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RESEARCH PAPER

INCIDENCE AND ANTIBIOTIC SENSITIVITY PATTERNS OF HAEMOLYTIC STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM THE ENVIRONMENTS OF PAEDIATRIC WARDS OF SELECTED HOSPITALS IN OSOGBO, OSUN STATE, NIGERIA

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***Staphylococcus aureus* is one of the leading microorganisms responsible for nosocomial infection in paediatrics. This study evaluated the incidence and antibiotic sensitivity patterns of haemolytic strains of *S. aureus* isolated from the environments of paediatric wards of Corner Stone Hospital (CSH), Osun State Hospital (OSH), and Emmanuel Hospital (EH), Osogbo. A total of 263 bacterial isolates were obtained from the hospitals. They were distributed as follows: 21.67%, 52.47% and 14.45% from CSH, OSH and EH respectively. *S. aureus* was predominant on the floor (47.15%) while the least was found in pillow (15.59%) in the hospitals. Sixty five (65) of the isolates were haemolytic and the distributions were 41.54%, 35.39% and 23.08% from CSH, OSH and EH respectively. The results showed that there were significant difference ($p \leq 0.05$) between the incidence of *S. aureus* and the location of the sampling. The antibiotic patterns shows that the isolates exhibited more susceptibility towards Gentamicin (100%) while the isolates exhibited more resistance towards Ampicillin (91.3%). The incidence of haemolytic and antibiotic resistant strain from locations where patients, health care personnel and workers have regular contact with represent a public health threat, therefore, safety measures to prevent nosocomial infections within the hospital environment should be put in place.**

Key words: *Staphylococcus aureus*, Paediatrics, Antibiotics, Hospitals, Nosocomial infections.

INTRODUCTION

Nosocomial infections remain a major problem in the hospital across the world and leads to high mortality. The infections results in complication during admission in the hospital and increase costs for patients, health centers (Behzadnia *et al* 2014). Organisms such as *Staphylococcus aureus*, *Streptococcus* spp., *Bacillus cereus*, *Cinetobacter* spp., coagulase negative *Staphylococci*, *Enterococci*, *Pseudomonas aeruginosa*, *Legionella* and members of the Enterobacteriaceae family like *Escherichia coli, Proteus mirabilis, Salmonella spp., Serratia marcescens* and *Klebsiella pneumoniae* cause nosocomial infections. However, the most frequently reported pathogens implicated in nosocomial infections are *E. coli, S. aureus, Enterococci, P. aeruginosa* and *Streptococcus* (Bereket *et al* 2012). *S. aureus* (**Figure 1**) has been one of the leading microorganisms responsible for the nosocomial infection in paediatrics (Balaban *et al* 2012) and several compounds have shown potential against this microorganism (Oyebamiji and Semire, 2017; Dahiya *et al* 2017).

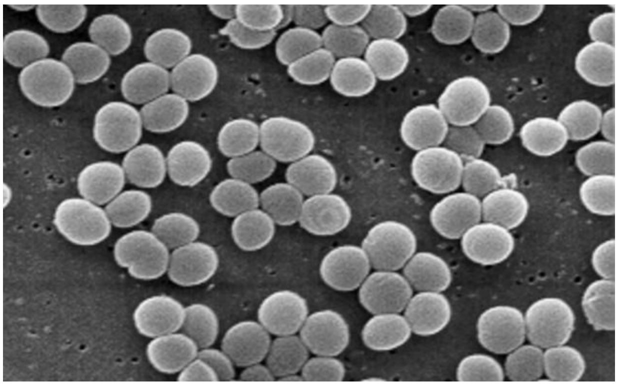

Fig. 1. *Staphylococcus aureus* bacteria

Neonates are particularly susceptible to infections from the environment because of their immature immune system. Although previous researchers have reported the isolation of *Staphylococcus aureus* in some Osun state hospitals (Onelum *et al* 2015), information on the prevalence of *Staphylococcus aureus* in paediatric wards in hospitals within Osun state is deplete. This research work therefore aims to provide information on the incidence of haemolytic strains of *S. aureus* isolated from the environments of paediatric wards of some selected hospitals in Osun State and their antibiotic sensitivity patterns.

