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ORIGINAL ARTICLE

VANCOMYCIN INTERMEDIATE RESISTANT STAPHYLOCOCCUS SPP. COLONIZATION IN HANDS OF HEALTH CARE WORKERS OF TERTIARY LEVEL HOSPITALS IN KOLKATA, INDIA

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ABSTRACT: AIMS: The emerging threat of widespread vancomycin resistance pose a serious public health concern. The objective of this study was to find the existence of vancomycin intermediate-resistant Staphylococcus spp (VIS) colonization in hands of health care workers. **METHODS:** 1284 isolates of Staphylococcus spp, from the fingers of 528 health care workers (HCW) of two tertiary level Government hospitals, were studied to identify the reduced susceptibility of vancomycin by minimum inhibitory concentrations (MIC) determination. **RESULTS:** The result indicated that 1058(82.39%) isolates have MICs of ≤4µg/ml and 226 (17.56%) isolates have MICs ≥4 µg/ml (but <32µg/ml), which is intermediate range between sensitive and resistance. **CONCLUSION:** This data revealed the significant presence of vancomycin intermediate-resistant staphylococci (VIS) in non-clinical isolates in Kolkata in the defined period of study.

KEYWORDS: Staphylococcus spp, vancomycin, intermediate resistant, health care workers.

INTRODUCTION: Vancomycin, a tricyclic glycopeptide antibiotic (C66H75Cl2N9O24) first isolated from a soil bacterium Actinobacteria species Amycolatopsis orientalis, acts by inhibiting the formation of the peptidoglycan polymers of the bacterial cell wall.[1]

Vancomycin also prevent the transfer and addition of the muramyl pentapeptide and minutely changes the permeability of cell wall of Gram-positive bacteria but is ineffective against Gram negative ones.[2] The achievable concentration of the antibiotic in plasma of man is 60µg/ml immediately after infusion and about 25µg/ml after 2 hrs when 1 gm dose is given intravenously.[1]

It is the drug of choice for treatment of gram-positive bacterial infections where penicillins and semi-synthetic penicillins such as methicillins are found as resistant or in treatment of those patients who have allergic response against penicillins. It has traditionally been reserved as a drug of last resort.[3]

Vancomycin intermediate-resistant Staphylococcus aureus (VISA) were first reported in Japan in 1996[4] and continue to be reported from various parts of the world. Resistance is mainly due to improper use of vancomycin and due to cross-resistance from other glycopeptides like teicoplanin[5]. So to maintain the effectiveness of the drug, it should be used very cautiously, and continuous surveillance for susceptibility is to be carried out.

As documented evidence of VIS in India are very few,[6] this study was intended to find out the vancomycin resistance pattern of the staphylococcal isolates in the hands of HCWs who are one of the potential source for harboring and transmission of the resistant strains to the patients.[7] This type of knowledge of the resistance pattern of staphylococcal isolates from the HCWs will also help to

prepare standard guidelines for infection control and prevention of transmission of the resistant strains in hospitals.

MATERIALS & METHODS:

Period of Study and places: The study was carried out from June 2012 to June 2013 at I.P.G.M.E.R/SSKM Hospital, Kolkata; and School of Tropical Medicine, Kolkata.

Case Selection: Samples were collected from hands of hospital workers (indoor) with no hand wash within three hours of sample collection.

Isolation and identification of isolates: The samples were collected from the finger tips underneath the nail blades of the individuals by applying one loopful of sterile normal saline and scrubbing the area with bacteriological loop. Immediately culture was done on 10% mannitol salt agar (MSA)[8] at the site of collection. Inoculated plates were incubated aerobically at 37˚C for 18hrs.

Gram positive, irregular cluster forming cocci, grown as yellow colonies in 10% MSA, showing positive reaction in catalase test were considered as genus Staphylococcus. Positive reaction by an isolate in anaerobic fermentation of mannitol, phosphatase and tube coagulase test were considered Staphylococcus aureus (SA), otherwise the isolate was considered coagulase negative staphylococci (CONS).[9]

Screening of vancomycin susceptibility: This was done by Kirby Bauer method [10] using 30µg/ml discs (Hi Media). The zone diameter of 15mm or more was considered as sensitive.

MIC determination: MIC of vancomycin was determined by agar dilution method as described elsewhere.[11] Briefly, gradient plates of Mueller-Hinton agar (Hi-Media, India) were prepared with vancomycin (2–32 µg/ml). By direct colony suspension method 0.5 McFarland equivalent inoculum were prepared in normal saline from 18–24 h agar plate culture. The suspension was further diluted to achieve desired inoculum concentration of 10^5 CFU/ml.

MIC values of vancomycin (Sigma, USA) were determined by spot inoculation of 1-2µL of the inoculums (~0.5 Mc Farland) on Mueller Hinton agar plates,[12] containing different concentration of the antibiotic (2, 4, 6, 8, 10, 12, 14, 16, 18 upto 32µg/ml), and incubating for 18 hrs at 37°C. The MIC of all isolates was measured in triplicate and the median data were selected. S. aureus (ATCC 29213) was taken as reference along with the test strains in all experiments.

