RESEARCH ARTICLE

The Comparative Study Among the MRSAcin, Nisin A and Vancomycin, on Biofilm Formation by Methicillin Resistance *Staphylococcus aureus* Isolated from Food Sources

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ABSTRACT

Biofilms formation by pathogens microbial Control considered important in medical research because it is the hazarded virulence factor leading to becoming difficult to treat because of its high resistance to antimicrobials. Glycopeptide antibiotic a (Vancomycin) and the commercial bacteriocin (Nisin A) were used to comparative with purification bacteriocin (MRSAcin) against MRSA biofilm. One hundred food samples were collected from Baghdad markets from July 2016 to September 2016, including (cheese, yogurt, raw milk, fried meat, grilled meat, and beef burger). All samples were cultures; *S. aureus* was confirmation by macroscopic culture and microscopic examination, in addition to biochemical tests. Methicillin resistance *S. asureus* (MRSA) were identification by antibiotic sensitivity test (AST), Vitek 2 system. The result shown the 60(60%) isolate were identified as *S. aureus* and 45(75%) gave positive result as MRSA isolate, M13 isolate was chosen as MRSA isolates highest biofilm formation for treatment with MRSAcin, Nisin A(bacteriocin) and Vancomycin (antibiotic) to compared the more antimicrobial have bacteriocidal effect. The sensitivity test uses to determine the effect of MRSAcin, Nisin A, and Vancomycin MIC on MRSA planktonic cell by (WDA). The new study shows the impacts of new kind Pure Bacteriocins (MRSAcin) from methicillin-resistant *S. aureus* (MRSA) highly effects then (Vancomycin and Nisin A) at different concentration. In a current study aimed to suggest new Bacteriocin is potent highly for the treatment of resistant bacteria biofilm infections in food preservatives.

Keywords: Bacteriocin, Biofilm, MRSA, Nisin, Vancomycin.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) have a gene that makes for resistant to most all (beta-lactam antibiotics) and resistance to other type's antibiotics is also common, especially in hospital-associated MRSA.[1] A biofilm is a community of (Bacterial and Fungal) that adheres to solid surfaces also (biotic and abiotic), in a biofilm, cells form a cluster and aggregation embedded in a self-produced (extracellular or intracellular) matrix consisting of bio-molecules, such as (polysaccharides, nucleic acids, proteins, and lipids.[2] In biolm bacterial cells mostly resistance to the immune host's and antibiotic treatments, chronic or relapsing Biofilm-associated infections; therefor successful prevention and treatment of these infections are significant issues.[3] (*S. aureus* and *Pseudomonas aeruginosa*) are amongst the most dominant pathogens isolated from nosocomial infections. Exo-polysaccharides (EPS) increase the production of Biofilm formation.[4] The number of cases of antibiotic resistance in recent years increases in has encouraged scientists to found alternative therapeutic options.[5] There is some teixobactin, as the relatively recent discovery, which exhibits activity against (gram-positive pathogens) including methicillin-resistant (MRSA) and mycobacteria which has a mode mechanism inhibition of peptidoglycan synthesis.[6] One option bacteriocins to compensate for the dearth of novel antibiotics to introduce as therapeutic options in clinical cases are ribosomally-synthesized antimicrobial peptides produced by many types bacteria and can exhibit narrow spectra of activity (targeting members of the same species), whereas others display broader activity spectra (targeting other species and genera).[7] Some lantibiotics significantly have been shown to possess activity against (antibiotic-resistant) targets such as (VRE)

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vancomycin-resistant enterococci and MRSA.[8] Classified Bacteriocins are broadly into class I and class II groups. The most studied lantibiotics extensively subclass of bacteriocins, which includes (staphylococcin C55, nisin, mersacidin, and lacticin) amongst others, Several lantibiotics exhibit potent activity against clinically relevant and food-borne pathogens.[9] Bacteriocins strong activity their target strains, often in the nanomolar range, rendering them more potent than their antibiotic counterparts in certain cases. Thus, bacteriocins on their own have potential for use in clinical applications.[10] In this review, we outline to compared among the MRSAcin with Nisin A ans Vancomycin against MRSA and suggest use MRSAcin as a food bio preservative.