MATERIALS AND METHODS

Study location

This study was carried out in 3 selected hospitals in Osun State, namely (Corner Stone Hospital, Osogbo; Emmanuel Hospital, Osogbo; Osun State Hospital, Osogbo) Osun State is an inland state in south-western Nigeria. The state is situated in the tropical rain forest zone. It lies approximately on Latitude 40˚ N of the equator and longitude 7.34˚ E of the Greenwich meridian and about 1100m above the sea level. Prior to the commencement of this study, permissions were obtained from all the three hospitals' management.

Sample collection

Samples were taken from different spots at different locations (Table, Bed bar, Chair, Floor, Pillow, Bed Spread, Door knob and equipment table) in the hospital environment. Sterile swab sticks with their envelopes were labelled accordingly and aseptically kept in a cooler containing ice and transported to Corner Stone Hospital, Osogbo; Emmanuel Hospital, Osogbo; Osun State Hospital, Osogbo at different times then, taken to paediatric ward in the hospitals' environment(s). The locations vary from one hospital to the other. A total of 27 samples were then obtained. Samples were collected in sterile containers and stored at 40°C prior to analysis.

Detection and enumeration of haemolytic strain of S. aureus

The detection of *S. aureus* was determined according to the method described in literature (Samie and Shivambu, 2011). Biochemical characterization which includes Gram staining, catalase and coagulase test were carried out on the suspected colonies according to the method described (Cheesbrough, 2002). The hemolytic activity testing of the *S. aureus* isolates was performed according to the method described (Jimenez *et al* 2008) and the strains were classified into non-hemolytic, alpha (α) hemolytic and beta (β) hemolytic.

Antibiotic susceptibility test

The antibiotic susceptibility of haemolytic strain of *S. aureus* was carried out according to the methods standardized by the Clinical and Laboratory Standards Institute, 2011. The following panel of antimicrobial disks was used for each test isolates: Ampicillin (10 μg), Ciprofloxacin (5 μg), Gentamycin (3 μg), Chloramphenicol (30 μg), Tetracycline (30 μg), Sulphamethoxazole (30 μg), Erythromycin (30 μg). The zone of inhibitions were measured and interpreted with the break point standards for zone of diameter provided by CLSI, 2011 guidelines, to classify the antibiotic sensitivity of each isolates as 'susceptible', 'intermediate' or 'resistant'.

Distribution of Staphylococcus aureus per location in the hospital

The distribution of *Staphylococcus aureus* per sample spot in different location in the hospital was calculated by dividing the number of *S. aureus* per location in the hospital by the cumulative number of *S. aureus* positive isolates in the hospital environment; which was then multiplied by 100 and expressed as the percentage distribution.

Statistical analysis

The distribution of *S. aureus* per sample spot in different locations, the distribution of hemolytic *S. aureus* to non-hemolytic strain and distribution of α-hemolytic strain to β-hemolytic *S. aureus* from each location within the three hospitals environments were compared statistically with SAS (Statistical Analysis

Software 92.2) using Pearson chi-square analysis. Statistical significance was defined by a p value <0.05.

RESULTS

Distribution of Staphylococcus aureus in the hospitals

Table 1 shows the distribution of *S. aureus* within the environment of the three hospitals studied. There is higher percentage (59.7%) of *S. aureus* from floor in Corner Stone Hospital. In Osun State Hospital, there is higher percentage (47.1%) of *S. aureus* from floor, door knob (32.6%), chair (7.3%), and pillow (13.04%). In Emmanuel Hospital, floor and door knob has the same percentage distribution (36.8%), chair (14.7%), equipment table having 11.8%. In all of the hospitals, floor has the highest distribution.

Table 1. Distribution of *S. aureus* within the environment of the three hospitals

Location	Number of *S. aureus* from spot of sample collection within location			p value
	CSH	OSH	EH	
Table	0(0.00)	0(0.00)	0(0.00)	0.05
Bed	0(0.00)	0(0.00)	0(0.00)	0.05
Chair	0(0.00)	10(10.00)[d]	10(10.00)[d]	0.05
Floor	34(33.5)[a]	65(65.50)[a]	25(24.0)[a]	0.05
Pillow	23(23)[b]	18(17.50)[c]	0(0.00)	0.05
Bed spread	0(0.00)	0(0.00)	0(0.00)	0.05
Door knob	0(0.00)	45(44.50)[b]	25(24.00)[a]	0.05
Equipment table	0(0.00)	0(0.00)	8(8.00)[c]	0.05
Σ^b	57(56.50)	138(137.5)	68(66)	0.05

[a,b,c] Percentage distribution of *S. aureus;* Σ^b Total percentage of distribution of *S. aureus* per hospital;
CSH: Corner Stone Hospital; OSH: Osun State Hospital; EH: Emmanuel Hospital.

