INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

ANTIBACTERIAL ACTIVITY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* BACTERIOCIN (MRSACIN) AND ITS THERAPEUTIC EFFECTS COMPARED WITH VANCOMYCIN IN EXPERIMENTAL SKIN INFECTION IN MICE

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ABSTRACT
This study aimed to determine the optimum condition of MRSAcin production from Methicillin resistant *Staphylococcus aureus* (MRSA) and compared its therapeutic effects with vancomycin on experimental MRSA skin infection. Bacterial samples from hospital contaminated burned skin were obtained for identification of MRSA. A total of 40 bacterial samples (out of 100) were diagnosed to be *Staphylococcus aureus*, only 33 of them were MRSA. The optimum conditions for MRSAcin production include pH 6, temperature 37°C for 48 hr and inoculums size 6×10^8 cell/ml, while the best stability condition was pH 5-7 and the resistant temperature 50-75 °C for 10-30 minute. *In vivo* study showed that MRSA contaminated wound revealed severe inflammation, necrosis, edema and hemorrhage, the lesion extended to the subcutaneous tissue. Treating the infected skin with MRSAcin lead to localization of the lesion to incision site with complete epithelial regeneration and proliferation of connective tissue, while vancomycin treated group showed severe necrosis, marked inflammatory reaction, and the lesion extended to the S/C tissue. In conclusion local treatment of skin wound infected with MRSA using a crude MRSAcin lead to localization of the lesion and enhanced the healing process compared with vancomycin, which showed less therapeutic effect.

KEYWORDS: MRSA, Bacterocin, vancomycin, skin.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a Gram positive bacterium acquired a gene (mecA gene) that makes it resistant to nearly all beta-lactam antibiotics. Resistance to other antibiotics is also common especially in hospital-associated MRSA. This organism is serious nosocomial pathogen, and finding an effective treatment can be a big challenge. Strains of community-associated MRSA are originated outside hospitals, while these organisms have generally been easier to treat, some strains have transferred into hospitals and become more resistant to drugs other than beta-lactams (Chaudhary *et al*., 2017; Hussein, 2016). Strains of MRSA are usually resistant to wide spectrum of antibiotics and also intrinsic resistance to ß- lactam antibiotics, it shows a particular ability to spread in hospitals and now present in many countries including Iraq (Hussein, 2016). The most important risk factors for the hospital acquisition of MRSA include prolonged hospitalization that stay in an intensive care unit, chronic diseases such as malignancy and chronic renal failure, surgery, prior exposure to antibiotics and contact with a patient colonized or infected with MRSA (Abdulgader *et al.*, 2015). Bacteriocins are peptides natural released by different varieties of bacteria and archea that are active against other bacteria and the producer has a specific immunity mechanism (Dobson *et al.*, 2012). Bacteriocins which have inhibitory effects towards sensitive strains are produced by both Gram positive and negative bacteria, Bacteriocins from lactic acid bacteria are used as food preservative like in dairy products. It has a useful use in health care products and cosmetics for treatment of acne, also being used in toothpaste to inhibit dental caries and in mouthwashes for the periodontal diseases (Indira *et al.*, 2011). *S. aureus* produce a wide variety of inhibitory substances including Staphylococcin, which showed very potent activity against many clinical isolates of *Mycobacterium tuberculosis*, many antimicrobial substances produced by staphylococci have been categorized as true bacteriocins (Coelho *et al.*, 2014). Vancomycin is an antimicrobial agent worldwide used in the treatment of MRSA infection (Levine, 2006). Recently, decreasing vancomycin susceptibility among *S. aureus*, the appearance of non-susceptible MRSA strains and the frequent vancomycin failure in treatment of MRSA infection regardless of the minimum inhibitory concentration of isolate, provides evidence of the need for more effective therapies and therapeutic approaches (Choo and Chambers, 2016). However, the current study aim to determine the *in vitro* and *in vivo* antibacterial effect of crude MRSAcin produced by MRSA and compared their therapeutic effects with vancomycin.

MATERIALS & METHODS

Clinical isolates

One hundred clinical samples of bacterial isolates from hospital contaminated burns wound at Al-Yarmouk

Teaching Hospital and Baghdad Teaching Hospital (Baghdad-Iraq) were obtained during the period November 2015 to January 2016 in order to search for *S. aureus*.

Diagnosis of *S. aureus* isolates

All samples cultured on mannitol salt agar and incubated at 37ºC for 24 hr and then colony morphology, Gram stain reaction and biochemical characteristics (ie: Oxidase reagent, Coagulase, Catalase reagent) was examined to diagnose *S. aureus* and MRSA strain was confirmed by vitek 2 system.

Antibiotic Resistance Test

Antibiotic resistance test was performed on Mueller-Hinton agar plates at 37 °C for 18 hr in the presence of 10 different types of antibiotics disc including Methicillin (5 µg), Oxacillin (1 µg), Clindamycin (15 µg), Tetracycline (30 µg), Erythromycin (15 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Penicillin G (10 µg) and Vancomycin (30 µg) (Bioanalyse®, USA). The zone of inhibition (in mm) around each disk was documented and compared with a standard interpretive chart (Clinical and Laboratory Standards Institute) (Wright, 2014).

