Multidrug and vancomycin resistance among clinical isolates of *Staphylococcus aureus* from different teaching hospitals in Nigeria.

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Abstract

Backgrounds: *Staphylococcus aureus* has emerged as a major public health concern because of the occurrence of multi-drug resistant strains. This study aimed at investigating the multi-drug and vancomycin resistance profile of *S. aureus* from different infection sites in some teaching hospitals in Nigeria.

Methods: Swabs were collected from different infection sites from out-patients in three teaching hospitals from October 2015 to May, 2016. The antibiotic-susceptibility test was carried out with selected antibiotics usually administered anti-microbials in the treatment of infections in these hospitals. The prevalence of multi-drug and vancomycin resistance strains of *S. aureus* from clinical samples was determined using disk diffusion and agar dilution methods respectively.

Results: The result showed (165)82.5% of the isolates were resistant to ≥3 antibiotics tested. They were highly resistant to ceftazidime 180(90%), cloxacillin 171(85.6%) and augmentin 167(83.3%), but susceptible to ofloxacin 150(75%), gentamicin 142(71.7%), erythromycin 122(61.1%), ceftriaxone 111(55.6%) and cefuroxime 103(51.7%). All the isolates from the HVS were all multidrug resistant strains. While (56)90.16% were multidrug resistant (MDR) in urine samples, followed by (8)88.89% MDR strains in sputum, (37)88.81% MDR strains in semen, (49)71.64% MDR strains in wounds and (6)60% MDR strains in ear swabs samples. Although (147)73.5% of the isolates were vancomycin susceptible *S. aureus* (VSSA), (30)15% were vancomycin intermediate resistant *S. aureus* (VISA) and (89)44.5% of the isolates were considered vancomycin resistant *S. aureus* (VRSA).

Conclusions: The high percentage of the VRSA could have resulted from compromising treatment options and inadequate antimicrobial therapy. The implication, infections caused by VRSA would be difficult to treat with vancomycin and other effective antibiotics of clinical importance. Ensuring proper monitoring of drug administration will, therefore, enhance the legitimate role of vancomycin as an empiric choice for both prophylaxis against and treatment of *staphylococcal* infections.

Keywords: Bacterial resistance, vancomycin resistant *S. aureus*, susceptibility studies, agar dilution.

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Introduction

Staphylococcus aureus is frequently found in the human respiratory tract and on the skin. It is estimated that 20% of the human population are long-term carriers of *S. aureus*[1] whereas it is a transient normal flora of human skin and mucosal surfaces in 20 to 90% of healthy population. To establish its pathogenic potential, *S. aureus* produces toxin and extracellular membrane compounds.[4] It produces various virulence factors including coagulase to clot plasma and coats the bacterial cells to probably prevent phagocytosis,[5] hyaluronidase and DNAse to break down hyaluronic acid and DNA respectively to help in its systemic spread[6] as well as staphylokinase to dissolve fibrin.[5] While these virulence factors allow its attachment to host's cells, invade tissues and evade the host's immune system, Silva and Gandra[4] indicated that enzymes like coagulase and catalase produced by *S. aureus* are responsible for the invasion of the immune system.

S. aureus infects wounds,[7] cause ascending urinary tract colonization and infection[8] and atopic dermatitis.[9] While it is responsible for necrotizing pneumonia, skin and soft tissue infections, bacteraemia as well as food poisoning

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through enterotoxin production[10-12] and may occur as commensals,[13] this organism can infect tissues when the skin or mucosal barriers have been breached[9], to cause infections associated with increased burden on health-care resources[14] in community and hospitals[15]. The unrestricted use of antibiotics and inadequate compliance to antibiotic regime along with inadequate surveillance for anti-microbial resistance are some of the imperative reasons accrued to the emergence of its highly resistant strains.[16,17]

Since the emergence of penicillin and methicillin resistant *S. aureus* strains in 1948 and 1961 respectively[18,19] and virtually all strains of *S. aureus* are, today, resistant to natural penicillins,aminopenicillins and antipseudomonal-penicillins,[20,21] it becomes necessary to find alternative antibiotics to treat *staphylococcal* infections.[22] Consequently, vancomycin, a tricyclic glycopeptide antibiotic, is used to treat Gram-positive infections involving methicillin resistant *S. aureus* (MRSA).[23,24] This antibiotic interferes with bacterial cell wall synthesis, as does penicillin, to lyse the cell.[25] However, soon after its introduction, reduced susceptibility to vancomycin was reported in Japan by Hiramatsu.[26] This was quickly followed by isolation of vancomycin intermediate resistant *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) isolates from France,[27] United Kingdom,[28] Brazil,[29] USA,[30,31] Germany,[32] India[33,34] and Belgium[35,36] to confirm that the emergence of these strains is a global challenge. From patients treated with glycopeptides and in patients with suspected or confirmed MRSA, vancomycin intermediate and a few vancomycin-resistant strains have been isolated.[37-39] While Assadullah et al.[33] and Khadri and Alzohairy[40] indicated that VRSA is not widely seen and a low level of resistance to vancomycin is being reported, the knowledge of the prevalence of VRSA and their antibiotic susceptibility pattern becomes fundamental in the selection of appropriate empirical treatment especially in hospital settings in the third world countries like Nigeria. This study, therefore, aimed at investigating the multi-drug and vancomycin resistance profile of *S. aureus* from different infection sites in some teaching hospitals in Nigeria. This is to detect VRSA as potential risk factor that could pose challenges to the effectiveness of anti-microbial therapy in the treatment of *staphylococcal* infections in developing countries like Nigeria.

