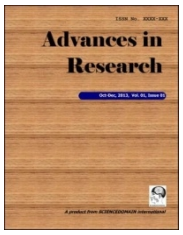

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Prevalence and Conjugal Transfer of Vancomycin Resistance among Clinical Isolates of *Staphylococcus aureus*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GFMG and RMAE designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author RMAE and HRA managed the literature searches, collection of samples, identification of VRSA, Antimicrobial susceptibility testing and the molecular studies. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) has been of great concern in clinical settings. Our study aimed to detect vancomycin resistance and the possibility of its conjugal transfer among clinical isolates of *S. aureus*.
Study Design: Medical microbiology.
Place and Duration of Study: This study was carried out in the department of microbiology, Faculty of pharmacy, between October 2011 and January 2013.
Methodology: Two hundreds and seven samples were collected from different types of infections and examined for *Staphylococcus aureus* using standard bacteriological procedures, Antimicrobial susceptibility testing against vancomycin and some antimicrobials was done by agar dilution method, resistance to methicillin was determined by disc diffusion test using cefoxitin (30µg), Vancomycin resistance gene transfer was tested by broth matting procedure and confirmed by plasmid and DNA product analysis.
Results: Sixty-three samples were positive for *Staphylococcus aureus*. All isolates were resistant to penicillin while the lowest resistance was to amikacin and vancomycin. One isolate was VRSA (MIC ≥ 16 µg/ml and positive for *vanA gene* 1032bp) while 5 isolates

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were VISA. All VRSA and VISA isolates were MRSA and showed a high resistance to other examined antibiotics. Vancomycin resistance was plasmid mediated and successfully transferred from VRSA donor to a vancomycin sensitive recipient isolate (MIC 2 µg/ml). The MIC of vancomycin for the transconjugant strain was 32 µg/ml. PCR product analysis for the transconjugate was positive for *vanA* gene.

Conclusion: Low incidence of vancomycin resistance, All VRSA and VISA were methicillin resistant, and mostly isolated from skin infections. Also, vancomycin resistance was plasmid mediated and successfully transferred to susceptible strains which is alarming. Skin colonization by VRSA strains harboring plasmid carrying resistance genes may result in widespread of these strains that could lead to outbreaks of staphylococcal diseases in both hospital and community.

Keywords: Vancomycin; resistance; Staphylococcus aureus; vanA gene; plasmid.

1. INTRODUCTION

Clinicians are continually being challenged by infections caused by *Staphylococcus aureus.* Not only is *S. aureus* a major cause of both community-acquired and health care–associated infections, but the treatment of suspected *S. aureus* infections is becoming increasingly more complicated [1].

It is reported that the incidence of *S. aureus* nosocomial infections associated with oxacillin and methicillin resistance surpassed 50% in 1999 among patients in the intensive care unit according to the Centers for Disease Control and Prevention's (CDC) and National Nosocomial Infections Surveillance (NNIS) system. Of great concern are reports of community-acquired infections associated with MRSA, such as those that contributed to the deaths of 4 children in 1999 [2]. Also, the emergence of high levels of resistance to methicillin, oxacillin, nafcillin, macrolides, tetracyclines, and aminoglycosides has made the therapy of staphylococcal disease a global challenge [1,3].

In 1980s, due to the widespread occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA), empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions. Vancomycin use in many countries also increased during this period because of the growing numbers of infections with *Clostridium difficile* and coagulase-negative staphylococci in health-care facilities [4]. Thus, the early 1990s saw a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually led to the emergence of strains of *S. aureus* and other species of staphylococci with decreased susceptibility to vancomycin.

As a result of antibiotic resistance, vancomycin has been the drug of last resort. Vancomycin, a glycopeptide antibiotic, acts against Gram-positive bacteria only, by inhibiting the incorporation of NAM-NAG-polypeptide into the growing peptidoglycan (PG) chain. It inhibits this process by reacting with D-Ala-D-Ala, which consequently blocks the release of terminal D-Ala and intrachain bond formation [5]. Vancomycin-resistant *Enterococcus faecium* harbors the *vanA* operon, which contains five genes, van S, -R, -H, -A and -X [6]. *vanS* and *vanR* are the regulator genes [7]. VanH is a D-hydroxy acid dehydrogenase that reduces pyruvate to D-lactate [8], which could be used by *VanA* ligase in conjunction with ATP and D-Ala to make a D-Ala-D-lactate depsipeptide, which is incorporated into the peptidoglycan layer. Vancomycin binds to N-acyl- D-Ala-D-lactate with an affinity 1000-fold

lower than that of N-acyl-D-Ala-D-Ala [9]. VanX is a dipeptidase required for the hydrolysis of D-Ala-D-Ala [10].

