COMPARATIVE STUDY OF DIFFERENT CLINICAL SAMPLES USED FOR THE DIAGNOSIS OF STAPHYLOCOCCAL SYSTEMIC INFECTIONS IN APPARENT HEALTHY STUDENTS

Iheukwumere, I.H[1]., Iheukwumere, C. M.[2], Chude, C. O.[1], Nwaolisa, C. N.[1], Ukoha, C. C.[1], Nwakoby, N. E.[1], Egbuna, C.[3,4] and Egbe, P.A.[5]

[1]Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria

[2]Department of Applied Microbiology& Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

[3]Department of Biochemistry, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State- 431124, Nigeria

[4]Nutritional Biochemistry and Toxicology Unit, World Bank Africa Centre of Excellence, Centre for Public Health and Toxicological Research (ACE-PUTOR), University of Port-Harcourt, Rivers State, Nigeria.

[5]Department of Anatomy, Faculty of Basic Medicine, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria

Email: ikpower2007@yahoo.com

ABSTRACT

Several studies have shown that the major problem that limits the diagnosis of infectious disease in the specimen used. This study was undertaken to compare two different clinical samples for the diagnosis of *Staphylococcus aureus* systemic infections in apparent healthy students of Chukwuemeka Odumegwu Ojukwu University. A total of 100 samples each of blood and stool were collected from apparent healthy students under sanitary conditions, and aseptically plated 1.0 ml of each sample in a mannitol salt agar using pour plate methods. The isolates obtained were appropriately characterized. The occurrences of different strains of the organism were recorded based on the age, sex and residence of the studied group. The results of the study revealed that 36% of the stool and 14% of the blood samples were positive to *S.aureus*. *S.aureus* I (Sa I), *S.aureus* II (Sa II) and *S.aureus* III (Sa III) were encountered from the studied samples. *S. aureus* was significantly ($p < 0.05$) detected most in the samples collected from 19-21 years (41.67%) students, female students (80.56%) and those students living in the school hostel (69.44%). Therefore the study has shown that *S. aureus* systemic infection can be diagnosed effectively using stool sample, and *Staphylococcus aureus* strain I was detected most in the studied samples.

INTRODUCTION

Staphylococcus was first identified as bacterial pathogen in 19th century. In 1880, Alexander Ogston first observed grape-like clusters of bacteria in pus from a surgical abscess in a knee joint and named them as *Staphylococcus* (Greek Staphyle, a bunch of grapes; kokos, grain or berry). In 1884, German physician Friedrich Julius Rosenbach was able to grow the organisms in pure culture and categorized them as per their colour production (Mohammad, 2017).

S. aureus is considered to be a major pathogen that colonises and infects both hospitalised patients with decreased immunity, and healthy immuno-competent people in the community. This bacterium is found naturally on the skin and in the nasopharynx of the human body (Harris *et al.,* 2012). It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening.

S. aureus has the ability to adapt to different environments and it may colonize the human skin, nails, nares and mucus membranes and may thereby disseminate among recipient host populations via physical contact and aerosols (Stark, 2013) Colonization with *S. aureus* is an important risk factor for subsequent S. aureus infection. *S. aureus* causes a wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia, endocarditis, septic arthritis and septicaemia. This bacterium is also one of the most common species in nosocomial infections. In addition, *S. aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins (Stark, 2013).

In identification of *Staphylococcal* infections different diagnostic measures are carried out such as culture of different clinical samples like blood, pus, urine, throat swab, sputum to detect the presence or absence of *S. aureus*. Rapid diagnostic tests using real time polymerase chain reaction (PCR) and quantitative PCR can also be used to determine the presence or absence of selected virulence or control genes. Identification of toxins using agglutination tests that are determined by clumping of the latex particles and assay studies which helps to determine the specific susceptibility to antibiotics of the infected strain provides another alternative for such diagnosis. In all these samples, *S. aureus* will be effectively detected provided that the organism is present. In the university community especially Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria, where Staphylococcal systemic infection is likely seen, blood or stool samples automatically become the clinical sample of choice for such case. Therefore, this study will compare the two clinical samples to ascertain which of them will be the first choice for such diagnosis.

