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PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN A TERTIARY CARE HOSPITAL AND SIGNIFICANCE OF LINCOSAMIDE SUSCEPTIBILITY IN MACROLIDE RESISTANT MRSA.

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Abstract: **Context:** Methicillin resistant *Staphylococcus aureus* (MRSA), specifically multiple drug resistant MRSA, are a real threat to the clinician and therapeutic option for these isolates has to be worked out. **Objective:** The objective of the present study was to assess the prevalence of MRSA in our hospital and further to identify inducible resistance to macrolides (erythromycin) among these MRSA, for therapeutic purpose. **Setting and Design:** This study was conducted over a period of three years. The clinical samples received from the patients admitted in various ICU's and wards of Dayan and Medical College & Hospital, Ludhiana, were included in the study. **Material and Method:** MRSA were detected using 1 µg disc of oxacallin. Two hundred thirty five MRSA which were erythromycin (macrolide) non susceptible but clindamycin susceptible, were tested using 15 mm disc approximation test (D-test) to evaluate erythromycin inducible (*erm*mediated) and non-inducible (*msrA* mediated) resistance. **Results:** prevalence of MRSA was found to be Out of 235 MRSA tested using D test, 51/235 (21.8%) showed negative D test and resistant to erythromycin in these isolate may be mediated by *msrA* genes. **Conclusion:** MRSA isolates with negative D-test will not develop resistance during the treatment with lincosamides. Patients with infection caused by multiple drug resistance MRSA with *msrA* genes may be treated with lincosamides without any fear of developing resistance and clindamycin may be kept as a reserve drug in severe MRSA infections or in cases where the patient is known to have hypersensitivity to β-lactam drugs, depending upon the results of the D-test.

Keywords: MRSA, multi drug resistance, lincosamide, macrolides, D-test

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) infections are a major cause of morbidity and mortality, particularly in hospitalized patients.[1, 2] Nosocomial strains of *S. aureus* in sharp contrast to community acquired strains, exhibit high rate of resistance to antibiotics and are frequently multi-drug resistant.[3] Multidrug resistant *S. aureus* isolates, esp. methicillin resistant strains (MRSA) are high priority for hospital epidemiologist, since these strains are virulent and much more difficult and expensive to treat.[4,5] It is difficult to identify the factors which contribute to the persistence of MRSA carriage but increasing number of patients at risk of acquisition, inadequate isolation facilities, inadequate antibiotic and admission policy and problems in identifying the source of outbreak are some of the major problems faced while controlling MRSA in the tertiary care hospital environment.[6] Accurate laboratory identification of methicillin resistance is therefore a paramount importance. Optimal therapy for MRSA infection has yet to be determined, but this matter is further complicated by macrolide-lincosamide-streptogramin B resistance (MLSB) either inducible or constitutive. However resistance due to active efflux encoded by *msrA* genes is also found in *Staphylococcal aureus*(MS phenotype), which results into resistance to macrolides and streptogramin B antibiotis, but not lincosamides and clindamycin is active against such isolates. Hence, clindamycin can be used in controlling few erythromycin and methicillin resistant *S. aureus*strains (MS phenotype) with *msr-A* gene.[7,8,9]

The objective of the present study was to assess the prevalence of MRSA in various wards and ICU of DMC&H, Ludhiana. Further to identify *msrA* mediated resistance to hmacrolides (erythromycin) among these MRSA for therapeutic purpose.

MATERIALS AND METHODS

This study was conducted over a period of three years. The clinical samples like blood, urine, pus and swabs (conjunctival swab, throat swab, wound swab, tracheostomy, vaginal swab), sputum, endotracheal secretions and catheter tips etc. which were received from the patients admitted in various ICU's and wards of Dayanand Medical College & Hospital, Ludhiana, were included in the study.

Isolation and Identification of *S. aureus*: Standard procedures were followed for the isolation and identification of *S. aureus* from clinical specimens[10] i.e. Gram staining, catalase, slide & tube coagulase and mannitol fermentation tests.

Antimicrobial Sensitivity Testing: Isolates were tested for susceptibility to antimicrobial agents by disc diffusion test according to CLSI guidelines.[11] *S. aureus* ATCC 25921 was used for quality control.

Screening for MRSA: MRSA screening was done using 1µg of oxacillin disc.[11]

D- Test (Phenotypic Testing): All the MRSA which were resistant to erythromycin and sensitive to clindamycin were tested by disc approximation test according to CLSI guidelines. A clindamycin disc (2µg) and erythromycin disc (15µg) were placed on inoculated Mueller – Hinton agar plate at a distance of 15 mm apart. Plates were incubated at 35˚C for 16-18h. Test showing flattening of the clindamycin zone adjacent to erythromycin disc were classified as D-test positive while those with circular zone were classified as D-test negative.