Conjugative transfer of high-level vancomycin resistance from *Enterococcus faecalis* to *Staphylococcus aureus* [11], and transfer of glycopeptide- and macrolide-resistance genes by transconjugation among enterococci and from *E. faecalis* to *S. aureus* [12] have been reported. Vancomycin-resistance gene (*vanA* gene) acquisition by *S. aureus* from *E. faecium* in the clinical environment has also been reported by Weigel et al. [13].

In 1997, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan [14]. The vancomycin minimum inhibitory concentration (MIC) results reported for this isolate was in the intermediate range (8 µg/mL) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards (NCCLS) [15]. This report was quickly followed by similar ones from other countries, including the United States [16], Belgium [17], Germany [18]. These strains were called vancomycin-intermediate *S. aureus* (VISA). The first clinical infection with vancomycin-resistant *S. aureus* (VRSA) (MIC ≥ 32µg/mL) was reported in July 2002 from Michigan [19] with a second case in Pennsylvania reported shortly thereafter [20]. Though, there have been only a few reports of VRSA, the high prevalence of MRSA and vancomycin use, both thought to be risk factors for VRSA, make the widespread dissemination of these organisms an alarmingly realistic possibility [21]. Such resistance could result in serious clinical and public health consequences because, currently, few licensed alternatives to vancomycin are available to treat serious resistant *S. aureus* infections [22]. Furthermore, there is an equally alarming threat of the risk of transmission of these organisms between patients [23].

The emergence of VRSA underscores the need for programs to prevent the spread of antimicrobial resistant microorganisms and to control the use of antimicrobial drugs in health-care settings.

In this study, we have shown the emergence of vancomycin resistance in our country, Egypt and its ability to undergo a conjugative transfer from one clinical strain to another.

2. MATERIALS AND METHOD

2.1 Isolation and Identification

The study included 63 strains of *S. aureus* isolated from clinical specimens obtained from 207 patients with wound infections, respiratory tract infection, urinary tract infections and eye infections. Identification of *S. aureus* was based upon colony morphology, positive Gram stain, DNase, catalase and coagulase tests, and fermentation of Mannitol [24,25].

2.2 Antibiotic Susceptibility Testing and MIC Determination

Antimicrobial susceptibility for methicillin was determined by the disk agar diffusion (DAD) technique using cefoxitin (30 µg) discs according to the guidelines recommended by CLSI [26].

MIC determination and antimicrobial susceptibility testing for the following antibiotics: Penicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, cefazolin, cefuroxime, gentamicin,

amikacin, ciprofloxacin, levofloxacin, vancomycin were performed using agar dilution method as recommended by CLSI [26].

2.3 Isolation of Plasmid DNAs

Plasmids were isolated by alkaline lysis techniques, separated by electrophoresis on 0.8% agarose (Sigma), and visualized with UV light after treatment with ethidium bromide [27].

2.4 Transfer of Vancomycin Resistance by Mating Procedure

Broth mating was performed as follows. Single colonies of donor and recipient cells were inoculated separately in Luria–Bertani (LB) broth and grown overnight at 37°C with shaking. These overnight cultures were diluted 1:100 in fresh medium, and each was grown to early exponential phase (OD_{600}~0.6). Mating mixture was prepared by adding 0.1ml of donor cells to 0.9ml of recipient cells, and was swirled gently for a few minutes and then incubated at 37°C for 6h (without shaking), followed by plating (0.2ml per plate) on Luria Bertani agar (LB) medium containing appropriate selective antibiotics (16µg/ml vancomycin and 2.5µg/ml ciprofloxacin). Colonies were picked up after 48-72h of incubation. Donor and recipient cells were also plated separately to check their disability to grow on the vancomycin plus ciprofloxacin plate, because the donor was ciprofloxacin-sensitive and the recipient was susceptible to vancomycin and ciprofloxacin resistant [28]. Then, MIC determination for vancomycin and plasmid profile was done for the transconjugate.