MATERIALS AND METHODS

Study Area: The study was carried out in Uli town. Uli is of historic importance situated at the extreme south east corner of Ihiala Local Government Area of Anambra State in Nigeria. Its neighboring towns are Amaofuo (formally a village in Uli town), Ihiala, Amorka, Ubulu, Ozara,

Egbuoma and Ohakpu. Uli town extends westward to the conference of the rivers of Atamiri and Enyinja, and across Usham Lake down on the lower Niger region. Its coordinates are 5° 47'N 6° 52' E and 5.783° N6.876°E . It occupies a landmass of 99sq.mile (256 km^2). The people of Uli are basically traders and farmers. The climate of the town is typically an equatorial rainforest type characterized by two main seasons; the rainy season which lasts between April and October and the dry season which lasts between November and March, with temperature which is usually high throughout the year and average minimum temperature at about 32°C and 25°C respectively.

Sample collection: A total of 100 samples each of stool and blood were aseptically collected randomly from different students from Chukwuemeka Odumegwu Ojukwu University. The samples were collected using sterile, cleaned, dried and grease-free containers that did not contain barium salt or any form of toilet paper. The samples were collected from diarrhoeic healthy students that were not in any form of medication. The samples were carefully labelled and transported to the laboratory for examination.

Isolation and characterization of organisms: The samples were first inoculated on sterile peptone water and incubated at 35±2°C for 24 h. This was aseptically plated in mannitol salt agar (MSA) and then incubated in inverted position at 35±2°C for 48 h. The isolates obtained from these culture plates were aseptically streaked on sterile nutrient agar (BIOTECH) plates to obtain pure cultures. The pure culture plates were then morphologically (size, edge, elevation, colour, Gram reaction, motility, shape) and biochemically (catalase and coagulase production, glucose, maltose, mannitol, dulcitol and inositol utilization) characterized using the procedures described by Chesbrough (2000). The Bergey's manual of determinative bacteriology was used to ascertain the organism using the version described by Whitman *et al*. (2012).

Occurrences of the characterized isolates: The characterized isolates were quantified to determine their occurrences based on the demographic classification done on the studied samples, and these were recorded in percentage. The most predominated strain of *S. aureus* from the studied samples was noted and recorded.

Statistical Analysis: The data obtained from the study were presented in percentage (%) and these were then represented in tables. The statistical significance of the study was carried out using one way analysis of variance (ANOVA) at 95% confidence level. The statistical implication of the demographic factors was done using Chi square. Pairwise comparison was done using student "t" test (Iheukwumere *et al*., 2018)

RESULTS

The colonial description, morphological and biochemical characteristics of the isolates are shown on Table 1. Their golden yellow appearance and sharp border edges on MSA is a true identity of *Staphylococcus aureus*. The isolates are non-motile and have the ability to produce catalase enzyme which are a true identity of *Staphylococcus aureus*, the strains of the isolates were traced

by their abilities to ferment inositol, maltose, dulcitol, glucose and mannitol as indicated in Table 1.

The occurrence of *Staphylococcus aureus* in the studied samples are shown in Table 2. The total occurrence of *S. aureus* I was significantly ($p < 0.05$) most in both the blood and stool samples. It was also observed that the occurrence of *S. aureus* in the stool sample were significantly ($p < 0.05$) higher than that of the blood samples.

The occurrences of different strains of *S.aureus* in blood and stool samples based on the age group are shown in Table 3. The study revealed that the total number of *S. aureus* was significantly ($p< 0.05$) detected most from the stool samples of students from 19-21 years whereas students from 16-18 years recorded the least occurrence. The study also revealed that the total number of *S. aureus* was significantly ($p < 0.05$) most from the blood samples of students from 16-18 and 19-21 years, whereas students from 21and above recorded the least occurrence. Sa1 was most encountered in both stool and blood samples. The occurrences of different strains of *Staphylococcus aureus* in blood and stool sample based on sex are shown below in Table 4. The study revealed that the total number of *S.aureus* was significantly ($p < 0.05$) detected most from both the blood and stool samples of female students whereas the male students recorded the least occurrence. The occurrences of different strains of *S. aureus* was significantly ($p< 0.05$) detected most from both the stool and blood samples collected from students living in the hostel whereas students living off the campus recorded the least occurrence.

