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Original Research Article

Methicillin Resistant *Staphylococcus Aureus*: A Cause of Concern

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ABSTRACT

Background: *Staphylococcus aureus* can cause superficial skin infections such as boils, furuncles, styes etc. to life threatening systemic illness like surgical site infections, meningitis, pneumonia, blood stream infections, osteomyelitis. Methicillin Resistant *Staphylococcus aureus* (MRSA) was first reported in 1961after 1 year of its clinical use. Presently MRSA is a major cause of Health care associated and community acquired infection worldwide. MRSA strains can readily develop resistance to most of the antimicrobials in clinical use. Methicillin resistance occurs mostly due to altered Penicillin binding protein (PBP2a) and mainly mediated by *mec*A gene. Cefoxitin resistance is a surrogate marker of *mec*A gene mediated Methicillin resistance.
Aim: To detect the incidence of MRSA strains in our hospital and to study the antibiotic susceptibility profile of MRSA strains.
Material & methods: 100 *Staphylococcus aureus* strains isolated from different clinical samples such as pus, blood, urine etc. were studied. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method and Methicillin resistance was detected by using Cefoxitin 30µg disc as per CLSI Guidelines, 2016.
Result: 56% *Staphylococcus aureus* strains were MRSA. All MRSA strains were sensitive to Vancomycin and Linezolid and resistant to Penicillin. Conclusion: Detection of MRSA strains should be done in Clinical Microbiology Laboratory for effective patients' treatment and implementation of Infection Control measures in Health Care Setup to prevent its spread.
Key words: Methicillin Resistant *Staphylococcus aureus* (MRSA).

INTRODUCTION

Staphylococcus aureus can cause simple superficial skin infections like boils, furuncles, to life threatening systemic illness e.g. pneumonia, meningitis, bacteraemia leading to endocarditis, osteomyelitis, septic arthritis and abscess formation in lungs, kidneys and brain etc. [1] Antibiotic resistance in *Staphylococcus aureus* has become an ever-increasing problem. In 1944 Penicillin resistance & in 1961 Methicillin resistance in Staphylococcus was recognized first. [2] The detection of antimicrobial susceptibility of a clinical isolate is crucial for effective antimicrobial therapy of patients with infectious disease. This is especially important considering the emergence of antibiotic resistant bacteria. [3] Antimicrobial resistance in *Staphylococcus aureus* is a cause of concern for clinicians and Microbiologists. *Staphylococcus aureus* cause hard-to-treat infections because they can develop resistance to most of the commonly used antibiotics such as β-lactamase, aminoglycosides, macrolides, clindamycin etc. The development of

antimicrobial resistance in *Staphylococcus aureus* occur due to various mechanisms like β-lactamase production, altered receptors, active efflux pump and ribosomal modifications. With emergence of MRSA, Vancomycin became the last resort to treat infected patients. But Vancomycin resistance has also been reported in 2002. [4] The emergence of Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin resistant *Staphylococcus aureus* (VRSA), Vancomycin intermediate *Staphylococcus aureus* (VISA) have posed therapeutic challenge of treating patients infected with *Staphylococcus aureus*. The resistance in MRSA and VRSA occur due to production of β-lactamase enzymes. Hence, it is clear that these antibiotics should be used as a reserve drug to those cases in which they are absolutely necessary. Prompt and accurate detection and antibiotic susceptibility profile is also important for prevention and control of drug resistant *Staphylococcus aureus* infections in the hospital and community. Methicillin resistance occurs due to altered Penicillin binding protein (PBP2a) and mainly mediated by *mec*A gene. Cefoxitin resistance is a surrogate marker of *mec*A gene mediated Methicillin resistance. [5]

Hence, the present study was undertaken to detect the incidence of MRSA strains isolated from different clinical samples in our hospital and to study the antibiotic susceptibility profile of MRSA strains.

MATERIALS AND METHODS

The present study was conducted in Department of Microbiology and was approved by Institutional Ethical Committee (IEC). The type of study was short term cross sectional study. 100 *Staphylococcus aureus* strains isolated from clinical samples such as pus, blood, medical devices, body fluids etc. and characterized by conventional tests [6] only was included in the study. The different clinical samples were received from the Indoor Patient Department (IPD) of our Hospital.