2.5 PCR- based Detection of Vancomycin Resistance Genes (vanA) in VRSA Strain and the Transconjugate Strain

Bacterial cultures on selective media plates were collected and suspended in sterile, deionized distilled water and heated in a boiling water bath for 10 min. The samples were cooled immediately on ice for 5-10 min and centrifuged at 13000 × g for 5 min. The supernatants were used as DNA templates for PCR [5]. Oligonucleotide primers for *vanA* (*vanA* F 5'CATGAATAGAATAAAAGTTGCAATA3') and (*vanA* R 5'CCCCTTTAACGCTAATACGACGATCAA3') gene.

2.5.1 PCR amplification of vanA

The PCR amplification mixture contained the following components: Phusion GC buffer containing 1.5 mM MgCl2, 200 mm each dNTP, 2 mm each primer, 0.1 mg template DNA, 3% (v/v) DMSO and 1 U Phusion DNA polymerase (Finnzymes). The amplification conditions were initial denaturation at 98°C for 2 min, followed by 35 cycles of denaturation at 98°C for 10 s; annealing at 50°C for 1 min; polymerization at 72 °C for 60 s for vanA, and final extension at 72°C for 5 min for all. The PCR product (5 µl aliquot) was separated by electrophoresis in 1 % agarose gel at 100V for 40 minutes in Tris-acetate buffer visualized by ethidium bromide staining illuminated by UV transilluminator [29,30].

3. RESULTS

A total of 63 *S. aureus* isolates were obtained from 207 samples collected from different infections (Table 1). Most of the isolated *S. aureus* isolates were isolated from skin infections followed by respiratory tract infections. Antibiotic resistance pattern revealed that all *S. aureus* isolates were penicillin resistant. Isolates showed high resistance to cefuroxime

(61.9%) and amoxicillin/clavulenic acid (50.8%) but showed low resistance to amikacin and vancomycin (1.6% each) (Fig. 1). It was found that one isolate was resistant to vancomycin (MIC≥ 16µg/ml) and 5 isolates were vancomycin intermediate (VISA) (2 isolates, MIC 4 µg/ml and 3 isolates, MIC 8 µg/ml) (Table 2, Fig. 1).

Table 1. Incidence of *Staphylococcus aureus* isolated from patients suffering from different types of infections

Infection type	No. of samples N= 207	No. of *S. aureus* N= 63	%*
Skin infection	65	30	46.2
Respiratory tract infections	85	25	29.4
Urinary tract infection	45	6	13.3
Eye infections	12	2	16.7

