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Prevalence of *Staphylococcus aureus* within the Hospital Environment

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ABSTRACT: The prevalence of *Staphylococcus aureus (S. aureus)* in the hospital environment was studied using swab samples from different parts of General Hospital, Umuguma Owerri, Imo State, Nigeria. A total of forty five (45) samples were collected from different parts of the hospital environment and analyzed aseptically in the Microbiology laboratory of the hospital, within thirty minutes of collection. Twenty five (25) representing 55.6% samples/ parts of the hospital environment were suspected to haboured *S. aureus*. Two samples i.e. 4.0% had non-significant growth of the organism while the rest 13 i.e. 28.9% did not seem to habour the organism. The morphological characteristics of the organisms on both media used (Mannitol salt agar and MacConkey agar) were used as presumptive identification while standard biochemical tests were used to confirm the isolates as *S. aureus.* Result analysis revealed a significant prevalence of *Staphylococcus aureus* within the hospital environment. There is therefore need for routine surveillance of the hospital environment to ensure that the organism does not continue to increase the rate of nosocomial infections.

KEYWORDS: *Staphylococcus aureus*, prevalence, hospital, environment, swab samples

ORIGINAL ARTICLE

INTRODUCION

Nosocomial infections [i.e. infections acquired from the hospital environment secondary to the patients' original condition] have posed a scourge to patients, staff and visitors to the hospitals over the years. According to Singleton (1999), nosocomial infection has been recognized ever since man first gathered patients under the same roof for the diagnosis and treatment of disease. The hospital exists as a closed community, it is therefore not surprising that certain microorganisms become predominant and may cause disease. It seems obvious that the occurrence of Nosocomial infection is related to hospital milieu. Various microorganisms have been implicated in Nosocomial infections, one of the most common causative agents being the ubiquitous St*aphylococcus aureus (S. aureus) among others.*

S. aureus is a Gram positive coccus that occurs in grape-like clusters. It is a eubacterium that is found on the surface of the human skin and mucous membranes (Ogbulie et al., 1999; Prescott et al., 2005). It also is found in other areas of human contact such as air, dust and food products (Ogbulie et al., 1999; Prescott et al., 2005). Cheesborough (1994) describes the *Staphylococcus* species as a group of non motile, non capsulate, Gram positive cocci of uniform size (about 1um in diameter) that occur characteristically groups but also singly and in pairs. *S. aureus* is an opportunistic pathogen in man and animals and is the most frequent cause of hospital and community infections (Prescott et al., 2005). The bacterium is a short term lived contaminant, short term resident or long term colony forming, non spore forming catalase and coagulase positive organism (Prescott et al.,2005).

Staphylococci are widely distributed in the environment. They form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract (Cheesborough, 1994). *S. aureus* is a member of the group of Gram positive bacteria called pyogenic [pus-producing] cocci (Sainsbury and Singleton, 2001). These cause various surpurative scalded skin syndrome (Prescott et al., 2005). As earlier said the organism is an opportunistic pathogen. Most strains become infectious usually when the skin or mucous membrane is punctured by variety of objects such as needles, blades, surgical devices etc. (Cheesborough, 2004; Prescott et al., 2005). Little wonder then, that it is a serious threat to hospitalized patients.

Generally, the organism presents three broad disease types; a variety of superficial infections such as pimples, boils and toxic epidermal necrolysis [characterized by outer layer of the skin separating from the deeper layers]; systemic infections such as endocarditis [inflammation of heart valves], osteomyelitis [inflammation of bone or bone marrow] and toxinoses

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such as food poisoning or toxic shock syndrome (Landolo, 2000). Archer and Crossley (1997) opined that the pathogenicity of the bacterium depends upon a number of virulence factors: a variety of surface protein on the bacterium's cell membrane which allow attachment and colonization of the bacterium within the cellular and extracellular material of the host; cellular proteins, proteases and toxins that inhibit phagocytosis and interfere with the ability of the host to actively hinder bacterial populations in the invasion of host tissues. Cheesborough, (1994) confirmed this opinion by suggesting that the versatility of *S. aureus may be traced to the array* of enzymes and toxins it produces. These enzymes and toxins include;

- Coagulase, an enzyme that clots plasma and coats staphylococcal cells which probably prevents their being engulfed by phagocytes.
- Leucocidin, a toxin that kills white blood cells
- Lytic toxins; these are exotoxins that destroy red cells and platelets.
- Deoxyribonuclease (DNAse), an enzyme that destroys deoxyribonucleic acid (DNA)
- Hyaluronidase, an enzyme that helps the organism to spread in tissues
- Lipases, these break down fats
- Staphylokinase, causes fibrinolysis
- Exfoliatin causes peeling of the human skin
- Enterotoxin B, causes food poisoning
- Beta-lactamases are enzymes that inactivate antibiotics. They are the cause of penicillin resistance by *S. aureus.*

Cui *and* Hiramatsu (2003) further identified accumulation of resistance factors by the bacterium making it immune to a variety of commonly used commercially available antibiotics thus increasing the ability of the organism to survive and multiply even in hostile environment as also accounting for its versatility.

