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Detection of Methicillin Resistance and Methicillin Sensitive Staphylococcus Aureus from Various Clinical Specimens in Tertiary Care Hospital

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ABSTRACT

INTRODUCTION:

Staphylococcus aureus is one of the most common Gram-positive pyogenic bacteria responsible for variety of disease. Methicillin resistance *Staphylococcus aureus* (MRSA) has become a major cause of hospital associated infection. MRSA can also acquire resistance to multiple alternative antimicrobials, further complicating treatment of infection. For the severe infection of MRSA, vancomycin considered one of the last treatments relatively new agents such as Linezolid and Daptomycin is used.

MATERIAL AND METHODS: 141 Staphylococcus aureus were isolated from samples such as pus, sputum, urine, blood, body fluid, wound swab received in microbiology department of Krishna institute of medical sciences, karad. They were conformed by microscopy, culture, and biochemical reaction. Then MRSA were detected by using disc diffusion test using cefoxitin (30ug) disc.

RESULTS: Majority of Staphylococcus aureus were isolated from pus samples 71 (50.35%). Out of 141 isolates, 81 (57.44%) were MRSA and 60 (42.55%) were MSSA. In MRSA and MSSA isolates, higher percentage of resistance was seen in Penicillin 78 (96.29%) and 55 (90.16%) respectively.

CONCLUSION: The present study emphasized the burden of MRSA in patients at our hospital. The determination of antibiotic sensitivity patterns and screening for MRSA among *Staphylococcus aureus* isolates are important for controlling the clinical *Staphylococcal* infection. The antibiotic sensitivity pattern helps in judicious use of antimicrobial agents.

INTRODUCTION

Staphylococcus aureus is one of the most common Gram-positive pyogenic bacteria responsible for variety of disease that range in severity from mild skin soft tissue infections to like threatening conditions such as endocarditis pneumonia and sepsis.[1]

In *Staphylococcus* species Penicillin and Methicillin resistant was first recognized in 1994 and 1961 AD respectively. Emerging resistance to methicillin in this organism has left us with very few therapeutic alternatives to treat the infections caused by them.[2]

Methicillin resistance *Staphylococcus aureus* (MRSA) has become a major cause of hospital associated infection. Methicillin resistant *Staphylococcus aureus* first discovered in Britain in the 1960 soon after the beta-lactam methicillin was introduced for clinical use against *Staphylococci*.[14] Early reports indicated

that methicillin resistant *S. aureus* (MRSA) were heterogeneous in their expression of resistance to beta lactam agent. Large difference in the degree of resistance were seen among the individual cell in a population. Also, there may be a variable interaction between the various factors are affecting, NaCl concentration effect is depending on the medium and the temperature of incubation.[15]

MRSA is one of the Drug-resistance pathogens of human. MRSA produce an Alternative penicillin binding protein (PBP2A) which is encoded by mecA gene produced by MRSA carried on the *Staphylococcal* cassette chromosome mec (sccmec). By the antibiotics, PBP2A is not inhibited therefore the cell continues to produce peptidoglycan and maintains a stable cell wall.[16]

While methicillin is no longer used clinically or even produced commercially, the term MRSA has persisted. Furthermore, the term methicillin resistance manifests as resistance to virtually all beta-lactams with the exception of the latest generation of cephalosporin beta-lactams. MRSA can also acquire resistance to multiple alternative antimicrobials, further complicating treatment of infection. For the severe infection of MRSA, vancomycin considered one of the last treatments relatively new agents such as Linezolid and Daptomycin is used. [17]

MATERIAL AND METHOD

The study was conducted over the period of November 2019– November 2021 in the microbiology laboratory of Krishna Institute of Medical Sciences and Krishna Hospital and Medical Research Centre, Karad. The study was done in 141 non repetitive isolates of *Staphylococcus aureus* from various samples i.e. pus, sputum, wound swab, blood, urine and body fluids from patients with active infection collected from all age group and both the sexes admitted to Krishna Hospital and Medical Research Centre Karad, which is a tertiary care hospital attached to Medical College during December 2020 to April 2021.