** Percentage was correlated to the number of samples collected from each type of infection.*

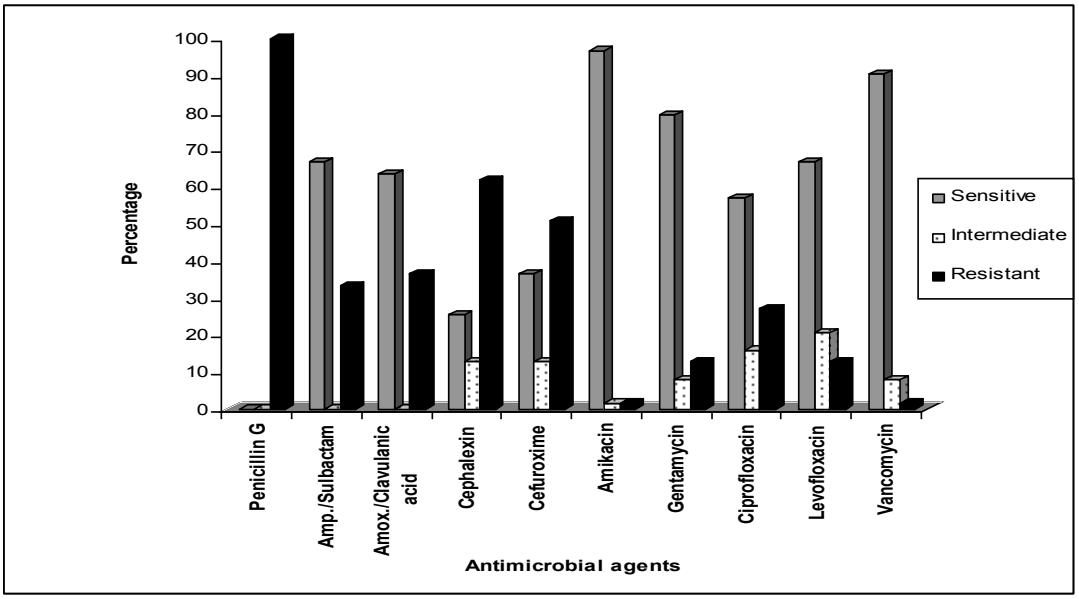

Fig. 1. Resistance pattern of the isolated *S. aureus* to different antimicrobial agents

Table 2. MICs of vancomycin for 63 isolates of *Staphylococcus aureus*.

MIC(µg/ml)	No. of strains	%*
0.25	14	22.2
0.5	17	27
1	16	25.4
2	10	15.9
4	2	3.2
8	3	4.7
16	1	1.6
Total	63	100

** Percentage was correlated to the total number of isolates.*

Plasmid analysis for VRSA revealed the presence of one plasmid (Fig. 2) which is confirmed by the presence of PCR product band positive for *vanA* gene at 1032bp (Fig. 3).

Disc agar diffusion test using Cefoxitin (30µg) discs revealed that out of 63 *S. aureus* isolates, 16 (25.4%) were methicillin resistant (≤ 21mm). Table 3 showed the resistance pattern of VRSA and VISA to other antimicrobials. As it revealed that VRSA isolate (Skin swab) and VISA isolates were all methicillin resistant. It also showed that these strains were all resistant to all tested β-lactams antibiotics. VRSA was found to be susceptible to ciprofloxacin and amikacin. The table showed also that VRSA and VISA were mostly isolated from skin.

Table 3. Resistance pattern of VISA and VRSA isolates to the tested antimicrobial agent

Strains No.	MIC Vancomycin (µg/ml)	Specimens	Resistant	Susceptible
VISA1	4	Throat swab	Pen., Amox.\calv., Amp.\sulb., Gen., Cip., Cefa., Cefu., Meth.	Amik., Levo.
VISA2	4	Urine	Pen., Amox.\calv., Amp.\sulb., Cefa., Cefu., Meth.,Amik., Levo.	Gen., Cipro.
VISA3	8	Skin swab	Pen., Amox.\calv., Amp.\sulb., Gen., Cip., Cefa., Cefu., Meth., Levo.	Amik.
VISA4	4	Throat swab	Pen., Amox.\calv., Amp.\sulb., Gen., Cip., Cefa., Cefu., Meth., Amik.	Levo.
VISA5	8	Skin swab	Pen., Amox.\calv., Amp.\sulb., Gen., Cefa., Cefu., Meth., Livo.	Amik., Cipro.
VRSA1	16	Skin swab	Pen., Amox.\calv., Amp.\sulb.	Cip., Amik.

Pen: penicillin; Amox.\calv: amoxcillin\calvulanic acid; Amp.\sulb: ampicillin\sulbactam; Amik: amikacin; Gen: gentamycin; Cip: ciprofloxacin; Lev: levofloxacin; Cefa: cefazolin; Cefu: cefuroxime; Meth: methicillin.

Transconjugant colonies were found on LB plates containing appropriate selective antibiotics taking VRSA (ciprofloxacin-sensitive) as a donor, and vancomycin-sensitive *S. aureus* (ciprofloxacin-resistant) as a recipient. No growth of recipient *S. aureus* strain and the donor strain VRSA, was observed on the above LB medium when inoculated separately. MIC of vancomycin was determined for the transconjugate and it was found that it increased from 2 µg/ml to 32 µg/ml. on the other hand, plasmid profile for transconjugate revealed the presence of one plasmid which was not found in the recipient strain before conjugal transfer (Fig. 2).

