A PROSPECTIVE STUDY OF ANTIBIOGRAM OF VANCOMYCIN RESISTANT (VANA) GENE POSITIVE METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS ISOLATES IN RURAL AND URBAN TERTIARY CARE SETUPS

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ABSTRACT

BACKGROUND

Vancomycin resistant *Staphylococcus aureus* isolates are innately resistance to other routinely used antibiotics. The present study was developed in identifying the vancomycin A (vanA) gene loci among methicillin sensitive *Staphylococcus aureus* (MSSA) isolates collected from rural and urban tertiary care setups.

MATERIALS AND METHODS

Staphylococcus aureus was confirmed by gram staining biochemical tests. Vancomycin resistance identified by agar dilution, E-test, disc diffusion and PCR methods were followed by antibiogram.

RESULTS

In this study 16665 samples were analysed, out of which 6538 were from rural and 10127 were from urban regions. In rural regions, Methicillin Sensitive *Staphylococcus aureus* (MSSA) constituted 401 (6.1%) and Methicillin Resistant *Staphylococcus aureus* (MRSA) 531 (8.1%); whereas in urban MSSA constituted 524 (5.2%) and MRSA 771 (7.6%). By using agar dilution method, Vancomycin Sensitive *Staphylococcus aureus* (VSSA) isolated in rural setup was 359 (89.5%), vancomycin intermediate resistant *Staphylococcus aureus* (VISA) was 35 (8.7%) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) was 7 (1.8%), but in case of urban setup VSSA isolated was 451 (86.1%), VISA 52 (9.9%) and VRSA 21 (4.0%). VISA and VRSA strains obtained from Agar dilution method were further confirmed by E-Test, disc diffusion and PCR methods. E-Test results for rural and urban setup were VSSA 2 (4.7%) and 1 (1.4%); VISA 33 (78.6%) and 51 (69.8%); VRSA 7 (16.7%) and 21 (28.8%), whereas disc diffusion results shown for VISA was 31 (73.8%) and 66 (90.4%), but in case of PCR VISA was 32 (76.2%) and 46 (63.0%), and VRSA 7 (16.7%) and 21 (28.8%). Cloxacillin (COX), Azithromycin (AZM), Gentamicin (G), Ciprofloxacin (CIP), Gatifloxacin (GAT), Tetracycline (TE), Cefoperazone (CPZ) and Piperacillin with tazobactam (PIT) were found to be highly significant ($P < 0.05$) and Cefuroxime (CXM) and Linezolid (LZ) were found to be not significant ($P > 0.05$) in Rural and Urban tertiary care centres.

CONCLUSION

Highest drug resistance was found in VRSA and then VISA strains of both setups. Piperacillin + tazobactam, tetracycline and linezolid were found to be useful drugs in treating VISA and VRSA infections.

KEYWORDS

VISA, VRSA, Agar Dilution, E-Test, VanA Gene, Drug Resistance.

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BACKGROUND

Staphylococcus aureus (SA) is responsible for a sizable number of infections globally^[1]; in 1960's methicillin was the drug of choice; soon after discovery it got resistance in 1961, then alternative effective anti-Staphylococcal antibiotics such as vancomycin were introduced.^[2] This is the drug of choice for the infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) and often used empirically for the patients in critical care settings with infections like severe sepsis, pneumonias and those under umbilical catheterisation

etc. in India. However, the first report of vancomycin resistance *Staphylococcus aureus* (VRSA) was by Hiramatsu et al (1997a, 1997b) from Japan in 1997.^[3,4] Since then, there are many reports of reduced susceptibility to vancomycin from all over the world including India.^[5,6,7]

There is a necessity of surveillance for *S. aureus* with reduced vancomycin susceptibility (SA-RVS); however, there are roadblocks since most Microbiology Laboratories perform disc diffusion test for antibiotic susceptibility which is not reliable for vancomycin testing.^[7] A survey by Centres for Diseases Control and Prevention (CDC) published in 2000, indicated that many laboratories participating in Emerging Infections Program were not using methods that can detect SA-RVS.^[8] Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for diagnosis of vancomycin susceptible *S. aureus* (VSSA), vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) are based on minimum inhibitory concentration (MIC) by microdilution or agar dilution methods and molecular methods.^[6,8,9] Reduced sensitivity to vancomycin in *S. aureus*

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occurs due to several genetic and phenotypic alterations in wild-type bacteria including altered expression of regulatory genetic elements, thickness of cell wall, changes in the penicillin binding protein (PBP) profiles and decreased cell wall autolysis.^[10]

We therefore aimed to determine the prevalence of drug resistance of VISA and VRSA among methicillin sensitive *Staphylococcus aureus* (MSSA) in rural and urban tertiary care setups.

