



Original Research Article

Phenotypic Detection of Inducible Clindamycin Resistance among the Clinical Isolates of *Staphylococcus Aureus* by Using D-Test

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Received: 01/01/2014

Revised: 27/01/2014

Accepted: 18/02/2014

ABSTRACT

Purpose: Resistance to antimicrobial agents among staphylococci is an increasing problem. This has led to renewed interest in the usage of MLS_B group of antibiotics to treat *Staphylococcus aureus*. In-vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to the expression of *erm* genes resulting in treatment failure, thus necessitating the need to detect such resistance by simple D-test on a routine basis.

Materials and methods: 392 *Staphylococcus aureus* isolates were subjected to antibiotic susceptibility testing using erythromycin and cefoxitin disc by Kirby Bauer disc diffusion method. Those isolates which were erythromycin resistant were further subjected to D-test as per CLSI guidelines.

Result: A total of 392 *Staphylococcus aureus*, 176 were erythromycin resistant, out of these, 46(26.13%) isolates were inducible clindamycin resistant, 103(58.52%) were constitutive resistant, while remaining 27(15.34%) showed MS_B phenotype. Inducible and constitutive resistances were found to be higher in MRSA (30% and 62.85% respectively) as compared to MSSA (11.11% and 41.66% respectively).

Key words: Clindamycin resistance, MLS_B phenotypes, MRSA

INTRODUCTION

Staphylococcus aureus is common cause of both community and nosocomial acquired infections. [1,2] Infections range from minor skin infections to life threatening conditions such as endocarditis, pneumonia, and septicemia. Increasing antimicrobial resistance in *Staphylococcus aureus* is one of the major concerns. [1-4]

Emergence of methicillin resistance in *Staphylococcus aureus* (MRSA) has left us very few therapeutic alternatives to treat staphylococcal infections. The macrolide-lincosamide-streptogramin-B (MLS_B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties. [5-9]

Widespread use of MLS_B antibiotics in serious staphylococcal infections results in emergence of increased number of strains acquiring resistance to MLS_B antibiotics. [10,11] Most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either constitutively (constitutive MLS_B phenotype) or inducibly (inducible MLS_B phenotype). [8,12-15]

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 these discs are not placed adjacent to each other. In such case, *in vivo* therapy with clindamycin may select constitutive *erm* mutants leading to clinical therapeutic failure. [15]

In case of another mechanism of resistance mediated through *msrA* gene i.e. efflux of antibiotics, Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vivo* and *in vitro* and the strain does not typically become clindamycin resistant during therapy. These isolates known as MS phenotype and clindamycin can be safely given in infections with this phenotype and there no risk of clinical failure. [8,14-16]

So it is mandatory to detect such strains for the better outcome of the patient who is on clindamycin therapy. Inducible MLS_B strains to clindamycin among the erythromycin resistant staphylococcal isolates can be easily detected by a simple D-test according to Frebelkorn *et.al.* [15]

MATERIALS AND METHODS

The study was conducted at Department of Microbiology, MGM Medical College, Aurangabad from January to December 2012. A total of 392 isolates of *Staphylococcus aureus* isolated from various clinical specimens like pus, sputum, body fluids, endotracheal tube aspirates, blood,

and urine were tested. The isolates were first identified as *Staphylococcus aureus* and then subjected to susceptibility testing by Kirby-Bauer disc diffusions method on Muller Hinton Agar (MHA) plates using erythromycin (15µg) disc and cefoxitin (30µg) disc to detect erythromycin resistance and MRSA respectively. D-test was performed on all the erythromycin resistant isolates, briefly; Erythromycin (15µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2µg) disc on a MHA plate previously inoculated with 0.5 McFarland bacterial suspensions. Following overnight incubation at 37⁰C was done and D-test results were interpreted and staphylococcal isolates were labeled as MS_B phenotype, inducible MLS_B phenotype, & constitutive MLS_B phenotype as per CLSI guidelines. [15,17] Criteria used are given below.

MS_B phenotype: Staphylococcal isolates exhibiting resistance to Erythromycin (Zone size ≤ 13 mm) while sensitive to Clindamycin (Zone size ≥ 21 mm) and giving circular zone of inhibition around Clindamycin was labeled as having this phenotypes

Inducible MLS_B phenotype: Staphylococcal isolates exhibiting resistance to Erythromycin (Zone size ≤ 13 mm) while sensitive to Clindamycin (Zone size ≥ 21 mm) and giving D shaped zone of inhibition around clindamycin was labeled as having this phenotypes

Constitutive MLS_B phenotype: Staphylococcal isolates exhibiting resistance to both Erythromycin (Zone size ≤ 13 mm) and Clindamycin (Zone size ≤ 14 mm) with giving circular shape of zone of inhibition if any around clindamycin. [15,17]

Quality control: Quality control of Erythromycin and clindamycin discs was checked using *Staphylococcus aureus* (ATCC 25923) strain according to the standard disc diffusion QC procedure. [18]

RESULTS

Total 392 Staphylococcal isolates were tested for susceptibility to erythromycin and cefoxitin to detect erythromycin resistance and MRSA respectively. Out of these, 176(44.90%) were resistant to erythromycin, and among them, 140(79.54%) isolates were MRSA and 36(20.45%) isolates were MSSA. D- test was performed for these isolates and it was observed that 46(26.13%) isolates were

inducible MLS_B phenotype(D-test positive), 27(15.34%) isolates were MS_B phenotype (D-test negative) and 103(58.52%) isolates were constitutive MLS_B phenotype.

