Inducible Clindamycin Resistance among Clinical Isolates of Staphylococcus aureus in a Tertiary Care Centre, Kerala, India

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ABSTRACT

Drug resistance among Staphylococcus aureus is an increasing problem. Clindamycin is one of the effective antibiotic for treating both methicillin sensitive and resistant Staphylococcal infections. A major concern regarding the use of clindamycin therapy is the presence of inducible resistance. In vitro routine tests for clindamycin susceptibility may fail to detect inducible resistance resulting in treatment failure. The present study aimed to find out the inducible clindamycin resistance in the clinical isolates of Staphylococcus aureus using D test. The study was conducted at the Department of Microbiology in Sree Narayana Institute of Medical Sciences, Kerala over a period of one year. All of the total 220 S. aureus isolates during the period were included in the study. D test was done in the erythromycin resistant strains as per CLSI guidelines. Inducible clindamycin resistance was observed in 12.7%, constitutive resistance in 8.1% and MLSB phenotype in 41.8% of the total isolates. The rate of MRSA and MSSA were 41.8% and 58.2% respectively. Inducible resistance and constitutive resistance were higher in MRSA (34.8% and 10.9% respectively) as compared to MSSA where the inducible resistance was only 3.1% and no constitutive resistance was noticed. 19.6% of MRSA and 21.9% of MSSA showed MS phenotype. Inducible clindamycin resistance was more common than constitutive resistance in our hospital. Both inducible and constitutive resistance showed higher incidence among MRSA than MSSA. The trends in resistance may vary from place to place. So D-test should be done routinely to delineate different resistant phenotypes in the laboratory to help clinicians for the judicious use of clindamycin in order to avoid therapeutic failure as well as irrational use of higher antibiotics.

Keywords
Clindamycin, D test, MLSB phenotype, Staphylococcus aureus.

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Introduction

Staphylococcus aureus is one of the most common cause of both community acquired and nosocomial infections. It includes minor cutaneous infections to life threatening conditions such as endocarditis, pneumonia and septicaemia (Lt.Col. Mahima Lall et al; 2013, Naima Fasih et al; 2010). Emergence of methicillin resistant S. aureus (MRSA) which are resistant to β-lactams as well as other classes of antibiotics often
presents difficulty in treatment. The Macrolide- Lincosamide- Streptogramin B (MLS\textsubscript{B}) class of antibiotics is commonly used in the treatment of both MRSA and methicillin sensitive \textit{S. aureus} (MSSA) infections (Prakash Sah \textit{et al} ;2015). Among these drugs clindamycin is an attractive therapeutic option especially in skin and subcutaneous infections as they are available both as oral and intravenous preparations, have excellent tissue penetration, are relatively inexpensive and require no renal dose adjustments. It also inhibits the production of certain toxins and virulence factors by \textit{S. aureus}. It is a useful choice in penicillin allergic patients. (Coyle EA \textit{et al} ;2003, Prakash Sah \textit{et al} ;2015). But the emergence of clindamycin resistance especially of inducible type due to the inappropriate use of MLS\textsubscript{B} antibiotic is becoming a major problem in its utility.

In \textit{Staphylococcus}, Macrolide resistance arises either by ribosomal modification or efflux mechanism. The efflux pump is encoded by msr A gene which affects the macrolides and streptogramins and produces the MS phenotype.

The ribosomal modification is mediated by 23S r RNA methylase encoded by the \textit{erm} genes. This methylase confers resistance to the MLS\textsubscript{B} class of antibiotics as the gene encodes methylation of the 23 S r RNA binding site that is shared by these drugs (Edward JE ;2010). Phenotypically such resistance may be constitutive (cMLS\textsubscript{B}) or inducible (iMLS\textsubscript{B}). In cMLS\textsubscript{B} phenotype methylase is always produced but in iMLS\textsubscript{B} phenotype it is produced only in the presence of an inducer, chiefly a macrolide. It is also possible for ribosomal mutations to occur spontaneously that transforms iMLS\textsubscript{B} strains to cMLS\textsubscript{B} phenotype without the presence of a macrolide inducer during clindamycin therapy (Leclercq R \textit{et al} ;2002).