Extraction of Bacterocin (MRSAcin)

MRSA isolate which showed the widest inhibition zone in antibiotic resistance test was selected to produce and extract of MRSAcin. The isolates cultured on Tryptic Soay broth (TSB) (2)% inoculated with 6×10^8 cell/ml of MRSA and incubated at 37 °C for 24 hours under aerobic conditions (Ali, 2010). Cells were harvested by centrifugation at 6000 rpm for 15 minutes, the cell-free supernatant was referred to as crude MRSAcin extract which heated to 80° C for 10 minutes, then cooled and centrifuged at 6000 rpm for 15 minutes (Powell *et al.*, 2007) followed by filtration of the supernatant through a 0.2 µm pore-size nylon syringe filter. The supernatant was stored at -20 °C.

MRSAcin activity assay

The crude MRSAcin activity was tested by detect its inhibitory effects on the growth of indicator organisms such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (indicator bacteria was obtained from Department of Biology, College of Science, University of Baghdad, Iraq). The antibacterial activity of crude MRSAcin was examined using agar well diffusion (AWD) and spot disc method.

Bacteriocin concentration

The best activity for MRSAcin was detected at a concentration of 62.50 µg\ml, the concentration determined according Lowry *et al.* (1951) method.

Effect of pH on MRSAcin

MRSAcin solution was mixed with 10 mM potassium phosphate buffer and the pH values was adjusted to ranging from 2 to 12 (at increments of one pH unit) using NaOH (1 N) or HCl (1N) and incubated at 37°C for 30 minutes.

Effect of Temperature

Samples of MRSAcin solution were exposed to different temperature degrees (20, 25, 30, 37 and 40)°C respectively for 30 minutes, also the activity tested at 121°C for 15 minutes. The protein concentration in each sample was measured before and after the temperature test using Lowry *et al.* (1951).

Experimental design

Seventy mice were divided into 5 groups, the mice in the 1st, 2nd, 3rd and 4th groups (n=15 for each group) were anesthetized with an intraperitoneal injection of a mixture of xylazine (5 mg/kg) and ketamine (75 mg/kg), then the hair of the right flank was shaved (3×2 cm) using electrical shaver and the remaining hair was shaved using disposable hand shaver. The shaved area was cleaned by soap and sterile D.W., after drying skin wound was induced using sterile lancet in which 3 parallel line of superficial skin wound was made. The 5th group (n=10) considered as control negative group.

The mice in the 1st group were considered as positive control group and the injured skin did not receive any treatment, while the injured skin of mice in the 2nd, 3rd and 4th group was contaminated by MARS using one drop containing 0.5×10^6 cfu/ml (McPherson *et al.*, 2005). Infected skin of mice in the 2nd group did not receive any treatment. The injured skin of mice in the 3rd group was treated locally with a 0.1 ml drop of MRSAcin (conc. 62.50µg\ ml) after 2 hrs of infection and the treatment repeated every 12 hr, while mice in the 4th group treated locally with a vancomycin ointment (Elbe Pharma Nig. Ltd company) after 2 hr post infection and treatment repeated every 12 hr.

Histopathological Study

Five mice were euthanized from each group at 24, 48 and 72 hr post injury and samples (1×2 cm) of injured skin were taken and fixed immediately in 10% formalin solution for 48 hrs, then the samples were processed routinely and sectioned by microtome (thickness 4-6 micron) and the slides stained by Hematoxyline and Eosin stain (Presnell and Schreibman, 1997). For scoring the microscopic lesions, the current study depends on parameters and semiquantitative scoring system designed by Kugelberg *et al.* (2005) with modifications, as following: for scoring of the inflammation (in the dermis and S/C tissue, separately): 0, no inflammation present; 1, little inflammation present; 2, moderate inflammation present; and 3, severe inflammation present. For scoring of the presence of neutrophils: 0, no neutrophils present; 1, a few neutrophils present; 2, moderate occurrence of neutrophils; and 3, abundant occurrence of neutrophils. For scoring of the presence of MNCs: 0, no MNCs present; 1, a few MNCs present; 2, moderate occurrence of MNCs; and 3, abundant occurrence of MNCs. For scoring of the presence of necrosis: 0, no necrosis; 1, mild necrosis; 2, moderate necrosis; and 3, severe necrosis. For scoring of the presence of fibrin: 0, no fibrin deposition; 1, little fibrin deposition; 2, moderate fibrin deposition; and 3, abundant fibrin deposition. For scoring of the presence of Epithelial regeneration: 0, complete regeneration; 1, moderate regeneration; 2, little regeneration; and 3, no regeneration. For scoring of the presence of hemorrhage: 0, no hemorrhage;1, mild hemorrhage; 2, moderate hemorrhage; and 3, severe hemorrhage.

Statistical analyses

The Statistical Analysis System - SAS (2012) program was used to analyze the difference between groups. Least

significant difference –LSD test (ANOVA) was used to significant compare between means in this study.

RESULTS

Identification of clinical isolates

Antibiogram of bacterial isolation from hospital indicates that from 100 sample, 40 (40%) bacterial isolate diagnosed as *Staphylococcus aureus* and only 33 (82.5%) out of 40 were diagnosed as MRSA strain. The diagnosis of MRSA isolates was confirmed using Vitek 2 system; all 33 isolates gave positive result with probability of 98-99%. MRSA isolates were resistant to all tested antibiotics especially Erythromycin, Gentamicin, Cefoxitin and Chloramphenicol, while it is sensitive to vancomycin (Table 1).

