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Extraction and Characterization of Staphylococcins from *S. aureus* Isolated from Selected Environments within Akure Metropolis and Evaluation of Their Antibacterial Activity

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Authors' contributions

This work was carried out in collaboration between both authors. Author TAO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author TTA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Staphylococcins are bacteriocins produced by *Staphylococcus aureus* and it has been proven that they have antibacterial potential. The purpose of this study was to compare the staphylococcin producing potential of *S. aureus* isolated from different environments and the antibacterial potential of the staphylococcins extracted. Using standard microbiological techniques *S. aureus* was isolated from different environments. The isolated *S. aureus* from the selected environments were screened for antibacterial potentials against the bacterial species (*Pseudomonas aeruginosa, Proteus* sp, *Bacillus subtilis* and *Klebsiella pneumoniae*) isolated from wound samples using agar well diffusion assay. The staphylococcins extracted were partially purified and molecularly characterized using Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The partially purified

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staphylococcins (PPS) were then screened for growth inhibitory activity against the isolated bacterial species from different wound samples while the effects of temperature and pH on the antibacterial activity of PPSs were also assessed using standard methods. Partially Purified Staphylococcin (PPSs) extracted from *S. aureus* isolated from leg wound (SALW), *S. aureus* from human stool (SAHS) and *S. aureus* isolated from cow dung (SACD) however displayed better inhibitory activity on most of the multi antibiotic resistant isolated bacteria with SALW. The inhibitory activities of the extracted staphylococcins were maximal at neutral pH and temperature of 27±2ºC while their potency reduced at extreme pH and increased temperatures. The molecular weight of the extracted staphylococcins ranged from 21kDa to 83kDa. From this study, the antibacterial activity of the extracted staphylococcins from *S. aureus* on bacteria isolated from wounds varied based on the environment from which the *S. aureus* was isolated. Staphylococcins SALW, SAHS and SACD were observed to show higher antibacterial activity than that of the antibiotics used on most of the bacteria isolates. Therefore, in sourcing for alternative therapy, SALW, SAHS and SACD should be exploited for the treatment of wound infections.

Keywords: Staphylococcins; S. aureus, antibiotic resistance; antibacterial activity; P. aeruginosa; Proteus sp; B. subtilis; Klebsiella pneumonia and Wounds.

1. INTRODUCTION

Wound infection is regarded as the most common nosocomial infection especially in patients undergoing surgery [1]. Post-caesarean wound infection is a disturbing occurrence in spite all the techniques and measures to ensure aseptic conditions [2], which results from increase in the resistance of the bacteria that are responsible for the condition to conventional antibiotics [3]. This infection has led to prolonged hospital stay, high hospital bills, as well as other morbidities and mortality [2]. The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics [4]. Bacteria such as *S. aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella* spp*., Proteus* spp*., P. aeruginosa, Bacteroides fragilis, Peptostreptococcus* sp and *Propionibacterium* spp. are mostly isolated from wounds [4].

Bacteriocins are ribosomally synthesized antimicrobial natural peptides (proteinaceous compounds) that are widely distributed in nature and are secreted by several bacteria [5]. Some bacteria such as Lactic Acid Bacteria (LAB), *Staphylococcus* sp, *Escherichia coli*, *Clostridium* sp. have been revealed to produce bacteriocins [5]. Staphylococcins are bacteriocins produced by *Staphylococcus* sp. with examples that include staphylococcin C55 (Class I), aureocin (Class II) and Lystostaphin (Class III) [5]. This type of bacteriocin has been shown to have antibacterial potential on both Gram positive and Gram negative bacteria which makes it to be a broad spectrum antibacterial agent [6]. Several research works have been done on the antibacterial potential of staphylococcins considering different strains of *S. aureus* [5]. However, consideration has not been given to the possible effect of environment of isolation of this organism on the antibacterial activity of staphylococcins. Therefore, the focus of this study was to compare the staphylococcin producing potential of *S. aureus* isolated from different environments and the antibacterial potential of the staphylococcins extracted on clinical isolates from wound samples.

2. METHODOLOGY

2.1 Study Area

Wound samples were obtained at State Specialist Hospital, Akure, Ondo State.

2.2 Collection of Wound Swabs

Wound swabs (102 samples) were collected using sterile swab stick from the post natal department of Mother and Child Hospital, Akure, Ondo State. The samples were stored in ice pack and transported immediately to the Microbiology Laboratory in Federal University of Technology Akure, Ondo State (FUTA) where they were analyzed.

2.3 Ethical Clearance

Permission was sought from the Hospital's Ethical Committee and Ministry of Health Ondo State before the commencement of the investigation.

2.4 Isolation and Identification of Bacteria Present in Wound Samples

Isolation of bacteria in wound samples was carried out using streaking method. The samples were streaked on Eosin methylene blue agar (EMB agar), Nutrient agar, Cystein Lactose Electrolyte Deficient Agar (CLED), Chocolate agar, Mannitol Salt Agar (MSA).Bacterial isolates were identified based on their morphological characterization on the selective culture media and observed after 24 hours of incubation. Parameter used in differentiating each isolate included colonial characteristics (edges, texture, elevation, colour, pigmentation, and size; cell morphology (Shape, arrangement and Gram reaction). Bacteria isolated from each plated Petri dishes were sub-cultured to get pure isolates [7]. The pure colonies isolated were inoculated on agar slant for biochemical characterization.

