Resistance pattern of *Staphylococcus aureus* isolated from surfaces at the State specialist Hospital, Osogbo, Osun State, Nigeria

Idowu, J Adeosun[1*], Titilayo, A Ajayeoba[1], Oluwatosin, A Ajibade[1], Elijah, K Oladipo[1] and Olubukola, M Oyawoye[1]

Abstract

Background: *Staphylococcus aureus* is one of the most highly resistant bacterial pathogens that have been increasingly reported around the world. This study focused on the isolation and antibiotic sensitivity patterns of *Staphylococcus aureus* isolated from Osun State Specialist hospital, Asubiaro environment, Osogbo, Osun state.

Methods: A total of 36 swab samples were collected from different spots within the units/wards of the hospital and susceptibility test was carried out on identified *S.aureus* using disc diffusion method. The susceptibility results were interpreted using CLSI, 2011 criteria.

Results: Out of a total of 269 *S.aureus* isolates, 47 isolates demonstrated hemolysis. 32(68.08%) strains out of 47 hemolytic isolates demonstrated beta-hemolysis while 15(31.91%) strains demonstrated alpha-hemolysis. The percentage distribution of antibiotics susceptibility test showed that the hemolytic strains were mostly resistant to Ampicillin (100.00%), Tetracycline (78.12%), Chloramphenicol (56.25%), Ciprofloxacin (34.38%) and the least was Gentamicin (15.62%).

Conclusion: There is the need for consistent on-going antimicrobial surveillance for important and commonly isolated clinically significant pathogens of *Staphylococcal* species to form the basis for developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a possible impending public health problem. The study therefore evaluated the incidence of hemolytic strain and antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from the hospital environment.

Keywords: *Staphylococcus aureus,* Antibiotics, Hospitals

Highland Med Res J 2019;19(1&2):24-28

Introduction

The genus *Staphylococcus* is among the numerous pathogens that have undergone significant changes in antimicrobial susceptibility in recent decades.[1] Particularly, *Staphylococcus aureus* being one of the most highly resistant bacterial pathogens have been increasingly reported around the world. More than 70% of *S. aureus* isolated from hospital environment are resistant to at least one of the drugs most commonly used to treat these infections.[2] *S. aureus* employs quite a number of virulence factors that enhance its pathogenicity. Some of such factors include production of toxins, enzymes, adhesins and microcapsules.[3]

Antimicrobial resistance is an increasing problem worldwide.[4]It has been termed a mounting problem especially in the developing countries and has become a growing concern. Previous reports according to Samie and Shivambu[5]established antibiotic resistance of *Staphylococcus aureus* to antibiotics such as ampicillin and the beta lactam antibiotics in general, which can be attributed to indiscriminate use of antibiotics**.** In Nigeria, reports have been centered on Methicillin resistant *Staphylococcus aureus* strains, [6]isolation from human, [7-8] and characterization of *Staphylococcus aureus* isolated from both human and animal sources. [9]Although previous authors have reported the isolation of *S. aureus* in Osun state, [7-8]information on the antibiotic susceptibility profile of hemolytic *S. aureus* isolated from environmental samples within hospital are deplete. This study therefore evaluated the antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from the hospital environment.

Methods

Study Location

This study was carried out in Osun state specialist hospital, Asubiaro, Osogbo. Osogbo is situated in the tropical rainforest belt of South-Western part of Nigeria. It lies approximately on Latitude 7º 46' 12"N and longitude 4º 34' 54"E. Prior to the commencement of the study, permission was obtained from the Osun State Hospitals Management Board, Osogbo, Osun State.

Sample collection

Sampling was carried out October, 2014. The unit/wards of the hospital sampled include Intensive care unit (ICU), male ward (MW), female ward (FW), emergency

[1]Department of Microbiology, Laboratory of Molecular Biology, Bioinformatics and Immunology, Adeleke University, Ede, Osun State, Nigeria.

All correspondences to:
Idowu, J Adeosun.
Email: adeosunidowu6@gmail.com,
adeosun.idowu@adelekeuniversity.edu.ng

unit (EU), Dressing room (DR) and labour ward (LW). Surface swabs were aseptically taken from different spots (bed bar, door knobs, bed sheet, table, chair, and floor) with labelled sterile swab sticks from each unit/ward. A total of 36 samples were collected in sterile containers and stored at 4°C prior to analysis.

