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Inducible Clindamycin Resistance among *Staphylococcus aureus* Isolates in Government Medical College, Aurangabad, India

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A B S T R A C T

Staphylococcus aureus is one of the most common human pathogens with ability to cause wide range of infections. The increasing incidence of a variety of infections due to hospital acquired and community associated methicillin-resistant *Staphylococcus aureus* (MRSA) has led to emphasis for need of safe and effective agents. Clindamycin is commonly used drug for MRSA as it is safe, effective, less costly and can be given orally. Due to extensive use of this antibiotic, it has developed resistance by this mechanism and hence it is important to detect resistance to clindamycin. The Clinical laboratory Standards Institute (CLSI) recommends D test for detecting inducible resistance phenotypically. Aim of the study was to see the Clindamycin resistance pattern in *S.aureus* (*Staphylococcus aureus*) isolates. During a period of one year i.e. in 2015,724 *S.aureus* isolates from various clinical samples were evaluated and methicillin resistance was determined using Cefoxitin (30 mcg) disc and inducible resistance to clindamycin was detected by D-test as per CLSI guidelines (2014). We observed that among 724 *S.aureus* isolates inducible resistance was found in 124(17.12%); 94 (19.02%) of 494 Methicillin Resistant *S.aureus* (MRSA) isolates and 30 (13.04%) of 230 Methicillin Sensitive *S.aureus* (MSSA) isolates showed inducible resistance. Study showed that D test should be used as mandatory method in routine disc diffusion testing to detect inducible Clindamycin resistance for optimum treatment of patients.

Introduction

Staphylococcus aureus is recognized as one of the most common organisms causing nosocomial and community-acquired infections in every region of the world (Yilmaz *et al.,* 2007). Staphylococci are responsible for several suppurative types of infections. They have a differential ability to spread and cause outbreaks in hospitals which is now recognized as an extremely successful human pathogen. Resistance to methicillin emerged shortly after the drug's introduction and is a factor that has helped *Staphylococcus aureus* become established as a nosocomial pathogen (Barber, 1961). The increasing prevalence of resistance to most antimicrobial agents in staphylococci,

especially spread of resistant strains in the community, signify the need for new effective agents to treat staphylococcal infections (Lewis *et al*., 2005). Methicillin-resistant *Staphylococcus aureus* (MRSA)are increasingly being reported as multidrug resistant with high resistance to macrolides (Erythromycin, Clarithromycin) and lincosamides (Clindamycin, Lincomycin) leaving very few therapeutic options (Srinivasan *et al.,* 2002).

Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible. The most common mechanism for such resistance is target site modification mediated by *erm* genes, which can be expressed either constitutive macrolide-lincosamide-streptogramin B (constitutive MLSB phenotype) or inducible macrolide-lincosamide-streptogramin B (inducible MLSB phenotype). Strains with inducible resistance to clindamycin 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. In case of another mechanism of resistance mediated through msrA genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant and Clindamycin-sensitive both *in vivo* and *in vitro* and the strain do not typically become Clindamycin resistant during therapy (Deotale *et al*., 2010).

Newer antibiotics like vancomycin, linezolid, and quinupristin-dalfopristin have been advocated in the management of such isolates, but recent reports of resistance to these agents raise real concerns over how long these uniform susceptibilities will hold good (Johnson *et al.,* 2003). This has led to renewed interest in the usage of MLSB antibiotics to treat *S.aureus* infections with, clindamycin being the preferred agent due to its excellent pharmacokinetic properties.

Clindamycin has excellent tissue penetration except for the central nervous system (Sivapalasingam *et al.,* 2010). Macrolide – induced Clindamycin resistance was observed among the clinical isolates of Staphylococcus since 1968 which could not be detected by the routine disc diffusion method. From such isolates constitutively resistant mutants are emerged and results in treatment failure with clindamycin in vivo which would be demonstrated on D-test (Frank *et al.,* 2002).

The main aim of this study to inducible clindamycin resistance amongst *S.aureus* isolates. And to study the utility of D test for the detection of inducible clindamycin resistance in *S.aureus* isolates.