MATERIAL AND METHODS

Isolates and Culture media

One hundred food samples were collected from Baghdad markets from July 2016 to September 2016, including (cheese, yogurt, raw milk, fried meat, grilled meat, and beef burger). All samples were primary cultured on Baird parker agar (BPA), and purification on Mannitol Salt agar (MSA) were incubated at 37Cº for 24 h and identification using macroscopic examination, microscopic examination and biochemical characteristics (Coagulase, Catalase reagent, Oxidase reagent and β-hemolysis).[11]

Confirmation of MRSA by Antibiotic Susceptibility Testing

All presumptive *S. aureus* isolates were confirmed as MRSA by AST. The cultures were grown on Muller Hinton Agar (MHA) plates and incubated at 37 °C for 18 hrs with the following antibiotics: Penicillin G (PG,10), Cefotaxiitin (FOX, 30), Clindamycin (CD, 2), Nitrofurantoin, (NI, 300), Ciprofloxacin, (CIP, 5) ,Oxacillin (OX, 10), Erythromycin E (15) , Gentamycin, GN (10) Tetracycline, T (30) and Vancomycin, VA (30). The inhibition zone around disk were recorded and compared with the Clinical and Laboratory Standards Institute.[12]

VITEK 2 System

All presumptive *S. aureus* isolates confirmation using vitek 2 system which automatically performs each one of the steps needed for identification and AST after first inoculums has been performed and standardized.[13]

Biofilm formation screening for MRSA isolates

All MRSA isolates that confirmation by AST and Vitek 2 system were biofilm formation screening according to[14]

Purification of MRSAcin (125) µg\ mL

Obtained of Purification Bacteriocin by two-step included (Ion exchang and Gel filtration) from (College of Science / University of Baghdad).[15]

Nisin A powder

Nisin A was obtained from Cayman Chemical Company a commercial preparation Nisin A (Sigma Nisaplin 2/5%) was performed through dissolved with 100mg from Nisin A powder by 10 ml HCl (0.02 N) to give10^4 IU/ml (40 IU=1 g) concentration. After that, the solution was passed throughout 0.45 filters to sterilization then was maintain at-20.[16]

Prepared Vancomycin powder stock solution:

Described by Andrews[17] The Vancomycin stock suspension (50 mg/ml) was performed via solute 0.5g from vancomycin powder in 10ml D.W.

Determination of the minimum inhibitory concentration (MIC) of Nisin A, Vancomycin using Agar Well Diffusion Assay (AWD)

Determination the bacteriocidal effect of the Nisin A, vancomycin and MRSAcin on MRSA planktonic cell using AWD method was applied according to.[18]

Comparative the bacteriocidal effect of purified MRSAcin with different concentration of Nisin A, and Vancomycin on the MRSA biofilm.

- The effect of pure MRSAcin, Nisin A, and Vancomycin against MRSA biofilm formation was tested using (MT) method according to.[19] The sterile 96-well flat bottomed plastic plate, the first line filled with 200 µl of broth only without bacteria, contains 1% glucose as a negative control. And the second well line filled with 200l from MRSA suspension only as control positive. Then MRSA isolates suspensions were putted 200L in three lines from the plate with for treatment. Next, the plate was covered and put in aerobic condition to incubated to (18–24) hours at 37. After that, an unattached content was removed, washed three times via 250 µl of sterile phosphate buffer saline, filled the wells by 200 µl from different concentrations of Nisin A, Vancomycinand 200µl from pure MRSAcin except the control negative and control positive. After 24 hrs The treated were washed three times with sterile D.W. times. Stain the plate with 200 µl 2% C.V. placing the plate under influx tap water to dye elimination. Left the plate was air-dried. Finally day solute by 96% (v/v) from ethanol and incubated to at 30 to 15 min. then optical density (OD) was measured by using automated Stat fax ELISA reader at 492 nm.

RESULT AND DISCUSSION

Isolation and Identification *S. aureus*: the result recorded 60(60%) from 100 food samples as *S. aureus* isolates were confirmed by microscopic examination after culture on BPA, and purification on MSA which appeared cream colony surround with yellow halo zone presumptive *S. aureus* isolates .Thus, this medium was considered as selective and a differentiated medium to *Staphylococcus spp.*also the 60(60%) isolates gave positive result to catalase and coagulase test but appeared negative to oxidase test. While the isolates on Blood agar showed, yellow-gray colonies are (4-3) mm in diameter on the zones of β-hemolysis. This description is mentioned by[20] (Figure 1).

