, and statistical significance was carried out using GraphPad Prism version 9 (GraphPad Software Inc., La Jolla). ,CA).

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Results

The identification 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 a narrow zone of beta-hemolysis around the colonies on blood agar (Table 3) and (Fig. 1).

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

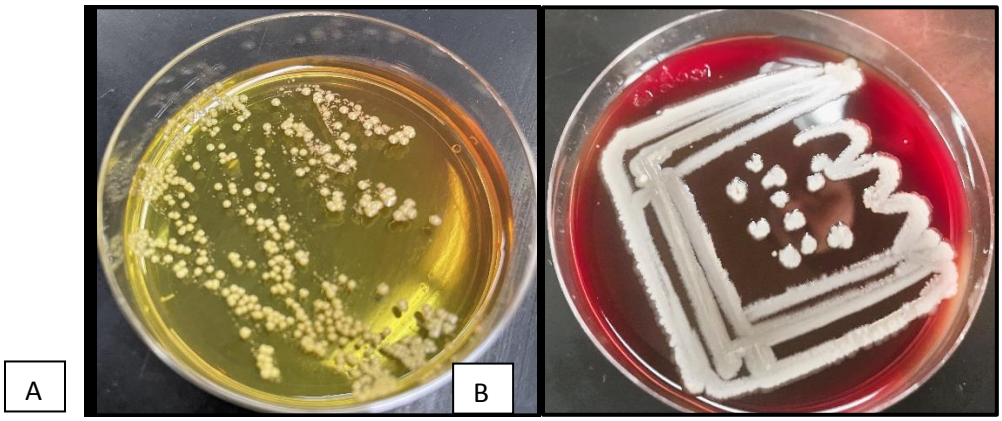

Figure (1): VRSA on (A) MSA (Mannitol fermenters isolate and (B) Blood agar at 37°C for 24 hrs.

The isolates of VRSA showed different antimicrobial agents used. There is resistance to 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. The results showed that all *S. aureus* isolates were susceptible

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to chloramphenicol at 95% and linezolid and Gentamicin at 100%, but that all isolates were resistant to Ciprofloxacin at 80%, Cefoxitin at 60%, and Trimethoprim at 50%.

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 other VRSA isolates as the preferable producer of crude VRSAcin with a wide inhibition zone reaching 15 mm (Fig. 2).

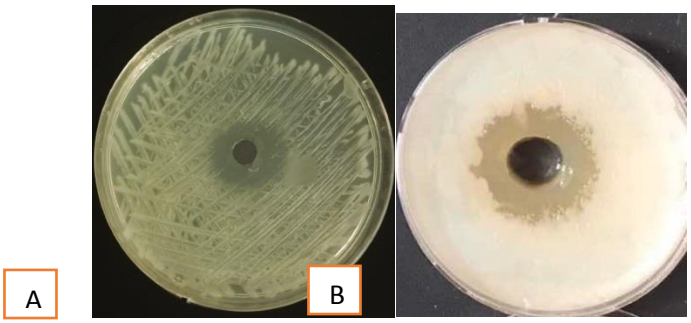

Figure2: Screening of crude VRSAcin from VRSA against A) *E.coli* B) *Staphylococcus aureus* on MHA at 37°C for 48 hrs.

Effect of Incubation Temperature

Temperature plays an important role in bacterial growth; it is considered an important factor influencing the lag phase of bacterial growth. Moreover, the activity of stain bacteriocins 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 has been achieved at 37 °C with an inhibition zone of 18mm in diameter against tested bacteria, while the bacteriocin product at 40°C gave a 13 mm inhibition zone, while other temperatures (20, 25, 30) showed a minimum inhabation zone of 8, 10, and 12 mm, respectively.

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Effect of Medium pH

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

Purification of Bacteriocin

The concentrated bacteriocin was successively subjected to ion exchange chromatography (IEC) on the DEAE-cellulose column. The elution profile (Fig. 3) 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. The ion exchange chromatography with DEAE cellulose enhanced the protein concentration of the bacteriocin to 0.88 mg/ml at zones of inhibition of 25.2 mm (Table 3).

