Journal of Advances in Biology & Biotechnology
5(1): 1-7, 2016; Article no.JABB.20465
ISSN: 2394-1081

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Toxicological Potential of *Staphylococcus* Species from Specific Environment in the Federal University of Technology, Akure, Nigeria

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Authors' contributions

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

Article Information

DOI: 10.9734/JABB/2016/20465
Editor(s):
(1) Deepak Tomar, Lowance Center for Human Immunology Emory University School of Medicine 615 Michael Street, Atlanta, Georgia.
Reviewers:
(1) Abdullah Yusuf, National Institute of Neurosciences & Hospital, Sher-E-Bangla Nagar, Bangladesh.
(2) S. Thenmozhi, Periyar University, India.
Complete Peer review History: http://sciencedomain.org/review-history/11900

Original Research Article

Received 28th July 2015
Accepted 15th September 2015
Published 19th October 2015

ABSTRACT

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 µm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters. They are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. The present animal that has been infected with *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis (S. epidermidis)* to study their pathogenicity. Nine wistar albino rat were divided randomly into three groups (each group contain 3 animals): group (1) (control group) were kept without inoculation. The second and third group (treated group) were inoculated intra-dermal with (0.5 ml) of bacterial inoculum (2.6 x 10^5) cfu/ml. The blood samples of the animals were taken to test for their blood parameters which include packed cell volume, red blood cell, white blood cell counts, neutrophil, lymphocytes, monocytes, eosin, and basophil. The clinical signs noticed in infected groups were decreased appetite, mild fever and abscess formation in one or two of the animals at site of injection. Histopatological investigations were carried out on the kidney, lungs and liver. The statistical analysis revealed that there was significant increase in red blood

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cell, ($p<0.05$) in infected group as compared with control while the packed cell volume, neutrophil, white blood cell, lymphocytes, monocytes, eosin, and basophil showed no significant differences when compared with the control group (Table 1). Histopathological study revealed that there were: vacuolization of glomerular (VG) and vacuolization of the tubules (VT), distruption of glomerular capillaries (DG) and distruption of congested glomerular with vacuolar appearance (CGV) in the kidney cell, in liver: there were ruptured vein (RV), mild Kuffer cells infiltration (MK) and necrotic effects (N) and in the lungs there were edema (E), hemorrhage (H), necrotic effects (N), hemorrhage and necrosis (HN) in the infected wistar albino rat. In conclusion the colonization with strains of *S. epidermidis* and *S. aureus* cause devastating effects on certain vital organs such as kidney, liver and lungs which, depending on their severity, which could be fatal.

Keywords: Pathological; haematological; Staphylococcus; environment; potential.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) are common commensal pathogens that are found on various parts of the human body as well as in different places of the environment. These are also dangerous pathogen, causing diseases like bacteraemia, pneumonia, toxins, exotoxins, toxic shock syndrome and staphylococcal Food Poisoning [1,2]. Staphylococci have been cultured from body cavities, including eyes, mouths, vaginas [3], and respiratory tracts [3]. In hospital environments, specimen contamination and iatrogenic infections are involved primarily by Staphylococci; furthermore it is well known that endogenous and nosocomial infections are often caused by staphylococci. *S. aureus* is a common pathogen associated with multiple disease processes, an important nosocomial and community-acquired pathogen [4] and one of the major bacterial agents causing foodborne diseases in humans worldwide [3,1]. These microorganisms have a formidable range of potential virulence determinants [5]. Coagulase negative staphylococci (CNS), in particular *Staphylococcus epidermidis*, were considered as harmless skin commensal, but in recent decades, they became important human pathogens. CNS are increasingly recognized as etiological agents of hospital acquired infections and *S. epidermidis*, in particular, is considered a low pathogenic microorganism, responsible for infections only in people with lowered immune system [6]. The pathogencity of both *S. aureus* and *S. epidermidis,* comes from their production of an impressive repertoire of virulence factors [7] that includes: surface proteins, that promote colonization of hosts tissues; invasions, that promote bacterial spread in tissue [8]; surface factors, that inhibit phagocyte engulfment [9]; biochemical proprieties that enhance their survival in phagocytes, as the catalase production; immunological disguises and membrane-damaging toxins that lyse eukaryotic cell membranes [10]; exotoxins that damage host tissues or otherwise provoke symptoms of disease [7]. With regard to *S. epidermidis* not much is known about mechanisms of pathogenesis [11]. A characteristic found in many pathogenic strains is the production of slime, resulting in biofilm formation. The slime is predominantly a secreted teichoic acid, normally found in the cell wall of the staphylococci. Slime production, with the ability to form biofilm on various surfaces, is considered a significant virulence factor for some staphylococci isolated from clinical samples [12,13]. The host defence system often forms a fibrin clot surrounding the site of invasion of the microorganism to prevent it to spread through the body. In contrast to many other parasites *S. aureus* promotes the fibrin clotting by enzyme coagulase to protect it from the host defence mechanism, and makes localised invasion [6]. During growth the microorganism releases toxins which result in cell damage leading to diseases of the host [6,14]. A few pathogenic bacteria do not invade the host but only produce toxins. This study was carried out to reveal the pathogenesis of *Staphylococcus aureus* and *S. epidermidis* in wistar albino rat which include ÷ clinical signs, changes in blood parameters and histopathological studies in some organs like ÷ kidney, lungs and liver.