Identification of MRSA

The result showed 45(75%) from 60 (60%) *S. aureus* gave positive results as MRSA by AST and Vitek 2 System. The

Figure 1: *S. aureus* A) On Mannitol Salt Agar B) Blood Agar at 37°C for 24 hrs.

result appeared the high significant in (P <0.05) between cheese and dairy product samples, while non-significant in (P <0.01) among other samples. Table 1

Antibiotic susceptibility test (AST) of *S. aureus*

Figure 2 show various levels of susceptibilities to different antibiotics among isolates that were observed by the Disk diffusion method. Out of 60 (60%) of *S. aureus* isolates 45(75%) isolates were multi-resistance for antibiotics with the highest level against Oxacillin, Cefoxitin, Penicillin G and Erythromycin reached to 54 (90%), 53 (88.3%), 50 (83.3%) and 22 (36.6%),respectively, while the sensitivity antibiotics were vancomycin, Nitrofurantoin Clindamycin. Ciprofloxacin 51(85%), 45(75%), 45(75%) and 40(66.6%), respectively, also appear resistance level to Tetracycline and Gentamycin 15(25%) and 11(18.3%), this result was similar to that obtained by[21] reported multi-antibiotic resistance were appeared against β lactam antibiotics as PG, OX, FOX, and E, while seemed to be high sensitivity toward VA, CIP, CD and NI, against MRSA isolates from food samples.

Screening of MRSA biofilm formation:

Microtiter plate (MTP) method were used to screening the MRSA ability to biofilm formation the result shown different positive results to have ability for biofilm formation ranged from moderate to highest strong biofilm category at 24(53%), while 21(46%) gave weak biofilm formation. The highest strong biofilm isolate (M13) were choose to treated with Nisin A, Vancomycin and MRSAcin.

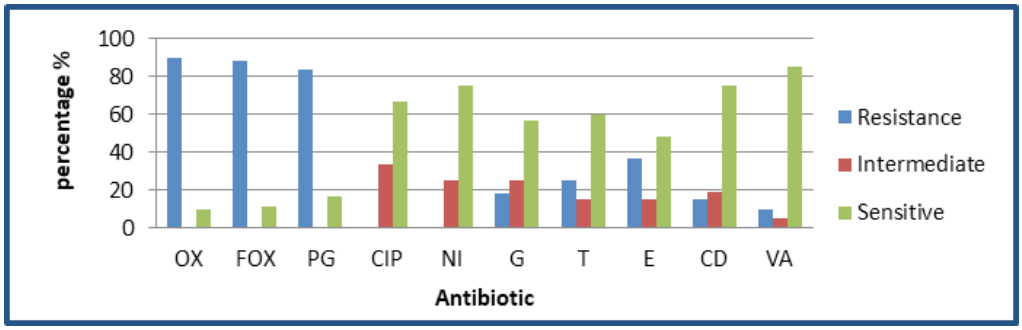

Figure 2: Antibiotic susceptibility test of *S. aureus*

Table 1: Prevalence of MRSA isolates among Food Samples

Sample type	*No. Sample*	*No. isolate*	*No. (%) of S. aureus*	*No. (%) of MRSA*	*Chi-squaree value*
Cheese	20	16	(26.7)	11(18.3)	4.39 *
Dairy product	20	15	(25)	10 (16.7)	4.55 *
Row milk	20	17	(28.3)	15 (25)	1.07 NS
Ice cream	10	2	(3.3)	1(1.7)	0.84 NS
Fried meat	10	3	(5)	3(5)	0.00 NS
Grilled meat	10	3	(5)	3(5)	0.00 NS
Borger	10	4	(6.7)	2 (3.3)	1.18 NS
Chi-squaree value	---	---	8.91 **	8.56 **	–

* (P<0.05), ** (P<0.01), NS: Non-Significant.

Sensitivity test for determination the MIC of Nisin A, Vancomycin and MRSAcin against MRSA planktonic cell

Nisin A:

For detection MRSA sensitivity toward nisin A was used WDA. Different concentration of nisin A the result recorded in Table (2), Figure (3). The result agree with[22] investigated that Nisin was activity against MRSA strain with.

