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Original Research Article

Incidence of *Staphylococcus aureus* Wound Infection amongst Patients Attending University of Port Harcourt Teaching Hospital, Rivers State, Nigeria

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Abstract

Staphylococcus aureus is a common hospital and community-acquired pathogen known to be frequently associated with wound infections. Therefore, the aim of the research was to determine the incidence of *Staphylococcus aureus* in patients with wound infection at University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. A total of 150 specimens from different types of wounds (traumatic wound, caesarean section, scrotal wound, surgical wounds, burns, diabetic foot, and plastic surgery) were collected from the Hospital for a period of six months and processed for isolation of *S. aureus*, following standard microbiological procedures. Samples were cultured on sterile mannitol salt agar (MSA) plates and characterized phenotypically based on cultural and biochemical identities. Data obtained showed 38.7% of the wound cases were contaminated with *Staphylococcus aureus isolates.* It was observed that the organism mainly occurred in burns (52.63%), while the lowest incidence was related to plastic surgery (18.2%) contamination. The study showed that 10.3% of isolates were associated with biofilm formation, while gamma hemolysis and beta-hemolysis was observed in 93.1% and 6.9% of isolates, respectively. The results of this study represent serious public health concerns, thus emphasizing the need for proper wound management.

Keywords: Biofilm formation, hemolysis, incidence, patients, *Staphylococcus aureus*, wound infection.

1.0 INTRODUCTION

Staphylococcus is responsible for a large amount of diseases in humans and animals. *S. aureus* is a non-motile, facultative Gram-positive aerobic bacterium that occurs in pairs [1]. "It is a major pathogen that colonizes and infects both immunocompromised hospital patients and healthy immunocompetent people in the community. These bacteria are found naturally on the skin and in the nasopharynx of the human body. If there is a break in the skin due to injury or surgery, or if a person's immune system is suppressed, the presence of *Staphylococcus aureus* on the skin can cause an infection [2]. *S. aureus* occurs mainly on mucosal surfaces [3] and is often involved in many nosocomial infections [3].

Healthy skin forms a formidable obstacle against microorganisms and many other bacteria, but once this defense process is tampered with, creating a wound, bacteria have an ideal environment for growth and reproduction. Wound contamination occurs because of a dynamic host-pathogen interplay such that the sum of the pathogen load is greater than the host's immune defenses, resulting in a systemic immune response [4].

Wound infection happens because of a unique interaction between humans and disease causing microbes [4]. Wound contamination is defined as the appearance of the organism on the wound area [5]. When the number of bacteria in a wound is low (contamination), there is no problem with wound healing. However, as the number of bacteria in the wound increases, the chance of infection increases. In critical colonization, the bacterial load in the wound becomes unbalanced, leading to infection if the amount of bacteria is not managed fervently [6]. Different types of pathogens are associated with wound infection some of these pathogens include *Staphylococcus aureus, Streptococcus species*, *Pseudomonas aeruginosa*, and anaerobes [7].

Infection can occur in acute wounds, such as surgical wounds (surgery site infections), and in chronic wounds, such as pressure ulcers, diabetic foot ulcers, and leg ulcers, which are more likely to be colonized by bacteria due to infection [8]. Infection will complicate surgical wound healing and is significantly more common [9]."Wound infections can be superficial (skin only), deep (muscle and tissue), or spread to the organ or site where the surgery was performed [9]. Wound infection and wound healing is influenced by several factors. Bacterial colonization and the pathogenic potentials of the colonizing bacterial agent is one of such factors that determine wound healing. Studies on this agent cannot be overemphasized due to the different virulent features, including formation of biofilms and hemolysis, amongst others factors associated with the pathogenesis of the organism. The aim of the study was therefore, to determine the incidence of *Staphylococcus aureus* wound infection patients attending University of Port Harcourt Teaching Hospital, Rivers State, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Description of Study location

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), East West Road Port Harcourt, Rivers State, Nigeria. The hospital lies within 4.8998^{o} North and 6.9292^{o} East. It is a major tertiary healthcare and research facility in Rivers State, which consists of various departments for distinct health cases, and a great number of patients from many geographical regions accessing it.

2.2 Study Design and Sample Collection

A cross-sectional (prevalence) study design was implemented to determine the incidence of *Staphylococcus aureus* associated with wound infection at the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria. The study sampled one hundred and fifty (150) wound cases collected randomly from patients with different wound types in the hospital using sterile swab sticks. The samples were taken aseptically and transported to the Microbiology Laboratory, Rivers State University.