MATERIALS AND METHODS

Study Settings

The prospective study was carried out during August 2013 to November 2015 for a period of 2 years 4 months and the approval was given by the Institutional Ethical Committee (IEC) at Saveetha University, Chennai, Tamilnadu. The experiment was conducted in two centres: 1) Rural setup or rural tertiary care centre, Rajiv Gandhi Institute of Medical Sciences (RIMS), Srikakulam, Andhra Pradesh; and 2) Urban setup or urban tertiary care centre, Andhra Medical College (AMC), Visakhapatnam, Andhra Pradesh.

Sample Selection

A total number of 16665 samples were analysed; 6538 (39.2%) were from rural and 10127 (60.8%) were from urban setups. Among these total samples, pus constituted 3106 (47.5%) and 4961 (48.9%) in rural and urban setups respectively. Percentage of *Staphylococcus aureus* isolated from pus samples was more in rural setup (14.3%) with predominance of MSSA compared to the urban setup.

Confirmation and Storage of *S. aureus* Isolates

Gram's staining was performed for all the samples and the likely organisms were determined. Then the pus samples were inoculated onto Blood agar, MacConkey agar and selective media (Mannitol Salt Agar) obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai and incubated at 37°C for 24 hours. The suspected colonies of *Staphylococci* were taken and Gram's staining was performed. All the Gram-positive cocci in clusters were further confirmed using a battery of standard biochemical reactions including the production of bound and free coagulase enzymes using slide and tube coagulase tests based on standard methods. A known coagulase positive strain *Staphylococcus aureus* ATCC-25923 was used as control. All confirmed *Staphylococcus aureus* strains were subsequently tested for Methicillin resistance based on recommendations of Clinical Laboratory Standard Institute (CLSI). Cefoxitin disc (30 µg) 15 was obtained from Hi-Media Laboratories Pvt. Ltd. to isolate MRSA and MSSA. If the zone of inhibition was 22 mm or less, isolates were considered as Methicillin resistant *Staphylococcus aureus* (MRSA) and the zone more than 22 mm was considered as Methicillin sensitive *Staphylococcus aureus* (MSSA).

Determination of Minimum Inhibitory Concentrations (MIC)

Agar dilution and E-test: As per National Committee for Clinical Laboratory Standards (NCCLS), *staphylococci* with MIC of vancomycin < 4 µg/ µL is susceptible, while for which the MIC is 8 - 16 µg/ µL are intermediate and those with MIC > 32 µg/ µL are resistant. Briefly, gradient plates of Mueller-Hinton agar (Hi-media) were prepared with vancomycin (2 -

32 mg/L, Sigma-Aldrich); 0.5 McFarland equivalent inoculum was prepared using 18 - 24 hrs. old culture was spotted on to gradient plates. Plates were incubated overnight at 35°C for 24 hrs. and assessed the visible growth. E-test (A and B) was obtained ranging from 0.016 to 256 µg based on the clear inhibitory zone calculated MIC. Clear intermediate resistant and resistant isolates were inoculated into the semi-solid nutrient agar and stored at -20°C until further study.

Genomic DNA Preparation

Frozen *Staphylococcus aureus* isolates were thawed and sub-cultured into brain-heart infusion broth (Hi-Media, Mumbai) and incubate at 37°C overnight. Genomic DNA was extracted using Macherey-Nagel Nucleospin food kit. GDNA concentration was measured using Thermo Scientific NanoDrop 8000. The average concentration for every sample was about 90 - 100 ng/ µL.