A selected transconjugant colony was picked up for PCR amplification that revealed the presence of a band for *vanA* gene at 1032bp (Fig. 3).

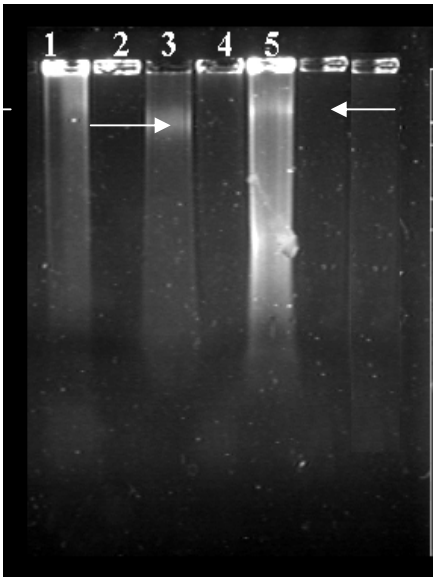

Fig. 2. Agarose gel electrophoresis of plasmid DNAs isolated from VRSA, VSSA and transconjugate strain. Lane 2 & lane 4: VSSA; Lane 3: VRSA (donor strain); lane 5: transconjugant strain

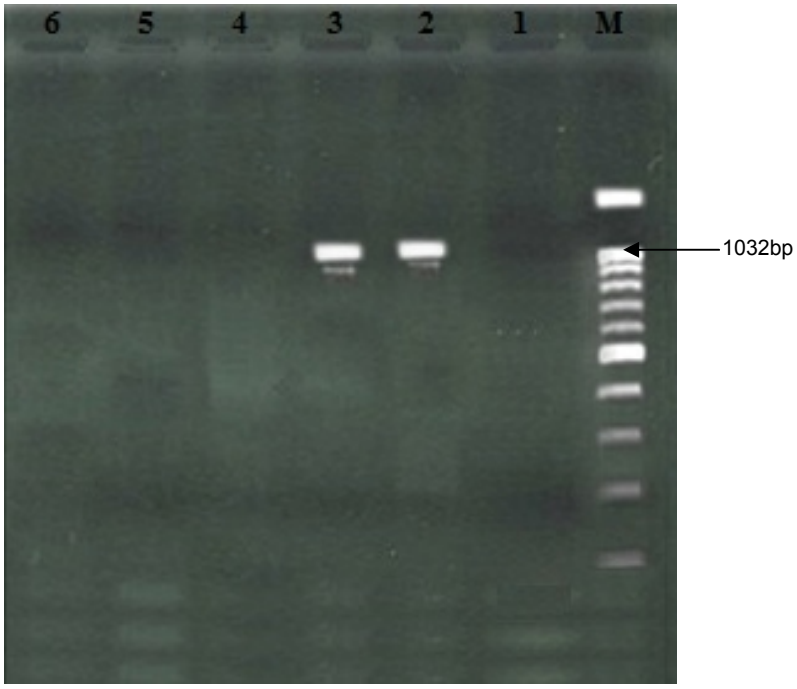

Fig. 3. PCR amplification of the *van*A gene for vancomycin resistant *S. aureus*. Lane 2 *van*A positive VRSA; Lane 3: van A positive transconjugant strain; lane 4: *van*A negative VSSA (the recepient strain befor gene transfer by transconjugation); and lanes 5,6 *van*A negative VISA strains , M-100 bp ladder 100-1500

4. DISCUSSION

As strains of *S. aureus* with reduced susceptibility continue to emerge and evolve, perhaps to full resistance, there is a clinical need to fully characterize them and conduct well designed research and epidemiological studies. The current vancomycin resistant staphylococci in hospital as well as in community are alarming situation to the clinicians. The development of antibiotic resistance in developing countries like ours seems to be very much related to the irrational antibiotic usage due to its easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries [31]. Widespread use of vancomycin to treat infections caused by MRSA and other gram-positive cocci has led to the emergence of vancomycin resistance. The large scale of development and subsequent spread of resistance to vancomycin has been perceived as a fearsome threat to the already challenging therapy of MRSA.

