SP-7

Antibiotic Resistance Pattern of Staphylococcus Aureus Causing Skin and Soft Tissue Infections

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Abstract: *Staphylococcus aureus*, though a normal commensal of our skin, the most predominant pathogen isolated from most cases of Skin and Soft Tissue Infections (SSTIs) both in the hospital and community settings. Methicillin-Resistant *Staphylococcus aureus* (MRSA) has become a major public health problem in hospitals and communities, accurate information about the changing trends of its resistance patterns is necessary for therapeutic decision making. *S. aureus* (n= 35) isolated from pus samples of patients with skin and soft tissue infections were included in the study. Kirby Bauer disc diffusion method was adopted to determine the antibiotic susceptibility pattern of the *S. aureus* isolates. Resistance to vancomycin was assessed by vancomycin (6 µg/mL) agar screen method. *mecA* mediated oxacillin (methicillin) resistance among the isolates were identified using cefoxitin disc (30 µg) as the surrogate marker (CLSI, 2020). D test was employed to determine inducible clindamycin resistance. Among the 35 Staphylococcal isolates tested, 17(48.9%) were found to be MRSA while, 18(51.4%) were MSSA ($p < 0.05$). None of the *S. aureus* (MRSA & MSSA) isolates exhibited resistance towards vancomycin. All the MRSA isolates were found to be resistant to penicillin. Amongst the MRSA isolates (n=17), 93.33%, 82.35% were susceptible to linezolid, clindamycin respectively. Nevertheless, only 85.71%, 77.7% of the MSSA isolates exhibited susceptibility to linezolid, clindamycin respectively. In our study, 5 isolates exhibited inducible clindamycin resistance. Lesser prevalence of linezolid resistance among MRSA and MSSA suggests that linezolid may be the drug of choice in the treatment of SSTIs caused by staphylococci.

Key words: *Staphylococcus aureus,* Antibiotic resistant, Skin and Soft Tissue Infections, MRSA.

INTRODUCTION:

Skin and soft tissue infections (SSTIs) is a commonly encountered infection worldwide that requires immediate medical attention that imposes a heavy burden in the health care settings. Among the various organisms causing SSTIs, *Staphylococcus aureus* is the most predominant pathogen isolated from most cases of SSTIs both in the hospital and community settings. *S. aureus* though a normal commensal of the external nares is also associated with a range of infections from mild soft tissue infections to severe infections such as septicaemia, pneumonia, infective endocarditis, deep-seated abscess, and toxic-shock syndrome.[1-2] The multi-drug resistance exhibited by the pathogen and its ability to evade the immune system accounts for the difficulty in implementing appropriate antibiotics for treatment.[3] *S. aureus* which was once susceptible to most of the antibiotics has now evolved resistance to most of the antibiotics. This negative transformation is likely to be attained due to its inherent virulence and its potential to habituate to these antibiotics to which it was once susceptible.Over the years, *S. aureus* has acquired resistance to a wide range of antimicrobial classes.[4] Out of the various classes of antibiotics to which the *S. aureus* species has attained resistance, Methicillin (MRSA) and Vancomycin resistance (VRSA and VISA) is the most significant. Methicillin resistance is attained when the methicillin-susceptible *S. aureus* (MSSA) strains acquires *mecA* gene by horizontal gene transfer from staphylococcal cassette chromosome(SCC) which is a mobile genetic element. These SCCs carrying the *mecA* gene (named as SCCmec) are incorporated in the chromosomes of MRSA strains. These SCCmec comprises of mec-gene complex which encodes the mecA gene which is regulated by the regulator genes namely mecR1, mecI and ccr-gene complex which encodes for cassette chromosome recombinase(CCR) that mediates the incorporation and excision of SCCmec into the MSSA strains.[5-6] Currently, Clinical laboratory Standards Institute (CLSI), recommends the use of cefoxitin (30ug) as the surrogate marker for the detection of Methicillin resistance in *Staphylococcus* species.[7] Also, the development of MDRSA (Multiple Drug Resistant *Staphylococcus aureus)* strains may be attributable to the over- use or inappropriate use of antibiotics, deficient investigation facilities and a lack of proper antibiotic policy in clinical practice. A variety of phenotypic variations do exist between MRSA strains isolated from the community (community acquired MRSA, CA-MRSA) and hospital set ups (Hospital acquired MRSA, HA-MRSA). The community acquired MRSA strains exhibit resistant to newer generation penicillins, oxacillin while they exhibit susceptibility to macrolides and lincomycins, erythromycin and clindamycin respectively. Nevertheless, nosocomial MRSA strains exhibit resistance towards multiple antimicrobial classes.[2] Regardless of the boundless initiatives taken by the infection control committees all over the world, there is a substantial increase in the number of MRSA.Various efforts that have been adopted to control the emergence of resistance among *S. aureus* is often hindered by - increased virulence, escalating treatment cost, high budget options available commercially. Thus, timely investigations of the resistance pattern of *S. aureus* species is necessary.[1] The emergence of rapid resistance of *S.aureus* to most of the antibiotics pose a challenge to the treatment options used for the same. Thus, to avoid any treatment failures care must be taken in selection of antibiotics for treating *S.aureus* infections.[5] The present study was designed to determine the antimicrobial

