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Presence of multiple *van* genes among glycopeptide non-susceptible *Staphylococcus aureus* exhibiting *in vitro* MIC creep phenomenon: A study from north-east India

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***Background & objectives*: The global prevalence of vancomycin-resistant *Staphylococcus aureus* (VRSA) has increased two fold since 2010, accounting for 2.4 per cent of *S. aureus* infections. The emerging hVISA isolates and their increasing trends pose a serious therapeutic challenge. The present study investigated *in vitro* vancomycin and teicoplanin minimum inhibitory concentration (MIC) creep in *S. aureus* and assessed their revertants.**

***Methods*: A total of 845 isolates were collected for this study, and 246 were confirmed as *S. aureus*. Molecular characterization of vancomycin resistance was carried out by PCR assay targeting genes types *viz*: *vanA*, *vanB*, *vanC*, *vanC2/C3*, *vanD*, *vanE*, and *vanG*. MIC was determined for vancomycin and teicoplanin by agar dilution method. MIC creep and revertant analysis were done by broth dilution method in the presence and absence of antibiotics.**

***Results*: PCR assay confirmed 12 isolates were harboured *vanA*, followed by *vanD* (n=8) and *vanB* (n=7). The study showed 69 isolates were screened positive for glycopeptide non-susceptibility. While analyzing vancomycin MIC creep, four isolates showed a significant increase in MIC, whereas no creep phenomenon was observed for the rest. In the case of teicoplanin, seven isolates showed the MIC creep phenomenon. Revertant analysis of all the isolates that showed MIC creep phenomenon for vancomycin and teicoplanin reverted to their original MIC when the antibiotic pressure was withdrawn.**

***Interpretation & conclusions*: In the present study setting, glycopeptide non-susceptibility was found in eight per cent of the isolates, and the present study found the occurrence of multiple *van* genes from isolates calculated from a single study center will impose a serious challenge in infection control and antibiotic policy. This study also underscores that heterogenic resistant isolates, upon exposure to vancomycin and teicoplanin at a minimum level, exhibited an increase in MIC, which will impact individuals receiving glycopeptide therapy.**

Key words Vancomycin-resistant *Staphylococcus aureus* (VRSA) - MIC creep - *van* gene – vancomycin - teicoplanin

Staphylococcus aureus is one of the predominant pathogens causing severe infections in community

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and hospital settings. The severity of infections leads to increased morbidity, higher healthcare costs, prolonged hospitalization, and increased risk of death[1]. The acquisition of resistance determinants by *S. aureus* has presented the greatest challenge to treating and controlling staphylococcal infections. Based on data from the Centre for Disease Control and Prevention (CDC), vancomycin-resistant *Staphylococcus aureus* (VRSA) is considered a serious threat to world health care[2]. Glycopeptide resistance is mainly due to acquiring *van* genes *viz*. *vanA, vanB, vanC, vanC2/C3, vanD, vanE, vanG*[3]. Reportedly, *vanA* harboring isolates exhibit a high level of inducible resistance, whereas a modest level of resistance in the case of *vanB* carrying isolates and low-level resistance is shown by *van*C[4]. In a study from India[5], *vanA* mediated vancomycin resistance was reported from a tertiary care hospital. From northeastern India, methicillin-resistant *S. aureus* (MRSA) associated infection was reported in 2009[6], where its prevalence rate was found to be 34.78 per cent. Although there is a lack of data on VRSA from this part of the country, vancomycin and vancomycin-resistant enterococci have recently been reported from a tertiary referral hospital in Assam[7]. Heterogeneous vancomycin-intermediate *S. aureus* (hVISA) exhibits vancomycin MIC within the susceptible range but has a subpopulation that confers resistance. VRSA and hVISA-associated infection pose a higher risk of treatment failure, prolonged hospital stays, higher treatment costs, and mortality. Treatment options for *S.aureus*-associated infections have become challenging in recent years with traditional antibiotics due to their biofilm-forming ability and acquisition of multiple resistance determinants. Besides therapeutic failure, slow clinical response and increased morbidity and relapse rate are consequences of this phenomenon[8]. It is also advocated that this problem should be dealt with locally with continuous evaluation of susceptibility and monitoring MIC patterns[9].