TABLE 1: Antibiotic susceptibility test of *S. aureus*

Antibiotic	Resistance No. (%)	Intermediate No (%)	Sensitive No. (%)
Methicillin	33 (82.5%)	1 (2.5%)	6 (15%)
Oxacillin	33 (82.5%)	3 (7.5%)	4 (10%)
Clindamycin	31 (77.5)	2 (5%)	7 (17.5%)
Penicillin G	30 (75%)	3 (%7.5)	7 (17.5%)
Tetracycline	30 (75%)	3 (%7.5)	7 (17.5%)
Erythromycin	28 (70%)	0 (0%)	12 (30%)
Gentamicin	27 (67.5%)	3 (% 7.5)	10 (25%)
Cefoxitin	27 (67.5%)	2 (5%)	11 (27.5%)
Chloramphenicol	26 (65%)	4 (10%)	10 (25%)
Vancomycin	17 (42.5%)	2 (5%)	21 (52.5%)

Screening for crude MRSAcin

One of the MRSA isolate was selected among other MRSA isolates as the best producer of crude MRSAcin according to their wide inhibition zone that reached 15 mm when tested on the basic indicator bacteria (Fig. 1).

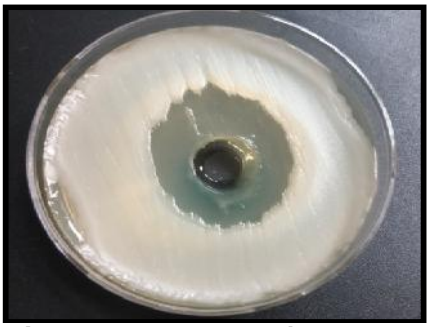
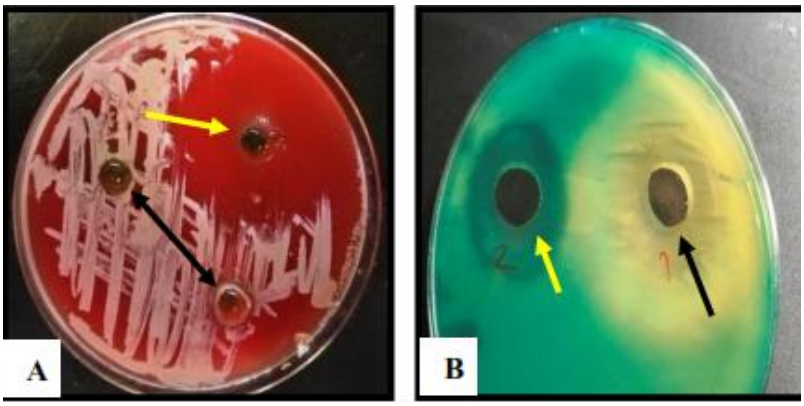


FIGURE 1: Screening of crude MRSAcin against *E. coli* on Nutrient agar at 37° C for 24-48 hrs.

Comparison between antimicrobial activities for crude bacteriocin using agar methods

Comparing the two methods (WDA and spot disc methods) used in this research, both showed antibacterial activity of crude MRSAcin against indicator bacteria of *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Fig. 2), the WDA method give higher diameter of inhibition zone ranged between 12 to15 mm in which is significantly ($p<0.05$) higher when compared with spot disc diffusion methods in which inhibition zone ranged between 5-6 mm (Fig. 3).



FIGRURE 3: Antibacterial activity of MRSAcin (WDA method) for 24-48 hr at 37°C
A. *S. aureus* on mantiol salt agar, control well (black arrow), crude MRSAcin (yellow arrow).

B. *P. aeruginosa* on nutrient agar, control well (black arrow), crude MRSAcin (yellow arrow).

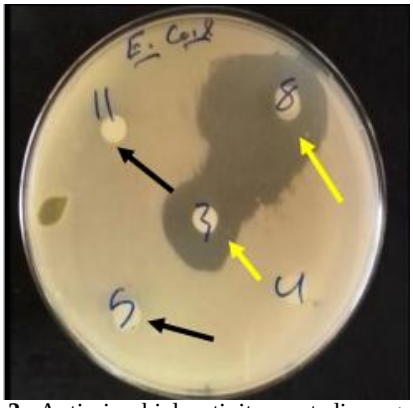


FIGURE 3: Antimicrobial activity spot disc agar method against *E. coli* in Mueller Hinton agar for 24-48 hrs at 37 °C, control disc (black arrow), MRSAcin disc (yellow arrow).

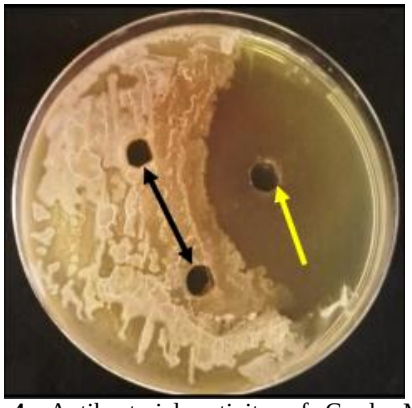


FIGURE 4: Antibacterial activity of Crude MRSAcin using 70% ammonium sulphate, control (black arrow), MRSAcin (yellow arrow).

Precipitation of MRSAcin

Crude MRSAcin was heated to denaturant any proteases and heat-sensitive proteins. Ammonium sulphate 70% was used to precipitation bacteriocin to obtain partial purification and increased the diameter of inhibition zone compared with crude bacteriocin. The inhibition zone diameter reached 17 mm (Fig. 4).

PH Stability of Crude MRSAcin

The activity of crude MRSAcin in different pH ranges showed that crude MRSAcin was stable and remained active at pH value ranged between 2 to 7, while the optimum pH was 6 which achieved the maximum antibacterial activity of crude MRSAcin, and the bacteriocin completely loss its antibacterial activity below 1 and above 8 (Fig. 5).