Conventional PCR

A Conventional PCR was performed following modified PCR analysis by Murugkar et al (2003)^[11] to the MicroAmp® 96-Well Reaction Plate (0.2 mL), added 3 µL buffer, 2 µL dNTPs, 0.3 µL Taq DNA polymerase (NEB, USA), 2 µL 5M Betaine, template 2µL, 20 picomoles concentration of Primer forward 2 µL, primer reverse 2 µL and HPLC water 6.7 µL and sealed accordingly with the applicator.

The ±1030 bp product of Vanco gene (*vanA*) was amplified by using primer set Forward- 5' ATGAATAGAATAAAAAGTTGC 3' and Reverse- 5' TCACCCCTTTAACGCTAATA 3' (Eurofins Genomics India Pvt. Ltd.) was used. Amplification was conducted in Applied Biosystems Veriti 96 well Thermal Cycler system. The amplification by Conventional PCR process was started with an initial denaturation step (94°C, 5 mins). Each cycle consisted of three steps (denaturation, annealing and extension). Each PCR reaction consisted of 35 cycles of amplification (initial 10 cycles was denaturation at 94°C for 1 min, annealing at 57°C for 1 min and DNA chain extension at 72°C for 1 min, last 25 cycles was denaturation at 94°C for 30 secs annealing at 62°C for 10 secs and DNA chain extension at 72°C for 30 secs). A final extension cycle was performed at 72°C for 5 mins (Applied Biosystems Veriti Thermal Cycler).

PCR products were detected by using Agarose gel electrophoresis. Electrophoresis was performed with 2% Agarose gel (Hi-Media) prestained with 0.5 µL/ 100 mL of Ethidium bromide (10 mg/mL). Gels were run at 80V using 1X TAE buffer and then photographed under UV illumination by using a Gel documentation system (UVITEC Cambridge).

Detection of susceptibility to other antimicrobial agents by disc diffusion method (Kirby-Bauer Disc diffusion method).

Antimicrobial susceptibility testing of the isolated strains of *Staphylococcus aureus* to various antimicrobial discs was carried out according to CLSI (2007)^[12] disc diffusion method. The *S. aureus* standard strain ATCC-25923 was used for quality control. All the antibiotic discs were obtained from Hi-Media Pvt. Limited, Mumbai, India. The following antibiotics like penicillin, cloxacillin, erythromycin, azithromycin, amikacin, gentamicin, ciprofloxacin, gatifloxacin, tetracycline, cephalexin, cefuroxime, cefoperazone, linezolid and combination drug like piperacillin + tazobactam were used and the diameter of zone of inhibition was measured with the help of special scale provided by Hi-Media, Mumbai, India.

Statistical Analysis

Data were registered in Microsoft Excel 2010 and analysed with Statistical Package for Social Science (SPSS) version 17 statistical software. Chi-square (χ^2) test allowed us to compare the prevalence of intestinal parasites according to age and sex.

RESULTS

In this study 16665 samples were analysed, out of which 6538 were from rural and 10127 were from urban regions. In rural setup, MSSA constituted 401 (6.1%) and MRSA constituted 531 (8.1%). Whereas in urban setup, MSSA constituted 524 (5.2%) and MRSA constituted 771 (7.6%) (Table 1). Percentage of *Staphylococcus aureus* (14.3%) isolated from pus samples was more in rural setup with a predominance of MRSA (8.1%) when compared to urban setup. Isolation of MSSA 43% and 40.5% and MRSA 57% and 59.5% in rural and urban setups respectively were found to be not significant.

In rural setup, out of 401 MSSA the percent of isolated was VSSA 359 (89.5%), VISA 35 (8.7%) and VRSA 7 (1.8%). In urban setup, out of 524 MSSA the percent of isolated was VSSA 451 (86.1%), VISA 52 (9.9%) and VRSA 21 (4.0%). In urban setup, percentage of VISA and VRSA isolated were compared with rural setup concentrations of vancomycin by agar dilution and distribution of VSSA. VISA and VRSA from MSSA in rural and urban tertiary care setups were found to be not significant ($P > 0.05$) by chi-square test (Table 2).

