Vancomycin intermediate resistant *Staphylococcus aureus* in the nasal cavity of asymptomatic individuals: a potential public health challenge

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Abstract

Background: The potential of transmitting multidrug resistant *Staphylococcus aureus* from asymptomatic individuals to healthy individuals could constitute a great challenge to antimicrobial therapy.

Methods: The antibiograms of the *S. aureus* from asymptomatic individuals were determined by disk diffusion and agar dilution assay techniques with different antibiotics and vancomycin.

Results: Of the 152 *S. aureus* isolated, (59)38.8% isolates were multi-drug resistant strains. Streptomycin was the most effective and inhibited (135)88.82% of the isolates while ceftazidime inhibited (24)15.8% of the isolates. While (82)54.0% of the isolates inhibited by cefuroxime had resistant colonies within their inhibition zones (Rc) and ofloxacin inhibited (100)65.8% of the isolates without having resistant colonies within the inhibition zones, ceftazidime inhibited (7)4.6% of the isolates with resistant colonies within the inhibition zones. Subjecting the isolates to vancomycin showed that (27)17.8% were resistant to 2 µg/ml, (43)28.3% were resistant to 4 µg/ml and (27)17.8% of the isolates were simultaneously resistant to both concentrations of vancomycin. Although (100)65.8% of the isolates had MAR_{index} ≥0.2, (52)34.2% of the isolates had MAR_{index} ≤ 0.2 and (65)428% of the isolates were considered multidrug resistant strains.

Conclusion: The isolation of multi-drug and vancomycin intermediate resistant strains of *S. aureus* in high percentage, in this study, presents a great threat to clinicians and general populace. The vancomycin intermediate resistant *S. aureus* (VISA) in asymptomatic individuals could be a critical concern to the therapeutic dilemma to be added to the presence of multi-drug resistance. A more sustainable therapy must be in place to prevent its dissemination or the outbreak of its infection.

Keywords: Antibacterial activity, multidrug resistance, VRSA, VISA, vancomycin

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Introduction

Staphylococcus aureus, being the most important *Staphylococcaceae* species, can be found in both healthy and immunocompromised individuals.[1] Its ecological niches are the anterior nares and most of invasive *S. aureus* infections are assumed to arise from nasal carriages.[2] Both methicillin-resistant and methicillin-sensitive strains can be found as normal commensals on the skin, the nasopharynx and anterior nares of many asymptomatic individuals who can, possibly, transmit methcillin-resistant *S. aureus* (MRSA) to healthy and immunocompromised persons in hospitals[1,3] by direct contact.

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The emergence of high levels of penicillin resistance has made the therapy of staphylococcal disease a global challenge. Methicillin-resistant *Staphylococcus aureus* (MRSA), so far restricted to hospitals,[4] was first reported in 1961 soon after methicillin was introduced in 1951 to treat penicillin - resistant *staphylococci*.[5] In the 1980s, due to widespread methicillin use, MRSA became a major problem globally[6] and glycopeptides became antibiotics of choice for the empiric treatment of infections caused by MRSA in many health-care institutions.

Despite the fact that vancomycin resistant strains of coagulase–negative *staphylococci* have been a cause of concern,[7] there was a temporary relief to the medical community when vancomycin, a glycoprotein able to inhibit the growth of all strains of MRSA, was discovered and used in many countries. However, the first strain of *S. aureus* with reduced susceptibility to vancomycin and teicoplanin was soon reported in Japan[8] while vancomycin-resistant *S. aureus* (VRSA) was later reported in Belgium, Germany, United States, Brazil and Jordan. [9-12] The early 1990s saw a discernible increase in vancomycin use, and established selective pressure that resulted in the emergence of resistant strains of *S. aureus* and other species of *staphylococci* with decreased susceptibility to vancomycin. Resistance of VRSA to many antimicrobial agents also posed a great danger to the patients because of the virulence of the organism.[13]

