Final Report of Major Research Project

F No. 43-473/2014 (SR) dated 17-11-2015

(01/07/2015 to 30/06/2018)

"A Study on Methicillin and Vancomycin Resistant *Staphylococcus aureus* Carrying mecA and vanA Gene Complex from Tertiary Care Centers."

Submitted

To

ज्ञान - विज्ञानं विमुक्तये

University Grants Commission

BAHADUR SHAH ZAFAR MARG

New Delhi -110 002

Submitted

By

Principal Investigator

Dr. V. S. Wadhai, M.Sc.,Ph.D.

Associate Professor and Head

Department of Microbiology,

Sardar Patel Mahavidyalya, Chandrapur,

(M.S.) India 442402.

Declaration

I, **Dr. Vijay S. Wadhai** hereby declare that the work presented in this Final report **"A study on methicillin and vancomycin resistant *Staphylococcus aureus* and carrying mecA and vanA gene from tertiary care centers."** is the outcome of my study under University Grants Commission's Major Research Project. It has not been submitted previously in part or full to anywhere for the award of any degree.

PRINCIPAL INVESTIGATOR
DR. V.S.WADHAI, M.Sc., Ph.D.
ASSOCIATE PROFESSOR AND HEAD
DEPARTMENT OF MICROBIOLOGY
SARDAR PATEL MAHAVIDYALAYA
CHANDRAPUR, (M.S.) INDIA.

Acknowledgement

I feel grateful indebted to the University Grant Commission, New Delhi for the award of Major Research Project and the financial assistance to pursue the research project. I convey my sincere thanks to Principal **Dr. R.P.Ingole, Sardar Patel Mahavidyalaya Chandrapur** for providing basics infrastructure facilities in the department of **Microbiology** to carry out the project.

Principal Investigator
UGC Major Research Project

PRINCIPAL INVESTIGATOR
DR. V.S.WADHAI, M.Sc., Ph.D.
ASSOCIATE PROFESSOR AND HEAD
DEPARTMENT OF MICROBIOLOGY
SARDAR PATEL MAHAVIDYALAYA
CHANDRAPUR, (M.S.) INDIA.

Annexure – III

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator : **Dr. Vijay Shamrao Wadhai**
2. Deptt. of Principal Investigator : **Microbiology**
   University/College : **Sardar Patel Mahavidyalaya, Chandrapur-442402**
3. UGC Approval Letter No. and Date : **F.No.43-473/2014 (SR)** dated on **17/11/2015**
4. Title of Research Project : **"A study of methicillin and vancomycin resistance *Staphylococcus aureus* isolates caring *mecA* and *vanA* gene complex from tertiary care centers ".**
5. Effective date of starting the projects : **01/07/2015**
6. a. Period of Expenditure : From **01/02/2016** to **30/06/2018**

   b. Details of Expenditure:

Sr. No.	Item	Amount Allocated (Rs.)	1st Amount Approved (Rs.)	2nd Amount Approved (Rs.)	Total Grant (Rs.)	Expenditure Incurred (Rs.)
1.	Books & Journals	30,000/-	30,000/-	Nil	30,000/-	30,000.00/-
2.	Equipment	4,50,000/-	4,50,000/-	Nil	4,50,000/-	4,49,937.00/-
3.	Contingency	30,000/-	15,000/-	12,000/-	27,000/-	37,714.00/-
4.	Field Work/Travel (Give details in the proforma at Annexure-IV)	50,000/-	25,000/-	20,000/-	45,000/-	49,382.00/-
5.	Hiring Service	40,000/-	20,000/-	Nil	20,000/-	45,400.00/-
6.	Chemical & Glassware	3,00,000/-	1,50,000/-	1,20,000/-	2,70,000/-	2,70,562.00/-
7.	Overhead Charge	97,000/-	97,000/-	Nil	97,000/-	97,000.00/-

MANDWAR & CO. SEAL CHANDRAPUR

c. Staff : **Mr. Ashish A. Ashtankar**

Date of Appointment : **01/02/2016**

Sr. No	Item	From	To	Total Expenditure (Rs.)	Amount Approved (Rs.)	Amount Received (Rs.)	Expenditure Incurred (Rs.)
1.	Honorarium to PI (Retired Teachers) @ Rs. 18,000/- p.m.	Nil	Nil	Nil	Nil	Nil	Nil
2.	**Project Fellow:** **i) NET/GATE** Qualified Rs.16, 000/- p.m. for initial 2 years and Rs.18, 000/-p.m. for the third year.	Nil	Nil	Nil	Nil	Nil	Nil
	ii) NonNET/NonGATE qualified- Rs. 14,000/- p.m. for initial 2 years and Rs. 16,000/- p.m. for the third year.	01/02/2016	30/06/2017	238000/-	4,16,000/-	3,74,000/-	4,34,000/-
		1/07/2017	30/6/2018	196000/-			

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. If as a result of check or audit objection some irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of **Rs. 13,13,400/- (Rupees Thirteen Lakh Thirteen Thousand Four Hundred Only)** received from the University Grants Commission under the scheme of support for Major Research Project entitled "**A study of methicillin and vancomycin resistance *Staphylococcus aureus* isolates caring *mecA* and *vanA* gene complex from tertiary care centers.**"vide UGC letter **No. F. No.43-473/2014(SR) dated 17/11/2015** out of which an amount of **Rs.14,13,995/- (Rupees Fourteen Lakh Thirteen Thousand Nine Hundred Ninety Five Only)** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Principal Investigator
UGC Major Research Project

REGISTRAR/PRINCIPAL
(Seal)

Principal
Sardar Patel Mahavidyalaya
Chandrapur

STATUTORY AUDITOR
(Govt. Internal Auditor/ Chartered Accountant)
(Seal)

For, MAMIDWAR & CO.
CHARTERED ACCOUNTANTS

MAMIDWAR & CO. SEAL CHANDRAPUR

SIGNATURE OF THE CO-INVESTIFGATOR

AJAY D. MAMIDWAR
(PROPRIETOR)

Annexure – IV

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

Name of the Principal Investigator : **Dr. Vijay Shamrao Wadhai**

Name of the Place visited	Duration of the Visit		Mode of Journey	Expenditure Incurred (Rs.)
	From	To		
1.Civil Hospital Gondpipri	10/03/16	11/03/16	Hired Taxi	1170+500(Halting Charges) =1670/-
2.Civil Hospital Mul,Chandrapur	30/03/16	31/03/16	Hired Taxi	=810/-
3.Civil Hospital Gadchiroli	14/05/16	15/05/16	Hired Taxi	1422+500(Halting Charges) =1922/-
4.Civil Hospital Chandrapur	01/06/16	02/06/16	Personnel Vehicle	0.00
5.Civil Hospital Aheri	06/06/16	07/06/16	Hired Taxi	2250+500(Halting Charges) =2750/-
6.Civil Hospital Nagpur	08/06/16	09/06/16	Hired Taxi	2970+500(Halting Charges) =3470/-
7.Civil Hospital Bhamragad	07/07/16	08/07/16	Hired Taxi	3150+500(Halting Charges) =3650/-
8.Civil Hospital Amravati	23/08/16	24/08/16	Hired Taxi	4770+500(Halting Charges) =5270/-
9.Civil Hospital Sironcha	27/08/16	28/08/16	Hired Taxi	4050+500(Halting Charges) =4550/-
Mid Term Expenditure:			**Total Amount**	**24092/-**
10.Civil Hospital Amravati	10/09/17	11/09/17	Hired Taxi	=4320/-
11.Civil Hospital Chandrapur	23/11/17	23/11/17	Personnel Vehicle	0.00/-
12.Civil Hospital Amravati	04/01/18	05/01/18	Hired Taxi	4860+1000(Halting Charges) =5860/-
13.Civil Hospital Dhanora	23/01/18	23/01/18	Hired Taxi	=4680/-
14.Civil Hospital Bhamragad	05/02/18	06/02/18	Hired Taxi	4680+1000(Halting Charges) =5680/-
15.Civil Hospital Sironcha	19/02/18	19/02/18	Hired Taxi	=4750/-
Final Expenditure:			**Total Amount**	**49,382/-**

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF PRINCIPAL INVESTIGATOR
Principal Investigator
UGC Major Research Project

REGISTRAR/PRINCIPAL
(Seal)
Principal
Sardar Patel Mahavidyalaya
Chandrapur

SIGNATURE OF THE CO-INVESTIGATOR

STATUTORY AUDITOR
(Govt. Internal Auditor/ Chartered Accountant)
(Seal)
For, MAMIDWAR & CO.
CHARTERED ACCOUNTANTS
AJAY D. MAMIDWAR
(PROPRIETOR)

Annexure –V

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization Certificate

Certified that the grant of **Rs. 13,13,400/- (Rupees Thirteen Lakh Thirteen Thousand Four Hundred Only)** received from the University Grants Commission under the scheme of support for Major Research Project entitled **"A study of methicillin and vancomycin resistance *Staphylococcus aureus* isolates caring *mecA* and *vanA* gene complex from tertiary care centers"** Vide **UGC letter No. F.No. 43-473/2014 (SR)** dated on **17/11/2015** out of which an amount of **Rs.14,13,995/- (Rupees Fourteen Lakh Thirteen Thousand Nine Hundred Ninety Five Only)** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down the University Grant Commission.

If as a result of check or audit objection irregularly is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR
Principal Investigator
UGC Major Research Project

REGISTRAR/PRINCIPAL
(Seal)
Principal
Sardar Patel Mahavidyalaya
Chandrapur

Sardar Patel Mahavidyalaya ★ SEAL ★ Chandrapur

MAMIDWAR & CO. ★ SEAL ★ CHANDRAPUR

STATUTORY AUDITOR
(Govt. Internal Auditor/
Chartered Accountant)
(Seal)
For, MAMIDWAR & CO.
CHARTERED ACCOUNTANTS
AJAY D. MAMIDWAR
(PROPRIETOR)

SIGNATURE OF THE CO-INVESTIGATOR

Annexure-VI

PROFORMA FOR SUPPLYING THE INFORMATION IN RESPECT OF THE STAFF APPOINTED UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

UGC FILE NO.F.43-473/2014 (SR) (HRP) YEAR OF COMMENCEMENT 0 1 | 0 7 | 2 0 1 5

TITLE OF THE PROJECT: **"A study of methicillin and vancomycin resistance *Staphylococcus aureus* carrying *mecA* and *vanA* gene complex from tertiary care centers."**

1.	Name of the principal Investigator	**Dr. Vijay Shamrao Wadhai**				
2.	Name of the University/College	**Sardar Patel Mahavidyalaya, Chandrapur**				
3.	Name of Research Personal Appointed	**Mr. Ashish A. Ashtankar** **Appointment Date: 01 Feb. 2016**				
4.	Academic Qualification	Sir. No.	Qualification	Years	Marks	%age
		1.	**M.Sc. (Microbiology)**	**2006**	**584**	**58.40**
		2.	**M.Phil**	**-**	**-**	**-**
		3.	**Ph.D.**	**-**	**-**	**-**
5.	Date of Joining	**01 Feb. 2016**				
6.	Date of Birth Research Personal	**26 Oct. 1983**				
7.	Amount of HRA, if drawn	**-Nil-**				
8.	Number of Candidate applied for the Post	**06 (Six Numbers)**				

CERTIFICATE

This is certified that all the rules and regulations of UGC Major Research Projects outlined in the guidelines have been followed. Any lapses on the part of the university will liable to terminate of said UGC project.

Principal Investigator
Principal Investigator
UGC Major Research Project

Head of Deptt.
Dr. V. S. Wadhai
Head Deptt. of Microbiology
S. P. College, Chandrapur

Register/Principal
Principal
Sardar Patel Mahavidyalaya
Chandrapur

Annexure-VII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

MAJOR RESEARCH PROJECT COPY OF THE SPECIMEN OF HOUSE RENT FOR PROJECT FELLOW

Certified that Shri/Dr. Not Appliable is paying House Rent of Rs.-_____ and is eligible to draw House Rent Allowances @_____ as per University Rules.

Sardar Patel Mahavidyalaya SEAL Chandrapur

Registrar/Principal
(Signature with Seal)
Principal
Sardar Patel Mahavidyalaya
Chandrapur

Certified that Shri/Dr. Not Appliable is not staying independently andtherefore is eligible to draw House Rent @ of Rs. _____p.m. minimum admissible to a Lecturer as per University Rules.

Sardar Patel Mahavidyalaya SEAL Chandrapur

Registrar/Principal
(Signature with Seal)
Principal
Sardar Patel Mahavidyalaya
Chandrapur

Certified that Shri/Dr. Not Appliable has been provided accommodation in the Hostel. But he/she could not be provided with single seated flat type accommodation as recommended by the Commission, Hostel fee @ Rs._____ per month w.e.f. _____ is being charged from him/her.

Patel Mahavidyalaya SEAL Chandrapur

Registrar/Principal
(Signature with Seal)
Principal
Sardar Patel Mahavidyalaya
Chandrapur

Annexure – VIII

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

~~Annual~~/Final Report of the work done on the Major Research Project

(Report to be submitted within 6 weeks after completion of each year)

1.	Project report No. ~~1st~~ /~~2nd~~ /~~3rd~~/Final ✓	:	**Final Report**
2.	UGC Reference No.F.	:	**F.No.43-473/2014 (SR)**
3.	Period of report	:	**(03years)**from **01/07/2015** to **30/06/2018**
4.	Title of research project	:	**"A study of methicillin and vancomycin resistance *Staphylococcus aureus* isolates caring *mecA* and *vanA*gene complex from tertiary care centers".**
5.	(a) Name of the Principal Investigator	:	**Dr. Vijay Shamrao Wadhai**
	(b)Deptt.	:	**Department of Microbiology**
	(c) University/College where work has progressed	:	**Sardar Petal Mahavidyalaya, Ganjward Chandrapur-442402**
6.	Effective date of starting of the project	:	**01/07/2015**

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved Rs.	:	**Rs.14,13,000/-** (Rupees Fourteen Lakh Thirteen Thousand)
b. Total amount received	:	**Rs.13,13,400/-** (Rupees Thirteen Lakh Thirteen Thousand Four Hundred Only)
c. Total expenditure Rs.	:	**Rs.14,13,995/-** (Rupees Fourteen Lakh Thirteen Thousand Nine Hundred Ninety Five Only)
d. Balance amount	:	**Rs. 99,600/-** (Rupees Ninety Nine Thousand Six Hundred Only)
e. Report of the work done:		**Please attach a separate sheet Encl. 1**

Encl.1

REPORT OF THE WORK DONE

i. **Brief Objective of the project** :

Multidrug resistant of *Staphylococcus aureus* is a major cause of nosocomial and community acquired infection and is on the rise. The glycopepdides Vancomycin has been proposed as the drug of choice for treating such infections. The present study aimed at identifying the Vancomycin resistance phenotypically among the MRSA isolates and molecular studies based on identification of *mecA* and *vanA* gene together with gene sequencing, from tertiary care Centre.

The objective of present study is mention as below:

- To study the patterns of antimicrobial susceptibilities of MRSA and VRSA isolates to the commonly prescribed antibiotics in Vidarbha region of Maharashtra.
- Study of current prevalence rate of Methicillin resistance among isolates of *Staphylococcus aureus.*
- Demonstration of Vancomycin resistance among MRSA isolates.
- To check the isolates for carrying *mecA* and *vanA* gene and their sequencing.

On the basis of above background the **objectives** of this study are mentioned as below:

1. To study the susceptibility and resistance pattern of *Staphylococcus aureus* isolates.
2. Determination of prevalence rate of MRSA isolate on disc diffusion method and minimal inhibitory concentration (MIC).
3. To determine reduced susceptibility to vancomycin among those MRSA isolates.
4. To detect *mecA* gene by using polymerase chain reaction (PCR) from MRSA Strains identified on disc diffusion and Minimal Inhibitory Concentration (MIC.)
5. To detect *vanA* gene by using Polymerase Chain Reaction from VRSA strain.
6. To evaluate efficacy of commonly used methods of antimicrobial susceptibility on disc diffusion and MIC by comparing with DNA-PCR method for methicillin and vancomycin resistance.

ii. **Work done so far and results achieved and Publication, if any resulting from the work (Give details of the papers and name of the Journals in which it has been published or accepted for publication:**

Yes, the progress has been according to the original plan of the work and toward achieving objectives.

Sample Collection

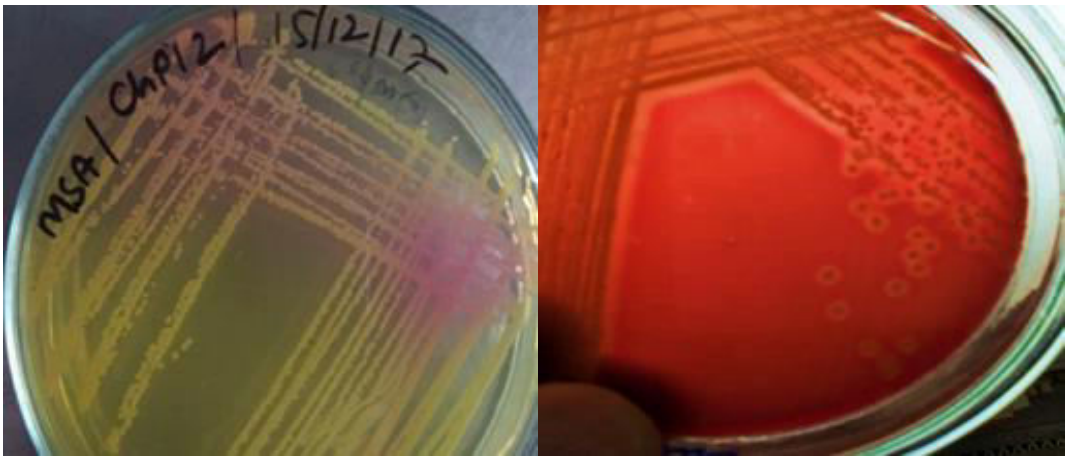
The research processed a total number of 1425 clinical samples (Pus, Blood and Urine) of admitted patients in tertiary care hospital in Vidarbha regions including **40.0%** clinical samples collected from civil hospital Chandrapur district including Mul, and Gondpipri. Total **38.94%** of clinical samples collected from government hospital Gadchiroli district including Aheri, Bhanragad and Sironcha and **13.26%** samples from government hospital Nagpur and **7.78%** sample collected from government hospital Amravati.

