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Prevalence and antimicrobial susceptibility pattern of Methicillin-Resistant *Staphylococcus aureus* isolated from various clinical samples of the patients attending in a Tertiary Care Teaching Hospital in Puducherry

Vamsi Muni Krishna P*[1], Sreenivasulu Reddy V[2], Praveen Kumar V[1], Suresh P[1]

[1]Research Scholar, Bharath University, Agaram Road, Selaiyur, Chennai – 600073, Tamil Nadu, India
[2]Department of Microbiology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry - 605502, India

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ABSTRACT

Staphylococci spp has been reported as a major cause of hospital and community-associated infections. *Staphylococcus aureus* is the most common pathogen causing a variety of infections relatively begins minor skin in sections (abscess. cellulitis, staphylococcal scalded skin syndrome) to live threating systemic infections like endocarditis, septic arthritis, pneumonia joint, and bone infections, toxic shock syndrome. Methicillin resistance was reported in 1961 and emerged in the last several decades as one of the most important nosocomial pathogens which were reported just one year of the launch of methicillin. MRSA now a day a big problem is because it is creating life threating problems medical institutions. The knowledge of MRSA prevalence and current antibiogram profile is necessary for the selection of appropriate treatment for related infections. Isolation and identification of *Staphylococcus aureus* were done by standard conventional microbiological methods. The Methicillin-resistant *Staphylococcus aureus* strains were tested by using Cefoxitin 30µg disc on Mueller - Hinton agar and antibiotic susceptibility testing were done by Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Insititute guidelines (CLSI). All the 164 MRSA (100%) strains were sensitive to Tigecycline, Vancomycin, Teicoplanin followed by Linezolid (92. 68%). Tigecycline, Vancomycin, Teicoplanin has until now excellent activity against clinical isolates of Methicillin-resistant *Staphylococcus aureus.*

* Corresponding Author

Name: Vamsi Muni Krishna P
Phone: +91-7780512600
Email: omomom005@gmail.com

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INTRODUCTION

Staphylococcus was first observed in human pyogenic lesions by Von Recklinghausen in the year of 1871. Louis Pasteur in 1880 was first observed the liquid culture of cocci from pus produced abscess by inoculating them into rabbits. Sir Alexander Ogston, a Scottish surgeon in 1880 who established the causative role of the cocci in surgical abscesses and other suppurative lesions conclusively. Sir Alexander Ogston gave the name *Staphylococcus* (Staphyle in Greek meaning bunch of grape and Kokkos meaning a berry) due to the typical occurrence of the cocci in grape-like clusters in pus and in the culture medium. Sir Alexander Ogston had noticed that non-virulent staphylococci were also present on skin surfaces. (Ogston. A, 1882) Most of the staphylococcal strains from pyogenic lesions were found to produce golden-yellow colonies, and the strains from normal skin produce white–colonies. In 1884 Anton Julius Friedrich Rosenbach named two species of staphylococci based on their pigmentations and

colonies as *Staphylococcus aureus* (golden-yellow colonies) and *Staphylococcus Albus* (white–colonies). Later in 1885 J. Passet named a third species as *Staphylococcus citrus* (lemon - yellow colonies). (Lowy. FD, 1998).

Staphylococcus aureus (S. aureus) is Gram-positive cocci; it is a catalase–positive, coagulase-positive, facultative anaerobe, oxidase-negative, non-sporing, non-motile, and occasionally capsulated bacterium. It occurs in grape-like clusters when viewed through a microscope, and it is a large golden yellow colony with beta- haemolysis when it grew on blood agar media. (Ogston. A, 1884).

Staphylococcus aureus is responsible for a broad range of clinical infections, most commonly found in skin, axillae and nasal passage of humans. Now a day's *S. aureus* is having been reported as a common cause of serious infections in both hospitals and community worldwide. In 1940 and through 1950, *Staphylococcus. aureus* developed resistance to antibiotic penicillin. Methicillin, it is a form of penicillin, was introduced to counter the problem of penicillin-resistance *Staphylococcus. aureus*. Methicillin was one of the most common types of antibiotic used to treat *S. aureus* infection. This was the so-called birth of MRSA. (Chambers. HF., 2001; Priya Datta *et al.,* 2011). In 1961 Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported by British scientists, and 1968 first outbreak human case of MRSA reported in the USA (Ridley M. *et al*, 1970; Cafferkey. MT, *et al.,* 1985) MRSA developed resistant to an entire class of penicillin and penicillin-like antibiotics called beta-lactams. This class of antibiotics includes penicillin, methicillin, oxacillin, amoxicillin and others (Lakshmi R. *et al.,* 2014). MRSA grouped under is most common in healthcare-associated – MRSA (HA-MRSA), community-associated MRSA (CA-MRSA) and livestock-associated (LA-MRSA) infections were reported in the late 1980 and early 1990. (Naimi, TS. *et al.,* 2003) The main etiology of MRSA is the production of an altered penicillin-binding protein, a 78 kDa protein termed as PBP2a; it has a low affinity for beta-lactam antibiotics. Penicillin-binding protein 2a (PBP2a) is mediated by the *mecA* gene present in MRSA. (Sharon J. *et al.,* 2005). The objectives of the present our study aimed to the prevalence and Antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolated from various clinical samples of the patients attending in Sri Lakshmi Narayana Institute of Medical Sciences (SLIMS), Puducherry, India.