Materials and methods

Samples were collected from 200 patients attending three teaching hospitals in Ogun State, Nigeria. These patients were being treated at out-patient Units of Babcock University Teaching Hospital, Ilisan-Remo, Olabisi Onabanjo Teaching Hospital, Sagamu and Federal Medical Center, Idi-Aba, Abeokuta, all in Ogun State, Nigeria from October 2015 to May 2016. Patients being treated with systemic antibiotics in the last 4 weeks were excluded. The test samples collected were taken by carefully rolling swabs saturated with sterile peptone water in the different infection sites from different teaching hospitals in Nigeria. The swabs were tightly sealed and immediately transported to the laboratory. The collected infection swab sticks were streaked on mannitol salt agar (MSA) and nutrient agar which were incubated overnight at 37°C for 24-48 h.[41] The bacterial colonies were subjected to established procedures such as Gram staining, microscopic appearance, colony morphology and biochemical tests such as tube DNase, catalase and coagulase tests for the characterization of the strains.[42-44]

Antibiograms of the isolates using multi-disc antibiotics

Each of the isolates was standardized using colony suspension method. Each strain's suspension was matched with 0.5 McFarland standards to give a resultant concentration of 1.5×10^6 cfu/ml. The antibacterial activity was determined using agar diffusion assay technique according to the modified Kirby–Bauer diffusion technique[43] by swabbing the Mueller-Hinton agar (MHA) (Oxoids UK) plates with the adjusted overnight culture of each of the test isolates. Multi-discs (Abtek) containing different antibiotics including ofloxacin (5 μg), augmentin (30 μg), ceftazioime (30μg), cefuroxine (30 μg), gentamicin (10 μg), ceftrioxone (30 μg), erythromycin (5 μg) and cloxacillin (5 μg) were aseptically placed on the inoculated agar plates and incubated at 37°C for 24 h. After 24 h of incubation, the plates were examined for inhibition zones.[45] The diameter of the inhibition zones produced by each antibiotic disk was measured to the nearest millimeter, recorded and interpreted using the Clinical and Laboratory Standard Institute Zone Diameter Interpretative Standards.[46] Each bacterial isolate was classified as susceptible (S), intermediate (I) and resistant (R) to antibiotics according to the zone diameter interpretation standard recommended by the Clinical Laboratory Standards Institute.[47]

Susceptibility of the isolates to vancomycin

The susceptibility of the different strains of *S. aureus*

to vancomycin was further determined by agar dilution method using CLSI guidelines.[48] Here, gradient plates of Mueller Hinton agar were prepared with different concentrations, 1 μg/ml, 2 μg/ml, 4 μg/ml, 8 μg/ml and 16 μg/ml, of the vancomycin by dissolving vancomycin tablets (Mast Diagnostics, Mast Group Ltd., Merseysidem UK, Lot: 311380, Exp: 2016 – 06) in 200 ml of sterilized molten agar maintained at a temperature of 50°C. The conical flasks containing the vancomycin tablets were then mixed gently till the tablets were completely dissolved. The antibiotic-containing agar was then dispensed aseptically into Petri dishes labeled according to the various concentrations of the vancomycin prepared and allowed to solidify. 0.5 McFarland equivalent inoculums prepared using 18 h old culture to give a resultant concentration of 1.5×10^6 cfu/ml was inoculated by streaking and stabbing the concentration gradient vancomycin-containing agar plates. The plates were incubated overnight at 35°C before being assessed for visible growth. Appearance of growth indicated vancomycin resistance.[49] Hence, the points of streaking and stabbing that showed bacterial growth were referred to as being vancomycin resistant while the points that did not show bacterial growth were referred to as vancomycin susceptible.

Results

A total number of 200 clinical strains of *S. aureus* were isolated from wound, urine, semen, ear swabs, sputum and high vaginal swabs (HVS) and characterized from different teaching hospitals in Lagos state. From the sample distribution, the highest incidence of *S. aureus* was in wounds 69(34.5%) followed by urine 62(31%), semen 42(21%), ear swabs 10(5%), sputum 9(4.5%) and HVS 7(3.5%) as shown in Figure 1.