Vancomycin, a tricyclic glycopeptide antibiotic, has been the cornerstone for treating MRSA infections.[6] Over recent years, however, there has been a gradual decrease in the susceptibility of MRSA for a number of reasons. These include relatively poor tissue penetration, slow bacterial killing and the potential for its toxicity all of which have limited the use of vancomycin in the management of MRSA infections.[14] Its efficacy against MRSA was reported to be inferior to that of beta-lactams used against methicillin susceptible *S. aureus* (MSSA) due to its slower in vitro bactericidal activity with a lower clinical response.[15] Thus, vancomycin treatment failures have been reported in patients infected with susceptible isolates with MIC values of 1.5 to 2.0 μg/ml[16,17] in addition to the presence of vancomycin-intermediate *S. aureus* (VISA) and heteroresistance VISA being associated with horrific clinical outcomes.[18,19] Considering the clinical relevance of multi-drug resistance amongst bacteria and the possible complications that may be caused by the development of VISA and VRSA, there is a need to determine the presence or absence of multi-drug, VISA and VRSA in these individuals that might constitute a reservoir for the dissemination of this pathogen. This is to forestall their treatment failures and transmission from asymptomatic to symptomatic individuals. This study, therefore, investigated multi-drug resistance pattern of *S. aureus* from the nares of asymptomatic individuals and determined their degree of susceptibility to vancomycin.

Materials and methods

Sample collection

After signing the informed consent previously approved by the Babcock University Research and Ethics Committee (BUREC), samples were collected from 250 asymptomatic individuals in Babcock University, Ogun State, Nigeria between September 2016 and May 2017. Individuals who have been treated with any antibiotic in the last 4 weeks were excluded. The samples were carefully collected by rolling swab saturated with sterile peptone water in the nostrils of 250 undergraduate healthy individuals after seeking and obtaining their individual verbal and signed written consent. The swabs were tightly sealed and immediately transported to the laboratory. The collected nasal swab sticks were streaked on mannitol salt agar (OXOID CM0085 – Oxoid Ltd. Wade Road, Basingstoke, Hants, RG24 8PW, UK (MSA)) and nutrient agar (LAB M -620 Lesher Place, Lansing, MI 48912 USA) which were incubated overnight at 37°C for 24 – 48 h.[20] The bacterial colonies were subjected to Gram staining, microscopic appearance, colony morphology and biochemical tests such as tube coagulase test, catalase test and DNase test according to standard protocols.[21-23]

Antibiogram study of the isolates from the nasal swabs using multi-disc antibiotics

The standard disc diffusion test was performed according to the recommendations of the Clinical Laboratory Standards Institute.[24] Four separated colonies of each of the isolates from nutrient agar were homogenized with inoculating loop in 2 mL sterile normal saline and vortexed to obtain uniform bacterial suspensions. Each strain's suspension was adjusted to 0.5 McFarland Standards by adding more organisms if the suspensions were too light or by diluting further with sterile saline if the suspensions were too heavy to give a resultant concentration of 1.5×10^6 cfu/ml. The antibacterial activity was determined according to the modified Kirby–Bauer disk diffusion technique.[25] The Mueller-Hinton agar (MHA) (OXOID CM0085 – Oxoid Ltd. Wade Road, Basingstoke, Hants, RG24 8PW, UK) plates were swabbed with the resultant adjusted culture of each of the test isolates. With sterile forceps, commercial antibiotics (Abtek) containing different antibiotics including ceftazidime (Cef) (30 μg), cefuroxime (Cfx) (30 μg), gentamycin (Gen) (10 μg), ceftriaxone (Cft) (30 μg), erythromycin (Ery) (5 μg), cloxacillin (Clx) (5 μg), ofloxacin (Ofl) (5 μg), augmentin (Aug) (30 μg), cotrimoxazole (Cot) (25 μg), streptomycin (Str) (10 μg), tetracycline (Tet) (10 μg) and chloramphenicol (Chl) (10 μg) were aseptically placed on the inoculated agar and incubated at 37°C for 24 h. After 24 h of incubation, the plates were examined for inhibition zones.[37] The diameter of

the inhibition zones produced by each antibiotic disk were measured to the nearest millimeter, recorded and interpreted using the Clinical and Laboratory Standard Institute Zone Diameter Interpretative Standards[24] Resistant colonies (Rc) isolated from within inhibition zones were further identified to be coagulase positive *S. aureus*. 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[24]

Multiple antibiotic resistance index (MAR_{index}) of *S. aureus* from nasal cavity

The Multiple Antibiotic Resistance (MARindex) of each isolate was calculated as the number of antibiotics to which the isolate is resistant divided by the total number of antibiotics to which it is exposed as indicated by Krumperman.[26] According to Adeleke and Omafuvbe,[27] isolates with MAR_{index} ≤0.2 might have come from individuals using antibiotics infrequently while one with MAR_{index} >0.2 may have come from individuals using antibiotics more frequently.