The true mechanism of vancomycin resistance in *S. aureus* is not known. It was initially feared that *S. aureus* would acquire the *van* gene that code for vancomycin resistance in *Enterococcus s*pp; this phenomenon was successfully accomplished in the laboratory [11]. Further, Showsh et al. [32] have demonstrated the presence of sex pheromone in *S. aureus* that promotes plasmid transfer in *Enterococcus* spp. Release of these pheromones by *S. aureus* with proximity to vancomycin-resistant enterococci causes the transfer of plasmids encoding *van* gene to the *S. aureus*.

The present study encountered one vancomycin resistant strain (1.5%) with MIC 16*µ*g\ml that was confirmed by PCR (showed band positive for *vanA* gene) and 5 isolates were VISA (7.9%). Many researchers reported vancomycin resistance; Bathaineh has reported VRSA strains from Jordan [33]. Assadulla et al. [34] have reported some strains of vancomycin intermediate *S. aureus* (VISA) from India. Song et al. [35] have also reported the emergence of heterogeneous vancomycin resistant *S. aureus* strains from India and its neighboring countries. Also, Saha et al. [5] reported that out of 57 *S. aureus* strains, one isolate was VRSA which agree with our results. On the other hand, Saderi et al. [36] reported that 3.5% of *S. aureus* isolates were VRSA which is higher than that reported in our study while Hakim et al. [37] reported a higher incidence of VISA (13%). It was found that methicillin resistance represented 25.4% of isolates that is lower than reported by Japooni et al. [38]. vancomycin resistant isolate and all vancomycin intermediate isolates were resistant to methicillin and mostly isolated from skin infection. Similar results were obtained by Denis et al. [17] and Alzolibani et al. [39]. On the hand, Dhanalakshmi, *et al.* reported that no VISA or VRSA were found among methicillin resistant strains which represent 31.3% of staphylococcal isolates [40]. VRSA and VISA showed resistance to penicillin, amoxicillin/clavulanic, ampicillin/sulbactam, cefazolin, cefuroxime. Also, VRSA showed resistance to gentamicin and levofloxacin but showed susceptibility to ciprofloxacin and amikacin. Multi-drug resistance of VRSA and VISA was also reported by many studies [5,37,41].

Six major phenotypes of vancomycin resistance *(VanA, VanB, VanC, VanD, VanE, and VanG*) were known [42,43]. *VanA* and *VanB* phenotypes are both common and transferable. The *VanA* phenotype confers high level resistance to vancomycin and teicoplanin, whereas the *VanB* phenotype exhibits variable levels of resistance to vancomycin, but not teicoplanin. The *VanC* phenotype is not transferable and is limited to *Enterococcus gallinarum* and *Enterococcus casseliflavus* (*Enterococcus flavescens*). *VanD*, *VanE*, and *VanG* phenotypes are uncommon [42,44].

Resistance in VISA isolates is typically mediated via mechanisms that develop in the presence of vancomycin and are not readily transferrable to other strains. The potential for spread of these isolates is low in the absence of vancomycin therapy. In contrast, VRSA isolates uniformly contain the *vanA* gene derived from *Enterococcus.* The *Van*A phenotype is transferable to other MRSA strains and across microbial species, with a much greater potential for spread, even in the absence of vancomycin therapy [45]. These results agree with our study which showed that vancomycin resistant isolate carried a plasmid that agree with Shriram et al. [46] who proved that vancomycin resistance was plasmid mediated as upon curing test they became sensitive to low concentrations of the antibiotic. Our study showed that plasmid mediated VRSA was positive for *vanA* gene and by testing the ability of resistance gene transfer, a conjugative transfer of vancomycin resistance gene from one clinical strain to another was observed. Transconjugate showed an increase in the MIC of vancomycin and was positive for *vanA* gene which agrees with the results obtained by Saha et al. [5].

