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Detection of methicillin resistance in *Staphylococcus* species from clinical samples

S. Jayashree[1], Dr. Mita D. Wadekar[*2], Pooja C.[3], Dr. Sathish JV.[4]

Professor and Head, Department of Microbiology, Chamarajanagar Institute of Medical Sciences, Chamarajanagar, Karnataka, India

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Abstract

Staphylococcus aureus that are resistant to many antibiotics are known as as Methicillin-resistant *Staphylococcus aureus* (MRSA). Infections caused by MRSA can cause severe infection especially in immunocompromised individuals. Compared to ordinary Staphylococcus infections, MRSA infectios are more difficult to treat. This is because the strains of staphylococcus known as MRSA do not respond well to many common antibiotics. The objective of the study is to detect MRSA in Staphylococcus species isolated from various clinical samples. A retrospective study of all staphylococcus species was conducted. Total of 161 isolates were isolated from various samples. They were processed and identified by standard Microbiological procedures. The antibiotics susceptibility testing was performed by Kirby- Bauer disc diffusion method using CLSI guidelines. MRSA was detected by disc diffusion test using Cefoxitin (30µg) disc. Of 161 samples processed, 74 Methicillin-resistant Staphylococcus species were isolated. Methicillin resistance were found to be high in pus (41.8%) samples followed by urine 29(39.1%) and blood 14 (18.9%) and they showed higher sensitivity towards vancomycin followed by linezolid. Methicillin resistance in Staphylococcus species are increasing rapidly and becoming a major problem. Disk diffusion method can be routinely employed to detect these resistant strains to understand the drug resistance patterns of Methicillin resistance producing staphylococcus species.

[*] **Corresponding Author:** Dr Mita D Wadekar ✉ drmdw20@gmail.com

Introduction

Staphylococci are the common commensal flora of skin and also colonizes nasal cavity. Conventional division includes two groups of Staphylococcus based on the coagulase reaction. Those are the coagulase-positive and coagulase-negative staphylococci. Among these two, coagulase-positive staphylococci are most commonly isolated (Gandhiraj *et al*., 2018). Methicillin-resistant *Staphylococcus aureus* (MRSA) are a special type of staphylococcus and they show higher resistance to various antibiotics. They are normal commensal of skin and nose, but will not cause any problems like other bacteria. MRSA is different from other types of staphylococcus because it is very difficult to treat with certain antibiotics such as methicillin (Biswajit Batabyal *et al*., 2012).

Methicillin resistance in *S. aureus* is mediated by a chromosomally coded gene called mecA gene, which alters penicillin-binding protein (PBP) present on *S. aureus* cell membrane to PBP-2a. MRSA are mainly divided into two types namely, either community or hospital associated MRSA (Apurba Sankar Sastry *et al*., 2016). In Gram-positive bacteria, drug resistant *S. aureus* is a serious and has a major global health concern (Ali Hassoun *et al*., 2017).

MRSA is becoming public health problem worldwide because of high mortality and morbidity associated with it and also increased healthcare costs (Bart N. Green *et al*., 2012). The major risk factors for MRSA has been reported and includes immunocompromised state, patients on dialysis, extremes of age, prolonged hospital stay, indwelling devices etc.(Eyob Yohaness Garoy *et al*., 2019). In 1940s, penicillin introduction to the market was a cornerstone in treating staphylococcal infections which was soon followed by the emergence of beta-lactamase producing strains (Emranaskari *et al*., 2012).

From recent health economic research based on pharmaceutical sales data, which showed increasing trend by 103% in antibiotic consumption (specifically towards last resort drugs) in India during 2000 and 2015. In countries where the prevalence of MRSA is high, suffering from cost of treatment, long-term hospitalization and the psychological stress will effect on the healthcare systems and economy (Ram Prabhoo *et al*., 2019). In all World Health Organization (WHO) regions reports of MRSA is documented to have exceeded 20% and 80% in some regions (Frederick K. Wangai *et al*., 2019).

Skin and soft tissue infections (SSTIs) are generally caused by *S. aureus* and also causes toxin-mediated infections like toxic shock syndrome, food poisoning and staphylococcal scalded skin syndrome. Most commonly reported invasive MRSA related conditions include septic shock, pneumonia, endocarditis, bacteremia, and cellulitis (Bart N. Green *et al*., 2012 and Nawfal Hussein *et al*., 2019).