Susceptibility test of samples to vancomycin

The susceptibility of coagulase positive isolates was further determined by agar dilution method according to the guidelines of CLSI.24 The isolates were subjected to susceptibility testing with 2 μg/ml and 4 μg/ml concentrations of vancomycin prepared by dissolving 0.4 mg of vancomycin into 200 ml of sterilized Mueller Hinton agar maintained at a temperature of 50°C. The overnight broth culture of each isolate was then adjusted to match up with 0.5 McFarland standards to give a resultant concentration of 1.5×10^6 cfu/ml before being inoculated and incubated at 37°C for 24 h by streaking the vancomycin-containing agar surfaces.

Results

In this study, 152 coagulase positive isolates were identified as *Staphylococcus aureus*. Being resistant to three classes of antibiotics (59)38.8% of these isolates were multi-drug resistant strains. The susceptibility of the isolates to the different antibiotics showed that streptomycin was the most effective. It inhibited (135)88.8% of the isolates. This was followed in a descending order by chloramphenicol (133)87.5% > ofloxacin (124)81.6% > erythromycin (112)73.7% > gentamicin (108)71.1% > cotrimoxazole (81)53.3% > cefuroxime (78)51.3% > tetracycline (76)50% > ceftriaxone (75)49.3% > augmentin (52)34.2% > cloxacillin (51)33.6% > ceftazidime (24)15.8%. Although these antibiotics inhibited the isolates with some of the isolates having resistant colonies (Rc) within their inhibition zones, ofloxacin inhibited the highest percentage (100)65.8% of the isolates without having resistant colonies within the inhibition zones. The ofloxacin was followed in a descending order by streptomycin (98)64.5% > chloramphenicol (93)61.2% > gentamicin (76)50% > erythromycin (59)38.8% > ceftriaxone (38)25.0% > cotrimoxazole (28)18.4% > cefuroxime (22)14.5% > ceftazidime (7)4.4%) which inhibited the least number of isolates without resistant colonies within the inhibition zones. Comparatively, highest number (109)71.7% of strains were resistant to ceftazidime. This is followed by (92)60.5% for cloxacillin > (74)48.7% for augumentin > (66)43.4% for tetracycline > (63)41.5% for cefuroxime > (60)39.5% for cotrimoxazole > (36)23.7% for ceftriaxone > (19)12.5%) for gentamicin and ofloxacin > (11)7.3% for chloramphenicol. Considering the susceptble isolates with resistant colonies (Rc) within their inhibition zones, (82)54.0% of the isolates inhibited by cefuroxime had resistant colonies within their inhibition zones. This was followed in a descending order by ceftriaxone (63)41.5% of the isolates with resistant colonies within their inhibition zones when inhibited by ceftriaxone > gentamicin (61)40.1% > cotrimoxazole (59)38.8% > erythromycin (58)38.2% > tetracycline (56)36.8% > augumentin (45)29.6% > chloramphenicol (43)28.3% > cloxacillin (38)25% > ofloxacin (37)24.3% > ceftazidime (33)21.7% > streptomycin (22)14.5% as shown in Figure 1.