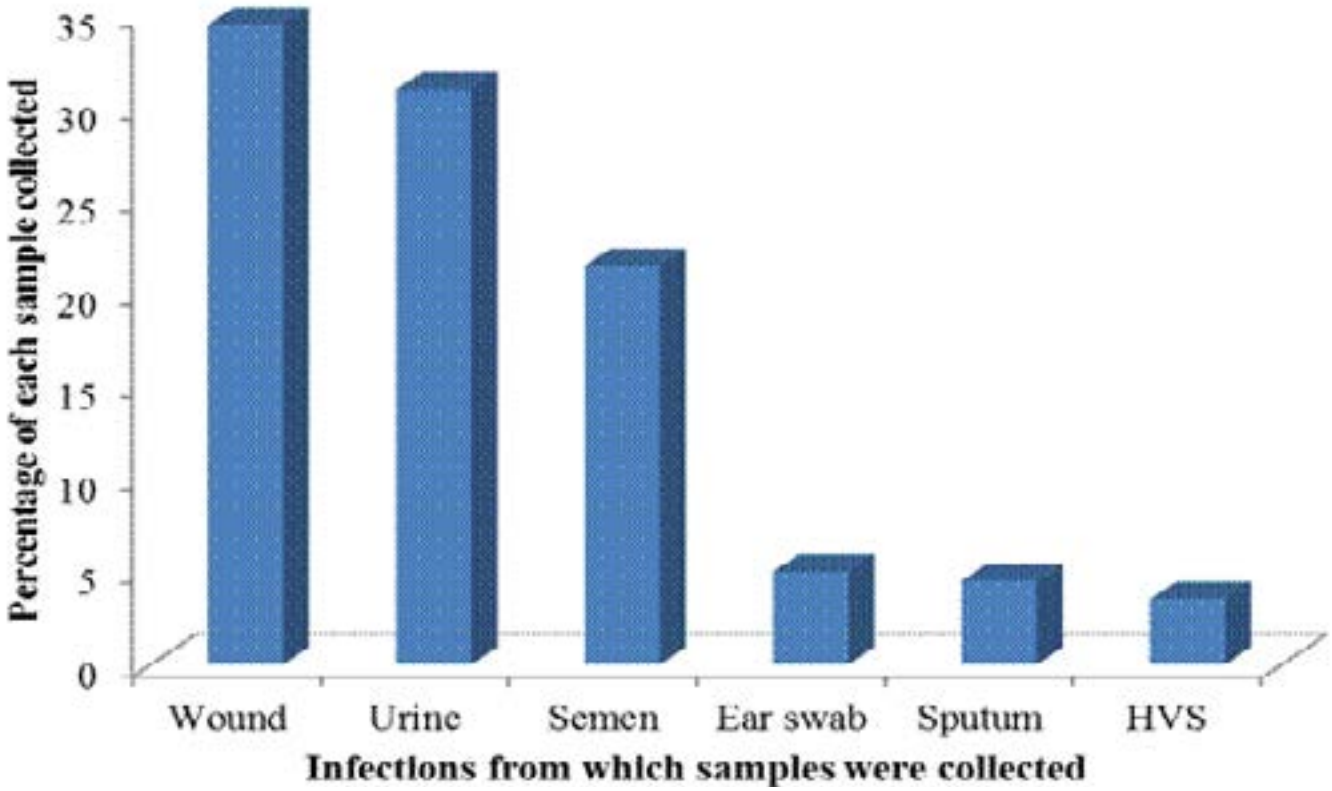

Figure 1: Percentage distribution of isolates from different infection sources

Though these isolates were highly resistant to ceftazidime 180(90%), Cloxacillin 171(85.6%) and augmentin 167(83.3%), (150)75% of the isolates were susceptible to ofloxacin, followed by gentamicin 143(71.7%), erythromycin 122(61.1%), ceftriaxone 111(55.6%) and cefuroxime 103(51.7%) in a descending order. However, (165)82.5% of the isolates exhibited multidrug resistance by being resistant to ≥3 of the test antibiotics as shown in Figure 2.

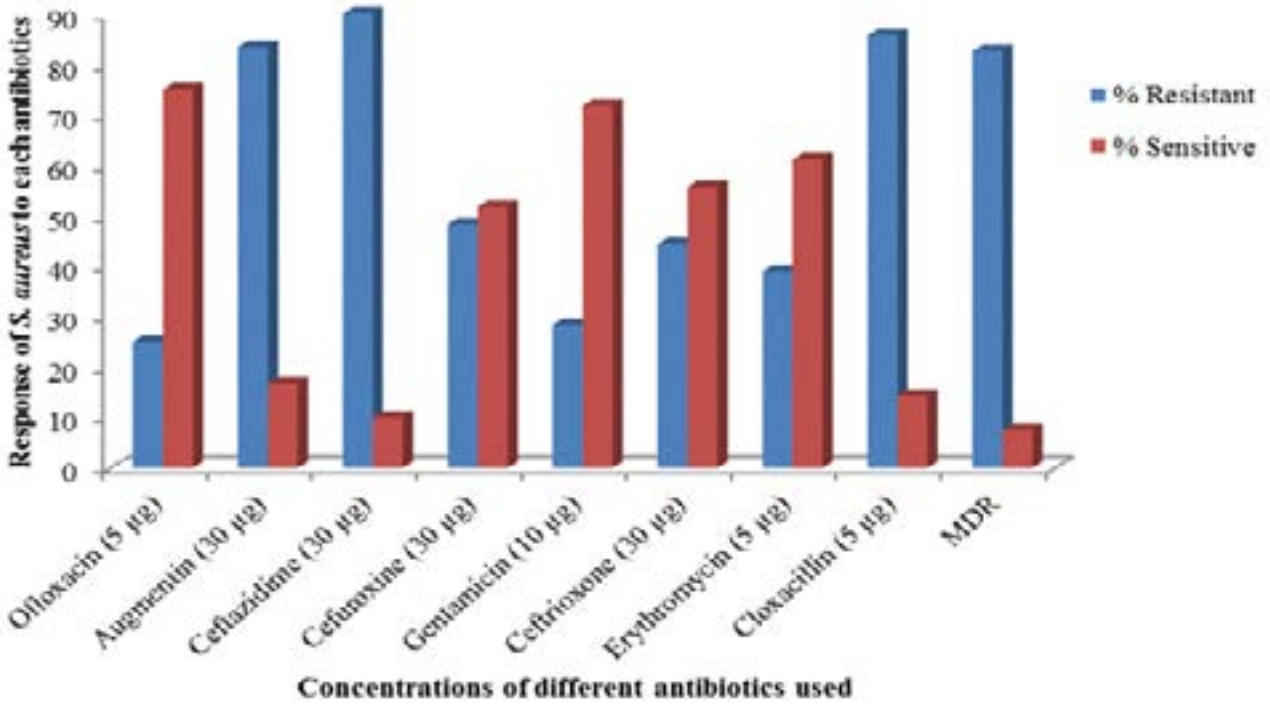

Figure 2: Resistance and Sensitivity profiles of *Staphylococcus aureus* **to different antibiotics**

From the susceptibility of these isolates to different concentrations of vancomycin used in this study as shown in Table 1, (97)48.5% were susceptible to all the concentrations of the vancomycin used while (34)17% were resistant to all the concentrations of the vancomycin used. Considering the number of strains susceptible at each concentration used, (153)76.5%, (149)74.5%, (137)68.5%, (123)61.5% and (111)55.5% of the *S. aureus* isolates were susceptible at 1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml and 16 µg/ml respectively. Considering the number of isolates susceptible at concentrations less than or equal to each of the concentration used, (147)73.5%, (15)7.5% and (15)7.5% of the isolates were susceptible at concentrations of ≤2 µg/ml, ≤4 µg/ml and ≤8 µg/ml respectively but different percentages of isolates were resistant at higher concentrations above the respective concentrations at which they were susceptible. Considering the number of isolates that were initially resistant to vancomycin but later became susceptible to this antibiotic, (2)1% of the isolates were resistant at 1 µg/ml but susceptible to other concentrations, (5)2.5% of the isolates were resistant at ≤2 µg/ml but susceptible to all the other concentrations and (3)1.5% were resistant at ≤8 µg/ml but susceptible only at 16 µg/ml but none of the isolates was resistant at concentration before 4 µg/ml.