This study is particularly focus on Chandrapur, Nagpur, Amravati and Gadchiroli district area of Vidarbha. Limited reports were available on development of methicillin and vancomycin resistant *Staphylococcus aureus* from this part of India Particularly Gadchiroli and Chandrapur. The purpose of present study was to evaluate current antimicrobial susceptibility patterns of *Staphylococcus aureus* and prevalence of MRSA along with this; it also concentrates on to study reduced susceptibility of Vancomycin against MRSA isolates.

Figure: 1 Sample collection by Government hospital Staff.

Bacterial Isolation

In current study, Out of 1425 clinical specimen receive for microbiological examination,765coagulase positive *Staphylococcus aureus* isolates were isolated randomly from clinical specimen such as Pus, blood and urine of admitted patients in tertiary care hospitals, in Vidarbha region between **February 2016 to June 2018.**The study was carried out at Department of Microbiology, Sardar Patel Mahavidyalaya, Chandrapur (M.S.), India. *Staphylococcus aureus* isolates were identified by colony morphology; Gram stain, DNase Positive test, and Coagulase positive tests and fermentation of Mannitol or β-heamolysin in blood agar and reduction of tellurite black colonies around zone appear in BPA by conventional methods.



A. B.

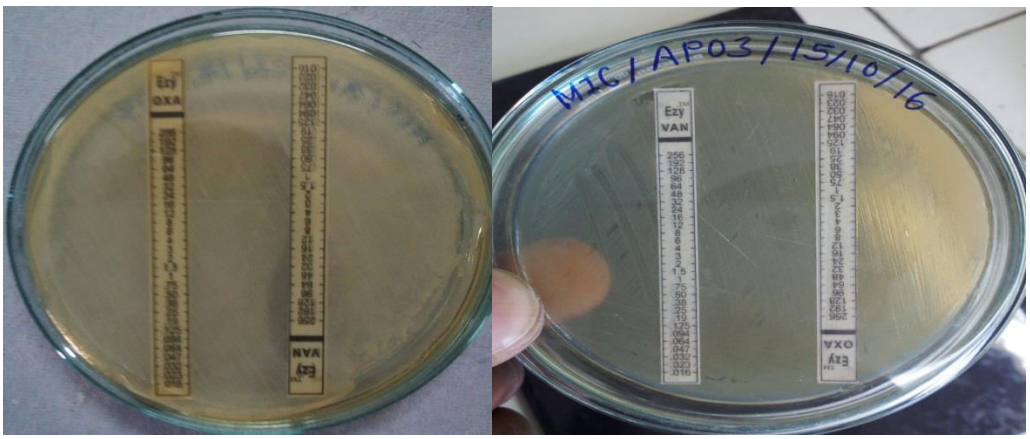
Figure: 2(A) *S. aureus* on MSA Agar Medium (Yellow color colonies shows Mannitol fermentation)(B) S. *aureus* on Blood Agar (Beta Haemolysis)

Antimicrobial Susceptibility Test

The antibiotic resistance profile was determined by the Disc Agar Diffusion (DAD) technique using different antimicrobial agent use in this project; Amikacin (30µg), Ceproflaxacin (5µg), Chloramphenicol (30µg), Eruthromycin (15µg), Gentamycin (10µg), Lincomycin (2µg), Methicillin (30µg), Penicillin G (10µg), Trimethoprime (5µg), Tetracycline (30µg), Tobramycin (10µg), and Vancomycin(30µg) Hi-media, Mumbai India, according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) and standard *Staphylococcus aureus* strains NCIM 5522 and 5521 were used as reference strains for MRSA.

Determination of MIC

Minimal inhibitory concentration of methicillin and vancomycin resistance was determined by E-test and disc diffusion method using CLSI guidelines. Briefly, plates of Hi-sensitivity agar (Hi-media) were prepared with forming lawn of inoculums prepared using 18-24hr old cultures was spotted with placing gradient strip 0.5mcg to >265mcg/ml of oxacillin and vancomycin on respectively. Plates were incubated overnight at 35^0C for 24hr before assessing the visible growth (CLSI) performance standards for antimicrobial susceptibility testing M100-S (latest edition); CLSI methods for Dilution Antimicrobial Susceptibility Test for bacteria that grow aerobically, Approved Standard M7-A; CLSI methods for Dilution Antimicrobial Susceptibility Test of Anaerobic Bacteria Approved Standard M11-A (Latest edition).



A. **B.**

Figure: 3 E-test with Oxacillin MIC Strip and Vancomycin MIC Strip (No Zone of Inhibition)

Preparation of culture suspension for DNA extraction

For preparation of suspension, a loopful of culture was taken from the culture plate/slant and suspended in 1ml of sterile syringe water and vortexed. The suspension was then stored at 4^0C until further use.

Isolation of Genomic DNA by CTAB method

Sample obtained was used for genomic DNA isolation by using modified CTAB protocol which was used further in PCR reaction. The samples were re-suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). The eluted DNA was again sequenced by Sanger Sequencing Method at the SaiBiosystem Privet Limited, Nagpur, India.

PCR based 16s rRNA gene amplification and Sequencing

Bacterial DNA was to amplify 16s rRNA gene applying following 16s universal primers. Sequencing reactions were performed using a ABI PRISM®BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq®SNA polymerase (FS enzyme) (Applied Biosystems).

16s Universal Primer for *S.aureus*

Table:1 Universal primer used for detection of *S.aureus*

Genus	Primer Name	Primers Sequence (5'-3')	Product Size
Staphylococcus aureus	**F**	AGAGTTTGATCCTGGCTCAG	1500bp
	R	AAGGAGGTGATCCAGCCGCA	

Table: 2 Specific Primers for *mecA* and *vanA* gene detection

Target gene	Primer Name	Primers Sequence (5'-3')	Product size
	F	GTAGAAATGACTGAACGTCCGATAA	
mecA	**R**	CCAATTCCACATTGTTTCGGTCTAA	310bp
vanA	**F**	CATGAATAGAATAAAAGTTGCAATA	
	R	CCCCTTTAACGCTAATACGATCAA	1030bp

Results

In this study, out of **1425** samples **765 (53.68%)** coagulase positive *Staphylococcus aureus* were obtained randomly from clinical specimen such as pus, blood and urine of admitted patients in tertiary care hospitals, in Vidarbha region between **February 2016 to June 2018**.All the *Staphylococcus aureus* isolates were further analyzed for their pathogenic characterization along with antibiotic sensitivity test (Disc Agar Diffusion Method) and resistance confirmed with E-test was found to be **281(36.73%)** of MRSA positive strains among Coagulase positive *S.aureus* and **41(14.59%)** of VRSA positive strains among MRSA isolates.The clinical samples were collected from both gender Males are more than the Females in Vidarbha region (Male; **61.56%** and Female; **38.43%**)(**Table: 3)[Figure: 4]**. The prevalence of positive cultures was more in pus samples (**81.04%**) as compare to Urine samples (**9.93%**) and Blood samples (**9.13%**). In respect to departmental unit the prevalence of positive CoPSA was more at surgical ward (**66.19%**), ICU ward (**12.45%**) and pediatrics ward (**9.25%**). Also the age group 41-50 years and 51-60 years were more prone to *S.aureus* infections.

Table: 3 Different age group and Sex wise distribution of *S. aureus* infection

Age Group	Male		Female		Total	
	No.	%	No.	%	No.	%
00-10	23	4.88%	15	5.10%	38	4.96%
11-20	38	8.06%	36	12.24%	74	9.67%
21-30	96	20.38%	44	14.96%	140	18.30%
31-40	86	18.25%	32	10.88%	118	15.42%
41-50	93	19.74%	69	23.46%	162	21.17%
51-60	57	12.10%	46	15.64%	103	13.46%
61-70	78	16.56%	52	17.68%	130	16.99%
Total	**471**	61.56%	**294**	38.43%	**765**	100.0%

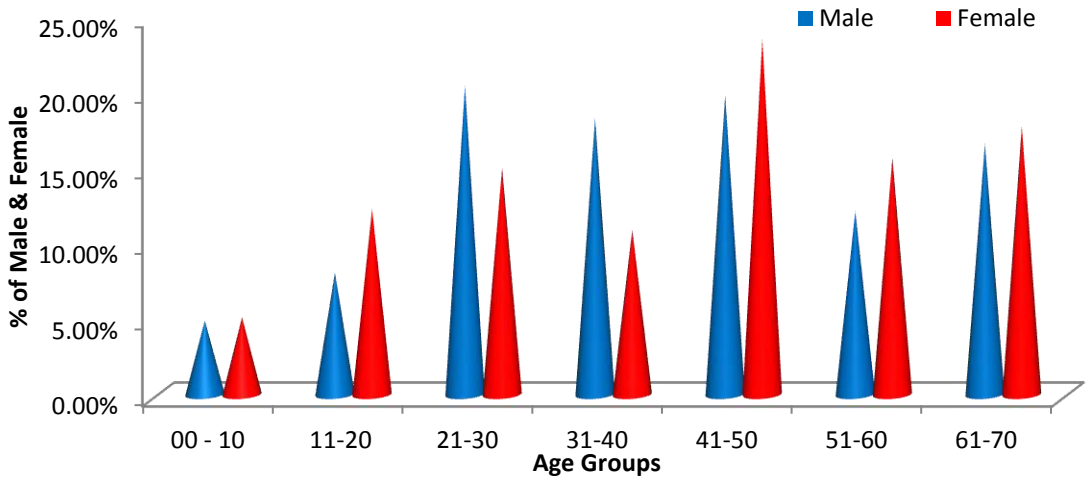


Figure: 4Sex wise distributions of *S.aureus* infections among patients With various age groups

In current study particularly focus on Chandrapur, Nagpur, Amravati, and Gadchiroli district area of Vidarbha region. Limited reports were available on development of Methicillin and Vancomycin resistant *Staphylococcus aureus* from this part of India particularly Gadchiroli and Chandrapur district. The MRSA prevalence is increasing worldwide and has become a serious public health issue. Where, the prevalence of MRSA among *S.aureus* isolated from admitted patient in tertiary care hospital of Vidarbha region including Chandrapur **(38.69%),** Nagpur **(36.27%),** Amravati **(38.35%)** and Gadchiroli **(34.95%)** respectively. In an average MRSA prevalence rate was found to be **36.73%** in Vidarbh region.On the basis of various study repot the prevalence rate of MRSA is **25%** to **50%** in India. A Gadchiroli and Chandrapur district of Vidarbha region is tribal and economically backward. MRSA prevalence data is not available from this part of India.

All the MRSA were subjected to antimicrobial susceptibility study to the recommended panel of 15 antimicrobial agents. In the present study the populations of four different districts were

in consideration. In Chandrapur region higher resistance in MRSA was found to multiple antibiotics including penicillin, Erythromycin, Tobramycin, Tetracyclin, Norfloxicin, Trimethoprim, Ciproflaxicin, and lower resistance was found to be Vancomycin, Amikacin, Gentamycin, and Nitillin. The antimicrobial susceptibility patterns of MRSA in Nagpur region revealed higher resistance to penicillin, erythromycin, tobramycin, Trimethoprime and most of the antibiotics shown lower resistance to Vancomycin, Amikacin, Chloramphenicol, Gentamycin and Nitillin. In Amravati region antimicrobial susceptibility study of MRSA had showed higher resistance to penicillin, Erythromycin, Lincomycin, Tetracycline, Chloramphenicol and lower resistance to Vancomycin, Nitillin and Amikacin.

In Gadchiroli antimicrobial susceptibility study of MRSA had showed higher resistance to penicillin, erythromycin, tobramycin, tetracycline and lower resistance to Vancomycin, and Nitillin. Over all, similar types of antibiogram patterns were observed in all districts under consideration. This study revealed Nitillin and Amikacin antibiotics were most effective drug for the treatment of MRSA and VRSA infections.

Among 281 MRSA strains, 41 strains (14.59%) were shown resistance towards Vancomycin antibiotics. All the VRSA were subjected to antimicrobial susceptibility study to the recommended panel of 15 antibiotics. In current study Chandrapur, Nagpur, Amravati, and Gadchiroli district were shown prevalence of VRSA among MRSA isolates was found to be**11.88%, 16.21%, 17.85%,** and**15.65%**respectively. In an average VRSA prevalence rate was found to be **14.59%** in Vidarbha region. Antibiogram patterns of VRSA isolates had shown Multi-resistant to most of most of tested antibiotics except some isolates which was sensitive to Nitillin, Chloramphenicol and Amikacin. This is the first report of fully developed VRSA strains in geographically central part of India. Vancomycin has been the drug of choice for over last few decades. However, the appearance and spreading of resistance to this glycopeptide among clinically important *Staphylococcus aureus* has made it problematic to manage severe infections caused by such pathogens. It is very essential to look for alternative to Vancomycin and other glycopeptides in the treatment of serious *Staphylococcus aureus* infections. It is also equally important to prevent the spread an emergence of glycopeptide resistance by taking proper infection control measures, so that we may not fall back into pre-antibiotic era.

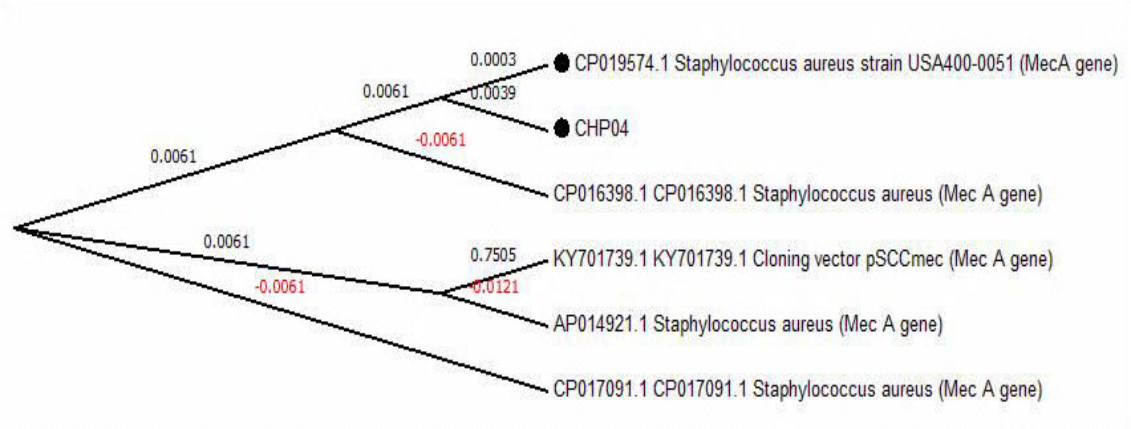


Figure: 5 Phylogenetic tree showing the relationship of the isolate ChP04 to closely related USA400-0051 (Accession number AP014921).

All the tested *S.aureus* isolates were reconfirmed using RT-PCR. PCR amplification of 16S rDNA of the clinical isolates ChP04, ChP12, ChP15, AmP05, AmU03, and ChP16 using universal primer for identification of *Staphylococcus aureus* strains. All the 16s rRNA positive isolated strain were aligned and a phylogenetic tree was constructed. The output was visualized in CLUSTAL alignment was used for the phylogram built up by using the MEGA5 software**[Figure: 5]**. Isolate ChP04 was found to be closely related with highly reported strain USA400-0051. These bacterial strains are community associated MRSA infections, particularly skin and soft tissue infections became common in the United State after year 2000. These isolates were first reported in Australia and named as Western Australia-1 clone. Later, ST1-SCCmecIV MRSA, carrying lukSF-PV genes encoding the Panton-Valentine Leucocidin (PVL) were detected in the USA and caused severe infection among America Indian children. After reemerge in USA and Canada as an important cause of skin-soft tissue infection and was renamed as USA400 were USA 400-0051 which is prototype of the USA400. The isolate ChP12 was observed to be closely related to strain S167. Where the four isolated strain shows negative results may be due to mutation. As mecA and vanA gene detection technique are very expensive so we were unable to perform mecA gene detection test on all the MRSA and VRSA isolates so we were found oxacillin and vancomycin resistant on disc diffusion method and MIC by E test. MIC test considered the gold strand of MRSA confirmation.

In the present study, mecA genes were detected by using the PCR based amplification technique in the selected MRSA isolates which was initially characterized methicillin resistant by 1µg oxacillin disc diffusion test. In current study, the one selected MRSA strains

were positive for mecA gene and one selected VRSA isolates were positive for vanA genes. ChP04 MRSA isolates was positive for mecA and ChP12 isolates was positive for vanA genes. This mecA gene detection by RT-PCR was found to be a rapid and reliable method for single-step identification of cultures of MRSA and may prove to be useful for direct application on clinical specimens. The current study had shown the emergence of VRSA carrying vanA gene in central region Vidarbha of India and although this study was restricted in four district of Vidarbha of Maharashtra state, there might be possibility of development of vancomycin resistance against MRSA in other part of India as antibiotic misuse is equally common in whole country especially backward region. Still, vancomycin is the last choice for the treatment of MRSA infection. Hence, there is immediate need of nationwide antimicrobial surveillance programme against pathogenic *Staphylococcus aureus* including all tribal region of each state of India to control the further emergence and spreading of VRSA strains. A strict regulation on irrational antibiotic usages might be a suitable and effective method in this direction.

