Research Paper Science

Emergence of Vancomycin Intermediate Staphylococcus aureus (VISA) and Heteroresistant Vancomycin Intermediate Staphylococcus aureus (hVISA) from Nagpur region of Central India.

*Vaishali U Thool

*Post Graduate Department of Microbiology, RTM Nagpur University, Nagpur

ABSTRACT

Vancomycin antibiotic is the ultimate treatment for vulnerable methicillin resistant Staphylococcal and multidrug resistant infections in orthopaedic departments. S.aureus with reduced susceptibilities is slowly spreading tentacles across the globe and causing treatment failures. This study aims at determining the presence of Vancomycin Intermediate Staphylococcus aureus (VISA) and Heteroresistant Vancomycin Intermediate Staphylococcus aureus (hVISA) in orthopaedic departments where antibiotic abuse is well known. Identification of S.aureus was confirmed by Gram staining and standard biochemical tests. Antibiotic susceptibility test and Ezy MIC Test Method were carried out in accordance with Clinical and Laboratory Standard Institute (CLSI) guidelines. Eleven VISA and three hVISA were identified from 51 S.aureus cultures tested. Emergence of resistance suggests nosocomial spread, antibiotic abuse and selective pressure.

Key Words : VISA, hVISA, MIC, E test, India

comycin antibiotic is a complex glycopeptide discovered in 1950s but was not used until 1980s until the emergence of Methicillin Resistant Staphylococcus aureus (MRSA). The hVISA phenotype is considered to be the precursor of VISA. In 1996 the first case of hVISA was reported in Japan 1. Currently the prevalence of hVISA varies because of difficulty of detection 2. Generally prior MRSA infection with high bacterial load, prior vancomycin exposure, nosocomial spread and outbreaks caused by VISA and hVISA are considered to be the risk factors for VISA and hVISA infections. VISA has distorted cellular physiology such as reduced peptidoglycan cross-linking, cell-wall turnover and autolysis causing the thickening of cell wall due to mutations in regulatory systems. This altered physiology changes the cell wall metabolism resulting in increased numbers of D-Ala-D-Ala residues which are the binding sites for vancomycin thus causing dense accumulation of vancomycin and resulting in delay in inhibition of cell wall synthesis 3.

CLSI revised the vancomycin minimal inhibitory concentration (MIC) interpretive criteria for S. aureus in 2006 owing to increasing reports of vancomycin treatment failures with MRSA infections. The values in parentheses are prior to 2006. Vancomycin susceptible ≤ 2 mcg/ml (≤4 mcg/ml), Vancomycin intermediate (VISA) 4 to 8 mcg/ml (8 to 16 mcg/ml), Vancomycin resistant (VRSA) ≥ 16 mcg/ml (≥ 32 mcg/ml). EUCAST (European Union Committee on Antibiotic Susceptibility Testing) have considered the Broth microdilution MIC of ≥ 4µg/ml as resistant, not considering intermediate susceptibility.

Material and methods:

Staphylococcal Isolates: A total 51 staphylococcal isolates were investigated for the period of one year from July 2010 to June 2011. The strains were collected from pus samples of patients from ambulatory settings of orthopaedic departments of Nagpur region. Pus samples were collected from dirty wounds on transport media swabs and brought to the microbiology laboratory immediately.

Media and culture conditions: On reception the swabs were inoculated on Brain Heart Infusion broth and incubated at 37°C for 24 hrs. The broth culture was then subcultured on Mannitol Salt Agar (Hi-Media, India) and Baird Parker Agar (Hi-Media, India). All the plates were incubated at 37°C for 24–48 hrs. Mannitol fermentation was observed and recorded from Mannitol Salt Agar plates whereas black colored colonies indicating tellurite reduction was noted from Baird Parker Agar plates for S.aureus. Gram staining was performed from colonies obtained on Mannitol Salt Agar and Baird Parker Agar and S.aureus was confirmed. Staphylococcus Agar No 110 W/Azide, MeReSa agar (Hi-Media, India) was also used for selective isolation of S.aureus.