The outcome of any staphylococcal infection depends on the population of the infecting organism and the host's defense or degree of resistance (Smith et al., 1999). Virulence which refers to the degree of pathogenicity is determined by three main characteristics: - invasiveness, infectivity and pathogenic potential. However, a few organisms can cause diseases if they are extremely virulent or the host's resistance is low (Ogbulie et al.,1999). A hospitalized patient's resistance can drop so much that its own micro biota may cause disease [i.e. endogenous infection] (Prescott, et al., 2005). This can be a serious problem among hospitalized patients whose immunity level is compromised. Hence *S. aureus* can be graded as a menace in the hospital environment and need to be contained if not curbed. *S. aureus* and its consequent infections especially Nosocomial infections may be curbed if such measures as the following are taken as recommended by Smith et al. (1999):

- Implementing active surveillance of the organism in the laboratory and health care environment
- Educating health care providers/workers about infection transmission and control measures
- Putting in place ,improved laboratory methods for ready identification of the organism
- Strictly following infection control protocols in treating those infected with the pathogen especially resistant ones.

Prescott, *et al.* (2005) adds that the organism can be curbed in the hospitals if:

- Patients are screened upon admission to prevent contact of carriers of drug resistant *S. aureus with* non carriers and exposure to infected surface.
- A new drug target at the organism is developed

Other long term preventive measures include proper sanitation, public health education and vaccination (Okeke et al., 1999). The above measures if put in place is expected to improve the situation in the near future but for them to be effective a proper knowledge of the prevalence/ abundance of the organism is required such that the measures taken will be adequately structured to curb the menace of Nosocomial infections caused by *S. aureus.*

This study is therefore aimed at assessing/ finding out the prevalence of *S. aureus* within the hospital environment with a view of alerting and educating the hospital community; patients, patients' relatives and hospital workers as well as the entire populace of the presence and abundance of this organism in their midst as well as its public health importance. This awareness is expected to create consciousness towards putting in place necessary preventive and control measures against the organism in our hospitals thereby preventing spread of secondary/ Nosocomial infections within the hospital community and the larger populace.

MATERIALS AND METHODS

Location of Study

This study was carried out in Owerri, Imo State, Nigeria with the consent of the hospital management and laboratory scientists of Imo State General Hospital, Umuguma, Owerri.

Sample Collection and Preparation

A total of 50 samples were collected randomly from different parts of the hospital environment such as laboratory bench, toilet sinks, ward floor, surgical materials etc using Evepon sterile swab sticks. Collection was done aseptically by opening the swab sticks just before collection, rubbing it over the sample area and immediately replacing it in the container. These were labeled appropriately and immediately carried to the General hospital laboratory for culturing.

Isolation and Characterisation of Organism

Mannitol salt agar and MacConkey agar culture media were used for culturing the specimens. The streaking culture technique as described by Ogbulie et al., (1998) was used for isolation of the organism. The method is as follows;

- With the swab stick containing the sample, make a pool at the edges of the two culture media plates in duplicates respectively
- With a sterile [flamed] wire loop, streak the sample from the loop to the entire surface of the Petri dish, flaming and cooling the wire loop in between streaks to obtain discrete colonies
- Incubate the plates upside down at 37^{o}C for 24 hours
- Observe the plates for microbial growth noting the morphological characteristics.

Identification of Isolates

Gram staining and other appropriate biochemical tests; catalase, coagulase, motility, mannitol utilization and oxidase tests as prescribed and described by Cheesborough (1994) for identification of *S. aureus* were carried out as follows;

Gram Staining: A smear of the isolate was made on a grease-free slide using a sterilized wire loop. The smear was air dried and heat fixed by passing the underside of the slide thrice over Bunsen burner flame. The fixed smear was then covered with crystal violet for 30- 60 seconds and washed with clean water. It was again covered with lugols iodine for 30 – 60 seconds, washed and rapidly decolourized using acetone. It was then washed again, after which it was counterstained with neutral red for 1 -2 minutes and washed off. The back of the slide was wiped and it was places in a draining rack for air drying. After drying, the stained slide was examined under the X100 objective of the light microscope using immersion oil.

Catalase Test: Some drops of hydrogen peroxide were placed in a clean test tube. A sterile wooden stick was used to pick a colony from the plate. This was inserted into the hydrogen peroxide. Instant appearance of bubbles within ten seconds was indicative of positivity.

Coagulase Test: A drop of physiological saline was placed on each end of a grease free sterile glass slide. A flamed and cooled wire loop was used to pick a single colony. This was emulsified in each drop of saline to make a thick suspension. A drop of plasma was added to one of the suspensions while the other acted as a control without the plasma. The slide was rocked gently. Clumping/agglutination of the suspension with plasma within 10 seconds indicate positive result.