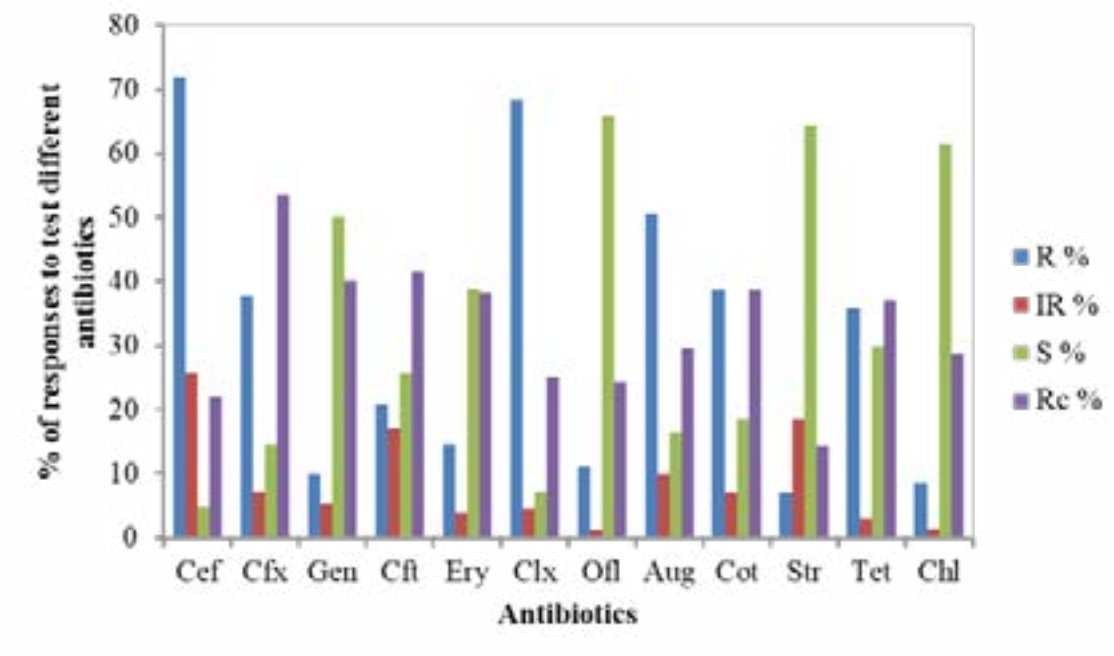

Figure 1: Susceptibility profile of *Staphylococcus aureus* isolated from nasal cavity of asymptomatic individuals

Key: Cef =Ceftazidime, Cfx =Cefuroxime,Gen= Gentamicin, Cft = Ceftriaxone, Ery = Erythromycin, Clx = Cloxacillin, Ofl = Ofloxacin, Aug = Augmentin, Cot = Cotrimoxazole, Str = Streptomycin, Tet = Tetracycline, Chl = Chloramphenicol, R = Resistance, I = Intermediate Resistance, S = Sensitive, Rc = Susceptible isolates with resistant colonies within inhibition zones

The antibiotics, number of classes of antibiotics and multidrug resistance index (MAR_{index}) of the *S. aureus* from asymptomatic nasal cavity are presented in Table 1. In this table, (54)35.5% of the isolates had $MAR_{index} \leq 0.2$ while (98)64.5% of the isolates had MAR $\geq$0.2. Of the (54)35.5% with MAR $\leq$0.2, (28)53.9% had their MAR_{index} equal to 0 implying that these isolates were not resistant to any of the antibiotics. While the MAR_{index} of 0.125 showed that (26)17.1% of the isolates was resistant to at least two antibiotics and MAR_{index} of 0.25 showed that (28)18.4% of the isolates was resistant to three antibiotics, MAR_{index} of 0.375, 0.5, 0.625, 0.75 and 1.0 showed that (27)17.7%, (11)7.2%, (13)8.6%, (9)5.9% and (12)7.9% of the isolates were resistant to at least three to six antibiotics, respectively. However, being resistant to three antibiotics, (65)42.8% of the isolates were considered multidrug resistant strains.

Table 1: Susceptibility profile and multidrug antibiotic resistance index of *Staphylococcus aureus* isolated from asymptomatic nasal cavity

Number of Isolates	MAR_{index}	Antibiotics	No. of classes of antibiotics	Number of Isolate(s)	MAR_{index}	Antibiotics	No. of classes of antibiotics
28	0	None	None	1	0.375	Cfx-Ery-Clx	3
4	0.125	Cef	1	1	0.375	Cot-Tet-Chl	3
1	0.125	Cfx	1	3	0.375	Cef-Cfx-Clx	2
2	0.125	Gen	1	7	0.375	Clx-Aug-Cot	3
1	0.125	Ery	1	8	0.375	Cef-Clx-Aug	3
8	0.125	Clx	1	1	0.5	Ery-Clx-Aug-Chl	4
2	0.125	Ery	1	1	0.5	Cef-Cft-Clx-Aug	3
2	0.125	Cot	1	1	0.5	Cef-Cfx-Ery-Clx	3
2	0.125	Tet	1	1	0.5	Cef-Cfx-Cft-Clx	2
4	0.125	Chl	1	1	0.5	Cef-Gen-Clx-Ofl	4
1	0.25	Cef-Clx	1	1	0.5	Cfx-Ery-Clx-Aug	4
2	0.25	Cef-Cfx	1	1	0.5	Ery-Clx-Str-Tet	4
1	0.25	Gen-Ofl	2	2	0.5	Cef-Cfx-Clx-Aug	3
1	0.25	Clx-Chl	2	2	0.5	Clx-Aug-Cot-Tet	4
4	0.25	Str-Chl	2	1	0.625	Ery-Clx-Cot-Tet-Chl	5
2	0.25	Cef-Ery	2	1	0.625	Gen-Clx-Aug-Cot-Tet	5
2	0.25	Ery-Clx	2	1	0.625	Clx-Cot-Str-Tet-Chl	5
2	0.25	Ery-Cot	2	1	0.625	Cef-Cfx-Clx-Ofl-Cot	4
3	0.25	Clx-Aug	2	2	0.625	Cef-Cfx-Clx-Ofl-Aug	4
4	0.25	Clx-Tet	2	2	0.625	Ery-Clx-Aug-Cot-Chl	5
6	0.25	Clx-Cot	2	2	0.625	Cef-Cfx-Ery-Clx-Aug	4
1	0.375	Ery-Clx-Tet	3	3	0.625	Cef-Cfx-Cft-Clx-Aug	3
1	0.375	Cef-Cfx-Cft	1	1	0.75	Cef-Cfx-Cft-Clx-Ofl-Aug	4
1	0.375	Clx-Cot-Chl	3	1	0.75	Cef-Cfx-Gen-Cft-Clx-Aug	4
1	0.375	Aug-Cot -Tet	3	2	0.75	Ery-Clx-Aug-Cot-Str-Tet	6
1	0.375	Cef-Cft-Ery	2	2	0.75	Gen-Ery-Clx-Aug-Cot-Chl	6
1	0.375	Ery-Clx-Aug	3	3	0.75	Cef-Cfx-Cft-Ery-Clx-Aug	4
1	0.375	Gen-Clx-Cot	3	1	1	Cef-Cfx-Gen-Cft-Ery-Clx-Ofl-Aug	6