5. CONCLUSION

Our study showed that vancomycin resistant and intermediate strains were mostly isolated from skin infections, multi-drug resistant. Also resistance genes were plasmid mediated (transferred successfully to sensitive strains) which is alarming and may lead to rapid dissemination of these strains among health care workers and patients by direct contact. This may soon become a global problem, unless antimicrobial agents are used more prudently. So, it becomes a must to start to implement infection-control precautions to prevent the spread of VRSA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med. 1998;339:520–32.
2. Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*: Minnesota and North Dakota, 1997–1999. MMWR Morb Mortal Wkly Rep. 1999;48:707–10.
3. Maranan MC, Moreira B, Boyle-Vavra S, Daum RS. Antimicrobial resistance in staphylococci. Epidemiology, molecular mechanisms, and clinical relevance. Infect Dis Clin North Am. 1997;11:813 – 849.
4. Ena J, Dick RW, Jones RN, Wenzel RP. The epidemiology of intravenous vancomycin usage in a university hospital: a 10-year study. JAMA. 1993;269:598 – 602.
5. Saha B, Singh AK, Ghosh A, Bal M. Identification and characterization of a vancomycin resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). J Medical Microbiol 2008;57:72–79.
6. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J Bacteriol 1993;175:117–127.

7. Wright GD, Holman TR, Walsh CT. Purification and characterization of *VanR* and the cytosolic domain of VanS: a two component regulatory system required for vancomycin resistance in *Enterococcus faecium* BM4147. Biochemistry 1993;32:5057–5063.
8. Bugg TDH, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochemistry. 1991a;30:10408–10415.
9. Bugg TDH, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Identification of vancomycin resistance protein VanA as a D-alanine : D-alanine ligase of altered substrate specificity. Biochemistry. 1991b;30:2017–2021.
10. Reynolds PE, Depardieu F, Dutka-Malen S, Arthur M, Courvalin P. Glycopeptide resistance mediated by enterococcal transposon Tn1546 requires production of vanX for hydrolysis of D-alanyl-D-alanine. Mol Microbiol 1994;13:1065–1070.
11. Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to Staphylococcus aureus. FEMS Microbiol Lett. 1992;72:195–198.
12. Młynarczyk A, Młynarczyk G & Łuczak M. Conjugative transfer of glycopeptide and macrolide resistant genes among enterococci and from *Enterococcus faecalis* to Staphylococcus aureus. Med Dosw Mikrobiol. 2002;54:21–28.
13. Weigel LM, Donlan RM, Shin DH, Jensen B, Clark NC, McDougal LK, Zhu W, Musser KA, Thompson J, other authors. High-level vancomycin-resistant *Staphylococcus aureus* isolates associated with a polymicrobial biofilm. Antimicrob Agents Chemother. 2007;51:231–238.
14. 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.
15. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 6th ed. Approved standard, M7-A6. Wayne, Pennsylvania. 2003
16. Smith TL, Pearson ML, Wilcox KR, et al. Emergence of vancomycin resistance in *Staphylococcus aureus*: Glycopeptide-Intermediate *Staphylococcus aureus* Working Group. N Engl J Med. 1999, 340:493 – 501.
17. 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.
18. 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.
19. No authors listed. *Staphylococcus aureus* resistant to vancomycin—the United States, MMWR Morb Mortal Wkly Rep. 2002;51:565 – 567.
20. No authors listed. Vancomycin-resistant *Staphylococcus aureus*—Pennsylvania,. MMWR Morb Mortal Wkly Rep. 2002;51:902.
21. Perl TM. The threat of vancomycin resistance. Am J Med. 1999;106:26S – 37S.
22. Schweiger ES, Scheinfeld NS, Tischler HR, Weinberg JM. Linezolid and quinupristin/dalfopristin: novel antibiotics for Gram-positive infections of the skin. J Drugs Dermatol. 2003;2:378 – 383.
23. Srinivasan A, Dick JD, Perl TM. Vancomycin resistance in staphylococci. Clin Microbiol Rev. 2002;15:430–438.