2.3 Sample Size determination

The sample size for the study was determined by the formula:

$N = [Z^2(pq)]/d^2$ [10]

Where:

N= the desired sample size

Z= Normal standard distribution that corresponds to confidence interval as 1.96

p= Prevalence of *Pseudomonas* species

q = 1-p

d= degree of accuracy / precision expected at 0.05

2.4 Isolation of *Staphylococcus aureus*

The different swab sticks specimens were plated on sterile Mannitol Salt Agar (MSA) plates using the streak plate method and incubated at 37ºC for 24 hours.

2.5 Purification and Preservation of Isolates

The distinct tentative *Staphylococcus* colonies on the MSA plates were further purified on freshly prepared Nutrient Agar (NA) plates by repeated subculturing until pure colonies (isolates) were obtained. Obtained pure isolates were inoculated aseptically into nutrient agar slants in Bijou bottles and incubated for 24 hours at 37ºC. After incubation, agar slants were then refrigerated at 4^{o}C to preserve the isolates.

2.6 Characterization and of *Staphylococcal* Isolates

This was done to identify the presumptive *Staphylococcus* isolates obtained from the different wound specimens analyzed. Characterization was done phenotypically on the basis of their colonial morphology, microscopy (Gram stain) and biochemical attributes. Prior to characterization, the preserved presumptive Staphylococcal isolates were first subcultured onto freshly prepared NA plates in order to resuscitate them and make them metabolically active.

2.7 Biofilm Production Test

This test was carried out to determine staphyloccocal isolates that are capable of producing biofilms (accumulation of mono-microbial populations held together by self-produced polymer matrix mainly composed of polysaccharides, proteins and extracellular DNAs). In order to determine this, a loopful of each isolated *Staphylococcus* species was inoculated each on different glucose-nutrient broth (5 ml) in test tubes. The tubes were incubated aerobically at 37ºC for 24 hours. After incubation, each tube was washed with 9 ml phosphate buffer saline and allowed to air dry. Crystal violet (10 ml) was further added to each tube and left at room temperature for 30 minutes, after which it was discarded. The tubes were rinsed with water and allowed to dry at room temperature in inverted position. Biofilm formation was detected by the presence of visible film on the wall and bottom of the tube [11].

3.12 Haemolysis Screening

The ability of species to induce haemolysis, (i.e. lyses of red blood cells); and the type of haemolysis caused was determined in this test. Blood agar (5% defibrinated blood in nutrient agar preparation) was used as test medium. The different identified isolates of *Staphyloccocus aureus* were introduced each into different blood agar plates and incubated for 18 – 24 hours at 37ºC. After incubation, a dark and greenish agar colouration indicated and α-haemolysis; lightened yellow and transparent, a β-haemolysis; while no colouration indicated a γ-haemolysis (no haemolysis).

3.0 RESULTS

4.1 Prevalence of *Staphylococcus aureus* in the wound sources

A total of 150 wound specimens were obtained from different types of wounds as shown in Figure 1. The data revealed that burns (B) were the most frequent wound type causing hospital visits. Traumatic wound (T/W) - 16%; caesarean section (C/S) - 14%; scrotal wound (ScrW) - 12%; surgical wound (SgW) - 4%; Accident and emergency (A/E) - 13.4%; diabetic foot ulcer (DFU) - 2.6% and plastic surgery (P/S) - 12% were also observed to be the other sources of wound causing hospitalization.

From the data obtained, it was observed that while 41% of wound cases from men were infected, a lower incidence was noted to be associated with wound cases from women (Figure 2).

As shown in Table 1, most of the staphylococcal isolates were obtained from burns (52.6%), followed by DFU, 50%; SCW, 44.4%; A/E, 37.5%; C/S, 36.38%; T/W and SgW, 33.3 % each, while plastic surgery (P/S) had the least incidence (18.2%) of *S. aureus* wound infection.

In the overall, the study recorded the presence of *S. aureus* in 58 specimens, representing a prevalence of 37%, as shown in Figure 3.

4.2 Biofilm Production and Haemolytic pattern of the isolates

The data obtained, as shown in Figure 4, revealed that 10 % of the isolates were able to produce biofilms. On the other hand, 54 isolates (representing 93 %) showed a beta-hemolytic pattern, while 7% showed a beta-hemolytic pattern (Figure 5).

