Vancomycin resistant *Staphylococcus aureus* from Clinical Isolates in Benin City, Nigeria

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Abstract
Multidrug resistant *Staphylococcus aureus* has increasingly been implicated as an agent of clinical infections worldwide. This study investigated the prevalence of Vancomycin resistant *S. aureus* (VRSA) among *S. aureus* causing infections in Benin City, Nigeria. A total of 400 non-repetitive *S. aureus* isolates were obtained from clinical specimens in the University of Benin Teaching Hospital. These isolates were identified by standard microbiological tests. Antimicrobial susceptibility tests and screening for vancomycin resistance (using phenotypic method) was carried out for all isolates. Eighteen isolates (4.5%) were resistant to vancomycin with 10 (2.5%) VRSA isolates recovered from females while 8(2.0%) were isolated from males (p = 0.9981). Isolates from catheter tip had the highest prevalence of VRSA (33.3%) and showed statistical significance in comparison with other clinical specimens (p = 0.0036). Vancomycin resistant *S. aureus* isolates showed poor susceptibility to commonly used antibacterial agents (< 50%). In order to mitigate the effect of selective pressure on the survival and proliferation of resistant strains, prudent use of vancomycin and other antimicrobial agents is advocated. The need for continuous surveillance, susceptibility testing and screening cannot be overemphasized.

Keywords: Vancomycin, *Staphylococcus aureus*, antimicrobial, resistance

Introduction
Staphylococcus aureus is one of the most common cause of bloodstream infections, skin and soft tissue infection, surgical site and wound infection, nosocomial infections, endocarditis as well as pneumonia (Kumurya and Yahaya, 2016).

Infections caused by *Staphylococcus aureus* is on the increase and has been reported to be associated with very high morbidity and mortality rates (Akanbi and Mbe, 2013). The advent and use of antibiotics such as penicillin and methicillin in the mid-20th century initially proved effective against *S. aureus*. However, *S. aureus* rapidly acquired resistance to these antibiotics and infections with penicillin-resistant *S. aureus* (PRSA), and in turn methicillin-resistant *S. aureus* (MRSA), were difficult to treat (McGuinness *et al.*, 2017).

The ability of *S. aureus* to outwit the immune system, above and beyond its multidrug resistance (MDR) phenotype makes it one of the most intractable pathogenic bacteria in the history of antibiotic chemotherapy (Hiramatsu *et al.*, 2014). Infection by *Staphylococcus aureus* was well controlled using methicillin until the isolation of the first strain of methicillin resistant *Staphylococcus aureus* (MRSA) in 1961 (Rashedul *et al.,* 2016).

In the 1980s, due to the widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to the glycopeptide vancomycin in many health-care institutions (Otobo *et al.*, 2018). For many years after its initial use, vancomycin was traditionally reserved as a drug of last resort after other antibiotics has failed. This is largely as a result of its high toxicity. However, with the increase in the prevalence and endemicity of MRSA strains, its use became more frequent (Alo *et al.*, 2013). The use of vancomycin in many countries increased because of its reliability as a therapeutic agent against *Staphylococcus aureus*. As a result, selective pressure was established that eventually led to the emergence of strains or

Staphylococcus aureus with decreased susceptibility to vancomycin.

In 2002, the first vancomycin-resistant *S. aureus* (VRSA) strain (with a vancomycin MIC value greater than 100 μg/ml) was reported in the United States (Sievert *et al.,* 2008). Resistance to vancomycin is accomplished by horizontal transfer of a plasmid-borne transposon carrying *van*A gene from vancomycin resistant *Enterococcus* to *Staphylococcus aureus* crosswise the genus barrier (De Niederhausern *et al*. 2011). The emergence of VRSA is thus of great concern considering the fact that *Staphylococcus aureus* is a notorious cause of human infections.

The aim of this study is to detect the presence and determine the prevalence VRSA among clinical isolates of *Staphylococcus aureus* in a tertiary healthcare facility in Benin City, Nigeria.

Materials and Methods
Isolates collection
Clinical isolates suspected to be *Staphylococcus aureus* obtained from various clinical specimens in a tertiary hospital (UBTH) in Benin City, Nigeria, were collected between November 2019 and June 2020 and used for this study. A total of 400 isolates were obtained. The isolates were purified by sub-culturing into nutrient agar plates for 24hrs. The isolates were identified by standard microbiological tests including colonial morphology, Gram staining, catalase test and coagulase test (Barrow and Feltham, 2003). The isolate was identified as *Staphylococcus aureus* if it was Gram positive, cocci, catalase positive and coagulase positive (slide or tube). The identified *Staphylococcus aureus* isolates were stored on nutrient agar slants and preserved at 4°C for further analysis.