2.5 Isolation and Identification of *S. aureus* from Selected Environments

Isolates from selected environments (Microbiology Laboratory air of Federal university of Technology, Akure, Poultry sewage, poultry soil, Surgical wound, wound from leg, cow dung, abattoir soil, apparently healthy human skin, abattoir air, toilet water, Hospital air from Federal University of Technology, Akure, Health centre, Wasp House, Human Stool, Sewage and Refuse dump) were collected aseptically inside peptone water and were inoculated by pour plating into already prepared Mannitol Salt Agar (MSA). The culture medium was incubated at 37ºC for 24 hours. Morphological and biochemical characteristics were observed and further biochemical tests were carried out for the identification of the bacteria.

2.6 Standardization of Test Organisms

A loopful of the bacterial culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours. An aliquot (0.2 ml) was pipetted from the 24 hours broth culture of the test organism and was dispensed into 20 ml sterile nutrient broth and incubated for another 4 hours to standardize the culture to 0.5 McFarland's standard (10^6cfu/ml) before use as described by Oyeleke et al. [8].

2.7 Screening of *S. aureus* Isolates for Bacteriocin Production

Agar spot tests and agar well diffusion assays were used to evaluate the staphylococcin activity of the *S. aureus* strains isolated from the selected environments on Brain Heart Infusion (BHI) agar. For this purpose, four reference strains were used to check sensitivity to the antimicrobial substances produced by the *S. aureus*. These are *S. aureus*, *Proteus* sp, *K. pneumoniae* and *P. aeruginosa*, as described by Narayanapillai [9]. *S. aureus* was grown in BHI broth at 37ºC for 24 hours. The indicator strains (reference strains) were cultured in peptone water at 37ºC for 24 hours. For the agar spot test, overnight cultures of *S. aureus* strains (3 µml) was spotted onto the surface of BHI agar plates and incubated at 37ºC for 24 h to allow the development of colonies. Volumes of 100 µl of the indicator strains, with a total approximately 2.8 x 10^6cfu/ml were inoculated into 7 ml of semi-solid peptone (broth plus 0.75% bacteriological agar), kept at a temperature of 45ºC for 20 minutes. After agitation, they were poured over the plate of BHI agar on which the *S. aureus* strains under test had grown. The plates were incubated at 37ºC for 24 h and checked for inhibition zones. Inhibition was considered positive when the inhibition halo of the indicator strain above the *S. aureus* colonies was more than 2 mm [10].

For the agar well diffusion assay, an overnight culture of indicator strains grown in peptone water at 37ºC was diluted to a turbidity equivalent to that of a 0.5 MacFarland standard. A lawn of an indicator strain was made by spreading the cell suspension over the surface of Mueller Hinton agar (MHA, LabM, Lancashire, UK) plates with a cotton swab. The plates were allowed to dry and a sterile cork-borer of diameter 6.0 mm was used to cut uniform wells in the agar plates. *S. aureus* strains were grown in BHI broth at 37ºC for 48 h. Cultures were centrifuged at 4000 g for 20 minutes at 4ºC. The cell-free supernatant (CFS) was collected, adjusted to pH 6.5 with 1 M NaOH. Each well in the MHA plates was filled with 80 µl of Whatmann filter-sterilized cell-free supernatants of the potential staphylococcins producer strains. The plates were kept at 4ºC for 2 h, to ensure diffusion of the supernatant fluid into the agar, and then incubated at 37ºC for 24 h. The antimicrobial activity was determined by measuring the diameter of zone of inhibition around the wells.

2.8 Partial Purification and Characterization of Staphylococcin

2.8.1 Partial purification

The Staphylococcin extracted from the isolated *S. aureus* that displayed antibacterial activity on bacterial isolates on wound samples (staphylococcins extracted from *S. aureus* isolated from stool (SAHS), staphylococcin extracted from *S. aureus* isolated from cow dung (SACD), staphylococcin extracted from *S. aureus* isolated from toilet water (SATW), Staphylococcin extracted from *S. aureus* isolated from leg wound (SALW), and staphylococcin extracted from *S. aureus* isolated from Abattoir soil (SAAS) were grown separately in different flasks containing 1 litre BHI broth for 48 h at 30ºC. Following incubation, the cultures were centrifuged at 6000 rpm for 20 min at 4ºC to remove the cells. This cell-free culture supernatant was brought to a final ammonium sulphate concentration of 55% saturation by slow addition of the salt, and was stirred overnight at 4ºC. Then, the mixture was centrifuged (6000 rpm, 4ºC, 15 min) and the surface pellicles and bottom pellets were harvested and re suspended in 20 ml of 0.05 M potassium phosphate buffer (pH 7.0). The mixture was stirred for 24 h at 4ºC, after which the suspension was dialyzed in a tubular cellulose membrane (1000 cut off) against 2 L distilled water for 24 h (Hena, 2013). This partially purified staphylococcin (PPS) was stored at 4ºC until use.

2.8.2 Protein quantification of PPS

Protein concentration of PPS was determined by Bradford method as documented by Hena (2013) [11] using a protein assay kit from BioRad Laboratories (California, USA) was used.

2.9 Molecular Weight Estimation of PPS Using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The molecular weight of the PPS was determined using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Hena. Sterile glass plates were assembled, 20 ml of 15% resolving gel were dispensed, 2 ml of butanol was then overlaid onto the gels, allowed to polymerize. After which the overlay was poured off and the gel surface rinsed with deionized water. To the gel, 8 ml of 5% stacking gel was overlaid and fixed in an electrophoresis apparatus. To the electrophoresis wells, equal volumes 20 µl of 1 x SDS and test sample preheated at 100ºC in a test tube for 30 min and marker (17671 – 103142 Da) respectively were loaded in the gel. The gel was then run 100 V for 5 h, after which it was stained with Coomassie brilliant blue R-250. The apparent molecular weights of the samples were determined by comparison with the mobility of the standard weight protein markers.