Material & Methods

The study was conducted in the Microbiology Department of Government Medical College Aurangabad during period January 2015to December 2015.Various clinical samples like pus, wound swabs, aspirates, blood, urine, sputum, tracheal aspirate, umbilical cord, catheter tip and body fluids were evaluated and included in our study. Total number of organisms isolated were 3,096. Among the organisms 724 (23.38%) were *Staphylococcus aureus* isolates. *S.aureus were* isolated by using conventional bacteriological methods such as colony morphology, Gram staining, catalase, coagulase test and mannitol fermentation test. All samples were processed as per standard procedures (Baird, 2008). Isolates were subjected to Antibiotic susceptibility testing by Kirby Bauers disk diffusion method on Muller Hinton agar (MHA) according to the Clinical and Laboratory standards Institute (CLSI -2014).

A 0.5 McFarland suspension of staphylococci was inoculated on Mueller Hinton agar plate, antibiotic discs applied were Gentamicin (10µg), Clindamycin (2µg), Erythromycin (15µg), Tetracycline (30µg), Cotrimaxazole (25µg), Amoxicillin Clavulanate (30µg), Ciprofloxacin (5 µg)and Cefoxitin (30 µg).

Methicillin resistance was detected by using Cefoxitin disc diffusion. MRSA & MSSA strains were categorized by phenotypic criteria.494 (68.23%) were MRSA and 230 (31.76 %) were MSSA as shown in (Figure 1).

D test was also noted on these erythromycin resistant strains for detection of various clindamycin resistance patterns on the same Antibiotic sensitivity plate.

Method for D test

For detection of inducible Clindamycin resistance (iMLSB), a disk approximation test was performed by placing a Clindamycin disc 15-26 mm away from the edge of erythromycin discover the MHA which is inoculated with the test organism. Following overnight incubation at 37^0C, Erythromycin diffuses, it induces, resistance to Clindamycin and results in flattening of the Clindamycin zone just next to the Erythromycin disk, making a D shape, which is interpreted D-test positive, whereas complete zone indicates D-test negative.

ATCC Controls used

*Staphylococcus aureus (*ATCC 25923) strains.

*Staphylococcus aureus (*ATCC 43300) MRSA strains

In house strains of *Staphylococcus aureus* showing D-test positive.

Three different phenotypes were appreciated after testing and interpreted as follows:

Erythromycin and Clindamycin sensitive isolates –Staphylococcal isolates sensitive to both erythromycin (zone size ≥23mm) and Clindamycin (zone size ≥21mm) were labelled as having this phenotype. (Refer Figure 2)

MS Phenotype – Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to Clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype. (Refer Figure 3)

Constitutive MLSB Phenotype – this phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin. (Refer Figure4)

Inducible MLSB Phenotype – Staphylococcal isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size≥21mm) and giving D shaped zone of inhibition around Clindamycin with flattening towards erythromycin disc were labelled as having this phenotype. (Refer Figure 5)

Results and Discussion

Among 724 *Staphylococcus aureus*; 494 (68.23%) were MRSA and 230 (31.76%) were MSSA. MRSA and MSSA both were showing D test positive (Refer Figure 6 and 7). Total D test positive were 124 (17.12%) of which 94 (19.02%) cases in MRSA and 30 (13.04%) cases in MSSA shows D test positive. Among MRSA and MSSA, D test negative were 149 (30.16%) and

41(17.82%) respectively. Samples showing both Erythromycin and Clindamycin sensitivity were 202(40.89%) and 144(17.82%) and 292 (59%) samples of MRSA were found to be erythromycin resistant and 86 (37.4%) samples of MSSA were found to be erythromycin resistant. Refer (Table.1) and (Figure 8)

Percentage of both inducible and constitutive resistance was found to be higher in methicillin resistant isolates as compared to methicillin sensitive staphylococci strains

Initial susceptibility report:

1. Erythromycin-R&Clindamycin-R - Resistant to Clindamycin
2. Erythromycin-S &Clindamycin-S - Susceptible to Clindamycin
3. Erythromycin-R but Clindamycin-S- Need D test to confirm Clindamycin resistance

D test interpretation:

1. D test Negative-Clindamycin susceptible (efflux mechanism of erythromycin resistance)
2. D test Positive-Inducible clindamycin resistance (iMLSB)

Report of D test positive was informed to the Physician of the Hospital and they were advised to withhold clindamycin therapy for that patient.

