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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* IN TERTIARY CARE CENTER AT SOUTHERN RAJASTHAN

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ABSTRACT: The objective of this study was to determine antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* in tertiary care center at Southern Rajasthan. Modified Kirby Baurer disc diffusion method was used for antimicrobial sensitivity testing of all samples. Maximum resistance among *Staphylococcus aureus* isolates (n=400) was observed against Methicillin (59%), Amikacin (53%) followed by other antimicrobial agents. Earlier Vancomycin, Linezolid and Pristinamycin were 100% sensitivity to MRSA. But results of this study shows that among MRSA isolates 13 % were found resistant to Vancomycin (VRSA), 12 % isolates found resistant to Linezolid and 9 % isolates Pristinamycin resistant. Pristinamycin was found sensitive in 75% of MRSA which were also Vancomycin resistant (VRSA) and Linezolid resistant (LRSA). The emergence of drug resistance and it's dissemination in MRSA is worrisome. So we need to develop newer agents as well as slow down the spread of resistant strains by various measures.

INTRODUCTION: Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the eminent public health concerns of the 21st century. Antibiotic resistance is a form of drug resistance whereby some sub-populations of a microorganism, usually a bacterial species, are able to survive exposure to one or more antibiotics [1]. During the past four decades, a bacterium known as Methicillin-Resistant *Staphylococcus aureus* (MRSA) has evolved from a controllable nuisance into a serious public health concern. MRSA is responsible for several difficult-to-treat infections in humans [2].

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A gene, mec-A is responsible for this Methicillin resistance. This alters the site at which Methicillin binds to kill the organism [3]. MRSA infections are also resistant to many antibiotics other than Methicillin, because the same cellular process is used by bacteria to be resistant to other antibiotics. [4].

MRSA can be categorized according to where the infection was acquired: Hospital-acquired MRSA (HA-MRSA) or Community-acquired MRSA (CA-MRSA). HA-MRSA has increased during the past decades due to a number of factors like increased number of immune-compromised and elderly patients, an increase in the number of invasive procedures and failures in infection control measures such as hand washing prior to patient contact and removal of non-essential catheters.

CA-MRSA infections typically occur as skin or soft tissue infections, but can develop into more invasive, life-threatening infections.

CA-MRSA tends to occur in conditions where people are in close physical contact, such as athletes involved in football and wrestling, soldiers kept in close quarters, inmates, childcare workers, and residents of long-term care facilities [5].

The present study was conceived with a view to determine the in vitro pattern of susceptibility and emergence of resistance in MRSA to newer Antimicrobial agents such as Vancomycin, Linezolid & Pristinamycin and to compare the results with other antimicrobial agents used in the treatment of such infections in southern Rajasthan.

MATERIAL AND METHODS: The present study was a prospective laboratory based study conducted on 400 consecutive isolates of *Staphylococcus aureus* species received at Microbiology Department of R.N.T. Medical College, Udaipur and Aravali Path lab from various departments of R.N.T. Medical College, Udaipur. Clinical samples with pure and predominant growth of *Staphylococcus aureus* species (coagulase positive) were selected for the present study. All patients with *Staphylococcus aureus* infection irrespective of site of infection, age, sex, religion, socioeconomic status were included in study. All contaminated samples with other bacteria's including coagulase negative *Staphylococcus aureus* isolates were excluded. Identifications and species characterization was done with the help of standard biochemical tests and on the basis of colony characters in MacConkey and Blood agar.

Procedure for antibiotic sensitivity screening: The antimicrobial agent sensitivity testing of all samples were performed by the modified Kirby Baurer disc diffusion method [6]. Similar looking 3-5 colonies isolates were selected from 18-24 hour incubated agar plate. Now the top of each colony touched with a loop and a saline suspension was prepared. The turbidity of suspension was adjusted to match 0.5 McFarlands turbidity standard by comparing both against a card with a white background and contrasting black lines. After adjusting the turbidity of the inoculum suspension within 15 minutes, a sterile cotton swab was dipped inside, rotated several times and pressed firmly on the inside wall of the tube above the fluid level.

The dried surface of a Muller-Hinton agar plate was inoculated by streaking the swab three times over the entire sterile agar surface, rotating the plate at 60° each time to ensure an even distribution of inoculums. Finally the rim of the agar was swabbed and the lid of the agar plate replaced.