Conclusion

From the results this is concluded that, *Staphylococcus aureus* were shows higher prevalence in pus and next in urine samples. The resistant pattern shown by these isolates where checked by the standard Disc agar diffusion test and E-test was shown the prevalence of MRSA among coagulase positive *S.aureus* isolated from admitted patient in tertiary care hospital of Vidarbha region including Chandrapur **(38.69%),** Nagpur **(36.27%),** Amravati**(38.35%)** and Gadchiroli **(34.95%)** respectively. In an average MRSA prevalence rate was found to be **36.73%** in Vidarbh region. In current study Chadrapur, Nagpur, Amravati, and Gadchiroli district were shown prevalence of VRSA among MRSA isolates was found to be **11.88%, 16.21%, 17.85%,** and**15.65%** respectively. In an average VRSA prevalence rate was found to be **14.59%** in Vidarbha region. From the above results shows selected one isolated MRSA strains ChP04 were positive for *mec*A gene and ChP12 isolates was positive for *vanA* genes. This *vanA* and *mecA* gene detection by RT-PCR was found to be a rapid and reliable method for single-step identification of cultures of MRSA and may prove to be useful for direct application on clinical specimens. The vancomycin resistant among the MRSA as well as increase in methicillin resistance among multi drug resistant *Staphylococcus aureus* and excessive use of antimicrobial agents have worsened the sensitivity. Large studies need to be done in various geographical regions of the country to better define the epidemiology,

mechanism of methicillin resistance as well as vancomycin resistant in *Staphylococcus aureus* and its clinical implication.

iii. Has the progress been according to original plan of work and to words achieving the Objective? If not, State reasons : **Yes!**

iv. Please indicate the difficulties, if any : **No**
Experienced in implementing the projects

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the Period (Annual basis) may please be sent to the Commission on a separate Sheet: **The Project is completed in the given tenure of the project.**

vi. If the Project has been completed, please enclose a summary of the finding of the study. One bound copy of the final report of work done may also be sent to University Grant Commission: **Summery of the finding of the study is attached in Annexure IX.**

vii. Any other information which would help in evaluation of work done on the project. At the completion of the Project, the first report should indicate the output, such as

a) Manpower trained : **Yes!** Project fellow appointed and trained

b) Ph.D. awarded : **No!**

c) Publication of results : **Yes!**

1) ***There YW, Wadhai****VS. Multidrug resistant Staphylococcus aureus: A global challenge. Drug Discovery, 2013, 7(18), 13-18*

2) ***There, Y.W. and Wadhai, V.S., Bhandari P.R****., A Study on Antimicrobial Susceptibility patterns of Staphylococcus aureus from tertiary care Cenre Chandrapur (M.S.) I J R B A T, Issue (3) Vol. II, May 2015, 358-361 [Impact Factor- 4.935]*

3) ***There, Y.W., Wadhai V.S. and Bhandari, P.****Prevalence of Vancomycin resistance Staphylococcus aureus among MRSA isolates from District Hospital Gadchiroli (M.S.) India. I J R B A T, 2016 Vol. 1 (3), 214-218 [Impact Factor- 4.935]*

4)***Wadhai, V. S. and Ashtankar, A. A.*****(2017).***"A Study on methicillin and vancomycin resistant Staphylococcus aureus from tertiary care hospitals, in Vidharbha region, India."International Journal of Current Research, 9, (01)*

5)***Shende, S.P., and Wadhai, V.S.*** **(2017).***Antibiotic Resistance Profiling of Staphylococcus aureus Isolated from Clinical Specimen from Tertiary Care Hospital.* (ICEMTE-2017) ISSN: 2321-8169 Volume: 5 Issue: 3 06.

Manuscript under Preparation: 02

1)***Wadhai, V.S., Sarkar, P.P. and Ashtankar, A. A.*** *The life threatening resistance superbug, MRSA and VRSA is currently horizons. In Communication for publication.*

2)***Wadhai, V.S., Sarkar, P.P. and Ashtankar, A. A.*** *A Study on methicillin and vancomycin resistant staphylococcus aureus from tertiary care centre, chandrapur (m.s.) india. In communication for publication..*

d) Other impact, if any : **Yes!**

Project Fellow present poster and paper in national and international conferences.

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

Principal Investigator
UGC Major Research Project

REGISTRAR/PRINCIPAL
(Seal)

Principal
Sardar Patel Mahavidyalaya
Chandrapur

SIGNATURE OF THE CO-INVESTIGATOR

Annexure – IX

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THEFINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	Title of the Project	:	**"A study of methicillin and vancomycin resistance *Staphylococcus aureus* isolates caring *mecA* and *vanA* gene complex from tertiary care centers".**
2.	NAME AND ADDRESS OF THE PRINCIPAL: INVESTIGATOR		**Dr. Vijay Shamrao Wadhai Department of Microbiology Sardar Patel Mahavidyalaya, Chandrapur-442402**
3.	NAME AND ADDRESS OF INSTITUTION :		**Sardar Patel Mahavidyalaya, Ganjward, Chandrapur 442402**
4.	UGC APPROVAL LETTER NO. AND DATE	:	**F.No.43-473/2014 (SR)** dated on **17/11/2015**
5.	DATE OF IMPLEMENTATION	:	**01/07/2015**
6.	TENURE OF THE PROJECT	:	**(03 Years) 01/07/2015 to 30/06/2018**
7.	TOTAL GRANT ALLOCATED	:	**Rs.14,13,000/- (RupeesFourteen Lakh Thirteen Thousand Only)**
8.	TOTAL GRANT RECEIVED	:	**Rs. 13,13,400/- (Rupees Thirteen Lakh Thirteen Thousand Four Hundred Only)**
9.	FINAL EXPENDITURE	:	**Rs.14,13,995/- (Rupees Fourteen Lakh Thirteen Thousand Nine Hundred Ninety Five Only)**

10. TITLE OF THE PROJECT: **"A study of methicillin and vancomycin resistance *Staphylococcus aureus* isolates caring *mecA* and *vanA* gene complex from tertiary care centers".**

11. OBJECTIVES OF THE PROJECT :

The objectiveof present study is mention as below:

- To study the patterns of antimicrobial susceptibilities of MRSA and VRSA isolates to the commonly prescribed antibiotics in Vidarbha region of Maharashtra.
- Study of current prevalence rate of Methicillin resistance among isolates of *Staphylococcus aureus.*
- Demonstration of Vancomycin resistance among MRSA isolates.
- To check the isolates for carrying *mecA* and *vanA* gene and their sequencing.

On the basis of above background the **objectives** of this study are mentioned as below:

1.To study the susceptibility and resistance pattern of *Staphylococcus aureus* isolates.
2.Determination of prevalence rate of MRSA isolate on disc diffusion method and minimal inhibitory concentration (MIC).
3. To determine reduced susceptibility to vancomycin among those MRSA isolates.
4.To detect *mecA* gene by using polymerase chain reaction (PCR) from MRSA Strains identified on disc diffusion and Minimal Inhibitory Concentration (MIC.)
5.To detect *vanA* gene by using Polymerase Chain Reaction from VRSA strain.
6.To evaluate efficacy of commonly used methods of antimicrobial susceptibility on disc diffusion and MIC by comparing with DNA-PCR method for methicillin and vancomycin resistance.

12. WHETHER OBJECTIVES WERE ACHIEVED : **Yes, We have successfully completed the objectives for which details are given in the Annexure VIII**

13. ACHIEVEMENTS' FROM THE PROJECT : **Yes!**

The main achievement of the projects is as follows.

- We successfully characterize methicillin and vancomycin resistance *Staphylococcus aureus* carrying mecA and vanA gene isolated from clinical sample (pus, urine and

blood) at genetic level. The clinical isolates of *S,aureus* was explored and determined the prevalence of MRSA in hospital care infections and demonstration the vancomycin resistance among MRSA isolates obtained from tertiary care centres in Vidarbha

- From this study different region of Vidarbha under consideration i.e. Chandrapur, Nagpur, Amravati and Gadchiroli where the prevalence of MRSA among *S.aureus* isolates was **38.69%, 36.27%, 38.65%** and **34.95%** respectively. In an average MRSA prevalence rate was **36.73%** in Vidarbha region and the prevalence rate of VRSA among MRSA, Chandrapur, Nagpur, Amravati and Gadchiroli were shown **11.88%, 16.21%, 17.85%,** and **15.65%** respectively.
- The main achievement from this study is to detect mecA and vanA gene among methicillin and vancomycin resistant strain isolated from clinical samples collected from tribal region of Vidarbha. In this study, one selected MRSA strains ChP04 were positive for mecA gene and one selected VRSA isolates ChP12 were positive for vanA genes. As mecA and vanA gene detection technique are very expensive so we were unable to perform mecA gene detection test on all the MRSA and VRSA isolates so we were found oxacillin and vancomycin resistant on disc diffusion method and MIC by E test. MIC test considered the gold strand of MRSA confirmation.
- From current study datathe high prevalence of MRSA infection and the emerging of *van*A gene containing of VRSA in Vidarbha. So, it is must to implement antibiotic policy in all the healthcare institution. The physician should be advice for microbiologist to determine the methicillin susceptibility of suspected patients and carries. So, that the emergence and spreading of methicillin and vancomycin resistance *S.aureus* can be prevented. A strict regulation on irrational antibiotics usages might be a suitable and effective method in this direction.
- The project has trained project fellow in the field of project handling, and various technique for isolation, sequencing of the DNA material.

14. **SUMMARY OF THE FINDING (IN 500 WORDS):**

Health care associated infections are the main reason for thehigher morbidity and mortality rate and the management of all theseconditions has been critically compromised by the appearance and rapidspread of antimicrobial resistance among the organisms floating in thehospital. MRSA infections have become a major problem worldwide. Theproblem is not restricted to developed countries. The last two decadehas seen an alarming increase in MRSA

infections in Indian hospitals. Vidarbha is the part of Maharashtra state of India, which include tribaldistrict i.e. Chandrapur and Gadchiroli. These two district of Vidarbha region are tribal and Naxalites (Maoists) prone area with least healthawareness, low socioeconomic status and lack of healthcare facilities.Also it is neglected part of Vidarbha in the antimicrobial surveillancestudy by Indian Network for Surveillance of Antimicrobial Resistance(INSAR) group, India in 2013. A very few reportswere available regarding Methicillin and Vancomycin resistance *Staphylococcus aureus* from thispart of India.In the present study the state of resistance exists amongclinical isolates of *S. aureus* was explored and determined theprevalence of MRSA in hospital care infections and demonstrate the vancomycin resistance among MRSA isolates obtained from tertiary care centres. The other purpose of our study was to characterize MRSA andVRSA isolated strains at genetic level, in order to efficiently supporttherapy and eradication of the pathogen.

An attempt was made in the present study with an aim to isolatecoagulase positive *S. aureus.* Total 765 coagulase positive *S. aureus*were isolated from pus, urine and blood clinical samples obtained from tertiary care centres of Vidarbha region during February 2016 to June 2018.The clinical samples were collected from both gender Males are more than the Females in Vidarbha region (Male; **61.56%** and Female; **38.43%**). The prevalence of positive cultures was more in pus samples (81.04%) as compare to Urine samples (**9.93%**) and Blood samples (**9.13%**). In respect to departmental unit the prevalence of positive CoPSA was more at surgical ward (**66.19%**), ICU ward (**12.45**%) and pediatrics ward (**9.25%**). Also the age group 41-50 years and 51-60 years were more prone to *S.aureus* infections. In current study four different region of Vidarbha under study i.e. Chandrapur, Nagpur, Amravati and Gadchiroli where the prevalence of MRSA among *S.aureus* isolates was **38.69%, 36.27%,38.65%** and **34.95%** respectively. In an average MRSA prevalence rate was **36.73%** in Vidarbha region. On the basis of various studies report the prevalence rate of MRSAis 25% to 50% in India. Gadchiroli and Chandrapur districts of Vidarbha region are tribal and economically backward. MRSA prevalence data isnot available from this part of India. Our studies had shown prevalence rate in Chandrapur and Gadchiroli district was 38.69% and 34.95% which very high as compare to other part of our country. The reason might be due to lack of medical facilities illiteracy among the tribal people, unavailability of antibiotics, unhygienic conditions, etc. The study has found higher rates of MRSA infections in elder age population 41-50 years and 51-60years were with a majority of male over females.

All the MRSA were subjected to antimicrobial susceptibility study to the recommended panel of 15 antibiotics. In the present study the populations of four different districts were in consideration i.e. Chandarpur, Nagpur, Amravati and Gadchiroli. In Chandrapur region higher resistance in MRSA was found to multiple antibiotics including Penicillin, Erythromycin, Tobramycin, Tetracyclin, Norfloxicin, trimethoprim, Ciproflaxicin, and lower resistance was found to be Vancomycin, Amikacin, gentamycin, and Nitillin.The antimicrobial susceptibility patterns of MRSA in Nagpur region revealed higher resistance to penicillin, erythromycin, tobramycin, Trimethoprimeand most of the antibiotics shown lower resistance to Vancomycin, Amikacin, Chloramphenicol, gentamycin and Nitillin.In Amravati region antimicrobial susceptibility study of MRSA had showed higher resistance to penicillin, Erythromycin, Lincomycin, Tetramycin, Chloramphenicol and lower resistance to Vancomycin, Nitillin and Amikacin.In Gadchiroli antimicrobial susceptibility study of MRSA had showed higher resistance to penicillin, erythromycin, tobramycin, tetracycline and lower resistance to Vancomycin, and Nitillin. Overall similar type antibiogram patterns were observed in all districts under consideration. These studies revealed amikacin, chloramphenicol and Nitillin antibiotics were most effective drugs for the treatment of MRSA and VRSA infections.

The current Study was carried out the antibiogram patterns of MSSA strains.In Chandrapur district region MSSA among the *S.aureus* isolates was **61.30%.**Higher resistance in MSSA was found to Penicillin, Erythromycin, Trimethoprime and Tobramycin, and most of the antibiotics lower resistance was found to Oxacillin/Methicillin, vancomycin, Gentamycin, Nitillin, Amikacin, Nirfloxacin and chloramphenicol. However, the prevalence rate of MRSA was found to be highest in Vidarbha region.In Nagpur region methicillin sensitive among the *S.aureus* isolates was **63.72%.** The antimicrobial susceptible patterns of MSSA was found to be higher resistance to penicillin, Erythromycin, and Trimethoprime and Nirfloxacinand most of the antibiotics shown good sensitive Vancomycin, Oxacillin, Tobramycin, Nitillin, Methicillin, Chloramphenicol and Gentamycin.In Amravati region methicillin sensitive among the *S.aureus* isolates was found to be **61.64%.** The antimicrobial susceptible patterns of MSSA was found to be higher resistance to penicillin, Erythromycin and Trimethoprime, and most of the antibiotics shown low resistance including oxacillin, vancomycin, Amikacin, Gentamycin, Tetracyclin, Ntillin, Chloramphenicol, and Tobramycin. In Gadchiroli district methicillin sensitive among the *S.aureus* isolates was found to be **65.4%.** This region of Vidarbha is mostly affected with Naxalites and tribal people around Gadchiroli. In this study from this region we found lower rate of MRSA

prevalence as compare to other part of Vidarbha. The antimicrobial susceptibility study of MSSA had showed higher resistance to penicillin, Trimethoprime, and Erythromycin and most of the antibiotics lower resistance to Vancomycin, Oxacillin/Methicillin, Chloramphenicol, Nitillin and Amikacin. In the entire four districts in Vidarbha,vancomycin and methicillin resistance were not seen in methicillin sensitive *Staphylococcus aureus* strains.

Among 281 MRSA strains 41 strains (14.59%) were shown resistance towards vancomycin antibiotics drugs. All the VRSA were subjected to antimicrobial susceptibility study to the recommended panel of 15 antibiotics. In current study, Chandrapur, Nagpur, Amravati and Gadchiroli were shown the prevalence rate of VRSA among methicillin resistance *S.aureus* isolates was **11.88%, 16.21%, 17.85%,** and **15.65%** respectively. In an average VRSA prevalence rate was **14.59%** in Vidarbha region. Antibiogram pattern of VRSA isolates had shown multi-resistant to most of tested antibiotics except some isolates which was sensitive to Amikacin, Nitillin and Chloramphenicol. This report of fully developed VRSA strains in geographically central part of India. Vancomycin has been the drug of choice for serious beta-lactum resistant Staphylococci positive infections for over last few decades. However, the appearance and spreading of resistance to this glycopeptides among clinically important *Staphylococcus aureus* has made it problematic to manage severe infections caused by such pathogens. It is very essential to look for alternative to vancomycin and other glycopeptides in the treatment of serious Staphylococcus infections. It is also equally important to prevent the spread and emergence of glycopeptides resistance by taking proper infection control measures, so that we may not fall back into pre-antibiotic era.