2.10 Lyophilization

The samples were transported in an ice packed container to the Department of Biochemistry, Federal Polytechnic Ado-Ekiti, for lyophilization. Lyophylisation procedure is used to preserve a liquid, creamy or a solid product by withdrawing the water through sublimation under vacuum, after having frozen the product. The partially purified staphylococcins were freezed and turned into ice. The intracellular water was then sublimated and resolidify on cold condensor plates.

2.11 Determination of Antibacterial Activity, Effect of Temperature and pH on the Antibacterial Activity and MIC of PPS on Bacterial Isolates from Wound Samples

Using agar well diffusion assay, an overnight culture of the bacteria grown in peptone water at 37ºC was diluted to a turbidity equivalent to that of a 0.5 MacFarland standard. A lawn of an indicator strain was made by spreading the cell suspension over the surface of Mueller Hinton agar (MHA, LabM, Lancashire, UK) plates with a glass spreader. The plates were allowed to dry and a sterile cork-borer of diameter 6.0 mm was used to cut uniform wells in the agar plates. Extracted staphylococcins SALW, SAHS, SATW, SACD and SAAS were dissolved in phosphate buffer of pH 7.0 to make concentration of 50 mg/ml, 25 mg/ml, 10 mg/ml and 5 mg/ml. Each well in the MHA plates was filled with 80 µl of extracted staphylococcins (PPS). The plates were kept at 4ºC for 2 h, to ensure diffusion of the supernatant fluid into the agar, and then incubated at 37ºC for 24 h. The antimicrobial activity was determined by measuring the diameter of zone of inhibition around the wells. Using the same procedure, the effect of temperature and pH was determine using

temperatures of 25ºC, 40ºC, 100ºC and 121ºC and pH buffer (acetic buffer- pH 2 - pH 4; citrate buffer- pH 5 - pH 6; phosphate buffer – pH 7; and trisHCl buffer- pH 8 - pH 10).

3. RESULTS

3.1 Types of Bacteria Isolated from Different Wound Samples

Different strains of bacteria were isolated from various wound samples. These bacterial species include; *S. aureus, P. aeruginosa, Proteus mirabilis, Proteus vulgaris, B. subtilis, K. pneumoniae, Staphylococcus epidermidis* and *Escherichia coli.* The morphological identification and biochemical characteristics can be found in Table 1.

3.2 Primary Screening of cell free Supernatant of *S. aureus* Isolated from Selected Sources for Antibacterial Activity on Wound Pathogens

Out of the *S. aureus* isolated from selected environments used in this study, only the ones isolated from human stool (SAHS), cow dung (SACD), abattoir soil (SAAS), water from water closest (SATW) and wound swab from leg (SALW) showed antagonistic activity on the organisms isolated from wound samples. This is shown in Table 2. These organisms were selected as potential source of staphylococcins.

3.3 Antibacterial Activity of Partially Purified Staphylococcin (PPS) with Conventional Antibiotics Used, and Effects of Concentration on Its Antibacterial Activity

Out of the fie partially purified staphylococcins (PPSs) used in this study, three of the staphylococcins, staphylococcins SALW, SACD and SAHS exerted growth inhibitory effect on five bacterial species *S. aureus, P. aeruginosa, Proteus* sp, *K. pneumoniae* and *B. subtilis.* Staphylococcins SAAS inhibited three organisms; *P. aeruginosa, Proteus* sp and *B. subtilis* while SATW inhibited the growth of only one organism, *Proteus* sp (Table 3). This organism i.e. *Proteus* sp was the only bacterium observed to be susceptible to the inhibitory activity of all the five staphylococcins used. *P. aeruginosa* and *B. subtilis* were susceptible to four of the PPSs (SALW, SACD, SAAS and SAHS). *S. aureus* and *K. pneumoniae* were susceptible to only three of the PPSs (SALW, SACD and SAAS). SALW was the most effective on *S. aureus* and *Proteus* sp, SACD on *P. aeruginosa,* SAHS on *K. pneumoniae* while SAAS was the most effective *B. subtilis.* Most of the bacterial isolates from wounds that displayed multidrug resistance to the conventional antibiotics used were susceptible to staphylococcin SAHS, SACD and SALW while few isolates were sensitive to SATW and SAAS. For example, SAHS displayed highest zone of inhibition of the growth of *P. aeruginosa* (23.0 mm) followed by SACD (16.5 mm) while SALW was 15.0 mm. Gentamycin on the other hand only showed inhibitory effect of 12.o mm) on the same bacterium while other antibiotics; tetracycline, ampicillin and Septrin did not inhibit the growth of microorganisms at all (Table 3). For *B. subtilis* however, staphylococcin SAAS had the highest. The other antibiotics used; tetracycline, anpicillin and Ampiclox did not inhibit the growth of the bacterium (Table 3).A similar trend was observed on *S. aureus* that was resistant to all the test antibiotics.

On the effect of concentration on its antibacterial activity, staphylococcin SAHS were observed to exact growth inhibition on most of the susceptible bacterial isolates from wounds even at low concentration of 5 mg/ml (Fig. 1). Staphylococcin SACD also had inhibitory activity on all the susceptible isolates but had reduced at 5 mg/ml (Fig. 2). Staphylococcin SATW on the other hand, had reduced inhibitory at all the concentration used and it only inhibited the proliferation of *Proteus* sp (Fig. 3). Staphylococcin SALW however was effective at various concentrations but was not effective at 5 mg/ml on a particular strain of *Proteus* sp and *S. aureus* (Fig. 4) while staphylococcin SAAS also had reduced effectiveness as the concentration reduced to 5 mg/ml as shown in Fig. 5.