Interpretation of MIC values was done according to CLSI guidelines [13] which states the MIC value of ≤4µg/ml, 8-16µg/ml and ≥32 µg/ml to be considered as susceptible, intermediate sensitive and resistant respectively.

STATISTICAL ANALYSIS: Statistical analysis was done with student t test.

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RESULTS:

Source	Doctors		Nurses		Attendants		Total	
	No. of Finger samples	No. of isolates (%)	No. of Finger samples	No. of isolates (%)	No. of Finger samples	No. of isolates (%)	No. of Finger samples	No. of isolates (%)
SSKM	200	SA 125 (62.5%)	225	SA 138 (61.3%)	300	SA 191 (63.6%)	725	SA 454 (62.6%)
		CONS 75 (37.5%)		CONS 87 (38.6%)		CONS 109 (36.3 %)		CONS 271 (37.3%)
STM	160	SA 104 (65%)	190	SA 123 (64.7%)	209	SA 141 (67.4%)	559	SA 368 (65.8%)
		CONS56 (35%)		CONS 67 (35.2%)		CONS 68 (32.5%)		CONS 191 (34.1%)

Table 1: Distribution of cases and corresponding isolation rates

The present data indicate that colonization of SA was more in attendants (63.6% and 67.4%), followed by doctors (62.5% and 65%) and nurses (61.3% and 64.7%) in both hospitals. Statistical analysis was done with student t test and the difference was found to be statistically non-significant (p=ns).

SOURCE		Doctors					Nurses					Attendants				
			MIC (µg/ml)					MIC (µg/ml)					MIC (µg/ml)			
		No	≤4 (%)	>4-<8 (%)	8-16 (%)	>16-32 (%)	No	≤ 4 (%)	>4-<8 (%)	8-16 (%)	>16-32 (%)	No	≤ 4 (%)	>4-<8 (%)	8-16 (%)	>16-32 (%)
SSKM	SA	125	109 (87.2)	5 (4)	11 (8.8)	0 (0)	138	99 (71.7)	5 (3.6)	34 (24.6)	0 (0)	191	179 (93.7)	3 (1.5)	9 (4.7)	0 (0)
	CONS	75	54 (72)	0 (0)	21 (28)	0 (0)	87	77 (88.5)	3 (3.4)	7 (8)	0 (0)	109	87 (79.8)	0 (0)	22 (20.1)	0 (0)
STM	SA	104	87 (83.6)	8 (7.6)	9 (8.6)	0 (0)	123	93 (75.6)	11 (8.9)	19 (15.4)	0 (0)	141	128 (90.7)	4 (2.8)	9 (6.3)	0 (0)
	CONS	56	47 (83.9)	0 (0)	9 (16)	0 (0)	67	43 (64.1)	0 (0)	24 (35.8)	0 (0)	68	55 (80.8)	0 (0)	13 (19.1)	0 (0)

Table 2: MIC value for vancomycin of staphylococcal isolates

Staplylococcus aureus (SA)
Coagulase negative staphylococci (CONS)

By disc diffusion method all strains of Staphylococcus spp emerged as vancomycin sensitive by the inhibitory zone diameter (≥15mm). They were further analyzed by determination of their MIC values for vancomycin (Table 2). MIC breakpoint value indicates 1058 (82.39%) strains were sensitive and 226 (17.56%) were intermediate-resistant among the total (n=1284) isolate.

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Among 822 SA and 462 CONS isolates 127(15.45%) and 99 (21.42%) were vancomycin intermediate sensitive respectively.

The data shows difference of vancomycin resistance in Staphylococcus aureus and CONS is statistically significant ($p<0.05$) indicating CONS are usually and naturally more resistant to antibiotics.

DISCUSSION: In addition to S. aureus, CONS are now recognized to be important cause of infection including nosocomial bacteremia and infection of foreign bodies such as indwelling catheters and prosthetic heart valves.[8,9] Similar to S. aureus, resistance to methicillin and other microbial agents has been increasing among CONS. So, vancomycin is becoming the drug of choice in treatment of infection due to them.[15] The present study reports the emergence of vancomycin intermediate resistant staphylococcal colonization among the HCWs.

The present investigation is a clear reflection of the fact that as many as 63.75 % doctors, 63% nurses and 65.5 % attendants are colonized in their fingers by SA. The data in Table 1 also indicates the situation is almost comparable in both the tertiary level hospitals included in the study. Similar study carried out by Sobha et al[14] in India revealed a colonization rate of 31.7% by CONS in HCW but no SA. The present study revealed 35.9% and 64% colonization of CONS and SA respectively. This may be due to the sample collection method adopted by the present group by direct use of saline full loop and inoculation on MSA at collection site, yielding better isolation.[15]

Previous reports revealed vancomycin resistant S.aureus in Northern and Southern India,[16,17] though none was reported from Eastern part of the country. The present study revealed alarming data of VIS being 17.56 % of the total isolates, as the risk of transmission of these organisms between the patients is concerned. Among these intermediate- resistant strains 21.42 % were CONS.