All the tested *S.aureus* isolates were reconfirmed using RT-PCR. PCR amplification of 16S rDNA of the clinical isolates ChP04, ChP12, ChP15, AmP05, AmU03, and ChP16 using universal primer for identification of *Staphylococcus aureus* strains. All the 16s rRNA positive isolated strain were aligned and a phylogenetic tree was constructed. The output was visualized in CLUSTAL alignment was used for the phylogram built up by using the MEGA5 software.Isolate ChP04 was found to be closely related with highly reported strain USA400-0051. These bacterial strains are community associated MRSA infections, particularly skin and soft tissue infections became common in the United State after year 2000. These isolates were first reported in Australia and named as Western Australia-1 clone. Later, ST1-SCCmecIV MRSA, carrying lukSF-PV genes encoding the Panton-Valentine Leucocidin (PVL) were detected in the USA and caused severe infection among America Indian children. After re-emerge in USA and Canada as an important cause of skin-soft tissue infection and

was renamed as USA400 were USA 400-0051 which is prototype of the USA400. The isolate ChP12 was observed to be closely related to strain S167. Where the four isolated strain shows negative results may be due to mutation. As mecA and vanA gene detection technique are very expensive so we were unable to perform mecA gene detection test on all the MRSA and VRSA isolates so we were found oxacillin and vancomycin resistant on disc diffusion method and MIC by E test. MIC test considered the gold strand of MRSA confirmation.

In the present study, mecA genes were detected by using the PCR based amplification technique in the selected MRSA isolates which was initially characterized methicillin resistant by 1µg oxacillin disc diffusion test. In current study, the one selected MRSA strains were positive for mecA gene and one selected VRSA isolates were positive for vanA genes. ChP04 MRSA isolates was positive for mecA and ChP12 isolates was positive for vanA genes. This mecA gene detection by RT-PCR was found to be a rapid and reliable method for single-step identification of cultures of MRSA and may prove to be useful for direct application on clinical specimens. The current study had shown the emergence of VRSA carrying vanA gene in central region Vidarbha of India and although this study was restricted in four district of Vidarbha of Maharashtra state, there might be possibility of development of vancomycin resistance against MRSA in other part of India as antibiotic misuse is equally common in whole country especially backward region. Still, vancomycin is the last choice for the treatment of MRSA infection. Hence, there is immediate need of nationwide antimicrobial surveillance programme against pathogenic *Staphylococcus aureus* including all tribal region of each state of India to control the further emergence and spreading of VRSA strains. A strict regulation on irrational antibiotic usages might be a suitable and effective method in this direction.

15. **CONTRIBUTION TO THE SOCIETY (GIVE DETAILS):**

Since, available treatments against MRSA infection are not sufficient strategies to combat with the rising prevalence of MRSA and VRSA; there is strong need for early diagnosis and detection of the people at high risk of developing resistance against *Staphylococcus aureus* most living in remote interior region of Vidarbha in Maharashtra State.

It has been established that genetic factors confer risk to develop resistance against *S.aureus.*

Development of drug resistance is supposed to as it is, then there has possibility of the nation may fall back into pre-antibiotic era. So, it is essential take immediate action to protect our patient from dangerous MRSA and VRSA infections. Therefore, following specific recommendation are prepared on the basis of data presented in the current study.

The high prevalence of MRSA infection and the emerging of *van*A gene containing of VRSA in Vidarbha seems to be related to overused andmisused of antibiotics. So there is need of immediate establishment ofstrict implementation of antibiotic policy. Following points should beconsidered for establishment of policy.

1) Prescription of antibiotic medicine should be according to antimicrobial susceptibility report as suggested from this study to medical professional.
   - Use of narrow spectrum antibiotics is to control the diseases at initial stages of infections.
   - It is most important to complete the course of antibiotic therapy given by the physician.
   - Ban the selling of antibiotic without prescription.
   - Regulate the usage of antibiotics for both humans and animals
2) The facility for molecular diagnostic technique (by Polymerase Chain Reaction) for the detection of resistant genes should be available to all tertiary care centers of the Vidarbha is an important recommendation of the current study.
3) The present data discovered the emergence of VRSA strain in tribal district Gadchiroli and Chandrapur was alarming situation and immediate action should be taken by government to control the spreading of resistance gens in this region. Also, I recommend more research to be carried out on topic in the near future, especially in Gadchiroli and Chandrapur district.
4) Regular orientation programme must be carried out including awareness of surveillance of nosocomial infection, update on development of antimicrobial resistance, and aggressive antibiotic control programme.

16. WHETHER ANY PH.D. ENROLLED/~~PRODUCED~~: **Yes! (01)**
OUT OF THE PROJECT

17. NO. OF PUBLICATIONS OUT OF THE PROJECT: **(05) Five**

1) There YW, Wadhai VS. *Multidrug resistant Staphylococcus aureus: A global challenge. Drug Discovery, 2013, 7(18), 13-18*

2) ***There, Y.W. and Wadhai, V.S., Bhandari P.R****., A Study on Antimicrobial Susceptibility patterns of Staphylococcus aureus from tertiary care Cenre Chandrapur (M.S.) I J R B A T, Issue (3) Vol. II, May 2015, 358-361 [Impact Factor- 4.935]*

3) **There, Y.W., Wadhai V.S. and Bhandari, P.** *Prevalence of Vancomycin resistance Staphylococcus aureus among MRSA isolates from District Hospital Gadchiroli (M.S.) India. I J R B A T, 2016 Vol. 1 (3), 214-218 [Impact Factor- 4.935]*

4) **Wadhai, V. S. and Ashtankar, A. A.(2017).** *"A Study on methicillin and vancomycin resistant Staphylococcus aureus from tertiary care hospitals, in Vidharbha region, India."International Journal of Current Research, 9, (01)*

5)**Shende, S.P., and Wadhai, V.S. (2017).** *Antibiotic Resistance Profiling of Staphylococcus aureus Isolated from Clinical Specimen from Tertiary Care Hospital.* (ICEMTE-2017) ISSN: 2321-8169 Volume: 5 Issue: 3 06

Manuscript under Communication for Publication: 2

*1) **Wadhai, V. S., Sarkar, P. P. and Ashtankar, A. A.** The life threatening resistance superbug, MRSA and VRSA is currently horizons. In Communication for publication.*

*2) **Wadhai, V.S., Sarkar, P.P. and Ashtankar, A. A.** A Study on methicillin and vancomycin resistant staphylococcus aureus from tertiary care centre, chandrapur (m.s.) india. In communication for publication.*

(PRINCIPAL INVESTIGATOR)
Principal Investigator
UGC Major Research Project

(REGISTRAR/PRINCIPAL)
(Seal)
Principal
Sardar Patel Mahavidyalaya
Chandrapur

(CO-INVESTIGATOR)

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DRUG DISCOVERY

Multidrug resistant *Staphylococcus aureus:* A global challenge

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ABSTRACT

Multidrug resistance is one of the serious problems faced by public health at the beginning of twenty first century. It is usually associated with significant morbidity, longer hospitalization, excess costs and mortality. Staphylococcus aureus is a common pathogen in hospital and community acquired disease that causes a wide range of infection such as skin and soft tissue infection to life threatening disease like respiratory tract infection, meningitis, endocarditis, bacteraemia, musculoskeletal infection and urinary tract infection. Approximately 90% of Staphylococcus strains are resistant to penicillin. In 1961 S. aureus developed resistance to Methicillin (MRSA), invalidating almost all antibiotics, including the most potent β-lactams. Quinolones, such as ciprofloxacin with increased anti-staphylococcal activity are available but their use may become limited due to the rapid development of resistance and allergic reaction during therapy. Vancomycin, a glycopeptides antibiotic, was used for the treatment of MRSA in 1980. Vancomycin resistant S. aureus (VRSA) first detected in the USA in 2002. The microorganisms employ different mechanisms in attaining multidrug resistance such as enzymatic deactivation of antibiotics, decreased cell wall permeability to antibiotics, altered target sites of antibiotic, efflux mechanisms to remove antibiotics, etc. Increasing resistance of S. aureus to last line of drug i.e., vancomycin, highlights the need for either development of new therapeutic agents or implementation of new strategies to control the resistance. Optimal use of existing antimicrobial agents, using alternative treatment options, reducing the used for antimicrobials by increasing immunity, application of nanoparticles in drug delivery, education of health professionals and patients, antibiotic policies and implementation of infection control measures.

Keywords: Methicillin, Vancomycin, Resistance, Nanoparticles.

1. INTRODUCTION

Staphylococcus aureus is a bacterium that belongs to the family of *Micrococcacea*. The bacteria are commensal organism mainly found in normal flora of the skin, intestine, upper respiratory tract and vagina (Lowy, 1998). *Staphylococcus aureus* can become pathogenic when physiological conditions such as pH, temperature and nutrient

availability are altered and become favourable for overgrowth (Mims et al. 2004). The Pathogenicity of *S. aureus* is determined by the production of toxins, such as the 33-kd protein-alpha toxin, exfoliatin A, exfoliatin B and Panton-Valentine leukocidin (PVL) toxins (Lowy, 1998). These toxins can be harmful to the host and cause various type of skin diseases like carbuncles, boils, folliculitis and impetigo and other complications, such as endocarditis, meningitis as well as toxic shock syndrome (TSS) (Mims et al. 2004). In 1878, Koch first noted that Gram-positive cocci responsible for different diseases depending on whether they formed pairs, chains or clusters. The *staphylococci* were identified as grape-like clusters of bacteria isolated from the pus of human abscesses (Ogeston, 1881). In 1884, Rosenbach differentiated species of *staphylococci* based on pigmentation. *Staphylococcus aureus* produced a golden yellow pigment are disease causing, whereas the non-disease causing strain was generally white (Rosenbach, 1884).

2. GLOBAL EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS*

In healthy individuals, the carrier rate of *S. aureus* range between 15% to 35% with a risk of 38% of individuals developing infection followed by a further 3% risk of infection when colonized with Methicillin susceptible *Staphylococcus aureus* (MSSA) (File, 2008) (Chakraborty et al. 2012). Some groups of individuals are more susceptible to *S. aureus* colonization than others including health-care workers, nursing home inhabitants, prison inmates, military recruits and children's (Ben-David et al. 2012) (Ho et al. 2008). In a study, conducted in 2007 by the University of the Witwatersrand and the University Hospital of Geneva, health-care workers are responsible for 93% of personnel to patient transmission of Methicillin resistant *S. aureus* (MRSA) (Albirich et al. 2008). Previously several outbreaks have been reported in Northern-Taiwan in 1997 that suggested MRSA transmission associated with health-care workers, including surgeons (Wang et al. 2001). Grundmann and colleagues reported a prevalence of > 50% in countries such as Singapore (1993-1997), Japan (1999-2000) and Colombia (2001-2002) while countries with a prevalence of 25% to 50% included South Africa (1993-1997), Brazil (2001), Australia (2003), Mexico and United States. The lowest prevalence of less than 1% was found in Norway, Sweden and Iceland (1993-1997) (Grundmann et al. 2007). In 2007, a prevalence of more than 50% of MRSA strains isolated from Cyprus, Egypt, Jordan and Malta was reported by Borg and colleagues. This high prevalence was due to overcrowding and poor hand hygiene facilities in the hospitals (Borg et al. 2007). From India, recently report 80% of MRSA strains were multidrug resistant. However all were uniformly sensitive to Vancomycin and Linezolic (Khan et al. 2007).

3. *STAPHYLOCOCCUS AUREUS* CARRIAGE AND DISEASE

Staphylococcus aureus is found as a commensal organism on the squamous epithelium of the anterior nares up to 20% of the population at any one time, however, it has been estimated that *S. aureus* can transiently colonize up to 60% of the human population (Foster, 2004). *S. aureus* can cause various types of infections ranging from minor skin abscesses to more serious invasive diseases. *S. aureus* commonly causes skin infection like boils, carbuncles, furuncles and impetigo, but after gaining access to the blood, it may be result in a major cause of endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicaemia (Lowy, 1998). Many invasive staphylococcal infections are correlated with nasal carriage of infecting strains. Although immunocompromised patients may be at greater risk for developing an invasive *Staphylococcal* infection, healthy individuals may be also susceptible, especially if they are carriers (Peacock et al. 2001).

4. ANTIBIOTICS

Antibiotics are hailed as the greatest medicinal achievement of the 20th Century. Before their discovery, there was higher mortality rate due to microbial infection. The earlier development of vaccination had introduced immunity to some diseases and sterilization had helped to reduce the chance of infection from surgery. With the subsequent formation of germ theory and the work identify the role of specific bacteria in the diseases anthrax and tuberculosis, the search for a cure began (Kaufmann et al. 2005). In 1929, Fleming noted that the growths of bacteria could be inhibited by the presence of a mould, *Penicilium notatum*. This effect was caused by a metabolic product from the mould that was interacting with the staphylococcal culture (Fleming, 1929). Penicillin was the first of the family of β-lactam that now form the largest share of the antibacterial market.

4.1. Treatment and prevention of *S. aureus* infections

Penicillin is still the main drug of choice for *staphylococcal* infections as long as the isolate is sensitive to it (Kowalski et al. 2007). Cephalosporin, such as cefazolin or cephalothin can be administered as an alternative choice of treatment to the patient with delayed- type penicillin allergy. Semisynthetic penicillin, such as Methicillin, is used for patients with β-lactamase producing staphylococcal isolates. Patients who have an MRSA infection are treated with a glycopeptides known as vancomycin. Vancomycin is the empirical drug of choice for the treatment of MRSA (Michel et al. 1997). Patients who are intolerable to vancomycin are treated with a fluoroquinolone (ciprofloxacin); lincosamide

(clindamycin); tetracycline (minocycline) or trimethoprim-sulfamethoxazole, which is also known as co-trimoxazole (Lowy, 1998).

Novel quinolones, such as ciprofloxacin with increased antistaphyloccal activity are available but their use may become limited due to the rapid development of resistance during therapy[1]. Several antimicrobial agents with activity against MRSA are currently evaluated and include: (i) oritavancin, a semisynthetic glycopeptide; (ii) tigecycline, a monocycline derivative (Guay, 2004) and (iii) DW286, a fluoroquinolone (Kim et al. 2003). Amongst these three antibiotics, tigecycline has been approved by the Food and Drug administration (FDA) in June 2005 (Stein et al. 2006).

Recently, an evaluation of glycosylated polyacrylate nanoparticles showed to have *in vitro* activities against Methicillin-resistant *Staphylococcus aureus* (Abeylath et al. 2007). Other recent investigative drugs include, silver nanoparticles, oleanolic acid extracted from *Salvia officinalis* (Sage leaves) (Yuan et al. 2008). Two novel antibiotics, neocitreamicins I and II, isolated from a fermentation broth of a *Nocardia* strain have shown to have *in vitro* activity against *S. aureus* and vancomycin-resistant *Enterococcus faecalis* (VRE) (Peoples et al. 2008). Accurate empirical therapy against *S. aureus* infections would be an important step towards the reduction of the development of resistance in the different strains.

4.2. Mechanism of action of Antibiotics

Antibiotics work in variety of ways. Some antimicrobial agents inhibit bacterial cell wall synthesis. These agents include β-lactam compounds such as penicillins (e.g. penicillin G, ampicillin and methicillin), cephalosporins and carbapenems, as well as monolactams and β-lactamase inhibitors. β-lactams inhibit the final stage of murein synthesis. This, by some undetermined mechanism, triggers murein hydrolases to lyse the cell. A related group of antibiotics that prevent a different step in cell wall synthesis are the glycopeptides, vancomycin and teicoplanin. Other agents have an antibacterial effect by inhibiting protein synthesis. Representatives of this group include the aminoglycosides, tetracyclines, macrolides and chloramphenicol which interfere with ribosome function. In addition, there are antibiotics that inhibit DNA synthesis, including quinolones, fluoroquinolones and sulfonamides (Normark et al. 2002).

5. MECHANISM OF ANTIBIOTICS RESISTANCE

Antimicrobial resistance is natural phenomenon & its effects, amplified by continuing and unnecessarily increase exposure to antimicrobials. Microorganisms have a distinct property to develop resistance against antimicrobials for their survival. They are enabling to carryout changes at genetic level & inherited these changes to next generation. They can also transfer these changes between same or different species thus contribute significantly in dissemination of resistance. In general mechanisms antimicrobial resistance come in four forms (www. tufts.edu).

- Enzymes that destroy or modify the antimicrobial substrate.
- Target site alteration like alteration of DNA gyrase, a target of fluoroquinolones.
- Bypass pathways that substitute for a metabolic pathway.
- Barrier to penetration or efflux pumps that exclude the agent.

5.1. Beta-Lactam Drugs

In Penicillins there are two main mechanism of resistance: (i) Cleavage of the β-lactam ring by β-lactamases/penicillinases, (ii) Alterations in the target PBPs that reduce their affinity to the penicillins. These two mechanisms are especially important to β-lactam resistance in *S. aureus*. Inactivation of β-lactam drugs: β-lactamase production appears to be the most common mechanism of resistance, with the discovery and identification of more than 100 distinct β-lactamases (Chambers et al. 1998). In terms of β-lactamase mediated resistance, the action of penicillin is prevented when the β-lactam ring of the antibiotic is hydrolyzed by β -lactamase. These molecules are extracellular enzymes which are divided into four types, A through D. In *S. aureus*, serotypes A and C have high activity. Genes for β-lactamase production, *blaZ oxpenP* are usually plasmid encoded, but these resistance genes may sometimes be found on the chromosome of the bacteria. *blaZ* is the gene that codes for the β -lactamase enzyme. In *S. aureus, blaZ* is carried by plasmids and is located on mobile genetic elements acquired from other bacteria. Three *S. aureus* transposons carry the *blaZ* gene: Tn4001, Tn4002 and Tn552. Tn552 encoded β-lactamase resistance is the most common in *S. aureus* plasmid.