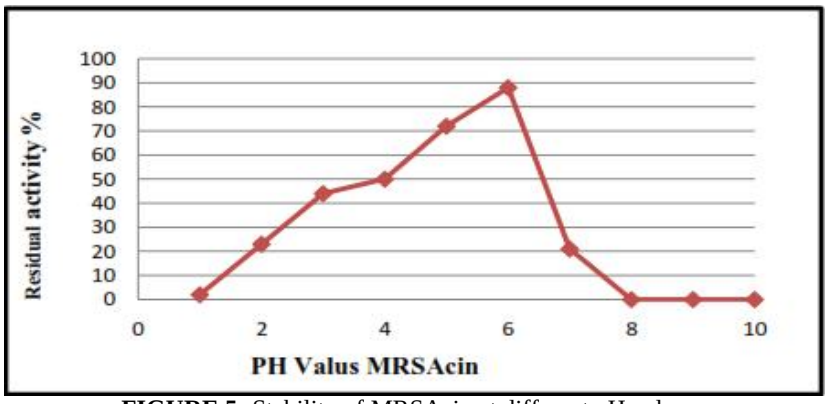


FIGURE 5: Stability of MRSAcin at different pH values.

Thermostability for MRSAcin

Thermostability for crude MRSAcin was assayed at different temperatures. As shown in (Fig. 6), the MRSAcin is resistant to temperature ranged from 50 to 75 °C for 10 and 30 minute, while MRSAcin loss its themostability at 100°C for 10 minutes.

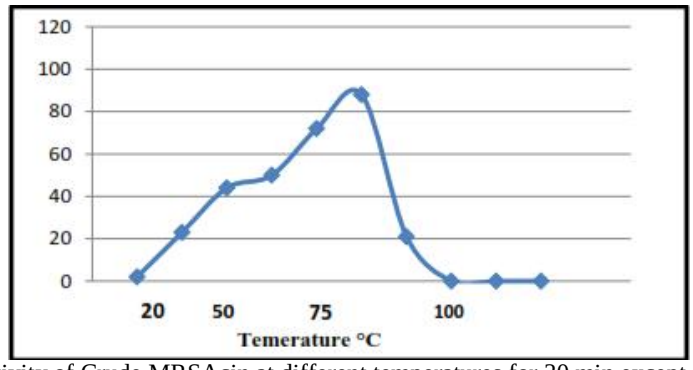


FIGURE 6: Residual activity of Crude MRSAcin at different temperatures for 30 min except at 121 °C for 15 minutes.

Histopathology

The score system showed that the 1st group (positive control) revealed the lowest score among other groups followed by the 2nd (MRSAcin) and the 3rd (vancomycin) groups, respectively, while the 2nd (MRSA infected) group showed the highest score. The scores of the skin response in different groups were summarized in table 2.

TABLE 2: Groups score of skin response.

Scoring system	Groups											
	G1 (n=15)			G2 (n=15)			G3 (n=15)			G4 (n=15)		
	24h (n=5)	48h (n=5)	72h (n=5)	24h (n=5)	48h (n=5)	72h (n=5)	24h (n=5)	48h (n=5)	72h (n=5)	24h (n=5)	48h (n=5)	72h (n=5)
Inflam. (skin):												
- Dermal	1.4	1.6	1.4	3	3	3	2.6	2.2	2	2.6	2.6	2.4
- S/C	0	0	0	1.2	2	2.6	0	0.4	0.6	0	0.4	0.6
Neutrophils	1.4	2.2	2.2	2.6	2.6	3	2	2.2	2	2.4	2.6	2.2
MNCs	0.8	1.2	1.4	0.8	1.4	2.4	0	1.2	2	0.4	1.8	2.2
Hemorrhage	0	0	0	1.8	2.2	2.2	0	0	0	0	0	0
Deg. and necrosis	2	2	1.8	3	3	3	2.4	2	1.8	2.8	2.6	2.2
Fibrin	0	0	0	1.2	1.2	1.6	0	0	0	0.4	0.8	1.2
Epithelial regeneration	3	1.8	0.4	3	3	3	3	2	0.8	3	2.6	2.6
Total Sum	8.6	8.8	7.2	16.6	18.4	20.8	10	10	9.2	11.6	13.4	13.4
(sum÷8)	1.08	1.1	0.9	2.08	2.3	2.6	1.25	1.25	1.15	1.45	1.68	1.68
Mean		3.08 c			6.98 a			3.65 c			4.81 b	
Score (Mean÷3)		1.03 c			2.33 a			1.22 c			1.60 b	
LSD value	0.362 **											

** (P<0.01). Means with the different letters in row differed significantly.

The histological changes in the 1st group (control positive) revealed the following, at 24 hrs post injury, there is necrotic debris of epithelial layer (epidermis) in the injured area with few neutrophils in the dermis layer (Fig. 7A), while at 48 hr post injury, there is signs of regenerated epithelia under the necrotic tissue, also there is moderate neutrophils infiltration between the collagen fibers in the dermis layer (Fig. 7B). At 72 hr post injury, there is almost complete regeneration of the epithelial cells of the epidermis with mild infiltration of neutrophils in dermis layer (Fig. 7C).