Constitutive resistant strains are easily identified by the routine antibiotic susceptibility test as they show resistance to both erythromycin and clindamycin. But the inducible clindamycin resistance is often missed unless D-test is done because the isolates show resistance to erythromycin but sensitive to clindamycin in the routine testing. Thus reporting iMLS\textsubscript{B} phenotypes as clindamycin sensitive by the microbiologist will mislead the clinician to start with clindamycin and treatment failure due to spontaneous mutations (Lewis JS \textit{et al}; 2005). On the other hand a negative result of inducible clindamycin resistance helps the clinicians to consider clindamycin as a therapeutic option for erythromycin resistant strains (Nita Gangurde \textit{et al}; 2014). A negative D test can also help the clinician to spare confidently the higher antibiotics like vancomycin and teicoplanin for treating non- life threatening MRSA infections. CLSI recommends D test as a simple, reliable and inexpensive test to perform along with the routine susceptibility testing in order to detect inducible clindamycin resistance in vitro (CLSI ;2013, Gade N \textit{et al}; 2013).

The prevalence of inducible clindamycin resistance varies by geographic location and bacterial species but reports are scanty from Kerala. The present study was undertaken to determine the percentage of inducible clindamycin resistance among \textit{S.aureus} isolates in our locality by incorporating D test as a routine procedure in the clinical microbiology laboratory, in order to guide our clinicians for appropriate clindamycin therapy.

**Materials and Methods**

The study was conducted in a tertiary care centre, Kerala over a period of one year (March 2015- March 2016). All of the total
220 *Staphylococcus aureus* isolates during the study period (from various clinical specimens like pus, wound swab, aspirates, blood and other sterile fluids) were included under the study. Antibiotic susceptibility testing was done by Kirby Bauer’s disc diffusion method on Mueller Hinton agar using Penicillin (10 U), Cefoxitin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), Cotrimoxazole (25 µg), Linezolid (30 µg) and Vancomycin (30 µg) as per CLSI guidelines (CLSI; 2013). Quality check for the disc were performed with ATCC *Staphylococcus aureus* 25923.

Isolates with cefoxitin zone size ≥22mm were considered as methicillin sensitive and those with zone size ≤ 21mm were considered as methicillin resistant. The isolates that were found to be erythromycin resistant (zone ≤ 13mm) were further studied for inducible clindamycin resistance using ‘D test” placing Erythromycin (15 µg) and Clindamycin (2 µg) disc at 15 mm apart. A flattening of the zone of inhibition adjacent to erythromycin discs after 18- 24 hours of incubation was considered as inducible clindamycin resistance (CLSI ;2013, Gade N et al; 2013).

Three different phenotypes were noted:

**MS phenotypes** : resistant to erythromycin (zone ≤ 13mm) and sensitive to clindamycin (zone ≥ 21mm)

**iMLS\(_B\) phenotype** : erythromycin resistant (zone ≤ 13mm) and clindamycin sensitive (zone ≥21mm) showing D shaped zone around the clindamycin disc with flattening adjacent to erythromycin disc

**cMLS\(_B\) phenotype** : both erythromycin (zone ≤ 13mm) and clindamycin are resistant (zone size ≤ 14mm)

**Results and Discussion**

Out of the 220 *S.aureus* isolates,138 isolates (62.7%) were erythromycin resistant and 168 isolates (76.4%) were clindamycin sensitive by routine antibiotic sensitivity testing. 54.5% of the total isolates were erythromycin resistant and clindamycin sensitive. By incorporating D test we got the following phenotypes- MS phenotype in 92 isolates (41.8%), c MLS\(_B\) in 18 isolates (8.1%) and i MLS\(_B\) in 28 isolates (12.7%). Methicillin resistance was noticed in 92 isolates (41.8%) and 128 isolates (58.2%) were MSSA. Among the MSSA, 32 isolates (25 %) were erythromycin resistant. Out of the total MSSA, MS phenotype was observed in 28 isolates (21.9%), i MLS\(_B\) phenotypes in 4 isolates (3.1%) and no cMLS\(_B\) phenotype was isolated. Sixty isolates (65.2 %) of MRSA were erythromycin resistant. MS phenotype was noticed in 18 isolates (19.6 %), c MLS\(_B\) in 10 isolates (10.9 %) and i MLS\(_B\) in 32 isolates (34.8%) out of the total MRSA.

The increasing frequency of Staphylococcal infections and changing pattern in antibiotic resistance have led to renewed interest in the use of clindamycin to treat such cases. Clindamycin is one of the effective agents for the treatment of skin and subcutaneous infections caused by MRSA and MSSA infections. (Lt.Col.Mahima Lall et al;2013, Naima Fasih et al ;2010). Inducible resistance to MLS\(_B\) antibiotics especially to clindamycin needs special concern as spontaneous constitutively resistant mutants have been selected from such isolates both in vitro and in vivo during clindamycin therapy ( Venkata Raghavendra Rao et al; 2012, Kalpana Date et al ;2012).