Methicillin resistance is another important β-lactam drug resistance mechanism in *S. aureus.* Methicillin is a semi-synthetic penicillin derivative. Resistance to this β-lactam drug in *S. aureus* is of great concern to medical and scientific personnel. The genes for methicillin resistance are located on the chromosome. The genes in the signaling pathway for methicillin resistance are *mecA mecRl, mecR2* and *mecl. mecA* codes for a penicillin-binding protein, PBP2a (also called PBP2) which has a lower binding affinity for β-lactam drugs than regular PBPs. PBPs are transpeptidases involved in the construction of the bacterial cell wall. The regulation of methicillin resistance resembles that of β-lactamase expression. The chromosomally located gene, *mecRl,* like the plasmid located *blaRl,*

codes for a sensor-transducer that is part of a two-component signalling system. *mecI* is a repressor of *mecA.* When *mecI* is bound to DNA, PBP2a is not produced. When *mecI* is unbound, then *mecA* is transcribed and PBP2a is produced. Lewis and Dyke found that *mecI* is also an effective regulator of *blaZ* and *blaI. mecR2*, like *blaR2*, is an accessory molecule involved in regulating PBP2a production (Lewis et al. 2000).

5.2. Aminoglycosides

The first method of resistance to aminoglycoside is via an alteration in the ribosomal target site. Mutations in the genes encoding ribosomal receptor proteins can result in changes in the structure of the ribosome such that it no longer binds the antibiotic or these receptor proteins may be absent. A second mechanism of resistance is impaired uptake of the antibiotic that diminishes the effective intracellular concentration of the antibiotic. It has been proposed that membrane impermeability may be a result of genotypic changes such as mutations in or deletions of porin proteins or other proteins involved in the transport and maintenance of the electrochemical gradient. Another suggested reason for impermeability is a phenotypic change owing to growth conditions under which the oxygen-dependent transport process is not functional (Chambers et al. 1998). The third mechanism of resistance is the most common and is due to the chemical inactivation of the amino glycoside by specific enzymes. Aminoglycosides may be acetylated at secondary amino groups by aminoglycoside acetyltransferases (AAC), adenylated at hydroxyl groups by aminoglycoside adenyltransferases (AAD) or phosphorylated at hydroxyl groups by phosphotransferases (APH). Modified aminoglycoside antibiotics no longer bind to ribosomes and accordingly are unable to inhibit proteins synthesis.

5.3. Tetracyclines

There are three main mechanisms of resistance to tetracyclines. 1) Decreased intracellular accumulation of the drug due to impaired influx or increased efflux via an active transport protein pump. 2) Ribosome protection due to the production of proteins which interfere with the tetracycline binding to the ribosome. 3) Enzymatic inactivation of tetracycline by chemical modification. In *S. aureus*, resistance is due to active efflux of the antibiotic out of the cell. Tetracycline resistance determinants may be chromosomally-encoded or plasmid-encoded (Lyon et al. 1987).

5.4. Glycopeptide

Vancomycin is most important member of this class which is last choice to treat *Staphylococcal* infection. There are two forms of vancomycin resistance have been demonstrated. The first form involves changes in the peptidoglycan synthesis (Walsh et al. 2002). There is a visible irregularly shaped & thickened cell wall, due to increased amount of peptidoglycan. There is decrease in cross linking of peptidoglycan strands resulting in the exposure of more D-Alanyl-D-Alanine residues (Hiramatsu et al. 1998). The second mechanism of resistance due to *vanA* operon, which is result of conjugation process between *E. faecalis* & MRSA strains. The *vanA* gene together with its regulator genes*, vanSR*, from vancomycin resistance *Enterococcus faecalis*(VRE) is carried by transposon, Tn1546 which is result into alteration of target site; the D-Ala-D-Lac instead of D-Ala-D-Ala (Gonzalez-Zorn et al. 2003).

5.5. Fluoroquinolone

This class include ciprofloxacin, ofloxacin, Norfloxacin, levofloxacin, grepafloxacin, trovafloxacin, etc and kill bacteria by inhibiting the DNA synthesis (Hooper, 2002). This class initially developed for the treatment of Gram-negative and Gram-positive bacteria other than *S. aureus*, thus exposure of S*. aureus* to fluroquinolone are minimal. *S.aureus* resistance to fluroquinolones is suggested to be as a result of exposure of the bacteria to fluroquinolone in mucosal and cutaneous surface of nasal cavity (Blumberg et al. 1991). Recently, a study reported an 85% fluroquinolone-resistance in MRSA strain (Udo et al. 2008). *S.aureus* develop resistance against fluoroquinolones by altering the target site i.e. DNA gyrase and topoisomerase, which are responsible for DNA replication.

6. DIAGNOSTIC IDENTIFICATION

The *S.aureus* identification is based on the phenotypic & genotypic investigation (Fluit et al. 2001). Phenotypic identification of *S.aureus* include Gram-staining, Catalase, Coagulase, DNAse, culture on Mannitol salt agar or blood agar & sugar fermentation test (Waldvogel, 2000). Upon identifying *S.aureus* by Gram staining(Gram positive cocci), Catalase(positive), fermentation test (oxidase positive) & tube coagulase(positive) or DNAse test (positive), the sample is grown on Mannitol salt agar or blood agar at 37^{O}C for 18 to24h . The colonies appear yellow on MSA & creamy white on blood agar. *S.aureus* colonies are subjected to antimicrobial susceptibility testing by Kirby Bauer disk diffusion method, automated methods such as the Vitek (bioMerieux, France) & Microscan (Dade Microscan, West Sacramento, CA) systems or conventially available method including Latex agglutination assay kits (Brown et al. 2005). Various molecular techniques have been implemented for the rapid identification of MRSA and VRSA strains is based

on the amplification of *mecA* & *vanA* gene respectively, which confer resistance to Methicillin & vancomycin (Mcclure et al. 2006).

7. CONCLUDING REMARK

The evolving of resistance in *Staphylococcus aureus* is continue to currently available antimicrobial drugs by changing in their genetic information by various mechanism. *Staphylococcus aureus* was developed resistance to almost all class of antibiotics. Vancomycin from glycopeptides category is last resort of drug. Hence there is need of implementation of new strategy and policies to controlled antibiotic resistance problem so that we may not fall back into pre-antibiotic era. The most effective way to prevent emergence of antibiotic resistance is by continuous surveillance of antibiotic resistance profile, reduce misused of antimicrobial drug by proper diagnostic procedure, development of significant new antimicrobial agents and also need of effective education & training to the public about the limitation of antibiotics so that they can utilized it carefully & also the need to adopt a personal hygiene.

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Antibiotic Resistance Profiling of Staphylococcus aureus Isolated from Clinical Specimen from Tertiary Care Hospital

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Abstract: In past two decade broadcasting and spread of antibiotic resistance in staphylococcus aureus micro-organism infect skin and soft tissue posing a great therapeutic challenge. Particular methicillin resistance S.aureus [MRSA] diverse mechanism of resistance based on phenotyping and genotyping. Typing of MRSA is vital to understand epidemiological swing and to initiate infection with control strategies. Also a certain group of S. aureus depute vancomycin resistance chronic creation of VRSA upon exposure to vancomycin. VRSA acquired by mutation based on thickening of cell wall due to accumulation of excess amount of peptidoglycan.

Keyword *MRSA, VRSA, S. aureus, Phenotyping, Genotyping, Resistance*

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I. INTRODUCTION

Staphylococcus aureus is one of the members of genus *staphylococcus*. That occurs ubiquitous and forms the most common cause of localized superlative lesions in human being having ability to developed resistance to penicillin. *Saureus* infection is wide spread concern due to its increasing resistance to miscellaneous type of antibiotic. Although soft-tissue infection are habitual, latest trouble in both inpatient and outpatient with uncomplicated peripheral infection on folliculitis, cellulitis and abscesses ,that intricate infection such as necrotizing fascilitis burn infection and diabetic foot, having consequential role blooming the antimicrobial resistance and illegitimate treated SSTI (skin and soft tissue infection) may primacysedevalution of endocarditis, asteomyclitis brain-lungs abscess or meningitis and toxic shock syndrome, due to familiar organism mannerly encountered in soft tissue infection are gram positive cocci, golden-grapes like cluster , non-motile, non-spore forming which measure around 0.7 to 1.2 um in diameter under microscope notably as *S. aureus*].(1)

THE FASCINATING DISCOVERY OF ANTIBIOTIC

Therapeutically fascinating story of development and evidenced of penicillin, it already design by Ernest Duchesne, (French medical student) but his work was forgotten. Penicillin rediscover by Scottish physician Alexander Fleming. During first world word Fleming had been enthusiastic to discovered unspecified think that would kill pathogen on wound infection

One day in September 1928 *penicillium notatum* spore unexpectedly landed on the surface of an exposed petri dish before it had been inoculated with staphylococci and new medical epoch was born. Although the precise event are still unclear. Ronald Hare has suggested that Fleming left the contaminated plate on a laboratory bench while he was on vacation, the first few days of vacation were cool, the fungus grew more rapidly than the bacteria and produced Penicillin. When the weather then turned worm, the bacteria began to grow and were lysed. On his returned Fleming noticed that a penicillin colony was growing at one edge and the *staphylococci* surrounding it had been destroyed, rather than discarding the contaminate plate, he correctly deduced that his mold contaminant was producing a diffusible substance lethal to *staphylococci*. He found that broth from a penicillium culture contained penicillin and that the antibiotic could destroy several pathogenic bacteria. Unfortunately Fleming next experiment convinced him that penicillin would not remain active in the body long enough after injection to destroy pathogen. After reading Fleming paper on penicillin one of Floreysco worker, Ernst chain in 1940 obtained the penicillium culture from Fleming and set about culture from Fleming and set about culturing it and purifying penicillin. When the purified penicillin was injected into mice they survived. Later Selman Waksman announced in 1944 that he got new antibiotic streptomycin produced by actinomycete streptomycin griseus. (8)

Implementation of Antibiotic in treatment of *S. aures* infection.

In 1880 era S.aureus bacterium perceived as *staph* at this epoch *S. aureus* infection inducement for painful skin and soft tissue. In 1940 penicillin born against *S. aureus*. In late 1940-1950 *S. aureus* strain resistance to penicillin, then high levels of penicillin resistance followed by the development and spread of strain resistance to the semi synthetic penicillin (methicillin, orocillin a nofcillin in 1961. In 1968 the first human case of MRSA reported in United States subsequently new strain evolution become resist to drags designed assist to

attack infection MRSA are resistant to all betalactum antibition. In 1996 the first MRSA to acquire resistance to vancomycin was isolated from a Japanese patient. In 2002 US documented that *S. aureus* resistant to the vancomycin so called Vancomycin resistant *S*. aureus

VRSA Development of Drug Resistance–

MRSA announced for resistive disparate antiseptics due to cellular changes that negatively affect the accumulation of antiseptic agents' comparise cell envelope expressed effiux mechanism. In *S. aureus*maltidrug resistance while quac A/B/G/H are multidrug family found in plasmid. (12)

Efflux Mechanism of MRSA and Drug development

MRSA strain fetch mecA gene encodes for low affinity penicillin binding protein (PBP) designated PBP2a. Predominantly mecA gene is part of chromosomally integrated mobile genetic element Known as *staphylococcal* cassette chemosemec (SCC mec) .This PBP2a possess Peptidoglycan transpeptidase activity yet low affinity for B-lactum antibiotic PBP2a exhibit not only constant rate of reducing acylation but also replitedalissociation constant by B. lactum so called as Betalactumase resistance.(10)

Vancomycin Resistance S.aureus and their Mechanism

MRSA has accounted in many Countries since its discovery in 1961, however in recent era the increasing frequency of MRSA infection. Due to in unequivocal cell-killing affect, and capable of eliminating MRSA from the patient body. So increased use of vancomycin, thus drug with rather weak cell-killing Potency against prevailing MRSA. Two class of vancomycen resistance Strain .VRSA that has a vancomycinmnimum inhibitory concentration (MIC Breckpoinout) of 8mg/l and hetro VRSA that spontaneously generates VRSA within the cell population (9)

Mechanism of VRSA

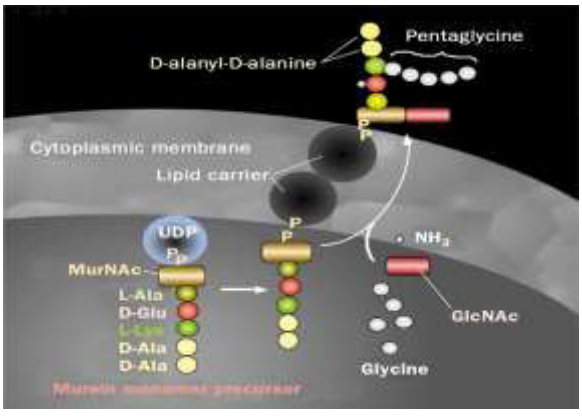

Figure 1. Synthesis of murein monomer (monomeric component of peptidoglycan). Murein monomer is composed of two amino sugars (N-acetyl muramic acid [MurNAc] and N-acetyl glucosamine [GlcNAc]) and ten amino acids. Murein monomer precursor is composed of MurNAc and stem peptides (L-alanine, D-glutamine acid, L-lysine, and two D-alanines). It is synthesized in the cytoplasm and attaches to a lipid carrier in the cytoplasmic membrane. Then, during its transfer to the outer surface of the cytoplasmic membrane, GlcNAc and five glycine are added, and its isoglutamic acid is amidated to become mature mureinmonomer.

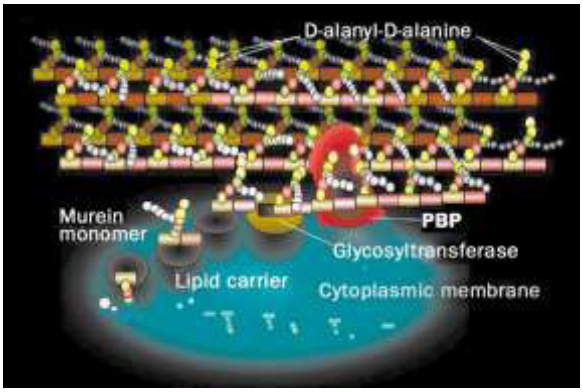

Figure 2. Assembly of peptidoglycan viewed from outside of the cell. In blue is the cytoplasmic membrane. Glycosylic transferees polymerizes therein monomer to produce a nascent peptidoglycan single chain. Penicillin-binding protein (PBP) grasps at the D-alanyl-D-alanine residues of stem peptide and cleaves in between the residues to ligate the penultimate D-alanine to the pentaglycine of the neighboring peptidoglycan chain. The twisting of peptidoglycan chains is omitted from the illustration for visual simplicity.

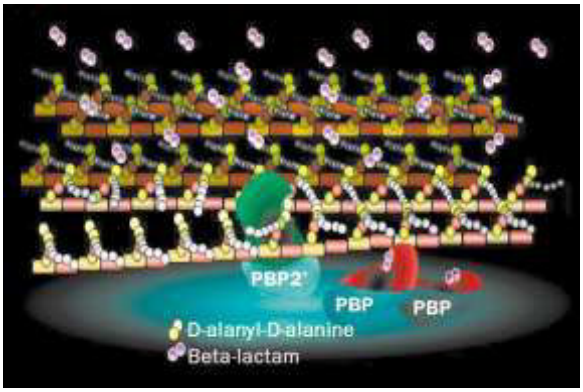

Figure 3. Action of beta-lactam: Beta-lactam (purple double cubes) is a structural analogue of D-alanyl-D-alanine residues. It inactivates S aureus PBPs (in red), but cannot bind to PBP2_ (in green; MRSA-specific PBP) with high affinity. Therefore, MRSA can continue peptidoglycan synthesis in the presence of beta-lactams whereas methicillin-susceptible S aureus cannot.

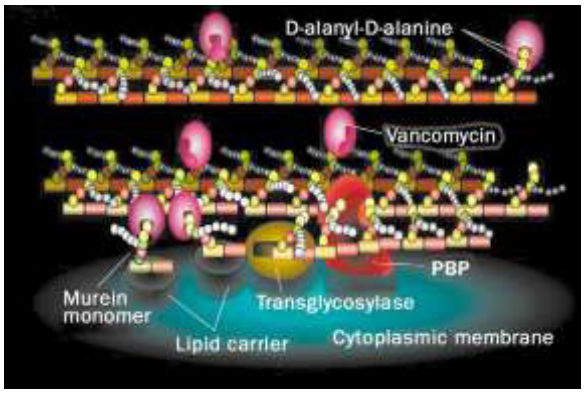

Figure 4. Action of vancomycin and teicoplanin. Drug binds to D-alanyl-
D-alanine residues of murein monomer. The murein monomer bound by vancomycin does not serve as a substrate for glycosyltransferase.

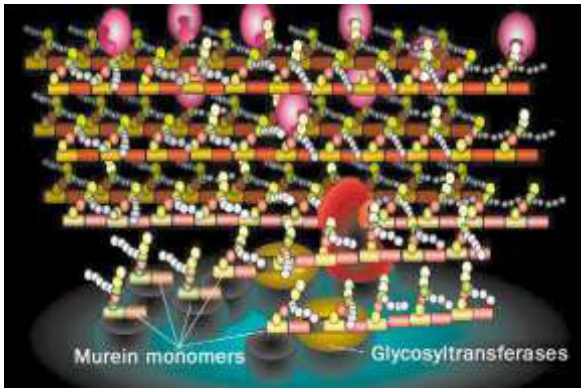

Figure 5: Thickened cell wall of Mu50. Affinity trapping mechanism of resistance Mu50 has 30–40 layers of peptidoglycan. Supply of mureinmonomer is increased and more monomers are incorporated into nascentpeptidoglycan chains. Increased D-alanyl-D-alanine residues are present in the completed peptidoglycan layers. More vancomycin molecules are trapped in the peptidoglycan layers and less reach the cytoplasmic membrane than usual.