susceptibility pattern of the *Staphylococci* isolated from pus samples and to assess the prevalence of MRSA and inducible clindamycin resistance.

MATERIAL AND METHODS:

A total of 35 *Staphylococcus aureus* isolated from pus samples of patients with skin and soft tissue infections attending a tertiary care Hospital in Chennai were included in the study. Identification of the Staphylococcal species was carried using standard microbiological techniques *viz.,* Gram staining, catalase, oxidase, Coagulase test- (slide & tube method), and further confirmed by plating on Mannitol salt agar (HiMedia laboratories Pvt Ltd, India). Kirby Bauer disc diffusion method was adopted to screen for the susceptibility the *S. aureus* isolates to following antibiotics, ciprofloxacin (5 mcg), chloramphenicol (30 mcg), co-trimoxazole (25 mcg), tetracycline (30 mcg), erythromycin (15 mcg), Teicoplanin (30mcg), Linezolid (30mcg) by as per CLSI guidelines, 2020.[7] Resistance to vancomycin was assessed by vancomycin (6 µg/mL) agar screen method. *mecA* mediated oxacillin (methicillin) resistance among the isolates were identified using cefoxitin disc (30 µg) as the surrogate marker (CLSI, 2020). Isolates with the zone of inhibition ≤ 21mm were classified as MRSA and those which exhibited a zone ≥ 22 was considered as MSSA strains[7]. Inducible clindamycin resistance was determined by Disc approximation test (D test)- clindamycin (2 µg/disc) and erythromycin (15 µg/disc) discs (HiMedia laboratories Pvt Ltd, India) were placed 15 mm apart on Muller Agar plates and were incubated at 37°C for 18 hrs. A D-type flattening of the zone of inhibition of clindamycin zone towards the erythromycin disc was scored as inducible clindamycin resistance, D-test positivity. *S. aureus* ATCC 25923 was included as the standard control.[7]

RESULT:

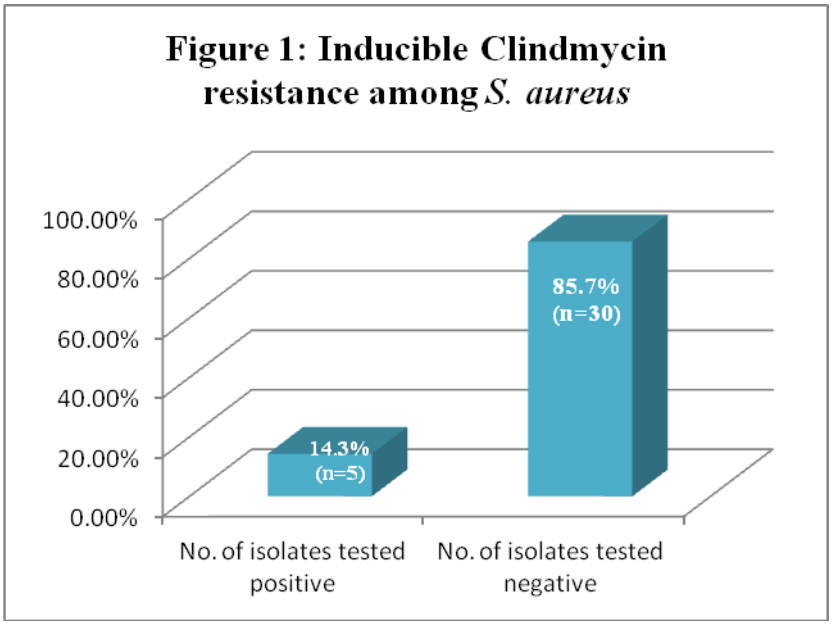
The methicillin resistance in the Staphylococcal species was then determined by performing antibiotic susceptibility testing using cefoxitin as the surrogate marker using Kirby-Bauer disc diffusion method. Among the 35 Staphylococcal isolates tested, 17(48.9%) were found to be methicillin resistant while 18(51.4%) were found to be methicillin susceptible ($p < 0.05$). None of the *S. aureus* (MRSA& MSSA) isolates exhibited resistance towards vancomycin. All the MRSA isolates were found to resistant to penicillin. Amongst the MRSA isolates(n=17), 93.33%,82.35% were susceptible to linezolid, clindamycin respectively. Nevertheless, only 85.71%, 77.7% of the MSSA isolates exhibited susceptibility to linezolid, clindamycin respectively. Of the 17 MRSA isolates tested, the susceptibility to the antimicrobials tested is as follows, tetracycline (61.54%), co-trimoxazole (56.25%), teicoplanin (33.3%) and tigecycline (18.75%) (Table 1). In contrast, higher rate of susceptibility was exhibited by the MSSA isolates (n=18) towards clindamycin (83.3%), tetracycline (85.7%), co-trimoxazole (75.0%), teicoplanin (57.14%) and tigecycline (5.5%) (Table 2). Inducible clindamycin resistance was exhibited by 5(29.4%) isolates, all of which were MRSA stains (Figure 1).

Table 1: Antibiotic susceptibility pattern of the MRSA isolates

MRSA isolates	**Resistance Pattern**		
	Sensitive	**Intermediate**	**Resistant**
Penicillin	-	-	100%
Linezolid	93.33%	-	6.67%
Clindamycin	82.35%	11.77%	5.88%
Co-trimoxazole	56.25%	18.75%	25.00%
Tetracycline	61.54%	15.38%	23.08%
Teicoplanin	33.33%	53.34%	13.33%
Ciprofloxacin	13.33%	6.67%	80.00%
Erythromycin	-	42.10%	57.90%
Tigecycline	18.75%	-	81.25%
Vancomycin	100%	-	-

Table 2: Antibiotic susceptibility pattern of the MSSA isolates.

MSSA isolates	**Antibiotic susceptibility Pattern**		
	Sensitive	**Intermediate**	**Resistant**
Penicillin	11.11%	-	88.9%
Linezolid	85.70%	-	14.30%
Clindamycin	77.78%	-	22.20%
Co-trimoxazole	75.00%	16.67%	8.33%
Tetracycline	85.70%	-	14.30%
Teicoplanin	57.14%	35.72%	7.14%
Ciprofloxacin	25.00%	-	75.00%
Erythromycin	47.06%	11.76%	41.18%
Tigecycline	5.56%	-	94.44%
Vancomycin	100%	-	-



DISCUSSION:

S. aureus is the most predominant pathogen causing pyogenic infections. Traditionally, MRSA has been recognized as a virulent nosocomial pathogen However, community-associated strains of MRSA (CA-MRSA) have emerged over the past several years among healthy young patients without significant health-care contact.[8] The clinical spectrum of CA-MRSA associated SSTIs includes furuncles, carbuncles and abscesses. CA-MRSA, however owing to the presence of unique virulence factors may cause invasive infections including, potentially lethal necrotizing pneumonia. CA-MRSA carry a distinct molecular makeup and lack the MDR genes harbored by HA-MRSA. CDC suggests that MRSA need to be considered in the differential diagnosis of SSTIs, especially those that are purulent (fluctuant or palpable fluid-filled cavity, yellow or white centered, central point or "head," draining pus, or possible to aspirate pus).[9] MRSA is a significant cause of morbidity and mortality. Prompt diagnosis and effective treatment of MRSA skin infections is essential as SSTIs if not treated may lead to more serious infections. Also, follow-up of patients with MRSA SSTIs is recommended by CDC especially if symptoms do not improve within 2 days / worsening of local symptoms/ if the patient develops systemic complications.[9] In our study, 48.6% of the *S. aureus* isolates were found to be MRSA while 51.4% were MSSA. This is line with the report various Indian reports.[10-13] MRSA exhibits resistance to all the currently available beta-lactam agents, penicillins and cephalosporins. Also, fluoroquinolones (e.g., ciprofloxacin, levofloxacin) and macrolides (erythromycin, clarithromycin, azithromycin) are not recommended for therapeutic management of MRSA SSTIs as resistance to these antibiotics is common or may develop rapidly. Nevertheless, clindamycin has been approved by the FDA to treat serious *S. aureus* infections. The centre for Disease Control (CDC) emphasizes that for the erythromycin-resistant isolates, D-zone test need to be performed to identify inducible clindamycin resistance. In our study, 14.3% of the *S. aureus* isolates exhibited inducible clindamycin resistance. This in line with an Iranian report.[14] Nevertheless, an Indian report by Venkata et al, has documented a very high (75.27%) prevalence of MRSA in 2012.[15] A seven year analysis (2009-2015) by Jakribettu *et al* had revealed a steady increase in resistance rate of *S. aureus* to cefoxitin, erythromycin, amikacin, levofloxacin, ciprofloxacin (62.17%) and clindamycin (17.8%).[1] In the present study we report a slightly higher incidence of cefoxitin, ciprofloxacin and clindamycin resistance respectively (48.6%, 80% and 20%). A recent report by Singh et al., which documented a higher resistance of 82. 3% in Tamil Nadu, in the contrary, we report only 20% of the *S. aureus* isolates to be resistant to clindamycin.[16] Linezolid has been approved by the FDA for the treatment of complicated skin infections, including those caused by MRSA (CDC). In our study, 93.3% of the MRSA isolates were susceptible to linezolid. However, administration of linezolid needs to be done with caution. Consultation with an infectious disease specialist is ideal as prolonged therapy linezolid may be associated with myelosuppression, neuropathy and lactic acidosis. But more clinical studies need to be done to evaluate clinical outcome in the patients after treatment with these antibiotics.

ETHICAL CLEARANCE:

This study protocol was reviewed and approved by the institutional ethical committee, Rajas Dental College &Hospital, Kavalkinaru, Tirunelveli district, South India. (EC No: RDCH/EC/09/2018)

CONCLUSION:

In our study a higher incidence (48.6%) of MRSA is documented among SSTIs. Also, we report a slightly higher incidence of cefoxitin (48.6%), ciprofloxacin (80%) and clindamycin (20%) resistance. Higher linezolid susceptibility rate (93.3%) among *S. aureus* isolates in our study, suggests linezolid to the drug of choice in the treatment of skin and soft tissue infections caused by MRSA.

CONFLICT OF INTEREST

Conflict of interest declared none.

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