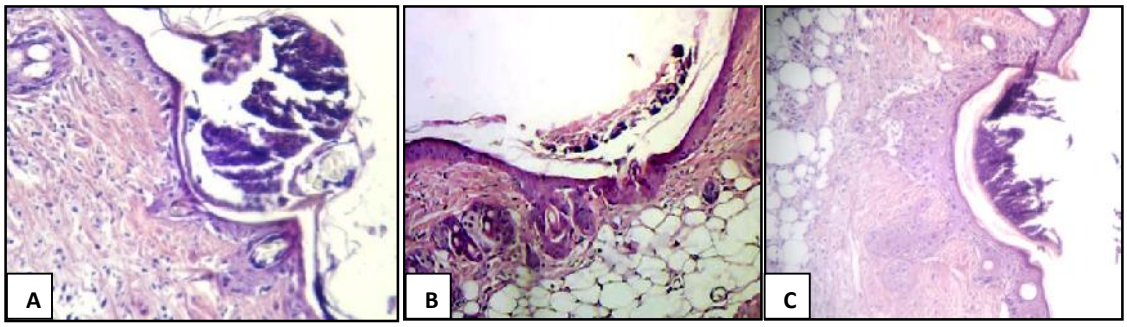


FIGURE 7: Histopathological section in the skin of mouse in group 1 (control positive) (H & E Stain)

A. 24 hrs post injury: necrotic debris of epithelial layer (epidermis) in the injured area with few neutrophils in the dermis layer (200X).
B. 48 hr post injury shows narrowing incision site with contact of epithelial cells from each edge of incision under cellular debris (100X).
C. 72 hrs post injury: complete regeneration of the epithelial cells of the epidermis with mild infiltration of neutrophils in dermis layer (100X).

The histopathological changes in skin of 2nd group (MRSA infected) showed the following, after 24 hr there is edema, hemorrhage and neutrophils infiltration in the subcutaneous tissue (Fig. 8A), in addition, other sections showed deposition of fibrin network in the incision site and neutrophils infiltration extended to the S/C tissue. After 48 hours, the previous changes became more severe; in addition, there is necrotic tissue in the incision site with neutrophils and MNCs infiltration extended to dermal layer (Fig. 8B). At 72 hours, the lesion was very severe, in which the edema and neutrophils infiltration became more abundant in the dermis layer (Fig. 8C).

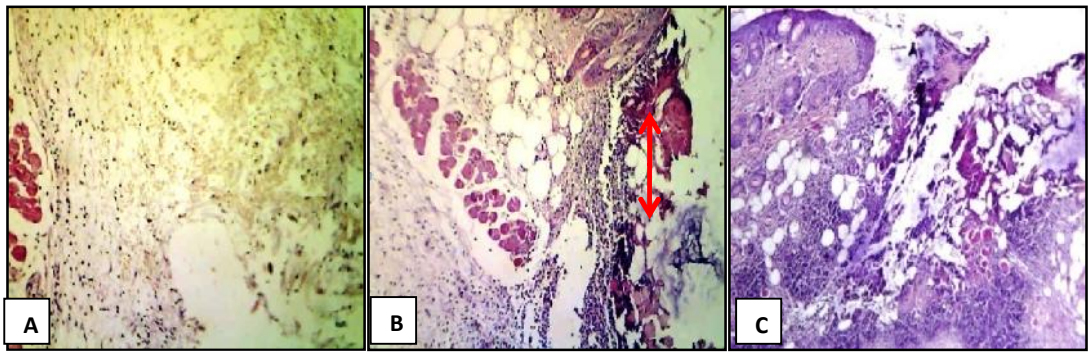


FIGURE 8: Histopathological section in the skin of mouse infected with MRSA (H & E stain):

A. 24 hr post infection: edema, hemorrhage and neutrophils infiltration in the submucosal layer (400X).
B. 48 hr post infection: necrotic tissue in the incision site (arrow) with neutrophils infiltration extended to dermal layer (100X).
C. 72 hr post infection shows cellular debris and neutrophils infiltration and abscess in the incision site, dermis and S\C tissue with abscess formation (100X).

In the 3rd group (infected MRSA and treated with MRSAcin), after 24 hr there is necrotic tissue with neutrophils infiltration in the incision site (Fig. 9A) and dermal layer, while after 48 hr the lesion revealed necrotic tissue with neutrophils infiltration in the incision site with moderate edema and inflammatory cells infiltration in the dermal and subcutaneous tissues with initiation of epithelial cell proliferation (Fig. 9B). At 72 hr the skin showed moderate mononuclear cells infiltration under complete regeneration of epidermal layer, the lesion did not extended to the adipose tissue (Fig. 9C). Other section showed thickness of epithelial layer under necrotic tissue with edema and few to moderate mononuclear cells infiltration in the dermal layer with connective tissue proliferation in the dermal layer and adipose tissue.

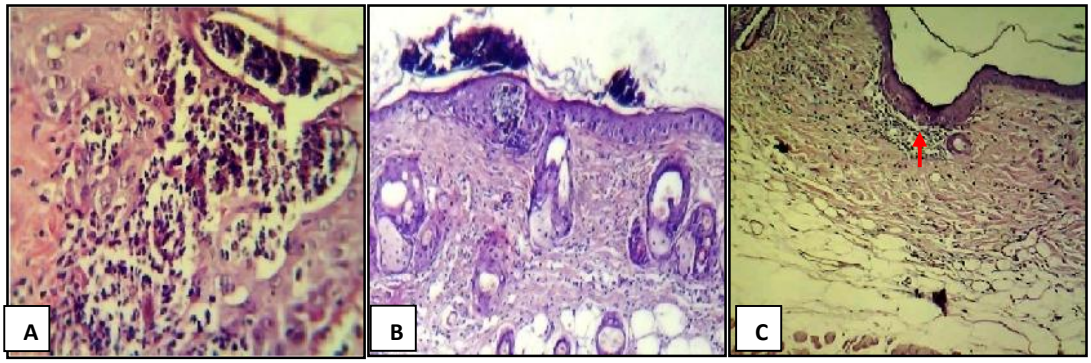


FIGURE 9: Histopathological sections of mice skin infected with MRSA and treated with MRSAcin showed (H& E Stain):

