PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCI

A.Venkata Raghavendra Rao[1], A.Kavitha[2], K.S.Seetha[3]

ABSTRACT

Background: The resistance to antimicrobial agents among *Staphylococci* is an increasing problem. Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to macrolide, lincosamide and streptogramin antibiotics.

In-vitro, routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D-test on a routine basis.

Aim: The aim of the study was to assess the frequency of inducible clindamycin resistance in clinical isolates of *Staphylococci* by using D-test on a routine basis to guide therapy.

Materials & Methods: A total of 108 *Staphylococcus species* were isolated from various clinical samples received in the microbiology department over a period of one year (February 2011 – January 2012) and were subjected to the study. Inducible clindamycin resistance was tested in these isolates by D-test as per CLSI guidelines.

Results: Among the 108 *Staphylococci* isolates, 32 (29.62 %) showed inducible clindamycin resistance and belonged to $iMLS_B$ phenotype. All 32 isolates were Methicillin Resistant *Staphylococcus aureus* (MRSA).

Conclusion: The 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 proper treatment of the patients.

KEY WORDS: Inducible clindamycin resistance, MRSA, $iMLS_B$ phenotype, D-test

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a common cause of both community and nosocomial acquired infections. Infections range from minor skin infections to life threatening conditions such as endocarditis, pneumonia and septicaemia[1]. The growing interest in the resistance of Staphylococcus aureus to various antibiotics in the last two decades, especially methicillin, has led to the use of alternative agents such as clindamycin[2]. Clindamycin belongs to the macrolide, lincosamide and streptogramin (MLS) family that act through inhibition of protein synthesis[1]. Clindamycin has several advantages in the treatment of staphylococcal infections. It can be administered intravenously and orally with good bioavailability. It penetrates the skin and soft tissue easily, exerts an inhibitory action on toxin production, and is relatively inexpensive[2]. Clindamycin, further more, is a useful choice in cases of penicillin allergy. Again development of resistance especially inducible resistance is a major barrier in its usage[1].

Bacterial resistance to antimicrobial agents generally involves drug inactivation, target site modification, impermeability or efflux mechanisms. Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confers resistance to macrolide, lincosamide and streptogramin antibiotics. Macrolide antibiotic resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CONS) may be due to an active efflux mechanism encoded by *msrA* (conferring resistance to macrolides and type B streptogramins only) or may be due to ribosomal target modification affecting macrolides, lincosamides and type B streptogramins (MLS_B resistance). *erm* genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA reducing binding by MLS agents to the ribosome[3].

Isolates with inducible clindamycin resistance are found to be resistant to erythromycin but susceptible to clindamycin when these discs are not placed adjacent to each other during antimicrobial sensitivity testing. Consequently, laboratory identification of such isolates is often missed, resulting in inappropriate therapeutic use of clindamycin and treatment failure. These isolates can

[1]PG Student, [2]Assistant Professor, [3]Professor & Head, Department of Microbiology, VMKV Medical College, Seeragapadi, Salem - 636308

be detected by the D-test, a disc diffusion test in which induction of clindamycin resistance by erythromycin is tested[4].

Phenotypic detection of inducible resistance can be done by this double disc diffusion test.

(D-test). D-test is simple, reliable, inexpensive and easy to interpret with high sensitivity and specificity. Clindamycin is a good option but prevalence of inducible resistance should be known, as it varies by geographical location and bacterial species. So the aim of this study was to assess the frequency of phenotypic expression of inducible *erm* gene expression in clinical isolates of *S.aureus* by D-test[5].

The present study was also aimed to find out the percentage of *Staphylococci* isolates having inducible clindamycin resistance ($iMLS_B$) in our geographical area using D-Test.

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology at V.M.K.V.Medical College, Salem from Feb 2011 to Jan 2012. A total of 108 *Staphylococcus spp.* were isolated from various clinical specimens like pus, wound swab, aspirates, blood & sterile fluids and tested[5]. All isolates were identified morphologically and biochemically by standard laboratory procedures[6]. Antibiotic susceptibilities were studied by modified Kirby Bauer's disc diffusion method on Mueller Hinton Agar plates using Ampicillin (10 µg), Penicillin G (10 units), Co-trimoxazole (25 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), Linezolid (30 µg), Oxacillin (1 µg) and Cefoxitin (30 µg) as per CLSI guidelines[5,7].

The isolates that were found to be erythromycin resistant (zone size $\leqslant$ 13mm) were further studied for inducible clindamycin resistance[8].

Detection of methicillin resistance: An inhibition zone of 10 mm or less around oxacillin disc (1 µg) and 19 mm or less around cefoxitin disc (30 µg) indicates MRSA[5].