Detection and enumeration of hemolytic strains of Staphylococ-cus aureus

The detection of *S. aureus* was determined according to the method described by Samie and Shivambu.[5] Biochemical chara-cterization which includes Gram staining, catalase and coagulase tests were carried out on the suspected colonies according to Cheesbrough.[10] The hemolytic activity testing of the *S. aureus* isolates was performed according to the method described by Jimenez *et al.,*[11] and the strains were classified into non-hemolytic, alpha (α) hemolytic and beta (β) hemolytic.

Determination of antimicrobial susceptibility of S.aureus

Antibiotic sensitivity discs (Oxoid, Thermo Scientific, UK) containing the following antimicrobials were used to test the susceptibility of the isolates: Ampicillin (AMP) (10μg), Ciprofloxacin (CIP) (5 μg), Gentamicin (GEN) (10 μg), Chloramphenicol (CHL) (30 μg) and Tetracycline (TET) (30 μg). *S. aureus* ATCC 25923 was used as positive control. The diameters of the zones of inhibition of the isolates were measured and interpreted according to the Clinical and Laboratory Standards Institute, 2011.

.

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 *Staphylococcus aureus* per location in the hospital by the cumulative number of *Staphylococcus aureus* positive isolates in the hospital environment; which was then multiplied by 100 and expressed as the percentage distribution.

Statistical analysis

The distribution of *Staphylococcus 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 Asubiaro hospital environment *were* compared statistically with SAS (Statistical Analysis Software 92.2) using Pearson chi-square analysis. Statistical significance was defined by a p value less than 0.05.

Results

Isolation and distribution of Staphylococcus aureus

Out of a total of 382 isolates of suspected *S. aureus* obtained from the swab samples, 269 isolates tested positive to all the confirmatory test used in this study. The prevalence of *S. aureus* within the units in the hospital were significantly different ($p<0.05$)and the highest isolation was FW (21.93%), followed by MW (21.56%), followed by LW (16.36%), followed by DR (14.13%), followed by ICU (13.75%) and the least was in EU (12.27%) respectively. Furthermore, the incidence of *S. aureus* within the sampling spots in the hospital were significantly different ($p<0.05$) and the highest isolation was bed sheet (23.05%) and the least was found on table (11.90%). The distribution of *S. aureus* in the different units and the sampling spots within the hospital is shown in Table 1.

Table 1: Distribution of *S. aureus* within Asubiaro hospital environment

Sampling Location	Number of S. aureus from sampling spot						Cumulative total of S.aureus/ location	p value
	Bed sheet	Bed bar	Floor	Door knob	Table	Chair		
ICU	7(2.60)a	10(3.71)	5(1.86)	4(1.49)	5(1.86)	6(2.23)	37(13.75)	0.05
EU	7(2.60)	4(1.49)	8(2.97)	3(1.12)	5(1.86)	6(2.23)	33(12.27)	
LW	9(3.35)	6(2.23)	10(3.71)	7(2.60)	4(1.49)	8(2.97)	44(16.36)	
FW	13(4.83)	12(4.46)	6(2.23)	15(5.57)	8(2.97)	5(1.86)	59(21.93)	
MW	20(7.43)	9(3.34)	10(3.71)	5(1.86)	6(2.23)	8(2.97)	58(21.56)	
DR	6(2.23)	8(2.97)	3(1.12)	9(3.34)	4(1.49)	8(2.97)	38(14.13)	
' [b]	62(23.04)	49(18.22)	42(15.61)	43(15.99)	32(11.90)	41(15.24)	269/382	

[a] Percentage distribution of *S. aureus*

' [b] Total number of *S. aureus* per spot of collection within location

ICU= Intensive care unit, EU= Emergency unit, LW= Labour ward, FW= Female ward, MW= Male ward, DR= Dressing room

Table 2: Distribution of hemolytic *S. aureus* within Asubiaro hospital environment

Sampling Location	Number of hemolytic S. aureus from sampling spot						Cumulative total of S.aureus/ location	P value
	Bed sheet	Bed bar	Floor	Door knob	Table	Chair		
ICU	3(1.12)a	4(1.49)	1(0.37)	1(0.37)	0(0.00)	0(0.00)	9(3.34)	0.008
EU	2(0.74)	2(0.74)	0(0.00)	3(1.12)	5(1.85)	1(0.37)	13(4.83)	
LW	2(0.74)	0(0.00)	2(0.74)	3(1.12)	0(0.00)	0(0.00)	7(2.60)	
FW	0(0.00)	4(1.49)	0(0.00)	0(0.00)	4(1.49)	2(0.74)	10(3.71)	
MW	2(0.74)	0(0.00)	0(0.00)	4(1.49)	0(0.00)	2(0.74)	8(2.97)	
DR	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	
' [b]	9(3.34)	10(3.72)	3(1.11)	11(4.10)	9(3.34)	5(1.85)	47/269	