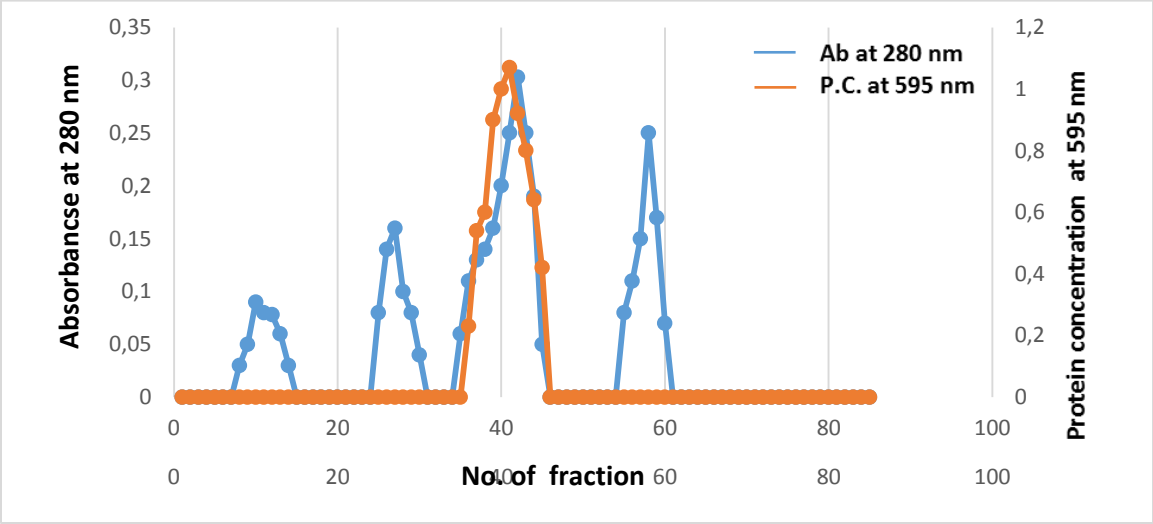

Figure (3): Ion exchange chromatography for VRSAcin using DEAE-cellulose column (2×40cm).

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Table (3): The purification steps of VRSAcin from VRSA

Purification step	Volume (ml)	Zones of inhibition (mm)	Protein concentration (mg/ml)
Culture filtrate	400	16	0.32
$(NH_4)_2SO_4$ precipitate	70	17.5	0.65
DEAE-cellulose	30	25.2	0.88

Minimum inhibitory concentration of vancomycin

In order to battle vancomycin-resistant gram-positive infections, researchers are also investigating non-traditional antimicrobials, such as antimicrobial peptides, bacteriophages, and nanoparticles. In order 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 brought on by gram-positive bacteria because it is particularly efficient against these bacteria. Among the most prevalent gram-positive infections that vancomycin is used to treat are skin infection

Presents a comparative analysis of the inhibition zone diameter of VRSA following treatment with varying vancomycin concentrations the lowest concentration (4 µg/ml) and the lowest concentration *S. aureus* bacteria demonstrated considerable inhibition against each other. Using the Well Diffusion Assay method (WDA), the inhibitory zone size of 15 mm is widened with an increased concentration of 32 µg/ml against test.

Antimicrobial Activity VRSAcin and vancomycin

The crude VRSAcin, whether alone or in combination, recorded activity by WDA against different pathogenic bacteria (gram-positive and gram-negative). The highest inhibition zone recorded against gram-positive bacteria *(S. aureus*) when tested with bacteriocin reached 25 mm, causing bacteriocin to be active against a cross-related type strain compared to vancomycin active against *Pseudomonas. aeruginosa* reached an inhibition zone of 12 mm, whereas vancomycin reached

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inhibition zones of 25 and 15 mm, respectively. The statistical analysis emphasized a significant difference ($p < 0.05$) in the dissemination mean zone of bacterial inhibition after concentrations of vancomycin 32 µg/mL compared **to** VRSAcin. Where it is possible to notice significant differences against Gram-negative bacteria compared to *S. aureus*, the differences were less significant.

Table (5): Antimicrobial activity VRSAcin and vancomycin against different spp bacteria

Bacterial Isolate	Zone of bacterial inhibition in mm (Mean±SD)		*p* Value
	Vancomycin (32 µg/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 **

Different letters (a, b, c, d, e) consider significant differences at $p < 0.05$ between groups in row

Histopathology

The lowest score was seen in the positive control group (0.79), followed by the treated group (1.1) and antibiotic linezolid-treated group (1.47), while the VESA-infected group showed the highest score (2.37). Details of the score in all groups were summarized in Table 7

Table 7: Group score of skin response.