Tube coagulase Test: Three to four pure colonies of culture were emulsified in 1ml of 1:6 diluted rabbit plasma and the tubes were incubated at 37°C. Clot formation was observed at 1 hr, 2 hrs, 3 hrs, and 4 hrs and further incubated overnight at room temperature if no clot formation was observed. S.aureus ATCC 29213 was used as control strain.

Latex agglutination test (HiStaph Latex kit): Latex agglutination test of all clinical S.aureus isolates was performed with HiStaph Latex kit according to the protocols supplied by the manufacturer (Hi Media, India Ltd, Mumbai, India).

Determination of Minimum Inhibitory Concentration (MIC): MIC of oxacillin, vancomycin and teicoplanin (Hi-Media, India) were determined by Ezy MIC test. All MICs were determined according to the instructions given by the manufacturer (Hi-Media Laboratories, Pvt Ltd, India). Briefly, plates of Mueller-Hinton agar (Hi-Media, India) were prepared. Inoculum were prepared by suspending two to three colonies in Brain Heart Infusion broth and incubated at 37˚C for two to three hours. The suspension was adjusted to 0.5 McFarland, corresponding to approximately 108cfu/ml and inoculated onto Mueller-Hinton agar plates. For detection of hVISA a higher inoculum (2 McFarland standard inoculum) and a prolonged incubation period of 48 hrs was used 2. The antibiotic Etest strips were placed on the inoculated Mueller-Hinton agar plates and the plates were incubated at 35°C for 24 hrs. The plates were read when sufficient growth was seen. MIC was read where the ellipse intersects the MIC scale on the strip and at the point of complete inhibition of all growth including hazes and isolated colonies using magnifying glass. Isolates were categorized as susceptible or resistant according to the breakpoints given by CLSI. S.aureus ATCC 29213 was used as vancomycin susceptible control.

Results:

MIC of all 51 *S.aureus* strains against oxacillin had shown that

40 *S.aureus* strains were resistant to oxacillin (MIC ≥ 4 µg/ml for *S. aureus*). Out of 40 oxacillin resistant *S.aureus* strains, nine strains of *S.aureus* were found to be VISA (MIC range 4-8 µg/ml) and three *S.aureus* strains were hVISA with high teicoplanin resistance and vancomycin MIC of 4 µg/ml. The detailed description of all the VISA and h/VISA are given in Table 1.

Table 1: MIC values of VISA and hVISA for different antibiotics used

S.aureus isolates	MIC values (µg/ml) of antibiotics used			VISA / hVISA
	Vancomycin	Teicoplanin	Oxacillin	
SA3	6	4	4 (R)	VISA
SA4	4	4	NZ (R)	VISA
SA22	4	4	12 (R)	VISA
SA26	4	4	NZ (R)	VISA
SA39	8	2	6 (R)	VISA
SA40	12	4	NZ (R)	VISA
SA59	16	4	NZ (R)	VISA
SA61	6	4	NZ (R)	VISA
SA63	4	4	32 (R)	VISA
SA29	4	32	6 (R)	hVISA
SA49	4	32	8 (R)	hVISA
SA50	4	36	8 (R)	hVISA

R - Resistant

Discussion:

With the emergence of vancomycin-resistant enterococci (VRE) in the late 1980s, S. aureus with diminished vancomycin susceptibility was speculated. The possible mechanism of vancomycin resistance in S.aureus was plasmid-mediated transfer of the vanA gene cluster from VRE to MRSA influenced by the release of sex pheromones by S.aureus. Therefore, with the first reported case of VISA in a clinical isolate of S. aureus in 1997 was mediated not via acquisition of vanA by a strain of methicillin-resistant S. aureus (MRSA), but by an unusually thickened cell wall containing D-Ala-D-Ala residue capable of binding vancomycin, thereby reducing availability of the drug for intracellular target molecules the researchers came into action4.

The present study encountered four VISA strains with vancomycin and teicoplanin MIC of 4µg/ml, three VISA strains with vancomycin MIC of 8, 12 and 16µg/ml and two VISA strains with vancomycin MIC of 8µg/ml. Assadullah et al have reported some strains of VISA from India 5. Two *S. aureus* strains were reported to be vancomycin and teicoplanin resistant (one strain with MIC 32 µg/ml and the other strain with MIC 64 µg/ml); six strains of *S. aureus* have shown to be VISA from northern part of India 6.