After drying the inoculated plate for 3-5 minutes, the antibiotic disks for Methicillin, Amoxicillin-Clavulanic Acid, Ceftizoxime, Ofloxacin, Amikacin, Azithromycin, Clindamycin, Vancomycin, Linezolid, Pristinamycin were placed at equidistance using a sterile forceps, so that they were no closer than 24 mm from center to center. The disks were gently pressed down to ensure complete contact with the agar surface. The plate were inverted and incubated at 35°C in ambient air within 15 minutes of disk application. After 18-24 hours of incubation the plates were viewed with unaided eye using reflected light, for the presence or absence zone of inhibition around each of the antibiotic disk. Antibiotic disks were obtained from Himedia Labs.

The zones of inhibition around each disk (including the diameter of the disk) was measured to the nearest in whole millimeter. The zone sizes were interpreted as per National committee on clinical laboratory standards recommendations 2007 (NCCLS 2007) as sensitive, intermediate or Resistant to the agents tested.

ZONE SIZE INTERPRETATIVE CHART (NCCLS 2007)

Antimicrobial agent	Disk content	Resistant	Intermediate	Sensitive
Methicillin	5 µg	≤ 9	10-13	≥ 14
Amoxicillin-clavulanic acid	20 /10 µg	≤ 19	-	≥ 20
Ceftizoxime	30 µg	≤ 14	15-19	≥ 20
Ofloxacin	5 µg	≤ 14	15-17	≥ 18
Amikacin	30 µg	≤ 14	15-16	≥ 17
Azithromycin	15 µg	≤ 13	14-17	≥ 18
Clindamycin	2 µg	≤ 14	15-20	≥ 21
Vancomycin	30 µg	-	-	≥ 15
Linezolid	30 µg	-	-	≥ 21
Pristinamycin	15 µg	≤ 15	16-18	≥ 19

OBSERVATION AND RESULTS: Rate of isolation of *Staphylococcus aureus* from 400 clinical samples was 65% from pus, 21% from sputum, 8% from urine, 2% from stool, 2% from blood and 2% from body fluids.

Maximum resistance among *Staphylococcus aureus* isolates (n=400) was observed against Methicillin (59%), Amikacin (53%) followed by other antimicrobial agents such as ofloxacin (51%), Amoxicillin-clavulanic acid 48%, Clindamycin 36%, Azithromycin 31%, Ceftizoxime 27% ,Vancomycin 13%, Linezolid 12% and Pristinamycin 9% as shown in **Table 1**.

Out of 236 pure MRSA species Vancomycin, Linezolid and Pristinamycin were found sensitive in 77.9%, 79.6%, and 88.1% isolates respectively presented in **Table 2**.

Among 52 pure MRSA species which were also resistant to Vancomycin (VRSA), 69.2% found sensitive to Linezolid and 92.3% Pristinamycin sensitive as shown in **Table 3**.

Pristinamycin was found sensitive in 75% of pure MRSA which were also Vancomycin resistant (VRSA) and Linezolid resistant (LRSA) as shown in **Table 4**.

TABLE 1: ANTIMICROBIAL SENSITIVITY PATTERN OF SOME OTHER ANTIMICROBIAL AGENTS TO *STAPHYLOCOCCUS AUREUS* ISOLATES (n=400)

Antibiotic Disk	Sensitive		Intermediate		Resistant	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
Methicillin	132	33	32	8	236	59
Amoxicillin-Clavulanic acid	188	47	20	5	192	48
Ceftizoxime	272	68	20	5	108	27
Ofloxacin	168	42	28	7	204	51
Amikacin	112	28	36	9	212	53
Azithromycin	232	58	44	11	124	31
Clindamycin	220	5	36	9	144	36
Vancomycin	332	83	16	4	52	13
Linezolid	344	86	8	2	48	12
Pristinamycin	356	89	8	2	36	9

TABLE 2: INCIDENCE OF SENSITIVITY AND RESISTANCE OF VANCOMYCIN, LINEZOLID AND PRISTINAMYCIN AMONG MRSA (n= 236)

Antibiotic	Sensitive		Resistant	
	No. of isolates	(%)	No. of isolates	(%)
Vancomycin	184	77.9	52	22.0
Linezolid	188	79.6	48	20.3
Pristinamycin	208	88.1	28	11.8

TABLE 3: INCIDENCE OF SENSITIVITY AND RESISTANCE OF LINEZOLID AND PRISTINAMYCIN AMONG VANCOMYCIN RESISTANT MRSA (n=52)

Antibiotic	Sensitive		Resistant	
	No. of isolates	(%)	No. of isolates	(%)
Linezolid	36	69.2	16	30.7
Pristinamycin	48	92.3	4	7.6

TABLE 4: INCIDENCE OF SENSITIVITY AND RESISTANCE OF PRISTINAMYCIN AMONG LINEZOLID, VANCOMYCIN RESISTANT MRSA (n=16)