Before initiating the antimicrobial therapy of infected individuals, the antimicrobial susceptibility testing for clinical isolates is performed to avoid indiscriminate usage of antibiotics on trial and error basis. This is particularly important considering the increase of resistance and the emergence of multidrug resistant organisms in Staphylococcus infections. Production of methylase and efflux proteins is the most widespread and important mechanism of resistance among Staphylococci, conferring resistance to MLSB group of antibiotics.

In our study Methicillin resistance was identified in 68% isolates of *Staphylococcus aureus*, similar studies as Gupta *et al.,* and Sharma *et al.,* in India. High rate of methicillin resistance is noted among *S.aureus* isolates in developed nations (Chelae *et al*., 2009).

Among them inducible Clindamycin resistance (D test positive) and constitive MLSB was found to be more in MRSA than MSSA, this was in concordance with a few studies reported before (Sanchez *et al*., 1993).

In MRSA and MSSA isolates of Staphylococcus aureus Erythromycin resistance was found in 292 (40%) cases of 494 and 86 (37.4%) cases of 230respectively.

Drug of choice in treatment of MRSA is Vancomycin, but it has some limitation. In such cases Clindamycin should be considered for the management of skin and serious soft tissue infections that are sensitive to Clindamycin due to good oral absorption and as a follow-up after intravenous therapy as its efficacy is not affected by high bacterial load at the site of infection and dose adjustment is not required even in severe hepatic or renal dysfunction.

It is less expensive than some of the newer agents with good oral absorption, making it an attractive substitute for outpatients therapy (Sanchez *et al*., 1993).

Table.1 Showing antibiotic susceptibility pattern of four different Phenotypes in MRSA and MSSA

Table – 1: Distribution of isolates			
Sr.no	**Susceptibility pattern (Phenotype)of Erythromycin and Clindamycin**	**MRSA (%)**	**MSSA (%)**
1	ERY-S, CL-S	202 (40.89)	144(62.60)
2	ERY-R, CL-R (constitutive MLSB)	49 (9.91)	13 (5.66)
3	ERY-R, CL-S, D test negative (MS)	149 (30.16)	41 (17.82)
4	ERY-R, CL-S, D test positive (inducible MLSB)	94 (19.02)	30 (13.04)
	Total	494(68.23)	230 (31.76)

Fig.1 Showing percentages of MRSA and MSSA.

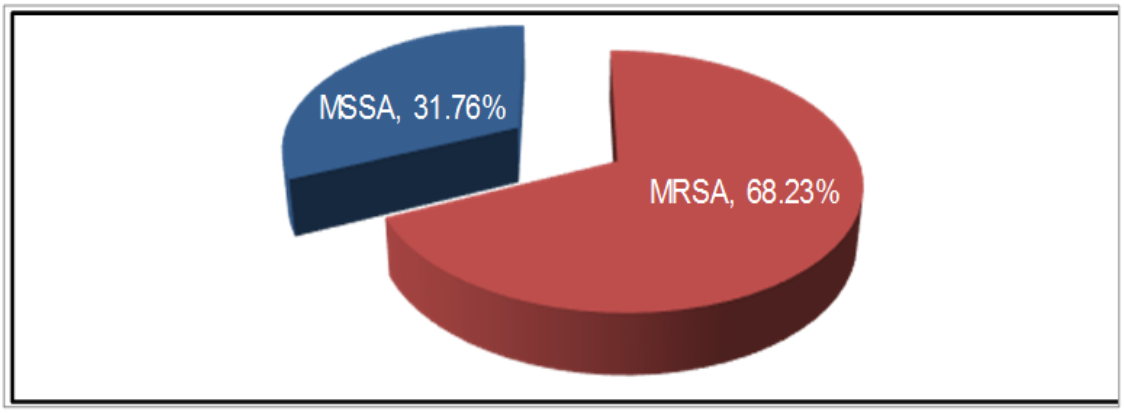


Fig.2 Staphylococcal isolate sensitive to both erythromycin and clindamycin.