S. aureus are often reported to show vancomycin MIC creep phenomenon which triggers the susceptible or intermediate *S. aureus* isolates towards resistance when antibiotic exposure is prolonged. MIC creep is defined as an increase in the distribution of higher vancomycin MIC values within the susceptible range[10]. This increasing trend of vancomycin MIC is a serious emerging threat and is reported across the globe. Many institutions have reported most MRSA strains that are susceptible to vancomycin to show a creep in the MIC of vancomycin concentration, and many authors have also reported individuals with MRSA bacteria treated with vancomycin found higher rates of clinical failure due to this phenomenon[11]. The current study was carried out to determine the occurrence of glycopeptide non-susceptible *S. aureus* in a tertiary care hospital in the northeastern part of India and *in-vitro* analysis of vancomycin and teicoplanin MIC creep phenomenon among the study isolates.

Material & Methods

This study was undertaken at the department of Microbiology, Silchar Medical College and Hospital, Silchar, Assam, India from September 2018 to August 2022 after obtaining the protocol approval from the Institutional Ethics Committee.

Collection of bacterial isolates: A total of 845 consecutive non-duplicate clinical isolates were collected from the individuals admitted or attended outpatient department of the Silchar Medical College. All isolates were subjected to Gram staining and cultural characteristics and were identified by VITEK® 2 compact instrument (bioMérieux, Marcy-I'Étoile, France).This sophisticated, advanced, and automated system can identify isolates efficiently within a shorter time frame. *S. aureus* ATCC 25923 was used as a control. The collection of isolates and their identification was done aseptically, maintaining a sterile environment.

Screening of glycopeptide non-susceptibility and minimum inhibitory concentration (MIC) determination: Screening of glycopeptides non-susceptibility was done using 6 µg/ml vancomycin and 10 µg/ml of teicoplanin (Cipla) in Brain Heart Infusion Agar (HiMedia Laboratories Pvt Ltd., Mumbai) by agar dilution method[12,13]. The agar dilution method has the advantage of detecting bactericidal concentration. It can rule out the presence of any subpopulation of secondary mutants that may potentially exhibit a resistance phenotype (hVISA). Isolates grown in the concentration mentioned above were suspected to be glycopeptide non-susceptible. MIC was determined for vancomycin and teicoplanin by agar dilution method according to CLSI guidelines 2017, 2020, and 2021[14-16]. For each isolate, colonies from an overnight growth were transferred to sterile saline. The suspension was adjusted to 0.5 McFarland standards and inoculated on Muller Hinton agar (HiMedia Laboratories Pvt Ltd., Mumbai) containing 1, 2, 4, 8, 16, 32, and 64 µg/ml of vancomycin and teicoplanin individually. *S. aureus* ATCC 25923 was used as a negative control.

Previously, laboratory-confirmed vancomycin non-susceptible *S. aureus* (vancomycin MIC 8 µg/ml) was taken as positive control.

Molecular detection of vancomycin resistance gene: All the screened positive isolates were further subjected to molecular characterization of vancomycin resistance by multiplex PCR targeting genes type *viz*: *vanA, vanB, vanC, vanC2/C3, vanD, vanE, vanG*. PCR was performed under the following conditions: initial denaturation at 95°C for 3 min, denaturation at 95ºC for 25 sec, annealing at 50ºC for 40 sec, and extension at 72ºC for 1min, final extension at 72ºC for 5 min followed by 32 cycles. The primers used in the study were provided as Supplementary Table[17].

In vitro MIC creep analysis: This study was done on selected eight isolates, taking two each from the MIC range of 2, 4, 8, and 16 µg/ml towards vancomycin. The isolates were subjected to serial passage in Luria Bertani broth (HiMedia Laboratories Pvt Ltd., Mumbai) containing a higher concentration of vancomycin and teicoplanin than the previous concentration. Any isolate that failed to grow in a higher antibiotic concentration was allowed to grow on the same prior concentration of vancomycin and teicoplanin. The duration of each passage was 24 h. Colonies were isolated from an overnight growth and transferred to saline for each isolate. The suspension was adjusted to 0.5 McFarland standard and inoculated in tubes containing 1, 2, 4, 8, 16, 32, 64 and 128 µg/ml of vancomycin and teicoplanin, and the process was continued for 30 days[18]. MIC was determined every day for each isolate for 30 days duration.

Analysis of revertant: The isolates that showed MIC creep were subjected to serial passage in LB broth at 1:1000 dilutions without antibiotic stress for 30 consecutive days. After each passage, MIC was checked against vancomycin and teicoplanin, respectively[18]. All the data were recorded digitally for automated instruments VITEK® 2 (bioMérieux, France) and PCR and manually for other experiments.