Results have been cross checked by E-Test, Disc diffusion and PCR methods for VISA and VRSA strains obtained from Agar dilution method. E-Test were VSSA 2 (4.7%) and 1 (1.4%); VISA 33 (78.6%) and 51 (69.8%); VRSA 7 (16.7%) and 21 (28.8%). Disc diffusion were only VISA 31 (73.8%), 66 (90.4%) and PCR were VISA 32 (76.2%), 46 (63.0%) and VRSA were 7 (16.7%) and 21 (28.8%) in rural and urban setups respectively. But none of the isolates were VRSA from disc diffusion method. Comparison of VISA and VRSA strains by Agar dilution, E-test and PCR methods in Rural and Urban tertiary care centres were found to be not significant ($P > 0.05$) (Table 3).

In rural setup, drug resistance less than 50% is seen. In VISA for amikacin (34.29%), tetracycline (25.71%), linezolid (0%) and piperacillin combined with tazobactam (22.86%). In VRSA for tetracycline (42.86%), linezolid (0%) and piperacillin combined with tazobactam (42.86%). In urban setup, drug resistance less than 50% is seen. In VISA for amikacin (34.62%), gentamicin (48.08%), tetracycline (34.62%), linezolid (0%) and piperacillin combined with tazobactam (26.92%). In VRSA for amikacin (42.86%) tetracycline (38.10%), linezolid (9.52%) and piperacillin combined with tazobactam (47.62%). In both the setups, VISA and VRSA are sensitive to amikacin, tetracycline, linezolid and piperacillin combined with tazobactam. Antibigram of Penicillin (P), Erythromycin (E), Amikacin (AK) and Cephalexin (CN) of VISA and VRSA were found to be significant ($P < 0.05$). Cloxacillin (COX), Azithromycin (AZM), Gentamicin (G), Ciprofloxacin (CIP), Gatifloxacin (GAT), Tetracycline (TE), Cefoperazone (CPZ) and Piperacillin with Tazobactam (PIT) were found to be highly significant ($P < 0.05$) and Cefuroxime (CXM) and Linezolid (LZ) were found to be not significant ($P > 0.05$) in Rural and Urban tertiary care centres (Table 4).

DISCUSSION

We have observed vancomycin resistance (vanA) gene loci in MSSA indicated that there was no relation of methicillin resistance or sensitivity of bacteria. So far there were huge published literature regarding the vancomycin intermediate or resistance among MRSA and association of SCCmec (methicillin resistant) gene, but the present study exhibited standard methods for detection of VISA and VRSA. VRSA could detect any one of the method of agar dilution, E-test and PCR. But in case of VISA standard method for detection is PCR compared to agar dilution, E-test and disc diffusion methods. In this study, we have observed 9 strains of VISA were phenotypically positive but genotypically (vanA gene loci) negative, this might be due to altered expression of regulatory genetic elements, thickness of cell wall and changes in the penicillin-binding protein (PBP) profile factors.

One control strain and the two VRSA strains from two setups were subjected to PCR amplification and Sanger sequencing. Sanger sequencing data uploaded in standard nucleotide data bank of Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology Information (NCBI). The search portal results were compatible with vancomycin-resistant enterococci (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA). GenBank numbers were allotted by NCBI as rural tertiary care setup MS-VRSA Banklt1890159 Seq1_SKLM3 KU641697 and urban tertiary care setup MS-VRSA Banklt1890159 Seq1_OMC3 KU641696.

Reduced sensitivity to vancomycin in *S. aureus* occurs due to several genetic and phenotypic alterations in wild-type bacteria including altered expression of regulatory genetic elements, thickness of cell wall, changes in the penicillin-binding protein (PBP) profiles and decreased cell wall autolysis (Hanaki et al, 1998). In the present study, isolation rate of VISA and VRSA by agar dilution in rural setup was 35 (83.3%) and 7 (16.7%), whereas in urban setup was 52 (71.2%) and 21 (28.8%) respectively. E-Test results showed VISA 33 (78.6%) with VRSA 7 (16.7%) and VISA 51 (69.7%) with VRSA 21 (28.8%) in rural and urban setups respectively. PCR results showed VISA 32 (76.2%) with VRSA 7 (16.7%) and VISA 46 (63.0%) with VRSA 21 (28.8%) in rural and urban setups respectively. By disc diffusion method VRSA was 0% in both centres, VISA 31 (73.9%) and 66 (90.4%) in rural and urban setups respectively. So we have concluded that the gold standard methods are agar dilution, PCR and E-test compared to disc diffusion method, which is not reliable. In VRSA similar results were found in the three methods. VISA strains cross checked by E-test and detected 33 and 51 after PCR amplification. VISA 32 and 46 were positive. Both the strain exhibited bands at 1032 bp position comparing with 100 bp ladder in an agarose 2% gel.

