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Comparative Study Of Inducible And Constitutive Clindamycin ResistanceAmong Methicillin Resistant *Staphylococcus aureus* Isolates.

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ABSTRACT

Staphylococcus aureus is the commonly encountered pathogen isolated from clinical specimens. Methicillin Resistant *Staphylococcus aureus* (MRSA)causes variety of human infections resulting in high rate of mortality and morbidity. Clindamycin, lincosamide antibiotic is a good option for clinicians to treat MRSA infections. The aim of the study was to screen for MRSA by disc diffusion method with cefoxitin and oxacillin discs and to determine the prevalence of inducible clindamycin resistance and constitutive clindamycin resistance in MRSA and compare them 200 *Staphylococcus aureus* were isolated from samples like pus, blood, sputum, vaginal swab, urine and body fluids received in microbiology department of Government Cuddalore Medical College, Chidambaram, Cuddalore, Tamil Nadu, India .They were confirmed by microscopy, culture and biochemical reaction. Then MRSA were detected by disc diffusion test using Cefoxitin (30µg) and Oxacillin(1µg) discs. Clindamycin resistance were detected by performing D-test by placing erythromycin 15µg and clindamycin 2µg discs at 15-20mm interdisc distance. Majority of *Staphylococcus aureus* were isolated from pus samples (87.5%). *Staphylococcus aureus* was highly sensitive to linezolid (100%) and vancomycin (99%) and 100% resistant to penicillin. Cefoxitin disc detected higher percentage (26%) of MRSA than oxacillin disc (24%). Analysis of clindamycin resistance in 52 (26%) MRSA isolates showed 42.30% of inducible clindamycin resistance, 30.76 % of constitutive clindamycin resistance and 26.92% were sensitive to both erythromycin and clindamycin. Detection of MRSA is very important for treating patients and to prevent its spread. MRSA isolates exhibiting inducible clindamycin resistance are seemed to be susceptible to clindamycin in vitro but resistant invivo resulting in treatment failure. So 'D'test is suggested along with routine antibiotic susceptibility testing to detect inducible clindamycin resistance.

Keywords: *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus*, Community Acquired Methicillin Resistant *Staphylococcus aureus.*

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INTRODUCTION

Staphylococcus aureus is the most frequently encountered pathogen isolated from clinical specimens. *Staphylococcus aureus* has the ability to asymptomatically colonize the normal population either persistently or transiently. 30% of humans are likely to be nasal carriers. Person to person contact or contact with fomites plays a role in its transmission [1]. Loss of normal skin barrier & presence of predisposing factors such as diabetes and HIV complicates infection. *Staphylococcus aureus* causes variety of human infections ranging from minor skin diseases such as furuncles, cellulitis, abscesses to life threatening infections like toxic shock syndrome, staphylococcal scalded skin syndrome, endocarditis, pneumonia & septicemia. Penicillin was the drug of choice to which *Staphylococcus aureus* developed resistance by producing the enzyme betalactamase [2]. Betalactam agents bind to PBP in cell wall of *Staphylococcus aureus* resulting in disruption of peptidoglycan synthesis & bacterial cell death. The mecA gene coding for PBP2A in cell wall of MRSA harbored by mobile SCCmec chromosome is responsible for methicillin resistance.CA-MRSA possess a small SCCmectype IV, V, or VII, which is transferred easily by transduction than the larger SCCmec types I, II, and III in HA-MRSA Detection of MRSA can be performed by an oxacillin or cefoxitin disc diffusion test. Cefoxitin is a strong inducer of mec A gene and thus helps in detection of MRSA. Alternatively the macrolide- lincosamide streptogramin B group of antibiotics can be used for treating MRSA infection. Clindamycin, a lincosamide antibiotic has become an attractive option for clinicians because of its bioavailability both in oral & intravenous formulations.[3]It has excellent tissue penetration.It is the treatment of option in individuals with penicillin allergy and renal impairement. Clindamycin has been used to treat pneumonia, soft-tissue and musculoskeletal infections due to MRSA. It can be used both in adults and children [3]. However, fear of appearance of clindamycin resistance during therapy has discouraged some clinicians prescribing it. The mechanism of inducible clindamycin resistance ($iMLS_B$) is due to target site modification mediated by erm gene which can be expressed by an inducer like erythromycin or constitutively ($cMLS_B$) [4]. The overlapping binding sites of macrolides, lincosamides, and streptogramins B in 23S rRNA accounts for the cross resistance to the 3 classes of drugs. The D-test is performed by placing clindamycin and erythromycin discs at an edge-to-edge distance of 15 to 20mm and looking for flattening of the clindamycin zone nearest the erythromycin disc. If D- test is positive it suggests the presence of an erm gene that could result in clindamycin resistance [5]. Strains with inducible clindamycin resistance are difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive in vitro when not placed adjacent to each other. In such cases, in vivo therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. But mutations in the promoter region of erm gene allows the production of methylase without an inducer [6]. These mutants are stably erythromycin and clindamycin resistant (Constitutive resistance). MRSA constitute a major health care problem with a strong potential for dissemination and high rate of mortality and morbidity. So the availability of sensitive and specific methods for detecting antibiotic resistance in these pathogens accurately has become a significant tool in clinical diagnosis [7]. In PCR by amplification of the mecA gene, MRSA is detected. PCR is highly, sensitive, and specific. But it requires advanced equipment's & moreover it is costly. So, it is not possible for routine testing in clinical laboratories. Incidence of clindamycin resistance in MRSA isolates varies widely by hospital and geographic region [8]. Errors in the detection of methicillin resistance can have serious adverse clinical consequences. False susceptibility results may result in treatment failure and the spread of MRSA if appropriate infection control measures are not applied. Conversely, false resistance results may increase healthcare cost following unnecessary isolation precautions and may lead to overuse of glycopeptides [9,10].