Apparently these intermediate strains were found to be sensitive to vancomycin in the disc diffusion method, indicating necessity of MIC determination to detect vancomycin intermediate or resistant Staphylococcus spp. Previous study has already proved the existence of vancomycin intermediate and resistance among the Staphylococcus spp isolated from stethoscopes [18] which is an important source of nosocomial infection.

It is alarming to note that in the present study that VIS were found in the isolates of Government sector hospitals, where the use of this high priced antibiotic is comparatively lower. So it can be assumed that in different Government hospitals it might be occurring through the transmission of the resistant gene from one bacterial strain to another.

The development of VIS emanates from the widespread occurrence of vancomycin resistant strains of Enterococci[19] probably playing the role of resistance transmission and supports our conclusion. Proper identification of the sources of infection, use of proper disinfectants, hand washing etc are important in controlling this type of nosocomial infections. Unfortunately, once these strain have developed, they become more difficult to deal with.

So it is very much important to monitor appropriate vancomycin dose to ensure complete eradication of the bacteria. It is also necessary to adopt alternative therapeutic approaches such as combined chemotherapy etc, to control staphylococcal as well as other bacterial infection in addition to institution of stringent infection control measures and surveillance of isolates from cases and colonization areas.

REFERENCES:

1. Martindale, the Complete drug Reference, 35th Edition, edited by Sean C. Sweetman. Moellering, RC Jr. (2006). "Vancomycin: A 50-Year Reassessment". Clin Infect Dis 42: S3–S4.
2. Vancomycin-Wikipedia, the free Encyclopedia.
3. CDC. Reduced susceptibility of Staphylococcus aureus to vancomycin - Japan 1996. MMWR 1997; 46: 624-6.
4. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of Satphylococcus aureus heterogeneously resistant to vancomycin. Lancet 1997; 350: 1670-3.
5. Sonavane A, De A, Mathur M. Screening for vancomycin intermediate - resistant Staphylococcus aureus among clinical isolates of MRSA. Indian Journal of Medical Microbiology. 2007: 25 (1): 79-80.
6. Assadullah S, Kakru DK, Thoker MA, Bhat PA, Hussain N, Shah A. Emergence of low level vancomycin resistance in MRSA. Indian J Med Microbiol 2003; 21: 196-8.
7. R. Sq UERI, O.C. GRILLO, V. LA FAUCI. Surveillance and evidence of contamination in hospital environment from meticillin and vancomycin-resistant microbial agents. J prev med hyg 2012; 53: 143-145.
8. J G Collee A G Fraser B P Marmion A Simmons. Mackie & Mccartney Practical Medical Microbiology, 14th ed. Churchill Livingstone: New York; 1996.
9. Karchmer, A. W., G. L. Archer, and W. E. Dismukes. 1983. Staphylococcus epidermidis causing prosthetic-valve endocarditis: microbiologic and clinical observations as guides to therapy. Ann. Intern. Med. 98: 447-455.
10. Ml ckelsen, P. A., J. J. Plorde, K. P. Gordon, C. Hargiss, J. Mc Clure, F. D. Schoenknecht, F. Condie, F. C. Tenover, and L. S. Tompkins. 1985. Instability of antibiotic resistance in a strain of Staphylococcus epidermidis isolated from an outbreak of prosthetic valve endocarditis. J. Infect. Dis. 152: 50-58.
11. National Committee for Clinical Laboratory Standards: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A5. In NCCLS. 5th edition. Wayne, PA, USA; 2000.
12. Working Party of the British Soceity for Antimicrobial Chemotherapy 1991. A guide to antibiotic sensitivity testing. Journal of Antimicrobial Chemotherapy 27: Supplement D, 1-50).
13. Interference Testing In Clinical Chemistry; Approved Guideline- Second Edition. EP7-A2. Vol.25 NO.27. Clinical and Laboratory Standards Institute Guidelines.
14. KL Shobha, PS Rao, J Thomas. Survey of Staphylococcus isolates among hospital personnel, environment and their antibiogram with special emphasis on Methicillin resistance. Indian Journal of Medical Microbiology, (2005) 23 (3):186-188.
15. Arjun Srinivasan, James D. Dick, and Trish M. Perl. Vancomycin Resistance in Staphylococci. Clinical Microbiology Reviews, July 2002, p 430-438.
16. Hare K Tiwari and Malay R Sen. 2006. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious Disease 2006, 6:156

DOI: 10.14260/jemds/2014/3049

17. Venubabu Thati, Channappa T. Shivannavar & Subhaschandra M. Gaddad. 2011. Vancomycin resistance among methicillin resistant Staphylococcus aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res 134, November 2011, pp 704-708.
18. Denis O, Nonhoff C, Byl B, Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of Vancomycinintermediate Staphylococcus aureus in a Belgian hospital: microbiological and clinical features. J Antimicrob Chemother. 2002; 50: 383 – 391.
19. Joseph Baran Jr., Kathleen M. Riederer, Jambunathan Ramanathan, and Riad Khatib. Recurrent Vancomycin-Resistant Enterococcus Bacteremia: Prevalence, Predisposing Factors, and Strain Relatedness. Clinical Infectious Diseases Volume 32, Issue 9. Pp. 1381-1383.

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