A. 24hrpost treatment: the incision site filled with inflammatory cells and cellular debris.(400X).
B. 48 hrs post treatment: necrotic tissue with neutrophils infiltration in the incision site with moderate edema and inflammatory cells infiltration in the dermal layer with initiated proliferation of epithelial cells (200X).
C. 72 hrs post treatment: mononuclear cells infiltration under complete regeneration of epidermal layer (arrow) without extended to adipose tissue (100X).

In 4th group (infected with MRSA and treated with vancomycin), after 24 hr there is severe necrotic tissue with marked neutrophils infiltration in the incision site (Fig. 10A), and after 48 hr the neutrophils infiltration seen in the dermal layer and reached the muscular region (Fig. 10B). At 72 hrs post treatment the skin showed severe necrotic tissue with marked neutrophils infiltration in the incision site, dermis and S\C tissue (Fig. 10C) with formation of granulation tissue.

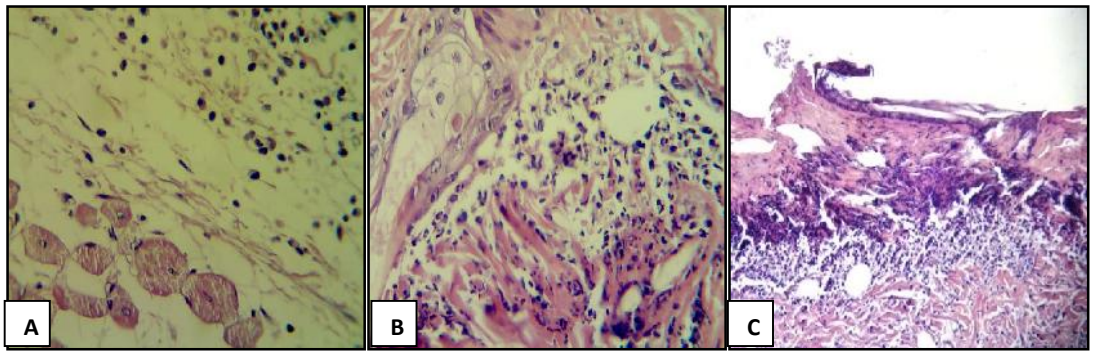


FIGURE 10: Histopathological sections of mice skin infected with MRSA and treated with vancomycin showed (H& E Stain):

A. 24 hrs post treatment: severe necrotic tissue with marked neutrophils infiltration in the incision site (400X).
B. 48 hr post treatment: severe neutrophils infiltration in the dermal layer reach muscular region (400X).
C. 72 hr post treatment: severe necrotic tissue with marked neutrophils infiltration in the incision site, dermis and S\C tissue (100X).

DISCUSSION

Hospital samples of contaminated burn wound revealed that 40% of the isolates were *S. aureus* and this refer to increase in the percentage of this bacterium in hospitals compared with previous study in Iraq in which only 24.4% of the hospital bacterial isolates were *S. aureus* (Alwan *et al.,* 2011). In addition, MRSA was resistant to most tested antibiotic except vancomycin and this contraindicated the results of Alwan *et al.,* 2011 whom mention *S. aureus* was less resistance to chloramphicol 28.57% among 65% in present study. Other study showed no (0%) resistance of MRSA to vancomycin and high resistance to gentamicin 100%, oxacillin (or cefoxitin) 100%, Erythromycin 100%, clindamycin 97.22% (Kaur and Chate, 2015). The variation in the results with other studies may be due to the unique ability of *S. aureus* to respond to new antibiotic with the development of a resistance mechanism, starting from penicillin and methicillin until the most recent antibiotics (Pantosti *et al.*, 2007).

The present results agree with Rahimifard and Naseri, 2016 who compared between 3 antimicrobial activity methods (spot on lawn, well diffusion and disk diffusion) using *Bifidobacteria infantis* and *Bifidobacteria bifidum* as probiotic bacteria against *Salmonella enterica* serotype *Enteritidis* and they found that the well diffusion assay (WDA) is the best method to identify the antagonism of bacteriocin of microorganisms compared with other methods. The results of antimicrobial activity of MRSAcin against indicator bacteria *S. aureus*, *E. coli* and *P. aeruginosa* in spot disc diffusion methods ranged between 5-6 mm, while study of Kaur *et al*. (2015) showed that the antibacterial activity of the bacteriocin of Lactobacillus strains showed inhibition zone reached 12-15 mm against indicator bacteria (*S. aureus*, *E. coli* and *P. aeruginosa*), also Sharma and Srivastava (2014) found that plantaricin peptides had anti-candida activity and the inhibition zone reached 9mm. According to the present study partial purification of bacteriocin by 70% ammonium sulphate increase the antibacterial activity of bacteriocin, a fact mentioned previously when antimicrobial activities of pyocin increased against food-spoiling bacteria and food-borne pathogens after partial purification by 70% ammonium sulphate precipitation (Naz and Rasool, 2013).