In our hospital, with the routine sensitivity testing, clindamycin sensitivity is found to be 76.4% which reveals a chance of more
clinical utility of clindamycin in our setting. 54.5% of this isolates were erythromycin resistant and clindamycin sensitive. But with D test, MS phenotype is only 41.8% of the isolates and the remaining 12.7% isolates is iMLS\textsubscript{B} phenotype. So fairly a big percentage would have been misinterpreted as clindamycin sensitive in the absence of the D test. Among the isolates, inducible clindamycin resistance is more common than constitutive resistance. The results of the present study is compared to some recent studies from India and other countries and is shown in Table:1. Even though there is slight difference in the percentages of resistance in different geographical areas majority of studies showed predominance of iMLS\textsubscript{B} than c MLS\textsubscript{B}.

41.8% of the total isolates were MRSA and 58.2% were MSSA. The prevalence of MRSA is different in various studies: Mehta \textit{et al}; 2007, Vekata Raghavendra \textit{et al}; 2012, Smita sood \textit{et al}; 2013, Nita Gangurde \textit{et al}; 2014, Prakash Sah \textit{et al}; 2015, B Sasirekha \textit{et al}; 2014, shows percentage of isolation of MRSA as 26.6%, 75.27%, 7.75%, 32%, 61.4% and 27.45% respectively. MRSA isolation is moderately high in our area. This difference in prevalence of MRSA among different countries and between different regions in a country may be due to varied population, geographical distribution and selection pressure in the community.

### Table 1: Inducible and Constitutive Clindamycin Resistance in Various Studies

| S. aureus n=220 | i MLS\textsubscript{B} (%) | c MLS\textsubscript{B} (%) |
|-----------------|-----------------|-----------------|
| Present study   | 12.7            | 8.1             |
| Gadepalli \textit{et al}; 2006 | 21              | 26.5            |
| Smita sood \textit{et al}; 2013 | 15.5            | 20.93           |
| Nita Gangurde \textit{et al}; 2014 | 13.53           | 12.61           |
| Prakash \textit{et al}; 2015 | 12.1            | 7               |

### Table 2: Inducible and Constitutive Clindamycin Resistance among MRSA and MSSA in Various Studies

| S. aureus n=220 | i MLS\textsubscript{B} (%) | c MLS\textsubscript{B} (%) |
|-----------------|-----------------|-----------------|
| MRSA            | MSSA            | MRSA            | MSSA            |
| Present study   | 34.78           | 3.1             | 10.87           | nil             |
| Venkata Raghavendra Rao \textit{et al}; 2012 | 45.71           | nil             | 2.85            | nil             |
| Smita sood \textit{et al}; 2013 | 62.5            | 60              | 38              | nil             |
| Nita Gangurde \textit{et al}; 2014 | 27.8            | 6.78            | 18.26           | 9.95            |
| Prakash Sah \textit{et al}; 2015 | 14              | 9.3             | 12.8            | nil             |
In our study both inducible and constitutive clindamycin resistance and MS phenotype were more in MRSA than MSSA. This is consistent with many studies from various regions. (Table:2 and Table:3). Almost all studies showed both inducible and constitutive resistance more in MRSA strains. In contrary, Schreckenberger et al and Levin et al reported higher incidence of inducible resistance in MSSA as compared to MRSA, 12.5% MRSA and 68% MSSA respectively (Nita Gangurde et al 2014). 54.4% of total MRSA isolates were both erythromycin resistant and clindamycin sensitive by routine test. But by incorporating D test, Ms phenotype is only 19.6% and 34.8 % are having inducible clindamycin resistance which can adversely affect the treatment with clindamycin. In MSSA also, 3.1% of isolates may be misinterpreted as sensitive and may lead to therapeutic failure.

So keeping in view of this relative high frequency of iMLS\textsubscript{B} phenotype especially among MRSA isolates, D test should be incorporated in the laboratory as a routine procedure. Providing a negative D test report from the lab will help clinicians to consider clindamycin for treating non life threatening Staphylococcal infections, especially for MRSA where there is only limited treatment options available. Thus use of higher antibiotics like vancomycin and linezolid can be reserved for complicated infections.

The incidence of resistance may be variable with geographical areas, study population and the hospital epidemiology. Hence there is definitely a need to monitor the local prevalence of these resistance phenotypes by the microbiologists to guide the physicians in treating such cases judiciously and effectively.

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