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Vancomycin Resistance Among Clinical Isolates of *Staphylococcus aureus* Obtained from Selected Hospitals in Sokoto Metropolis

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Abstract

The decreased vancomycin susceptibility and subsequent emergence of vancomycin resistant Staphylococcus aureus (VRSA) strains is a major public health problem. This study was aimed at detecting the prevalence of vancomycin resistant Staphylococcus aureus among clinical isolates obtained from patients attending Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto. A total of 80 S. aureus clinical isolates were obtained from the medical microbiology laboratories of the selected hospitals. Antibiotic sensitivity testing of the isolates was carried out using the agar dilution method and isolates were screened for vancomycin resistance using vancomycin agar screen method. Of the 80 S. aureus isolates studied, 69 (86.0%) were identified as vancomycin susceptible S. aureus (VSSA) with MIC value of ≤2 µg/ml, 11 (13.8%) were identified as vancomycin intermediate S. aureus (VISA) and had MIC value of 4-8 µg/mL (VISA) and none of the isolates was identified as vancomycin resistant S. aureus (VRSA). The study detects high prevalence rate of VISA in the study area and identifies the need for increased public awareness on the danger associated with the presence of drug resistant bacteria. Emphasis should be directed at discouraging practices such as the use of over the counter medications which increase the rate of development of drug resistant organisms.

Keywords: Vancomycin, Resistance, Staphylococcus aureus, MIC, VRSA

INTRODUCTION

The evolution of bacterial resistance is generating a serious public health problem due to the indiscriminate use of antibiotics, the application of non-optimal doses, the irregularity in the taking of medicines has led to the emergence of multiple drug resistance in some bacteria such as *Staphylococcus aureus* (Cárdenas *et al.*, 2019). *Staphylococcus aureus* are gram positive bacteria cocci in shape; appear in clusters and non-spore forming, is the leading cause of most *Staphylococcus* infections. They are commonly present as parasite of man, forming part of the normal flora of the skin, upper respiratory tract and intestinal tract. *Staphylococcus aureus* is usually carried in the nose of 20-40% of healthy individuals. *Staphylococcus aureus* causes infections such as; Conjunctivitis (especially in neonates), Abscess, boils and impetigo: it's a common cause of secondary infections of ulcers, burns, skin disorders and insect bite, Pneumonia, empyema, Bacteremia, endocarditis, osteomyelitis and food poisoning (Cheesebrough, 2009).

In the treatment of infections caused by *Staphylococcus aureus* in the early days, penicillin was used and at the time of introduction, was susceptible to penicillin. After the initial success, the ever increasing consumption of antibiotics over the past five decades has led to the emergence of drug resistance among *S. aureus* strains (David and Daum, 2017). Methicillin became the drug of choice, however, discovery of various strains of methicillin-resistant *Staphylococcus aureus* (MRSA) due to the *mec*A gene encoding penicillin binding protein PBP2a soon arose with the resistance to methicillin also resulting in resistance to all ß-lactam antibiotics such as penicillin and cephalosporins and non ß-lactams such as erythromycin, clindamycin, gentamycin and co-trimoxazole (Geitani *et al.*, 2019) thereby giving rise to multi- drug resistant *Staphylococcus aureus* (MDRSA).

Vancomycin became available for clinical use but in the 1990's several reports suggested that the susceptibility of *S. aureus* to vancomycin was changing. Emergence of clinical isolates of methicillin-resistant *Staphylococcus aureus* strains with decreased susceptibility to vancomycin (vancomycin intermediate-resistance *S. aureus* [VISA]) seen in Japan (Hiramatsu *et al.*, 2001, Asakura *et al.*, 2018)

and four (4) vancomycin resistant (vancomycin resistant *S. aureus* [VRSA]) strains found in the The resistance was found to be horizontally transmitted to the *S. aureus* where they co-exist with *E. faecalis* (Shettigar *et al.*, 2018).

Numerous factors including the practice of indiscriminate purchasing of drugs over the counter have led to the development of drug resistant bacteria. Thus there is the need to constantly detect the presence of not only drug susceptible isolates but also drug resistant isolates circulating in the population so as to adopt public health measures that will lead to the control and eradication of such organisms. This study was aimed at detecting vancomycin resistant *S. aureus* in clinical isolates obtained from patients attending Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto

MATERIALS AND METHODS

The study was carried out in two hospitals located within Sokoto metropolis; Specialists Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto. Sokoto is a city located in the extreme northwest of Nigeria, near the confluence of the Sokoto River and the Rima River. As of 2006 it has a population of Population: 563,861Latitude: 13° 03' 45.68" N Longitude: 5° 14' 35.59" E (www.google.com) A total of 80 isolates of *Staphylococcus aureus* were collected from the medical microbiology laboratories using nutrient agar slants and transported to the medical microbiology laboratory in the school of medical laboratory science, Usmanu Danfodiyo University Sokoto. Also, information regarding the isolates was obtained. Identification and purification of isolates was done using colony morphology, gram staining, catalase test, coagulase test and mannitol fermentation test (Abouelnour *et al.*, 2020).