METHODS

Total of 200 *Staphylococcus aureus* isolates from clinical samples including, pus, sputum, blood, vaginal swab and urine were included in the study. Samples were received from outpatients and in patients who attended microbiology department of Government Cuddalore Medical College, Chidambaram, Cuddalore, Tamil Nadu, India. Processing of samples. The received samples were checked for proper labelling with Name, Age, Sex and I.P/ O.P No. of the patient, date and time ofcollection of the sample and processed immediately. Direct smears were prepared from sample material like pus, sputum, urine and vaginal swab on a clean glass slide. Gram staining was done and examined under microscope. The findings were recorded. Blood samples sent in brain heart infusion broths were incubated for18 - 24hours and then sub cultured. All the above specimens were inoculated on to the nutrient agar plate, blood agar, and MacConkey agar, and incubated at 37º C for 18-24 hours aerobically and observed after incubation. All the suspected colonies were identified by colony morphology, gram staining was done and

the organism subjected to various biochemical tests to identify and characterize them. Further confirmation was done by slide and tube coagulase test, and growth on Mannitol Salt Agar.

RESULTS

The study included 200 *Staphylococcus aureus* isolates from samples like pus, blood, sputum, vaginal swab, urine and body fluids. Among 200 *Staphylococcus aureus* isolates the sample wise distribution was as follows. Pus constituted 175 (87.5%), urine 10 (5%), blood 6 (3%), sputum 4 (2%), vaginal swab 3 (1.5%) and synovial fluid 2 (1%), as given in Table1 .The above observation shows that *Staphylococcus aureus* was isolated maximally from pus Samples (87.5%) and only few were isolatedfrom urine, blood, sputum, vaginal swab and other body fluids. The resistant and sensitivity pattern of *Staphylococcus aureus* isolates to different antibiotic groups is given in Table2 .Out of the 200 *Staphylococcus aureus* isolates100% were sensitive to linezolid and 99% were sensitive to vancomycin.77 % were sensitive to amikacin, 73%were sensitive to doxycycline, 69% were sensitive to cotrimoxazole, 68.5 % were sensitive to cephalexin, 66.5 % were sensitive to amoxy clavulanic acid, 64 % were sensitive to cefotaxime, 59 % were sensitive to ciprofloxacin. *Staphylococcus aureus* strains were highly sensitive to linezolid and vancomycin. Moderate level sensitivity was seen in amikacin, doxycycline, cotrimoxazole, cephalexin, amoxy clavulanic acid, cefotaxime and ciprofloxacin. Table 2 lists the resistance pattern of *Staphylococcus aureus* isolates. Out of the 200 isolates 100% were resistant to penicillin G, 33.5% were resistant to ciprofloxacin, 33.5 % were resistant to amoxy clavulanic acid, 27.5 % were resistant to cephalexin, 27.5% were resistant to cotrimoxazole, 26.5 % were resistant to cefotaxime, 24.5% were resistant to doxycycline, 20.5 % were resistant to amikacin and 1% were resistant to vancomycin.