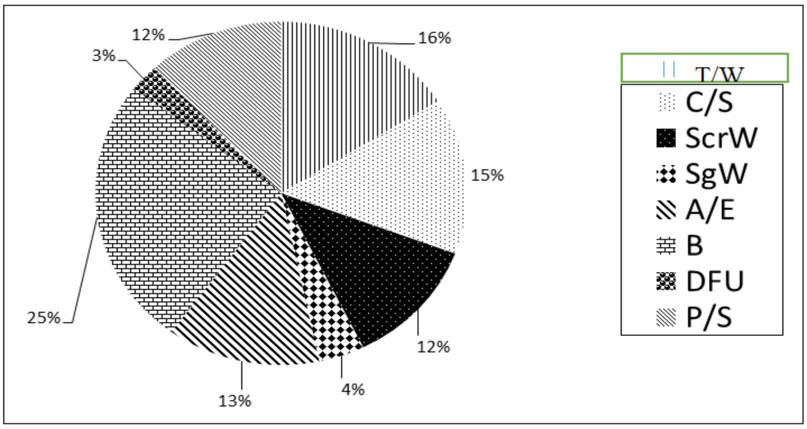

Figure 1: Percentage of different types of wounds studied

Key:
T/W = traumatic injury
C/S = caesarean section
ScrW = scrotal wound
SgW = surgical wound
A/E = accident and emergency
b = burns
DFU = diabetic foot ulcer
P/S = plastic surgery

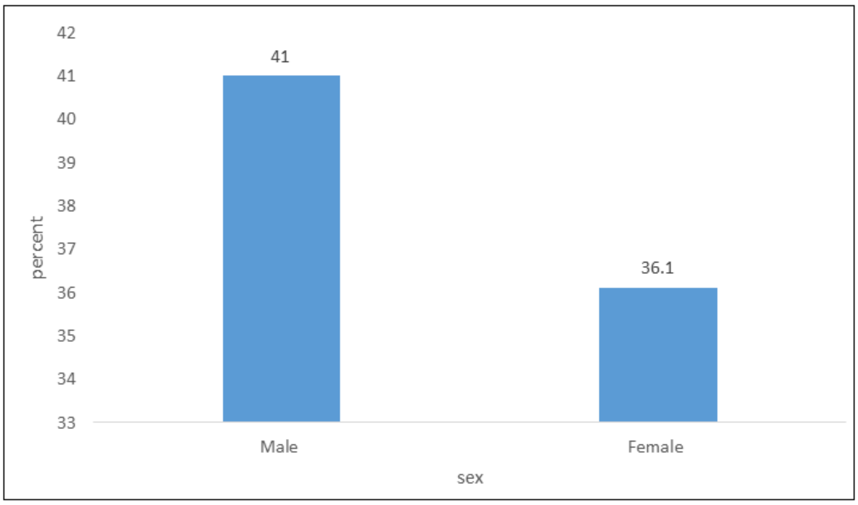

Figure 2: Sex based prevalence of *Staphylococcus aureus* contamination of wound

Table 1: Occurrence of *Staphylococcus aureus* based on sources of wound

Ward	No of specimens	No infected	% Occurrence
Accident and emergency	16	6	37.5
Traumatic wound	24	8	33.3
Scrotal wound	18	8	44.4
Plastic surgery	22	4	18.2
Ceasarean session	22	8	36.5
Diabetic foot ulcer	4	2	50
Surgical wound	6	2	33.3
Burns	38	20	52.6
Total	150	58	