3.4 Effects of Temperature on the Growth Inhibitory Activity of the Extracted Staphylococcins

All the extracted staphylococcins (SALW, SATW, SAAS, SAHS and SACD) subjected to a temperature of 25ºC for 15 minutes showed inhibitory activity on the susceptible isolates. However when subjected to higher temperature from 100ºC to 121ºC, only staphylococcin SALW was still effective. Fig. 6 shows the effect of temperature on the antibacterial activity of the extracted staphylococcins on using *Proteus* sp the most sensitive bacterium to the activity of the staphylococcins used.

Table 1. Morphological and Biochemical characteristics of isolated bacteria

Isolates	Colour	Shape	Elevation	Texture	Size	Cell shape (Arrangement)	Gram Reaction	Catalase	Coagulase	Oxidase	Citrate	H_2S	Mannitol	Lactose	Sucrose	Dextrose	Glucose/e	Probable Organisms
1	Blue green	Circular/ Regular	Flat	Smooth	Big	Bacilli (clustered)	-	+	-	+	-	+	+	+	+	+	+	*Pseudomonas sp*
2	Cream/ yellow	Circular/ Regular	Raised	Smooth	Tiny	Cocci (clustered/ chain)	+	+	+	-	+	+	+	+	+	+	+	*S. aureus*
3	Dirty cream	Circular/ Irregular	Flat	Rough	Tiny	Cocci (clustered)	-	+	-	-	+	+	+	+	+	+	+	*Proteus sp*
4	Cream	Circular/ Regular	Raised	Smooth	Small	Bacilli (clustered)	-	+	-	-	-	+	+	+	-	+	+	*K. pneumoniae*
5	Cream	Circular/ Regular	Raised	Smooth	Big	Bacilli (chain)	+	+	-	-	+	+	-	-	+	-	-	*B. subtilis*
6	Cream/ yellow	Circular/ Regular	Raised	Smooth	Tiny	Cocci (clustered/ chain)	+	+	-	-	+	+	+	+	+	+	+	*Staphylococcus epidermidis*

Table 2. Primary screening of cell free supernatant of *S. aureus* isolated from selected sources for antibacterial activity on wound pathogens

Sources	Diameter zones of inhibition (mm)				
	S. aureus	***Pseudomonas* sp**	***Proteus* sp**	***Klebsiella* sp**	***B. subtilis***
MLA	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
PSW	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
PS	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
SW	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
LW	0.00±0.00^{a}	0.00±0.00^{a}	10.50±0.50^{b}	0.00±0.00^{a}	13.50±0.50^{c}
CD	10.50±0.50^{b}	14.00±0.50^{b}	0.00±0.00^{a}	10.50±0.50^{b}	10.50±0.50^{b}
AS	0.00±0.00^{a}	0.00±0.00^{a}	10.50±0.50^{b}	11.00±0.00^{b}	11.50±0.50^{b}
SK	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
AA	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
TW	0.00±0.00^{a}	0.00±0.00^{a}	11.50±0.50^{b}	10.50±0.50^{b}	0.00±0.00^{a}
HA	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
WH	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
HS	11.50±0.50^{b}	0.00±0.00^{a}	0.00±0.00^{a}	13.50±0.50^{c}	14.00±0.50^{c}
S	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
RD	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values in the same row carrying the same superscript are not significantly different according to Duncan's multiple range tests at (P≤0.05).

Key: MLA: Microbiology Laboratory Air, PSW: Poultry Sewage, PS: Poultry Soil, SW: Surgical wound, LW: Wound from Leg, CD: Cow Dung, AS: Federal University of Technology, Akure, Abattoir Soil, SK: Skin, AA: Abattoir Air, TW: Water from Water Closet, HA: Hospital Air from Federal University of Technology, Akure, Health Centre; WH: Wasp House, HS: Human Stool, S: Sewage, RD: Refuse Dump

Table 3. Antagonistic activity of the partially purified staphylococcins (PPSs) (50 mg/ml) on multidrug resistant bacteria isolates from wounds

Sources	Diameter zones of inhibition (mm)					
	S. aureus	***Pseudomonas* sp**	***Proteus* sp**	***Klebsiella* sp**	***B. subtilis***	***E. coli* ATCC29522**
SALW	15.96±0.85^{c}	15.03±0.20^{c}	25.04±0.015^{c}	10.02±0.25^{b}	16.02±0.05^{c}	10.00±0.00^{b}
SACD	11.04±0.02^{b}	17.06±0.10^{d}	15.02±0.005^{b}	10.01±0.10^{b}	16.04±0.05^{c}	12.00±0.00^{b}
SAAS	0.00±0.00^{a}	10.07±0.15^{b}	11.00±0.20^{a}	0.00±0.00^{a}	25.04±0.15^{d}	0.00±0.00^{a}
SATW	0.00±0.00^{a}	0.00±0.00^{a}	15.06±0.005^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
SAHS	11.55±0.65^{b}	15.55±0.47^{c}	15.04±0.005^{b}	15.91±0.11^{c}	18.04±0.05^{c}	12.50±0.00^{b}
TETRACYCLINE	17.00±1.00^{c}	12.00±0.00^{b}	15.50±0.00^{b}	0.00±0.00^{a}	10.50±0.50^{b}	15.50±0.00^{b}
GENTAMYCIN	10.50±0.00^{b}	10.50±0.00^{b}	10.00±0.00^{b}	0.00±0.00^{a}	15.50±0.00^{c}	11.50±0.00^{b}

Values in the same row carrying the same superscript are not significantly different according to Duncan's multiple range tests at (P≤0.05).

SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound's swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

3.5 Effects of pH on the Growth Inhibitory Activity of the Extracted Staphylococcins

All the extracted staphylococcins (SALW, SATW, SAAS, SAHS and SACD) showed inhibitory activity on the susceptible isolates with *Proteus* sp being the most sensitive bacterium to the effect of staphylococcins used at pH of 7.0. However, at low pH of 2.0, staphylococcin SACD still had inhibitory activity on the bacterium while staphylococcin SAHS retains its inhibitory activity on the bacterium at alkaline pH of 10.0 (Fig. 7).

3.6 Protein Content of Partially Purified Extracted Staphylococcins

Staphylococcin extracted from human stool (SAHS) has the highest protein content (55%) while the staphylococcin extracted from wound (SALW) has the lowest protein content (10%) (Fig. 8). The absorbance value at 540 nm for the different extracted staphylococcin can be seen in Table 4.

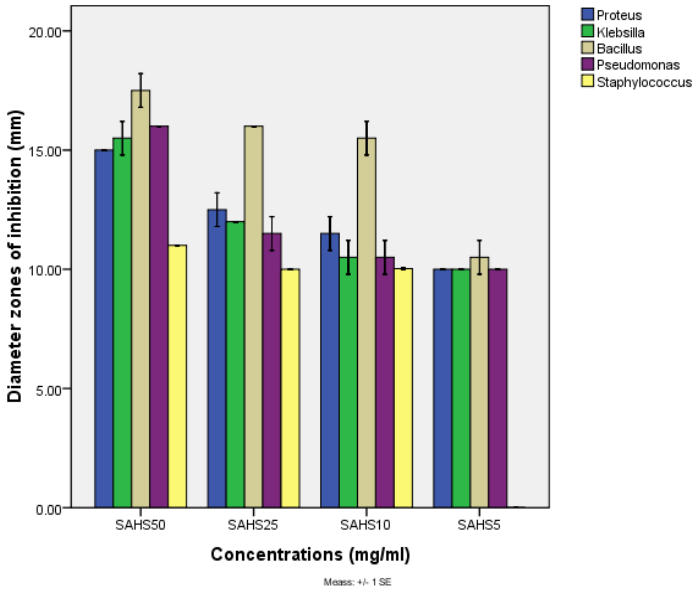

Fig. 1. Effect of concentrations of staphylococcin extracted from *S. aureus* isolated from stool sample (SAHS) on bacterial isolates from wounds

SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

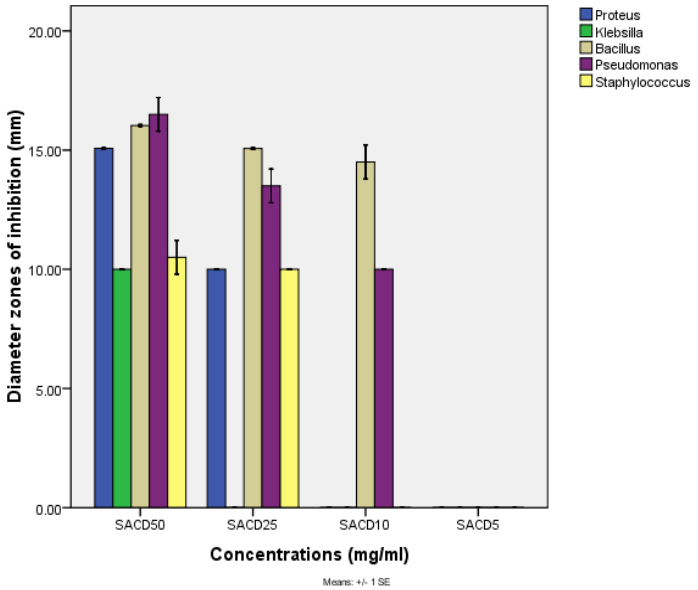

Fig. 2. Effect of concentrations of staphylococcin extracted from *S. aureus* isolated from cow dung (SACD) on bacterial isolates from wounds

SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

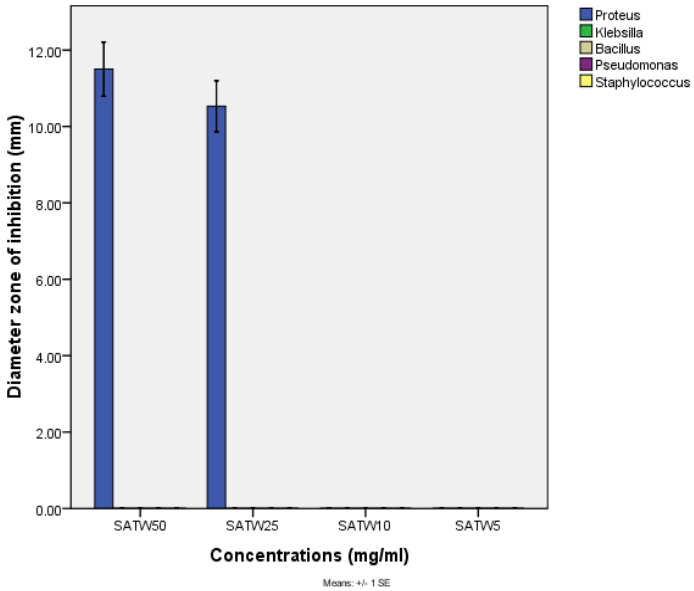

Fig. 3. Effect of concentrations of staphylococcin extracted from *S. aureus* isolated from toilet water (SATW) on bacterial isolates from wounds

SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

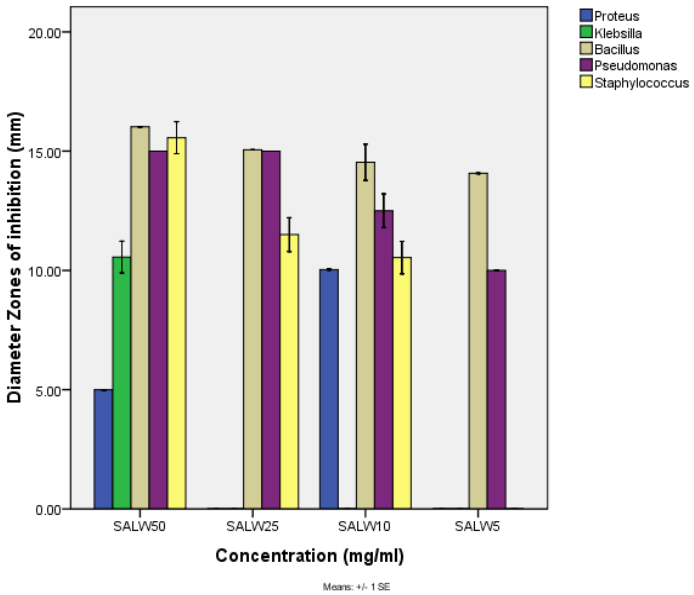

Fig. 4. Effect of concentrations of staphylococcin extracted from *S. aureus* isolated from wound sample (SALW) on bacterial isolates from wounds

SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from abattoir soil

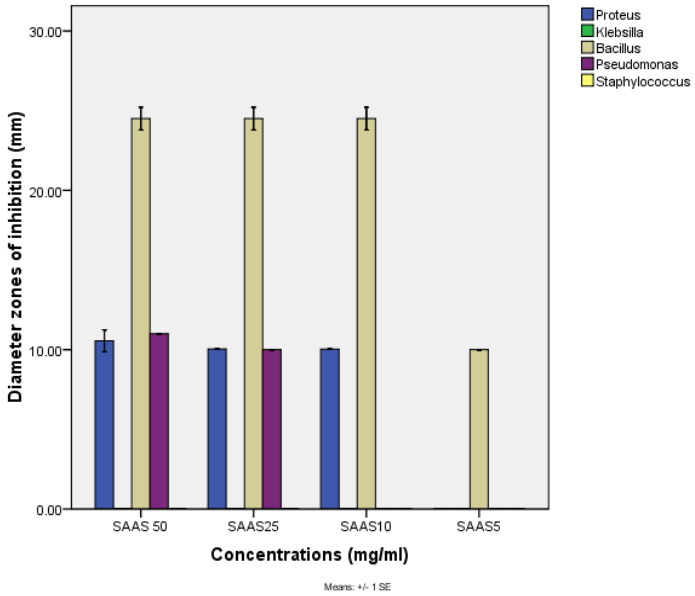

Fig. 5. Effect of concentrations of staphylococcin extracted from *S. aureus* isolated from abattoir soil (SAAS) on bacterial isolates from wounds

SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

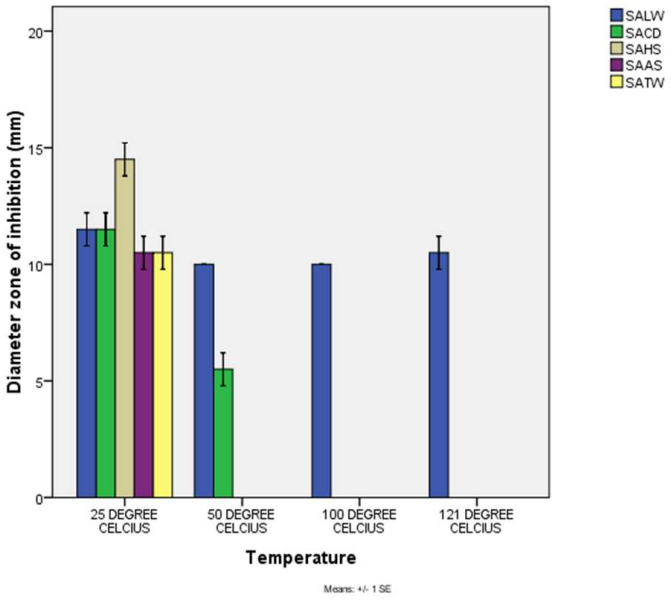

Fig. 6. Effects of temperature on the inhibitory activity of extracted staphylococcins on *Proteus mirabilis*

Key: SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

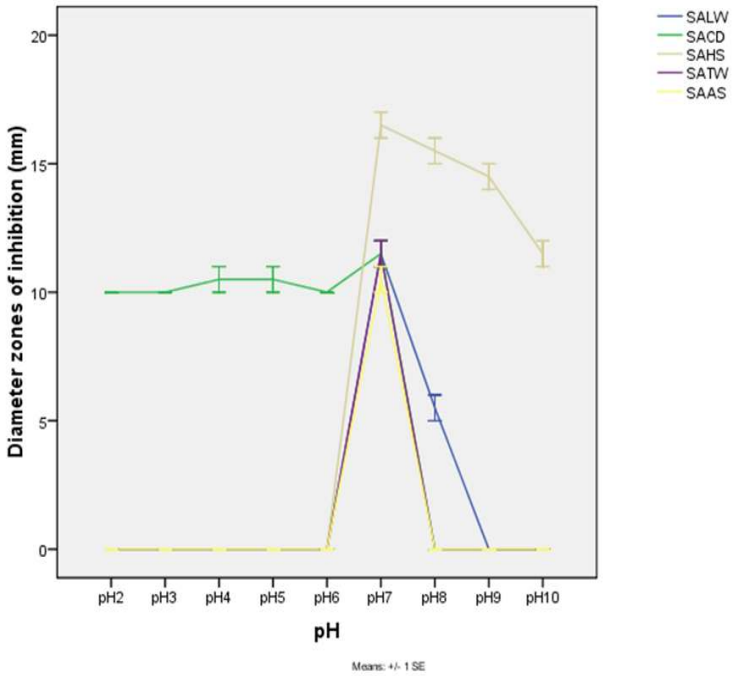

Fig. 7. Effects of pH on the inhibitory activity of extracted staphylococcins on *Proteus* sp.