Antibiotic Susceptibility Test: All 100 *Staphylococcus aureus* strains were tested for antibiotic susceptibility test by Kirby Bauer disc diffusion method [7] according to Clinical Laboratory Standard Institute (CLSI) Guidelines. [5] Lawn culture of the test strain (turbidity adjusted to 0.5 Mc Farland standard) was done on Mueller Hinton agar (MHA) plate and antibiotic discs were put with all aseptic precaution. Six antibiotic discs were put on a 90 mm diameter MHA plate. The plates were incubated at 37^0 C overnight. The antibiotic discs used were Penicillin (10 units), Erythromycin (15µg), Clindamycin (2µg), Tetracycline (30µg), Ciprofloxacin (5µg), Gatifloxacin (5µg), Pristinamycin (15µg), Vancomycin (30µg), Linezolid (30µg) etc. For urine sample, additional Nitrofurantoin (300µg) disc was put. The control strain used for antibiotic susceptibility test was ATCC *Staphylococcus aureus* 25923. All antibiotic discs, culture media and control strain used for this study was procured from Hi media Laboratories Pvt. Limited, India.

Detection of Methicillin Resistance: It was done by using Cefoxitin (30µg) disc according to CLSI Guidelines, 2016. [5] The zone of inhibition ≤ 21mm was considered resistant and hence Oxacillin resistant *Staphylococcus aureus* i.e. MRSA whereas the zone of inhibition ≥ 22 mm was considered as Oxacillin sensitive *Staphylococcus aureus* i.e. MSSA.

OBSERVATIONS & RESULTS

Out of 100 *Staphylococcus aureus* strains studied, 56% strains were Methicillin Resistant Staphylococcus aureus (MRSA) and 44% strains were Methicillin Sensitive Staphylococcus aureus (MSSA).

Table 1: Isolation of MRSA & MSSA strains from different clinical specimens (n=100).

Specimen	MRSA (no)	MSSA (no)
Pus & wound swab (56)	31	25
Blood (31)	17	14
Urine (06)	04	02
Others*(07)	04	03

Others*include Foley's catheter tip (4), Central line tip (2), Nasal swab (1)

Table 1 shows out of 100 *Staphylococcus aureus* strains studied, 56 strains were

isolated from pus and wound swab and 31 were from blood. 17(54.8%) strains isolated from blood and 31 (55.4%) strains isolated from pus and wound swab were MRSA respectively. Only 1 strain isolated from nasal swab was MRSA.

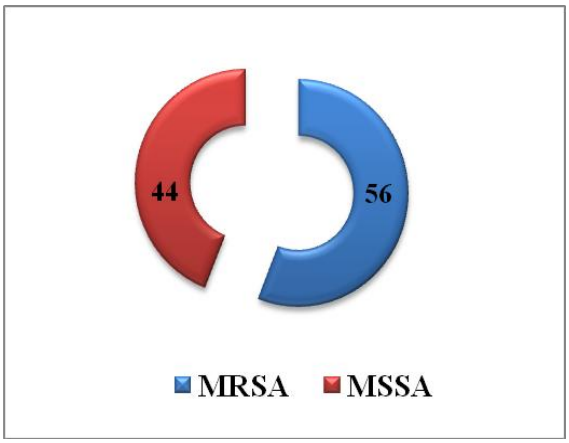
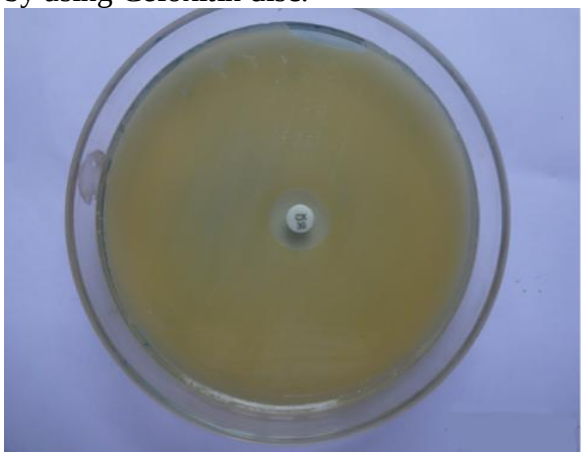


Figure 1: Incidence of MRSA and MSSA strains among Staphylococcus aureus strains studied (n=100).

Photograph 1 showing detection of MRSA by using Cefoxitin disc.