2. METHODOLOGY

2.1 Toxicological Analysis of *Staphylococcus* Species on Wistar Albino Rat

Wistar albino rats of 144.3-188.9 grams in weight obtained from Akure city were housed in the same environmental condition obtained for 2

weeks for adaptation; and were then divided randomly into three groups; (each group contained 3 animals); group one (control group) were kept without inoculation without given them any saline in which bacterial cells were suspended. The second and third group (known as treated group) were inoculated intra-dermal with (0.5 ml) of bacterial inoculum (2.6×10^5). The experiment lasted for 21 days, with daily investigation of clinical signs. At the end of the experiment, the animals were sacrificed and their haematological and histological parameters were examined.

2.2 Bacterial Strain

Overnight (18 hours) cultures of *S. aureus* and *S. epidermidis* from microbiological laboratory of Federal University of Technology Akure, Ondo State, were prepared by inoculating a single colony from mannitol salt agar plate into ten milliliters of nutrient broth. Cultures were centrifuged for 10 minutes at 3000 rpm and the supernatant was decanted. The bacterial pellet was re-suspended in a volume of sterile normal saline equal to the discarded supernatant. Serial tenfold dilution in normal saline was made from each inoculum. Bacteria were counted by incubating 0.1 ml portions on nutrient agar, incubated for 24 hours and the numbers of colonies were recorded as previously described in [15]. A measured volume of re-suspended cells, which regularly contain dose ($2.6x10^5$) cfu/ml were used for this experiment according to [16].

2.3 Haematological Analysis of *Staphylococcus* Species

The blood samples (3 ml) with anticoagulant were taken to evaluate blood parameters; including white blood cell counts, lymphocytes, monocytes, basophiles, eosinophil (count and percentage), red blood cell counts and, packed cell volume.

2.4 Histopathological Examination of *Staphylococcus aureus* and *Staphylococcus epidermidis*

Tissue samples from kidney, liver, and lungs from albino rat were removed and were fixed in 10% neutral buffered formalin and were processed for sectioning [17]. Sections (4-6 µm) were stained using haematoxylin and eosin (H and E) and Giemsa's stain techniques, respectively. All samples were then studied under a light microscope (Olympus BH-2) [18].

2.5 Statistical Analysis of Data

Data obtained were subjected to one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level using SPSS 15.0 version. Differences were considered significant at $p \leq 0.05$.