Key: SAHS- Staphylococcin extracted from S. aureus isolated from stool
SACD- Staphylococcin extracted from S. aureus isolated from cow dung
SATW- Staphylococcin extracted from S. aureus isolated from toilet water
SALW- Staphylococcin extracted from S. aureus isolated from leg wound swab.
SAAS- Staphylococcin extracted from S. aureus isolated from Abattoir soil

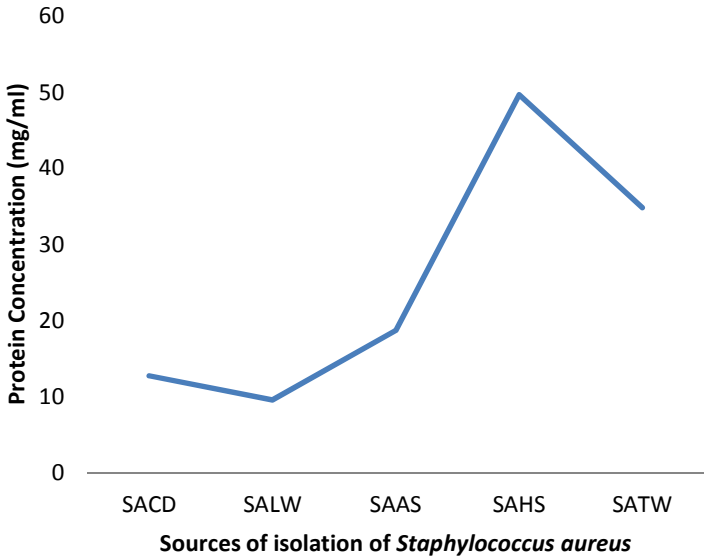

Fig. 8. Protein concentration of the partially purified extracted staphylococcin

Legend: SACD-Staphylococcin extracted from S. aureus isolated from air in cow dung
SAAS-Staphylococcin extracted from S. aureus isolated from abattoir soil
SALW-Staphylococcin extracted from S. aureus isolated from wound
SATW-Staphylococcin extracted from S. aureus isolated from water from water closet
SAHS-Staphylococcin extracted from S. aureus isolated from stool

3.7 Molecular Weight of the Extracted Staphylococcins

The molecular weight ranged from 22kDa to 83kDa (Table 5). The list of protein used as standards and the equation that was used to determine the molecular weight of the extracted staphylococcins can be seen in the Table 6.

Table 4. Absorbance of staphylococcins extracted from *S. aureus* isolated from different sources

Sources of Staphylococcin	Absorbance
SACD	0.1827±0.00953[a]
SALW	0.1387±0.00145[b]
SAAS	0.2707±0.00606[c]
SAHS	0.5047±0.01157[d]
SATW	0.3635±0.2223[e]

Values in the same row carrying the same superscript are not significantly different according to Duncan's multiple range tests at (P≤0.05).
Key: SACD: Staphylococcin extracted from S. aureus isolated from cow dung
SALW: Staphylococcin extracted from S. aureus isolated from wound
SAAS: Staphylococcin extracted from S. aureus isolated from FUTA abattoir soil
SAHS: Staphylococcin extracted from S. aureus isolated from stool
SATW: Staphylococcin extracted from S. aureus isolated from water in water closet

4. DISCUSSION

Bacteria isolated from these wounds samples are *S. aureus, P. aeruginosa, Proteus* sp*, B. subtilis, Klebsiella pneumonia* and *Staphylococcus epidermidis.* This is in agreement with the reports of Shittu et al*.* [4] and Valarmathi et al. [12]. The presence of *S. aureus* in wound infections may be due to the fact that skin being a predominant normal flora of the skin. Moreover, Nasal carriage of *S. aureus* has been identified as an important risk factor for the acquisition of *S. aureus* infection, although this may depend on an array of factors that may either be environmental or patient-related [3] while the presence of *Proteus species* can be due to contamination of wound with patient's endogenous flora [3]. The occurrence of *K. pneumoniae* in this study agrees with report of Valarmathi et al. [12], Opalekunde et al. [3] and Mwambete and Rugemalila [13], although it was not as high as reported by Opalekunde et al. [3]. The major threat caused by the implication of *K. pneumoniae* is prolonged stay in the hospital and excessive spending on drugs. The presence of the *B. subtilis* has been occasionally reported to be implicated with wound infections. Its presence could be from the clinical officials that are taking care of the patients. The pathogenic potential of *B. subtilis* is generally described as low or absent [14], bacteremia/septicaemia, endocarditis, meningitis and infections in wounds, the ear, the eyes, respiratory tracts, urinary tracts and gastrointestinal tracts [15]. Infections caused by *B. subtilis* are mostly associated with immunocompromised patients and can as well occur whenever its being displayed from its normal environment [14,16].