Results

Among 845 isolates, 246 were confirmed as *S. aureus* based on the VITEK® 2 compact instrument. Of the 69 were screened as glycopeptide non-susceptible. While conducting an MIC study, it was observed that 37 isolates were in the intermediate and 13 were in the resistant range against vancomycin (Table I). Towards teicoplanin, 39 isolates were within the intermediate range (16 µg/ml), and 30 were resistant (Table I). PCR assay confirmed a total of 12 isolates were harbouring *vanA*, followed by *vanD* (n=8) and *vanB* (n=7). Isolates with an MIC range of 2-16 µg/ml for vancomycin and 8-16 µg/ml for teicoplanin were selected for MIC creep analysis. Eight isolates were chosen for the study, covering each MIC range. While analyzing vancomycin MIC creep, four showed a significant increase in MIC (Fig. 1; Table II). In contrast, no creep phenomenon was observed for the rest of the four isolates. In the case of teicoplanin, seven isolates showed the MIC creep phenomenon (Fig. 2; Table III). While performing revertant analysis of all the isolates that showed MIC creep phenomenon for vancomycin and teicoplanin, it was observed that the isolates reverted to their initial MIC mostly in between 2-3 wk for vancomycin. The same phenomenon was observed for teicoplanin in 1-2 wk (Fig. 3 and 4; Table IV and V). However, when isolates were subjected to serial passages for 30 consecutive days without any antibiotic pressure, the MIC of vancomycin and teicoplanin came down to 1-0.25 µg/ml of the antibiotic.

Table I. Minimum inhibitory concentration (MIC) of *Staphylococcus aureus* isolates against vancomycin and teicoplanin

Antibiotics	Concentrations (µg/ml)								Total no. of isolates (n)
	≤2	2	4	8	16	32	64	≥64	
Vancomycin	1	11	8	9	5	0	2	0	36
Teicoplanin	0	0	0	1	17	11	6	1	36

Discussion

Vancomycin, the most commonly used glycopeptide antibiotic, is one of the empiric treatment options for MRSA infections[19]. Glycopeptide non-susceptibility among *S. aureus* is a serious concern that restricts treatment options within clinical settings. In the current study, glycopeptide non-susceptibility was found in eight per cent of the isolates, which agrees with a previous study where nine per cent of hVISA was recorded[20]. A study conducted in Italy 2012 by Tascini *et al*[21] revealed that out of 91 clinical isolates of *S. aureus*, 10 (9.9%) were resistant to teicoplanin, and 5 (5.5%) were resistant to vancomycin. Shariati *et al*[22] conducted a meta-analysis and systematic review on the prevalence of hVISA/VISA/VRSA. After data analysis from 82 studies, it was found that the overall prevalence of VRSA was 1.5 per cent, VISA was 1.7

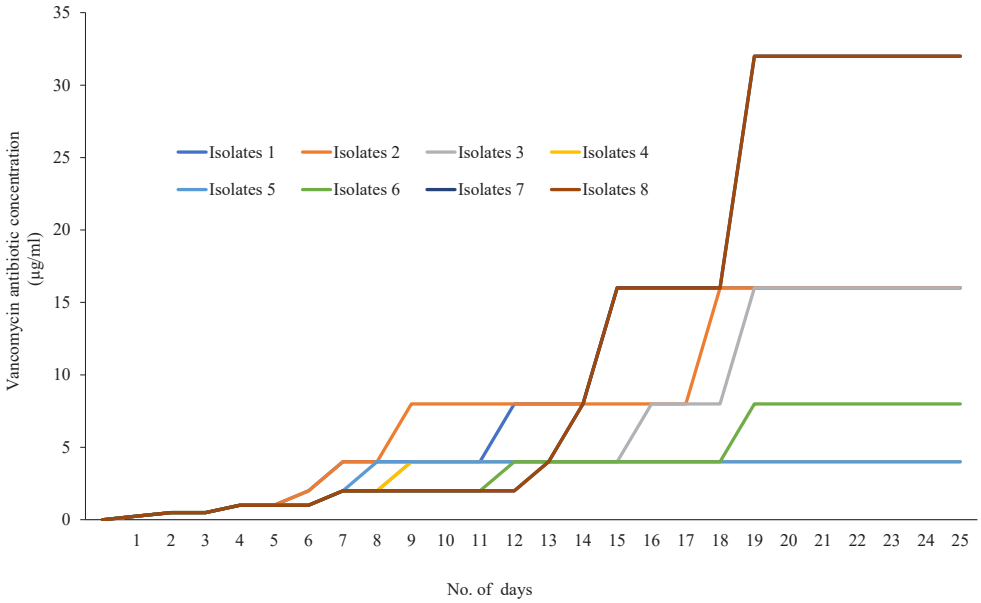

Fig. 1. Four *Staphylococcus aureus* isolates (isolates 1, 4, 6, 7) showing the minimum inhibitory concentration (MIC) creep phenomenon against vancomycin.