II. CONCLUSION

Theoretical research with the ultimate goal to develop and promote enhance diagnosis, better conceptual treatment and new vaccines that are consequence against MRSA/VRSA that gives increasing prevalence of MRSA/VRSA in both hospital and community setting. In this study reports that commonest organism like *S. aureus* encountered in skin and soft tissue infections. However, in this view varied bacteriology and antobiogram of SSTI. So on this based efflux mechanism of MRSA/VRSA discussed with antion of their drug resistance.

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PREVALENCE OF VANCOMYCIN RESISTANCE *STAPHYLOCOCCUS AUREUS* AMONG MRSA ISOLATES FROM DISTRICT HOSPITAL GADCHIROLI (M.S.) INDIA

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Abstract

Introduction: *Staphylococcus aureus is* the frequently isolated from patients with serious healthcare-associated infections (HAI). Methicillin & Vancomycin Resistant *Staphylococcus aureus* infections are a tremendous, and growing, burden for healthcare systems and hospitals, and are associated with significant healthcare costs. So there is need to continuous monitoring of development of resistant to avoid fall in pre antibiotic era. The main objectives of this study was to find out the antibiogram patterns of *S.aureus*, the prevalence of methicillin resistant *S.aureus* and demonstration of Vancomycin resistance among MRSA strains at the tribal region Gadchiroli (M.S.), India. **Method**: From Government district hospitals, Gadchiroli the total 234 clinical samples were collected from different source and 114 samples were positive for *S. aureus*. Out of these 78 clinical samples were positive for coagulase test. The pure isolates of coagulase positive *S.aureus* were screened on Oxacillin resistant screen agar and tested for antimicrobial susceptibility by using standard methods. **Results:** Out of 78 clinical specimens samples were found to be coagulase positive *S.aureus*. The antibiotics oxacillin, penicillin, erythromycin, gentamycin and tetracyclin had shown maximum resistance on disc diffusion. Amikacin, chloramphenicol and vancomycin antibiotics had showed high sensitivity to all resistant strains of MRSA. The noticeable result in this region was nitrofurantoin had shown around 50% resistance. Of the isolates 50 isolates were methicillin resistant *S.aureus* (MRSA). According to Disc Diffusion method the Prevalence rate of MRSA was found 64.10 % and Vancomycin resistant among MRSA isolates was found 16%. MIC By Etest method had shown MRSA (48.71%) and VRSA(13.15%). **Conclusion:** Our study emphasizes the need for continuous monitoring of the antimicrobial susceptibility pattern of *S.aureus* isolates including MRSA for the selection of appropriate therapy. Gadchiroli is the backward tribal region of Vidarbha, from the present findings it appears that the spread of MRSA in community is very high and the vancomycin which is the last choice for the treatment MRSA strains also shown resistant. So there is need of high alert in hospital settings and need of continuous monitoring and surveillance to control resistance.

Keywords: MRSA, VRSA, Antibiotics and Oxacillin

1 Introduction

Healthcare-associated methicillin-resistant S. *aureus* (MRSA) is a major cause of nosocomial infections worldwide, with significant attributable morbidity and mortality in addition to pronounced healthcare costs. Many hospitals struggle with increasing amounts of MRSA, which are "multi-resistant" against all beta-lactam antibiotics. Often, applicable antibiotics for treatment are only glycopeptides like vancomycin and teicoplanin[9]. Methicillin-resistance in *S.aureus* occurs when an isolate carries an altered penicillin binding protein, PBP2a, which is encoded by a 50 kb piece of *DNA-mecA* gene which generates the production of an altered cell wall component (PBP2a) to which penicillins and cephalosporins cannot attach. Methicillin resistant *Staphylococcus aureus* (MRSA) strains have become endemic in hospitals worldwide. Antibiotic resistant pathogen constitutes an important and growing threat to public health. Healthcare associated Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infection with significant attribute morbidity and mortality in addition to pronounced healthcare cost[1]. Many hospital struggles with increasing amount of MRSA which are multi-resistant against all beta-lactum antibiotics. Gadchiroli city is a Tribal region of Maharashtra state, limited reports were available on development of Methicillin and other antibiotics resistant *Staphylococcus aureus* form this part of India. The purpose of present study was to evaluate current antimicrobial susceptibility patterns of *Staphylococcus aureus* and prevalence of vancomycin resistant *Staphylococcus aureus* among MRSA isolates.

2 Materials and Methods

The present study was conducted on the clinical specimens collected from Government district hospitals, Gadchiroli the total 234 clinical samples were collected from different source and

114 samples were positive for *S. aureus*. Out of which specimen was found to be coagulase positive *Staphylococcus aureus*. Of the 78 clinical specimens, 48 specimens were from pus, 18 were from burn patient and 12 from sputum sample. Standard procedure was followed for isolation and identification of *S.aureus* and to perform antimicrobial activity[7].In brief the specimen was collected in sterile container and transport to the laboratory. The specimens were then immediately inoculated nutrient broth tube and incubate overnight at 37^{0}C for enrichment. Then the loop full of sample transfer to the Blood agar and the Mannitol Salt Agar and were incubated at 37^{0}C for 18-24 hours. The suspected isolated colonies were exposed to Gram's staining and other biochemical test. *Staphylococcus aureus* organisms were confirmed mainly by positive DNase test and coagulase tests. Confirmed coagulase positive *S.aureus* isolates were screened for methicillin resistant on Oxacillin resistant screen agar. Methicillin resistant *S. aureus* were further subjected to antimicrobial sensitivity testing by standard disk diffusion method and minimum inhibitory concentration by E-test method as per NCCLS standards[2,3,7,10]. All the culture media, antibiotics discs and E-test strips were obtained from Hi media laboratory.

3 Results

From Government district hospitals, Gadchiroli the total 234 clinical samples were collected from different source and 114 samples were positive for *S. aureus*. Out of these 78 clinical samples were positive for coagulase test **(Table 1) [figure 1]**. Out of total, 42 (53.84 %) and 36 (46.16 %) of *Staphylococcus aureus* isolates were isolated from males and females, respectively. The age wise distribution of total patients with Coagulase positive *S. aureus* infection were as follows; the age group 0-10 years included 19(24.36%), 11-20 years 02(02.56 %); 21-30 years 03(03.85 %); 31-40 years 05(06.41%); 41-50 years 22(28.21 %); 51-60 years 19(24.36 %); 61-70 years 08(10.26 %); and 71-80 years 00 (0.00 %). In this region 41-50 and 51-60 age groups population were extremely affected with *Staphylococcus aureus* infection.

3.1 Overall resistant patterns of *S. aureus*

A total of 78 viable strains of *S. aureus* from Gadchiroli region were tested for antimicrobial susceptibility by disc diffusion. The antibiotic susceptibility test by disc diffusion was done on each isolate by using 12 antibiotics; Oxacillin (OX), Amikacin (AK), Tetracycline (TE), Erythromycin (E), Gentamycin (GN), Methicillin (MET), Chloramphenicol (C), Penicillin (P), Tobramycin (TB), Norfloxacin (NX), Nitrofurantoin (NF) and Vancomycin (V).

The overall resistance pattern of each antibiotic tested was as follows; oxacillin 50 (64.10%), Amikacin 17 (21.79%), Tetracycline 42 (53.85%), Erythromycin 62 (79.49%), Gentamycin 46 (58.97%), Methicillin 50 (64.10%), Chloramphenicol 12 (15.38%), Penicillin 78 (100.00%), Tobramycin 59 (75.64%), Norfloxacin 53 (67.95%), Nitrofurantoin 44 (56.41%) and Vancomycin 08 (10.26%) **(Table 2) [Figure 2]**.

3.3 MIC of Methicillin and Vancomycin by E-test method

All methicillin resistant *S. aureus* strains found on Disc Diffusion method and ORSAB were further tested for Minimum Inhibitory Concentration (MIC) by Etest method for methicillin and vancomycin antibiotics. Moreover, the result of susceptible strains has MIC's to oxacillin of <4 mg/L. Oxacillin Etest were read after 24 hours and after 48 hours. After that, if the reading is negative, the sample was considered as MSSA strain.

The pattern of antimicrobial susceptibility using MIC's on two antibiotics was as follows; oxacillin was resistant to 38 (76.00%) and 12 (24.00%) sensitive; vancomycin was resistant to 05 (62.50%) and sensitive to 03 (37.50%) **(Table 3)**.

3.4 Incidence of MRSA and VRSA in Gadchiroli region

The incidence rate of methicillin resistance among 78 coagulase positive *S. aureus* isolates on disc diffusion and MIC was 64.10% and 48.71 % respectively as shown in **Table 4**. Antimicrobial susceptibility test by MIC is considered as gold standard; therefore the prevalence rate of MRSA in Gadchiroli region was 48.71%. According to MIC test the incidence rate of VRSA among MRSA isolates was found 13.15%.

Table 1: Coagulase positive & negative with number of samples of *S. aureus* in Gadchiroli

Sample	CoPSA	CoNSA	Total
Pus	48	14	62
Sputum	12	12	24
Burned Wound	18	10	28
Total	78	36	114

Table 2: Over all distribution of antimicrobial susceptibility of *S. aureus* on disc diffusion in Gadchiroli (Total No. 78)

Sr. No.	Name of Antibiotic	Resistant		Sensitive	
		No.	%	No.	%
1.	Oxacillin (Ox)	50	64.10%	28	35.90%
2.	Amikacin (Ak)	17	21.79%	61	78.21%
3.	Tetracycline (Te)	42	53.85%	36	46.15%
4.	Erythromycin (E)	62	79.49%	16	20.51%
5.	Gentamycin (GN)	46	58.97%	32	41.03%
6.	Methicillin (MET)	50	64.10%	28	35.90%
7.	Chloramphenicol (C)	12	15.38%	66	84.62%
8.	Penicillin (P)	78	100.00%	0	0.00%
9.	Tobramycin (TB)	59	75.64%	19	24.36%
10	Norfloxacin (NX)	53	67.95%	25	32.05%
11	Nitrofurantoin (NF)	44	56.41%	34	43.59%
12	Vancomycin (V)	8	10.26%	70	89.74%

Table 3: Antimicrobial susceptibility by MIC on methicillin resistant strains of *S. aureus* found on disc diffusion in Gadchiroli

Sr. No.	Name of Antibiotic	Antimicrobial Susceptibility test			
		MIC			
		Resistant		Sensitive	
		No.	%	No.	%
1	Oxacillin (OX) (**Total-50**)	38	76.00	12	24.00
2	Vancomycin (V) (**Total-08**)	05	62.50	03	37.50

Table 4: Comparison of antimicrobial susceptibility pattern of MRSA strains identified on disc diffusion with MIC in Gadchiroli

Sr. No	Name of Antibiotics	Antimicrobial Susceptibility Test							
		Disc Diffusion				MIC			
		Resistant		Sensitivity		Resistant		Sensitivity	
1	Oxacillin **(Total-78)**	50	64.10%	28	34.90%	38	48.71%	40	51.29%
2	Vancomycin **(Total-50)**	08	16.00%	42	84.00%	05	13.15%	33	86.85%

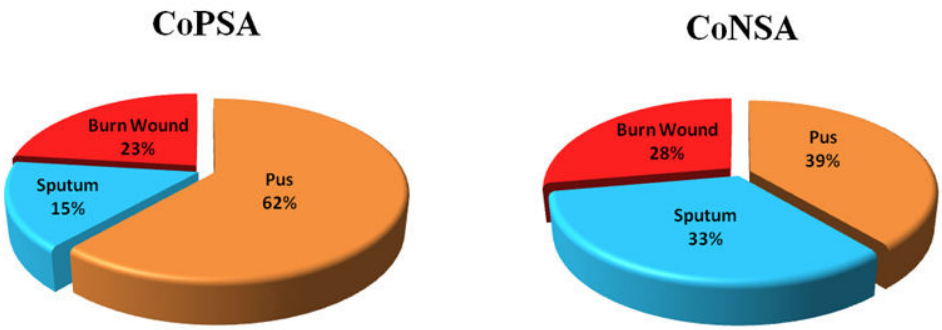

Figure 1: Sample wise Percentage of Coagulase positive *S. aureus* in Gadchiroli

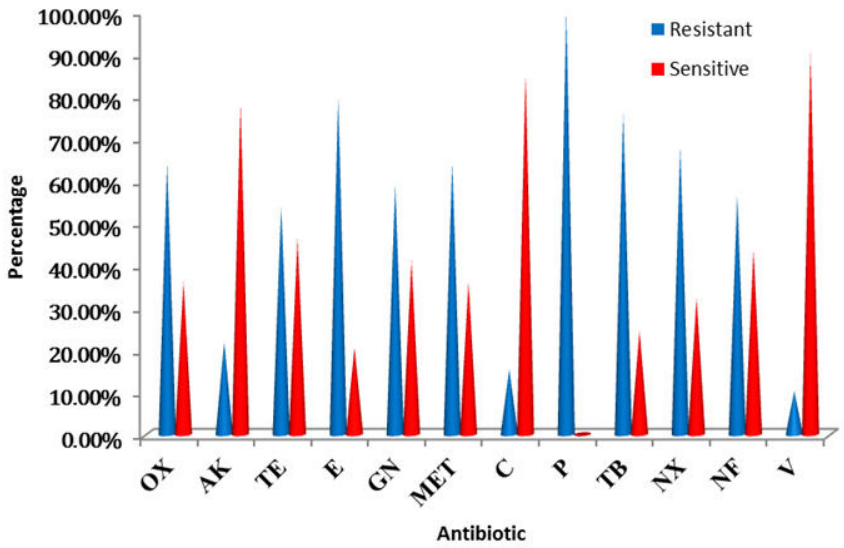

Figure 2 Overall distribution of antimicrobial susceptibility of *S. aureus* on disc diffusion in Gadchiroli

Discussion

This study was conducted at Gadchiroli district Hospital which is declared as tribal region. Very few reports are available on MRSA from this region. According to antimicrobial susceptibility by MIC Etest method, 38 methicillin resistant isolates were and the prevalence rate of MRSA is 48.71%. The result were shown that significant rise in antimicrobial resistance in this region as compare to other part of India. In other country the prevalence rate of MRSA was found different, in India (31-39%), Pakistan (84%), Malaysia (40%), USA (52%)[14-15]. The emergence Vancomycin resistance against MRSA strains are great concern. First case of VRSA resistance was reported in 2002(USA)[15], Then some other countries also reported VRSA resistance . In India Ashdulla et al have reported Vancomycin intermediate *S.aureus* (VISA) and many reports from north India also recorded the emergence of low level and intermediate vancomycin resistance[14-15].Venubabu et al reported VRSA strain from Hyderabad in 2009[16]. In our Study out of 38 MRSA strains 05 strains(13.50%) had shown vancomycin resistance. So it is quite alarming situation to the community and clinicians. The development of antibiotics resistance may be due to misused and uncontrolled used of drugs without proper diagnosis.

Conclusion

The present study first time exposed the vancomycin resistance in this part of India. Prevalence rate of MRSA and VRSA was found 48.71% and 13.15%. These findings are suggesting the need of regularly monitoring the antibiotic resistance patterns of MRSA and implementation of strict rules and regulation on antibiotic usages otherwise; we are supposed to be entering again into pre-antibiotic era. The most effective way to prevent emergence of antibiotic resistance is by continuous surveillance of antibiotic resistance profile,

reduce misused of antimicrobial drug by proper diagnostic procedure, development of significant new antimicrobial agents and also need of effective education.

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RESEARCH ARTICLE

A STUDY ON METHICILLIN AND VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM TERTIARY CARE HOSPITALS, IN VIDHARBHA REGION, INDIA

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Staphylococcus aureus,
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ABSTRACT

Multidrug resistant methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community acquired infections and is on the rise. The glycopeptides vancomycin has been proposed as the drug of choice for treating such infections. The present study aimed at identifying the methicillin and vancomycin resistance *staphylococcus aureus* from tertiary care hospitals in Vidarbha region, (M/S) India. This study presents the report of methicillin and vancomycin heteroresistance in *Staphylococcus aureus* isolate from clinical samples. The original isolate was resistant in vitro to methicillin and fewer to vancomycin. Resistance confirmed for all isolates with E-tests using strips of methicillin MIC of >265 mcg/ml and vancomycin MIC of >256 mcg/ml. MRSA were isolated and identified from different clinical samples using conventional methods. Antibiogram of the isolates and MIC were determined following CLSI guidelines. All Multi Drug resistant *Staphylococcus aureus* isolates were MRSA. Only four Vancomycin resistant *Staphylococcus aureus* were found, out of the all MRSA isolates. All MRSA had a Methicillin MIC > 265 mcg/ ml except one showing 5.0 mcg/ml that of three VRSA had a Vancomycin MIC > 265 mcg/ ml and one shows MIC 24 mcg/ml.