24. Bannerman TL. Staphylococcus, Micrococcus, other Catalase positive cocci that grow aerobically. In Manual of Clinical Microbiology. 2003;384–404. Edited by PR. Murray, EJ Baron, JH Jorgensen, MA Pfaller, RH Yolken. Washington, DC: ASM Press.
25. Turk DC, Porter IA. A Short Textbook of Medical Microbiology, 4th edn. London; 1978. Hodder and Stoughton.
26. Clinical and laboratory standards institutes. Performance standards for antimicrobial susceptibility testing. Twenty first informational supplement M100-S21; 2011. Wayne, PA: CLSI.
27. Birnboim. A rapid alkaline extraction method for the isolation of plasmid DNA, Meth. Enzymol. 1983;100:243-255.
28. Philippon AM, Paul GC, Jacoby GA. Properties of PSE- 2beta-lactamase and genetic basis for its production in *Pseudomonas aeroginosa*. Antimicrob Agents Chemother 1983;24:362–369.
29. Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U. S. hospitals. Antimicob Agents Chemother 1993;37:2311-2317.
30. Cohen ML. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science. 1992;257:1050-1055.
31. Holloway K. Antimicrobial resistance: the facts. Essential Drug Monitor WHO, 2000;28&29:7-8.
32. Showsh SA, De Boever RH, Clewell DB. Vancomycin resistance plasmid in *Enterococcus faecalis* that encodes sensitivity to a sex pheromone also produced by *Staphylococcus aureus*. Antimicrob Agents Chemother. 2001;45:2177-2178.
33. Bataineh AB. Resistance of *Staphylococcus aureus* to Vancomycin in Zarqa, Jordan. Pak J Med Sci. 2006;22:144-148.
34. 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;21:196-198.
35. Song JH, Hiramatsu K, Suh JY, Ko KS, Ito T, Kapi M, Kiem S, Kim YS,Oh WS, Peck KR, Lee NY. Asian Network for Surveillance of Resistant Pathogens Study Group: Emergence in Asian countries of *Staphylococcus aureus* with reduced susceptibility to vancomycin. Antimicrob Agents Chemother. 2004;48:4926-4928.
36. Saderi H, Owlia P, Shahrbanooie R. Vancomycin resistance among clinical isolates of *Staphylococcus aureus*. Arch Iranian Med; 2005;8:100–103.
37. Hakim ST, Arshed S, Iqbal M, Javaid SG. Vancomycin sensitivity of *Staphylococcus aureus* isolates from hospital patients in Karachi, Pakistan. Libyan J Med. 2008;176-179.
38. Japooni A, Alborzi A, Orafa F, Rasouli M, Farshad S. Distribution patterns of methicillin resistance genes (mecA) in *Staphylococcus aureus* isolated from clinical specimens. Iran Biomed J. 2004;8:173-78.
39. Alzolibani AA, Robaee AAA, Shobaili HAA, Bilal JA, Ahmad MI, Bin Saif G. Documentation of vancomycin-resistant Staphylococcus aureus (VRSA) among children with atopic dermatitis in the Qassim region, Saudi Arabia Acta Dermatovenerol APA. 2012;21:51-53.
40. Dhanalakshmi TA, Umapathy BL, Mohan DR. Prevalence of methicillin, vancomycin and multi drug resistance among *Staphylococcus aureus*. J Clin Diag Res. 2012;6:974-977
41. Wootton M, Howe RA, Walsh TR, Bennett PM, MacGowan AP. In vitro activity of 21 antimicrobials against vancomycin-resistant *Staphylococcus aureus* (VRSA) and hetero-VRSA (hVRSA). J Antimicrob Chemother. 2002;50:760–761.
42. Krcmery Jr V, Sefton A. Vancomycin resistance in Gram-positive bacteria. International J Antimicrob Agents. 2000;14:99–105.

43. Courvalin P. Vancomycin resistance in Grampositive cocci. Clin Infect Dis. 2006;42:S25–S34.
44. Murray BE. Vancomycin-resistant enterococcal infections. N Engl J Med. 2000;342:710–721.
45. Hageman JC, et al. Investigation and control of vancomycin-intermediate and - resistant *Staphylococcus aureus*: A Guide for Health Departments and Infection Control Personnel; 2006. Atlanta, GA. Available at: http://www.cdc.gov/ncidod/ dhqp/pdf/ar/visa_vrsa_guide.pdf.
46. Shriram V, Kumar V, Mulla J, Latha C. Curing of plasmid - mediated antibiotic resistance in multi drug resistant pathogens using Alpinia galanga rhizome extract, Advanced Bio Tech.; 2013. vol1.www.advancedbiotech.in.

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