Original Article

Microbiology

Prevalence and Phenotypic Detection of Erythromycin-Induced Resistance to Clindamycin in MRSA Isolates

VELVIZHI G, SUCILATHANGAM G, PALANIAPPAN N

ABSTRACT

Background and objectives: The present study was undertaken to determine the prevalence of MRSA in clinical samples in a tertiary care hospital and to demonstrate the *in vitro* ability of erythromycin to induce clindamycin resistance in clinical isolates of ***Staphylococci.***

Materials and Methods: A total number of 112 ***Staphylococcus aureus*** strains were isolated from clinical specimens and MRSA were detected by the routine antibiotic susceptibility testing methods including the oxacillin disk method, the Cefoxitin disc diffusion test and the Oxacillin screen agar method and the results were interpreted as per the standard guidelines. The clinical isolates of erythromycin-resistant (ER-R), clindamycin-susceptible Staphylococci (CL-S) were examined for inducible clindamycin resistance (ICR) by the erythromycin induction test by using the double disc susceptibility test (D-test). Strains which produced ICR showed flattening of the clindamycin disc zone which was adjacent to the erythromycin disc.

Results: Out of the 112 isolates, 29 (25.9%) Methicillin Sensitive Staphylococcus aureus (MSSA) and 83 Methicillin Resistant Staphylococcus aureus (MRSA) were identified by the Cefoxitin disc diffusion test and the Oxacillin screen agar method. Among the 112 *Staphylococcus aureus* strains which were studied, 67 (32.4%) were erythromycin resistant. These isolates, when they were subjected to the D test, showed 36 (32 %) constitutive MLSB (cMLSB) phenotypic strains which were resistant to both erythromycin and clindamycin and 31 isolates showed clindamycin sensitivity. Out of these,16 (14.2%) strains were D-zone positive i.e. of the inducible MLSB (iMLSB) phenotype, which were resistant to erythromycin and sensitive to clindamycin, while 15 were negative for the D test, thus indicating that they were of the MS phenotype. Of the 36 cMLSB phenotypic strains, 24 isolates were MRSA and 12 were MSSA, while all the iMLSB phenotype strains were MRSA.

Conclusions: We conclude that a significant number of ER-R CL-S strains were positive for ICR, among the MRSA isolates. These isolates should be reported as clindamycin resistant. Given the high rate of inducible resistance to clindamycin in the staphylococcal isolates, the test for inducible resistance to clindamycin should be included in the routine antibiotic susceptibility tests, as it will help in guiding the therapy.

Key Words: *Methicillin Resistant Staphylococcus aureus,* Cefoxitin disc diffusion test, Oxacillin screen agar, Inducible Clindamycin Resistance, MLSBi phenotype.

KEY MESSAGE

- *S.aureus* is the most common cause of nosocomial infections and is of increasing concern because of its tendency to develop multiple antibiotic resistance, which often complicates the treatment.
- The treatment of staphylococcal infections by using MLSB antibiotics is commonplace, but it is often accompanied by increased numbers of resistant strains.
- The use of the D test in a routine laboratory will enable us in guiding the clinicians regarding the judicious use of clindamycin in skin and soft tissue infections; as clindamycin is not a suitable drug for the D test positive isolates, while it can definitely prove to be a drug of choice in the case of the D test negative isolates.

INTRODUCTION

Nosocomial infections account for the morbidity and mortality of millions of patients annually, worldwide. [1] *Staphylococcus aureus,* especially Methicillin-resistance *S. aureus* (MRSA), is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonizations and infections. [1] MRSA is of concern not only because of its resistance to Methicillin, but also because it is generally resistant to many other chemotherapeutic agents [1]. Since the MRSA strains are also resistant to multiple antibiotics, there is a possibility of extensive outbreaks, which may be difficult to conclude [2]. The accurate detection of MRSA is an important prerequisite for the appropriate treatment and the epidemiological assessment of the nosocomial infections which are caused by these strains [2],[3].

Although the clinical significance of methicillin-resistance has been questioned in the past, there is now a widespread acknowledgement of the pathogenicity of MRSA. It has emerged as a significant cause of both nosocomial and community acquired infections. Furthermore, during the past decade, there has been a steady increase in the incidence of the infections which are caused by this bacterium [4].

Clindamycin is an alternative choice for mild to moderate MRSA infections, especially in the penicillin allergic patients. However, the subinhibitory concentration of erythromycin is a common inducer of inducible clindamycin resistance (ICR). [5-7].

To detect the inducible clindamycin resistance, there is a specific disk diffusion method that shows that the resistance is induced by erythromycin [5]. In this method, an erythromycin disk is placed next to a clindamycin disk. When erythromycin diffuses, it induces resistance to clindamycin and this results in the flattening of the clindamycin zone of inhibition, just next to the erythromycin disk, forming a D shape and so this method is called the D-test. Our study estimated the frequency of the D-test among the MRSA isolates.

