ANTIBIOGRAM STUDY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AND DEMONSTRATION OF VANCOMYCIN RESISTANCE AMONG MRSA ISOLATES FROM TERTIARY CARE HOSPITAL, NAGPUR (M.S.) INDIA

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ABSTRACT
Staphylococcus aureus has long been recognized as a major pathogen of hospital acquired infections. Over the last decade, 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. Nagpur region of Maharashtra state is a central part of our country, which have various tertiary care clinical centres. Limited reports were available on development of Methicillin and Vancomycin resistant Staphylococcus aureus form this part of India. Vancomycin from glycopeptides category is last resort of drug. Hence there is need of continuous surveillance of antibiotic resistance patterns of Staphylococcus aureus and there genotypic variation to controlled antibiotic resistance problem so that we may not fall back into pre-antibiotic era. From Indira Gandhi Medical College and hospitals, Nagpur the total 619 clinical samples were collected from different source and 418 samples were positive for S. aureus. Out of these 362 clinical samples were positive for coagulase test. In Nagpur region methicillin resistance among the S. aureus isolates was 61.60%. Higher resistance in MRSA was found to multiple antibiotics, penicillin, Tetracyclin, Erythromycin, nitrofurantoin, Tobramycin and gentamicin and lower resistance was found to Vancomycin, Amikacin and chloramphenicol. Prevalence rate of MRSA was found 61.60 % and Vancomycin resistant among MRSA isolates was found 27.20%. The increasing trend of antimicrobial resistance was observed in present study.
Keywords: MRSA, VRSA, Vancomycin, Methicillin

I. INTRODUCTION

Staphylococcus aureus is one of the members of genus *Staphylococcus*. The genus *Staphylococcus* is currently composed of 41 species and 21 subspecies[1]. The *Staphylococci* most frequently associated with human infection are *S. aureus, S. epidermidis* and *S. saprophyticus*. Other *Staphylococcus species* may also be associated with human infection[2]. *Staphylococcus aureus* has long been recognized as a major pathogen of hospital acquired infections. Over the last decade, 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. Many hospital struggles with

increasing amount of MRSA which are multi-resistant against all β-lactum antibiotics[3].

The global problem of increasing trend in antimicrobial resistance is particularly pressing in developing countries, where the methicillin resistant *Staphylococcus aureus* (MRSA) is often several causal agents in hospital-acquired infections. There is now increasing in difficulties to treat such patient because of emergence of resistance to all current antibiotic classes.

Nagpur region of Maharashtra state is a central part of our country, which have various tertiary care clinical centres. Limited reports were available on development of Methicillin and Vancomycin resistant *Staphylococcus aureus* form this part of India. Vancomycin from glycopeptides category is last resort of drug. Hence there is need of continuous surveillance of antibiotic resistance patterns of *Staphylococcus aureus* and there genotypic variation to controlled antibiotic resistance problem so that we may not fall back into pre-antibiotic era. 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.

I. METHODS AND MATERIAL

From Indira Gandhi Medical College and hospitals, Nagpur the total 619 clinical samples were collected from different source, out of which 362 specimen was found to be coagulase positive *Staphylococcus aureus*. Of the 619 clinical specimens, 308 specimens were from pus, 51 were from burn patient and 59 from sputum sample. Standard procedure was followed for isolation and identification of *S. aureus* and to perform antimicrobial activity[4]. In brief the specimen was collected in sterile container and transport to the laboratory. The specimen then immediately inoculated in nutrient broth tube and incubates overnight at 37^0C for enrichment. Then the loop full of sample transfer to the Blood agar and the Mannitol Salt Agar and were incubated at 37^0C 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. After confirmation of *S.aureus* isolates were subjected to antimicrobial sensitivity testing by standard disk diffusion method and minimum inhibitory concentration by E-test method as per NCCLS standards[4,5]. All the culture media, antibiotics discs and E-test strips were obtained from Hi media laboratory.

II. RESULTS AND DISCUSSION

From Indira Gandhi Medical College and hospitals, Nagpur the total 619 clinical samples were collected from different sources and 418 samples were positive for *S. aureus*. Out of these 362 clinical samples were positive for coagulase test **[figure 1].** Out of total, 198 (54.70 %) and 164 (45.30 %) 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 67(18.51%), 11-20 years 16(4.42 %); 21-30 years 29(8.01%); 31-40 years 447(12.98 %); 41-50 years 88(24.31 %); 51-60 years 53(14.64 %); 61-70 years 46(12.71 %); and 71-80 years 16(4.42 %) [**Figure 2**].