RESULT

During the study period a total of 16445 clinical samples were processed, out of which growth of *Staphylococcus aureus* was obtained in1551 samples excluding duplicate isolates from the same patient. Out of these 341 (21.9%) were identified as MRSA isolates. Figure1 shows the distribution of these MRSA. A total of 235 MRSA were erythromycin resistant but clindamycin susceptible, were included in the further studies. No clindamycin resistant and erythromycin sensitive isolates were found. Figure 2 shows the antimicrobial susceptibility of MRSA against the various antibiotics tested. The resistance of these MRSA towards other antibiotic tested varies between 0 - 86%. All the isolates were found susceptible to vancomycin and linezolid.184/235 (78.2%) isolates showed blunting of the growth around clindamycin disc towards erythromycin side i.e. D-test positive, indicating *erm* mediated MLSBi phenotype whereas remaining 51/235 (21.8%) did not show any blunting ie D test negative. indicating *msrA* mediated (efflux) erythromycin resistance phenotype.

DISCUSSION

A pathogen can be considered as multiple drug resistant if it is resistant to two or more unrelated antibiotics to which the pathogen is considered to be inherently susceptible.[11,12] Multidrug resistant MRSA are defined as the MRSA isolates resistant to three non ß- lactam antibiotics classes.[12,13] In the present study 61%, 70-81% and 83% of MRSA were resistant to flouroquinolones (ciprofloxacin), aminoglycosides (gentamicin, amikacin and netlilmicin) and macrolides (erythromycin) respectively. The multiple drug resistance in MRSA is of heightened concern because therapeutic alternative against these isolates i.e. vancomycin is a relatively toxic agent. In addition recent reports of vancomycin and other glycopeptide resistance among the MRSA can further accentuate the clinical problem.[14] However in the present study no MRSA was found resistant to vancomycin or linezollid.

Erythromycin (macrolide) and clindamycin (lincosamide) represent two distinct classes of antimicrobial agents that inhibit protein synthesis by binding to 23S ribosomal subunits of bacterial cells. The methylation of target site on 23S ribosomal subunit renders affected ribosomes incapable of binding macrolide, lincosamide and

streptogramin B (MLSB) antibiotics. The enzyme methylase responsible for methylatation of target site can be produced constitutively or is inducible. Further low level of erythromycin is the most effective inducer for the enzyme methylase. Under *in-vitro* conditions *Staphylococcusaureus* isolates with constitutive resistance are resistant to both macrolides and lincosamide and isolates with inducible resistance are resistant to erythromycin but appear susceptible to lincosamide since they show clear zone of inhibition around lincosamide disc in spite of blunted edge towards erythromycin side. Such resistance is typically mediated by *erm* genes. [15,16,17] Under *in-vivo* condition treatment of patient with such isolates using lincosamide may select for constitutive *erm* mutants leading to clinical failure.[9] Clindamycin and its derivatives are generally not prescribed in erythromycin resistance *S. aureus* infections even if it is susceptible to the drug *in vitro*.

In the present study we found that 235 of the MRSA isolates were sensitive to clindamycin but were resistant to erythromycin. Out of these 184 isolates represent *erm* mediated resistance, since they showed positive D-test i.e. flattening of the zone of inhibition towards erythromycin side. Hence these isolates should be reported as both erythromycin and lincosamide resistant

Resistance to macrolide can also occur by efflux which is typically mediated by *msrA* genes. Isolates with *msrA* genes are nonsusceptible to erythromycin but are susceptible to lincosamide by *in-vitro* test and these isolate do not develop resistant to lincosamide during therapy. [15, 18] In the present study we found that 51/235 MRSA isolates were reported as D-test negative since they did not show flattening of the susceptibility zone around the clindamycin disc towards erythromycin side, hence resistant to erythromycin in these isolate may be mediated by *msrA* genes. Patients with infection caused by multiple drug resistance MRSA with *msrA* genes may be treated with lincosamides without any fear of developing resistanceand clindamycin may be kept as a reserve drug in severe MRSA infections or in cases where the patient is known to have hypersensitivity to β-lactam drugs, depending upon the results of the D-test.

Therefore it is important for the laboratories and clinician to be aware of the prevalence of MLSBi isolates so that a conclusion can be made as to whether D-test is to be performed in routine or to report all erythromycin resistant isolates as lincosamide resistant. This study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance in *Staphylococci* for the optimum treatment of patients. Further D-test must be performed with multi-resistant MRSA where lincosamides is likely therapeutic option.