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INTRODUCTION

Staphylococcus aureus is one of the most common causes of nosocomial infections, especially pneumonia, surgical site infections and blood stream infections andcontinues to be a major cause of community and hospital acquired infections (Bhateja et al, 2005; Loon, 2000; Proctor RA And Peters G, 1998). Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected approximately 40 years ago and is still among the top three clinically important pathogens (Stewart et al, 1961). The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic penicillins (methicillin, oxacillin, and), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge (Wootton et al, 2001). The glycopeptides vancomycin was considered to be the best alternative for the treatment of multidrug resistant *Staphylococcus aureus*. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains exhibiting two different resistance mechanisms (Mathews AA et al, 2010;Saderi H et al, 2005). Initially vancomycin Intermediate *Staphylococcus aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997 was believed to be due to the thickened cell wall, where many vancomycin molecules were trapped within the cell wall. The trapped molecules clog the peptidoglycan meshwork and finally form aphysical barrier towards further incoming vancomycin molecules (Forbes BA et al, 2007). The second, noted in United States in 2002 among *Staphylococcus aureus*, was identical to the mechanism seen in vancomycin-resistant *Enterococcus*. Vancomycin resistant *Enterococcus faecium* harbours the vanA operon, which contains five genes, *Van*S, -R, -H, -A and –X8. But Tiwari and Sen have reported a VRSA which is *van* gene-negative. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil, France, United Kingdom, Germany, India, and Belgium has confirmed that the emergence of these strains is a global issue (Arthur et al, 1993). The aim of the present study was to identify the emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) and methicillin resistant *Staphylococcus aureus* (MRSA) among *Staphylococcus aureus* isolates from tertiary care hospitals in Vidarbha region, Maharashtra, India, and to determine the sensitivity of these isolates to different antimicrobial agents. Further search is also conducted for the mecA and vanA gene in MRSA and VRSA strains (Pierard D et al, 2004).

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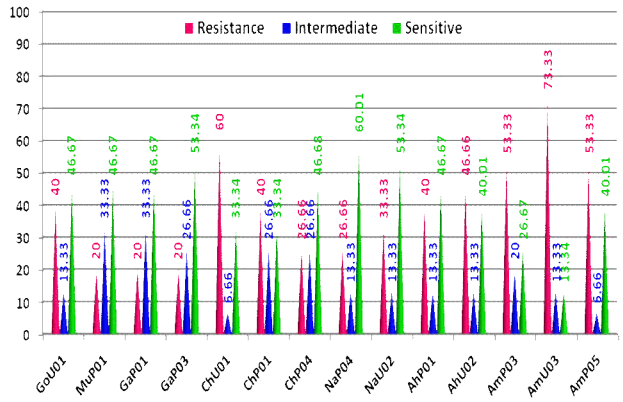


Fig. 1. Antibiogram of Staphylococcus aureus isolates showing different types of antibiotic resistance, intermediate and sensitive pattern in percentage

Prevelence of methicillin and vancomycin resistance in Staphylococcus aureus

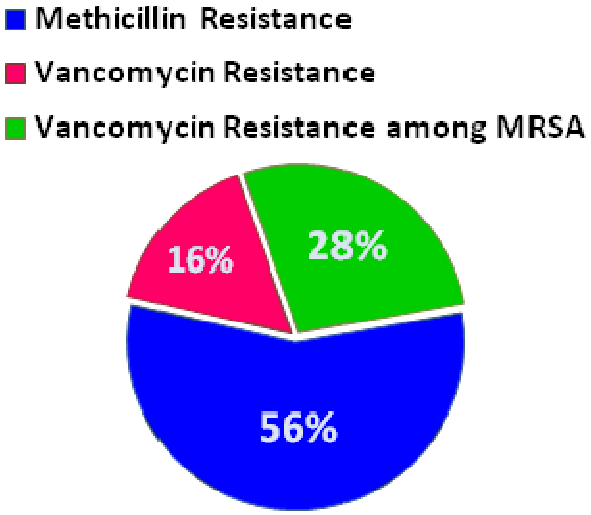


Fig. 2 Represent the Methicillin and vancomycin resistance pattern in percentage with MIC strip test (E-test)

MATERIALS AND METHODS

Bacterial isolates: A total of 21 numbers of *Staphylococcus aureus* isolates were obtained randomly from clinical samples (blood, urine and burn lesion swabs, wound swabs) of admitted patients in tertiary care hospitals, in Vidarbha region between February and September 2016. The study was doneat Centre for Higher Learning and Research in Microbiology, Sardar Patel Mahavidyalaya, Chandrapur (M.S.), India.

Staphylococcus aureus was identified by colony morphology, Gram stain, DNase, catalase and coagulase tests and fermentation of mannitol by conventional methods.

Antibiotic susceptibility testing

The antibiotic resistance profile was determined by the Disc Agar Diffusion (DAD) technique using different antimicrobial agents; Amikacin (30 µg), Ceprofaxacin (5µg), Chloramphenical (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Lincomycin (2µg), Methicillin (30 µg), Netillin (30 µg), Norfloxacin (10 µg), Oxacillin (1µg), Penicillin G (10 U), Trimethoprime (5µg) , Tetracycline (30 µg), Tobramicin (10 µg), Vancomycin (30 µg) (Hi-media, Mumbai India) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) (Wayne Pa, 2007, Wayne Pa, 2006). The standard *Staphylococcus aureus* strains NCIM 5522 and NCIM 5521 were used as reference strains (Centre for Higher Learning and Research in microbiology, Sardar Patel Mahavidyalaya, Chandrapur) for MRSA.

Determination of MIC

Minimal inhibitory concentration (MIC) of methicillin and vancomycin was determined by E-test of disc diffusion method using CLSI guidelines (Class II Special Control Guidance Document, 2009). Briefly, Plates of Hi-sensitivity agar (Hi-media) was prepared with forming lawn of inoculums prepared using 18-24 h old culture was spotted with placing gradient strip 0.5 mcg to > 265 mcg / ml of oxacillin and vanomycinon respectively. Plates were incubated overnight at 35^{o}C for 24h before assessing the visible growth (CLSI Performance standards for antimicrobial susceptibility testing M100-S (latest edition); CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard M7-A; CLSI Methods for Dilution Antimicrobial Susceptibility Tests of Anaerobic Bacteria Approved Standard M11-A (Latest edition).

RESULTS AND DISCUSSION

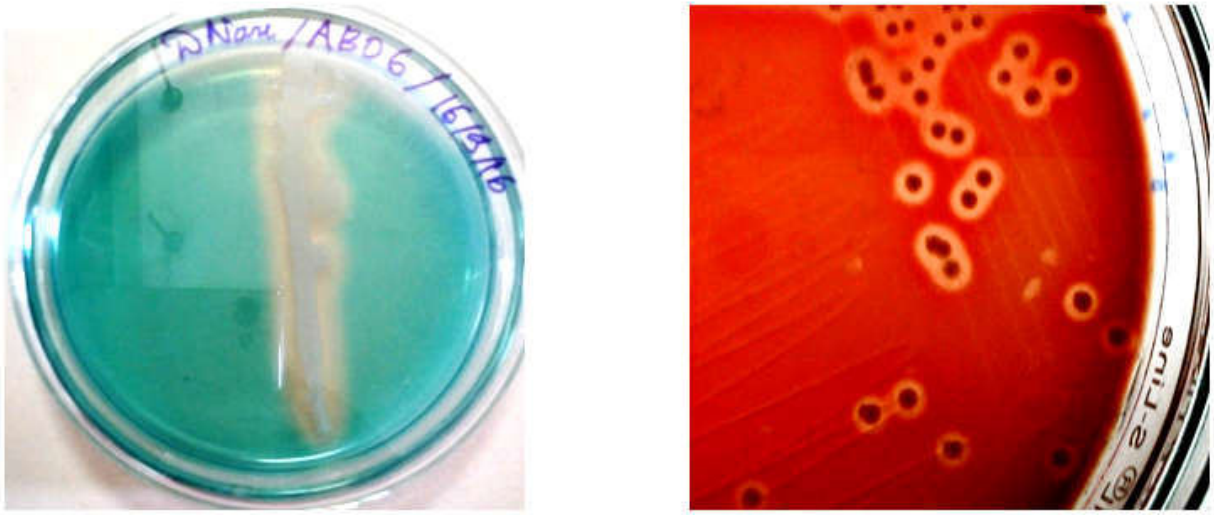
Various clinical samples were analyzed for the infection with *Staphylococcus aureus* and checked for their antibiotic resistance. Throughout the all samples 24 numbers of *Staphylococcus aureus* isolates confirmed including coagulase positive and coagulase negative strains. One of the limitations of the present studywas that, the detection of mecA or PBP 2a which is considered asthe gold standard for detecting the MRSA strains was not done because of technical and economic constrains (Rahbar et al, 2006).

Table No. 1: *Staphylococcus aureus* isolates from different location and different clinical samples

Name of Tertiary Care Hospital	Clinical Sample	S.aureus Isolates	No. of MRSA	No. of VRSA
Civil HospitalGondpimpri	Urine	02	01	NII
Civil Hospital Mul	Blood	01	NIL	NII
	Urine	03	NIL	NII
	Pus	03	01	NII
Civil Hospital Gadchiroli	Pus	02	02	NII
Civil Hospital Chandrapur	Urine	01	01	NII
	Pus	02	02	NII
Civil Hospital Nagpur	Pus	02	01	01
	Urine	01	01	NII
Civil Hospital Aheri &Bharmragad	Pus	02	01	01
	Urine	02	01	NII
Civil Hospital Amravati	Urine	01	01	NII
	Pus	02	02	02
Total		24	14 (58.33%)	04 (16.66%)

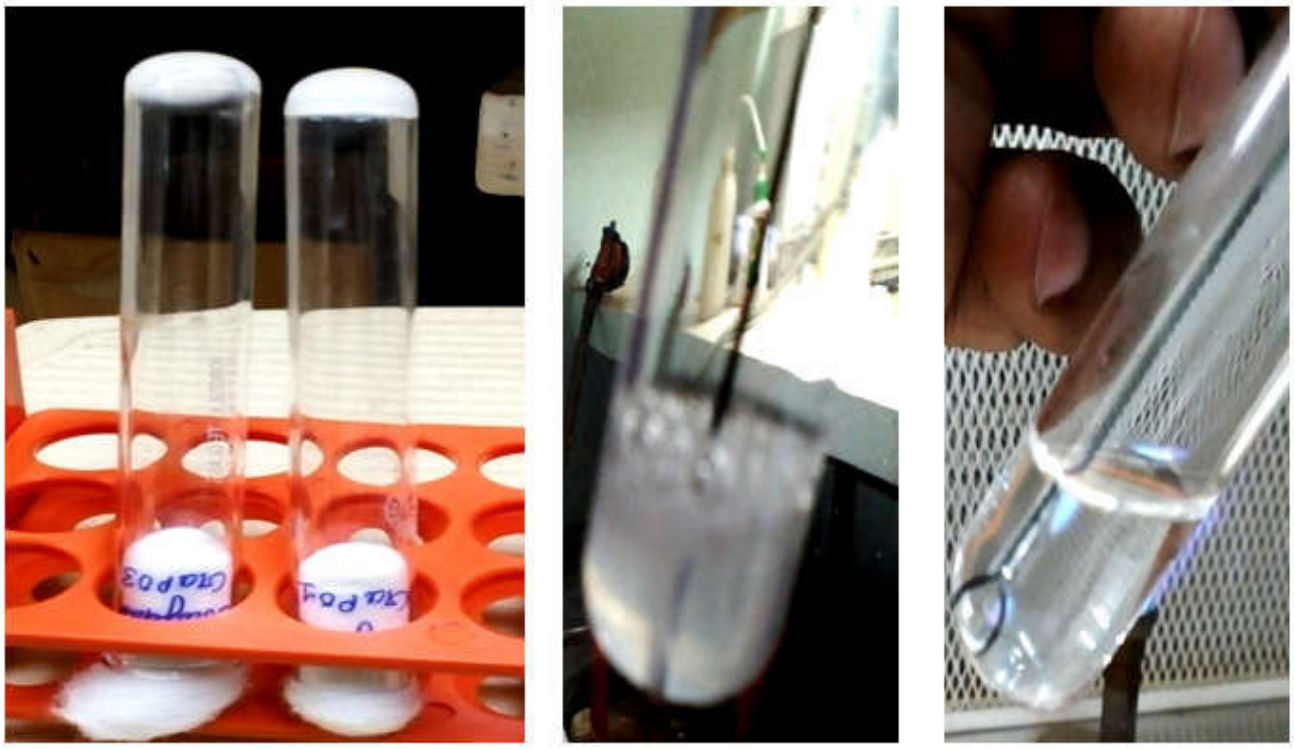
Table No. 2. Biochemical characteristics of MRSA and VRSA isolates

Codes of Isolates	DNase Test	Coagulase Test	Catalase Test
GoU01	Positive√	Negative	Negative
MuP01	Positive√	Negative	Negative
GaP01	Positive√	Positive√	Negative
GaP03	Positive√	Positive√	Negative
ChU01	Positive√	Negative	Negative
ChP01	Positive√	Positive√	Negative
ChP04	Positive√	Positive√	Negative
NaP04	Positive√	Positive√	Positive√
NaU02	Positive√	Positive√	Negative
AhP01	Positive√	Negative	Positive√
AhU02	Positive√	Positive√	Negative
AmP03	Positive√	Positive√	Positive√
AmU03	Positive√	Negative	Positive√
AmP05	Positive√	Positive√	Positive√



A. **B.**

Fig.1. (A) S. *aureus* on DNase Agar Medium (Zone of Clearance around the colonies)
(B) *S. aureus* on Blood Agar(Beta Haemolysis)



A. **B.** **C.**

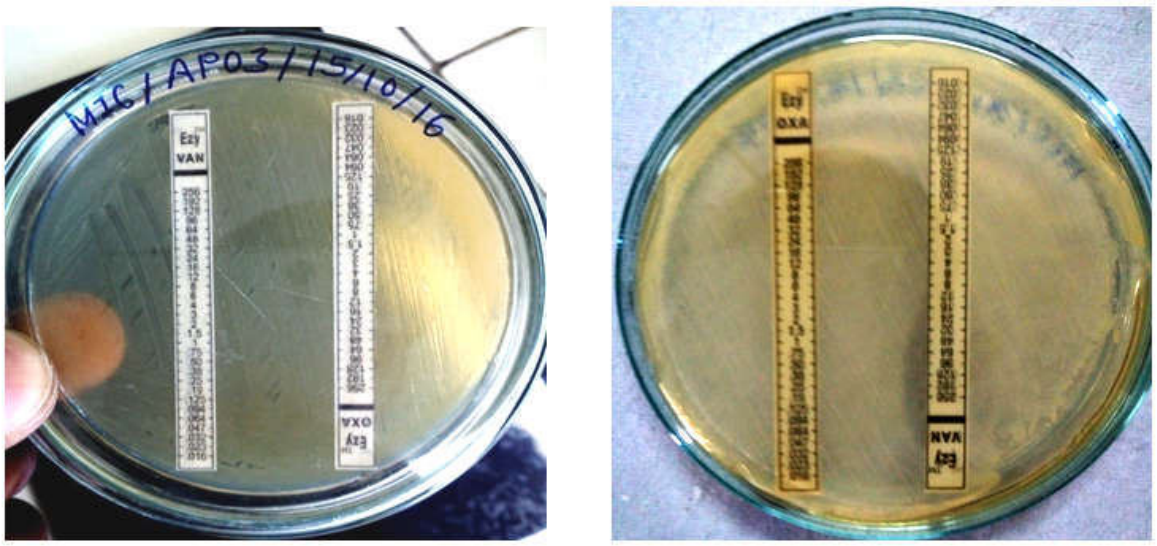
Fig.2. (A) Coagulase Test (with rabbit Plasma)
(B) Catalase tube test (with 3% H_2O_2) Positive, and (C) Negative

Table No. 3. Antibiogram of all *Staphylococcus aureus* Isolates

Antibiotics / S. aureus isolates	GoU01	MuP01	GaP01	GaP03	ChU01	ChP01	ChP04	NaP04	NaU02	AhP01	AhU02	AmP03	AmU03	AmP05
Oxacillin	Resistant	Inter-	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Methicillin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Ciproflaxacin	Resistant	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Inter-mediate	Inter-mediate	Resistant
Erythromycin	Resistant	Inter-mediate	Sensitive	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Penicillin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Tetracyclin	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Inter-mediate	Sensitive	Sensitive	Resistant	Resistant	Inter-mediate	Inter-mediate	Resistant	Sensitive
Amikacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Chloramphenicol	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Inter-mediate	Resistant
Gentamycin	Sensitive	Sensitive	Inter-mediate	Sensitive	Resistant	Inter-mediate	Inter-mediate	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Lincomycin	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant
Netillin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Trimethoprim	Sensitive	Sensitive	Inter-mediate	Inter-mediate	Resistant	Resistant	Resistant	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Resistant
Tobramycin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
Vancomycin	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Sensitive	Resistant	Sensitive	Resistant	Resistant	Sensitive
Norfloxacin	Inter-mediate	Inter-mediate	Inter-mediate	Inter-mediate	Resistant	Resistant	Inter-mediate	Sensitive	Sensitive	Sensitive	Inter	Resistant	Resistant	Inter-mediate