Table II. *Staphylococcus aureus* isolates showing the minimum inhibitory concentration (MIC) creep phenomenon against vancomycin

Initial MIC (µg/ml)	Number of days and isolates grown in respective concentration of antibiotic (µg/ml)																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
8	0.25	0.5	0.5	1	1	2	4	4	4	4	4	8	8	8	16	16	16	16	16	16	16	16	16	16	16
16	0.25	0.5	0.5	1	1	2	4	4	8	8	8	8	8	8	8	8	8	16	16	16	16	16	16	16	16
16	0.25	0.5	0.5	1	1	1	2	2	4	4	4	4	4	4	4	8	8	8	16	16	16	16	16	16	16
2	0.25	0.5	0.5	1	1	1	2	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
4	0.25	0.5	0.5	1	1	1	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
2	0.25	0.5	0.5	1	1	1	2	2	2	2	2	4	4	4	4	4	4	4	8	8	8	8	8	8	8
4	0.25	0.5	0.5	1	1	1	2	2	2	2	2	2	4	8	16	16	16	16	32	32	32	32	32	32	32
8	0.25	0.5	0.5	1	1	1	2	2	4	4	4	4	4	4	8	8	8	8	8	8	8	8	8	8	8

Table III. *Staphylococcus aureus* isolates showing the minimum inhibitory concentration (MIC) creep phenomenon against teicoplanin

Initial MIC (µg/ml)	Number of days and isolates grown in respective concentration of antibiotic (µg/ml)																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
16	0.25	0.5	0.5	1	1	2	2	4	8	8	8	16	16	32	64	64	64	64	64	64	64	64	64	64	64
16	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	16	16	16	16	16	16	16	16	16	16	16	16	16
16	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	16	16	16	16	16	32	32	32	32	32	32	32	32
16	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	32	32	32	32	32	32	32	32	32	32	32	32	32
16	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	16	16	16	16	16	32	32	32	32	32	32	32	32
16	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	16	32	32	32	32	32	32	32	32	32	32	32	32
16	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	16	32	64	64	64	64	64	64	64	64	64	64	64
8	0.25	0.5	0.5	1	1	1	2	4	8	8	8	16	16	32	64	64	64	64	64	64	64	64	64	64	64

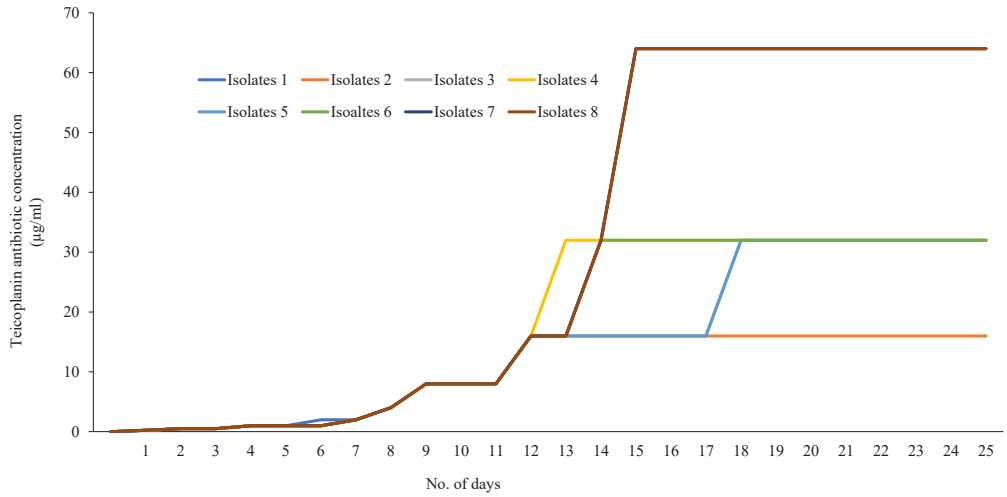

Fig. 2. Seven *S. aureus* isolates (isolates 1, 3, 4, 5, 6, 7, 8) showing the minimum inhibitory concentration (MIC) creep phenomenon against teicoplanin.