Antibiotic Susceptibility Testing

Standard inoculum of the isolates was prepared using sterile normal saline from colonies obtained from an 18-hour blood agar plate. The saline suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard containing approximately $1\text{-}2\times10^8$ colony-forming units (CFU)/mL (Abouelnour *et al.*, 2020).

Detection of vancomycin susceptibility using vancomycin agar screening

All *S. aureus* isolates were tested for vancomycin susceptibility using the CLSI agar USA (Weigel *et al.*, 2003, McGuiness *et al.*, 2017).

screen method with slight modification by replacing Brain Heart Infusion agar with Mueller Hinton agar (Oxoid, UK) containing 3 µg/mL (MHA-V3) and 6 µg/mL (MHA-V6) vancomycin (Oxoid, UK) prepared in-house in the laboratory where this study was conducted. To prepare vancomycin screening agar plates, Mueller Hinton agar was prepared according to manufacturer's instructions (Oxoid). Media was mixed well and dissolved by heating until complete dissolution. Sterilization was done by autoclaving at 121ºC for 15 minutes. Media was cooled to 45ºC, 3 µg/mL (MHA-V3) and 6 µg/mL (MHA-V6) Vancomycin was added aseptically followed by gentle homogenization and pouring into Petri dishes. Prepared medium was stored at 8-15°C (Périllaud *et al.*, 2019). Inoculum suspension from overnight culture of *S. aureus* was prepared and standardized to 0.5 McFarland standards (~1.5\108 CFU/mL). MHA-V3 and MHA-V6 plates were inoculated with 10 µL of the suspension and incubated aerobically at 37°C overnight. Growth of one or more colonies indicates isolates with reduced vancomycin susceptibility (CLSI, 2020).

Determination of MIC (Agar Dilution Method)

Minimum Inhibitory Concentrations of vancomycin on *S. aureus* isolates were also determined of the *S. aureus* isolates to vancomycin were also determined by by agar dilution method. Gradient plates of Mueller-Hinton agar were prepared with vancomycin (0.5-8µg/ml). Inoculation of the media plates were done by direct colony suspension method of 0.5McFarland equivalent inoculums prepared in sterile normal saline. Plates were incubated for 18hours at 35ºC and subsequently observed for any visible growth (CLSI, 2020). Results were read and interpreted according to CLSI guidelines.

RESULTS

The result of the study shows that based on CLSI interpretation 69 (86%) of the studied isolates had MIC of less than 2µg/ml and were identified as VSSA (Table 1). Table 1 also shows that 11 (13.8%) of the isolates had MIC of 4-8µg/ml and were identified as VISA. None of the isolates had a vancomycin MIC of >16, as such vancomycin resistance was not detected in the study.

Table 1: Vancomycin Susceptibility of *Staphylococcus aureus* isolates in the study hospitals

MIC (µg/mL)	No. of isolate (%)	CLSI Interpretation
<1.0	56(70.0)	Vancomycin Susceptible *Staphylococcus aureus*
2.0	69(86.0)	Vancomycin Susceptible *Staphylococcus aureus*
4 – 8	11(13.8)	Vancomycin Intermediate *Staphylococcus aureus*
>16	0(0.0)	Vancomycin Resistant *Staphylococcus aureus*
Total	**80**	

Table 2 revealed that out of the 80 samples studied 24 (30%) were collected from in-Patient and 56 (70%) samples from Out-Patient. The Table also shows that the distribution of VSSA and VISA differed in significantly among in-Patient with 19 (79%) and 5 (20.8%) identified as VSSA and VISA respectively compared to Out-Patient sample with 50 (89.2%) and 6 (10.7%) identified as VSSA and VISA respectively (P=0.056). Table 2 further revealed that the 61 (76.2%) samples collected from Specialists Hospital Sokoto were higher than the 19 (23.7%) collected from Maryam Abacha Women and Children Hospital Sokoto. The study also revealed that samples from in-patient of Specialists Hospital Sokoto had higher number of VSSA and VISA of 16 (80%) and 4 (20%) respectively compared to those from Maryam Abacha Women and Children Hospital Sokoto with 3 (75%) VSSA and 1 (25%) VISA respectively (Table 2). Similarly, among the out-patient samples, samples from Specialists Hospital Sokoto had higher number of VSSA and VISA of 37 (902%) and 4 (9.7%) compared to those from Maryam Abacha Women and Children Hospital Sokoto with 13 (86.6%) VSSA and 2 (13.3%) VISA respectively.