MATERIALS AND METHODS

It is a teaching hospital and laboratory-based study approved by Institutional Ethical clearance taken from Sri Lakshminarayana Institute of Medical Sciences, Pondicherry. Institutional Ethics committee (human studies) Ref. No. IEC/C-P/50/2014.

A total of 418 consecutive, clinically significant, non-repetitive clinical isolates of *Staphylococcus aureus* were isolated from various clinical specimens (Pus, Sputum, Blood, Eye swabs, catheter tip, Throat swabs, Wound swabs, urine, Body fluids and Ear swabs) received in the Microbiology Department, Sri Lakshmi Narayana Institute of Medical Sciences (SLIMS), Puducherry, during the period of January 2014 to December 2017 were included in this study. The samples all processed as per standard laboratory techniques procedures.

Specimen collection

All specimens were collected in sterile containers following aseptic measures and transported to the laboratory without delay and processed immediately. Each specimen was stained with Gram's staining, and findings are noted. All the specimens were cultured in nutrient agar, Mac Conkey's agar, 5% sheep blood agar and selective media including mannitol salt agar. All plates are incubated overnight at 37°C (Patricia M Tille, 2014).

Identification of *S. aureus*

Based on Gram' staining smears and Colony morphology observed these organisms having following characters consider to be *Staphylococcus aureus*: Gram-positive cocci in clusters, 2-4 mm in diameter, smooth, circular, opaque, with beta – haemolysis. Golden yellow colored colonies on nutrient agar were noted, and slide coagulase and tube coagulase were performed for differentiation of *S. Aureus* The biochemical tests like catalase, and coagulase, mannitol fermentation tests were performed and allocated to appropriate genera to the isolates. (Apurba Sankar Sastry *et al.,* 2016).

Identification of MRSA by Cefoxitin (30µg) disk diffusion method

All *Staphylococcus aureus* isolates were tested by using Cefoxitin disc diffusion using the 30µg disc on Muller Hinton agar media (Hi-Media Laboratories, Mumbai). One of the detective methods used for methicillin-resistance. The test strains were considered as sensitive if a zone was $\geq$22 mm and zone size was $\leq$21 mm was resistance based on Clinical Laboratory Standard Institute (CLSI) guidelines, previously National Committee for Clinical and Laboratory Standards (NCCLS). (CLSI-2013).

Antibiotic susceptibility by Kirby-Bauer disc diffusion method: Antibiotic sensitivity was tested using the on Mueller-Hinton agar according

Table 1: Distribution of Methicillin-resistant Staphylococcus aureus (MRSA) according to the Age wise

Characteristics Age (in years)	No. of MRSA isolates (n=164)	Percentage (100.00%)
1 -10	4	2.43%
11-20	18	10.97%
21-30	27	16.46%
31-40	24	14.63%
41-50	32	19.51%
51-60	37	22.56%
61-70	12	7.31%
71-80	8	4.87%
81-90	2	1.21%

Table 2: Gender wise distribution of Methicillin-resistant Staphylococcus aureus MRSA isolates: (n=164)

Gender	Total	MRSA isolates	
		No	Percentage
Male	263	96	58.53%
Female	155	68	41.47%
Total	418	164	100.00%

Table 3: Distribution of Methicillin-resistant Staphylococcus aureus (MRSA) from various clinical specimens (n=164)

Clinical specimens	No. of MRSA (n=164)	Percentage of MRSA (100.00)%
Pus	76	46.34%
Sputum	32	19.51%
Blood	6	3.66%
Eye swabs	3	1.83%
Catheter tip	1	0.60%
Throat swabs	8	4.88%
Wound swabs	19	11.59%
Urine	11	6.71%
Body fluids	5	3.05%
Ear swabs	3	1.83%