[a]Percentage distribution of hemolytic *S. aureus*
' [b]Total number of hemolytic *S. aureus* per spot of collection within location
ICU= Intensive care unit, EU= Emergency unit, LW= Labour ward, FW= Female ward, MW= Male ward, DR= Dressing room

Distribution of hemolytic isolates in Asubiaro hospital

Out of a total of 269 *S. aureus* isolates, 47 (17.47%) showed hemolysis on blood agar. The differences in the incidence of hemolytic isolates were significantly different ($p<0.05$) and the highest hemolytic *S. aureus* isolate was found in EU (*4.83*%), followed by FW (3.71%), followed by ICU (3.34%), followed by MW (2.97% each) and the least in LW (2.60%) respectively. However, hemolytic strain was not identified in the dressing room.

Furthermore, the highest hemolytic *S. aureus* within the sampling spots in the hospital was found from doorknob (4.10%) while the least from the floor (1.11%) respectively. Table 2 shows the distribution of hemolytic *S. aureus* strain in Asubiaro hospital.

Table 3: Distribution of beta hemolytic *S. aureus* within Asubiaro hospital environment.

Sampling location	Number of alpha hemolytic S. aureus from sampling spot						P value
	Bed sheet	Bed bar	Floor	Door knob	Table	Chair	
ICU	0	4	0	0	0	0	0.0002
EU	2	0	0	3	5	1	
LW	2	0	2	3	0	0	
FW	0	4	0	0	0	2	
MW	2	0	0	0	0	2	
DR	0	0	0	0	0	0	
' [b]	6	8	2	6	5	5	

' [b]Total number of beta hemolytic *S. aureus* per spot of collection within location
ICU= Intensive care unit, EU= Emergency unit, LW= Labour ward, FW= Female ward, MW= Male ward, DR= Dressing room

Table 4: Distribution of alpha hemolytic *S. aureus* within Asubiaro hospital environment.

Location	Number of alpha hemolytic S. aureus from sample spot						P value
	Bed sheet	Bed bar	Floor	Door knob	Table	Chair	
ICU	3	0	1	1	0	0	0.0028
EU	0	2	0	0	0	0	
LW	0	0	0	0	0	0	
FW	0	0	0	0	4	0	
MW	0	0	0	0	4	0	
DR	0	0	0	0	0	0	
' [b]	3	2	1	1	8	0	

' [b] Total number of alpha hemolytic *S. aureus* per spot of collection within location.
ICU= Intensive care unit, EU= Emergency unit, LW= Labour ward, FW= Female ward, MW= Male ward, DR= Dressing room

Differentiation of Staphylococcus aureus hemolytic strains

Out of 47 isolates that demonstrated hemolysis, 32 (68.08%) isolates exhibited beta hemolysis (complete hemolysis) while 15 (31.91%) isolates exhibited alpha hemolysis (incomplete hemolysis). The distribution of each type of hemolytic strain were significantly different ($p<0.05$) within the locations in the hospital and the sampling spots. The distribution of beta and alpha hemolytic *S. aureus* within Asubiaro hospital environment is shown in Table 3 and 4 respectively

Table 5: Antimicrobial sensitivity results of *S.aureus* isolates from Asubiaro hospital environment

Drugs	% of resistant isolate	% of Intermediate isolate
AMP	32(100)	0(0.0)
CHL	14(43.75)	4(12.5)
TET	19(59.37)	6(18.75)
CIP	5(15.63)	6(18.75)
GEN	4(12.5)	1(3.12)

AMP= Ampicillin, CHL= Chloramphenicol, TET= Tetracycline, CIP= Ciprofloxacin, GEN= Gentamicin

Antimicrobial Sensitivity of hemoly-tic Staphylococcus aureus

The antibiotics sensitivity test showed that all *Staphylococcus aureus* were resistant to Ampicillin (100%), followed by tetracycline (59.37%), chloramphenicol (43.75%), ciprofloxacin (15.63%) and the least resistant is gentamicin (12.5%). Table 5 shows the antimicrobial sensitivities of hemolytic *Staphylo-coccus aureus* isolated from Asubiaro hospital environment.