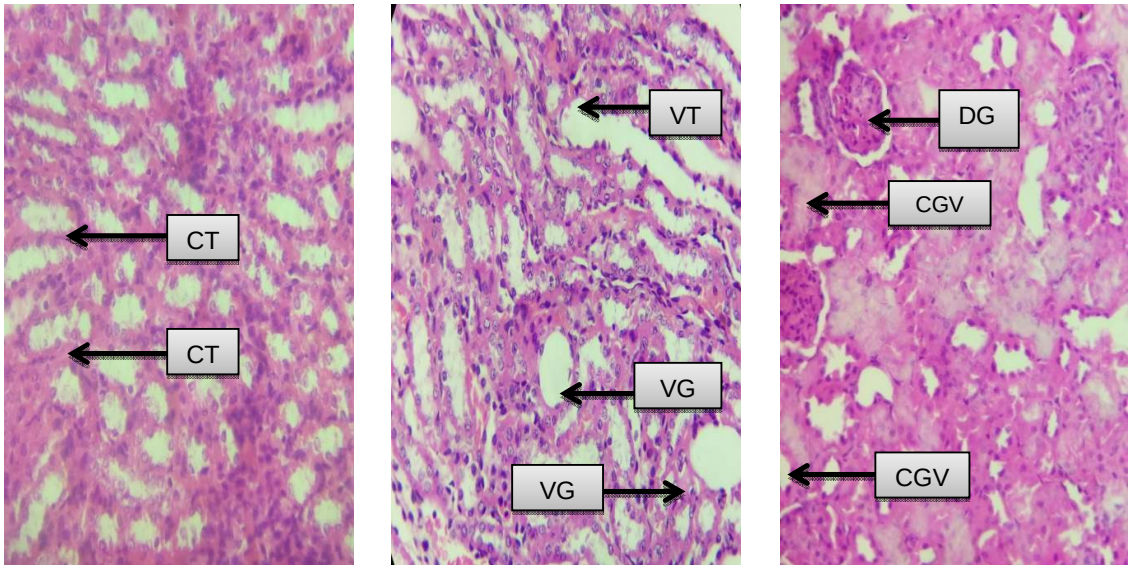
3. RESULTS

3.1 Haematological Profile of the Experimental Animals

In the context of the clinical signs: depression and decrease in appetite in the treated group while, no signs were noticed in the control group. There was significant increase between the treated group in the red blood cell mean with the control group 8±1.29 and the wistar rat infected with *S. aureus* having a mean value of 12±2.80 and *S. epidermidis* 11±1.76 while there was no significant difference between the packed cell volume, white blood cell, neutrophil, lymphocyte, monocytes, eosinphil and basophil (Table 1).

3.2 Histopathological Profile of the Experimental Animals

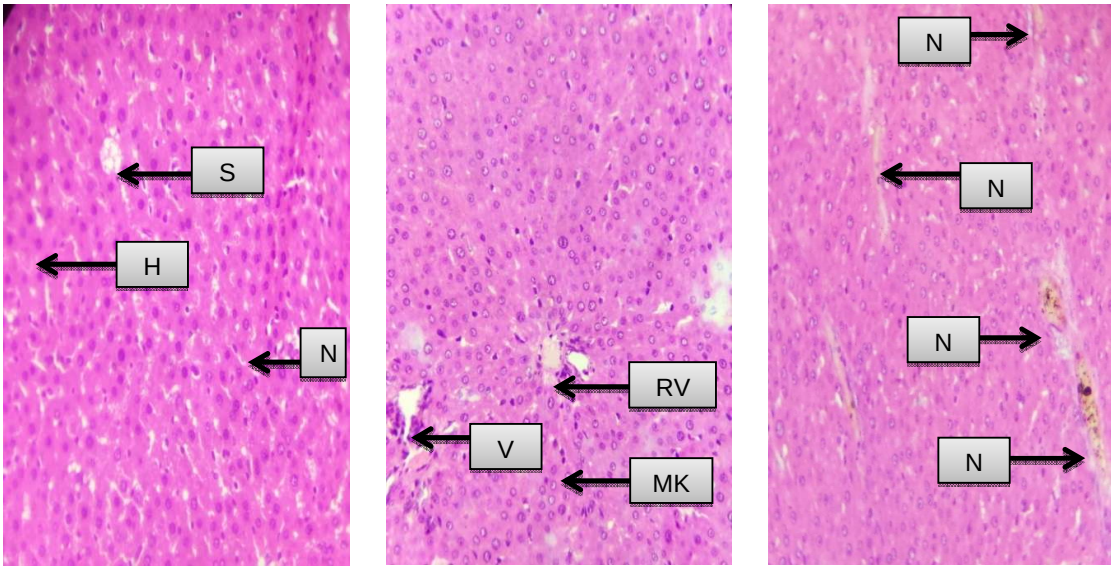
The kidney shows convoluted tubules in the control group while the SHA group shows vacuolization of glomerular and tubules, the SSU group show disruption of glomerular capillaries with vacuolar appearance. In the control group, there were normal liver cells showing hepatic cell, nucleus and sinusoids of the albino rat liver. In the SHA group, the liver showed ruptured vein, mild Kupffer cells infiltrations, vacuolization of the liver and necrotic effects were observed in the SSU group (Fig. 1). The lungs showed normal alveoli and alveolar sac in the control group while in the SHA group there was congestion, accumulation of the erythrocytes between the alveolar cells and necrosis, SSU groups shows edema, haemorrhage, necrotic effects and necrosis as shown in Fig. 2.