Table 2: Distribution of Vancomycin Susceptible (VSSA), Vancomycin Intermediate (VISA) and Vancomycin Resistant (VRSA) Isolates according to Patient Status

Patient Status	Vancomycin Susceptibility	S.H.S No (%)	MAWCH No (%)	Total No (%)
In Patients	VSSA	16 (80%)	3 (75%)	19 (79%)
	VISA	4 (20%)	1 (25%)	5 (20.8%)
	VRSA	0	0	0
		20 (25%)	**4 (5%)**	**24 (30%)**
Out-Patients	VSSA	37 **(90.2%)**	13 **(86.6 %)**	50 **(89.2%)**
	VISA	4 **(9.7%)**	2 **(13.3%)**	6 **(10.7%)**
	VRSA	0	0	0
Total		41 (51.2%)	**15 (18.7%)**	**56 (70%)**

Key: VSSA=Vancomycin Susceptible *Staphylococcus aureus*,
VISA= Vancomycin Intermediate *Staphylococcus aureus*
VRSA= Vancomycin Resistant *Staphylococcus aureus*
S.H. S= Specialists Hospital Sokoto
MAWCH=Maryam Abacha Women and Children Hospital Sokoto

Table 3 shows the distribution of VSSA, VISA and VRSA with respect to the source of samples. The highest number of samples of 39 (72.7%) was obtained from wound, followed by urine 15 (18.8%) and ear swab 13 (17.5%) and the least was from eye swab 1 (1.3%) (Table 3). Table 3 also show that urine, ear swab, HVS and wound samples had higher number of VSSA of 31 (93.3%), 8 (92.3%), 6 (85.7%) and 31 (79.5%) respectively (P=0.744). However, wound samples had higher number of VISA of 20.5% followed by HVS1 (14.3%), ear swab 1 (7.7%) and urine 1 (6.7%).

Table 3: Distribution of Vancomycin Susceptible (VSSA), Vancomycin Intermediate (VISA) and Vancomycin Resistant (VRSA) Isolates according to Source of Sample

Sample	VSSA No (%)	VISA No (%)	VRSA No (%)	Total No (%)
Wound	31 (79.5)	8 (20.5)	0 (0.0)	39 (72.7)
HVS	6 (85.7)	1 (14.3)	0 (0.0)	7 (8.8)
Urine	14 (93.3)	1 (6.7)	0 (0.0)	15 (18.8)
Ear swab	12 (92.3)	1 (7.7)	0 (0.0)	13 (17.5)
Eye swab	1 (100)	0 (0.0)	0 (0.0)	1 (1.3)
Blood	2 (100)	0 (0.0)	0 (0.0)	2 (2.5)
Urethral swab	3 (100)	0 (0.0)	0 (0.0)	3 (3.8)
Total	**69 (86.2)**	**11 (13.8)**	**0 (0.0)**	**80 (100)**

Key: VSSA=Vancomycin Susceptible *Staphylococcus aureus*,
VISA= Vancomycin Intermediate *Staphylococcus aureus*
VRSA= Vancomycin Resistant *Staphylococcus aureus*

DISCUSSION

The findings of this study revealed that 13.8% of the samples were identified as VISA. This could be related to the observations made by Park *et al*. (2019) who reported that with the increase in the prevalence and endemicity of MRSA strains, the use of vancomycin became more frequent resulting in the emergence of vancomycin intermediate and vancomycin resistant *S. aureus* leaving clinicians with few therapeutic options for treatment. Studies by Mathews *et al*. (2010); Watkins *et al*. (2019) revealed that *Staphylococci* still remain the commonest of all the clinical isolates which are responsible for several suppurative types of infections and capable of acquiring and utilizing multi resistance mechanisms. Compared to the findings of this study, other studies revealed higher prevalence of VISA of in 18% in Abakaliki, Nigeria (Moses *et al*., 2013) and lower prevalence of 4.3% (Ogbolu *et al*., 2015). In the south western part of Nigeria, a relatively high prevalence rate of 15.1% and 11.4% for VISA and VRSA respectively was reported (Bamigboye *et al*., 2018). Although, few isolates were involved in the study which may have resulted in the high prevalence rate, the high prevalence rate of VISA in this study could be attributed to the high rate of indiscriminate abuse of antibiotics (Park *et al*., 2019). No vancomycin resistance *Staphylococcus aureus* (VRSA) was discovered in this study which is similar to reports of other studieswith 0% prevalence rate for VRSA (Dhanalakshmi *et al*., 2012; Ogbolu *et al*., 2015 and Park*et al*., 2019). However, some studies reported higher and lower prevalence of 14.2% (Okolie *et al*., 2015), 5.3% (Moses *et al*., 2013) and 1.4% (Bamigboye *et al*., 2018).

