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Prevalence and in vitro susceptibility pattern of MRSA,VRSA, VISA isolates from various clinical samples in tertiary care hospital

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Abstract

Background: Staphylococcus is notorious for its ability to become resistant to antibiotics. MRSA emerged as nosocomial pathogen in the early 1960. Methicillin Resistant *Staphylococcus aureus* are implicated in serious infections and nosocomial infection outbreaks, thus limiting the treating options to very few agents such as vancomycin and teicoplanin. Vancomycin has been regarded as the first line drug for the treatment for MRSA but its irrational use lead to emergence of vancomycin resistance. The Aim was to determine the prevalence and resistance of MRSA ,VRSA,VISA isolates from various clinical samples in a tertiary care hospital. **Material & Methods:** This present prospective study was done in the Microbiology department of Government Medical College. The study was conducted for a period of one and half year i.e from January 2019 to June 2020. All the samples (pus, urine, blood, body fluids, sputum etc) were processed as per standard protocols. **Results:** Out of 26,471 samples, 6578(24.85%) were found to be culture positive. 1583 isolates were identified as Staphylococcus aureus. Among them 1278(80.7%) were MRSA, 21(1.3%) were VISA and 8(0.5%) were VRSA. Maximum number of MRSA isolates were obtained from orthopaedics ward (22.7%) and Intensive Care Unit and most of them were isolated from pus(45%) followed by blood (19.09%)samples. Among them highest resistance were observed against azithromycin (85.6%), followed by ciprofloxacin (63.5%) and least resistance to rifampicin and doxycycline. Majority of the VISA and VRSA strains were isolated from ICU followed by orthopaedics, surgery. Most of them were isolated from pus followed by blood and urine specimen and most were found to be multidrug resistant while they retained their sensitivity to Linezolid and Teicoplanin. **Conclusions:** As there is high prevalence of MRSA isolates so the treatment options are limited to vancomycin. Overuse of vancomycin can lead to emergence of VRSA strains. So the need for rational use in the infection-control practices to prevent transmission of MRSA as well as VISA strains. Strict implementation of hand hygiene, decolonization of MRSA carriers,and education of healthcare team will be quite helpful.

Keywords:- Antimicrobial resistance, Staphylococcus aureus, MRSA, VRSA & VISA.

INTRODUCTION

The genus Staphylococcus includes pathogenic organisms in which Staphylococcus aureus is the most important pathogen causing wide variety of infections ranging from mild skin and soft tissue infections to serious life-threatening infections.[1] The bacterium also

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causes toxin mediated diseases such as food poisoning, Toxic Shock Syndrome and Staphylococcal Scalded Skin Syndrome. Strains of S.aureus can also host phages, such as PVL (Panton Valentine Leukocidin),that increases its virulence. It is a major cause of nosocomial infections including pneumonia, post-operative wound infection bacteriemia and other infections.

Staphylococcus aureus is present as normal flora of skin and anterior nares in humans and upto two third of the population is colonized by it, responsible for causing a broard spectrum of diseases in hospital as well as community and and cause resistance to commonly used antibiotics.[2] Most notable example was the emergence of Methicillin Resistance Staphylococcus aureus (MRSA) which was just reported one year after the launch of methicillin. Today Methicillin Resistant Staphylococcus aureus is one of the commonest bacterium causing nosocomial infections and has now become a community pathogen.[3] Indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse and nasal carriage of MRSA are important risk factor for its acquisition.[4]

Key determinant of Methicillin resistance is the mecA gene, which is encoded in a novel penicillin binding protein 2a (PBP2a).[5] The mecA gene is carried on a genetic element designated as the staphylococcal cassette chromosome mec" (SCCmec), inserted near the chromosomal origin of replication.[6]

Hospital acquired MRSA (HA-MRSA) are typically the result of clonal spread by MRSA being transferred from patient to patient, frequently using healthcare personnel as intermediaries. HA-MRSA strains are multidrug resistant. MRSA is now emerged as a widespread cause of community infection as well.CA-MRSA can spread rapidly among healthy individuals. Most common .

presentation of CA-MRSA is soft tissue infection to the life threatening infections like necrotizing pneumonia.[7]

Vancomycin has been regarded as the first line drug for treatment of MRSA. Indiscriminate use of this drug lead to the emergence of resistance of VRSA strains and it was first reported in 1997 from Japan.[8] This is of great concern and hardly any treatment options left. So the aim of the study was to determine the prevalence of MRSA, VRSA,VISA isolates from various clinical samples in tertiary care hospital.

