DETECTION OF MRSA AND VRSA *STAPHYLOCOCCUS AUREUS*FROM TERTIARY CARE CENTER, CHANDRAPUR, MAHARASHTRA.

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ABSTRACT

The enlarge resistance factor of methicillin resistant *Staphylococcus aureus* (MRSA) strains to Vancomycin has been perceived as an alarmist threat in therapeutic fields. The most notorious multidrug resistance hospital pathogen has spread in global manner. MRSA and VRSA repeatedly turned down the dispute with large number of chemotherapeutics, harvest mordent green chemistry. This paper is intended to provide clinical presentation of the prevalence of methicillin and vancomycin resistance of *S.aureus.* Out of 102 isolates of *Staphylococcus aureus,* 86 isolates were found to be methicillin-resistant *S. aureus* (MRSA) and 16 isolate were found to be vancomycin resistant *S .aureus.* The Minimum inhibitory concentration (MIC) for 16 multi drug resistant VRSA isolates that showed intermediate and resistant results was determined using the MIC test strips according to CLSI guidelines The prevalence of MRSA was 84.32.% by Kirby Bauer disc diffusion method and 15.68% 0f VRSA by Antimicrobial Susceptibility test (AST). It was noticed that among the 102 isolates, 55 were from male patients and 47 were from female patients. Treatment of multi drug resistant MRSA is problematic because the choice of antibiotics in such cases in very limited. Sophisticated strategies and costly efforts to limit the growth of this epidemic known as "Superbug"

Keywords: - MRSA, VRSA, S.aureus, Chemotherapeutics Antibiotic resistance

1. Introduction

An antibacterial is substance that kills or inhibits the growth of bacteria. On the basis of mode of action, antibacterial are broadly classified into two broad categories as bactericidal that kill bacteria without leaving any option for their survival and bacteriostatic that cease all metabolic activities of bacteria that are important for their survival so they are called as growth inhibitor bacteria (TuazonCU*et, al.,1993*) *Staphylococcus* genus is a heterogeneous group of bacteria consisting of 30 species. *Staphylococcus aureus* has been found to be the most clinically important species, it has been recognized as one of the most common cause of human infections, such as skin infections, wound infections and bacteremia. The introduction of antibiotics has lowered the mortality rate of S. aureus infections. However, the bacteria have rapidly developed resistance mechanisms against many antimicrobial agents. (Shands KN, *et.al., 1980*) Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated and recognized more than 50 year ago. MRSA is a specific strain of the *S. aureus*, which is resistant to methicillin and all β-lactams. MRSA are all members of β-lactam antibiotics. (Gahin-Hausen B*et .al 1987*). Clinical isolates of vancomycin-resistant *S. aureus* (VRSA) have been reported recently .The emergence of *S. aureus* isolates resistant to vancomycin and other wide range of structurally un-related antibiotics have elevated MRSA into a multidrug- resistant rise a uniquely effective

antibiotic resistance mechanism that can protect the microorganism against 'Superbug", making it more and more dangerous than ever in a hospital environment and also the healthy community (Gahin-Hausen B*et al 1987*; Hiramatsu K*et al .,1997*) .

The global spread of MRSA and VRSA constitutes one of the most serious growing challenges in medical community that repeatedly turned down with large number of chemotherapeutics, harvest mordent green chemistry. It is not limited to medical facilities and healthcare institution anymore. (Hiramatsu K*et,al .,1997*)

1.1. Clinical presentation

The objectives of the present study were to detect the prevalence and identify the multi-drug resistant MRSA and VRSA from clinical specimens in tribal region and this cross section study was carried out at Chandrapur district Maharashtra, India.

