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Prevalence and Antibiogramof MERSA Isolated from Sample in Tertiary Care Center in Central India

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Abstract

Staphylococcus aureus is an important human pathogen causing various kinds of infections ranging from minor skin diseases to life- threating endocarditis. It has acquired resistance to previously effectiveantimicrobialsincluding the methicillin. Methicillin resistant Staphylococcus aureus (MRSA) is a prototype of resistant bacteria associated with greater lengths of hospital stay, higher mortality , increased costs and more troublesome to patient when compared with methicillin sensitive Stphylococcusaureus (MSSA). The present study was a prospective study conducted for a period 1 year 8 month (from Nov 2011- June 2013) all staphylococcal isolate in Microbiology Laboratory, Shri Aurbindo Medical college &P.G.instituteindoreindia. Gram staining of each specimen (except blood) was performed and findings noted. Each specimen was cultured onBlood agar and MacConkey's agar aerobically incubated overnight at 37˚c. Staphylococcal isolate were identified by phenotyping methods lke Gram stain, catalase test, slide and tube coagulase test growth on manitol salt agar, VP test Phosphates test and bacitracin susceptibility test .The antimicrobialsusceptibility testingwas performed by Kirby -Bauer disc diffusion method as per CLSI guidelines. In the present studyout of 649 Staphylococcus 245 (37.8%) strains were methicillin resistant Staphylococcus aureus (MRSA) and 404 (62.2%) were , methicillin sensitive Staphylococcus aureus (MSSA). Out of Total 649 S.aureus, 245 were MRSA. Highest isolation of MRSA was found in Tracheal aspirate (73.3%) , blood culture(70.97%) followed by suction tip (69.2%) , Sputum (63.2%) , body fluids/CSF(52.9%) , tissue(50%), broncho alveolar lavage(46.2%), pus & wound swab(32.6%), vaginal swab (26.7%)and urine(21.7%). MRSA strain were 100 % resistant to penicillin followed by erythromycin(96.7%), clindamycin (88.6%), ciprofloxacin (85.3%) , Cotrimoxazole (70.6%) , rifampicin (42.9%) and Nitrofurantoin (66.7%).

Key words: MERSA, Antibiogram, prevalence, India

Study Design: Prospective Observational Study.

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1. INTRODUCTION

Staphylococcus aureus is an important human pathogen causing various kinds of infections ranging from minor skin diseases to life- threating endocarditis. It has acquired resistance to previously effective antimicrobialsincluding the methicillin.[1]Methicillin resistant Staphylococcus aureus (MRSA) is a prototype of resistant bacteria associated with greater lengths of hospital stay, higher mortality , increased costs and more troublesome to patient when compared with methicillin sensitive Staphylococcusaureus (MSSA)[2-6]

β-Lactams are considered as the first-choice antibiotics totreat Staphylococcal infections. Currently, the increasingresistance against antibacterial drugs is a major publichealth concern and one of the biggest challenges faced byphysicians. In S. aureus, resistance to methicillin occursbecause of variations in the alteration of constitutivepenicillin-binding proteins (PBPs) or expression ofthe mecA.[7,8]This has triggered alarm of medical community as S. aureus causing life-threatening infections in hospitals and community. Now MRSA is one of the most common causes of nosocomial infections accounting for 40% to 70% of S. aureus infections in intensive care units (ICUs). [9]

The Clinical and Laboratory Standards Institute (CLSI) [11]has recommended the cefoxitin disk test for prediction of mecA–mediated resistance.Detection of mec A gene by Polymerase chain reaction (PCR) is considered to be gold standards but it is not yet available in all clinical laboratories. Therefore phenotypic methods still remain a method of choice in resource limited settings .[10]The present study was done to characterize and to determine the prevalence of MRSA isolates obtained from clinical specimens in a health care setup.

2. MATERIAL AND METHODS

The present study was a prospective study conducted for a period 1 year 8 month (from Nov 2011- June 2013) all staphylococcal isolate in Microbiology Laboratory, Shri AurbindoMedical College & P.G.Institute IndoreIndia. The specimens like pus and wound swab, blood, bronchoalveolar lavage, sputum, tracheal aspirate, suction tip, urine, vaginal swab, tissue, body fluids/ CSF submitted to microbiology laboratory were processed as per standard procedures. [12] Gram stainingof each specimen (except blood) was performed and findings noted. Each specimen was cultured onBlood agar and MacConkey's agar aerobically incubated overnight at37˚c . Staphylococcal isolate were identified by phenotyping methods like Gram stain, catalase test, slide and tube coagulase test growth on manitol salt agar, VP test phosphatase test and bacitracin susceptibility test.