Staphylococcus aureus isolates were 100% resistant to penicillin and 100% sensitive to linezolid. Moderate level of resistance was seen toamikacin, ciprofloxacin, doxycycline, co-trimoxazole, cephalexin, cefotaxime and amoxy clavulanicacid. Very minimal resistance was noted in vancomycin. As evident from Table 3 among 200 isolates of *Staphylococcus aureus*, 26% were resistant and 74 % were sensitive to cefoxitin whereas 24% were found to be resistant and 76% were sensitive to oxacillin as determined by disc diffusion method. Cefoxitin disc detected higher percentage of methicillin resistant *Staphylococcus aureus* by disc diffusion method. Among 200 *Staphylococcus aureus* isolates74% MSSA and 26% MRSA were observed as given in Table 4 .Age wise distribution as given in Table : 5 shows out of 200 S.aureus isolates taken for study, 15.50% between 1-12 years, 9.50% between 13-20 years, 42.50% between 21-40 years, 19.50% between 41-60 years and 13% more than 60 years of age. Out of 52 MRSA isolates 9.61% were between 1-12 years, 11.54% were between 13-20 years, 51.92% were between 21-40 years, 15.38% were between 41-60 years, and 11.54% were more than 60 years of age. From this it is inferred that maximum *Staphylococcus aureus* and MRSA isolates were from the age group between 21-40 years followedby 41-60 years age. Among 52 MRSA isolates sex ratio was found to be 65.38 % Males and 34.61 % Females. This is given in Table 6 indicating predominance of MRSA among males. out of 200 Staphylococcal isolates 40 % were isolated from wound infection, 9 % from cutaneous ulcer, 8 % from abscess, 7.5 % from cellulitis, 7.5 % from suppurative otitis media,6 % from pyoderma, 5% from urinary tract infection 4% from osteomyelitis, 3% from burns, 3% from septicemia, 2% from pneumonia 1.5 % from gangrene, 1.5 % from vaginal infection 1% from necrotizing fasciitis, and 1% from septic arthritis. MRSA were isolated from 44.23 % of wound infection, 11.54 % ofcutaneous ulcer, 9.62% of abscess, 7.69 % of cellulitis, 7.69 % of pyoderma , 5.77 % of osteomyelitis, and 3.85 % of urinary tract infection. Burns, septicemia, gangrene, necrotizing fasciitis, and suppurative otitis media cases constituted 1.92% of MRSA each. It is inferred from the above data that wound infections constituted higher percentage of MRSA. Analysis of clindamycin Resistance in 52 MRSA isolates showed 42.30% of inducible clindamycin Resistance, 30.76 % of constitutive clindamycin Resistance, and 26.92% were sensitive to both erythromycin and clindamycin. MS phenotype was not observed as given in Table 8 .Above observation shows that, inducible clindamycin resistance was reported in a higher percentage than constitutive clindamycin resistance.

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Table 1: Frequency Of *Staphylococcus aureus* Isolates Indifferent Specimens

Samples	Total no of S. aureus isolates	Percentage
Pus	175	87.5%
Urine	10	5%
Blood	6	3%
Sputum	4	2%
Vaginal swab	3	1.5%
Synovial fluid	2	1%

Table 2: Antibiotic Sensitivity Pattern Of *Staphylococcus aureus*

Drugs	Sensitive	Intermediate sensitive	Resistant
Linezolid	200 (100%)	-	-
Vancomycin	198 (99%)	-	2 (1%)
Amikacin	154 (77 %)	5 (2.5 %)	41 (20.5%)
Doxycycline	146 (73%)	5 (2.5%)	49 (24.5%)
Cotrimoxazole	138 (69%)	7 (3.5%)	55 (27.5%)
Cephalexin	137 (68.5%)	8 (4%)	55 (27.5%)
Amoxy clavulanic acid	133 (66.5%)	-	67 (33.5%)
Cefotaxime	128 (64%)	19 (9.5%)	53 (26.5%)
Ciprofloxacin	118 (59%)	15 (7.5%)	67 (33.5%)
penicillin G	-	-	200 (100 %)

Table 3: Detection Of Methicillin Resistance By Disc Diffusion Test Using Oxacillin And Cefoxitin Discs

Disc diffusion test	Cefoxitin(30µg) disc	Oxacillin (1µg)disc
Resistant	52 (26 %)	48 (24 %)
Sensitive	148 (74 %)	152 (76%)

Table 4: Prevalence Of MRSA Among *Staphylococcus aureus* Isolates

Total isolates	MRSA	MSSA
200	52 (26%)	148 (74%)

TABLE -5 AGE WISE DISTRIBUTION OF MRSA

Age in years	Total no of S. aureus Isolates (200)	MRSA (52)
1-12	31 (15.50%)	5 (9.61%)
13-20	19 (9.50%)	6 (11.54%)
21-40	85 (42.50%)	27 (51.92%)
41-60	39 (19.50%)	8 (15.38%)
> 60	26 (13.0%)	6 (11.54%)