Scoring system	Groups											
	G1 (n=9)			G2 (n=9)			G3 (n=9)			G4 (n=9)		
	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)

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Inflam. (skin): - Dermis - S/C		0.7 0	1.7 0	1.3 0	2.7 2	3 2.3	3 2.6	1.7 0	2.3 0.6	2 0.3	2.3 0	2.7 0.6	2.3 0.6
Neutrophils		2.2	1.3	1	2.7	3	3	2	1.7	1.3	2.3	2.7	2..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		

Histopathological changes in the positive control group one day post-injury showed mild infiltration of neutrophils in the dermis under necrotic epithelial (Fig. 4a). At day 5, there is slight regeneration of epidermal epithelium from the edges of the incision site; the new epithelium showed vacuolar degeneration (Fig. 4b); and at day 7, there is intact new epithelium in the incision site with regular collagen fibers (Fig. 4c).The VRSA group revealed the following: after 1 day post-infection, there is severe necrosis in the dermal layer, which extends to the dermis with loose collagen fibers (Fig. 5a), while on day 5, the necrosis extends to the deep dermis and hypodermis with hemorrhage, neutrophils, MNC infiltration, and multiple abscesses (Fig. 5b). At 7 days post-infection, the lesion became more severe, and a large abscess was seen in the dermis and hypodermis (Fig. 5c). In the treated group, at day 1, there is severe necrosis in the epidermis and dermis layers, necrotic tissue sloughed from the skin (Fig. 6a), and mild infiltration of neutrophils in the epidermal layer. On the 5^{th} day of treatment, there is mild regeneration of the epidermis and multifocal aggregation of MNCs, especially around necrotic hair follicles (Fig. 6b), while on day 7 there is complete regeneration of the skin epithelia, which separates the necrotic tissue from the skin, and the dermis shows irregular, dense collagen fibers (Fig. 6C). Linezolid-treated group at day 1 post-treatment showed necrotic in the epidermis and upper dermis (Fig. 7a); on day 5, the lesion extended to the hypodermis as a multiple abscess (Fig. 7b); and at 7 days, the lesion was characterized by the presence of a large abscess extended in the dermis and hypodermis (Fig. 7c).

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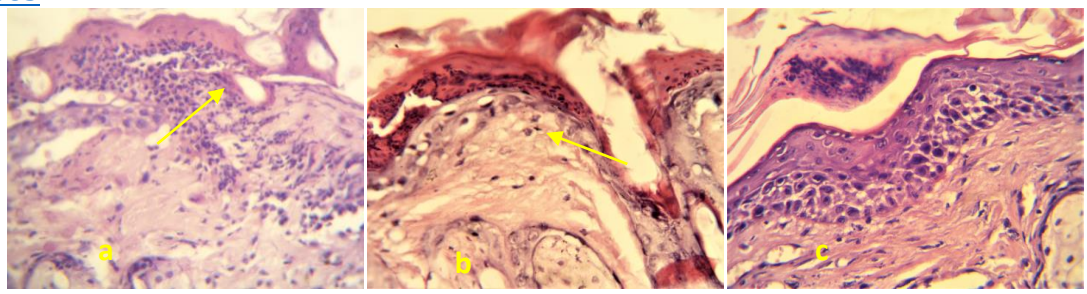

Figure 4: Skin sections of mice in positive control group (H & E Stain):

a- day 1 post injury: mild infiltration of neutrophils in the dermis under necrotic epithelial layer (arrow) (200×).
b- day 5 post injury: regeneration of epidermal epithelium from the edge of the incision under the necrotic tissue, the new epithelia showed vacuolar degeneration (arrow) (200×).
c- day 7 post injury: complete regeneration of the dermal epithelium under necrotic tissue with mild sub epidermal MNCs infiltration (200×).