Our study also documented three hVISA strains. Two strains showed vancomycin MIC of 4µg/ml and teicoplanin MIC of 32µg/ml and one strain showed vancomycin MIC of 4µg/ml and teicoplanin MIC of 36µg/ml. The exact frequency of hVISA isolates has been difficult to document due to lack of clear-cut norms of heteroresistance and a paucity of uniform criteria for screening. The clinical significance of hVISA is also not clear but some studies have reported treatment failures and mortality with hVISA infections7. In a review of 14 epidemiological studies, the overall prevalence of hVISA among clinical MRSA isolates was reported to be 2.2% 8. Song et al, 2004 have also reported the emergence of hVISA strains from India and its neighboring countries 9. Although broth microdilution method is considered to be the gold standard for measuring vancomycin MIC, it is a cumbersome test and generally not used in routine diagnostic laboratory. Presently Etest is widely used and hence our work was carried out using the same method.

MRSA are the commonest pathogen in orthopaedic hospitals. The ambulatory settings of the orthopaedic departments from where the clinical samples were collected were not hygienically maintained and resulting in nosocomial spread. In this study all the VISA and hVISA were MRSA, suggesting a greater concern. The development and subsequent spread of VISA/hVISA is a threat to the medical community who is already facing a challenge in MRSA treatment.

Conclusion:

The surfacing of VISA/hVISA represents the beginning of vancomycin resistance era and highlights the consequence of treatment failures of 'superbugs'. There should be an immediate action to check further spread of VISA strains. Checking irrational antibiotic usage, nationwide surveillance program, identifying potential areas which are under the threat of VRSA/VISA emergence, checking nosocomial spread should be the areas where attention should be focused by the concerning authorities to stop the menace.

REFERENCES

1.Hiramatsu, K., Aritaka, N., .Hanaki, H., Kawasaki, S., Hosoda, Y., Hori, S., Fukuchi, Y., Kobayashi, I. (1997). Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. Lancet 350, 1670-1673. | 2.Howden, B. P., Davies, J. K., Paul, Johnson, D. R., Stinear, T. P., Grayson, M. L. (2010) Reduced Vancomycin Susceptibility in Staphylococcus aureus, Including Vancomycin-Intermediate and Heterogeneous Vancomycin-Intermediate Strains: Resistance Mechanisms, Laboratory Detection, and Clinical Implications. Clin. Microbiol. Rev. 23(1) 99-139 doi: 10.1128/CMR.00042-09. | 3.Cui, L., Ma, X., Sato, K., Okuma, K., Tenover, F. C., Mamizuka, E. M., Gemmell, C. G., Kim, M. N., Ploy, M. C., El-Solh, N., Ferraz, V., Hiramatsu, K. (2003). Cell wall thickening is a common feature of vancomycin resistance in Staphylococcus aureus. J. Clin. Microbiol. 41, 5-14. | 4.Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T., Tenover, F. C. (1997). Methicillin resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother, 40,135-136. | 5.Assadullah, S., Kakru, D.K., Thoker, M. A., Bhat, F. A., Hussain, N., Shah, A. (2003). Emergence of low level vancomycin resistance in MRSA. Indian J Med Microbiol, 21,196-198. | 6.Tiwari, H. K., Sen, M. R. (2006). Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious Diseases, 6,156. doi:10.1186/1471-2334-6-156. | 7.Moore, M. R., Remington, F., Chambers, H. F. (2003). Vancomycin Treatment Failure Associated with Heterogeneous Vancomycin-Intermediate Staphylococcus aureus in a Patient with Endocarditis and in the Rabbit Model of Endocarditis. Antimicrob Agents Chemother. 47(4), 1262-1266. doi: 10.1128/AAC.47.4.1262-1266. | 8.Liu, C., Chambers, H. F. (2003). Staphylococcus aureus with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother, 47, 3040-3045. | 9. Song, J., Hiramatsu, K., Suh J. Y., Ko K. S., Ito. T., Kapi, M., et al. (2004). Emergence in Asian Countries of Staphylococcus aureus with Reduced Susceptibility to Vancomycin. Antimicrob Agents Chemother, 48(12), 4926-4928. |