High percentage of inducible and constitutive resistance was observed amongst MRSA isolates (30% and 62.85% respectively) as compared to MSSA isolates (11.11% and 41.66% respectively). MS phenotypes were identified more among MSSA (47.22%) as compared to MRSA (7.14%). (Table-1).

Table-1: Distribution of MLS_B phenotype among erythromycin resistant strains. (n=176)

E-R strains	Type of resistance			Total
	iMLS _B phenotypes	cMLS _B phenotypes	MS phenotypes	
MRSA	42(30%)	88(62.85%)	10(7.14%)	140(79.54%)
MSSA	04(11.11 %)	15(41.66 %)	17(47.22%)	36(20.45%)
Total	46(26.13 %)	103(58.52%)	27(15.34 %)	176

DISCUSSION

The prime step before initiating the antimicrobial therapy of infected individuals is performing the antimicrobial susceptibility testing for clinical isolates to avoid indiscriminate usage of antibiotics on trial and error basis. Empirical treatment for staphylococcal infection is more dangerous due to the emergence of multi drug resistant strains especially MRSA. In cases of failure to beta-lactam antibiotics, Clindamycin is preferred due its excellent pharmacokinetic properties.

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. [13,19] From such isolates constitutively resistant mutants are emerged and results in treatment failure with clindamycin in vivo which would be demonstrated by D-test. [13,20] So before declaring the clindamycin sensitivity among the clinical isolates of *Staphylococcus aureus*, it is mandatory to check for inducible resistance. Negative D-test among the erythromycin resistant isolates confirm

the sensitivity to clindamycin and possible to choose clindamycin as drug of choice in the treatment of staphylococcal infections.

In our study we found 176(44.89%) erythromycin resistant isolates. Amongst them 46(26.13%) were inducible clindamycin resistant (D-test-Positive), while rest of the isolates were negative for D-test, out of which 103(58.52%) isolates were shown to have constitutive clindamycin resistance and 27(15.34%) showed real susceptibility to clindamycin which were designated as MS phenotypes.

It was also observed that percentage of inducible resistance and constitutive resistance was higher amongst MRSA isolates (30%, 62.85% respectively) as compared to MSSA (11.11%, 41.66% respectively). MS phenotypes were identified more among MSSA (47.22%) as compared to MRSA (7.14%).

Shantala G.B *et.al.* [10] observed, higher incidence of inducible resistance in MRSA (32.53%) as compared to MSSA (15.38%). AH. Shruti *et.al.* [1] reported higher incidence of inducible resistant phenotype among MRSA (28.6%) as compared to

MSSA (2.4%). Deotale V *et.al.*^[15] reported, higher incidence of inducible resistance in MRSA (27.6%) as compared to MSSA (1.6%), which is in concordance with present study.

Some observations differ from present study. Very high incidences of inducible clindamycin resistance were noted by Veena manjunath *et.al.*^[6] (57.6% in MRSA and 16.22% in MSSA) and P.Shreenivasulu Reddy^[13] (46.2% in MRSA and 22.2% in MSSA). While lower incidences were noted by Kavita Prabhu *et.al.*^[2] (20% in MRSA and 6.15% in MSSA) and V Gupta *et.al.*^[18] (20% in MRSA and 17.3% in MSSA).

In present study, MS phenotypes were found to be higher amongst MSSA as compared to MRSA (47.22% & 7.14% respectively). This is in concordance with Veena Manjunath *et.al.*^[6] (62.1% in MSSA and 18.6% in MRSA).

The different patterns of resistance phenotypes observed in various studies are because of iMLS_B resistance varies by geographical region, methicillin susceptibility and even from hospital to hospital.^[21] Hence it should be determined in individual settings.

These observations suggest that had D-test not been performed on 46(26.13%) of erythromycin resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.

CONCLUSION

Reporting *Staphylococcus aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On other hand negative results for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option. Hence it should be mandatory to detect such resistance and for judicious use of

clindamycin. All D-test positive isolates should not be treated with clindamycin but it is the drug of choice for all D- test negative isolates (MS phenotypes).

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How to cite this article: Ghogare HS, Hatkar SS, Bansal MP. Phenotypic detection of inducible clindamycin resistance among the clinical isolates of staphylococcus aureus by using D-test. *Int J Health Sci Res*. 2014;4(3):149-153.