- **Bacterial isolates**

Our study included specimens that are collected between October 2017 and May 2018. A total of 150*S. aureus* isolates from male and female , all age group of out or inpatients were obtained randomly taken from various body sites of infection including blood, urine and throat swabs, wound and ear swabs in different wards from Government medical college & hospital Chandrapur (MS).The Centre for higher learning and research, microbiology department of Sardar Patel Mahavidyalaya (MS) India. *S.aureus* was identified by (1) Dependent variables (primary screening): Gram staining, Biochemical test ,Sugar fermentation test Catalase , DNAse, Coagulase culture on Mannitol salt agar or blood agar(2) Independent variables (secondary screening): Antibiotic susceptibilitytesting**,** Minimal inhibitory concentration**(**MIC**)** ,using standardcollection techniques (CLSI). A total 102 sample were positive for *S.aureus* out of which 60 clinical samples from pus, 40 clinical samplesfrom urine and 2 clinical sample from blood.

- **Antibiotic susceptibility testing**:

The antibiotic-resistance profile was determined by the Dis Diffusion Agar technique using different antimicrobial agents; penicillin (10 ug), oxcilling (10 µg), methicillin (10 µg), ampicillin (10 µg), ceftazidime (10 µg), gentamicin (10 µg), tetracycline (10 µg) ; vancomycin (10ug) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI).(3,5) The standard*S. aureus*strains NCTC 5522 and 5521 were used as reference strains (department of microbiology, S.P College Chandrapur.)

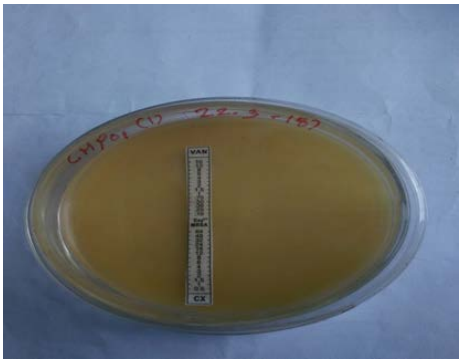


Fig. 1- AST for vancomycin against *S.aureus* strain

- **MIC Determination:**

Minimal inhibitory concentration **(**MIC**)**of vancomycin was determined by CLSIguidelines Brieflygradient plates of Mueller-Hinton agar (Hi-media) were prepared with vancomycin -Cefoxitin (VAN 0.19-16.0 mcg/ml &CX 0.5-64 mcg/ml).and Oxacilline - vancomycin (OXA: 0.064-8.0 mcg/ml & VAN: 0.19-16.0 mcg/ml) both are dual antibiotic strip, equivalent inoculum prepared using 18-24 h old culture was spotted on to gradient plates. Plates were incubated over night at 35°C for 24 h before assessing the visible growth.

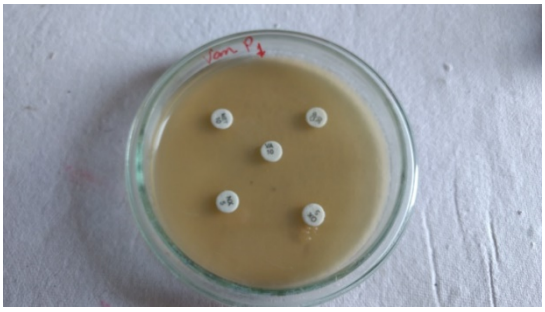


Fig. 2 - E-test for determination of MIC of vancomycin against *S. aureus* strain.

2. Result

*2.1*Only a few reactions are most commonly used that are medically important for distinguishing *S.aureus*. Which are as follows

Table 1 - Diagnostic Identification

Test	Interpretation	Result
Gram staining	Cocci , grapes	Gram positive
MSA	Yellow colour, Mannitol fermentation	Positive
Blood agar	Beta-haemolysis	Positive
ORSA	Intense and diffuse blue colour	Positive
BPA	Reduce telluride , black colonies	Positive
CLED agar Positive	Yellow colonies , lactose fermentation	Positive

2.2 Below is the list of various biochemical test which have great importance in research work

Table 2 – Biochemical test

Test	Result
MP-VP	Positive
Indol	Negative
Citrate	Positive
Urease	Positive
Catalase	Positive
Coagulase	Positive
DNAse	Positive
CLED agar	Positive

2.3 S. aureus ferment various sugars producing without gas. Mannitol fermentation positive which is of great importance in differentiating S.aureus.