Even with a 13.8% VISA prevalence rate, no isolate with total resistance to vancomycin was found in this study. The absence of VRSA in the study could be related to the cost, as vancomycin may not be in use in the peripheral rural setups, thus decreasing the selection pressure for vancomycin resistance. However, in contrast to the reports of this study, there have been reports of VRSA in other Northern regions of the country. For, instance, studies by Kumurya and Yahaya (2016) in Kano, Nigeria reported a VRSA prevalence rate of 14% although, this study focused on VRSA from MRSA which may be responsible for the high prevalence. A relatively high prevalence rates of VRSA of 44.5% among healthy women in Zaria and 89.2% in patients attending University Teaching Hospital, Abeokuta were reported (Onanuga *et al*., 2006; Olufunmiso *et al*., 2017). Another 5.4% VRSA prevalence in fresh and fermented milk from animals was also reported (Umaru *et al*., 2013). The high prevalence of VRSA reported in these studies may be related to fact that the interpretations of VRSA assessment was based on disc susceptibility which is not in line with the recommendation of various international guidelines on antibiotics or vancomycin susceptibility (CLSI, 2020).

The findings of these study report that most of the *Staphylococcus aureus* isolates in this study were susceptible to vancomycin with 70% having a MIC of less than 1µg/ml indicating high susceptibility rate and 86% had MIC of ≤2µ/ml and identified as VSSA. This is in line with a study in Abakaliki where a VSSA prevalence of 83.6% was reported (Moses *et al*., 2013). Another study in Osogbo reported a prevalence rate of 51% VSSA.

This finding has been corroborated by reports of high prevalence of VISA and VRSA colonization among health workers and patient in the hospital (Askari *et al*., 2012). Most isolates in the susceptible region (MIC ≤2µg/ml) with high susceptible MIC of 1.5µg/ml or 2µg/ml were from wound samples. Increased mortality and treatment failure have also been

reported in infections with VSSA isolates with an elevated vancomycin MIC in the fully susceptible range (Yang *et al*., 2018; Bouiller *et al*., 2018; Van Hal *et al*., 2012). Typically, these isolates have MICs near the susceptibility breakpoint such as 1.5 or 2µg/ml.

The findings of this study revealed that none of the isolates was identified as VRSA. An important finding of this study was that the identification of four isolates as VISA could have been missed if the isolates were evaluated using only MHA with 6 µg/mL of vancomycin, however the use of MHA with 3µg /ml of vancomycin established their identity as VISA. This is in line with CLSI guidelines considering its suggestion to define the level of sensitivity of the methods for VISA, for which the MICs are 4 µg/ ml (Dhanalakshmi *et al*., 2012). Saderi *et al*. (2005) noted that the availability of limited therapeutic alternatives in the treatment of the VRSA and VISA isolates has not helped therefore, the detection of vancomycin resistance should be done in the clinical microbiology laboratory with meticulous care, keeping in mind the sensitivity and the specificity of the methods which are used.

One limitation of this study is the absence of isolation of the *Van A/Van B* genes by Polymerase Chain Reaction (PCR). However, it was reported that these genes were absent in the VISA and VRSA strains isolated even in the presence of phenotypic resistance to vancomycin (Al-Tamimi *et al*., 2020). Hence, the presence or absence of the resistant *van A/B* genes does not necessarily rule out that strains are not VRSA. Also, the phenotypic expression of VRSA and clinical failure of the drug *in vivo* lends credence to the weight of the burden of this resistance in *S. aureus*. Vancomycin resistant *S. aureus* strains have increased the yearning of the world at large for the development of new drugs that will be effective in the treatment of multidrug resistant bacteria, *S. aureus* being one of them. Especially considering the widespread infection caused by *S. aureus* in developing countries.

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