MATERIAL AND METHODS

This prospective observational study was done in the Microbiology department of Government Medical College, Amritsar. The study was conducted for a period of one and half year i.e from January 2019 to June 2020. All the samples (pus, urine, blood, body fluids, sputum etc) from patient of all age groups and both genders admitted in the hospital and received in the Microbiology Department of Government Medical Collage, Amritsar was processed as per standard protocols.

Samples were processed and cultured on Blood and Mac Conkey agar and incubated for 24 hours aerobically at 37°C. Identification of Staphylococcus aureus were made based on the colony characteristics, Gram staining and motility and by using standard microbiological

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techniques.[9] Antimicrobial susceptibility was performed by Kirby Bauer Disk diffusion method as per CLSI guidelines.

Various antibiotics included were -Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (15µg), Aziyhomycin (30µg), (Norfloxacin (10µg), Nitrofurantoin (300µg) in case of urine), Doxycycline (30µg) linezolid (30µg), Teicoplanin (30µg), Quinupristin- dalfopristin (15µg), Clindamycin (2µg), Rifampicin (5µg).[10]

All the isolates were subjected to cefoxitin disk diffusion test using a 30 µg disk. A lawn culture was done on MHA plate. Plates wereincubated at 35°C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 21 mm was reported as Methicillin resistant and ≥ 22 mm. is sensitive.[11]

The susceptibility to vancomycin was screened by vancomycin screen agar method and further confirmed by broth dilution method as per CLSI guidelines.

RESULTS

During the study period a total of 26,471 samples received from the patients admitted in various indoor/outdoor departments of tertiary care hospital, Amritsar. Out of total clinical samples, 6,578 (24.85%) samples were found to be culture positive. Amongst them, 3565(54.75%) Gram-negative and 3013 (45.25%) Gram-positive isolates were identified.

Among the Gram positive 1583(52.53%) Staphylococcus aureus were isolated and were tested for Methicillin resistance and Vancomycin resistance screening and confirmatory methods and 305 (19.27%) isolates were MSSA, 1278 (80.73%) were MRSA, 21(1.3%) were VISA and 08(0.5%) were VRSA