Identification and differentiation of Staphylococcus aureus haemolytic strains

A total of 57 strains from Corner Stone Hospital, Osogbo, demonstrated haemolysis on 5% sheep blood agar from the *S. aureus* isolated from the hospital. 27 isolates exhibited beta haemolysis (complete haemolysis). In Osun State Hospital, 23 isolates exhibited beta haemolysis. In Emmanuel Hospital, 15 isolates exhibited beta haemolysis (**Table 2**).

Table 2. Distribution of beta haemolytic *S. aureus* within the environment of the three hospitals

Location	Number of beta haemolytic *S. aureus* from spot of sample collection within location			p value
	CSH	OSH	EH	
Table	0(0.00)	0(0.00)	0(0.00)	0.05
Bed	0(0.00)	0(0.00)	0(0.00)	0.05
Chair	0(0.00)	10(10.00)[d]	10(10.00)[d]	0.05
Floor	11(11)[a]	12(12.00)[a]	1(1.00)[c]	0.05
Pillow	16(16)[b]	7(7.00)[b]	0(0.00)	0.05
Bed spread	0(0.00)	0(0.00)	0(0.00)	0.05
Door knob	0(0.00)	4(4.00)[c]	11(11.00)[a]	0.05
Equipment table	0(0.00)	0(0.00)	3(3.00)[b]	0.05
Σ^b	27(35)	23(23.0)	15(15)	0.05

[a,b,c] Percentage distribution of *S. aureus;* Σ^b Total percentage of distribution of beta haemolytic *S. aureus* per hospital;
CSH: Corner Stone Hospital; OSH: Osun State Hospital; EH: Emmanuel Hospital.

Distribution of haemolytic isolates Corner Stone Hospital, Osun State Hospital and Emmanuel Hospital, Osogbo environment

After haemolytic test was conducted, 27 isolates of *S. aureus* exhibited beta-haemolysis from Corner Stone Hospital, with pillow having the highest percentage distribution (59.3%) and the floor with (40.7%). Twenty three (23) isolates of *S. aureus* were beta haemolytic from Osun State Hospital, with floor having the highest percentage distribution, 52.2%, pillow (30.4%) and Door knob (17.4%), and 15 were also beta haemolytic from Emmanuel Hospital with door knob having the highest percentage distribution (73.3%), Equipment table (20%) and floor (6.6%).

Antibiotic sensitivity testing result

The tables (**Tables 3-5**) below shows the antibiotics susceptibility distribution of *S. aureus* obtained from each of the hospitals, where Gentamicin showed the highest percentage of sensitivity (100%) and Ampicillin shows the highest percentage of resistance (100%) from Corner Stone Hospital. In Osun State Hospital, Gentamicin also show the highest percentage of sensitivity pattern (100%) and Ampicillin has the highest percentage of resistance (91.30%). In Emanuel Hospital, Gentamicin also has highest percentage of sensitivity and Ampicillin with the highest percentage of resistance (100%).

Table 3. Antimicrobial sensitivity results of *S. aureus* isolates from Corner Stone Hospital environment

Drugs	Number of samples susceptible (%)	Number of samples with intermediate susceptibility (%)	Number of samples resistant (%)	Total
Ampicillin	0(0.0)	0(0.0)	27(100)	27
Chloramphenicol	24(88.89)	0(0.0)	3(11.11)	27
Tetracyline	0(0.0)	0(0.0)	27(100)	27
Ciprofloxacin	26(96.3)	1(3.7)	0(0.0)	27
Gentamicin	27(100)	0(0.0)	0(0.0)	27
Sulphamethoxazole-Trimethroprim	17(62.96)	4(14.8)	6(22.22)	27
Erythromycin	20(74.07)	4(14.8)	3(11.11)	27

Table 4. Antimicrobial sensitivity results of *S.aureus* isolates from Osun State Hospital environment