(A) **(B)** **(C)**

Fig. 1. Histopathological results of kidney

A: Normal kidney showing the convoluted tubules (CT) of control albino rat, B: SHA Kidney showing vacuolization of glomerular (VG) and vacuolization of the tubules (VT), C: SSU Kidney showing distruption of glomerular capillaries (DG) and distruption of congested glomerular with vacuolar appearance (CGV), Mag =X100, Keys: SSU- Staphylococcus epidermidis from unpolluted soil, SHA- Staphylococcus aureus from hand



(A) **(B)** **(C)**

Fig. 2. Histopathological results of liver

A: Normal liver cells showing hepatic cell (H) and nucleus (N) of the liver and sinusoids (S) of the control albino rat, B: SHA Liver showing vacuolization (V), ruptured vein (RV) and mild kuffer cells infilteration (MK), C: SSU Liver showing necrotic effects (N) (x100), Mag =X100, Keys: SSU- Staphylococcus epidermidis from unpolluted soil, SHA- Staphylococcus aureus from hand

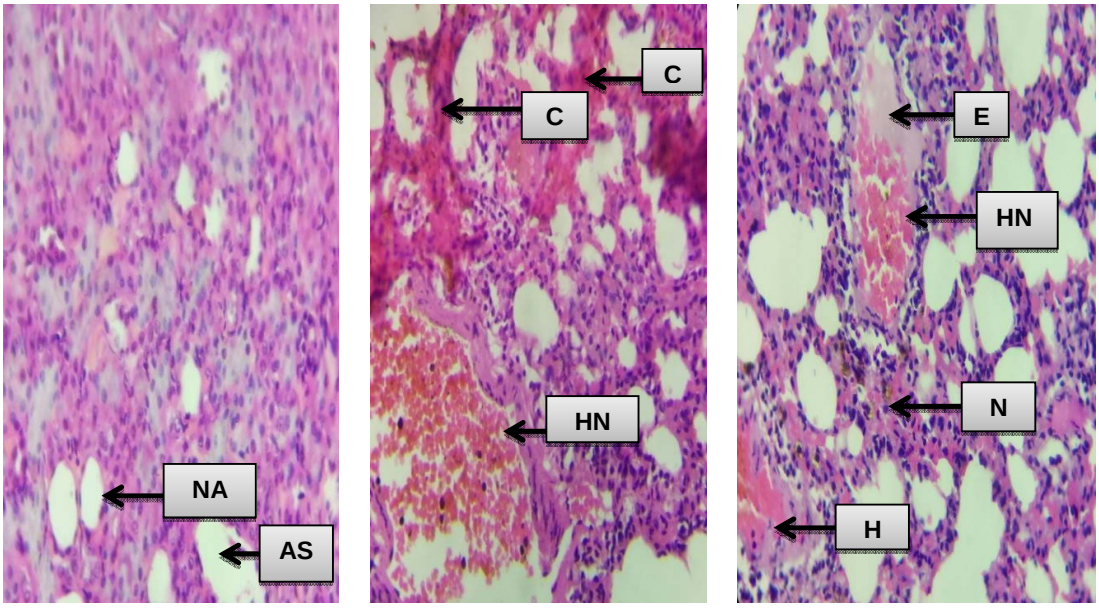


Fig. 3. Histopathological results of lungs

A: Lungs showing normal alveoli (NA) and alveolar sac (AS) of control albino rat, B: SHA LUNGS showing congestion (C) accumulation of erythrocytes between the alveolar cells, hemorrhage and necrosis (HN) ,C: SSU LUNGS showing edema (E), hemorrhage (H), necrotic effects (N), hemorrhage and necrosis (HN) , Mag =X100, Keys: SSU- Staphylococcus epidermidis from unpolluted soil, SHA- Staphylococcus aureus from hand