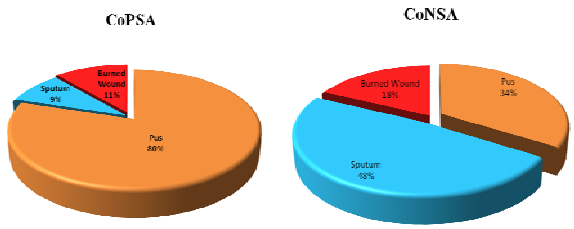

Figure 1: Sample wise distribution of Coagulase positive and negative *S. aureus* in Nagpur

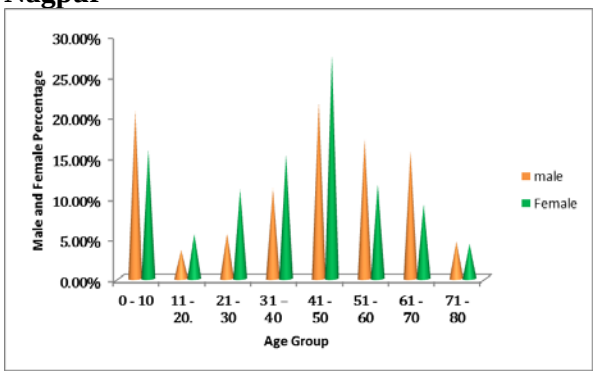

Figure 2: Sex wise distributions of *S. aureus* infections among patients with various age groups in Nagpur.

In Nagpur region 41-50 age group population were extremely affected with *Staphylococcus*

aureus infection with nearly equal ratio of both sex.

All clinical *S. aureus* samples were investigated by Disk diffusion method. Those isolates were shown resistant on disk diffusion which was further reconfirmed on Oxacillin resistant screen agar base (ORSAB) with 2 mg/L oxacillin 50 000 U/L polymyxin B and 5.5% NaCl as suggested by CLSI standards[6].

A total of 540 viable strains of *S. aureus* from Nagpur 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)

Table 1: **Overall distribution of antimicrobial susceptibility of *S. aureus* on disc diffusion in Nagpur (Total No – 362)**

Sr. No.	Name of Antibiotic	Resistant		Sensitive	
		No.	%	No.	%
1.	Oxacillin (Ox)	239	66.02	123	33.98
2.	Amikacin (Ak)	52	14.36	310	85.64
3.	Tetracycline (Te)	312	86.19	50	13.81
4.	Erythromycin (E)	308	85.08	54	14.92
5.	Gentamycin (GN)	274	75.69	88	24.31
6.	Methicillin (MET)	239	66.02	123	33.98
7.	Chloramphenicol (C)	67	18.51	295	81.49
8.	Penicillin (P)	358	98.9	4	1.105
9.	Tobramycin (TB)	289	79.83	73	20.17
10.	Norfloxacin (NX)	276	76.24	86	23.76
11.	Nitrofurantoin (NF)	302	83.43	60	16.57
12.	Vancomycin (V)	65	17.96	297	82.04

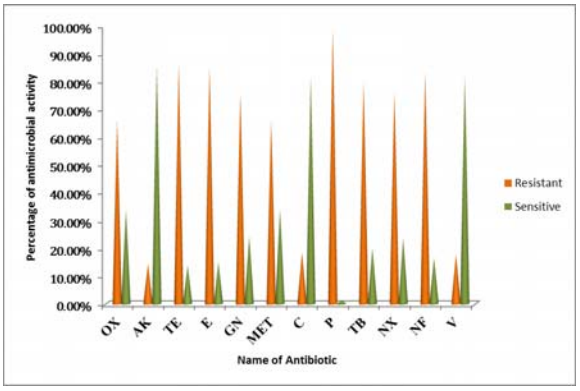

Figure 3: **Overall distribution of antimicrobial susceptibility of *S. aureus* on disc diffusion in Nagpur.**

The overall resistance pattern of each antibiotic tested was as follows; Oxacillin 239 (66.02%), Amikacin 52 (14.36%), Tetracycline 312 (86.18%), Erythromycin 308 (85.08%), Gentamycin 274 (75.69%), Methicillin 239 (66.02), Chloramphenicol 67 (18.52%), Penicillin 358 (98%), Tobramycin 289 (79.83%), Norfloxacin 276 (76.24%), Nitrofurantoin 302 (83.42%) and Vancomycin 65 (17.95%) (**Table 1**) [**Figure 3**].