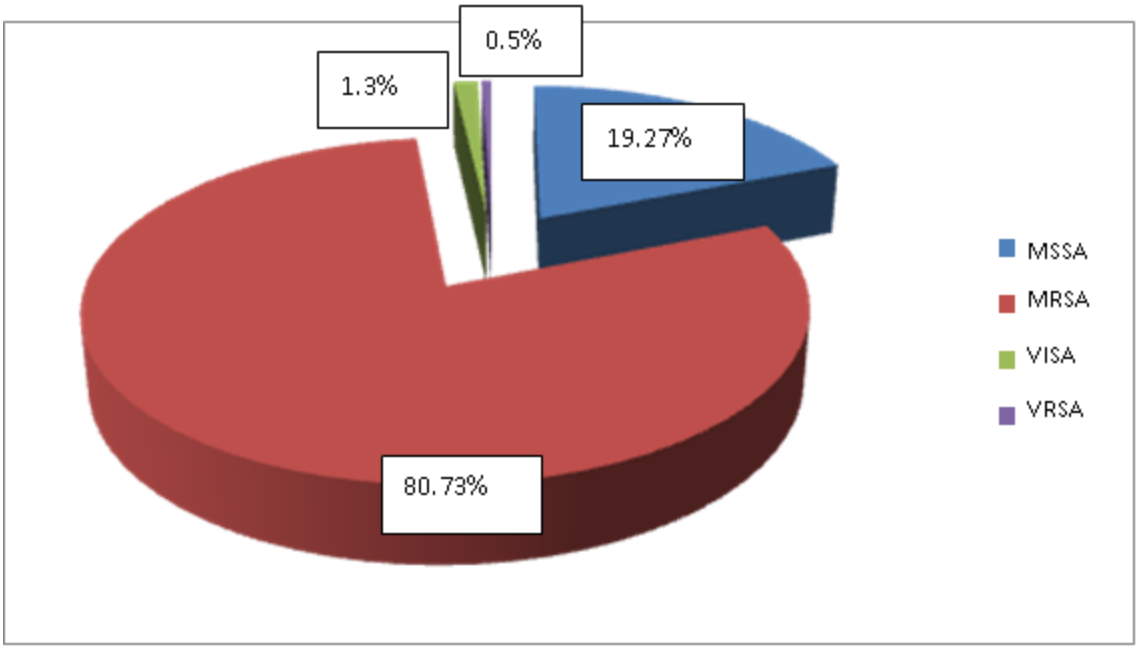

Figure 1: Prevalence of Staphylococcus aureus isolates

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Total 1583 isolates were tested to detect the Methicillin resistance by cefoxitin (30µg) disc by Kirby Bauer Disk diffusion method as per CLSI guidelines and found 1278(81%) to be Methicillin resistant.

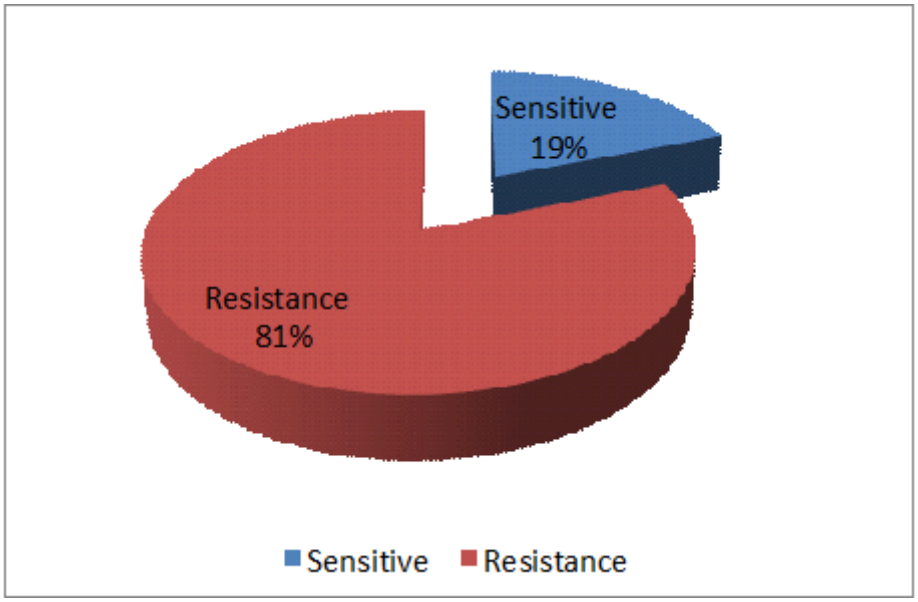

Figure 2: Testing of Methicillin Resistance among staphylococcus aureus

All Staphylococcus aureus were also screened for vancomycin resistance by agar dilution method. 36 Staphylococcus aureus isolates which were resistant to vancomycin by screening test were being confirmed for vancomycin resistance by macro broth dilution method as per CLSI guidelines and out of 36, 29 came out to be vancomycin resistant.