The detection of inducible clindamycin resistance was performed using the D-test. Briefly, an erythromycin disc was placed 15 mm (edge to edge) from a clindamycin disc in a standard disc diffusion test. A flattening of the zone of inhibition in the area between the discs where both drugs have diffused after 18-24 hours of incubation was considered to be inducible clindamycin resistance[8]. **[Figure.1 and 2]**

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

- MS Phenotype - Staphylococcal isolates exhibiting resistance to erythromycin (zone size $\leqslant$ 13mm) while sensitive to clindamycin (zone size $\geqslant$ 21mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype.
- Inducible MLS_B ($iMLS_B$) Phenotype - Staphylococcal isolates showing resistance to erythromycin (zone size $\leqslant$ 13mm) while being sensitive to clindamycin (zone size $\geqslant$ 21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype.
- Constitutive MLS_B ($cMLS_B$) Phenotype - this phenotype was labelled for those Staphylococcal isolates which showed resistance to both erythromycin (zone size $\leqslant$ 13mm) and clindamycin (zone size $\leqslant$ 14mm) with circular shape of zone of inhibition if any around clindamycin[5,9].

Quality control of the erythromycin and clindamycin discs was performed with ATCC *Staphylococcus aureus* 25923[8].

RESULTS

Among the 108 total isolates studied 93 (86.11%) were *Staphylococcus aureus* and 15 (13.89%) were coagulase-negative staphylococci (CONS).Among the 93 *S. aureus* strains, 70 (75.27%) were methicillin-resistant *S.aureus* (MRSA) and 23 (24.73%) were methicillin-sensitive *S. aureus* (MSSA).Among the coagulase-negative staphylococci (CONS), 13 (86.66%) were methicillin-sensitive (MSCONS) and 2 (13.33%) were methicillin-resistant (MRCONS).Of the 108 clinical isolates, 39 (36.11%) showed erythromycin resistance. Among the erythromycin-resistant isolates, 37 (94.87%) were *S. aureus* among which 34 were MRSA and 3 were MSSA. Among 39 erythromycin-resistant isolates, 32 (29.62%) of the total isolates were belonged to the $iMLS_B$ phenotype and showed inducible clindamycin resistance. [Figure 1 & 2]

This study revealed a relatively higher incidence of inducible clindamycin resistance among MRSA isolates i.e. 32/70 (45.71%). Only 2 (2.85%) among MRSA isolates were of $cMLS_B$ phenotype [Figure 3] and showed constitutive resistance.

The observed results among these study isolates are depicted in **Table-1**.

Table -1: Resistance Phenotypes of Isolates:

Total Isolates- 108

Figure.1: showing iMLSB phenotype - Erythromycin-R, Clindamycin-S, D-test-Positive in the routine antimicrobial susceptibility testing.

Figure.2: showing iMLSB phenotype - Erythromycin-R, Clindamycin-S, D-test- Positive

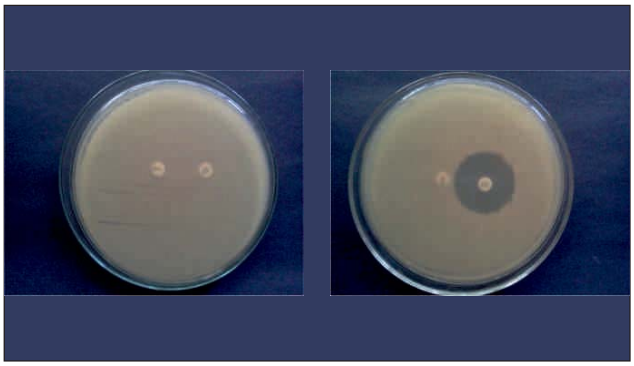

Figure.3: showing cMLSB phenotype - Erythromycin-R, Clindamycin-R

Figure.4: showing MS Phenotype - Erythromycin-R, Clindamycin-S, D test- Negative

Resistance Phenotypes of Isolates					
Organism isolated		**Erythromycin resistant** n=39 (36.11%)	**iMLS $_B$** n=32	**cMLS $_B$** n=2	**MS Phenotype** n=5
Staphylococcus aureus n=93 (86.11%)	**MSSA** n=23 (24.73%)	**3**	-	-	**3**
	MRSA n=70 (75.27%)	**34**	**32** (45.71%)	**2** (2.85%)	-
Coagulase-Negative Staphylococci (CONS) n=15 (13.89%)	**MSCONS** n=13 (86.66%)	**1**	-	-	**1**
	MRCONS n=2 (13.33%)	**1**	-	-	**1**

iMLSB - Erythromycin-R, Clindamycin-S, D test- Positive
cMLSB - Erythromycin-R, Clindamycin-R
MS Phenotype - Erythromycin-R, Clindamycin-S, D test- Negative

DISCUSSION

The increasing prevalence of MRSA infections especially with the spread of resistant strains in the community poses a challenge to physicians in terms of the use of alternative antibiotic agents. Although clindamycin has been considered an acceptable option for patients with community-acquired MRSA infections, reports on high rates of clindamycin-resistant community-acquired MRSA strains are limiting its use[2].

In recent times, clindamycin has become an excellent drug for some Staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients[5]. Clindamycin is a good substitute to treat soft tissue infections by both MRSA and MSSA infections. Its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production are its advantages[1]. Also, clindamycin has a good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics[5]. However, clindamycin resistance can develop in staphylococcal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both in vitro testing and in vivo during clindamycin therapy[5,7].