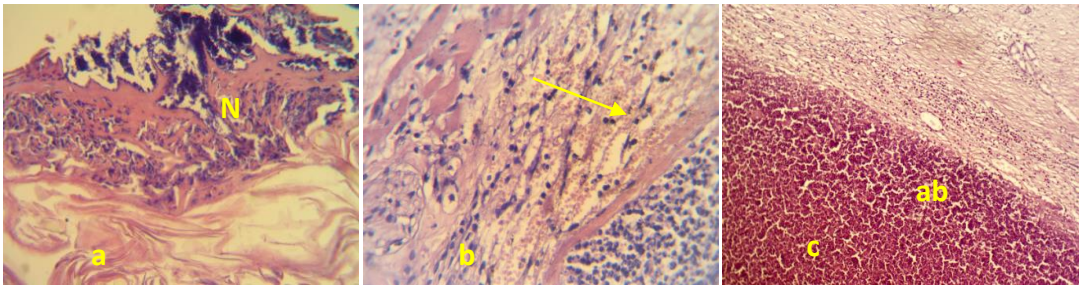

Figure 5: Skin sections of mice in VRSA infected group (H & E Stain):

a- day 1 post infection: severe necrosis (N) of the epidermal layer which extend to the dermal layer with loose collagen of the dermis (100×).
b- day 5 post infection: hemorrhage (arrow) in the dermal layer with abscess formation (200×).
c- day 7 post infection: large abscess (ab) in the dermis extend to subcutaneous tissue (100×).

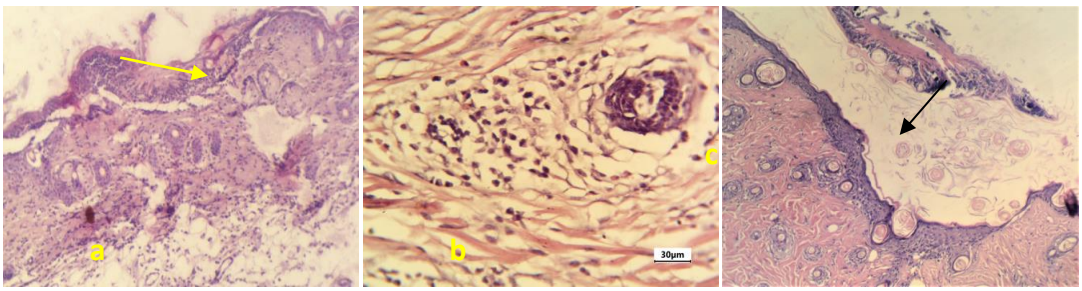

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Figure 6: Skin sections of mice in VRSAcin treated group (H & E Stain):

a- day 1 post treatment: Sever necrosis in the epidermis and dermis layers, the necrotic tissue sloughed from the skin (arrow) (100×).
b- day 5 post treatment: focal aggregation of MNCs around necrotic hair follicles (200×).
c- day 7 post treatment: showed healed ulcer characterized by complete regeneration of epidermal epithelium separate the necrotic tissue from the skin (arrow) (100×).

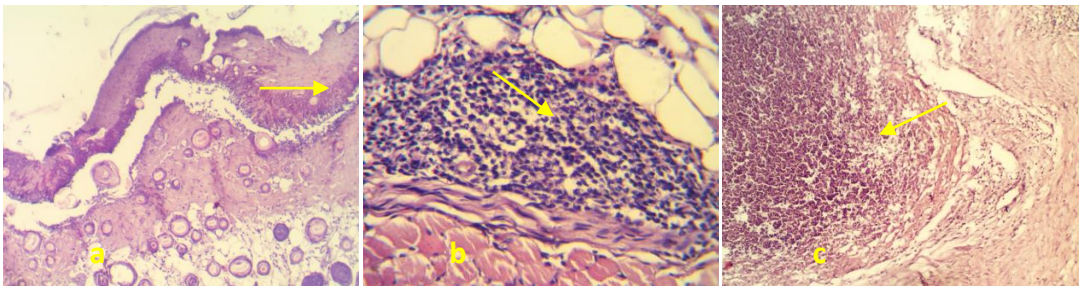

Figure 7: Skin sections of mice in linezolid treated group (H & E Stain):

a- day 1 post treatment: necrotic skin layer with inflammatory cells sloughed from host tissue (arrow) (100×).
b- day 5 post treatment: abscess in the hypodermis (arrow) (200×).
c- Day 7 post treatment: large abscess in the dermis (arrow) (100×).