In the present study piperacillin + tazobactam, tetracycline and linezolid were found to be useful drugs in treating VISA and VRSA infections and similar findings were observed by Rajadurai pandi K et al^[13] with 100% sensitivity for linezolid. In the study by Rajesh Kamtikar et al,^[14] highest resistance was seen with penicillin and least resistance to vancomycin and linezolid and alarming resistance to the routinely used antistaphylococcal antibiotics like erythromycin (75% - 85%), cephalosporins group (50% - 100%) and gentamicin (50% - 75%). Rubeena Hafeez et al^[15] and Maninder Kaur et al^[16] recorded similar observations. In the present study resistance of Penicillin (P), Erythromycin (E), Amikacin (AK), Cephalexin (CN), Cloxacillin (COX), Azithromycin (AZM), Gentamicin (G),

Ciprofloxacin (CIP), Gatifloxacin (GAT), Tetracycline (TE), Cefoperazone (CPZ) and Piperacillin with tazobactam (PIT)

were found to be highly significant of VISA and VRSA strains of both setups.

Name of Tertiary Care Centre	All Samples	Pus Samples	Staphylococcus Spp.	Staphylococcus aureus	MSSA	MRSA
Rural - Rajiv Gandhi Institute of Medical Sciences (RIMS) Srikakulam, AP	6538 (100)	3106 (47.5)	1920 (29.4)	932 (14.3)	401 (6.13)	531 (8.12)
Urban - Andhra Medical College (AMC) Visakhapatnam, AP	10127 (100)	4961 (49)	2553 (25.2)	1295 (12.8)	524 (5.17)	771 (7.61)

Table 1. Isolation of MSSA from all Samples (n= 401, n= 524)

Name of the Centre	Total Strains	VSSA		VISA		VRSA	
		≤ 2 mcg		4 - 8 mcg		≥16 mcg	
		No	%	No	%	No	%
Rural tertiary care setup	401	359	89.5	35	8.7	7	1.7
Urban tertiary care setup	524	451	86.1	52	9.9	21	4.0

Table 2. Associations of Isolation of VSSA, VISA and VRSA by Agar Dilution among Rural and Urban MSSA

Chi-square= 4.50, Df= 2, P value= 0.106 Not significant.

Rural Tertiary Care Setup	Agar Dilution		E-Test		Disc Diffusion		PCR for vanA Gene	
	(n= 401)		(n= 42)		(n= 42)		(n= 42)	
	No	(%)	No	(%)	No	(%)	No	(%)
VSSA	359	89.5	2	4.7	-	-	-	-
VISA	35	8.7	33	78.6	31	73.8	32	76.2
VRSA	7	1.8	7	16.7	-	-	7	16.7
Urban Tertiary Care Setup	Agar Dilution		E-Test		Disc Diffusion		PCR for vanA gene	
	(n= 524)		(n= 73)		(n= 73)		(n= 73)	
	No	(%)	No	(%)	No	(%)	No	(%)
VSSA	451	86.1	1	1.4	-	-	-	-
VISA	52	9.9	51	69.8	66	90.4	46	63
VRSA	21	4	21	28.8	-	-	21	28.8
chi-square= 4.50 Df= 2 P value= 0.106			chi-square= 3.06 Df= 2 P value= 0.217		-		chi-square= 2.28 Df= 1 P value= 0.131	
Table 3. Associations of VSSA, VISA and VRSA Strains by Agar Dilution, E-Test, Disc Diffusion and PCR for VanA Gene Methods in Rural and Urban Setups								