Discussion

Staphylococcus aureus as isolated in this study has been reported as one of the most common nosocomial pathogens usually isolated in a hospital environment. They present a threat to medical community because they can survive long periods in conducive environments[12] and can lead to fatal infections. The occurrence of *S. aureus* from different wards/units have been identified and reported by previous authors to cause significant epidemiologic and therapeutic challenges implicated in a wide variety of infections. [13]

The higher isolation of *S. aureus* from FW corroborates previous authors.[14-15]In Nigeria, it has been reported that women are prone to infection from microorganisms, especially *S. aureus*[16-17]and it could be resulting from poor hygiene of individual patients or lack of proper cleanliness of the hospital environment.

The higher isolation of *S. aureus* from bed sheet have also been reported. [18]People who carry *S. aureus* can shed the organism in large numbers on their skin during normal daily activities,[18]which makes the transmission of *S. aureus* contamination onto clothing/bed-linens possible. The least isolation of *S. aureus* (11.90%) from the tables in the hospital environment suggests that a larger percentage of microbial contamination are removed with the regular cleaning.

The high prevalence of hemolytic strain in the emergency unit and sampling spot requires attention for more precautionary measures to reduce the threat of nosocomial infection to patients and health workers. Ebrahimi *et al.,*[19]also reported the isolation of hemolytic strain of *S. aureus* within the hospital environment.

The results showed that *Staphylococcus aureus* was present in environments of Osun state specialist hospital, Asubiaro and are mostly resistant to Ampicillin. The high resistance of *S. aureus* to ampicillin have been reported.[20] This high resistance to ampicillin may be as a result of the thickening of the cell walls of the isolates which is also responsible for adaptive resistance of *S. aureus* to Amikacin.[21] The enzymes β-lactamases produced by some bacteria act in the hydrolysis of the ring β-lactamic of the penicillin which is transformed in acid neutralizing its bactericidal effect. The high sensitivity of *S. aureus* to gentamicin (84.38%) corroborates the work of by Authier *et al.,*[22] where the antibiotics sensitivity of *Staphylococcus aureus* isolated from canine samples were tested and gentamicin was found to inhibit *Staphylococcus aureus* the most as the drug is a large antimicrobial spectrum.[23]Testing the sensitivity of *Staphylococcus aureus,* on different kind of antibiotics, is very important as concerning the clinical picture of the disease generated by this pathogen agent.[23]

Limitations of the study

Some limitations were acknowledged in this study which include not carrying out molecular identification of the isolates as well as not determining if the isolates had resistant genes and/or virulent genes. Also, plasmid profiling was not carried out to determine if resistance in the isolates was plasmid mediated or chromosome mediated.

Conclusion

It is evident that *Staphylococcus aureus* is present in the hospital environment and it has virulent factors such as ability to lyse the red blood cells in their host. Also, Gentamicin can inhibit the growth of hemolytic strains of *Staphylococcus aureus.*

Conflict of Interest

All authors declare no conflict of interest.

References

1. Wu SW, Lencastre H, Tomasz A. Recruitment of the *mecA* gene homologue of *Staphylococcus aureus* into a resistance determinant and expression of the resistant phenotype in *Staphylococcus aureus*. J. Bacteriol. Res. 2001; 183: 17–24.
2. Centers for Disease Control and Prevention. *Campaign to Prevent Antimicrobial Resistance in Healthcare Settings: Why a Campaign?* Atlanta, GA: Centers for Disease Control and Prevention; 2001.Available at www.cdc.gov/drug resistance/healthcare/problem.htm. Accessed on 4th April, 2015.
3. Zecconi A, Scali F. *Staphylococcus aureus* virulence factors in evasion from innate immune system. J. Clin. Infect. 2013; 25:11-20.