Fig.3 Erythromycin resistant and clindamycin sensitive *Staphylococcal* isolate. (MS phenotype)

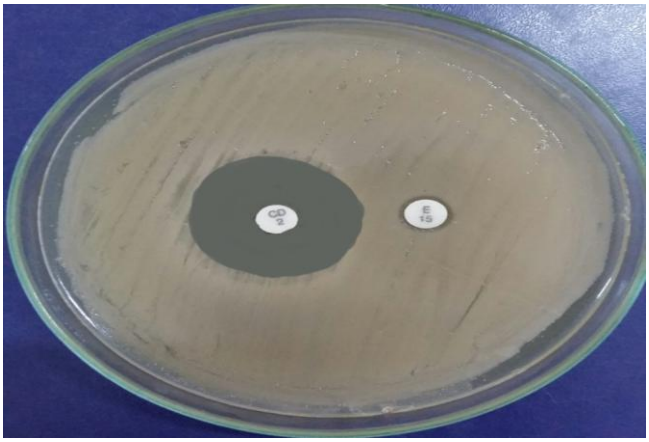


Fig.4 Staphylococcal isolate showing resistant to both erythromycin and clindamycin (Constitutive MLSB phenotype)

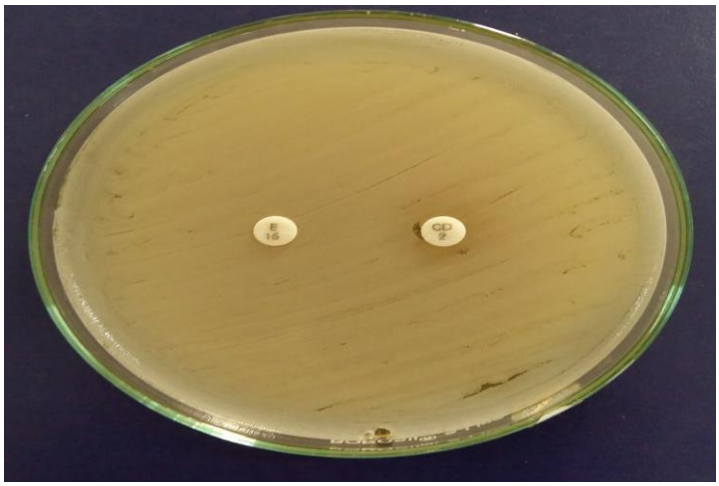


Fig.5 Staphylococcal isolate showing erythromycin resistant and clindamycin sensitive zone, with D shaped zone of inhibition around it with flattening towards erythromycin disc (inducible MLSB phenotype)

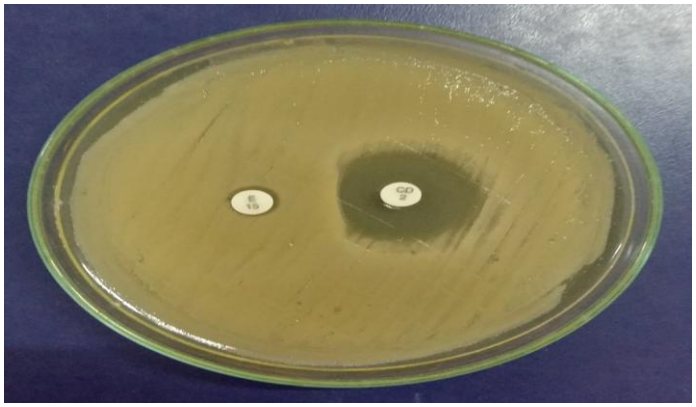


Fig.6 Showing D test positive in MRSA (Methicillin resistance Staphylococcus aureus.

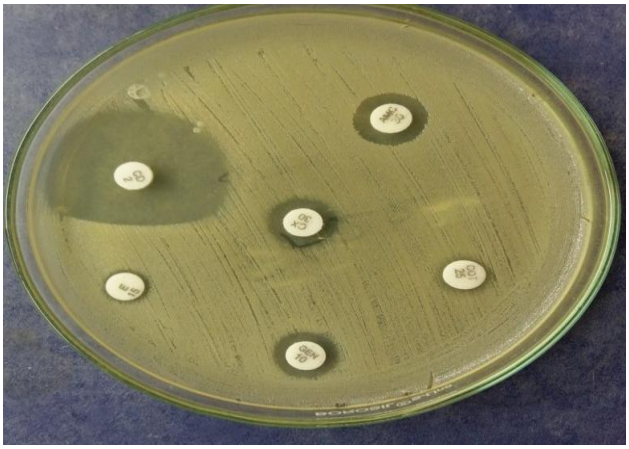


Fig.7 Showing D test positive in MSSA (Methicillin sensitive *Staphylococcus aureus*).