Isolation and identification

All specimens were inoculated onto Nutrient agar, Blood agar and MacConkey agar. The plates were incubated at 37^0 c for 24 hours. Identification of *S. aureus* was first done using colony morphology on 5% sheep blood agar. Cream to golden yellow colonies with or without haemolysis were further identified using Gram stain, catalase test and coagulase test by standard microbiological techniques.

Antibiotic susceptibility test

The antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per by CLSI guidelines 2020. Control strains used were *Staphylococcal aureus* ATCC -25923 and MRSA -43300. The drugs used were Amikacin (30µg), Ampicillin (10µg), Chloramphenicol (30µg), Ciprofloxacin(5µg), Clindamycin (2µg), Co-Trimoxazole (1.25/23.75µg), Erythromycin (15µg), Gentamycin (10µg), Levofloxacin (5µg), Linezolid (30µg), Nitrofurantoin (300µg), Penicillin (10µg), Tetracycline (30µg), Vancomycin (30µg) were dispensed onto the surface of the inoculated agar plate using sterile forceps.

Detection of methicillin resistance *Staphylococcus aureus* :

Cefoxitin disc diffusion test :

Methicillin resistance was detected by using Cefoxitin (30-ug) on Muller- Hinton agar supplemented with 2% NaCl followed by incubation at 35^0 c.

Zone diameter of 22 mm or more was taken as sensitive and 21 mm or less was considered as resistant. These resistant isolates were considered as MRSA.

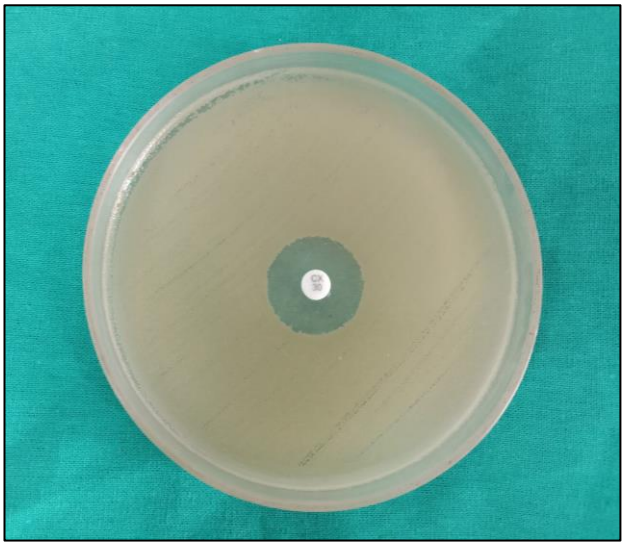

Fig No. 1: Methicillin Resistance *Staphylococcus aureus* (MRSA)

OBSERVATIONS AND RESULTS

Over a period of 1-year, 141 *Staphylococcus aureus* isolates were studied. These isolates were from patients admitted to inpatient departments, intensive care units and patients attending outpatient departments at Krishna Institute of Medical Sciences and Krishna Hospital, Karad.

TABLE NO. 1 SAMPLE WISE DISTRIBUTION OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM DIFFERENT SECTION OF HOSPITAL

Samples	Total IPD/OPD samples	Percentage (%)
Blood	22	15.60%
Urine	16	11.34%
Pus	71	50.35%
ETT	8	5.68%
Wound swab	11	7.80%
Vaginal swab	4	2.84%
Catheter tip	4	2.84%
Sputum	5	3.55%
Total	141	100%