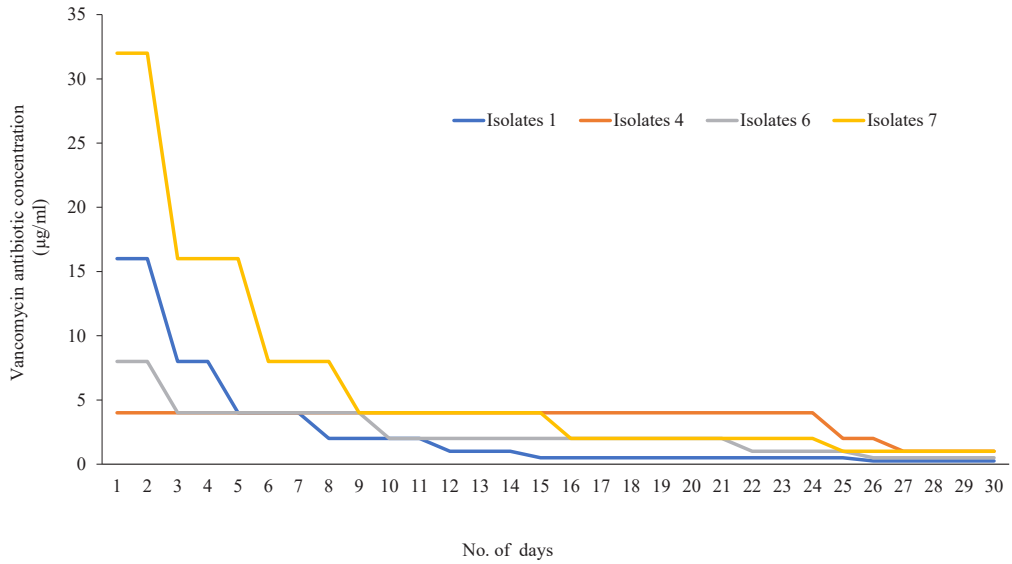

Fig. 3. Revertant analysis of four *S. aureus* isolates that showed MIC creep against vancomycin have reverted back to their respective original MIC on withdrawn of vancomycin pressure.

per cent, and hVISA was 4.6 per cent, and after 2010, it increased to 2.4, 4.3, and 5.3 per cent, respectively[22]. In India presence of *vanA* was reported from *S. aureus*[23]. Where as the current study reported the presence of multiple *van* genes. In this study, vancomycin and teicoplanin MIC creep was observed *in vitro* condition that supports prolonged exposure to vancomycin increases the MIC of susceptible isolates. Furthermore 69 out of 246 *S. aureus* isolates were glycopeptide non-susceptible, of which 37 were of the VISA phenotype. This imposes the risk of adverse clinical outcomes if not detected early. A variable MIC range of isolates towards vancomycin and teicoplanin was observed in the study. Total 29 isolates in the susceptible MIC range against vancomycin have a potential risk of attaining hVISA phenotype in the future, severely compromising the glycopeptide treatment option. A study conducted in China showed vancomycin MIC creep in *S. aureus* isolates throughout the five yr study period[24]. There are a few contemporary reports from India where hVISA was reported within diabetic and non-diabetic individuals. They observed an occurrence rate of 6.4 per cent hVISA within MRSA isolates[25]. Another Indian study observed reduced vancomycin susceptibility (11.6%) in *S. aureus*[26]. Similarly, another report from south India observed 12 per cent hVISA within diverse

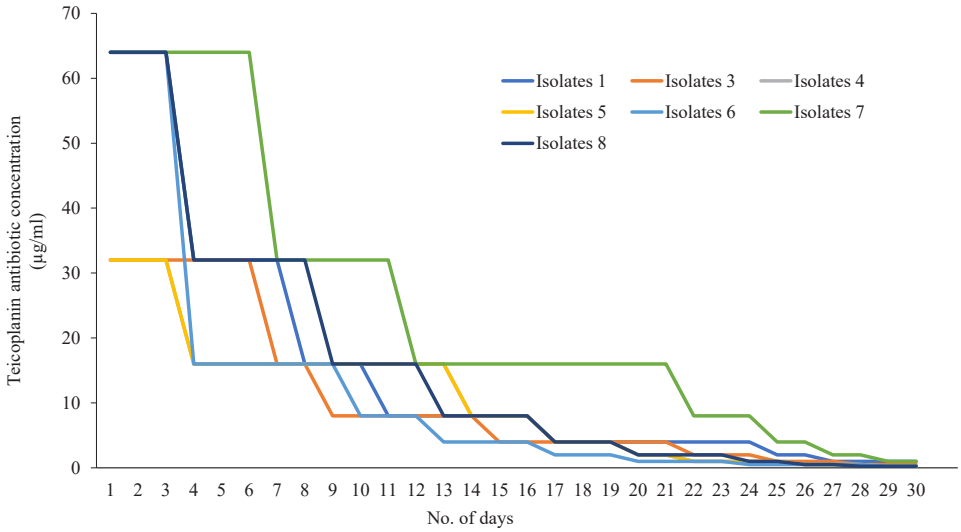

Fig. 4. Revertant analysis of seven *S. aureus* isolates that showed MIC creep against teicoplanin have reverted back to their respective original MIC on withdrawn of teicoplanin pressure.