Table 3 – Sugar fermentation test

Test	Result
Fructose	Positive
Glucose	Positive
Lactose	Positive
Mannitol	Positive
Maltose	Positive

2.4Out of 102 isolates of *Staphylococcus aureus*, 86 isolates were found to be methicillin-resistant *Staphylococcus aureus* (MRSA). And 16 isolate were found to be vancomycin resistance *Staphylococcus aureus* .The prevalence of MRSA was 84.32.% by disc diffusion method & 15.68% 0f VRSA by Antimicrobial susceptibility test(AST).

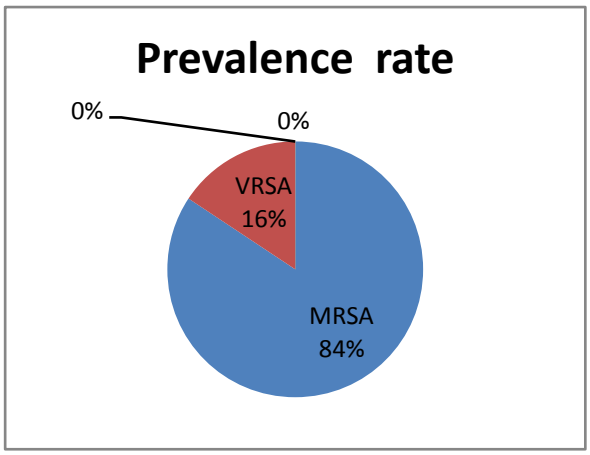


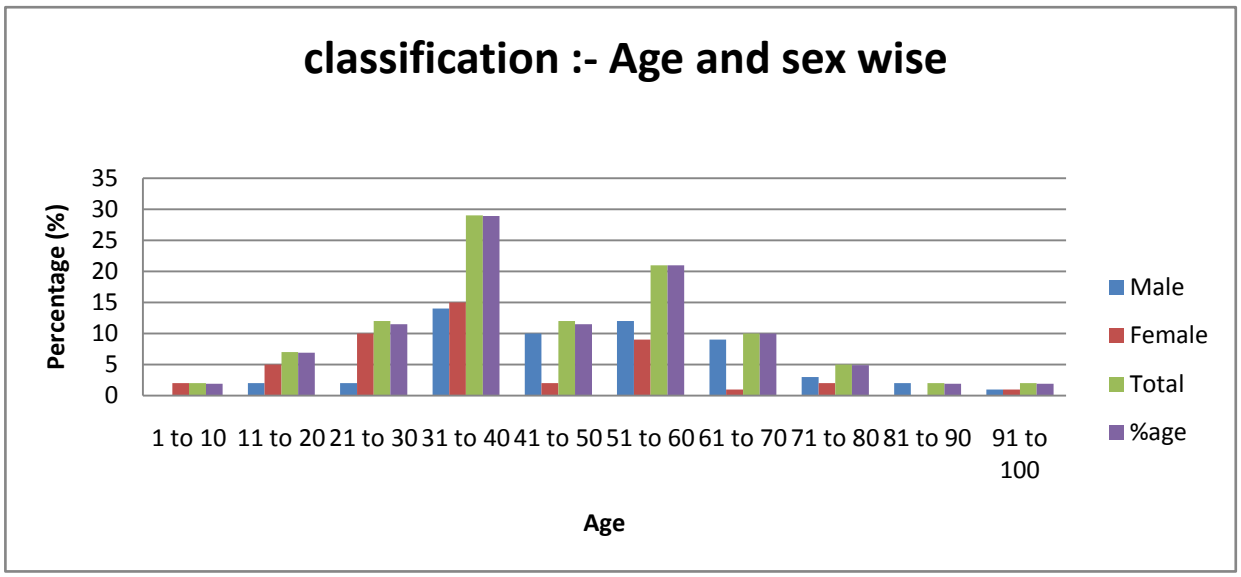
Fig. 3 - Represent the prevalence rate of MRSA and VRSA

*2.5*The age of the patients from whom MRSA were obtained ranged from 1 months to 100 years of age. It was noticed that amongst the 102 isolates, 55 were from male patients and 47 were from female patients.