Key: Cef = Ceftazidime, Cfx = Cefuroxime, Gen = Gentamicin, Cft = Ceftriaxone, Ery = Erythromycin, Clx = Cloxacillin, Ofl = Ofloxacin, Aug = Augmentin, Cot = Cotrimoxazole, Str = Streptomycin, Tet = Tetracycline, Chl = Chloramphenicol

On subjecting the coagulase positive *S. aureus* isolates to 2 μg/ml and 4 μg/ml vancomycin, (27)17.9% of the isolates were resistant to vancomycin at 2 μg/ml concentration while (43)28.5% of the isolates were resistant to this antibiotic at 4 μg/ml. Although (5)3.0% of the isolates were resistant at 2 μg/ml and sensitive at 4 μg/ml, (27)17.8% were resistant at 2 μg/ml, (43)28.3% were resistant at 4 μg/ml and (27)17.9% of the isolates were simultaneously resistant to both concentrations as shown in Figure 2. From case definition of CLSI [24] indicating vancomycin MIC of ≤ 2 μg/ml as vancomycin-susceptible *S. aureus* (VSSA), vancomycin MIC = 4-8 μg/ml as vancomycin-intermediate *S. aureus* (VISA) and vancomycin MIC ≥ 16 μg/ml as vancomycin-resistant S. aureus (VRSA), the isolates, in this study, may be grouped as being vancomycin sensitive *S. aureus* (VRSA) and vancomycin intermediate *S. aureus* (VISA).

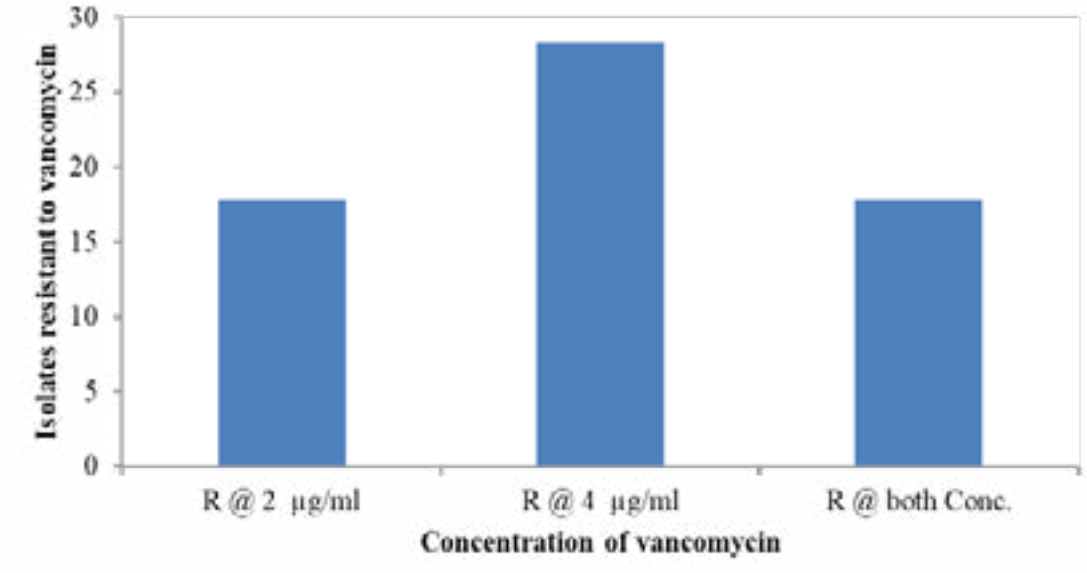

Figure 2: Percentage of *Staphylococcus aureus* resistant to different concentrations of vancomycin