Table 1. Haematological profile of the experimental animals

Samples	PCV	RBC	WBC	NEU	LYM	MON	EOS	BAS
SSU	34±5.29[a]	11±1.76[a,b]	4.57±3.00[a]	60±5.00[a]	39.3±4.04[a]	0.67±1.15[a]	0.00±0.00[a]	0.00±0.00[a]
SHA	37±8.32[a]	12±2.80[b]	4.00±1.42[a]	55±13.01[a]	44.0±13.11[a]	0.67±1.15[a]	0.00±0.00[a]	0.00±0.000[a]
Control	27±2.52[a]	8±1.29[a]	4.53±0.42[a]	48±8.54[a]	47.67±4.51[a]	1.00±1.73[a]	0.00±0.00[a]	0.00±0.000[a]

Keys: PCV- packed cell volume, RBC- red blood cell, NEU-neurophils, LYM-lymphocytes, MON- Monocytes, EOS- eosinophils, BAS-basophil, SSU- Staphylococcus epidermidis from unpolluted soil, SHA- Staphylococcus aureus from hand
Note: Values followed by different alphabets along the same rows are significantly different at p≤0.05

4. DISCUSSION

Pathogenicity test of *S. aureus* and *S. epidermidis* conducted on healthy wistar albino rats by intradermal routes revealed significant increase in red blood cell counts between infected and control groups, while there was no significant increase in packed cell volume, white blood cell, neutrophil, lymphocyte and monocytes counts between the treated groups, the control group was higher in packed cell volume, white blood cell, neutrophil, lymphocyte and monocytes counts than the treated wistar rat, for the eosinophil and basophil there was no significant difference in contrast to other study [5] that reported significant increase in white blood cell in infected animals. There was no death occurring in the first week of experimentally infected animals suggesting that there was no high toxigenic nature of the organism in contrast to the report by Persis and Kalaiarasi [19]. It was observed that the kidneys of the rats exhibited some histological alterations such as vacuolization of glomerular, vacuolization of the tubules, distruption of glomerular capillaries, distruption of congested glomerular with vacuolar appearance. The alterations observed in the kidneys of the albino rats are probably due to exposure to toxin or metabolites from *Staphylococcus* spp from different source. Similar results have been reported by Bengston et al. [20]. The liver of the albino rat also exhibited histopathological changes like vacuolization, ruptured vein, necrotic effect and Kupffer cells infiltration. Degeneration of liver tissue and necrosis could be due to the infiltration of leucocytes. According to Thangavel et al. [21] necrosis is the direct

toxic effect. Accumulation of erythrocytes between the alveolar cells, edema, hemorrhage and necrotic effects were the pathological observed on the lungs of infected albino rats. This could be due to respiratory infection probably caused by the *Staphylococcus* spp that was inoculated. The lung is the essential organ of respiration and receives the entire cardiac output. Also, the lung plays an important role in host defence and regulation of circulating levels of biologically active materials by extensive surface of pulmonary vascular bed [13]. The results of experimental infection was lower to the results reported by Abdel-Motelib and Salem [22] who found that *S. aureus* caused 100% mortality in experimentally infected rabbits with the same dose and route of inoculation.

5. CONCLUSION

In conclusion the colonization with strains of *S. epidermidis* and *S. aureus* cause devastating effects on certain vital organs such as kidney, liver, and lungs which, depending on their severity, could be fatal.

ACKNOWLEDGEMENT

Authors are very much thankful to Mr E. O Ogundare for helping financially. This study does not have any supporting funds or grants from any agencies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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