TABLE NO. 2 DISTRIBUTION OF MRSA AND MSSA IN *STAPHYLOCOCCUS AUREUS*

	No. of isolates	Percentage (%)
MRSA	81	57.44
MSSA	60	42.55
Total	141	100

TABLE NO. 3 ANTIBIOTIC RESISTANCE AMONG MRSA AND MSSA ISOLATES

Antibiotics	MRSA				MSSA			
	Sensitive	%	Resistant	%	Sensitive	%	Resistant	%
Amikacin (AK)	73	90.12 %	7	8.64%	60	98.36 %	1	1.63%
Ampicillin (AMP)	11	13.58 %	69	85.18 %	6	9.83%	55	90.16 %
Chloramphenicol (C)	74	91.35 %	6	7.40%	60	98.36 %	1	1.63%
Ciprofloxacin (CIP)	16	19.75 %	64	79.01 %	12	19.67 %	49	80.32 %
Clindamycin (CD)	77	95.06 %	3	3.70%	58	95.08 %	3	4.91%
Co-Trimoxazole (COT)	59	72.83 %	21	25.92 %	39	63.93 %	22	36.06 %
Erythromycin (E)	21	25.92 %	59	72.83 %	10	16.39 %	51	83.60 %
Gentamycin (GEN)	59	72.83 %	21	25.92 %	47	77.04 %	14	22.95 %
Levofloxacin (LE)	19	23.45 %	61	75.30 %	9	14.75 %	52	85.24 %
Linezolid (LZ)	75	92.59 %	5	6.17%	59	96.72 %	2	3.27%
Nitrofurantoin (NIT)	75	92.59 %	5	6.17%	58	95.08 %	3	4.91%
Penicillin (P)	2	2.46%	78	96.29 %	6	9.83%	55	90.16 %
Tetracycline (TE)	69	85.18 %	11	13.58 %	52	85.24 %	9	14.75 %
Vancomycin (VA)	67	82.71 %	13	16.04 %	48	78.68 %	13	21.31 %

Table no. 1 shows- Maximum isolates were from Pus 71 (50.35%) followed by Blood 22 (15.60%), Urine 16 (11.34%), Wound swab 11 (7.80%), ETT 8 (5.68%), Sputum 5 (3.55%), Vaginal swab 4 (2.84%) and Catheter tip 4 (2.84%)

In this method of detection of MRSA and MSSA in *Staphylococcus aureus*, out of 141 (100%) isolates, 81 (57.44%) were MRSA and 60 (42.55%) were MSSA.

Table No.3 shows antibiotic resistance among MRSA and MSSA isolates. In MRSA isolates, higher percentage of resistance was seen in Penicillin 78 (96.29%) followed by Ampicillin 69 (85.18%), Ciprofloxacin 64 (79.01%), Levofloxacin 61(75.30%), Erythromycin 59 (72.83%), Gentamycin 21 (25.92%), Co-trimoxazole 21 (25.92%), Vancomycin 13 (16.04%), Tetracycline 11 (13.58%), Amikacin 7 (8.64%), Chloramphenicol 6 (7.40%), Linezolid 5 (6.17%), Nitrofurantoin 5 (6.17%) and Clindamycin 3 (3.70%).

In MSSA isolates, higher percentage of resistance was seen in Penicillin 55 (90.16%), Ampicillin 55 (90.16%) followed by Levofloxacin 52 (85.24%), Erythromycin 51 (83.60%), Ciprofloxacin 49 (80.32%), Co-trimoxazole 22 (36.06%), Gentamycin 14 (22.95%), Vancomycin 13 (21.31%), Tetracycline 9 (14.75%), Nitrofurantoin 3 (4.91%), Clindamycin 3 (4.91%), Linezolid 2 (3.27%), Chloramphenicol 1 (1.63%) and Amikacin 1 (1.63%).

DISCUSSION

This study was undertaken in the Department of Microbiology, Krishna Institute of Medical Sciences, Deemed to be University, Karad to detect MRSA and MSSA from various clinical samples.

In the present study, *Staphylococcus aureus* was isolated from various clinical specimens. In this study, 141 samples were processed and results were analyzed.