Table 6: Gender Distribution Of MRSA

Sex	Total (200)	MRSA (52)
Male	120	34 (65.38 %)
Female	80	18 (34.61%)

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Table 7: Distribution Of MRSA Among Various Infections

Diseases	Total (200)	MRSA (52)
Wound infection	80 (40 %)	23 (44.23 %)
Cutaneous ulcer	18 (9 %)	6 (11.54 %)
Abscess	16 (8 %)	5 (9.62%)
Cellulitis	15 (7.5 %)	4 (7.69 %)
Pyoderma	12 (6 %)	4 (7.69 %)
Osteomyelitis	8 (4%)	3 (5.77 %)
Urinary tract infection	10 (5%)	2 (3.85 %)
Suppurative otitis media	15 (7.5 %)	1 (1.92%)
Burns	6 (3%)	1 (1.92%)
Septicemia	6 (3%)	1 (1.92%)
Gangrene	3 (1.5 %)	1 (1.92%)
Necrotizing fascitis	2 (1%)	1 (1.92%)
Pneumonia	4 (2%)	0 (0%)
Vaginal infection	3 (1.5%)	0 (0%)
Septic arthritis	2 (1%)	0 (0%)

Table 8: Clindamycin Resistant phenotypes of MRSA by D-test

Susceptibility pattern (phenotype)	MRSA (52)	Percentage(26%)
ERY R, CLI-S (D -Test positive; $iMLS_B$)	22	42.30%
ERY-R, CLI-R ($cMLS_B$)	16	30.76%
ERY-S, CLI-S (S - Phenotype)	14	26.92%
ERYR, CLI-S (D –Test negative;MSPhenotype)	Nil	0 %

DISCUSSION

MRSA is a major cause of hospital and community acquired infections. Clindamycin is an excellent drug to treat not only serious infections like sepsis, endocarditis, osteomyelitis, pneumonia, and staphylococcal scalded skin syndrome caused by MRSA but also MSSA [11]. It is less expensive compared to newer antibiotics. As it can be given orally it can be used in outpatient therapy. Drugslike tetracyclines and fluroquinolones are not advised for treating childrenand pregnant women due to side effects. But clindamycin is a treatmentof option in children and it can also be used in penicillin allergic individual [12].

It is very necessary to distinguish between staphylococci having inducible clindamycin resistance from those with MS Phenotype.Because MS Phenotype in staphylococcal strains does not result in failure of therapy, whereas it occurs in inducible clindamycin resistance· D test is a simple, reliable and significant test. Sensitivity of D test performed at 15mm disk spacing is 100% corelated with detection of erm genes by polymerase chain reaction [13]. *Staphylococcus aureus* has emerged as a major cause of nosocomial infections for quite some time. Clindamycin is a very useful drug in treating skin and soft tissue infections. It can be used in penicillin allergic individual. It is a promising therapeutic option in the era of drug resistance. The costly antibiotics like vancomycin can be reserved for severe illness [14].

In our study higher incidence of inducible clindamycin resistance was detected among isolates derived from outpatients (community acquired) as compared to inpatients or hospital acquired (58.40% and 41.60% respectively). This judgment was parallel to another study which also reported higher incidence of inducible clindamycin resistance from community (66.67%) than from hospital (33.33%) [15]. This may be outstanding to the fact that clindamycin being an oral drug has been increasingly prescribed by the physicians in outdoor clinical settings, thus leading to increased incidence of community-acquired inducible clindamycin resistance. In our study we also looked forward for handling options for inducible clindamycin resistant S. aureus isolates by detecting their antimicrobial susceptibility to a variety of other antibiotics [16].

The erythromycin resistant Staphylococcal isolates will be misidentified as clindamycin sensitive if D test is not performed. To avoid prescribing clindamycin to those who exhibit inducible clindamycin

resistance, D test must be done routinely. Giving false report that patient is infected with MRSA will lead to fatal consequences due to inadequate therapy, whereas wrongly labelling the patient infected with MSSA as MRSA will lead to unwanted usage of costly drugs like vancomycin [17-20].

CONCLUSION

In our study we also looked presumptuous for management options for inducible clindamycin resistant S. aureus isolates by detecting their antimicrobial susceptibility to a variety of other antibiotics. It was initiate that all isolates with iMLSB phenotype were 100% susceptible to linezolid and vancomycin, followed by reasonable susceptibility (71,14%) to gentamicin, cefuroxime and smallest amount susceptibility to doxycycline, ciprofloxacin (23.81% and 20.95% respectively). This judgment is in concordance to previous studies that also found that all the iMLSB isolates were homogeneously susceptible to linezolid and vancomycin

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