amino acid substitution in *tcaRAB*, *vra*SR and *gra*SR genes[27]. Recent studies from abroad also reported the presence of VISA/VRSA among MRSA strains[28]. A study from Saudi Arabia[29] has reported vancomycin MIC creep in *S. aureus* over three years, which declined subsequently over the next three years' time. VISA/hVISA phenotypes are reported to be associated with a mutation on *vraSR* and *graSR*, two-component regulation systems. It was observed that the constructs (mutants) demonstrated a remarkable increase in vancomycin MIC[30]. Thus, the *in vitro* gradual increase in vancomycin MIC in the current study might have a link with mutations in regulatory regions. A study from the USA[31] highlighted the MIC creep phenomenon over some time towards vancomycin. Recently, a study from India showed progressive MIC creep towards teicoplanin[32]. In the present study, all the isolates that showed MIC creep against vancomycin and teicoplanin reverted to a susceptible MIC range when antibiotic stress was withdrawn for 30 days. However, no study could be found to compare with the findings of the present study. Yeh *et al*[33] in 2012 showed increased vancomycin usage, resulting in vancomycin MIC creep in MRSA. It was also reported that vancomycin usage in 30 days before the isolation of a *S. aureus* culture had a higher MIC. However, this increase could not be correlated with higher mortality[33]. The findings of this study will augment global knowledge of antimicrobial resistance. Our observations on low vancomycin MIC (4 µg/ml) of *vanD* harboring isolates and moderate to high vancomycin MIC (8-64 µg/ml) of *vanA* and *vanB* harboring isolates could underscore how these resistance determinants confer different phenotypes. In the present study, the *in vitro* MIC creep phenomenon was observed in laboratory conditions, advocating studies to be undertaken over two to three yr to understand and correlate with glycopeptide usage and any increase in MIC. The findings of this study emphasize the need for a local epidemiological cut-off point for screening resistant pathogens. This warrants designing future diagnostics that can effectively detect heterogeneous resistant populations of bacteria. The current study could predict how these glycopeptide nonsusceptible isolates can attain a higher inhibitory concentration within the patient population when initiating glycopeptide treatment. This also highlights the adoption of testing facilities for glycopeptide non-susceptibility within routine microbiology laboratories. However, the information of the current investigation is restricted to *in vitro* analysis only.

Overall, the study found the prevalence of multiple *van* genes within a single study centre, which poses a severe challenge to treatment options. The presence of *van* genes among clinical isolates of *S. aureus* is a serious concern as the hospital environment acts as a reservoir for the resistance determinants. The emergence of hVISA is a significant threat that requires urgent screening and proper reporting.The present study is retrospective, and this advocates further need for prospective investigation under the umbrella of an antibiotic stewardship programme, thereby adopting control measures to contain this spread and detect resistant strains. This study also signifies that

Table IV. *Staphylococcus aureus* isolates that showed MIC creep against vancomycin have reverted back to their respective initial MIC on withdrawn of antibiotic pressure. Isolate 1 reverted on 3rd day of withdrawal of antibiotic exposure, whereas isolates4, 6 and 7 reverted on 25th, 10th and 9th day of withdrawal of antibiotic exposure

Isolates ID	Initial MIC (µg/ml)	Number of days and isolates showed growth in decreasing concentration of vancomycin (µg/ml)																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	8	16	16	8	8	4	4	4	2	2	2	2	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25
4	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	2	1	1	1	1
6	2	8	8	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	0.5	0.5	0.5	0.5	0.5
7	4	32	32	16	16	16	8	8	8	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1

Table V. *Staphylococcus aureus* isolates that showed MIC creep against teicoplanin have reverted back to their respective initial MIC on withdrawal of antibiotic pressure. Isolates 1, 3, 7 and 8 reverted on 8th, 6th, 12th and 13th day of withdrawal of antibiotic exposure, whereas isolates 4, 6 and 6 reverted on 4th day of withdrawal of antibiotic exposure