All Staphylococcusaureus isolates were tested for methicillin resistance by Cefoxitin (30 µg) disc diffusion test (CDD), Chromogenic agar method. E test to know minimum inhibitory concentration (MIC) of Oxacillin and Vancomycin, Latex agglutination test based on detection of PBP2a in 100 strains of S.aureus. Polymerase chain reaction (PCR) for detection of mec A gene in 100 cefoxitin resistant strains of MRSA.The antimicrobial susceptibility testing was performed by Kirby -Bauer disc diffusion method as per CLSI

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guidelines.[13]A lawn culture of the test strain was prepared on Mueller- Hinton agar (MHA), plate. With all aseptic precautions the antibiotics discs were placed on Mueller Hinton agar plate and incubated at 37˚c overnight. Following antibiotics discs (Hi Media Pvt. Ltd, Mumbai) were used according to CLSI guidelines 2010 and ATCC 25923 (MSSA), 43300(MRSA)strain was used as a control strain.[13]

3. RESULT

Table 01: Prevalence of methicillin resistant Staphylococcus aureus (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA) strains.

S. aureus	MRSA		MSSA	
	Number	Percentage	Number	Percentage
649	245	37.8%	404	62.2%

In the present studyout of 649 Staphylococcus 245 (37.8%) strains were methicillin resistant Staphylococcusaureus (MRSA) and 404 (62.2%)were, methicillin sensitive Staphylococcus aureus (MSSA).

Figure 01.: Prevalence of methicillin resistant Staphylococcus aureus (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA) strains

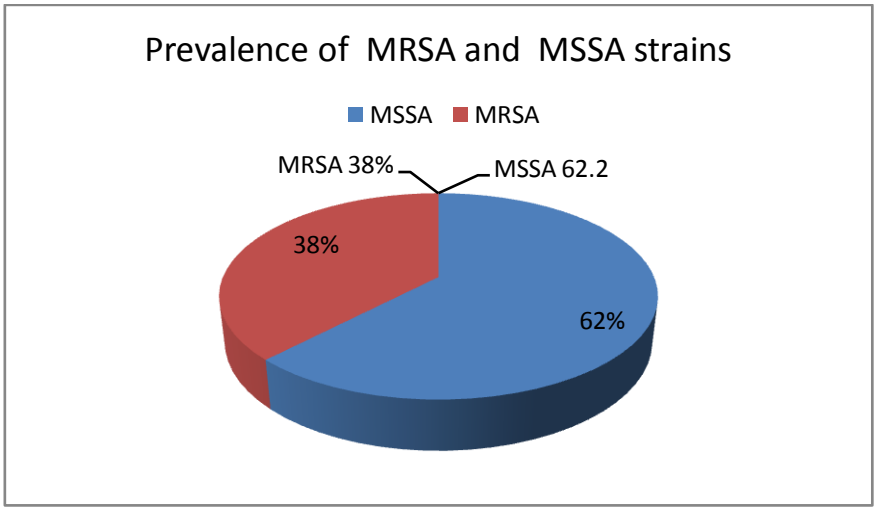

Table 02: Isolation of methicillin resistant Staphylococcus aureus (MRSA)

from different clinical specimens (n= 649)

Specimens	S. aureus	MRSA	
	N=649	N=245	Percentage
Pus & wound swab	484	158	32.6
Blood	31	22	70.97
BAL	26	12	46.2
Urine	23	5	21.7
Sputum	19	12	63.2

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Vaginal swab	15	4	26.7
Tracheal asp.	15	11	73.3
Suction tip	13	9	69.2
Tissue	6	3	50
Body Fluid/CSF	17	9	52.9
TOTAL	649	245	37.8

Out of Total 649 S.aureus, 245 were MRSA. Highest isolation of MRSA was found in Tracheal aspirate (73.3%) , blood culture(70.97%) followed by suction tip (69.2%) , Sputum (63.2%) , body fluids/CSF(52.9%) , tissue(50%), broncho alveolar lavage(46.2%), pus & wound swab(32.6%), vaginal swab (26.7%)and urine(21.7%).