The current results resample the results of the pyocin SA188 which is active at pH range from 4 to 10 (Naz and Rasool, 2013) also bacteriocin from Lactobacillus murinus AU06 still 100% active between pH 6-7 and retained 70% of activity at pH 4 and the effect of tempterture revealed that the bacteriocin was stable between 30-80 °C and retained more than 60% of its activity at 60 °C for 30 min and decreased afterwards (Elayaraja *et al*., 2014). Another important results showed by Wladyka *et al*. (2013) in which bacteriocin BacCH91 from *S. aureus* CH-91 showed complete bactericidal activity at pH 3-6, while pH 7 and higher causes loss of the bactericidal activity until pH 11 which showed complete inactivation of the bacteriocin, also they found that BacCH91 loss its activity when incubation at temperatures higher than 60°C for several hours, and maintained its activity about 1 hour at at temperatures more than 80 °C. the inactivation induced by temperature may related to the polypeptide chain hydrolysis as seen by HPLC and ESI-MS analyses (Wladyka *et al*. 2013).

The results of *in vivo* study, in the 1stgroup revealed that the first phase of normally proceeding wound healing is marked by the influx of inflammatory cellular infiltrate consisting of PMNs and macrophages under a fibrin plug. The control wounds group showed good influx of inflammatory cells, which consisted first mostly of PMNs. As expected, the number of PMNs peaked in the control wounds during the first 3 days, but after 72 there is decrease in neutrophils in tissue with few macrophages revealed a normal wound healing process and these results agreed with He *et al.* (2003).

In addition, the current results suggest that the complete regeneration of the epithelial cells and` the presence of fibrin plug which seen in this group especially at 72 hr post injury was a part of the skin healing process especially in immune component animal.

In the 2ndgroup (infected with MRSA), severe tissue damage (when compare with G1), including edema, hemorrhage and tissue necrosis, and these changes can be attributed to the highly pathogenic bacteria (MRSA) which known to be a highly resistance and capable to evade the

immune system as well as their virulence factors (enzymes, toxins, adhesion proteins, and cell surface proteins) which causes toxic affect (Chipolombwe *et al*., 2016), for example Labandeira-Rey *et al*. (2007) suggested that *S. aureus* strain express PVL protein caused necrotizing pneumonia and the current result may suggested the same idea to explain the severe necrosis in the skin, also the heavy infiltration of neutrophils in the infected area which extend to the dermis at 72 hrs post wound infection may suggest that neutrophils recruitment to the site of infection is required for an effective immune response against *S. aureus* in and during the first 24 hr, skin wounds of mice inoculated with MRSA developed large neutrophilic abscesses (Mölne *et al.*, 2000). According to the previous facts, the current results agreed with Kugelberg *et al.* (2005) who mentioned that superficial skin wound inoculated with *S. aureus* showed pronounced acute inflammatory response included most layers of the skin.

The histopathological changes in the 3rd group (infected with MRSA and treated with MRSAcin) showed that the lesion localized to incision area and better prognosis comparing with G2 with complete epithelial regeneration and C.T. proliferation at 72 hr post treatment and this may attribute to the *S. aureus* bacteriocin in which Lysostaphin may kill staphylococcal bacteria by cleaving the pentaglycine bridges in the cell wall, Lysostaphin has attracted renewed attention as a viable antimicrobial therapy in the light of increasingly prevalent multi-drug resistance (Kumar, 2008).

The result of the 4th group showed severe damage to skin tissue and the lesion extend to the S\C tissue with minimum healing process when compared with group 3, this unexpected results (since *in vitro* study showed that the bacterium was sensitive to vancomycin) contraindicated other previous studies in which vancomycin was very effective drug in systemic treatment (intravenous injection) of MRSA in horses (Orsini *et al.*, 2005), and subcutaneous or oral administration in mice (Guo *et al.*, 2013).

In conclusion, Treatment of MRSA infected skin with MRSAcin limited the skin damage and localized the lesion to the incision site with good healing process (relatively resemble normal healing process) compared with vancomycin treatment which failed to limit the infection and the lesion spread from the epidermis to the dermis and subcutaneous tissues.

REFERENCES

Abdulgader, S.M., Shittu, AO, Nicol, M.P., Kaba, M. (2015) Molecular epidemiology of Methicillin- resistant *Staphylococcus aureus* in Africa: a systematic review. Frontiers in microbiology. 2015;6:34810.3389/ fmicb. 2015. 00348.

Ali, W.S. (2010) "Production, purification and characterization of plantaricin from local strains of Lactobacillus plantarum", Ph.D thesis, College of Science, University of Baghdad.

Alwan, M.J., Lafta, I.J., Hamzah, A.M. (2011) Bacterial isolation from burn wound infections and studying their antimicrobial susceptibility. Kufa Journal For Veterinary Medical Sciences. 2(1).

Chaudhary, D., Subhash, A., Galvis, J., Guardiola, J. (2017) Bilateral thigh methicillin-resistant *Staphylococcus aureus* necrotising fasciitis in a man with newly diagnosed Human Immunodeficiency Virus (HIV). BMJ Case Rep. 2017. pii: bcr2016218468. doi:10.1136/bcr-2016-218468.

Chipolombwe, J., Török, M.E., Mbelle, N., Nyasulu, P. (2016) Methicillin-resistant *Staphylococcus aureus* multiple sites surveillance: a systemic review of the literature. Infection and drug resistance. 9:35-49.