Disc Susceptibility testing
The antimicrobial susceptibility testing for each of the isolates previously identified as *Staphylococcus aureus* was done using the modified Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2013). The following antimicrobial agents were used in their respective concentration: Cefoxitin (30 μg), Vancomycin (30 μg), Augumentin (30 μg), Cefuroxime (30 μg), Ceftazidime (30 μg), Erythromycin (5 μg), Piperacillin/Tazobactam (30/6 μg) and Azithromycin (15 μg) (Oxoid, U.K.). The isolates from the nutrient slants were streaked

on nutrient agar plates and incubated overnight at 37°C. A sterile inoculating loop was used to pick a colony of the isolate and transferred into 5ml normal saline and homogenized properly until slightly turbid. Turbidity of the suspension was matched with 0.5 MacFarland turbidity standard. The isolate suspension already matched with 0.5 MacFarland turbidity standard was used as inoculum to inoculate Mueller-Hinton agar plates using sterile swabs. The antibiotic discs were placed aseptically on the agar surface. The plates were incubated at 37°C for18- 24hrs and observed for zones of inhibition. The zone of inhibition was determined by measurement in millimeters using a calibrated ruler. The degree of susceptibility of the isolates to each antibiotic was interpreted as either sensitive or resistant according to Clinical and Laboratory Standards Institute (CLSI, 2013).

Detection of VRSA
The minimum inhibitory concentration (MIC) of *Staphylococcus aureus* to vancomycin was determined by the Macrobroth Dilution Method (CLSI, 2009). Dilutions to cover concentrations of 0.25μg/mL to 16 μg /mL of vancomycin were made with Mueller-Hinton Broth in tubes. By using a direct colony suspension method, 0.5McFarland equivalent bacterial inoculum was prepared in sterile distilled water after culturing for 24 hours on an agar plate. The suspension was further diluted to achieve the desired inoculum concentration of 5×10^5cfu/ml. All strains were added to the tubes of Muller-Hilton broth containing different concentrations of vancomycin. The tubes were incubated for 18-24 hours at 37°C and checked for growth in the form of turbidity. The drug concentration in the last tube showing no growth was taken as the MIC.

Data analysis
The data obtained was analyzed using INSTAT version 3.0 for Windows (Graphpad Company California, USA) and findings were presented using percentages, frequency distribution tables, charts etc. A p-vale of <0.05 was considered statistically significant.

Results
A total of 400 isolates of *Staphylococcus aureus* was used for this study. Of these, 18(4.5%) were resistant to vancomycin. Also 10(2.5%) VRSA isolates were obtained from females while 8(2.0%) were isolated from males. There was no significant difference in the prevalence of VRSA between males and females (p=0.9981). The highest prevalence rate in relation

to the various age groups was from participants within the age bracket of ≤ 1-10 years (12.5%). There was significant association between the age and prevalence rate of VRSA (p =0.0438) (Table 1). Isolates from catheter tip had the highest prevalence of VRSA (33.3%). There was a significant association between the nature of specimen and prevalence rate of VRSA isolates (p = 0.0036) (Table 2). The antibiotic susceptibility profile of VRSA showed that the isolates had low susceptibility to the antibiotics as none showed up to 50% antibacterial activity (Table 3).

Table 1: Prevalence of VRSA in relation to demography of participants

Characteristics	No of test	No of VRSA (%)	OR	95%CI	P- value
Gender					
Male	189	8 (4.23)	0.888	0.343, 2.300	0.9981
Female	211	10(4.74)			
Age (years)					0.0438
≤1-10	64	8 (12.5)			
11-20	19	1 (5.26)			
21-30	64	1 (1.56)			
31-40	97	4 (4.12)			
41-50	68	1 (1.47)			
51-60	47	1 (2.13)			
≥61	41	2 (4.88)			
Source of patient					
In-patients	296	14 (4.73)	1.241	0.399, 3.860	0.9212
Out-patients	104	4 (3.85)			