Discussion:

The colony morphology of *S. aureus* in this study was typical of most references, but some strains showed yellow fermenter colonies while others showed whitish pink fermenter ones [26]. The results of the antibiotic susceptibility test coincided with those of [27], who reported that *S. aureus* was resistant to Ciprofloxacin (54.2%) and Gentamicin (12.5%). According to [28], 37% and 29% are resistant to Ciprofloxacin and Gentamycin, respectively, while according to [29] and [30], 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. [31] MRSA is resistant to ciprofloxacin (51%), and gentamicin (72%). On the other hand, *S. aureus* and MRSA isolated from non-clinical specimens such as cosmetic tools showed resistance to most antibiotics, such as vancomycin and ciprofloxacin [32]. Screening for crude VRSAcin agreed with [33], who showed that the antimicrobial activity disappears under different laboratory conditions, and this indicates that the existence of the indicator strains in culture may induce bacteriocin production. On the other hand, estimation effects of S-Pyocin

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bacteriocin activity on acne and fungi [34]. while other results showed *Staphylococcus spp*. isolates resist many antibiotics: 99.99% of the isolates to CLR, P, and AMP; 92.30% of the isolates [35]. The effects of incubation temperature and medium pH in other studies revealed extracellular protease production at optimum temperatures of 25°C to 55°C [36], and the optimum temperature for *S. epidermidis* Y73 growth was 37°C [37]. The optimal pH for VRSAcin growth was investigated to cover all the potential possibilities since the normal pH value of the stratum corneum is 4.1–7. In addition, the pH values of physiological gaps that include axillae, groin, toe, and anus range between 6.1 and 7.4. Thus, using such a range is justified by the natural variety of the human pH [38]. Ion exchange (DEAE-Cellulose) features used in this step due to high-resolution power, Nisin A" bacteriocin from *Lactobacillus spp* synergistic with silver nanoparticles anti-bacterial isolation from the local food markets [39] [40]. The result showed that there is a decrease in the volume of purified salvaricin when treating DEAE-cellulose. The maximum activity of MAR-pyocin served in the fractions was 14, and the specific activity for these fractions was 1200 AU/mg protein with 1.2 purification folds and 17% yield. [41].According to the histopathological study, the positive control group revealed heavy neutrophils and moderate infiltration of MNCs, and this indicates a sequence of events of normally proceeding wound healing. The peak of neutrophils began to decline 5 days post-injury, and these results agreed with [42].

In the VRSA-infected group, the severe damage to tissue can be attributed to the virulence factors, which include toxins, enzymes, cell surface proteins, adhesion proteins, and other virulence factors, in addition to the capability of VRSA to evade the immune system [43]. Strains of *S. aureus* produce PVL protein, which may be responsible for severe tissue necrosis [44]. In addition, α-hemolysin (α-toxin) secreted by strains of *S. aureus* may cause superficial dermo necrosis when the lesion extends from the subcutaneous tissue [45]. Also, abscess formation in VRSA (induced large abscess), VRSAcin, and linezolid groups revealed that the infection extended to the dermis at day 3, and hypodermis at day 7 post-wound infection 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 S. aureus* and MRSA in wounded skin [46] [47]. The localization

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of skin lesions in the VRSAcin-infected group may be attributed to lysostaphin, which is one of the *S. aureus* bacteriocins that cleaved the cell wall pentaglycine bridge and killed staphylococcal bacteria[48]. The results of the linezolid-treated group showed moderate to severe damage to the skin, and the effectiveness of this antibiotic was lower than the effects of VRSAcin. This may suggest several differences between them, including the mechanisms of action and other properties, such as that bacteriocins are more temperature-stable compared to antibiotics and bear extreme pH. [49]. Results from additional research areas 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. [50]. The active bacteriocin as a probiotic in ointment or emul gel may offer 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. [51]

Conclusion

VRSA strains infection in human burned 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 by VRSAcin as an antibacterial agent that promotes the healing process with obvious superiority to linezolid ointment.

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Author's Declaration

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- Conflict of interest: None.
- We hereby confirm that all the Tables and Figures in this manuscript are ours.
- Authors sign on ethical considerations approval.
- Ethical Clearance: The project was approved by the local ethical committee in College of Veterinary medicine, University of Baghdad.

Author's Contributions

A. Q. Al-awadi performed the experimental part of this research and designed the score system for histopathology. M. E. Ahmed performed the clinical isolation of bacteria and prepared test the VRSAcin in vitro.

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