Antibiotic	Sensitive		Resistant	
	No. of isolates	(%)	No. of isolates	(%)
Pristinamycin	12	75	4	25

DISCUSSION: In recent years, MRSA becomes prevalent worldwide and are now an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide. Treatment options for MRSA are very limited and in today's clinical practice newly developed antibiotics like vancomycin, Linezolid and Pristinamycin are used to treat

MRSA. So the aim of this study was to determine the *in vitro* susceptibility of *Staphylococcus aureus* to relatively newer antimicrobial agent like Vancomycin, linezolid and Pristinamycin and to compare the results with other conventional antimicrobial agents used in the treatment of such infections.

In the present study maximum MRSA isolated from pus (65%) followed by sputum (21%), Urine (8%), Blood (2%) and body fluid (2%) etc. This pattern was also evidenced in other study done by Vidya Pai *et al*; 2010 and Nitish Kumar Sharma *et al*; 2013 [7-8]. This may be because *Staphylococcus aureus* accounts most of Skin and soft tissue infection (pus) and respiratory tract infection (sputum).

Various studies from India have shown MRSA incidence ranging from 46.57% to 54.14%. In the previous studies done at Varansi and Rohtak (India) the incidence of MRSA respectively was 46.5% in 2006 (Hare K Tiwari et al; 2006)[9] 54.1% in 2008 (Deep et al; 2008).[10] In this study at Udaipur (India) incidence was 59%. This supports that prevalence rate of MRSA is increasing with time.

Study from different parts of the world also show varying incidence of MRSA from 38% to 58%. (S. Hafiz *et al*; 2002, Alper Tünger *et al*; 2004, Syed Zahid Bukhari *et al*; 2011, Abdul rehman *et al*; 2011) [11-14]. The varying incidence in the different regions is probably related to the different Geographical conditions, environmental factors, antibiotic usage pattern and Prevalence and dissemination of the Methicillin resistant strains.

Glycopeptides such as Vancomycin are used as first line drug for treatment of MRSA. In this study, 83% *Staphylococcus aureus* were found Vancomycin sensitive, 4% were Vancomycin Intermediate (VISA) and 13% were Vancomycin resistant (VRSA). Among MRSA 77.9% were Vancomycin sensitive.

A study done by Hare K Tiwari *et al* in 2006 at Varanasi (India) found only 0.2% VRSA [9] and 7.8% prevalence rate is observed by S. Bhatawadekar *et al* in 2010 at Pune [15]. In this study, prevalence rate of VRSA significantly increased to 13%, it shows that prevalence rate of VRSA increasing with time in India may be due to various reasons. The concern regarding the development of VRSA is worrisome. Fortunately most strains of VRSA are not multi-resistant and there remain some therapeutic options available besides Vancomycin. Although there is possibility that these VRSA strains will become resistant to currently available agents with time like it occurred with MRSA in the past in hospitals.

The incidence of Vancomycin and Methicillin-resistant Gram-positive Infections continues to increase, so alternative options for treatment are necessary. Linezolid is found sensitive in 80% isolates of MRSA and in 69.2% in VRSA isolates. So Linezolid may provide new options to treat these patients.

Pristinamycin is found sensitive in 88.1% isolates of MRSA 92.3% of VRSA isolates and 75% isolates of LRSA. So Pristinamycin is even effective in treatment of LRSA.

The results demonstrated that the Linezolid and Pristinamycin have good *in vitro* activity against MDR Gram-positive cocci. These drugs are promising therapeutic options in an era of rapidly growing antibiotic resistance.

CONCLUSION: The emergence of drug resistance in MRSA is worrisome in the present therapeutic scenario. Earlier Vancomycin, Linezolid and Pristinamycin were 100% sensitivity to MRSA. Result of this study shows resistant has also occurs to these so called newer agents. The other concern, besides the development of resistance in MRSA strains to all available antibiotics (and those is in pipeline), is the ability of these strains to disseminate. So we need to develop newer agents as well as slow down the spread and amplification of resistant strains by good infection control, conservative measures, prudent use of antibiotics, and good hygiene and avoiding irrational use of newer agents.

It is interesting to find out that in few isolate where all newer agents were resistant, clindamycin and amoxicillin- clavulanic acid found sensitive. Similar finding was also observed by Bindiya Bagga *et al* in 2010 [16]. It suggests that the role of conventional antibiotics in treatment of resistant strain infection still persist.

So considerations of conventional antibiotics are also necessary with newer antibiotics during such treatment.

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