Currently, the treatment options for the MRSA infections are limited to very few and expensive drugs like teicoplanin, vancomycin and linezolid. Thus, the control of MRSA is essential to curtail the introduction and the spread of infection [8].

This study was undertaken to determine the prevalence of MRSA in clinical samples in a tertiary care hospital and to study the erythromycin-induced resistance to clindamycin in the MRSA isolates.

AIMS AND OBJECTIVES

To find out the prevalence of *Methicillin Resistant Staphylococcus aureus* (MRSA) in clinical samples in Tirunelveli.

To demonstrate the *in vitro* ability of erythromycin to induce clindamycin in erythromycin resistant and clindamycin susceptible clinical isolates of Staphylococci.

MATERIALS AND METHODS

Study cases

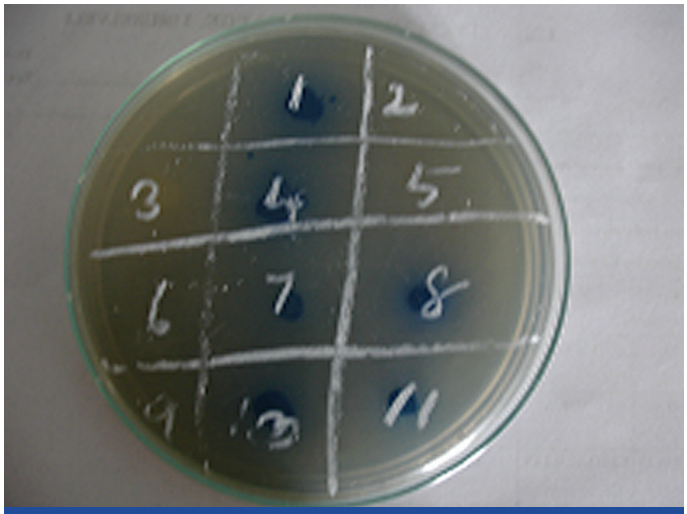
This study was conducted for a period of 9 months from February 2009 to October 2009. A total number of 112 *Staphylococcus aureus* strains were isolated and identified from clinical specimens such as pus, blood and urine in the Department of Microbiology by following standard procedures [9].

MRSA detection

Antibiotic susceptibility tests were performed by the Kirby-Bauer disc diffusion method [10]. Methicillin resistance was detected, based on the CLSI recommendations, by using a 1µg oxacillin disc, a cefoxitin (30µg) disc and oxacillin screen agar[11]. Oxacillin screen agar was performed by the direct colony suspension method and it was adjusted to match the 0.5 MacFarland's turbidity standard. The suspension was inoculated on the oxacillin resistance screening agar base (ORSAB), which is a selective medium which contains aniline blue to detect mannitol fermentation, resulting in intensely blue coloured colonies of *S. aureus*. The plates were incubated for 24 hours at 35°C. Any growth on the plate which contained oxacillin was considered to be resistant to methicillin [11,12]. [Table/Fig 1] *Staphylococcus aureus* ATCC 25923 was used as the control strain for the disc diffusion method.

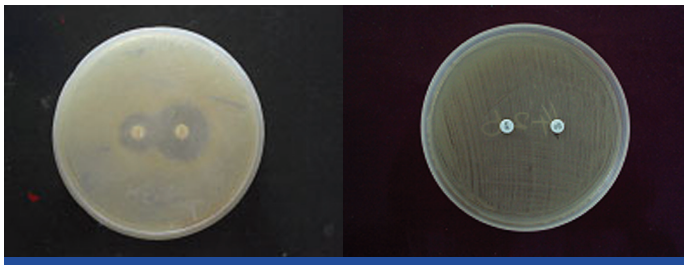
Erythromycin induction by using the double disc susceptibility test (D-test)

The Erythromycin and Clindamycin double disc susceptibility test (D-zone test) was performed as per the NCCLS guidelines of 2004



[Table/Fig-1]: Oxacillin screen agar with blue colonies

[Table/Fig-2]: Erythromycin resistant and clindamycin sensitive Staphylococcal isolate giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc suggestive of inducible MLSB phenotype



[Table/Fig-3]: (a) Erythromycin resistant and clindamycin sensitive Staphylococcal isolate with circular zone of inhibition around clindamycin suggestive of MS phenotype. (b) Both Erythromycin resistant and clindamycin resistant Staphylococcal isolate (c MLSB).

[12]. A disc containing erythromycin (15 µg) was placed 15mm from centre to centre of a clindamycin (2 µg) disc. The inducible resistance to clindamycin was manifested by the flattening or blunting of the clindamycin zone of inhibition, which was adjacent to the erythromycin disc, which gave a D-shape to the zone of inhibited growth.[Table/Fig 2]

RESULTS

A total of 112 *Staphylococcus aureus* isolates were obtained from various clinical samples. A maximum of 74 samples were obtained from the age group of 31-40 years, followed by the age group 21-30 years (28 samples). From the age groups of 1-10 and 61-70 years, seven and three samples were obtained respectively.