Vancomycin and MRSAcin:

For measure, MRSA sensitivity toward vancomycin MIC was used WDA. The current study found a higher inhibition zone diameter (24mm) against M13 strain comparative to Nisin A, vancomycin.The results were shown no significant ($P < 0.05$) among MRSA planktonic cell treatment. Table (2), Figure (4). The present result agrees with[23] were report vancomycin's intermediate and nonsusceptible against two MRSA isolates. Also, the present study acceptable with the previous study (15) was investigated the crude MRSAcin effective agent wide range of microorganisms involving Gram pathogeneses.

Comparative the bacteriocidal effect of purified MRSAcin with different concentration of Nisin A, and Vancomycin on the MRSA biofilm

The antibiofilm activity of (MRSAcin, Vancomycin, and Nisin A) at different concentration was carried out by using microtiter plate method, the results showed Purified MRSAcin at 125 µg/ml more affected against MRSA biofilm then Vancomycin and Nisin A. the result showed non-significant different at ($P < 0.05$) among MRSA biofilm treated. Table (3), Figure (5).

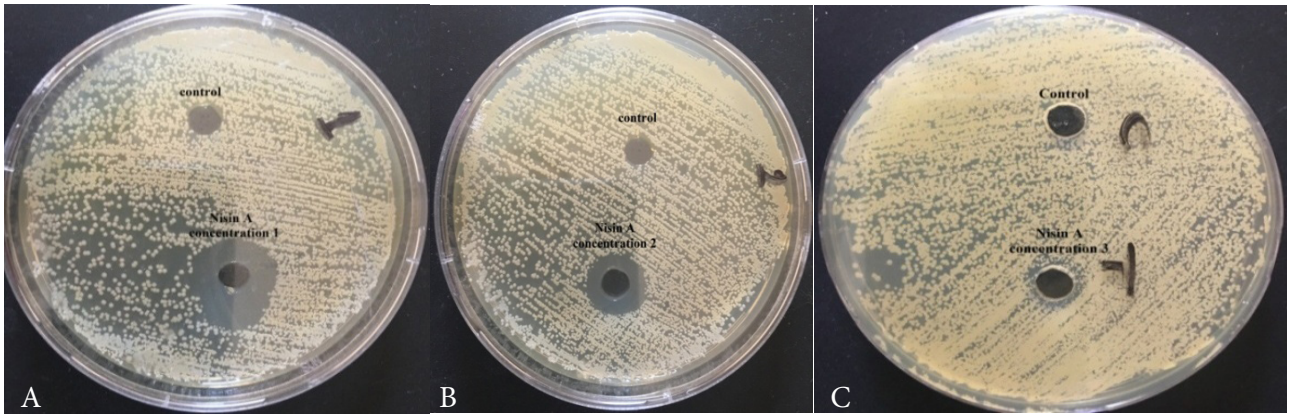

Figure 3: MIC for nisin at different concentration (A) concentration 1 (B) concentration 2(C) concentration 3 on Mueller Hinton agar at 37°C for 24 hours.

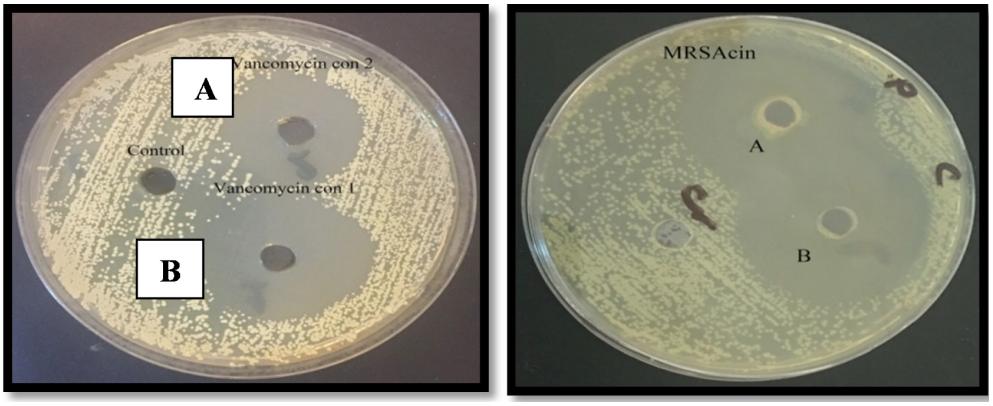

Figure 4: MIC for 1) Vancomycin at (3.0 and 3.5) g/ml(2) MRSAcin on 125 µg/ml A) Purified B) crude on Muller Hinton Agar at 37°C for 24 hr.