Figure 1: Distribution of MRSA (N=341)

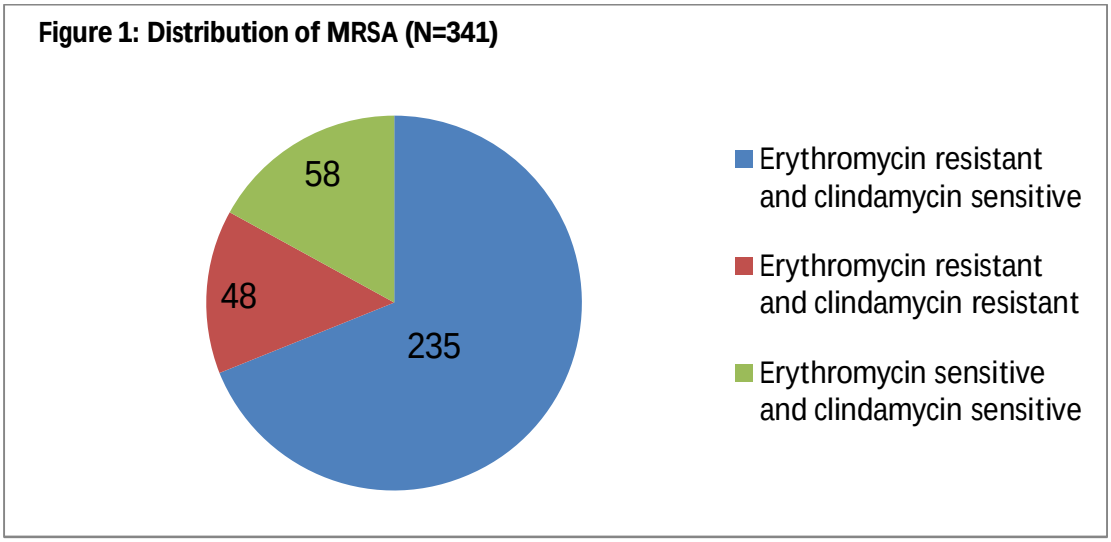
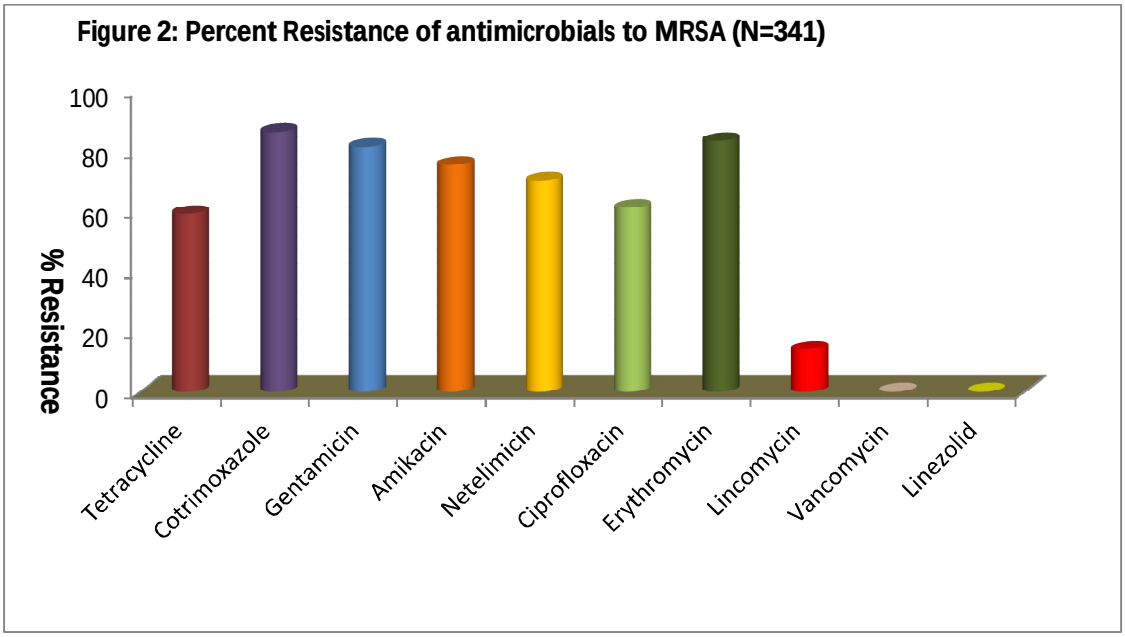


Figure 2: Percent Resistance of antimicrobials to MRSA (N=341)



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