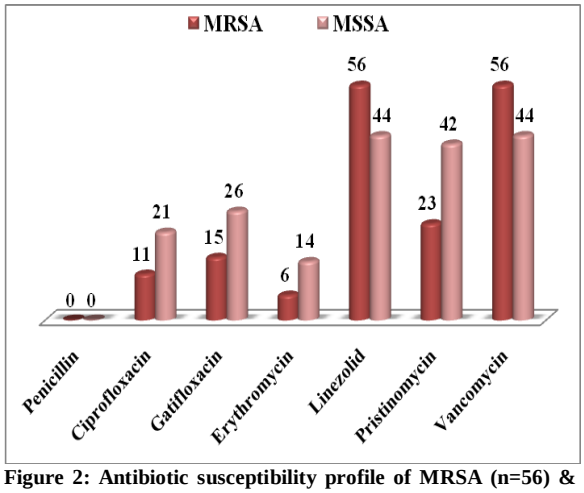


Figure 2: Antibiotic susceptibility profile of MRSA (n=56) & MSSA (n=44) strains studied.

Figure 2 shows the antibiotic susceptibility profile of 100 *Staphylococcus aureus* strains studied. It was observed that all (100%) strains were sensitive to Vancomycin & Linezolid but resistant to Penicillin. Out of 56 MRSA strains only 6 (10.7%) strains were sensitive to Erythromycin and 11 (19.6 %) strains were sensitive to Ciprofloxacin and23 (41.1%) strains were sensitive to Pristinamycin. Out of 44 MSSA strains isolated, 42 (95.5%) strains were sensitive to Pristinamycin. Only 1, out of 4 MRSA strains isolated from urine was sensitive to Nitrofurantoin, whereas 2 MSSA strains from urine were sensitive to Nitrofurantoin.

DISCUSSION

It is a cause of great concern about the rapid rise in antibiotic resistance of *Staphylococcus aureus* strains worldwide. In the present study, 100% *Staphylococcus aureus* strains including MSSA strains were resistant to Penicillin. Very high resistance was observed in Erythromycin, Ciprofloxacin and Gatifloxacin i.e. 80%, 68% and 59% respectively. Kaur et al also reported isolates of MRSA showed 100% resistance to penicillin and no strain was resistant to Vancomycin and Linezolid. [8] The most common reason for antibiotic resistance in *Staphylococcus aureus* strains and MRSA strains is indiscriminate use of antibiotics. There is a wide difference between antibiotic susceptibility profile of MRSA and MSSA strains. Hence, routine detection of MRSA by Cefoxitin disc should be done in Clinical Microbiology Laboratory for a better therapeutic outcome and implementation of Infection Control measure in Health care setup.

Table 2: Incidence of MRSA strains reported by various workers

Authors	Year	MRSA%
Basak & Deshpande [9]	1997	30.6
Verma et al. [10]	2000	80.9
Anuprabha et al. [11]	2003	54.6
Tiwari et al. [12]	2008	38.4
Sasirekha et al. [13]	2014	27.5
Poddar etal. [14]	2015	36
Present study	2016	56

In the present study, 56% *Staphylococcus aureus* strains were MRSA and 44% were MSSA. The incidence of MRSA shows a large variation from 30.6% to 81%. [9,10] This is mainly because of different methods used for detection of MRSA and different *Staphylococcus aureus* strains studied by different workers. In 2014, Sasirekha et al. have reported 27.5% MRSA strains [13] whereas Poddar et al. in 2015 reported that 36% *Staphylococcus aureus* strains were MRSA. [14] Our studies correlated well with studies done by Anuprabha et al. who have reported the incidence of MRSA strains as 54.8%. [11] In a study conducted in our department in 2007, the incidence of MRSA was reported as 51.8%. [15] Our hospital is a tertiary care hospital in a rural set up and caters to patients from villages of Vidarbha and adjoining aras of Madhya Pradesh, Chhattisgarh and Andhra Pradesh. Most of the patients attend 3-4 practitioners and take several classes of antibiotics before attending our hospital. Lack of awareness and improper use of antibiotics might be the contributory factor for high incidence of MRSA in our study.

CONCLUSION

MRSA strains should be detected in Clinical Microbiology Laboratory for effective patients' treatment and implementation of Infection Control measures in Health Care Setup to prevent its spread. Stopping MRSA is in our hand and hand hygiene practices should be followed strictly. CDC has given the dictum 'Wash Your Hands, Save Lives'!

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