Table1: **Characteristics and Identities of the Isolate**

Parameter	Isolate I	Isolate II	Isolate III
Appearance on Mannitol salt agar	Golden Yellow	Golden Yellow	Golden Yellow
Edge	Sharp border	Sharp border	Sharp border
Elevation	Convex	Convex	Convex
Size (mm)	3.10	2.90	2.70
Cell Morphology	Cocci	Cocci	Cocci
Gram Reaction	+	+	+
Motility	Non-motile	Non-motile	Non-motile
Catalase	+	+	+
Coagulase	+	+	+
Glucose	+	+	+
Maltose	+	-	-
Mannito	+	+	+
Dulcitol	+	+	±
Inositol	±	±	±
Possible organism	*Staphylococcus aureus*	*Staphylococcus aureus*	*Staphylococcus*

aureus

+ = Positive +/- = partially positive - = Negative

Table2: Occurrences of *Staphylococcus aureus* in the studied samples

Isolate	n=100	
	Stool (%)	Blood (%)
Sa I	24 (66.67)	6 (42.86)
Sa II	4 (11.11)	4 (28.57)
Sa III	8 (22.22)	4 (28.57)
Total	36 (100.00)	14 (100.00)

Table3: Occurrences of different strains of *Staphylococcus aureus* based on the age group

Age group	Stool				Blood			
	SaI (%)	SaII (%)	SaIII (%)	T (%)	SaI (%)	SaII (%)	SaIII %)	T (%)
16-18	6(16.67)	1(2.71)	2(5.56)	9(25.00)	2(14.29)	2(14.29)	1(7.14)	5(35.71)
19-22	10(2.78)	1(2.71)	4(11.11)	15(41.67)	2(14.29)	1(7.14)	2(14.29)	5(35.71)
22 & above	8(22.22)	2(5.56)	2(5.56)	12(33.33)	2(14.29)	1(7.14)	1(7.14)	4(28.57)
Total	24	4	8	36	6	4	4	14

Table 4: Occurrences of different strains of *Staphylococcus aureus* based on sex

Sex	Urine				Stool			
	SaI(%)	SaII(%)	SaIII(%)	T (%)	SaI(%)	SaII(%)	SaIII(%)	T (%)
Male	4(11.11)	1(2.78)	2(0.56)	7(19.44)	1(7.14	0(0.00)	2(14.23)	3(21.43)

Female	20(55.56)	3(8.33)	6(16.67)	29(80.56)	5(35.72)	4(28.57)	2(14.23)	11(78.57)
Total	24	4	8	36	6	4	6	14

Table 5: Occurrences of different strains of *Staphylococcus aureus* based on residence.

Residence	Urine				Stool			
	SaI(%)	SaII(%)	SaIII(%)	T (%)	SaI(%)	SaII(%)	SaIII(%)	T (%)
Hostel	16(44.44)	3(8.33)	6(16.67)	25(69.44)	3(21.43)	3(21.43)	2(14.29)	8(37.14)
Off the campus	8(22.22)	1(2.77)	(2.5.55)	11(30.56)	3(21.43)	1(7.14)	2(14.29)	6(42.86)
Total	24	4	8	36	6	4	4	14

DISCUSSION

The roles of *S. aureus* in cases of diseases has been established and reported to have substantial degree of morbidity and mortality among children and adults. The illness is characterized by severe sepsis, endocarditis, superficial skin infections to severe, and potentially fatal, invasive disease (Thapaliya *et al.,* 2017). The characteristic features of the isolates from blood and stool samples in this study are in line with the features described by Cheesbrough (2000). The presence of *S. aureus* in the studied blood and stool samples could be traced from improper personal hygiene, frequent eating from student government (SG) canteen, consumption of contaminated salads and rampant exchange of snacks among the students, and these activities can lead food intoxication and systemic infection. The occurrences of *S. aureus* in stool more than blood samples supported the findings of many researchers (Fletcher *et al*., 2015; Humphrey *et al*., 2015; Song *et al*., 2017; Kates *et al*., 2018). *S. aureus* is a gastro pathologic bacterium that has the ability to colonize the intestinal mucosa and induces diarrhoea.

The high occurrence of *S. aureus* in the stool samples of students from ages 19-21 years could be attributed to the fact that this age bracket consist of teenagers and adolescents, and these young

students are characteristically vulnerable to increased sexual activity which predisposes them to this organism. The frequent detection of isolate SaI in the studied samples could be attributed to genetic diversity, genetic variation and high adaptive mechanism of the strain. Studies have shown that this could be as a result of its ability to survive long in most unfavourable environments and the virulent nature of the organism which gives it the ability to overcome body defence mechanism and resistance to antibiotics.

The pronounced occurrence of *S. aureus* in the blood and stool samples of female students than the samples from male students supported the findings of Bhatt *et al*. (2014), Smith *et al*. (2017), Tacconelli *et al*. (2017) and Okonkwo *et al*. (2018). Female students leave in the school hostel where personal hygiene is not fully practiced whereas no male hostel exists in the institution. Also, the female students are prone to eating fruit salads, roasted meats, roasted fishes, African salads and all sorts of contaminated drinks like locally produced zobo drink.