Choo, E.J. and Chambers, H.F. (2016) Treatment of Methicillin-Resistant Staphylococcus aureus Bacteremia. Infection & chemotherapy, 48(4):267-73.

Coelho, M.L., Coutinho, B.G., da Silva Santos, O.C., Nes, I.F., de Freire Bastos, M.D. (2014) Immunity to the Staphylococcus aureus leaderless four-peptide bacteriocin aureocin A70 is conferred by AurI, an integral membrane protein. Research in microbiology, 165(1):50-9.

Dobson, A., Cotter, P.D., Ross, R.P., Hill, C. (2012) Bacteriocin production: a probiotic trait?. Applied and environmental microbiology.78(1):1-6.

Elayaraja, S., Annamalai, N., Mayavu, P., Balasubramanian. T. (2014) Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. Asian Pac J Trop Biomed, 4:s305-s311.

Guo, Y., Ramos, R.I., Cho, J.S., Donegan, N.P., Cheung, A.L., Miller, L.S. (2013) In vivo bioluminescence imaging to evaluate systemic and topical antibiotics against community-acquired methicillin-resistant Staphylococcus aureus-infected skin wounds in mice. Antimicrobial agents and chemotherapy, 57(2):855-63.

He, Z., Ong, C.H., Halper, J., Bateman, A. (2003) Progranulin is a mediator of the wound response. (2003). Nature medicine, 9(2):225-9.

Hussein, N.R. (2016) Prevalent Genotypes of *Staphylococcus aureus* Strains Isolated from Healthcare Workers in Duhok City, Kurdistan Region, Iraq. International Journal of Infection, 3(2). e35375.

Indira, K., Jayalakshmi, S., Gopalakrishnan, A., Srinivasan, M. (2011) Biopreservative potential of marine Lactobacillus spp. African Journal of Microbiology Research, 5(16):2287-96.

Kaur, D.C. and Chate, S.S. (2015) Study of antibiotic resistance pattern in methicillin resistant Staphylococcus aureus with special reference to newer antibiotic. Journal of global infectious diseases, 7(2):78.

Kaur, S., Kaur, H.P., Kanchan (2015) Evaluation of Antibacterial Activity of Bacteriocin Produced from

Lactobacillus sp. Isolated from Rhizosphere Soil. Int J Pure Appl Biosci, 3:136-142.

Kugelberg, E., Norström, T., Petersen, T.K., Duvold, T., Andersson, D.I., Hughes, D. (2005) Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes*. Antimicrobial agents and chemotherapy, 49(8):3435-41.

Kumar, J.K. (2008) Lysostaphin: an antistaphylococcal agent. Applied microbiology and biotechnology, 80(4): 555-61.

Labandeira-Rey, M., Couzon, F., Boisset, S., Brown, E.L., Bes, M., Benito, Y., Barbu, E.M., Vazquez, V., Höök, M., Etienne, J., Vandenesch, F. (2007) *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. Science, 315(5815):1130-3.

Levine, D.P. (2006) Vancomycin: a history. Clinical Infectious Diseases, 42(1):S5-12.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. (1951) Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193(1):265-75.

McPherson, S., Ellis, R., Fawzi, H., Panter, S.J. (2005) Postoperative methicillin-resistant Staphylococcus aureus enteritis following hysterectomy: a case report and review of the literature. European journal of gastroenterology & hepatology, 17(11):1225-7.

Mölne, L., Verdrengh, M., Tarkowski, A. (2000) Role of neutrophil leukocytes in cutaneous infection caused by *Staphylococcus aureus* Infection and immunity, 68(11): 6162-7.

Naz, S.A. and Rasool, S.A. (2013) Isolation, Production and Characterization of Bacteriocin Produced by strains form indigenous environment. Pak. J. Bot., 45:261-267.

Orsini, J.A., Snooks-Parsons, C., Stine, L. (2005) Haddock M, Ramberg CF, Benson CE, Nunamaker DM. Vancomycin for the treatment of methicillin-resistant staphylococcal and enterococcal infections in 15 horses. Canadian journal of veterinary research, 69(4):278.-286.

Pantosti, A., Sanchini, A., Monaco, M. (2007) Mechanisms of antibiotic resistance in Staphylococcus aureus. Future microbiology, 2(3):323-34.

Powell, L.M., Slater, S., Mirtcheva, D., Bao, Y., Chaloupka, F.J. (2007) Food store availability and neighborhood characteristics in the United States. Preventive medicine, 44(3):189-95.

Presnell, J.K., Schreibman, M.P., Humason, G.L. (1997) Humason's animal tissue techniques. Johns Hopkins University Press.

Rahimifard, N. and Naseri, M. (2016) Evaluation and comparison of three antimicrobial activity methods using Bifidobacteriabifidum and Bifidobacteria infantis as probiotic bacteria against *Salmonella enterica* serotype Enteritidis. J. Bacteriol. Mycol., 2:00024.

Sharma A. and Srivastava S. (2014) Anti-Candida activity of two-peptide bacteriocins, plantaricins (Pln E/F and J/K) and their mode of action. Fungal biology, 118(2):264-75.

Wladyka, B., Wielebska, K., Wloka, M., Bochenska, O., Dubin, G., Dubin, A., Mak P. (2013) Isolation, biochemical characterization, and cloning of a bacteriocin from the poultry-associated Staphylococcus aureus strain CH-91. Applied microbiology and biotechnology, 97(16):7229-39.

Wright, G.D. (2014) Something old, something new: revisiting natural products in antibiotic drug discovery. Canadian journal of micro biology, 60(3):147-54.