Table 1: The susceptibility of *Staphylococcus aureus* strains to different concentrations of vancomycin antibiotic

Susceptibility of *Staphylococcus aureus* strains to different concentrations of vancomycin antibiotic

S/N	*S. aureus*	Source	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml
1.	MDR	Wound	-	-	-	-	-
2.	MDR	Wound	+	+	+	+	+
3.	MDR	Wound	-	-	+	+	+
4.	MDR	Wound	-	-	-	+	+
5.	SS	Wound	-	-	-	+	+
21.	MDR	Wound	-	-	-	+	+
22.	MDR	Wound	-	-	-	-	-
23.	SS	Wound	-	-	-	-	-
24.	MDR	Wound	-	-	-	-	-
25.	MDR	Wound	-	-	-	-	+
26.	MDR	Wound	-	-	-	-	+
27.	MDR	Wound	-	-	-	-	-
28.	SS	Wound	-	-	-	-	-
29.	MDR	Wound	+	+	+	-	-
30.	SS	Wound	-	-	-	-	-
31.	MDR	Wound	-	-	-	-	-
32.	SS	Wound	-	-	-	-	-
33.	MDR	Wound	-	-	-	-	-
34.	MDR	Wound	-	-	-		
51.	MDR	Wound	+	+	+	+	+
52.	MDR	Wound	-	-	-	-	-
53.	MDR	Wound	-	-	-	-	+
54.	SS	Wound	-	-	-	-	-
55.	MDR	Wound	+	+	+	+	+
56.	SS	Wound	-	-	-	-	+
57.	MDR	Wound	-	-	+	+	+
58.	MDR	Wound	-	-	-	-	-
72.	MDR	Semen	-	-	-	-	-
73.	SS	Semen	-	-	-	-	-
74.	MDR	Semen	+	+	+	+	+
75.	MDR	Semen	-	-	+	+	+
76.	MDR	Semen	+	+	+	+	+
77.	MDR	Semen	+	+	+		
94.	MDR	Semen	+	+	+	+	+
95.	MDR	Semen	+	+	+	+	-
96.	MDR	Semen	-	-	+	+	+
97.	MDR	Semen	+	+	+	+	+
98.	MDR	Semen	-	-	-	-	-
112.	MDR	Semen	-	-	-	-	-
113.	MDR	Urine	-	-	-	+	+
114.	MDR	Urine	-	-	-	+	
130.	MDR	Urine	+	+	+	-	-
131.	MDR	Urine	-	-	-	-	-
132.	MDR	Urine	-	-	-		
151.	MDR	Urine	-	-	-	-	-
152.	SS	Urine	-	-	-	-	-
153.	MDR	Urine	-	-	-	-	-
154.	MDR	Urine	-	-	-	+	+
155.	SS	Urine	-	-	-	-	-
165.	SS	Urine	-	-	-	-	-
166.	MDR	Urine	-	-	-	+	+
167.	MDR	Urine	-	-	-	-	
182.	SS	Sputum	-	-	-	-	-
183.	MDR	Sputum	-	-	-	-	-
184.	MDR	Ear-swab	-	-	-	-	-
193.	SS	Ear-swab	-	-	-	-	-
194.	MDR	HVS	-	-	+	+	+
195.	MDR	HVS	-	-	-	+	+
196.	MDR	HVS	-	-	-	-	-
197.	MDR	HVS	-	-	-	-	-
198.	MDR	HVS	+	+	+	+	-
199.	MDR	HVS	-	-	-	-	-
200.	MDR						

According to the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS), *S. aureus* isolates for which vancomycin MIC are 4-8 µg/ml are classified as vancomycin-intermediate (VISA), and isolates for which vancomycin MIC´s are greater than 8 µg/ml are classified as vancomycin-resistant.[50] From case definition of Kluytmans et al.[51] indicating vancomycin MIC of ≤2 µg/ml as vancomycin-susceptible *S. aureus* (VSSA), vancomycin MIC = 4-8 µg/ml as vancomycin-intermediate susceptible *S. aureus* (VISA) and vancomycin MIC ≥ 16 µg/ml as vancomycin-resistant *S. aureus* (VRSA), (147)73.5% of the isolates were vancomycin susceptible *S. aureus* (VSSA). Combining those isolates susceptible at concentrations ≤4 µg/ml and those of susceptible at ≤8 µg/ml, (30)15% of the isolates were considered vancomycin intermediate resistant *S. aureus* (VISA). However, (34)17% of the isolates that were resistant to all the concentrations of the vancomycin used were considered VRSA in addition to (55)27.5% of the isolates that were resistant to vancomycin at the concentration of 16 µg/ml. Hence, (89)44.5% of the isolates were considered VRSA. Considering the distribution of *S. aureus* on the basis of their susceptibility to vancomycin with respect to isolates from the different sampling sources, (180)90% of the *S. aureus* from ear samples were susceptible to vancomycin while (20)10% of the isolates were vancomycin intermediate resistant *S. aureus* (VISA) and no vancomycin resistant *S. aureus*. In HVS, VSSA was (4)57.14%, VISA was (2)28.5% and VRSA was (1)14.3%.

In semen samples, VSSA was (22)53.5%, VISA was (3)7% and VRSA was (17)39.5%. In sputum samples, VSSA was (6)66.7%, VISA was (1)11.1% and VRSA was (2)22.2%. While VSSA was (18)29.0%, VISA was (6)9.7% and VRSA was (38)61.29% in urine samples, wound samples had VSSA 43(61.8%), VISA 6(8.8%) and VRSA 20(29.4%). Invariably, the percentages of VRSA in the various samples collected varied in a descending order from urine samples having VRSA 38(61.29%), followed by semen 17(39.5%) > wound 20(29.4%) > sputum 2(22.2%) > HVS 1(14.3%) > ear swabs 0(0%). While the isolates from the HVS were all multi-drug resistant strains, (59)90.2% of isolates from the urine samples were multi-drug resistant, followed by (8)88.9% MDR strains from sputum, (37)88.81% MDR strains from semen, (49)71.64% MDR strains from wounds and (6)60% MDR strains from ear swabs as shown in Figure 3.