Isolates ID	Initial MIC (µg/ml)	Number of days and isolates showed growth in decreasing concentration of teicoplanin (µg/ml)																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	16	64	64	64	32	32	32	32	16	16	16	8	8	8	8	8	8	4	4	4	4	4	4	4	4	2	2	1	1	1	1
3	16	32	32	32	32	32	16	16	16	8	8	8	8	8	8	4	4	4	4	4	4	4	2	2	2	1	1	1	0.5	0.5	0.5
4	16	32	32	32	16	16	16	16	16	16	16	16	16	16	8	8	8	4	4	4	2	2	1	1	1	1	0.5	0.5	0.5	0.25	0.25
5	16	32	32	32	16	16	16	16	16	16	16	16	8	8	8	8	4	4	4	2	2	1	1	1	1	1	0.5	0.5	0.5	0.25	0.25
6	16	32	32	32	16	16	16	16	16	16	8	8	8	4	4	4	4	2	2	2	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.25	0.25
7	16	64	64	64	64	64	64	32	32	32	32	32	16	16	16	16	16	16	16	16	16	16	8	8	8	4	4	2	2	1	1
8	8	64	64	64	32	32	32	32	32	16	16	16	16	8	8	8	4	4	4	4	2	2	2	2	1	1	0.5	0.5	0.25	0.25	0.25

the heterogenic resistance strains upon exposure to vancomycin and teicoplanin at a minimum level could increase MIC significantly, particularly in individuals receiving therapeutic intervention in real time.

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***Conflicts of Interest*:** None.

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References

1. Diaz R, Afreixo V, Ramalheira E, Rodrigues C, Gago BJ. Evaluation of vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* infections – a systematic review and meta-analysis. *Clin Microbial Infect* 2018; *24* : 97-104.
2. Mohanasoundaram KM, Lalitha MK. Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus*. *Indian J Med Res* 2008; *127* : 78-84.
3. Pootoolal J, Neu J, Wright GD. Glycopeptide antibiotic resistance. *Annu Rev Pharmacol Toxicol* 2002; *42* : 381.
4. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. *Clin Microbiol Rev* 2000; *13* : 686-707.
5. Phukan C, Lahkar M, Ranotkar S, Saikia KK. Emergence of vanA gene among vancomycin-resistant enterococci in a tertiary care hospital of North-East India. *Indian J Med Res* 2016; *143* : 357-61.
6. Saikia L, Nath R, Choudhury B, Sarkar M. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* in Assam. *Indian J Crit Care Med* 2009; *13* : 156.
7. Das PP, Nath R, Choudhury G, Sarmah A, Bora P. Trends in antimicrobial resistance in a tertiary care hospital of Assam, India. *J Pure Appl Microbiol* 2023; *17* : 1591-604.
8. Sharma R, Hammerschlag MR. Treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in children: a reappraisal of vancomycin. *Curr Infect Dis Rep* 2019; *21* : 1-8.
9. Kehrmann J, Kaase M, Szabados F, Gatermann SG, Buer J, Rath PM, *et al*. Vancomycin MIC creep in MRSA blood culture isolates from germany: A regional problem? *Eur J Clin Microbiol Infect Dis* 2011; *30* : 677-83.
10. Hsieh YC, Lin YC, Huang YC. Vancomycin, teicoplanin, daptomycin, and linezolid MIC creep in methicillin-resistant *Staphylococcus aureus* is associated with clonality. *Medicine (Baltimore)* 2016; *95*: e5060.
11. Rojas L, Bunsow E, Munoz P, Cercenado E, Rodriguez-Creixems M, Bouza E. Vancomycin MICs do not predict the outcome of methicillin-resistant *Staphylococcus aureus* bloodstream infections in correctly treated patients. *J Antimicrob Chemother* 2012; *67* : 1760-8.
12. Alos JI, García-Cañas A, García-Hierro P, Rodríguez-Salvanés F. Vancomycin MICs did not creep in *Staphylococcus aureus* isolates from 2002 to 2006 in a setting with low vancomycin usage. *J Antimicrob Chemother* 2008; *62* : 773-5.
13. Fitzgibbon MM, Rossney AS, O'Connell B. Investigation of reduced susceptibility to glycopeptides among methicillin-resistant *Staphylococcus aureus* isolates from patients in Ireland and evaluation of agar screening methods for detection of heterogeneously glycopeptide-intermediate *S. aureus. J Clin Microbiol* 2007; *45* : 3263-9.
14. Clinical and Laboratory Standards Institute. *M100. Performance standards for antimicrobial susceptibility testing 27th edition; 2017*. Available from: *https://clsi.org/media/1469/m100s27_sample.pdf*, accessed on October 15, 2022.
15. Clinical and Laboratory Standards Institute. *M100. Performance standards for antimicrobial susceptibility testing 30th edition*; *2020*. Available from: *https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf*, accessed on October 15, 2022.
16. Clinical and Laboratory Standards Institute. *M100. Performance standards for antimicrobial susceptibility testing 31st edition; 2021*. Available from: *https://clsi.org/media/z2uhcbmv/m100ed31_sample.pdf*, accessed on October 15, 2022.
17. Depardieu F, Perichon B, Courvalin P. Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. *J Clin Microbiol* 2004; *42* : 5857-60
18. Gajamer VR, Bhattacharjee A, Paul D, Deshamukhya C, Singh AK, Pradhan N, *et al*. *Escherichia coli* encoding blaNDM-5 associated with community-acquired urinary tract infections with unusual MIC creep-like phenomenon against imipenem. *J Glob Antimicrob Resist* 2018; *14* : 228-32.
19. Shariati A, Dadashi M, Moghadam MT, van Belkum A, Yaslianifard S, Darban-Sarokhalil D. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review and meta-analysis. *Sci Rep* 2020; *10* : 12689.
20. Othman HB, Halim RM, Gomaa FAM, Amer MZ. Vancomycin MIC distribution among methicillin-resistant *Staphylococcus aureus*. Is reduced vancomycin susceptibility related to MIC creep? *Open Access Maced J Med Sci* 2019; *7* : 12.
21. Tascini C, Flammini S, Leonildi A, Ciullo I, Tagliaferri E, Menichetti F. Comparison of teicoplanin and vancomycin *in vitro* activity on clinical isolates of *Staphylococcus aureus*. *J Chemother*. 2012; *24* : 187-90.
22. Shariati A, Dadashi M, Moghadam MT, van Belkum A, Yaslianifard S, Darban-Sarokhalil D. Global prevalence and distribution of vancomycin resistant, vancomycin intermediate