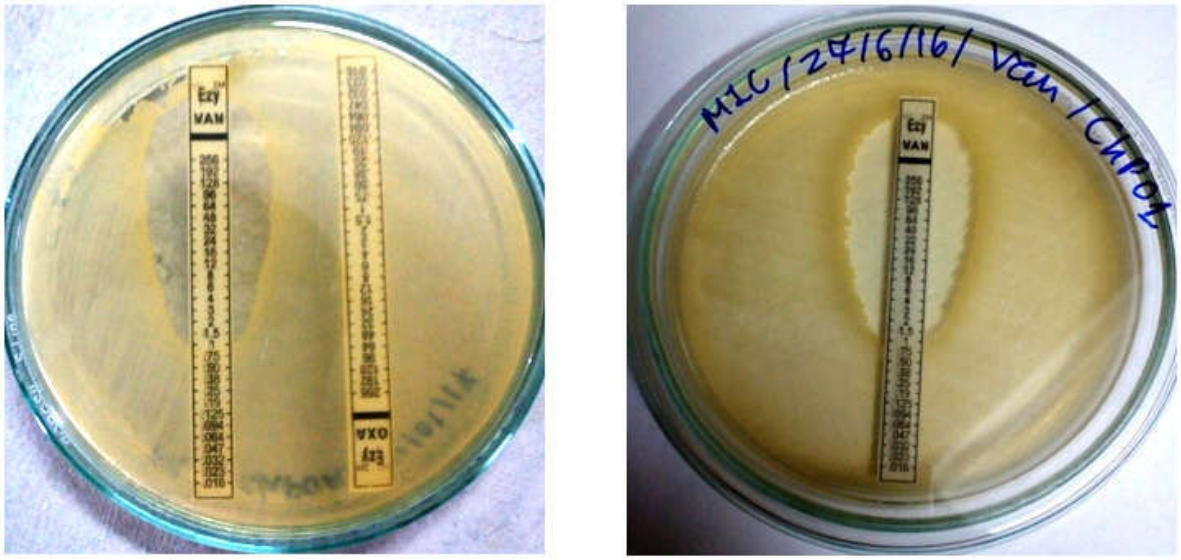
Table No. 4. Results obtained with E-Test of *Staphylococcus aureus* isolates

S. aureus Isolates	Oxacillin MIC Strip test	Vancomycin MIC Strip test	Conclusion
GoU01	> 265 mcg /ml	1mcg	Methicilin Resistant
MuP01	>265 mcg /ml	1.5 mcg /ml	Methicilin Sensitive
GaP01	> 265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
GaP03	>265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
ChU01	> 265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
ChP01	> 265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
ChP04	>265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
NaP04	>265 mcg /ml	24 mcg /ml	Vancomycin Resistant
NaU02	>265 mcg /ml	1.2 mcg / ml	Methicilin Resistant
AhP01	>265 mcg /ml	>265 mcg / ml	Vancomycin Resistant
AhU02	>265 mcg /ml	1.0 mcg /ml	Methicilin Resistant
AmP03	>265 mcg / ml	>265 mcg / ml	Vancomycin Resistant
AmU03	5.0 mcg / ml	>265 mcg / ml	Vancomycin Resistant
AmP05	>265 mcg / ml	1.0 mcg / ml	Methicilin Resistant



A. **B.**

Fig. 3. (A) & (B) E-test with Oxacillin MIC Strip And Vancomycin MIC Strip (No Zone of Inhibition)



C. **D.**

Fig. 4. (C) & (D) E-test with Vancomycin MIC Strip showing zone of inhibition

All the *Staphylococcus aureus* isolates were further analyzed for their pathogenic characterization along with antibiotic sensitivity test (Disc Agar Diffusion method) and resistance confirmed with E-test. Vancomycin resistance has been perceived as a fearsome threat to the already challenging therapy of MRSA and MDR-MRSA (Tiwari HS et al, 2008). Along with all representative data and the results obtained, it seen that a serious need for more study and research including molecular characterization of MRSA as well as VRSA (Prax M et al, 2013). Also there is a need to do more research and studies at the plenty of knowledge to knowing the exact mechanism of acquiring antibiotic resistance in *Staphylococcus aureus* along with proper treatment of patients against such an infection from *Staphylococcus aureus* in tertiary care hospitals.The morphological, biochemical and MIC test observation found for all among the MRSA and VRSA isolates as well as pictorial data as fallows.

Conclusion

From the above results and discussion this is concluded that, *Staphylococcus aureus* were shows higher prevalence in pus or wound swabs and next in urine samples. The resistant pattern shown by these isolats where checked by the standard Disc Agar Diffusion test as methicillin resistant *Staphylococcus aureus* (MRSA) were shown 58.33%and vancomycin resistant *Staphylococcus aureus* (VRSA) 16.66%out of all *Staphylococcus aureus* isolates and only 28.57% shows vancomycin resistant out of all MRSA. All the MRSA and VRSA isolates had shown multi drug resistance where MIC confirmed with E-test (CLSI, Approved Standard, M7-A (Latest edition) shows resistant against methicillin 92.85% and 28.57% against vancomycin. But all the MRSA and VRSA were found susceptible to Amikacin, Netilin and shows 100 % resistant against penicillin-G.

Acknowledgement

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Antibiotic Resistance Profiling of Staphylococcus aureus Isolated from Clinical Specimen from Tertiary Care Hospital

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Abstract: In past two decade broadcasting and spread of antibiotic resistance in staphylococcus aureus micro-organism infect skin and soft tissue posing a great therapeutic challenge. Particular methicillin resistance S.aureus [MRSA] diverse mechanism of resistance based on phenotyping and genotyping. Typing of MRSA is vital to understand epidemiological swing and to initiate infection with control strategies. Also a certain group of S. aureus depute vancomycin resistance chronic creation of VRSA upon exposure to vancomycin. VRSA acquired by mutation based on thickening of cell wall due to accumulation of excess amount of peptidoglycan.

Keyword *MRSA, VRSA, S. aureus, Phenotyping, Genotyping, Resistance*

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I. INTRODUCTION

Staphylococcus aureus is one of the members of genus *staphylococcus*. That occurs ubiquitous and forms the most common cause of localized superlative lesions in human being having ability to developed resistance to penicillin. *Saureus* infection is wide spread concern due to its increasing resistance to miscellaneous type of antibiotic. Although soft-tissue infection are habitual, latest trouble in both inpatient and outpatient with uncomplicated peripheral infection on folliculitis, cellulitis and abscesses ,that intricate infection such as necrotizing fascilitis burn infection and diabetic foot, having consequential role blooming the antimicrobial resistance and illegitimate treated SSTI (skin and soft tissue infection) may primacysedevalution of endocarditis, asteomyclitis brain-lungs abscess or meningitis and toxic shock syndrome, due to familiar organism mannerly encountered in soft tissue infection are gram positive cocci, golden-grapes like cluster , non-motile, non-spore forming which measure around 0.7 to 1.2 um in diameter under microscope notably as *S. aureus*].(1)

THE FASCINATING DISCOVERY OF ANTIBIOTIC

Therapeutically fascinating story of development and evidenced of penicillin, it already design by Ernest Duchesne, (French medical student) but his work was forgotten. Penicillin rediscover by Scottish physician Alexander Fleming. During first world word Fleming had been enthusiastic to discovered unspecified think that would kill pathogen on wound infection

One day in September 1928 *penicillium notatum* spore unexpectedly landed on the surface of an exposed petri dish before it had been inoculated with staphylococci and new medical epoch was born. Although the precise event are still unclear. Ronald Hare has suggested that Fleming left the contaminated plate on a laboratory bench while he was on vacation, the first few days of vacation were cool, the fungus grew more rapidly than the bacteria and produced Penicillin. When the weather then turned worm, the bacteria began to grow and were lysed. On his returned Fleming noticed that a penicillin colony was growing at one edge and the *staphylococci* surrounding it had been destroyed, rather than discarding the contaminate plate, he correctly deduced that his mold contaminant was producing a diffusible substance lethal to *staphylococci*. He found that broth from a penicillium culture contained penicillin and that the antibiotic could destroy several pathogenic bacteria. Unfortunately Fleming next experiment convinced him that penicillin would not remain active in the body long enough after injection to destroy pathogen. After reading Fleming paper on penicillin one of Floreysco worker, Ernst chain in 1940 obtained the penicillium culture from Fleming and set about culture from Fleming and set about culturing it and purifying penicillin. When the purified penicillin was injected into mice they survived. Later Selman Waksman announced in 1944 that he got new antibiotic streptomycin produced by actinomycete streptomycin griseus. (8)

Implementation of Antibiotic in treatment of *S. aures* infection.

In 1880 era S.aureus bacterium perceived as *staph* at this epoch *S. aureus* infection inducement for painful skin and soft tissue. In 1940 penicillin born against *S. aureus*. In late 1940-1950 *S. aureus* strain resistance to penicillin, then high levels of penicillin resistance followed by the development and spread of strain resistance to the semi synthetic penicillin (methicillin, orocillin a nofcillin in 1961. In 1968 the first human case of MRSA reported in United States subsequently new strain evolution become resist to drags designed assist to

attack infection MRSA are resistant to all betalactum antibition. In 1996 the first MRSA to acquire resistance to vancomycin was isolated from a Japanese patient. In 2002 US documented that *S. aureus* resistant to the vancomycin so called Vancomycin resistant *S*. aureus

VRSA Development of Drug Resistance–

MRSA announced for resistive disparate antiseptics due to cellular changes that negatively affect the accumulation of antiseptic agents' comparise cell envelope expressed effiux mechanism. In *S. aureus*maltidrug resistance while quac A/B/G/H are multidrug family found in plasmid. (12)

Efflux Mechanism of MRSA and Drug development

MRSA strain fetch mecA gene encodes for low affinity penicillin binding protein (PBP) designated PBP2a. Predominantly mecA gene is part of chromosomally integrated mobile genetic element Known as *staphylococcal* cassette chemosemec (SCC mec) .This PBP2a possess Peptidoglycan transpeptidase activity yet low affinity for B-lactum antibiotic PBP2a exhibit not only constant rate of reducing acylation but also replitedalissociation constant by B. lactum so called as Betalactumase resistance.(10)

Vancomycin Resistance S.aureus and their Mechanism

MRSA has accounted in many Countries since its discovery in 1961, however in recent era the increasing frequency of MRSA infection. Due to in unequivocal cell-killing affect, and capable of eliminating MRSA from the patient body. So increased use of vancomycin, thus drug with rather weak cell-killing Potency against prevailing MRSA. Two class of vancomycen resistance Strain .VRSA that has a vancomycinmnimum inhibitory concentration (MIC Breckpoinout) of 8mg/l and hetro VRSA that spontaneously generates VRSA within the cell population (9)

Mechanism of VRSA

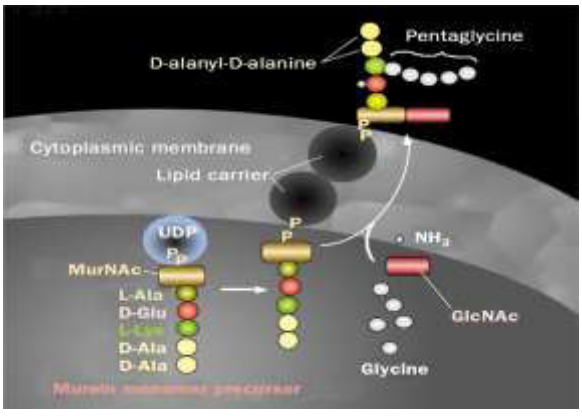

Figure 1. Synthesis of murein monomer (monomeric component of peptidoglycan). Murein monomer is composed of two amino sugars (N-acetyl muramic acid [MurNAc] and N-acetyl glucosamine [GlcNAc]) and ten amino acids. Murein monomer precursor is composed of MurNAc and stem peptides (L-alanine, D-glutamine acid, L-lysine, and two D-alanines). It is synthesized in the cytoplasm and attaches to a lipid carrier in the cytoplasmic membrane. Then, during its transfer to the outer surface of the cytoplasmic membrane, GlcNAc and five glycine are added, and its isoglutamic acid is amidated to become mature mureinmonomer.

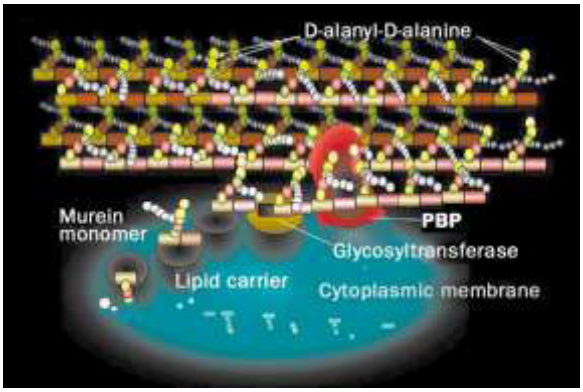

Figure 2. Assembly of peptidoglycan viewed from outside of the cell. In blue is the cytoplasmic membrane. Glycosylic transferees polymerizes therein monomer to produce a nascent peptidoglycan single chain. Penicillin-binding protein (PBP) grasps at the D-alanyl-D-alanine residues of stem peptide and cleaves in between the residues to ligate the penultimate D-alanine to the pentaglycine of the neighboring peptidoglycan chain. The twisting of peptidoglycan chains is omitted from the illustration for visual simplicity.

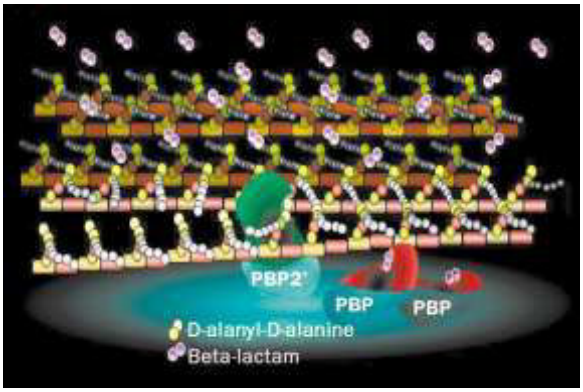

Figure 3. Action of beta-lactam: Beta-lactam (purple double cubes) is a structural analogue of D-alanyl-D-alanine residues. It inactivates S aureus PBPs (in red), but cannot bind to PBP2_ (in green; MRSA-specific PBP) with high affinity. Therefore, MRSA can continue peptidoglycan synthesis in the presence of beta-lactams whereas methicillin-susceptible S aureus cannot.

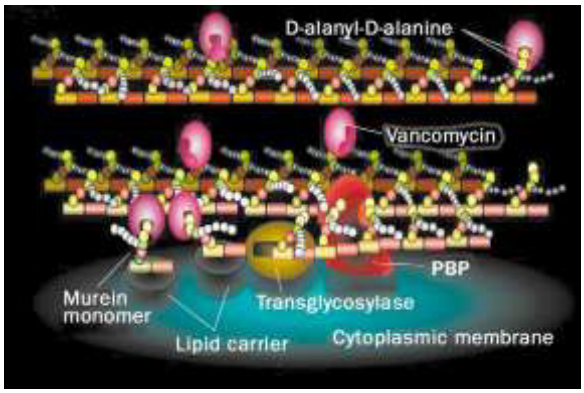

Figure 4. Action of vancomycin and teicoplanin. Drug binds to D-alanyl-
D-alanine residues of murein monomer. The murein monomer bound by vancomycin does not serve as a substrate for glycosyltransferase.

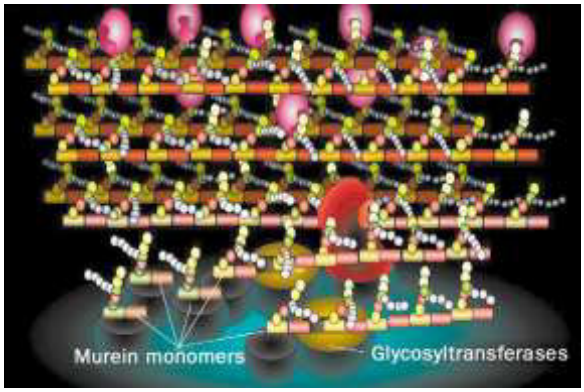

Figure 5: Thickened cell wall of Mu50. Affinity trapping mechanism of resistance Mu50 has 30–40 layers of peptidoglycan. Supply of mureinmonomer is increased and more monomers are incorporated into nascentpeptidoglycan chains. Increased D-alanyl-D-alanine residues are present in the completed peptidoglycan layers. More vancomycin molecules are trapped in the peptidoglycan layers and less reach the cytoplasmic membrane than usual.

II. CONCLUSION

Theoretical research with the ultimate goal to develop and promote enhance diagnosis, better conceptual treatment and new vaccines that are consequence against MRSA/VRSA that gives increasing prevalence of MRSA/VRSA in both hospital and community setting. In this study reports that commonest organism like *S. aureus* encountered in skin and soft tissue infections. However, in this view varied bacteriology and antobiogram of SSTI. So on this based efflux mechanism of MRSA/VRSA discussed with antion of their drug resistance.

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Annexure – XI

UNIVERSITY GRANT COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

FINAL REPORT ASSESSMENT/EVALUATION CERTIFICATE

(To be Members Expert Committee Not Belonging to the Institute of Principal Investigator)

(to be submitted with the final report)

It is Certified that the final report of Major Research Project entitled "**A study of methicillin and vancomycin resistance *S.aureus* isolates caring *mecA* and *vanA* gene complex from tertiary care centers**" by **Dr. Vijay Shamrao Wadhai, Dept. of Microbiology** has been assessed by the committee consisting the following members for final submission of the report to the UGC, New Delhi under the scheme of Major Research Project.

Comments/Suggestions of the Expert Committee:

Name & Signatures of Experts with Date:

Sir No.	Name of Expert	University/College Name	Signature with Date
1	Dr. P. H. Kumbhare	Gurunanak College of Science, Ballarpur	
2	Dr. S. S. Wankar	Janta Mahavidyalaya, Chandrapur	

It is certified that the final report has been uploaded on UGC-MRP portal on.....

It is also certified that final report, Executive summer of the report, Research documents, monograph academic paper provided under Major Research Projects have been posted on the website of the University/College.

Sardar Patel Mahavidyalaya SEAL Chandrapur

(Registrar/Principal)

Seal

Principal
Sardar Patel Mahavidyalay
Chandrapur