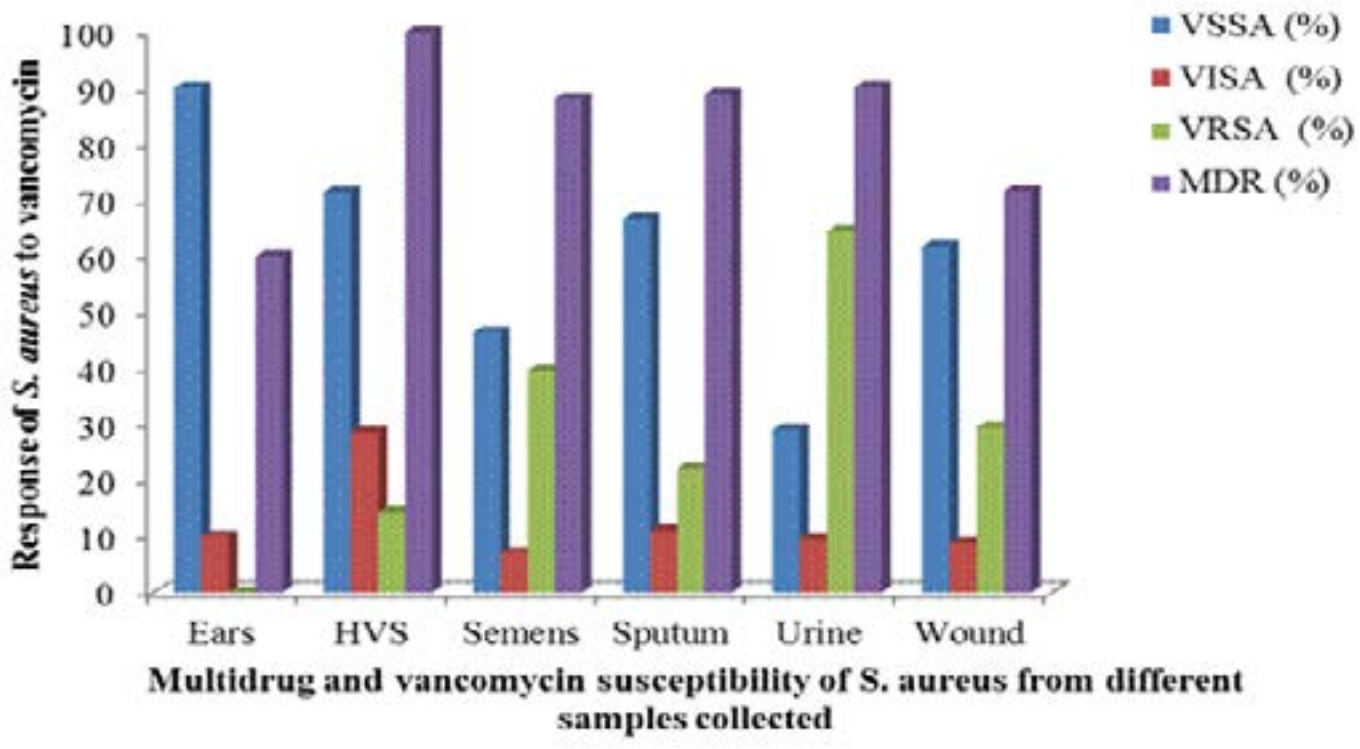

Figure 3: Percentage distribution of multi-drug and vancomycin susceptibility of *S. aureus* in each infection samples

Discussion

Staphylococcus aureus has been one of the most problematic nosocomial pathogens and a major threat to human health worldwide due to its anti-microbial resistance, infectivity and possession of virulence factors[52,53] as well as its ability to repeatedly acquire resistance to overcome the challenges presented by the new anti-*staphylococcal* antibiotics.[54] Although vancomycin is the main antimicrobial agent available to treat serious *staphylococcal* infections, especially those of MRSA, a decrease in vancomycin susceptibility of *S. aureus* and isolation of vancomycin intermediate and resistant *S. aureus* from many countries have been reported.[55-57] Since its first being reported in 1997, the threat of vancomycin resistance in *S. aureus* has been the topic of intensive research, discussion and cause for alarm in the health care community.[58] There is widespread concern that vancomycin-resistant *S. aureus* poses, by far, the greatest risk to patients, given the virulence of the organism.

In this study, the prevalence of VRSA was found to be (89)44.5% of the investigated *S. aureus* isolated. However, the (89)44.5 % vancomycin resistance rate of *S. aureus*, in this study, was higher than that of 21% reported by Flamm et al.[59] in Nepal, 3.6% reported in Iran,[60] 40% reported by Mimejad et al.[61] in Iran and the 16.4% reported by Godebo et al.[62] in Ethiopia. Although these variations in the degree of resistance are geographically based, these varied degrees of resistance to vancomycin have resulted in an increasing concern about its therapeutic effectiveness in serious *staphylococcal* infections. While the determination of the antimicrobial susceptibility is crucial for an optimal therapy, for epidemiological purposes and for infection control measures,[60,63] the treatment of the *S. aureus* infections has become problematic because of the emergence of resistance to methicillin, vancomycin and other antibiotics.[60,64]

In agreement with De Lassence et al.[65] who indicated that VRSA tend to be multi-drug resistant against a large number of currently available anti-microbial agents, compromise treatment options and increase the likelihood of inadequate anti-microbial therapy and a resultant increase in morbidity and mortality, VRSA, a trait assigned to *S. aureus* strains with vancomycin minimum inhibitory concentration greater than 8 μg/ml,[66,67] showed high percentages of resistance to a wide range of anti-microbial agents including augmentin 167(83.3%), cloxacillin 171(85.6%) and ceftazidime 180(90%). Consequently, treatment of *Staphylococcus* infections will become more difficult because (165)82.5% of the strains, in this study, were resistant to ≥3 antibiotics tested at the same time.[68] As the frequency of antibiotic-resistant bacteria among countries is proportional to their relative rates of antibiotic use,[69,70] a never-ending need to produce and market costlier new antibiotics to treat progressively more resistant infections is inevitable.[71]

As the case may be in Nigeria and some other developing countries, virtually all drugs are sold in drug stores called "Chemists" in the local parlance without obtaining antibiotic sensitivity test results from the medical laboratories or prescriptions from clinicians. These factors, according to Yah et al.[72], increase the rate of drug abuse and consequently increase the rate of development of bacterial resistance to antibiotics in a geometric rate higher than that in developed countries. In this study, the presence of VISA may be an important indicator of the insidious decline of the clinical effectiveness of vancomycin in the hospitals or injudicious use of vancomycin in hospitals for wrongly diagnosed or false positive MRSA. While (34)17% of the isolates were resistant to MIC ≥16 μg/ml of the vancomycin antibiotic showed a fast increasing rate of development of vancomycin resistant *S. aureus* especially among clinical isolates, having (89)44.5% VRSA is an indication that *S. aureus* has become more resistant to vancomycin in comparison to other reports. This may, probably, pose a big problem towards its use as the ultimate drug against MRSA. These isolates may have acquired resistance by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan.[73,74] The cell wall thickening may have caused vancomycin molecules to become trapped in the outer layers of the cell wall, clog the peptidoglycan meshwork and form physical barriers limiting its access to the cytoplasmic membrane where the functional targets of vancomycin are located.[75]