Table 3: The comparative effect of Nisin A, vancomycin in different concentration and purified MRSAcin bacteriocin on MRSA biofilm

Treatment	*Control Positive*	*Control Negative*	*Nisin A*	*Vancomycin*	*MRSAcin*	*LSD value*
Con 1	0.193	2.771	0.740	0.754	0.183	0.532 *
Con 2	0.177	2.776	0.844	0.769	0.152	0.644 *
Con 3	0.147	2.772	0.965	0..882	0.464	0.671 *
Con 4	0.199	2.674	0.913	0.972	0.368	0.659 *
Con5	0.193	2.733	1.242	0.993	0.213	0.504 *
Con 6	0.332	2.614	1.297	1.087	0.533	0.684 *
Con 7	0.192	2.643	1.299	1.09	0.154	0.691 *
Con 8	0.144	2.635	1.344	1.310	0.462	0.588 *
Con9	0.227	2.547	1.473	1.506	0.520	0.711 *
Con 10	0.193	2.771	1.492	1.626	0.318	0.635 *
LSD value	0.277 NS	0.503 NS	0.582 *	0.615 *	0.367 *	–

* ($P<0.05$), NS: Non-Significant.

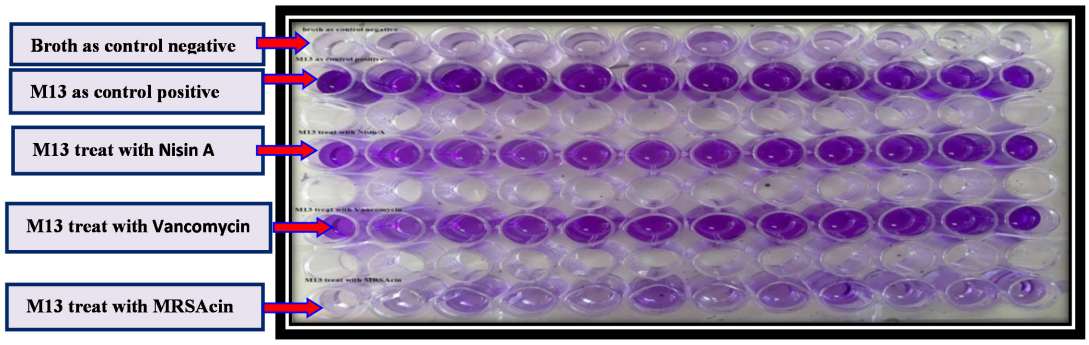

Figure 5: The comparative bactericidal effect among MRSAcin, antibiotic disk Vancomycin, and Nisin A on MRSA strain.

Table 2: The effect of Nisin A and Vancomycin and purified MRSAcinin different concentrations against MRSA by WDA.

Concentration (µg/ml)	*Nisin A inhibition zone diameter size (mm)*	*Vancomycin inhibition zone diameter size (mm)*	*MRSAcin inhibition zone diameter size (mm)*	*Control negative D.W*	*LSD value*
10^{-1}	22	3	4.8	0	5.03 *
10^{-2}	18	26	0	0	6.82 *
10^{-3}	0	17	0	0	5.26 *
10^{-4}	0	0	0	0	0.00 NS
LSD value	4.75 *	6.31 *	2.77 *	0.00 NS	–

* ($P<0.05$), NS: Non-Significant.

The result agrees with.[15,23] The result disagrees with[25] Shows the tested bacteriocins nisin A showed the highest bactericidal activity against both planktonic cells and biofilm cells. Result agree with[26] Vancomycin is widely used as therapy strain MRSA infections. We have shown that vancomycin exhibits bactericidal activity against growing planktonic cells of *S. aureus* there by leading to bacterial cell death by osmotic lysis.

CONCLUSION

These ours study we suggests the effects of Pure MRSAcin bacteriocins against most isolate of MRSA biofilm compare when using Nisin A and Vancomycin at different concentration The tested bacteriocins showed the highest bactericidal activation agent MRSA biofilm material and suggest that bacterocin from MRSA attacks biofilm cells more effectively than (Vancomycin) using Although is widely used at (first-line therapy) for difference MRSA infections.

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