Table 1: Confirmatory test for detection of vancomycin resistance

MIC of Vancomycin as per CLSI	Number of isolates of Staphylococcus aureus	Percentage
<2 µg/ml (VSSA)	7	98.17%
4-8 µg/ml (VISA)	21	1.33%
>16 µg/ml (VRSA)	08	0.5%
Total	36	100%

Table 2: Sample wise distribution of MRSA, VISA and VRSA isolates

Ward	MRSA(1278)	VISA(21)	VRSA(08)
Pus	329(25.74%)	09(42.85%)	04(50%)
Wound	246(19.24%)	02(9.52%)	01(12.5%)
Blood	24419.09%)	07(33.33%)	02(25%)
Urine	171(13.4%)	01(4.76%)	01(12.5%)
Vaginal	144(11.3%)	-	-
Catheter tip	89(6.96%)	02(9.52%)	-

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Sputum	18(1.40%)	-	-
Umblical catheter	16(1.25%)	-	-
Pleural /ascitic fluid	08(0.62%)	-	-
Ear discharge	07(0.55%)	-	-
CSF	06(0.47%)	-	-

Maximum number of MRSA isolates were obtained from Orthopaedics ward (22.7%) followed by Intensive Care Unit (21.90%) and Gynaecology ward (18.0%).
Majority of the VISA and VRSA strains were isolated from ICU(38.9% followed by orthopaedics (23.8%), NICU(13.5%).

Table 3: Antimicrobial Resistance pattern of MRSA,VISA,VRSA isolates

Antibiotics	**MRSA(1278)**	**VISA(21)**	**VRSA(08)**
Amikacin (30µg)	215 (16.8%)	07(33.3%)	05(62.5%)
Gentamicin (10µg)	537(42%)	11(52.3%)	06(75%)
Ciprofloxacin(15µg)	431 (63.5%)	21(100%)	08(100%)
Azithromycin (30µg)	946(85.6%)	20(100%)	05(75%)
Norfloxacin(10µg)	114 (66.7%)	01(4.76%)	01(12.5%)
Doxycycline(30µg)	111(8.7%)	12(57.2%)	07(87.5%)
Nitrofurantoin(300µg)	42(24.6%)	01(4.76%)	01(12.5%)
Clindamycin(2µg)	711(55.6%)	07(33.3%)	06(75%)
Rifampin (5µg)	161(12.6%)	12(57.2%)	05(62.5%)
Vancomycin (by macro broth dilution)	0	21(MIC in range of 4-8µg/ml)	08(MIC ≥16µg/ml)
Linezolid (30µg)	2(0.2%)	0	0
Quinopristin/ Dalfopristin (15µg)	1108(86.7%)	13(61.9%)	07(87.5%)
Teicoplanin(30µg)	0	0	0

Note-Norfloxacin & Nitrofurantoin is for urine isolates only Azithromycin is not for urine samples.

DISCUSSION

The global spread of resistant Staphylococcus aureus constitutes one of the most serious challenges to the treatment of hospital acquired infections. It represents a threat to the successful treatment of bacterial infections with subsequent adverse consequences of increased morbidity, mortality, length of hospital stays, and health care costs.[12] Resistance to Methicillin in S. aureus is associated with the resistance to multiple drug resistance (MDR). MRSA with MDR leaves a limited choice of antibiotics for treatment, and causes difficult-to-treat infections.[13]

In 1980 empiric therapy for nosocomial Staphylococcus infections was changed to vancomycin in MRSA. Due to increased vancomycin use Vancomycin Resistant Staphylococcus aureus (VRSA) has been reported now.

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Out of 26,471 samples, 6,578(24.85%) were found to be culture positive. Out of these 3013 were Gram positive, among them1583(52.5%) isolates were found to be Staphylococcus aureus which was the most common isolate in present study, concordance with the studyof Mamtora D et al.[14]

Among the total of 1583 Staphylococcus aureus, 1278 were resistant to methicillin i.e. 80.73% which was in concordance with the study of Verma et al where the prevalence of MRSA was 62.14 to 80.89% were reported. High prevalence could be attributed to poor infection control policies.[15]

Lower prevalence of MRSA in comparison to present study were reported by Subedi et al, Baral et al (26%) and Pandey et al (26.12%).[16,17,18] the difference in the prevalence of MRSA among different studies may be due to difference in location and time period of study and also differ from one hospital to another hospital.