Since the $iMLS_B$ resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location, D-test becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of *Staphylococcus aureus*. Failure to identify $iMLS_B$ resistance may lead to clinical failure of clindamycin therapy[10].

In our study, MRSA isolates showed higher incidence of inducible (45.71%) than constitutive resistance. It is also observed that there is no $iMLS_B$ resistance among MSSA isolates. This was in concordance with a few of the studies reported before as depicted in **Table-2**.

Table-2: Inducible clindamycin resistance ($iMLS_B$) resistance in various studies:

	iMLS B resistance among	
	MRSA %	MSSA %
Ajap et al [16]	5.7	3.6
Gadepalli et al [13]	30	10
Rahabar M et al [15]	22.6	4
Yilmaz et al [14]	24.4	14.8
Gupta V et al [10]	20	17.33
Ciraj AM et al [8]	38.4	12.9
Pal N et al [11]	43.56	6.93
Deotale et al [9]	27.6	1.6
Shantala GD et al [12]	32.5	15.53
Prabhu et al [5]	20	6.15
Our present study	45.7	-

So keeping in view of this relative high frequency of $iMLS_B$ resistance among MRSA isolates, D-test should be performed in the laboratory as a routine procedure. The D-test is an easy, sensitive and reliable test to perform along with routine susceptibility testing in clinical laboratory settings without specialized testing facilities to detect $iMLS_B$ resistance among *staphyloccocal* isolates which in turn help in proper effective treatment.

The incidence of resistance is highly variable with regard to geographic locality; hence the local data regarding inducible clindamycin resistance is helpful in guiding anti-staphylococcal therapy[6].

CONCLUSION

In the light of restricted antibiotic range available for the treatment of MRSA infections, clindamycin should be considered as a part of treatment regimen for managing serious soft tissue infections. Inducible clindamycin resistance ($iMLS_B$) resistance is a significant problem in *S. aureus* isolates, more so in MRSA as found in our study. Isolates that are erythromycin resistant but clindamycin susceptible should not be reported so, unless tested for $iMLS_B$ resistance in vitro. However double disc diffusion test (D-test) must be implemented in routine clinical laboratories to discriminate between inducible clindamycin resistance and clindamycin susceptibility. D-test can be used as a simple, auxillary and reliable method to delineate inducible and constitutive clindamycin resistance in routine clinical laboratories and helps the clinicians to treat the patients effectively without any treatment failure.

REFERENCES

1. Naima Fasih,Seema Irfan,Afia Zafar,Erum Khan,Rumina Hasan.Inducible clindamycin resistance due to expression of erm genes in Staphylococcus aureus: Report from a Tertiary Care Hospital Karachi, Pakistan: JPMA 2010; 60:750-53.
2. Shouval DS, Samra Z, Shalit I, Livni G, Bilvasky E, Ofir O, Gadba R, Amir J.Inducible clindamycin resistance among methicillin-sensitive Staphylococcus aureus infections in pediatric patients. Isr Med Assoc J. 2011 Oct; 13(10):605-8.
3. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disc diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase negative Staphylococci. J Clin Microbiol 2003; 41:4740-4.
4. Renushri, Saha A, Nagaraj, Krishnamurthy V. Inducible clindamycin resistance in Staphylococcus aureus isolated from nursing and pharmacy students. J Lab Physicians 2011; 3:89-92.
5. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians 2011; 3:25-7.
6. Baird D. Staphylococcus. Cluster forming gram positive cocci. Mackie and McCartney Practical Medical Microbiology.14 th ed. p. 245-58.
7. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. Vol.2, No.1 Clinical Laboratory Standards Institute; 2007.
8. Ciraj AM, Vinod P, Sreejith G, Rajani K. Inducible clindamycin resistance among clinical isolates of staphylococci. Indian J Pathol Microbiol2009; 52:49-51.
9. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian J Med Microbiol 2010; 28:124-6.
10. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in Staphylococcus aureus: A study from North India. J Postgrad Med 2009; 55:176-9.
11. Pal N, Sharma B, Sharma R, Vyas L. Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. J Postgrad Med 2010; 56:182-5.
12. Shantala G B, Adithi S Shetty,Rahul Rao K, Vasudeva, Nagarathnamma T. Detection of inducible clindamycin resistance in clinical isolates of Staphylococcus aureus by the Disc Diffusion Induction Test. Journal of Clinical and Diagnostic Research [serial online] 2011 february [cited: 2012 jul 1]; 5:35-37.
13. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhry R. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus. Indian J Med Res 2006; 123:571-3.
14. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol 2007; 56:342-5.
15. Rahabar M, Hajia M. Inducible clindamycin resistance in Staphylococcus aureus: A cross sectional report. Pak J Biol Sci 2007; 10:189-92.
16. Azap, O. K., Arslan, H., Timurkaynak, F., Yapar, G., Oruc, E. & Gagir, U.Incidence of inducible clindamycin resistance in staphylococci: ?rst results from Turkey. Clin Microbiol Infect 2005; 11: 582–584