Discussion

Epidemiologically, the increase in the drug-resistant virulent bacterial strains has become a serious problem in the treatment and control of staphylococcal infections. Vancomycin-resistant *Staphylococcus aureus* (VRSA), gradually becoming important pathogens and endemic[28,29] are on the increase worldwide.[30] Although there have been increase in the levels of resistance among bacteria isolated from patients with nosocomial infections,[31] with methicillin-resistant *S. aureus* increasingly becoming nosocomial pathogens, vancomycin treatment failure or a worse clinical outcome and increasing vancomycin minimum inhibitory concentrations (MICs) have been reported.[32] These have resulted in prolonged hospital stay, continued antibiotic therapy and rising health care expenses, morbidity and mortality.[33]

In this study, the isolates were more resistant to the β-lactam antibiotics having a broad spectrum of activity against Gram-positive and Gram-negative bacteria[34] but showed varied degree of resistance to the other classes of antibiotics. The resistance may be due to their exposure to cephalosporins,[35] vancomycin and many commonly used antimicrobial agents including semisynthetic penicillins, macrolides, tetracyclines and aminoglycosides,[36] production of low-affinity penicillin-binding proteins,[37] expression of drug-destroying enzymes such as β-lactamases,[38] altered drug targets as well as decreased bacterial permeability and increased drug efflux[39]. The levels of multi-drug resistance of these isolates from asymptomatic individuals are alarming and confirm the assertion that healthy members of the community represent the largest reservoir of bacteria resistant to antimicrobial agents. The resistant colonies recorded within inhibition zones suggested heterogeneous resistant nature of some of the isolates. Contrary to this study, Close et al.[40] indicated that the prevalence of the heterogeneously resistant strains may be up to 22% in some clinical isolates.

Although *S. aureus* colonize the skin and anterior nares, such colonization which can easily be transmitted by formites or direct contact with other healthy individuals[41] may be transient or persistent or spread faster during upper respiratory tract viral infections. Therefore, to prevent the development of untreatable staphylococcal infections due to spread of vancomycin resistance, it becomes inevitable to determine their susceptibility to glycopeptides after the resistance of *enterococci*[42] and *staphylococci* to such antimicrobials have been previ-

ously described.[43] Hence, while the vancomycin minimum inhibiory concentration required to inhibit most strains of *S. aureus* is typically between 0.5 and 2 μg/ml,[44], (27)17.86% were resistant at 2 μg/ml, (43)28.29% were resistant at 4 μg/ml and (27)17.86% of the isolates were resistant to both concentrations. Contrary to expectation, more strains were susceptible at lower concentrations of 2 μg/ml than was obtained at 4 μg/ml μg/ml. While Arthur et al.[45] and Reddy et al.[46] indicated that vancomycin resistance is mediated by vanA and vanB gene clusters altering the target for vancomycin from D-alanine-D-alanine to D-alanine-D-lactate which eventually results in blocking the release of terminal D-alanine and interchain bond formation, the mechanism of resistance in *S. aureus* has been linked to cell wall thickening limiting the access of vancomycin to the cytoplasmic membrane where the functional targets of vancomycin are located.[47,48]

Conclusion

The emergence and spread of multi-drug and vancomycin resistance is a threat to the already challenged therapy of staphylococcal infections. Its transmission to both immunocompetent and immunocompromised individuals could constitute a significant public health challenge. In this study, the presence of VISA in asymptomatic individuals is considered a critical concern to be added to the presence of multi-drug resistance hereby reported. Therefore, while minimizing antibiotic pressure and use to control the emergence of resistant strains in the hospital and in the community, clinicians and community must not only nurture culture resulting in infection prevention but also abide by practices that prevent transmission of potential pathogenic organisms. The study, therefore, indicates that asymptomatic individuals are carrying multi-drug and vancomycin intermediate resistant *S. aureus* to a great extent and a more sustainable therapy must be in place to prevent its dissemination or the outbreak of its infection to create significant public health challenges.

Conflicts of interest

Authors hereby declare that they have no conflict of interest

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