Detection of MRSA by disc diffusion test can be done by using cefoxitin or oxacillin discs. Other methods includes oxacillin-screening agar, PCR (detects mecA gene) and latex agglutination test (detects PBP -2a) (Apurba Sankar Sastry *et al*., 2016 and Ali Hassoun *et al*., 2017). In case of serious infections due to MRSA the drug of choice will be Vancomycin. Over use of vancomycin has lead to emergence of resistance to vancomycin. It may be of low grade resistance known as VISA (vancomycin intermediate *S. aureus*) or high-grade resistance known as VRSA (vancomycin resistant *S. aureus*) (Graeme R. Nimmo *et al*., 2007). Control measures involve screening of MRSA carriers and their treatment, stoppage of antibiotic misuse, ensuring proper infection control measures. Microbiology laboratory should detect these MRSA and also their antibiotic susceptibility pattern to provide reliable treatment options to clinicians for treating patients.

Materials and methods

Retrospective study was conducted at Microbiology laboratory, District hospital attached

to Chamarajanagar Institute of Medical Sciences for duration of 1 year from July 2018 to June 2019.All staphylococcus species isolated from various clinical samples (pus, blood, urine) were included. Culture and sensitivity results were collected from Microbiology Laboratory registers. Isolates were processed and identified by standard Microbiological procedures (J.G. Collee *et al*., 14th edition). The antibiotic susceptibility testing was performed by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI 2019).

The following antibiotics were tested:penicillin (10 units), gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg), cotrimoxazole (25µg), chloramphenicol (30µg), tetracycline (30µg), erythromycin (15µg), clindamycin (2µg), linezolid (30µg), vancomycin (10µg). Resistance data were interpreted according to Clinical laboratory Standards Institute (CLSI, 2019).

Detection of MRSA and MR-CONS by 30µg Cefoxitin disc

Lawn cultures of each strain were done on Muller-Hinton agar. The inoculated plates were incubates at 35°C for 16-18 hours. Zone of inhibition was measured and interpreted according to CLSI guigelines: For MRSA susceptible, ≥ 22mm□ resistant, ≤ 21mm and for MR-CONSsusceptible, ≥25mm and resistant, ≤ 24mm.

Data analysis

Data analysis was done using MS Excel.

Ethical considerations

Ethical clearance was obtained from the Institutional Ethical clearance committee of Chamarajanagar Institute of medical sciences, Chamarajanagar.

Results

Of 161 samples processed, 107 were *Staphylococcus aureus* and 54 were found to be CONS. Among those 107 samples 48 (44.8) were found to be MRSA strains, among 54 CONS 26 (48.1%) were found to be MR-CONS strains.

Table 1 shows total number of Methicillin-resistant staphylococcal isolates. Table 2 shows distribution of MRSA and MR-CONS in different clinical samples and resistance were found to be high in pus 31(41.8%) samples followed by urine 29(39.1%) and blood 14 (18.9%).

Table 3 shows that MRSA strains showed higher sensitivity towards vancomycin (100%) followed by linezolid (87.5%), erythromycin (68.7%), clindamycin (66.6%), amikacin (66.6%), ciprofloxacin (64.5%), tetracycline (60.4%), chloramphenicol (56.2%), gentamycin (45.8%), cotrimoxazole (39.5%) and very least sensitive to penicillin(16.6%).

Table 4 shows MR-CONS strains showed higher sensitivity towards vancomycin (100%) and linezolid (100%) followed by clindamycin (80.7%), chloramphenicol (73%), amikacin (69.2%), tetracycline (65.3%), gentamycin (61.5%), cotrimoxazole (57.6%), erythromycin (38.4%), ciprofloxacin (23%) and very least sensitive to penicillin(7.6%).

Table 1. No. of Methicillin resistant staphylococcal isolates.

Organism	Methicillin Resistance No.(%)
Staphylococcus aureus(n= 107)	48 (29.8)
CONS (n =54)	26(16.1)
Total (n = 161)	74(45.9)

Table 2. Distribution of Methicillin-resistant staphylococcus species in different samples.