By disc diffusion method the resistance pattern among 65 vancomycin resistant *S. aureus* strains was as follows; Amikacin 23 (35.36%), Tetracycline 63 (96.92%), Erythromycin 65 (100%), Gentamycin 54 (83.08%), Oxacillin 65 (100%), Chloramphenicol 28 (43.08%), Penicillin 65 (100%), Tobramycin 56 (86.15%), Norfloxacin 42 (64.62%), and Nitrofurantoin 42 (75.38%) (**Table 2**) [**Figure 4**].

Most of the tested antibiotics had shown higher resistance towards VRSA strains except amikacin and chloramphenicol. So, these two antibiotics are good choice for the treatment of *Staphylococcus aureus* infection.

Table 2: Antimicrobial resistance patterns of vancomycin resistant *S. aureus* strains on disc diffusion in Nagpur (Total No – 65)

Sr. No.	Name of Antibiotic	Resistant		Sensitive	
		No.	%	No.	%
1.	Vancomycin (V)	65	100.00%	0	0.00%
2.	Oxacillin (Ox)	65	100.00%	0	0.00%
3.	Amikacin (Ak)	23	35.38%	42	64.62%
4.	Tetracycline (Te)	63	96.92%	2	3.08%
5.	Erythromycin (E)	65	100.00%	0	0.00%
6.	Gentamycin (GN)	54	83.08%	11	16.92%
7.	Methicillin (MET)	65	100.00%	0	0.00%
8.	Chloramphenicol (C)	28	43.08%	37	56.92%
9.	Penicillin (P)	65	100.00%	0	0.00%
10.	Tobramycin (TB)	56	86.15%	9	13.85%
11.	Norfloxacin (NX)	42	64.62%	23	35.38%
12.	Nitrofurantoin (NF)	49	75.38%	16	24.62%

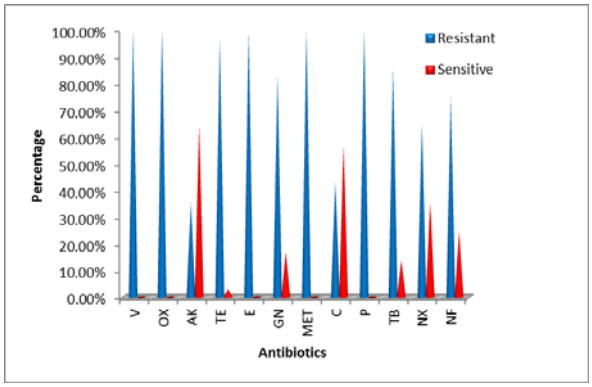

Figure 4: Antimicrobial resistance among vancomycin resistant S. aureus strains on disc diffusion in Nagpur (Total No. – 65)

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 223 (93.30%) and 16 (6.69%) sensitive out of 239 MRSA isolates obtained from disc diffusion test; vancomycin was resistant to 48 (73.84%) and sensitive to 17 (26.15%) out of 65 VRSA isolates (**Table 3**).

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

Sr. No.	Name of Antibiotic	Antimicrobial Susceptibility test			
		MIC			
		Resistant		Sensitive	
		No.	%	No.	%
1.	Oxacillin (OX) **(Total-239)**	223	93.30	16	6.69
2.	Vancomycin (V) **(Total-65)**	48	73.84	17	26.15

The incidence rate of methicillin resistance among 362 coagulase positive S. aureus isolates on disc diffusion and MIC was 66.02 % and 61.60 % respectively as shown in Table 4.13. Antimicrobial susceptibility test by MIC is considered as gold standard; therefore the prevalence rate of MRSA in current study was 61.60%. According to MIC test the incidence rate of VRSA among MRSA isolates was found 21.52 %.

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

Sr. No.	Name of Antibiotics	Antimicrobial Susceptibility Test							
		Disc Diffusion				MIC			
		Resistant		Sensitivity		Resistant		Sensitivity	
1	Oxacillin **(Total-362)**	239	66.02%	123	33.97%	223	61.60%	139	38.40%
2	Vancomycin **(Total-239)**	65	27.20%	174	72.80%	48	21.52%	175	78.48%