Name of the Drug	Drug Content	Interpretation	Rural Tertiary Care Setup (n= 42)					Urban Tertiary Care Setup (n= 73)				
			VISA (n= 35)		VRSA (n= 7)		Total	VISA (n= 52)		VRSA (n= 21)		Total
			No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Penicillin (P)	10U	R	35	100	7	100	389	52	100	21	100	494
		S	0	0	0	0	12	0	0	0	0	30
Total			35	100	7	100	401	52	100	21	100	524
Cloxacillin (COX)	5 µg	R	23	65.71	6	85.71	218	33	63.46	15	71.43	242
		S	12	34.29	1	14.29	183	19	36.54	6	28.57	282
Total			35	100	7	100	401	52	100	21	100	524
Erythromycin (E)	15 µg	R	29	82.86	7	100	320	42	80.77	19	90.48	386
		S	6	17.14	0	0	81	10	19.23	2	9.52	138
Total			35	100	7	100	401	52	100	21	100	524
Azithromycin (AZM)	15 µg	R	24	68.57	7	100	254	28	53.85	15	71.43	255
		S	11	31.43	0	0	147	24	46.15	6	28.57	269
Total			35	100	7	100	401	52	100	21	100	524
Amikacin (AK)	30 µg	R	12	34.29	6	85.71	105	18	34.62	9	42.86	172
		S	23	65.71	1	14.29	296	34	65.38	12	57.14	352
Total			35	100	7	100	401	52	100	21	100	524
Gentamicin (G)	10 µg	R	22	62.86	7	100	218	25	48.08	13	61.9	226
		S	13	37.14	0	0	183	27	51.92	8	38.1	298
Total			35	100	7	100	401	52	100	21	100	524
Ciprofloxacin (CIP)	5 µg	R	30	85.71	7	100	312	40	76.92	19	90.48	312
		S	5	14.29	0	0	89	12	23.08	2	9.52	212

Total			35	100	7	100	401	52	100	21	100	524
Gatifloxacin (GAT)	5 µg	R	32	91.43	7	100	334	38	73.08	16	76.19	371
		S	3	8.57	0	0	67	14	26.92	5	23.81	153
Total			35	100	7	100	401	52	100	21	100	524
Tetracycline (TE)	30 µg	R	9	25.71	3	42.86	46	18	34.62	8	38.1	127
		S	26	74.29	4	57.14	355	34	65.38	13	61.9	397
Total			35	100	7	100	401	52	100	21	100	524
Cephalexin (CN)	30 µg	R	21	60	7	100	209	36	69.23	15	71.43	275
		S	14	40	0	0	192	16	30.77	6	28.57	249
Total			35	100	7	100	401	52	100	21	100	524
Cefuroxime (CXM)	30 µg	R	18	51.43	4	57.14	210	29	55.77	16	76.19	265
		S	17	48.57	3	42.86	191	23	44.23	5	23.81	259
Total			35	100	7	100	401	52	100	21	100	524
Cefoperazone (CPZ)	75 µg	R	32	91.43	7	100	362	46	88.46	21	100	434
		S	3	8.57	0	0	39	6	11.54	0	0	90
Total			35	100	7	100	401	52	100	21	100	524
Linezolid (LZ)	30 µg	R	0	0	0	0	6	0	0	2	9.52	14
		S	35	100	7	100	395	52	100	19	90.48	510
Total			35	100	7	100	401	52	100	21	100	524
Piperacillin with tazobactam (PIT)	100/10 µg	R	8	22.86	3	42.86	62	14	26.92	10	47.62	67
		S	27	77.14	4	57.14	339	38	73.08	11	52.38	457
Total			35	100	7	100	401	52	100	21	100	524
P value < 0.05* significant												
Table 4. Associations of Drug Resistance of VISA and VRSA Strains in Rural and Urban Setups												

Table 4. Associations of Drug Resistance of VISA and VRSA Strains in Rural and Urban Setups

CONCLUSION

VISA or VRSA can be detected using any one of the method of agar dilution, E-Test or PCR, but not using by disc diffusion method because of its disc content of 30 µg. PCR is the more specific, sensitive and standard method in detecting even low copy number of organism. We have found that vanA gene loci can be identified in any gram-positive bacteria and which is not related to methicillin sensitive or resistant. Highest drug resistance found in VRSA, then VISA strains of both setups. Piperacillin + tazobactam tetracycline and linezolid were found to be useful drugs in treating VISA and VRSA infections.

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