Table 4: Antibiotic susceptibility pattern of and Methicillin-resistant Staphylococcus aureus : (n=164)

Antibiotic	Resistance	Percentage%	Sensitivity	Percentage%
Penicillin (10units)	164	100%	0	0.00%
Erythromycin (15µg)	151	92.07%	13	7.92%
Cephalexin(30 µg)	147	89.63%	17	10.36%
Azithromycin(15µg)	139	84.75%	25	15.24%
Co-trimoxazole(25 µg)	119	72.56%	45	27.43%
Cefuroxime(30 µg)	92	56.09%	72	43.90%
Ciprofloxacin(10µg)	89	54.26%	75	45.73%
Gentamycin(10 µg)	87	53.04%	77	46.95%
Tetracycline(30µg)	78	47.56%	86	52.43%
Clindamycin(2 µg)	72	43.90%	92	56.09%
Amikacin(30µg)	40	24.39%	124	75.60%
Linezolid(30 µg)	12	7.31%	152	92.68%
Tigecycline(30µg)	0	0%	164	100%
Vancomycin (30µg)	0	0%	164	100%
Teicoplanin (30µg)	0	0%	164	100%

to antibiotic Clinical and Laboratory Standards Institute disc susceptibility testing guidelines (2013). Bacterial suspension equivalent to 0.5 McFarland was prepared by mixing 3-5 well-isolated colonies in 3-4ml of sterile physiological saline. Each suspension was inoculated on Muller Hinton agar (Hi-Media Laboratories, Mumbai) using a sterile cotton swab and antibiotic disks were applied and incubated aerobically at 37°C.

Antibiogram was determined for the following antibiotics are Penicillin (10units), Erythromycin (15µg), Cephalexin (30µg), Azithromycin (15µg),

Co-trimoxazole (25µg) Cefuroxime (30 µg), Ciprofloxacin (10µg), Gentamycin (10 µg), Tetracycline (30µg), Clindamycin (2 µg), Amikacin (30µg), Linezolid (30 µg), Tigecycline (30µg), Vancomycin (30µg), Teicoplanin (30µg). (Hi-Media Laboratories, Mumbai) (Bauer, AW. *et al.,* 1966).

RESULTS

A total 418 clinical isolates, 263 (62. 9%) patients were male, and 155 (37. 08%) were female patients. The male to female ratio in the present study was 2:1. Majority of the patients were the age of 51-60 years, as shown in Table- 1 and Table-2.

All Sample processed were Pus 76 (46.34%), Sputum 32 (19. 51%), Blood 6 (3.66%), Eye swabs 3 (1.83%), catheter tip 1 (0.60%), Throat swabs 8 (4.88%), Pus Wound swabs 19 (11.59%), urine 11 (6.71%), Body fluids 5 (3.05%) and Ear swabs 5 (1.83%) shown in Table-3.

DISCUSSION

In our present study, the prevalence of MRSA was found to be 39.23%, which is a similar study by khandle SK. *et al.,* 2003; who reported MRSA prevalence of 39. 10% hospital in Solapur (south Maharashtra) similar prevalence rate of MRSA was re ported from other research workers. Rajaduraipandi K *et al.,* 2006 (31.1%); Mehta AA *et al.,* 1996 (31.8%); Pantazatou *et al.,* 2003 (33. 3%); Merlino *et al.,* 2002 (34%); Lahari *et al.,* 2009 (34.78%); Nishi V *et al.,* 2006 (35%); Renata L. Pacheco *et al.,* 2011 (39%); ouri *et al.,* 2001 (41.45%); Tyagi *et al.,* 2008 (42%); anumanthappa *et al.,* 2003 (43%); and Anupurba *et al.,* 2003 (54. 85%).