	URINE No.(%)	PUS No.(%)	BLOOD No.(%)
MRSA (n=48)	22 (29.7)	15 (20.2)	11 (14.8)
MR-CONS (n=26)	07 (9.4)	16 (21.6)	03 (4.05)
Total (n = 74)	29(39.1)	31(41.8)	14 (18.9)

Table 3. MRSA strains showing Resistance and Sensitivity to different antibiotics.

Antibiotics	Resistant No.(%)	Sensitive No.(%)
Penicillin	40 (83.3)	08 (16.6)
Gentamicin	26 (54.1)	22 (45.8)
Amikacin	16 (33.3)	32 (66.6)
Ciprofloxacin	17 (35.4)	31 (64.5)
Cotrimoxazole	29 (60.4)	19 (39.5)
Chloramphenicol	21 (43.7)	27 (56.2)
Tetracycline	19 (39.5)	29 (60.4)
Erythromycin	15 (31.2)	33 (68.7)
Clindamycin	16 (33.3)	32 (66.6)
Linezolid	06 (12.5)	42 (87.5)
Vancomycin	00 (0)	48 (100)

Table 4. MR-CONS strains showing Resistance and Sensitivity to different antibiotics.

Antibiotics	Resistant No.(%)	Sensitive No.(%)
Penicillin	24 (92.3)	02 (7.6)
Gentamicin	10 (38.4)	16 (61.5)
Amikacin	08 (30.7)	18 (69.2)
Ciprofloxacin	20 (76.9)	06 (23)
Cotrimoxazole	11 (42.3)	15 (57.6)
Chloramphenicol	07 (26.9)	19 (73)
Tetracycline	09 (34.6)	17 (65.3)
Erythromycin	16 (61.5)	10 (38.4)
Clindamycin	05 (19.2)	21 (80.7)
Linezolid	00 (0)	26 (100)
Vancomycin	00 (0)	26 (100)

Discussion

S.aureus has the ability to cause a wide range of diseases and capacity to adapt to diverse environmental forms, because of which it has a significant importance (Hafsatali Grema *et al*., 2015). MRSA causes both community-associated and hospital associated infections, hence considered as dangerous pathogen (INSAR group 2013). Among the causes of nosocomial infections worldwide, MRSA is the major cause causing 50% or more of hospital-acquired *S. aureus* infections. Community-associated MRSA causes infections in otherwise healthy people may have a serious or even fatal outcome (Nuno A. Faria *et al*., 2005).

Our study showed prevalence of MRSA is 44.8% which was in concordance with other studies (Shilpa Arora, *et al*., 2010 and Solmaz Dibah, *et al*., 2014) which showed 46% and 46.3% MRSA production respectively. Prevalence of MR-CONS showed 48.1% in our study which is similar with studies done by (Tekalighkejela, *et al*., 2013) which showed 52.2% MR-CONS production. Regarding sample distribution, Methicillin-resistant staphylococcus species were found to be high in pus (41.8%)samples followed by urine 29(39.1%)and blood 14 (18.9%) in our study which is concordance with other studies (Raghabendra Adhikari, *et al*., 2017 and Eyob Yohaness Garoy *et al*., 2019) which showed higher distribution in pus as 35.5% and 35.6% respectively. In present study vancomycin and linezolid showed higher sensitivity for Methicillin-resistant staphylococcus species which was found similar with studies done by Shilpa Arora, *et al*., (2010) and Solmaz Dibah *et al*., (2014).

Depending upon the knowledge of MRSA incidence in the patient location and evidence of patient colonization, the selection of an empiric agent for treatment of suspected MRSA can be done. Based on the presence of coexisting illness, prior treatment (including antibiotic therapy), and the duration of hospitalization, an empirical approach to the treatment of suspected nosocomial infection with possible MRSA can be given (A.S. Haddadin *et al*., 2002).

Conclusion

S aureus particularly MRSA causes community and hospital acquired infections and is a most common pathogen with increased morbidity and mortality. In our study the disc diffusion method by using cefoxitin disc appeared to be a simple and accurate method for the detection of methicillin resistance in *S. aureus*. For the treatment of MRSA infection, vancomycin and linezolid can be used. Good hand hygiene practices and strict adherence to infection control policies remain essential to control the spread of MRSA.

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Conflict of interest

The authors declare that there is no conflict of interest.

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