Five (33.3%) of the *S. aureus* isolated from selected sources within Akure metropolis (SACD, SATW, SAHS, SALW and SAAS) were observed to have different degree of antagonistic activity on the wound pathogens. This agrees with the documentation of Maria et al. [5]. Adelaide et al. [17] stated that the environment in which an organism proliferates can affect the metabolites it produces which resulting metabolites help the microorganisms to thrive in such an environment, especially in a very adverse condition. These metabolites include bacteriocins, hydrogen peroxide, catalase, diacetyl etc. The bacteriocins produced by *S. aureus* known as staphylococcins have been examined extensively and reported to have broad of inhibitory activities [10]. The report of this study also attests to this.

Staphylococcin SAHS, SACD and SALW had inhibitory activity on *Proteus mirabilis*, *S. aureus*, *K. pneumoniae, B. subtilis,* and *P. aeruginosa*; although, they had no inhibitory activity on *Proteus* sp and *Staphylococcus epidermidis.*

In comparing these results with that of conventional antibiotics, staphylococcins SAHS, staphylococcins SALW and staphylococcins SACD displayed higher inhibitory activities compared to the conventional antibiotics. The observed results agrees with the report of Narayanapillai et al. [9] who reported that bacteriocin had antibacterial activity on *E. coli, S. aureus, B. subtilis*, *P. mirabilis, P. aeruginosa* and *K. pneumonia.* The mode of action of gram positive bacteriocin produced by Gram positive bacteria such as *S. aureus* as reported by Juan and Antonio [18], Maria et al. [5] and Andreza et al. [10] is pore formation which reduces the energy flux of the bacteria.

Table 5. Molecular weight of the staphylococcins extracted

Sample	MW (Da)	DIST(cm)	RF	Log MW
SALW1	83327.86	1.6	0.2286	4.9207
SALW2	37898.72	3.9	0.5571	4.5786
SATW1	51578.68	3	0.4286	4.7124
SATW2	21165.08	5.6	0.8	4.32562
SAAS1	55240.81	2.8	0.4	4.7423
SAAS2	27839	4.8	0.6857	4.4446
SAHS1	48170.88	3.2	0.4571	4.6828
SAHS2	22667.84	5.4	0.7714	4.35541
SACD1	57168.25	2.7	0.3857	4.7571
SACD2	21903.57	5.5	0.7857	4.3405

Key: SACD-Staphylococcin extracted from S. aureus isolated from air in cow dung
SAAS-Staphylococcin extracted from S. aureus isolated from abattoir soil
SALW-Staphylococcin extracted from S. aureus isolated from wound
SATW-Staphylococcin extracted from S. aureus isolated from water from water closet
SAHS-Staphylococcin extracted from S. aureus isolated from stool

Table 6. Molecular weight of the standard used for the molecular weight determination of the staphylococcins

Normal standard	MW (Da)	DIST(cm)	RF	LOGMW
Phosphorylate	97000	1.3	0.1857	4.9867
bovine serum albumin	66000	2.1	0.3	4.8195
chicken ovalbumin	45000	3.5	0.5	4.6532
soybean trypsin inhibitor	30000	4.4	0.6286	4.477
bovine lactolobulin	20000	5.7	0.8143	4.301
lysozyme	17670	6.3	0.9	4.2472

The activities of staphylococcins used in this study are temperature dependent. At room temperature 27±ºC, the effectiveness of all the staphylococcins was at maximum. This agrees with the report of Narayanapillai et al. [9] who also reported that the activity of bacteriocin can be affected by the optimum temperature of the bacteria.

However, staphylococcin SALW still had inhibitory activity at increased temperature (≥100ºC). This means that this staphylococcin is thermo stabile. The thermo stability of staphylococcins has been reported and it is said to be due to their small molecular size [10,6].

The inhibitory activity of staphylococcins extracted was at maximum at neutral pH of 7. However, staphylococcins SACD was also active at acidic pH while staphylococcins SAHS was still potent at alkaline pH although at a reduced rate. This report is in agreement with Okpara et al. [6] who reported the pH stability of bacteriocin at pH of 4 – 8.

The molecular weight of the extracted staphylococcins used in this investigation ranged from 21kDa to 83kDa. Staphylococcins SAHS has molecular weight of 22kDa and 57kDa, staphylococcins SATW has molecular weight of 21kDa and 52kDa, staphylococcins SAAS has molecular weight of 22kDa and 48kDa and staphylococcins SALW has 83kDa and 38kDa.

The high molecular weight observed may be due to the presence of protein, carbohydrate, and lipid [5,10].

Staphylococcin extracted from *S. aureus* isolated from leg wound (SALW), from apparently healthy human stool (SAHS) and from *S. aureus* from cow dung (SACD) have superior antibacterial potential to conventional antibiotics against bacteria isolated from wounds; therefore they can be developed as potential therapy for wound infection associated with multi drug resistant bacteria until a better alternative is produced.

5. CONCLUSION

This study has shown the presence of some pathogenic bacteria in with wounds of patients attending State Specialist Hospital, Akure and Mother and Child Hospital, Akure. Some of these pathogens are multidrug resistant strains. The resistance of the isolated bacteria to most of the antibiotics tested is of major concern because so many complications can result after CS delivery and in accident or burns victims thereby increasing morbidity and mortality rate as a result infections that these bacterial species can cause in these patients.

Although staphylococcins have prominent antibacterial effect, staphylococcin extracted from *S. aureus* isolated from leg wound (SALW), from apparently healthy human stool (SAHS) and from *S. aureus* from cow dung (SACD) have superior antibacterial potential to conventional antibiotics against most of the bacteria isolated from wounds. Therefore, they can be developed as potential therapy for wound infection associated with multi drug resistant bacteria until a better alternative antibacterial agent is produced.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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