A total of 1,583 Staphylococcus aureus screened for vancomycin resistance by agar dilution method and 36(2.27%) were found to be vancomycin resistant.They were confirmed by macrobroth dilution method. Out of 36 screening positive S.aureus 21 isolates were having MIC 4-8µg/ml i.e. VISA(1.33%) and 08 isolates having MIC in range of >16µg/ml i.e. VRSA(0.55%) and there was an association between the screening and confirmatory resistance vancomycin test which was statistically significant(p value=<0.001), concordant with the study conducted by Randhir Kumar et al in which vancomycin resistance were screened by disc diffusion method and MIC were determined by agar dilution method,[8] and in this study out of 633, there was 63(9.95%) VISA and 24(3.75%) were VRSA.This emergence of glycopeptides resistance may be due to building of selective pressure of vancomycin, unjustified use of antibiotics, over the counter availability without prescription.

In present study maximum MRSA isolates were from pus 329(45%) and blood 244(19.09%) which was in concordant with the study done by LK Khanal et al where MRSA isolation rate was higher from the pus samples {wound swabs (76.9%),followed by purulent exudates (67.7%) and abscesses (64.1%).[19] The maximum isolates were from pus samples, could be due to the exposure of wound to micro-organisms in the environment and S aureus present on skin as a commensal make the wound more prone to infection.

Maximum MRSA was isolated from orthopedics 290 (22.7%) followed by ICU 280 (21.9%), Gynaecology 230(18%) and NICU 143 (11.19%).which is similar to study conducted by JB Sharma et al in which MRSA colonization rate was found to be 85.36% and 78.57% in orthopedics and ICU respectively.[20] This could be due to the reason that these patients are exposed to antimicrobials as prophylaxis which in every single case is invariably continued irrationally for several days. Immunosuppression in patients admitted in ICU may also lead to more chance to MRSA infection.

Among the VISA and VRSA strains maximum were isolated from pus (46.4%)specimens. This was found to be similar to the study conducted by Randhir kumar et al.[8] in which most of VRSA strains were isolated from pus 54%,

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urine 28.6%, blood 11.1% and vaginal swab 6.3% respectively.

Majority of the VISA and VRSA strains were isolated from ICU(38.9%) patients which was similar with the study conducted by Moses V et al,[21] in which most of the isolates were from ICU patients. Other study conducted Mohanty S et al,[22] most of the isolates were from surgery, followed by ICU and Pediatrics. Because most of the ICU patients have associated co-morbid conditions which decrease their immune response and they are more prone to infection.

MRSA isolates showed highest resistance to azithromycin (85.6%), and least resistance to rifampicin. This was in close similarity with the studies conducted by mazhar salim al-zoubi et al and Dhanalakshmi T.A et al.[10]

It was observed that 21 VISA (MIC in range of 4-8µg/ml) and 08 VRSA(MIC-≥16) were obtained out of 1583 staphylococcal isolates. Most of them were found to be multidrug resistant while they retained their sensitivity to Linezolid and Teicoplanin, similar findings were observed in the study conducted by Bhattacharya D et al, Venubabir thati et al, Mohanty S et al.[22,23,24]

CONCLUSIONS

As there is high prevalence of MRSA isolates in our institute, so the treatment options are limited with vancomycin being the most effective drug available. However, overuse of vancomycin can lead to emergence of VRSA strains. This is of great concern as hardly any treatment options are left. So, this study emphasizes the need for continuous monitoring of MIC levels of vancomycin in MRSA, and the importance of its rational use in the infection-control practices to prevent transmission of MRSA as well as VISA strains. Strict implementation of hand hygiene, decolonization of MRSA carriers, active surveillance of recently admitted at risk patients and education of healthcare team will be quite helpful in this regard.

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