Table 5: Comparision of MRSA prevalence with Indian studies

S. No.	Author and Year	Place	MRSA%
1	Chakravarthy *et al.,* 1988.	Delhi	6.8%
2	Pullimood TB *et al.,*1988.	Vellore	6.9%
3	Pal N *et al.,*1990.	Chandhigarh	22.8%
4	Madhur SK *et al.,*1994.	Delhi	32.8%
5	MRSA surveillance study group by Mehta *et al.,*1996.	Mumbai	26.6%
		Delhi	42.5%
		Banglore	47.1%
6	Verma *et al.,*2000.	Indore	80.89%
7	Majumder D *et al.,*2001.	assam	23.2%
8	Tahnkiwale *et al.,*2002.	Nagpur	19.56%
9	Anupurba *et al.,*2003.	Uttar Pradesh	54.85%
10	Khandle SK *et al.,*2003 .	Maharashtra	39.1%
11	Hanumanthappa *et al.,*2003.	Karnataka	43%
12	Mohanty *et al.,*2004.	Delhi	38.56%
13	Nishi V *et al.,*2004.	Mangalore	35%
14	Rajaduraipandi *et al.,* 2006.	Tamilnadu	31.1%
15	Dar JA *et al.,*2006.	Uttar Pradesh	54.85%
16	Tiwari HK *et al.,*2006.	Varanasi	40.61%
17	Mehta *et al.,* 2007.	Chandigarh	24%
18	Pai V *et al.,*2008.	Mangalore	29.7%
19	Tyagi A *et al.,*2008.	AIIMS, New Delhi	42%
20	Lahari S *et al.,*2009.	Assam	34.78%
21	Arora S *et al.,*2009.	Amritsar	46%
22	Anila *et al.,*2010.	Coimbatore	34%
23	Navneetkumar *et al.,* 2010.	Gujrat	16.27%,
24	T.Venu *et al.,*2011.	Hyderabad	79.6%
25	Minal Trivedi *et al.,* 2011.	Ahmedabad	20.25%
26	Shrikanth *et al.,* 2013.	Gulbarga	32.2%
27	Ankur Goyal *et al.,*2013.	Agra	32.6%
28	Nutanbala NG *et al.,* 2015.	Jamnagar	29.17%
29	Radhika *et al.,* 2016..	Vadodara	52%
30	Balamurali *et al.,*2016.	Visakhapatnam	34.2%
31	Pramodhini *et al.,* 2017.	Puducherry	33.3%
32	Rajesh TP *et al.,*2018.	Palakkad	42.9%
33	Surani *et al.,*2018.	Jamnagar	37.66%
34	The present study,2019.	Puducherry	39.23%

A higher prevalence rate as obtained by some other studies in their work like Borg M *et al.,* 2006 (65%); Dr. Kulkarni *et al.,* 2014 (70. 3%); T. venu *et al.,* 2011 (79. 6%) and Verma *et al.,* 2000 (80. 89%). The details of these findings given in Table-5.

A higher prevalence MRSA was isolated were from pus (46. 34%) followed by sputum (19. 5%) which correlates with Tyagi *et al.,* 2008 (44. 1%); Bala Murali *et al.,* 2016 (52%); Anupurba *et al.,* 2003 (53%); Dr. Kulkarni *et al.,* 2014 (64. 6%); Pai *et al.,* 2010 (74%). Whereas lower prevalence reported by Bilal Ahmed Mir *et al.,* 2013 (27. 5%); Tahnkiwal. SS *et al.,* 2002 (26. 92%); Ankur Kumar *et al.,* 2015 (21. 42%).

The drug resistance pattern of MRSA isolates from various clinical samples found to be variable. All the 164 MRSA (100%) strains were sensitive to Tigecycline, Vancomycin Teicoplanin followed by Linezolid (92. 68%). However, 164 MRSA (100%) strains were (100%) resistant to Penicillin (100%), Erythromycin (92.07%), Cephalexin (89. 63%), Azithromycin (84.75%), Co-trimoxazole (72.56%), Cefuroxime (56. 09%), Ciprofloxacin (54.26%), Gentamycin (53. 04%), Tetracycline (47. 56%), Clindamycin (43.90%) and Amikacin (24. 39%). These results are correlates with Rajesh TP. *et al.,* Palakkad, 2018; Surani, *et al.,* Jamnagar, 2018; Pramodhini *et al.,* Puducherry, 2017; Umadevi. S, Kurnool, 2017; Bala Murali *et al.,* Visakhapatnam, 2016; Radhika *et al.,* Vadodara, 2016; Maj puneeth Bhatt *et al.,* Pune, 2015; and Tiwari HK *et al.,* Varanasi, 2006.

CONCLUSION

In our present study shows that the MRSA is one of the nosocomial pathogens isolated from most of the clinical specimens like pus, Sputum, Blood, Eye swabs, catheter tip, Throat swabs, Wound swabs, urine, Body fluids and Ear Swabs. All isolates were sensitive to Tigecycline, Vancomycin; Teicoplanin hence remains the drug of choice for MRSA infections. This would help us to effective antibiotics and thus prevent the development of drug resistance, misusage of antibiotics.

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