The high occurrence of *S. aureus* in blood and stool samples of students living in the hostel than those living off the campus could be attributed to the lack of good personal and environmental hygiene, improper waste disposal and lack of well aerated rooms.

CONCLUSION

This study has revealed the presence of *S. aureus* in blood and stool samples gotten from apparent healthy students living in Hotel and off the campus of Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria. The studied isolates were significantly detected more in stool samples than the blood samples. It was observed that isolate SaI was the most predominant from the studied samples when compared to isolate Sa I, Sa II, and Sa III. The study has shown that stool sample is an effective sample used for the diagnosis of systemic *S. aureus* infection.

REFERENCES

Bhatt, C.P., Karki, B.M.S., Baral, B., Gavtam, S., Shah, A. and Chaudhary, A. (2014). Antibiotic susceptibility pattern of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital. *Journal of Pathology of Nepal* 4: 548–551.

Cheesbrough, M. (2000). District Laboratory Practices in Tropical Countries, Second Edition. Cambridge University Press, Cambridge, UK, pp. 58–62.

Fletcher, S., Boonwaat, L., Moore, T., Chavada, R. and Conaty, S. (2015). Investigating an outbreak of *Staphylococcal* food poisoning among travelers across two Australian States. *Western Pacific Surveillance and Response Journal* **6**(2): 17–21.

Harris, R.S. Cartwright, E.J.P., Torok, E. M., Holden, T.G. and Brown, M.N. (2012). Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*. *Lancet Infectious Diseases* **13**: 130–136.

Humphrey, H., Fitptrick, F. and Harvey, B.J. (2015). Gender differences in rate of carriage and blood infection caused by methicillin resistant *Staphylococcus aureus:* Are they real, do they matter and why? *Clinical Infectious Diseases* **61**(1): 1–7.

Joseph, O.A.S. (2017). *Staphylococcus aureus* surface colonization of medical equipment and environment, implication in hospital-community epidemiology. *Journal of Hospital & Medical Management* **3**:1–7.

Kates, A.E. Thapaliya, D., Smith, T.C. and Chorazy, M.L. (2018). Prevalence and molecular characterization of *Staphylococcus aureus* from human stool samples. *Antimicrobial Resistance and Infection Control* **7**: 42–51.

Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research* **8**: 21–33.

Mohammad, F. K. (2017). Brief history of *Staphylococcus aureus*: A focus to antibiotic resistance. *E C Microbiology* **5**(7): 36–39

Okonkwo, E.C., Orji, J.O., Aondoackae, A.D., Ugbo, E.N., Moses, I.B., Ogene, L. and Nwuna, E.N. (2018). Prevalence and antibiotic sensitivity pattern of *Staphylococcus aureus* isolate of non-hospital original. *Archives of Clinical Microbiology* **9**(1): 76–84.

Smith, J., Lopes-curtes, L.E., Kaasch, A.J., Sagaard, M., Thomsen, R.W., Schonheyder, H.C., Rodriguez-Bano, J. and Nielson, H. (2017). Gender differences in the outcome of community acquired *Staphylococcus aureus* bacteremia. A historical population-based cohort study. *Clinical Microbiology and Infection* **23**(1): 27–32.

Song, X.X., Fang, D.H., Quan, Y.Q. and Feng, D.J. (2017). The pathogenic detection for 126 children with diarrhea and drug sensitivity tests. *European Review for Medical and Pharmacological Sciences* **21**(4): 95–99.

Stark, S. (2013). *Staphylococcus aureus*: Aspect of Pathogenesis and Molecular Epidemiology. Department of Clinical Microbiology, Ryhor Country Hospital, Jonkoping, Sweden, pp.9–11.

Tacconella, E. and Foschi, F. (2017). Does gender affect the outcome of community- acquired *Staphylococcus aureus* bacteremia? *Clinical Microbiology and Infection* **23**: 23–25.

Thapaliya, D., Forshey,B.M., Kadariya,J., Quick, M.K., Farina, S. and Smith, T.C. (2017). Prevalence and molecular characterization of Staphylococcus aureus in commercially available meat over a one year period in Lowa, USA. *Food Microbiology* **65**: 122–129.

White, W.B., Good fellow, M., Kampfer, P., Busser, H., Trujillo, M.E., Luding, W. and Suzuki, K. (2012). Bergey's manual of systematic bacteriology, Second Edition, Volume 5, part A ans B. Springer-Verley New York, pp. 100–116.