In this study, that the percentages of VRSA varied in a descending order from urine samples having VRSA 38(61.26%), followed by semen 17(39.5%) > wound 20(29.4%) > sputum 2(22.22%) > HVS 1(14.29%) > ears (0%) is contrary to the report of Dhand et al.[76] who found no VRSA, VISA and VSSA in 250 *S. aureus* from clinical samples. On the other hand, while VRSA (4.7%), VISA (9.3%) and VSSA (86.0%) were reported by Ilang

et al.[77], 26.7% of VRSA in post-operative pus samples[78] and 36.1% of VISA in blood and body fluids[79] were reported. These differences might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure. Therefore, prolonged use of antibiotics and prolonged hospitalization are other important factors making hospitals an ideal place for transmission and perpetuation of VRSA.[80]

In conclusion, this study shows that there is a high prevalence of vancomycin-resistant *S. aureus* (VRSA) amongst isolates from the clinical samples investigated. The VRSA were multi-drug resistant against a large number of currently available anti-microbial agents. The high percentage of the VRSA could have resulted from compromising treatment options and inadequate anti-microbial therapy, a lack of sufficient knowledge on the danger of the wrong use of antibiotics, high proximity to a large number of unlicensed drug vendors and the inappropriate use of broad spectrum antibiotics in the medical practice. Efforts should, therefore, be made in ensuring proper monitoring of drug administration and its use to prevent drug misuse and abuse as well as to prevent or reduce the rate of anti-microbial resistance amongst clinical pathogens. These will, therefore, enhance the legitimate role of vancomycin as an empiric choice for both prophylaxis against and treatment of *staphylococcal* infections.

Conflict of interest

Authors hereby declare that they have no conflict of interest.

References

1. Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A, Ganz T. Determinants of *Staphylococcus aureus* by nasal carriage. *Clin Diagn Lab Immunol.* 2001; 8(6):1064–9

2. Foster TJ. The *Staphylococcus aureus* Superbug. *J Clin Invest.* 2004; 114:1693-6 PubMed . Doi: 10.1172/JCI200423825

3. Plata K, Rosato A, Wegrzyn G. *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. *Acta Biochem Polon* 2009; 56(4):597 PubMed -612.

4. Silva WP, Gandra EA. Estafilococos coagulase positiva: patógeno de importância em alimentos. *Hig Aliment.* 2004; 18(122):32 PubMed -40.

5. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev.* 2000; 13(1):16–34

6. Jarraud SC, Lyon GJ, Figueiredo AM, LinaG, Vandenesch F, Etienne J, Muir TW, Novick RP. Exfoliating-producing strains define a fourth agr specifically group in *Staphylococcus aureus*. *J Bacteriol.* 2000; 182(22):6517-22. PubMed

7. Henry JB. Clinical Diagnosis and Management by Laboratory Methods, 20th Edition. Philadelphia: W. B. Saunders Company, 2001.

8. Robert RM, Brennen C, John DR, Marilyn MW, Obman A, Stout JE, Victor L. Isolation of *Staphylococcus aureus* from the Urinary Tract: Association of Isolation with Symptomatic Urinary Tract Infection and Subsequent *Staphylococcal* Bacteremia Veterans Affairs Pittsburgh Healthcare System and University of Pittsburgh School of Medicine, Pennsylvania, 2006.

9. Birnie AJ, Bath-Hextall FJ, Ravenscroft JC, Williams HC. Interventions to reduce *Staphylococcus aureus* in the management of atopic eczema. The Cochrane database of systematic reviews 3, 2008.

10. Cavalcanti SM, de França ER, Vilela MA, Montenegro F, Cabral C, Medeiros AC. Estudo comparativo da prevalência de *Staphylococcus aureus* importado para as unidades de terapia intensiva de hospital universitário, Pernambuco, Brasil. *Rev Bras Epidemol.* 2006; 9(4):436 PubMed -46. http://dx.doi.org/10.1590/S1415-790X2006000400004

11. Cunha ML, Peresi E, Calsolari RA, Araújo Jr. Detection of enterotoxin genes in coagulase-negative *Staphylococci* isolated from foods. *Braz J Microbiol.* 2006; 37(1):70 PubMed -4. http://dx.doi.org/10.1590/S1517-83822006000100013

12. Santos AL, Santos DO, Freitas CC, Ferreira BL, Afonso IF, Rodrigues CR, et al. *Staphylococcus aureus*: visitando uma cepa de importância hospitalar. *J Bras Patol Med Lab.* 2007; 43(6):413-23. http://dx.doi.org/10.1590/S1676-24442007000600005

13. Cimolai N. MRSA and the environment: implications for comprehensive control measures. *Eur J Clin Microbiol Infect Dis.* 2008;27(7):481–93. DOI: 10.1007/s10096-008-0471-0

14. Mathei C, Niclaes L, Suetens C, Jansb B, Buntinx F. Infections in Residents of Nursing Homes, *Infect Dis Clin North Am.* 2007; 21:761–772. Doi:10.1016/j.idc.2007.07.005

15. Farhadian A, Behzadian Q, Shahin NP, Rahbar M, Farzam V. Determination of Vancomycin and Methicillin Resistance in Clinical Isolates of *Staphylococcus aureus* in Iranian Hospitals. *British Microbiol Res J.* 2014; 4(4):454-461. DOI: 10.9734/BMRJ/2014/4836

16. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. *The Lancet* 2006; 368:874–885. DOI: 10.1016/S0140-6736(06)68853-3
17. Stefani S, Goglio A. Methicillin-resistant *Staphylococcus aureus*: related infections and antibiotic resistance. *Int J Infect Dis*. 2010; 14(Suppl 4):S19–S22. Doi: 10.1016/j.ijid.2010.05.009
18. Jevons MP. Celbenin'-resistant *staphylococci*. *Br Med J*. 1961; 1:124-125 PubMed .
19. Mimica MJ, Mendes CMF. Diagnóstico laboratorial da resistência à oxacilina em *Staphylococcus aureus*. *J Bras Patol Med Lab*. 2007; 43:399-406.
20. Neu HC. The crisis in antibiotic resistance. Sci. 1992; 257:1064–1073.
21. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis*. 2001; 7:178 –182. DOI: 10.3201/eid0702.700178
22. Ricardo SB. Emergência de *S. aureus* meticilina-resistente (MRSA) na comunidade. *Prat Hosp*. 2004; 4(34):131 PubMed -4.
23. Moellering RC Jr,. Vancomycin: a 50-year reassessment. *Clin Infect Dis*. 2006; 42(suppl 1):S3-4. DOI:10.1086/491708
24. Lee DS, Kang MS, Hwang HJ, Eom SH, Yang JY, Lee MS, Lee WJ, Jeon YJ, Choi JS, Kim YM. Synergistic effect between dieckol from Ecklonia stolonifera and β-lactams against methicil¬lin-resistant *Staphylococcus aureus*. *Biotechnol Bioprocess Eng*. 2008; 13:758-764. DOI: 10.1007/s12257-008-0162-9
25. Barna JCJ, Williams DH. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. *Ann Rev Microbiol*. 1984; 38:339-357 PubMed .
26. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*. 1997; 40:135–136.
27. Poly MC, Grelaud C, Martin C, de Lumley L, Denis F. First clinical isolate of vancomycin-intermediate *Staphylococcus aureus* in a French hospital. *Lancet* 1998; 351:1212.
28. Howe RA, Bowker KE, Walsh TR, Feest TG, MacGowan AP. Vancomycin-resistant *Staphylococcus aureus*. *Lancet* 1998; 351:602.
29. Oliveira GA, Dell'Aquila AM, Masiero RL, Levy CE, Gomes MS, Cui L, et al. Isolation in Brazil of nosocomial *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Infect Control Hosp Epidemiol*. 2001; 22:443-8. DOI: 10.1086/501932
30. Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, Tenover FC, Zervos MJ, Band JD, White E, Jarvis WR. Emergence of vancomycin resistance in Staphylococcus aureus. Glycopeptide-Intermediate *Staphylococcus aureus* Working Group. *N Engl J Med*. 1999; 340:493–501. DOI: 10.1056/NEJM199902183400701
31. Sievert DM, Boulton ML, Stoltman G, Johnson D, Stobierski MG, Downes FP., et al. *Staphylococcus aureus* resistant to vancomycin, United States, 2002. MMWR 2002; 51(26):565 PubMed -7.
32. Bierbaum G, Fuchs K, Lenz W, Szekat C, Sahl HG. Presence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Germany. *Eur J Clin Microbiol Infect Dis*. 1999; 18:691–696.
33. Assadullah S, Kakru DK, Thoker MA, Bhat FA, Hussain N, Shah A. Emergence of low level vancomycin resistance in MRSA. *Indian J Med Microbiol*. 2003; 2:196-8.
34. Tiwari HK, Sen MR. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. *Infect Dis*. 2006; 6:156.
35. Denis O, Nonhoff C, Byl B, Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of vancomycin-intermediate *Staphylococcus aureus* in a Belgian hospital: microbiological and clinical features. *J Antimicrob Chemother*. 2002; 50:383–391.
36. Pierard D, Vandenbussche H, Verschraegen I, Lauwers S. Screening for *Staphylococcus aureus* with a reduced susceptibility to vancomycin in a Belgian hospital. *Pathologie Biologie*. 2004; 52:486-8.
37. Liu C, Chambers H. *Staphylococcus aureus* with heterogeneous resistance to Vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. *Antimicrob Agents Chemother*. 2003; 47(10):3040-3045.
38. Paul M, Kariv G, Goldberg E, Raskin M, Shaked H, Hazzan R, et al. Importance of appropriate empirical antibiotic therapy for methicillin-resistant *Staphylococcus aureus* bacteremia. *J Antimicrob Chemother*. 2010; 65:2658-2665.
39. Rehm S, Tice A. *Staphylococcus aureus*: Methicillin-Susceptible *S. aureus* to methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus*. *Clin Infect Dis*. 2010; 51(S2):176-182.
40. Khadri H, Alzohairy. Prevalence and antibiotic susceptibility pattern of methicillin-resistant and coagulase-negative *staphylococci* in a tertiary care hospital in In-