Table 4:- Age and sex wise distribution of patient Male to female ratio was 2:1. Maximum numbers of isolates were from age group 31 to 40 years.

Table 4 - Age and sex wise distribution of patient s

Age	Male	Female	Total	%age
1-10	0	2	2	1.9
11-20	2	5	7	6.9
21-30	2	10	12	11.5
31-40	14	15	29	28.9
41-50	10	2	12	11.5
51-60	12	9	21	21
61-70	9	1	10	10
71-80	3	2	5	4.9
81-90	2	0	O2	1.9
91-100	1	1	2	1.9
Total	55	47	102	100



*2.6*The MIC for 16 of 102 isolates (15.68 %) for vancomycin was unique MIC determination paper strip. which is coated with two different antibiotic on a signal strip in a concentration gradient manner The upper half has Vancomycin with a highest concentration tapering downloads and capable of showing MIC in range of 0.19-16.0 mcg/ml , whereas lower half is similarly coated with Cefoxitin concentration gradient to given MIC in the range of 0.5-64.0mcg/ml . out of Sixteen isolates nine showed an MIC range between 4-8 mcg/ml, indicating vancomycin intermediate resistance. For the remaining seven isolates, the MIC was in the range of 16-64 mcg/ml indicating that these seven isolates were vancomycin-resistant (VRSA).

Table-5 Description of VRSA including antibiotic susceptibility as determined by disc diffusion method. (Ca-ceftazidime; G-gentamycin; E-erythromycin,V-vancomycin;Me-methicillin; O-oxacillin;T-tetrcyline P-penicillin)

Isolate no	specimen	Resistance to	Susceptible to
VRSA 1	Wound swap	V,Me,O ,E	T,P,G,Ca
VRSA 2	Wound swap	Me,O,G,V	E,P,T,Ca
VRSA 3	Wound swab	V,O,E,Me	T,G,P,Ca
VRSA 4	Urine	T,E,G	V,Me,O
VRSA 5	Wound swab	V,O,Me	P,Ca,T,G
VRSA 6	Urine	Ca,T,G	Me,V,O
VRSA 7	Urine	V,O,Me	P,Ca,T

3. Discussion

Infections caused by methicillin-resistant *S. aureus* have been associated with high morbidity and mortality rates in hospital.This study was conducted at chandrapur district hospital which is publicize as tribal region so cosmic rang of malnutrition patient finding in tertiary care hospital.
As above report antimicrobial susceptility by MIC, Etest method 16 isolates of Vancomycin resistance ,and prevalence rate of VRSA is 15.69%,and 86 isolates were found to be methicillin-resistant The prevalence rate of MRSA was 84.32.% by disc diffusion method. A study has indicated that experiments with presence of at concentration of 10ug/ml of various antibiotics.

This dangerous antibiotic resistance development occurred close to another worrisome important milestone in the history of MRSA.Vancomycin is vital antimicrobial agent available to treat serious infections with MRSA but unfortunately, decrease in vancomycin susceptibility of *S. aureus*and isolation of vancomycin-intermediate and resistant *S. aureus* have recently been reported From manycountries. Vancomycin-resistant *S. aureus* tend to be multidrug resistant against a large number of currently available antimicrobial agents.

4. Conclusion

Treatment of multi drug resistant MRSA is problematic because the choice of antibiotics in such cases in very limited. The activity of all available drugs must be tested against the isolate to establish which ones could be used to treat these infections; in order to control the dissemination of such multidrug resistant bacteria.it is evolving into a growing epidemic, increasingly claiming victims. Sophisticated strategies and costly efforts to limit the growth of this epidemic are needed to stem the severe consequences known as “Superbug.

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