MRSA is recognized as a problem worldwide. It has appeared as a major hospital pathogen in various hospitals in Europe and America in 80's and continued to be so in 90's. The prevalence of MRSA has widely diverse from hospital to hospital in various countries. S. aureus infections acquired about 40%, in large US hospitals are methicillin-resistant. . In many American and European hospitals, the percentage of MRSA has ranged from 29% to 35% of all clinical isolates[7]. In India prevalence of MRSA in hospitals varies considerably from one region to another and among hospitals but the data on the prevalence of MRSA strains in most of the region especially tribal region in Indian are limited. In several Indian hospitals prevalence of MRSA varies from 22% to 68%, as per current Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group's report 2013[8]. Prevalence of MRSA has increased rapidly since 1993 at tertiary care centre from 12% in 1992 to 80.89% in 1999. The MRSA isolates showed resistance to several other therapeutic drugs. Incidence of MRSA was as low as 6.9% in 1988 in India and reached to 24% & 32.8% in Vellore & Lucknow reported in 1994[9-10]. Overall prevalence remained in the same range in Mumbai, Delhi & Bangalore in 1996 and Rohtak[11] & Manglore[12] in 1999. However, the situation appeared to be more alarming in Tata hospital in Mumbai[13] where, it reached to 87% in 1995 and tapered to 64% in 1996. In 2001 Vidhani *et. al*. found 51.6% MRSA prevalence in New Delhi[14]. The INSAR group[8], India reported MRSA prevalence was 42% in 2008 and 40 % in 2009. According to Khan, *et. al*., study prevalence of MRSA was found 32% in 2011[15]. Tambekar, *et. al*. were reported high level of HA-MRSA in Vidarbha region[15]. In current study, the prevalence of MRSA among *S. aureus* isolates was observed 61.60%. Bhuchande, S. (2014) reported 64.22% MRSA prevalence from Nagpur region in 2014[16] which is slight higher than our studies. Although it's enormously difficult to explain these conflicting data with respects to both time and place of study, the variation is possibly due to differential clonal expansion, drug pressure and indiscriminate use of antibiotics in community and hospitals.

The study has found higher rates of MRSA infections in elder age population 41-50 years of age with a majority of males over females. The older age 51-60 and child age 0-10 year's population found nearly equal rate of prevalence of MRSA with higher rate of males over females. Similarly higher male numbers among MRSA isolates was found in other studies. The median age of the patients with MRSA infection

in this study was 40 years (range 1 to 80) similar to Miller, et. al., (2005) where they reported median age of the patients to be 46 years (range, 28-68)[18].The extreme of ages explained the immune deficient individuals susceptible to infections. All these data compare with the other studies like Saxena, et. al., (2004)[19] that reported the same age group patterns of infection but differ from Madani, et. al.,(2001) where all age groups were equally affected[20].

In 1950 vancomycin antibiotic was discovered and approved by Food and Drug Administration in 1958[21]. It is rarely used in the treatment of Gram positive cocci because of its toxicity profile and less toxic beta-lactum drug was effectively working. Later after the emergence of methicillin resistant *Staphylococci* strains and extensive beta-lactum resistance this agent became prominence. In the late 1980s vancomycin resistance was reported against Enterococci in Europe and developing counties and spread to much of the developing world. The reduced susceptibility of vancomycin towards S. aureus was first reported in Japan[22] (1997) and fully resistant strains vancomycin resistant strains was identified in USA[23] (2002). The first incident of VRSA appearance from a tertiary care hospital in North India was reported by Tiwari and Sen[24]. In current study the prevalence of VRSA among methicillin resistant *S. aureus* isolates was observed 21.52%, which is moderately higher than the previous studies.

This is first report of fully developed VRSA strains in geographically central part of India. Vancomycin has been the drug of choice for serious beta-lactam-resistant *Staphylococci* positive infections 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 alternatives to vancomycin and other glycopeptides in the treatment of serious *Staphylococcus aureus* infections. It is also equally important to prevent the spread and emergence of glycopeptide resistance by taking proper infection control measures, so that we may not fall back into pre-antibiotic era.

III. CONCLUSION

The present study first time exposed the vancomycin resistance in this part of India and prevalence rate of MRSA and VRSA are 61.60% and 21.52%respectively. In current study, Vancomycin, Amikacin and Chloramphenicol were found to be the most effective antibiotics against the *S. aureus* isolates among the routinely used antibiotics in this region.

The increasing trend of antimicrobial resistance was observed in present study. 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. The foremost reason of such resistance was due to the overused, misused and interrupted treatment of antibiotics in critical areas of the hospitals. The leading factors responsible for overused and misused of antibiotics were over-prescription by physicians without accurate diagnosis of diseases, giving antibiotics to the colonized patients associated with risk factors in high risk areas, and treating mild skin infections with systemic antibiotics. In many developing countries, problems normally arise because antimicrobial agents are readily approachable and can be purchased as a commodity without the advice or prescription of a physician or other trained health care provider.

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