Table 2: Association of VRSA in relation to different clinical specimen

Specimen	No. tested	No. with VRSA (%)
Wound swab	166	5 (3.01)
Ear swab	29	0*
Eye swab	22	4 (18.18)
Throat swab	7	1 (14.29)
Nasal swab	1	0*
Urethral swab	13	0*
High vaginal swab	30	1 (3.33)
Endocervical swab	33	1 (3.03)
Abscess/pus	11	1 (9.09)
Aspirate	6	0*
Urine	73	3 (4.11)
Semen	3	0*
Catheter tip	6	2 (33.33)

*not included in statistical analysis; p= 0.0036

Table 3: Susceptibility profile of VRSA to some selected antibiotics

Antibiotics (µg)	Number Sensitive (%)
Augmentin (30)	7 (38.9)
Piperacillin/Tazobactam (30/6)	8 (44.4)
Cefuroxime (30)	4(22.2)
Erythromycin (5)	3 (16.7)
Ceftazidime (30)	1 (5.6)
Azithromycin (15)	4 (22.2)

N= 18

Discussion

Staphylococcus aureus has been one of the most problematic nosocomial pathogens and a major threat to human health globally. This is as a result of its antimicrobial resistance, infectivity and highly sophisticated virulence factors (Chambers, 2005; Klevens *et al.*, 2007) as well as its ability to repeatedly acquire resistance to overcome the challenges presented by new antibiotics against it (Tallent *et al.*, 2002). Although vancomycin is the main antimicrobial agent available to treat infections with MRSA, a decreased susceptibility of *S. aureus* to vancomycin and the isolation of VRSA from many countries including Nigeria have been reported (Olufunmiso *et al.*, 2017; Taiwo *et al.*, 2011). Rapid emergence of resistance in bacteria have been associated with overuse, misuse or indiscriminate use of antibiotics in both clinical and community settings.

In this study, the overall prevalence of VRSA among *S aureus* isolates was 4.5%, which is in line with the work of Alo *et al.* (2013) who recorded a prevalence of 5.3% among clinical isolates in Abakiliki, Nigeria. This prevalence is however higher than 1.4% reported in Osogbo by Bamigboye *et al.* (2018). The high prevalence rate can be due to high rate of indiscriminate abuse of antibiotics. In Nigeria most drugs including antibiotics are easily gotten from local pharmacies without prescription and control

leading to increased acquisition of resistance mechanisms by bacterial strains.

The prevalence of VRSA was significantly higher in age group $\leqslant$ 1-10years (p= 0.0438). The reason for this may be as a result of the fact that children are easily exposed to various pathogenic bacteria since their immunity is not that formidable. It is believed that the immunity of children is not properly developed at this stage to cope with bacterial infections hence they are vulnerable and easily infected especially when hospitalized (Simon *et al.*, 2015).

The frequency of VRSA in relation to nature of specimen shows that the highest prevalence was from catheter tips. This prevalence rate was found to be statistically significant (p= 0.0036). In a recent study carried out by Siddiqui *et al.* in 2017, strains of VRSA were recovered from catheter tips with a prevalence of 16.67%. It is also on record that the first ever VRSA was isolated from catheter specimen (Sievert *et al.*, 2008). The high prevalence rate of VRSA from catheter tips observed in this study may be as a result of the fact that catheters are a major reservoir of many pathogenic microorganisms which are prone to development of resistance. Microorganisms can adhere to catheter surface and embed themselves in a layer of biofilms which is a product of the human body and itself (Sedor and Mulholland, 1999). By forming

biofilm, bacteria protect themselves from host defense, disinfectants and antibiotics thus causing resistance (Sievert *et al*., 2008).

The susceptibility profile for VRSA isolates also showed that the VRSA isolates were highly resistant to the different antimicrobial agents used in this study. VRSA tend to be multi-drug resistant against a large number of currently available antimicrobial agents, compromising treatment options thus increasing the likelihood of inadequate antimicrobial therapy resulting in increased morbidity and mortality (De Lassence *et al*., 2006).

Conclusion
The overall prevalence of VRSA among *Staphylococcus aureus* isolates was 4.5%. There was a significant association between the prevalence of VRSA and nature of specimen with highest prevalence from catheter tips. The prudent use of vancomycin and other antimicrobial agents is advocated. The need for continuous surveillance, susceptibility testing and screening cannot be overemphasized. Regulations on availability of antibiotics and other prescription drugs to the public should be enacted. Efforts should also be channeled to development of new drugs so as to counter the menace of multidrug failure.

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