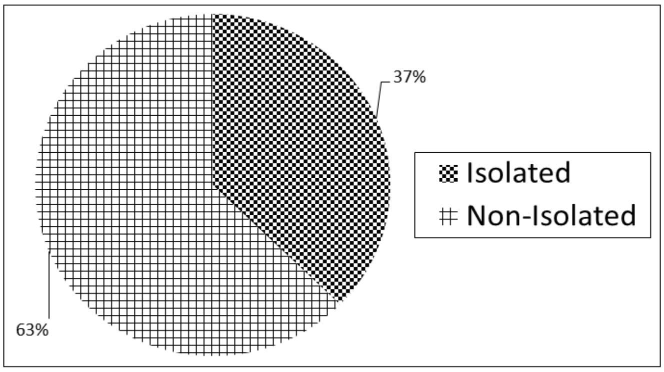

Figure 3: Overall prevalence of *Staphylococcus aureus* in wound samples

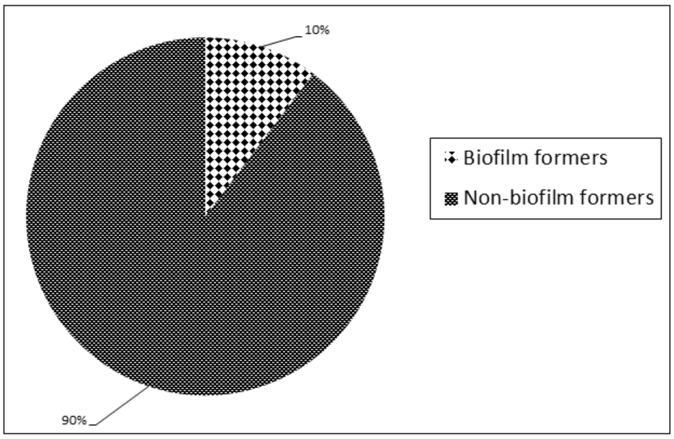

Figure 4: Biomembrane-forming *Staphylococcus aureus* from wound samples

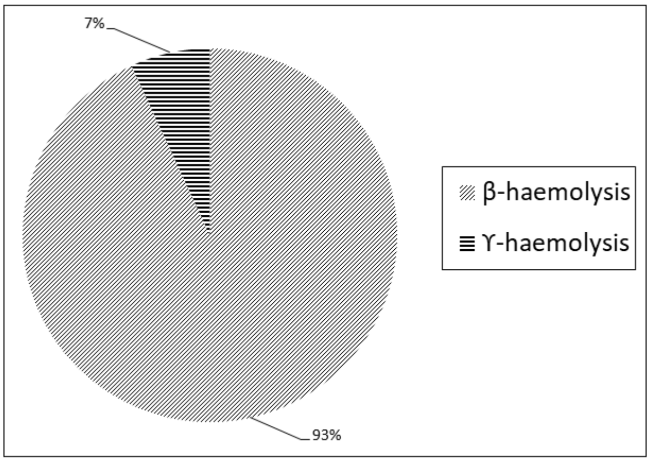

Figure 5: Haemolytic pattern of *Staphylococcus* Types of wound samples

4.0 DISCUSSION

Since *Staphylococcus aureus* is a normal skin flora, it was important to sample different types of wounds to decipher the pattern *of* staphylococcal wound contamination in hospital environment.

Previous researchers [12, 13] had implicated *Staphylococcus aureus* as the most frequent wound contaminant. This study has however reported the pattern of wound infection by *Staphylococcus aureus*, indicating an overall prevalence of 38.7% in wound cases in the study location. The study observed that majority of *Staphylococcus aureus* came from burns (52.6%), while the least amount came from plastic surgery (18.2%). The difference in the prevalence of this bacterial agent could be due to the environmental exposure rate and the degree of sterilization generally used in wound management [14]. This pattern of isolation is consistent with the study by Lipsky *et al.*, 2012 [15], which concluded that infection is more likely to occur in frequently exposed parts compared to isolated parts.

In this study, it was discovered that the prevalence was higher among the male gender compared to the female gender. However, other researchers elsewhere [16, 17] had reported gender variation and concluded that gender and age does not contribute to the pattern of microbial wound infection.

Only a small fragment of the isolated *Staphylococcus aureus* isolates (10.3%) were able to produce biofilm. Biofilms are assemblages of microbes suspended in a matrix of polyatomic extracellular substances: exogenous polysaccharides (EPS), extracellular DNA (electronic DNA), proteins, and amyloid proteins. Approximately 80% of chronic and recurrent microbial infections in the human body are caused by bacterial biofilms [6, 15]. Therefore, the presence of biofilm-forming *Staphylococcus aureus* in some wound samples may indicate a lengthy handling method.

Biofilms are typically formed by bacteria when exposed to harsh environmental conditions [5]. The implications biofilm in wound healing have been reported widely. Biofilm is known to represent one of the most complicated factors implicated in the healing of wounds. The infections associated with biofilms are devastating for patients since they can persist for months, causing patients to lose hope of recovery. In particular, biofilm has been previously detected in chronic leg ulcers [18, 19], diabetic foot ulcers [18], pressure ulcers [20], burns [21], malignant wounds [22] and surgical wounds [23].

Hemolytic pattern of staphylococcal species 93% and 7% have beta-hemolysis and gamma-hemolysis, respectively. Gamma hemolysis indicates the absence of hemolysis, while beta hemolysis indicates complete hemolysis [24]. This translates to 7% of the Staphylococcal species lacking the ability to hydrolyze red blood cells, while 93% of the isolates showed the ability to completely hydrolyze red blood cells. This breakdown is due to the effect of beta hemolytic, which is a highly active spingomyelinase against erythrocytes [25]. The presence of a large number (93.1%) of *beta-hemolytic Staphylococcus species* in wound samples indicates additional complications (eg, fungal infections, chronic myelitis, and respiratory tract infection).

4.0 CONCLUSION

With regards to the data generated from the entire course of study, it can be inferred that *Staphylococcus* is an evident component of the micro biome associated with infection of different wound types and that the incidence of biofilm- forming species is of a concern to healing rates and other biofilm-associated complications.

High prevalence of hemolytic species is indicative of high risk of disease formation from possible infections as a result of reduced oxygen and nutrient circulation that comes with decreased red blood cell counts. Wound cases must be managed appropriately to prevent bacterial colonization.

CONSENT AND ETHICAL APPROVAL

As per international standard guideline participant consent and ethical approval has been collected and preserved by the authors.

Competing Interests: Authors have declared that no competing interests exist.

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