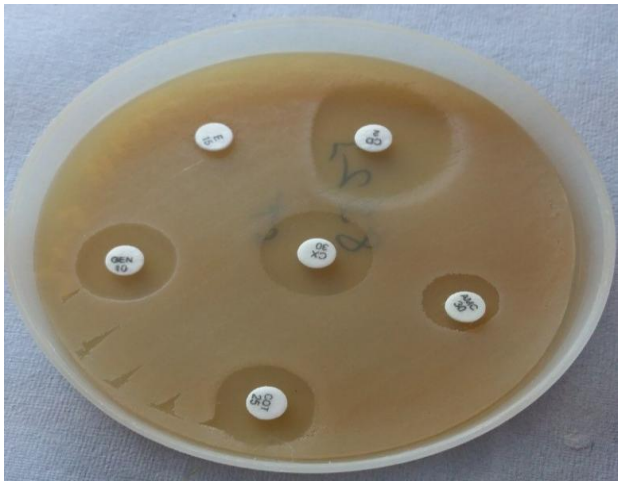
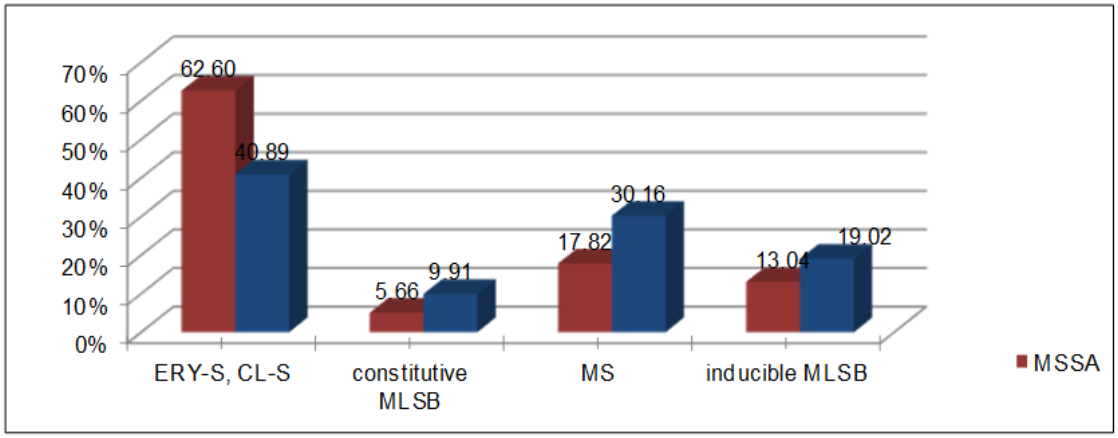


Fig.8 Bar Diagram showing different phenotypes in MRSA and MSSA



Simple laboratory testing, (i.e. erythromycin, clindamycin'D-zone' test) can separate strains that have the genetic potential to become resistant during therapy from strains that are truly susceptible to clindamycin. Without the double-disk test, all staphylococcal isolates with inducible MLSB would have been mistakenly interpreted as clindamycin-susceptible. On the other side, to categorically consider all erythromycin resistant staphylococci as clindamycin resistant would deny potentially safe and effective therapy for patients infected with isolates that carry only the export mechanism.

In conclusion, in hospital like ours where inducible MLSB strains is found more in both MSSA & MRSA, we recommend to perform D-test routinely. Inducible clindamycin resistance as noted by a positive D test should be reported as resistant. A comment should be added that - this isolate is presumed to be resistant based on detection of inducible resistance. Clindamycin may still be effective in some patients for empirical outpatient treatment options for staphylococcal infections have become more limited as concerns about the prevalence of MRSA have increased.

Clindamycin should be kept as a reserve drug and be usually advocated in severe MRSA infections depending upon the antimicrobial susceptibility results. Reporting *S.aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand, negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option.

Use of D test in a routine laboratory enables us in guiding the clinicians in judicious use of Clindamycin, as Clindamycin is not a suitable drug for D test positive MS phenotype resistance was almost equal among isolates; while it can definitely prove to be a drug of choice in case of D test negative isolates.

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