Drugs	Number of samples susceptible (%)	Number of samples with intermediate susceptibility (%)	Number of samples resistant (%)	Total
Ampicillin	2(8.69)	0(0.0)	21(91.30)	23
Chloramphenicol	21(91.30)	0(0.0)	2(8.69)	23
Tetracyline	7(30.44)	2(8.69)	14(60.87)	23
Ciprofloxacin	21(91.30)	0(0.0)	2(8.69)	23
Gentamicin	23(100)	0(0.0)	0(0.0)	23
Sulphamethoxazole-trimethroprim	18(78.26)	3(13.04)	5(21.74)	23
Erythromycin	5(21.74)	9(39.13)	9(39.13)	23

Table 5. Antimicrobial sensitivity results of *S.aureus* isolates from Emmanuel Hospital environment

Drugs	Number of samples susceptible (%)	Number of samples with intermediate susceptibility (%)	Number of samples resistant (%)	Total
Ampicillin	0(0.0)	0(0.0)	15(100)	15
Chloramphenicol	11(73.33)	0(0.0)	4(26.67)	15
Tetracyline	6(40.0)	0(0.0)	9(60.0)	15
Ciprofloxacin	8(53.33)	4(26.67)	3(20.0)	15
Gentamicin	15(100)	0(0.0)	0(0.0)	15
Sulphamethoxazole	4(26.67)	4(26.67)	7(46.67)	15
Erythromycin	7(46.67)	3(20.00)	5(33.33)	15

DISCUSSION

Staphylococcus aureus isolated from this study have been reported as one of the most common nosocomial pathogen usually isolated in a hospital environment. It is an important pathogen that causes significant epidemiologic and therapeutic challenges implicated in a wide variety of infections from numerous sources health care personnel, ICU and in paediatric wards (Chikere *et al* 2008).

From this study, a total of 263 isolates was suggestive of pathogenic Staphylococci by their fermentation of mannitol in selective mannitol salt agar medium. The selective Mannitol Salt Phenol red Agar (MSA) medium allows accomplishing a screening or selection of *S. aureus* from samples potentially contaminated (Smyth and Kahlmeter, 2005). Their high concentration of salt (NaCl 7.5g% *w/v*) tends to inhibit the growth of many microorganisms, however it has great importance to microorganisms of the Staphylococci group (Kampf and Kramer, 2004).

The least percentage of *S. aureus* was isolated from the pillow in corner stone hospital environment and from both Osun State Hospital and Emmanuel teaching hospital, chair has the least distribution. An analysis suggests that 99% removal is adequate in general circumstances to reduce the microbial risk from fomites to a very low concentration for a single touch, with no consideration of pathogen die-off in the environment. Therefore, constant cleaning of these formites especially tables has been able to kill off the pathogens it might be carrying, i.e. greater removal has been sought to provide more assurance of safety. Overall, in the three selected hospitals studied, *S. aureus* was more in pillow, floor, bed spread, equipment table in correlation with the work carried out earlier (Uneke *et al* 2010) where organisms involved in nosocomial infection had been isolated from the fabrics used in hospital especially the bedspreads.

S. aureus isolates showed 100% resistance to Ampicillin which is concord with the earlier work (Gabriel and Kebede, 2007) whose work reported 100% resistance of *S. aureus* that was isolated from among health workers of Jimma University Specialized Hospital to Ampicillin. High sensitivity percentage to gentamicin was observed in this study which is in accordance with earlier report (Uwaezuoke and Aririatu, 2004) who recorded a high susceptibility of 91.7% to gentamicin from a total of 100 different clinical specimens that were investigated with a yield of 48 *Staphylococcus aureus* isolates. This finding shows that staphylococcal infections could be treated with gentamicin.

The differences found in the antibiotic sensitivity pattern of isolates to commonly used drugs can be linked with the prevailing usage and abuse of the drugs. The high resistance rate to these commonly used drugs contrast with high sensitivity to Gentamicin which is less frequently used. A relationship can therefore be inferred between antibiotic usage and the level of drug resistance encountered in this study. The use of antibiotics with caution by health practitioners and efforts to control gaining access to antibiotics as well as appropriate use of antibiotics officially in the locality will probably help to limit the increasing rate of drug resistance in disease causing microorganisms (Shahriar *et al* 2010).

CONCLUSION

From the results obtained above, it can be concluded that *Staphylococcus aureus* causes Nosocomial infection and can be transferred through contact with unclean environment (*e.g.* floor, formites) of the hospital which affect the immune system of paediatrics. It can also be concluded that *S. aureus* is resistant to Ampicillin and Tetracycline but susceptible to broad spectrum antibiotics such as Gentamicin and Ciprofloxacin.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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