dia. *Int J Med Med Sci.* 2010; 2(4):116 PubMed -120.
41. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 12th ed. Mosby; 2007; p. 98-257.
42. Holt JG, Krieg NR, Sneath PHA, Williams ST. *Staphylococcus* spp. In: Bergey's manual of determinative bacteriology, 9th ed. Baltimore, MD: Williams & Wilkiins; 1994; p. 544-51.
43. Cheesbrough M. Medical Laboratory Manual for Tropical Countries, ELBS ed; Tropical health technology publications and Butterworth–Heinemann Ltd: Cambridge, UK, 2002; 2:2-392.
44. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2: Cambridge University press, Cambridge, 2009; pp. 62-69.
45. Bauer AW, Kirby WM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966; 45:493-496.
46. Clinical and Laboratory Standard Institute (CLSI). Performance standards for Anti-microbial susceptibility Testing Eighteenth informational supplement. M100-S18, 2008; 28(1):46-52.
47. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing.19th Informational Supplement. (M100-S19), Wayne, Pa, USA, 2009.
48. Clinical and Laboratory Standards Institute (CLSI). Performance standards for anti-microbial susceptibility testing, 17th informational supplement (M100-517). Wayne, Pa: Clinical and Laboratory Standards Institute, 2007.
49. Huber SK, Mohammad JM, Fridkinn SK, Gaynes RP, McGowan JE, Tenover FC. Glycopeptide intermediate Staphylococcus aureus: Evaluation of novel screening methods and results of a survey of selected US hospitals. *J Clin Microbiol.* 1999; 37:3590-3593.
50. [68] CLSI, 2011
51. Kluytmans J, Van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev.* 1997; 10(3):505–20.
52. Chambers HF. Community-associated MRSA—resistance and virulence converge. *New Engl J Med.* 2005; 352:1485– PubMed ;1487.
53. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *J Am Med Assoc.* 2007; 298:1763–1771. PubMed
54. Tallent SM, Bischoff T, Climo M, Ostrowsky B, Wenzel RP, Edmond MB. Vancomycin susceptibility of oxacillin resistant *Staphylococcus aureus* isolates causing nosocomial bloodstream infections. *J Clin Microbiol.* 2002; 40(6):2249 PubMed -2250.
55. Whitener CJ, Park SY, Browne FA, Parent LJ, Julian K, Bozdogan B, et al. Vancomycin-resistant *Staphylococcus aureus* in the absence of vancomycin exposure. *Clin Infect Dis.* 2004; 38(8):1049 PubMed -55.
56. Bataineh AB. Resistance of *Staphylococcus aureus* to Vancomycin in Zarqa, Jordan. *Pak J Med Sci.* 2006; 22:144-148.
57. Menezes GA, Harish BN, Sujatha S, Vinothini K, Parija SC. Emergence of vancomycin-intermediate *Staphylococcus* species in Southern India. *J Med Microbiol.* 2008; 57:911-2.
58. Chien JW, Kucia ML, Salata RA. Use of linezolid, an oxa¬zolidinone, in the treatment of multidrug-resistant Gram-positive bacterial infections. *Clin Infect Dis.* 2000; 30: 146-151 PubMed .
59. Flamm RK, Weaver MK, Thornsberry C. Factors associated with relative rates of antibiotic resistance in *Pseudomonas aeruginosa* isolates tested in clinical laboratories in the United States from 1999 to 2002. *Antimicrob Agents Chemother.* 2004; 7:2431–2436.
60. Saderi H, Owlia P, Shahrbanooie R. Vancomycin resistance among the clinical isolates of *Staphylococcus aureus. Arch Iran Med.* 2005; 8(2):100-03.
61. Mimejad R, Fallahi S, Kiani J, Jeddi F, Khoobdel M, Jonaidi N, Alaeddini F. Epidemiology assessment of bacterial agents in osteomyelitis and their antibiotic resistance pattern determination. *J Biol Sci.* 2008; 8:478–481.
62. Godebo G, Kibru G, Himanot T. Multidrug-resistant bacteria isolates in infected wounds at Jimma University Specialized Hospital. *Ethiopia Ann Clin Microbiol Antimicrob.* 2013; 12:17.
63. Perez LRR, Caierao J, Antunes ALS, de Azevedo PA. Use of the D test method to detect inducible clindamycin resistance in coagulase negative *Staphylococci. Braz J Infect Dis* 2007; 11(2):186-88.
64. Mathews AA, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistant *Staphylococcus aureus. Indian J Pathol Microbiol.* 2010; 53 (1):79-82.
65. De Lassence A, Hidri N, Timsit JF, Joly-Guillou ML, Thiery G, Boyer A, et al. Control and outcome of a large outbreak of colonization and infection with glycopep-

tide-intermediate *Staphylococcus aureus* in an intensive care unit. *Clin Infect Dis*. 2006; 42:170-8.
66. Palazzo ICV, Araujo M, Darini A. First Report of Vancomycin-Resistant *Staphylococci* Isolated from Healthy Carriers in Brazil. *J Clin Microbiol* 2005; 43:179-185 PubMed .
67. Rebiahi S, Abdelouahid D, Rahmoun M, Abdelali S, Azzaoui H. Emergence of vancomycin-resistant *Staphylococcus aureus* identified in the Tlemcen University Hospital (North-West Algeria). Médecine et maladies infectieuses 2011; 41:646–651.
68. Manfredi R. Update on the appropriate use of linezolid in clinical practice. *Ther Clin Risk Manag*. 2006; 2(4):455-464.
69. Cars O, Molstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet*. 2001; 357:1851– PubMed ;1853.
70. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005; 365:579– PubMed ;587.
71. Howard D, Rask K. The impact of resistance on antibiotic demand in patients with ear infections. In: Laxminarayan R, ed. Battling resistance to antibiotics and pesticides: an economic approach.Washington DC: RFF Press, 2002; 119–133 PubMed .
72. Yah, S.C., Enabulele, I.O., Eghafona, N.O. (2007). The screening of multi-drug resistance (MDR) susceptibilities of *Staphylococcus aureus* and *Staphylococcus epidermidis* to methicillin and vancomycin in teaching hospitals in Nigeria.
73. Hiramatsu K. Vancomycin-resistant *Staphylococcus aureus*: a newmodel of antibiotic resistance. *Lancet Infect Dis*. 2001; 1:147–155.
74. Gillespie S, Hawkey P. Principles and practice of clinical bacteriology, 2nd edition. John Wiley & Sons Ltd, England, 2005.
75. Cui L, Ma X, Sato K, et al. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. *J Clin Microbiol*. 2003; 41:5–14.
76. Dhand A, Bayer AS, Pogliano J, Yang SJ, Bolaris M, Nizet V, Wang G, Sakoulas G. Use of *antistaphylococcal* beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: Role of enhanced daptomycin binding. *Clin Infect Dis*. 2011; 53(2):158–163.
77. Ilang DC, Nworie OE, Ekuma UO, Egbule UCC. Detection of Vancomycin Resistant *Staphylococcus aureus* from Clinical Specimens in Federal Teaching Hospital Abakaliki I (FETHA I). *Int J Open Sci Res* (IJOSR). 2013; 1(5):66-74.
78. Chakraborty SP, Mahapatra S, Bal M, Roy S. Isolation and identification of vancomycin resistant *Staphylococcus aureus* from post operative pus sample. *Al Ameen J Med Sci*. 2011; 4(2):152 PubMed -168.
79. Salah UK, Mahmood AC, Abdul H. Antibiotic resistance pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical specimens. *J Pharm Biol Sci*. 2013; 4(6):37-40.
80. Chandrashekhar G, Unakal B, Kaliwal B. Phenotypic Characterization and Risk Factors of Nosocomial *Staphylococcus aureus* from Health Care Centers. *Advances in Microbiol*. 2012; 2:122-128