and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: A systematic review and meta-analysis. *Sci Rep* 2020; *10* : 12689.

23. Maharjan M, Sah AK, Pyakurel S, Thapa S, Maharjan S, Adhikari N, *et al*. Molecular confirmation of vancomycin-resistant *Staphylococcus aureus* with *vanA* gene from a hospital in kathmandu. *Int J Microbiol* 2021; 2021 : 3847347.
24. Chang W, Ma X, Gao P, Lv X, Lu H, Chen F. Vancomycin MIC creep in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from 2006 to 2010 in a hospital in China. *Indian J Med Microbiol* 2015; *33* : 262-6.
25. Sreejisha M, Shenoy MS, Shenoy MS, Dhanashree B, Chakrapani M, Bhat KG. Molecular and clinical features of heterogeneous vancomycin-intermediate *Staphylococcus aureus* in tertiary care hospitals in South India. *Sultan Qaboos Univ Med J* 2023; *23* : 447.
26. Mohanty S, Behera B, Sahu S, Praharaj AK. Recent pattern of antibiotic resistance in *Staphylococcus aureus* clinical isolates in Eastern India and the emergence of reduced susceptibility to vancomycin. *J Lab Physicians* 2019; *11* : 340-5.
27. Bakthavatchalam YD, Babu P, Munusamy E, Dwarakanathan HT, Rupali P, Zervos M, *et al*. Genomic insights on heterogeneous resistance to vancomycin and teicoplanin in Methicillin-resistant *Staphylococcus aureus*: A first report from South India. *PLoS One* 2019; *14* : e0227009.
28. Gohar NM, Balah MM, Sahloul N. Detection of vancomycin resistance among hospital and community-acquired methicillin-resistant *Staphylococcus aureus* isolates. *Egypt J Med Microbiol* 2023; *32* : 45-52.
29. Aljohani S, Layqah L, Masuadi E, Al Alwan B, Baharoon W, Gramish J, *et al*. Occurrence of vancomycin MIC creep in methicillin resistant isolates in Saudi Arabia. *J Infect Public Health* 2020; *13* : 1576-9.
30. Cui L, Neoh HM, Shoji M, Hiramatsu K. Contribution of vraSR and graSR point mutations to vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; *53* : 1231-4.
31. Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05. *J Antimicrob Chemother* 2007; *60* : 788-94.
32. Khurana S, Mathur P, Malhotra R. *Staphylococcus aureus* at an Indian tertiary hospital: Antimicrobial susceptibility and minimum inhibitory concentration (MIC) creep of antimicrobial agents. *J Glob Antimicrob Resist* 2019; *17* : 98-102.
33. Yeh YC, Yeh KM, Lin TY, Chiu SK, Yang YS, Wang YC, *et al*. Impact of vancomycin MIC creep on patients with methicillin-resistant *Staphylococcus aureus* bacteremia. *J Microbiol Immunol Infect* 2012; *45* : 214-20.

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