Among 141 isolates majority of the isolates were from Pus (50.35%), followed by Blood 15.60%, Urine 11.34%, Wound swab 7.80%, ETT 5.68%, Sputum 3.55%, Vaginal swab 2.84% and Catheter tip 2.84%. This finding can be correlated with the study conducted by Subasini Majhi et. al [1] in which they have reported maximum number of *Staphylococcus aureus* isolates from Pus 53.1%, followed by Urine 18.6%, Blood 11.5%. Similarly, Adhikari RP. et al. [2] reported that majority of isolates were from Pus (54.4%), followed by Blood (22.2%), Sputum (14%) and Urine (7.4%). Nilam R. Patil et.al [39] also reported that maximum isolation of *S. aureus* was from pus samples (78.8%). In a study conducted by Sangita Thapa et al. [40] highest isolation rate of *S. aureus* was seen in Blood (44.4%) followed by Pus (23.4%), Urine (11.7%) and Sputum (10.2%).

The present study showed higher percentage of antibiotic resistance were Penicillin 133 (94.32%) According to Gupta D. et.al. [45] 161 *Staphylococcus aureus* have been reported in which higher percentage of resistance was noted for penicillin 93.78%. Another study conducted by Nizami Duran et al. [42] also reported similar results where Penicillin 92.8%. The study conducted by Mojtaba Nikbakhta et.al, [43] Priyanka Kalbhor et.al [44] and Goudarzi M. et.al [46] showed similar result to our study.

The present study showed 57.44% of MRSA and 42.55% of MSSA among 141 *Staphylococcus aureus* isolates. Similarly, Ciraj AM et.al [48] reported that 57% of MRSA. The above data correlates with the result of Arora S. et. al [49] who have documented 54.8% MRSA. This is in accordance with study of Hassan AK. et.al [51] who have documented 55% of MRSA and 48% MSSA. Anupurba S et.al [53] 2003 has reported 54.8% of MRSA in their study. Eyob YG. et.al [52] 2019 has reported 72% highest prevalence of MRSA. In contrast, other studies have reported lowest prevalence i.e. Supriya S. Tahnkiwale et.al [50] reported has 19.56% of MRSA. Mehta M. et.al [47] reported has 24% of MRSA.

In present study, among MRSA isolates, higher percentage of resistance was seen in Penicillin 78 (96.29%) followed by Ampicillin 69 (85.18%), Ciprofloxacin 64 (79.01%), Levofloxacin 61 (75.30%), Erythromycin 59 (72.83%), Gentamycin 21 (25.92%), Co-trimoxazole 21 (25.92%), Vancomycin 13 (16.04%), Tetracycline 11 (13.58%), Amikacin 7 (8.64%), Chloramphenicol 6 (7.40%), Linezolid 5 (6.17%), Nitrofurantoin 5 (6.17%) and Clindamycin 3 (3.70%).

Anjali Kulshrestha et. al [55] has reported higher percentage of resistance in MRSA were Penicillin 88% followed by Ciprofloxacin 66%, Erythromycin 43%, Co-trimoxazole 43%, Levofloxacin 28%, Clindamycin 31%, Vancomycin 3%, Tetracycline 3%. Similarly, Mshana SE. et.al [56] has reported higher percentage of resistance in MRSA were Penicillin 96% followed by Co-trimoxazole 92%, Tetracycline 69%, Ciprofloxacin 54%. Gade ND. et.al [57] has also reported higher percentage of

resistance in MRSA were Penicillin 100% followed by Ciprofloxacin 92.5%, Co-trimoxazole 89.7%, Erythromycin 61.7%, Gentamycin 57%, Clindamycin 43.9% and Tetracycline 42.1%.

CONCLUSION

The present study emphasized the burden of MRSA in patients at our hospital. The determination of antibiotic sensitivity patterns and screening for MRSA among *Staphylococcus aureus* isolates are important for controlling the clinical *Staphylococcal* infection. The antibiotic sensitivity pattern helps in judicious use of antimicrobial agents.

ETHICAL APPROVAL

The study was approved with protocol number (043/20202021) by Ethics committee of Krishna Institute of Medical Sciences, Deemed to be University, Karad.

CONCENT

As per international standard or university standard, patients written consent has been collected and preserved by the author's.

DATA AVAILABILITY

The article contains the appropriate and proper data obtained during the experiment which supports the results, discussion and conclusion of the research article.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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