4. Uneke CJ, Ogbonna A, Oyibo PG, Onu CM. Bacterial contamination of stethoscopes used by health workers: public health implications. J. Infect. Dev. Countr.2010; 4(7): 36-41.
5. Samie A, Shivambu N.Biofilm production and antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from HIV and AIDS patients in the Limpopo Province, South Africa. Afr. J. Biotechnol. 2011; 10. 14625-14636.
6. Carlene A, Muto MD, John A, Jernigan MD, Belinda E, Ostrowsky, M.D, *et al*. SHEA Guideline for Preventing NosocomialTransmission of Multidrug-Resistant Strains of*Staphylococcus aureus* and *Enterococcus. Infect. Control Hosp Epidemiol.* 2003; *24:362-371.*
7. Olowe OA, Eniola KI, Olowe RA, Olayemi AB. Antimicrobial Susceptibility and Betalactamase detection of MRSA in Osogbo, SW Nigeria. *Int. J. Sci. Nat.* 2007; 5(3): 44-48.
8. Adekunle OC, Olatunji OM. Isolation and antibiotics susceptibility of *Staphylococcus aureus* obtained from nasal cavity from hospitalized patients. J. Pharm. Biomed. Sci. 2011; 10 (10): 10-15.
9. *El-Jakee J, Ata S, Nagwa M, Bakry G, Sahar A, Zouel F.* Characteristics of *Staphylococcus aureus* Strains Isolated from Human and Animal Sources. J. Agric.Environ. Sci.2008; 4 (2): 221-229.
10. Cheesbrough M. District laboratory practice in tropical countries. Part II; Cambridge University Press UK. 2002; pp. 136-142.
11. Jimenez E, Delgado S, Fernandez L, Garcia N, Albujar M, Gomez A, *et al*. Assessment of the bacterial diversity of human colostrums and screening of *Staphylococcal* and *Enterococcal* population for potential virulence factors. Afr.J. Biotechnol. 2008; 159: 595- 601.
12. Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. J. *Clin. Microbiol.* 2004; 17: 863-93.
13. Patel H, Vaghasiya Y, Vyas BRM, Chanda S. Antibiotic resistant *Staphylococcus aureus:* A challenge to researchers and clinicians. J. Bacteriol. Res. 2012; 2:23-45.
14. Kitara LD, Anywar AD, Acullu D, Odongo-Aginya E, Aloyo J, Fendu, M. Antibiotic susceptibility of *Staphylococcus aureus* in suppurativelesions in Lacor Hospital, Uganda. J. Afr. Health Sci. 2011;11: 1-6.
15. Khattak SU, Bacha N, Lutfullah G, Bakht J, Ali J, Khan, M. Study of the genetic traits associated with antibiotic resistance in *Staphylococcus aureus* isolated from Khyber paktunkhwa, Pakistan. Asian Pac. J. Trop. Dis. 2015; 5(5): 393-398.
16. Onanuga A, Oyi AR, Olayinka BO, Onaolapo JA. Prevalence of community-based multiresistant *Staphylococcus aureus* amoung healthy women in Abuja, Nigeria. Afr. J. Biotechnol. 2005; 4 (9):942-945.
17. Akortha EE, Ibadin OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amoung patients with urinary tract infection (UTI) in UBTH Benin City, Nigeria.Afr. J. Biotechnol. 2008; 7 (11): 1637-1640.
18. Bloomfield F, Martin E, Carlo S, Kumar J, Elizabeth A. The infection risks associated with clothing and household linens in home and everyday life settings,and the role of laundry.*Int.* J. Sci. forum on Hand Hygiene 2011; 12: 32-37.
19. Ebrahimi A, Ghasemi M, Ghasemi B. Some Virulence Factors of Staphylococci Isolated From Wound and Skin Infections in Shahrekord, Iran. Jundishapur J. Microbiol. 2014; 7(4): e9225. DOI: 10.5812/jjm.9225
20. Okwu M, Bamgbala S, Aborisade W. Prevalence of Nasal Carriage of Community-associated Methicillin resistant *Staphylococcus aureus* (CA-MRSA) among Healthy Primary School Children in Okada, Nigeria. *J. Nat. Sci. Res.*2012; 12(4): 61-65.
21. Yuan W, Hu Q, Cheng H.,Shang W, Liu N,Hua Z,Zhu J., Hu Zhen, Yuan J, Zhang X, Li S, Chen Z, Hu X, Fu, J and Rao X.(2013). Cell wall thickening is associated with adaptive resistance to amikacin in methicillin-resistant *Staphylococcus aureus* clinical isolates. J. Antimicrob. Chemother. 68:1089-1096
22. Authier S, Paquette D, Labrecque O, Messier S. Comparison of susceptibility to antimicrobials of bacteria isolates from companion animals in a verterinary diagnostic laboratory in Canada between 2 time points 10 years apart. Can. Vet. J. 2006; 47:774-778.
23. Filimon MN, Borozan AB, Goia SL, Popescu R, Gherman VD. Testing the sensitivity of *Staphylococcus aureus* antibiotics.J. Biomed. Sci. 2009; 2:70-73