Motility Indole Urease Test: A straight wire was used to collect a discrete colony of isolate. This was stabbed once into a freshly prepared motility indole agar. It was then incubated at 37^{o}C for 24 hours after which it was observed for growth. 0.5 ml of Kovacs reagent was then added into the suspension and observation made once more. Motility is indicated by diffused growth of organism with zone of turbidity while formation of deep red coloured ring on the surface of the agar upon addition of Kovac's reagent indicates indole production. Finally, a change of colour from yellow to pink shows urease production.

Mannitol and Lactose Fermentation

These were observed on the mannitol salt agar and MacConkey agar plates respectively after incubation.

Oxidase Test: A piece of heavy metal free-clean filter paper was placed on a clean Petri dish; 3 drops of freshly prepared Oxidase reagent were added on the filter paper. With a sterile wooden stick, a colony of the test organism was pick and smeared on the filter paper. Appearance of a blue purple colour within ten seconds (10 secs) indicates positivity and vice versa.

RESULTS

Out of a total of forty five (45) samples collected from different parts of the hospital environment, twenty five (25) representing 55.6% samples/ parts of the hospital environment which are: were suspected to habour *S. aureus* as all organisms that grew on mannitol salt agar as well as non lactose fermenting on MacConkey agar were taken as suspects. Two samples i.e. 4.0% had non significant growth of the organism while the rest 13 i.e. 28.9% did not seem to habour the organism.

The suspected organisms were then subjected to Gram staining and confirmatory biochemical tests and the result is shown in table 1 below:

Table 1. Result of Gram staining and confirmatory biochemical tests on all organisms suspected to be *S. aureus.*

Gram staining	Catalase test	Coagulase test	Oxidase test	Motility test	Indole test	Urease test	Mannitol utilization test	Organism
Positive cocci in clusters	Positive	Positive	Negative	Negative	Negative	Positive	Positive	*S. aureus*

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

From the results above, all the organism growing on mannitol salt agar proved to be *Staphylococcus aureus* this corroborates Cheesborough (1994) and Prescott *et al.*(2005) that *Staphylococcus aureus* is able to utilize the sodium chloride salt in mannitol salt hence can grow under such harsh environmental condition unlike other species of organisms. The biochemical tests results are also in line with the reports of other authors on characteristics of *Staphylococcus aureus* (Ogbulie et al., 1998; Cheesborough, 1994 and Prescott et al., 2005).

The result also shows that 59.6% of the samples tested haboured varying levels of *Staphylococcus aureus.* This is worthy of note because it implies a greater percentage of the samples tested and informs the fact that the organism is abundant in the hospital environment. This abundance may be traced to the versatility of the organism as well as the number of people visiting, relative to the size of the hospital which happens to be a closed community. Both symptomatic and asymptomatic carriers form part of the hospital community, this fact could be a cause of the high prevalence of the organism in the environment. It is also worthy to note that the organism is part of the normal microbial flora of the skin, upper respiratory and intestinal tract (Cheesborough, 1994) hence can be easily deposited in the hospital environment by patients, patients' relatives, hospital workers and visitors alike. This also confirms why the organism was found on the skin of laboratory staff, door handles, air and nasal swabs.

S. aureus is versatile and can grow in different environments. It is an opportunistic bacterium and only causes diseases when there is an abrasion in the normal host's external defences or in cases of low immunity. Patients with abrasions or that underwent surgery should be kept as far away as possible from possibly contaminated environment as well as carriers of the organism. People should be made aware of the presence of the organism in their midst and its mode of pathogenesis. It is noteworthy that the organism can be contained by various means.

It is necessary that active surveillance of the organism be made from time to time to ensure that the level of abundance does not increase in the environment. Specific steps targeted at greatly reducing the population of the organism in the hospital environment should also be put in place. External surfaces should be disinfected or sterilized as much as possible from time to time (i.e. as part of routine cleaning). It is also recommended that the antibiogram of the strains of the organism in the environment be carried out to estimate the level of resistant ones in the environment as well as their source of resistance i.e. whether resistance is plasmid mediated or chromosomal mediated such that new drug targets can be established as prescribed by Prescott *et al. (2005)*. Furthermore, health care providers should take appropriate precautions (maintain good hygiene and proper asepsis) to ensure that they do not transmit the organism from one patient to the other during the course of treatment. Public awareness of the presence of the organism, its mode of transmission and course of infection as well as preventive measures to avoid infection should be on-going in the hospitals as well as the entire community. *S. aureus* has serious public health import and is of epidemiological concern hence the hospital management and government should form policies to effect the above recommendation and even more protective measures.

In conclusion, if the above recommendations are put in place the menace of secondary / Nosocomial infections faced by patients in our hospitals especially those caused by *S. aureus* will be greatly reduced. This will not only be a relief to patients but also to the community at large.

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