Among the 112 *Staphylococcus aureus* strains, 83(74%) were Methicillin resistant *Staphylococcus aureus* (MRSA) and 29 (26%) were Methicillin sensitive *Staphylococcus aureus* (MSSA). The categorization of the isolates along with their sources is depicted in [Table/Fig 4].

A higher percentage of 65% of the MRSA isolates were obtained from the age group of 31-40 years and this was followed by the age groups of 21-30 years (32.5%) and 1-10 years (2.5%). No MRSA isolates were found among the age group of 61-70 years.

Among the 112 *Staphylococcus aureus* strains which were studied, 67 (32.4%) were erythromycin resistant. These isolates, when they were subjected to the D test, showed 36 (32 %) constitutive MLSB (cMLSB) phenotypic strains which were resistant to both erythromycin and clindamycin; 31 isolates showed clindamycin sensitivity. Out of these,16 (14.2%) strains were D-zone positive i.e. of the inducible MLSB (iMLSB) phenotype, which were resistant to erythromycin and sensitive to clindamycin, while 15 were negative for the D test, thus indicating that they were of the MS phenotype. Of the 36 cMLSB phenotypic strains, 24 isolates were MRSA and 12 were MSSA, while all the iMLSB phenotype strains were MRSA. [Table/Fig 5]

Sample	Total	MSSA	MRSA
Urine	5	3	2
Pus	100	21	79
Blood	7	5	2

[Table/Fig-4]: Sources and Categorization of staphylococcal isolates

Organ-ism	ERY-S, CL-S	iMSLB pheno-type	cMLSB pheno-type	MS phe-notype	Total
MRSA	35(42.1%)	16(19%)	24(29%)	8(9.6%)	83(74%)
MSSA	10(34.5%)	-	12(41%)	7(24.1%)	29(26%)

[Table/Fig-5]: Prevalence of Erythromycin-Induced Resistance to Clindamycin in *Staphylococcus aureus* isolates

DISCUSSION

In our study, 16(14.2%) *Staphylococcus aureus* strains were of the iMLSB phenotype, whereas Angel et al from CMC, Vellore, reported the presence of 23.2% strains of the iMLSB phenotype in their study [13]. Fiebelkorn et al reported that 28% [14] and Dizbay et al reported that 90% [15] of their Staphylococcus aureus strains were of the iMLSB phenotype. No MSSA strain was of the iMLSB phenotype in the present study. But other researchers found that 4% to 15% of their MSSA strains were of the iMLSB phenotype. [16-18] . In our study, out of the 83 MRSA strains, 16 (19%) were of the iMLSB phenotype, though several studies from different parts of India have reported that 30% to 64% of their MRSA strains were of the iMLSB phenotype [18-20].

Though the incidence of the cMLSB phenotype is quite high outside India, Angel et al have not found any cMLSB resistance in Staphylococcus aureus strains. [13,14,16]. We found 36 (32%) Staphylococcus aureus strains with the cMLSB phenotype, out of which 24 (29%) were MRSA strains and 12 (41.3%) were MSSA strains.

Though the confirmation of the iMLSB phenotype can be done by detecting the *erm* gene, the D-test is an easy test to perform for the detection of the iMLSB phenotype. All of our 16 iMLSB phenotype *Staphylococcus aureus* strains showed a false sensitivity zone by the routine Kirby-Bauer disc diffusion method. There are a few reports on the clindamycin treatment failure in infections with Staphylococcus aureus strains with inducible clindamycin resistance [21,22].

Clindamycin is commonly used to treat skin and bone infections which are caused by the MRSA strains, because of its tolerability and excellent tissue penetration and also, because no renal adjustments are needed. Its good oral absorption makes it an important option in the outpatients therapy or as follow-up after intravenous therapy [18]. But without the D-zone test, our 16 *Staphylococcus aureus* isolates with inducible clindamycin resistance would have been misclassified as Clindamycin sensitive, resulting in a therapeutic failure. This is where the D-zone test becomes significant and important.

As clindamycin is one of the most commonly used antibiotics for the MRSA strains, the increasing clindamycin resistance in the form of iMLSB and cMLSB, limits the therapeutic options for MRSA to the antibiotics like linezolid and vancomycin.

CONCLUSION

We hereby conclude that without the D-zone test, all *Staphylococcus aureus* isolates with inducible clindamycin resistance would have been misidentified as clindamycin susceptible by the routine antibiotic susceptibility testing methods, resulting in the misuse of clindamycin and treatment failure. Hence, all clinical microbiology laboratories should perform the D-zone test as per the CLSI guidelines, 2004, which is simple and inexpensive, when the Staphylococci appear to be erythromycin resistant and clindamycin susceptible by the routine tests.

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