Table 03: Antibiotic susceptibility profile of methicillin resistant Staphylococcus aureus (MRSA) (n=245)

Antibiotics	Methicillin Resistant Staphylococcus aureus (MRSA)			
	Sensitive		Resistant	
	Numbers	Percentage	Numbers	Percentage
Penicillin	0	0	245	100
Erythromycin	8	3.3	237	96.7
Clindamycin	28	11.4	217	88.6
Ciprofloxacin	36	14.7	209	85.3
Cotrimoxazole	72	29.4	173	70.6
Gentamycin	135	55.1	110	44.9
Rifampicin	140	57.1	105	42.9
Tetracycline	171	69.8	74	30.2
Chloramphenicol	204	83.3	41	16.7
Linezolid	245	100	0	0
Vancomycin	245	100	0	0
Nitrofurantoin (Urine samples =5) MRSA= 5 MSSA= 18	3	60	2	40

MRSA strain were 100 % resistant to penicillin followed by erythromycin(96.7%), clindamycin (88.6%), ciprofloxacin (85.3%) , Cotrimoxazole (70.6%) rifampicin (42.9%) and Nitrofurantoin (66.7%).

4. DISCUSSION

The distribution of MRSA varies according to factors such as population, areas studied , use of different culture techniques and different interpretation of guidelines.[14,15]In the present study a total of 649 Staphylococcus aureus strains were isolated fromdifferent clinical samples like pus and wound swab, blood, sputum, bronchoalveolar lavage, tracheal aspirate, body fluids , CSF etc. We also evaluated the antibiotic susceptibility

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pattern of these isolates and various phenotypic methods for detection of MRSA. The study was also conducted to detect mecA gene by PCR in 100 cefoxitin resistant MRSA strains .

Methicillin resistance in S. aureus restricts therapeutic options for clinical isolates and theincidence of MRSA is escalating in India. Antibiogram analysis has been found to be a good epidemiological marker for MRSA phenotyping.Currently, majority of S. aureus strains are beta-lactamase producer , hence resistant to penicillin. In our study all 245 MRSA and out of 404 , 99.3% MSSA isolates were resistant to penicillin while all MRSA and MSSA were sensitive to linezolid and vancomycin similar to **LoveenaOberoi et al 2013**[16] and **Sharma M et al 2013** [17]reported 100% of their MRSA isolates were resistance to penicillin and sensitive to Vancomycin and Linezolid .

Sashirekha B et al 2012 [14]reported that 7.14% of isolates were resistant to vancomycin.

In our study MRSA isolates showed higher resistance to erythromycin (96.7%), Clindamycin (88.6%) , ciprofloxacin (85.3 %), and cotrimoxazole (70.6%). Similar resistance has also beenreported by Sharma M 2013 [17]

Sharma JB et al 2010[18] found in their study that 100% of MRSA isolates were resistant toerythromycin , trimethoprim, ciprofloxacin, gentamycin and tobramycin; 85% to clindamycin and 96% were also resistant to tetracycline, co-trimoxazole and amikacin.**Tiwari et al (2008)** [19] reported 76.1% tetracycline resistance in MRSA.

In our study 8 3.3% MRSA were sensitive to Chloramphenicol , similar results (80%) havebeen reported by **Baddour MM et al. (2007)**[20] and 73.80% by **Sashirekha B et al (2012)**[14]Thus, ourstudy reported that antibiotics such as Chloramphenicol and Tetracycline can be promising if susceptibility testing is done, reserving vancomycin for life threatening infection.Considerable variations were found in the resistance profiles among MRSA isolated fromdifferent countries. The high level resistance of the isolates in the present study to

penicillin, cotrimoxazole, ciprofloxacin, and rifampicin can be attributed to the fact thatthese antibiotics are frequently used in treatment of common infections.Monotherapy is associated with increased resistance as compared to combination therapy.Therefore, combination treatment is advisable and proven to be beneficial in treatment anderadication of MRSA strains. [14]

An urgent need exists for more appropriate selection and use of antimicrobial drugs in the developed as well as in developing countries. The focus in developing countries should be on the availability of safe and effective drugs and on the enforcement of more responsible national drug policies. These issues must be addressed by the collective action of Governments, the pharmaceutical industry, health care providers, and consumers. The developed countries have an important stake in the ways in which antibiotics are used in developing countries because resistant microorganisms do not recognize national boundaries.

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