Original Article

The Prevalence Of Inducible Clindamycin Resistance Among Gram Positive Cocci From Various Clinical Specimens

Microbiology Section

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ABSTRACT

Clindamycin is an excellent drug which can be used for the treatment of skin and soft tissue infections which are caused by Gram positive cocci and it also serves as an alternative for penicillin in penicillin allergic patients. Clindamycin resistance may be inducible or constitutive. Inducible resistance cannot be detected by the routine antimicrobial susceptibility tests or by the disc diffusion method.

This study was undertaken to study the prevalence of inducible resistance in Gram positive cocci by the D test as per the CLSI guidelines.

73(42%) out of the 168 consecutive isolates of MRSA, MSSA, CONS, S.pneumoniae and S.pyogenes were erythromycin resistant. 27(16%) isolates showed inducible clindamycin resistance and a higher percentage was noted in MRSA (28%) as compared to MSSA (12%) and CONS (14%).

This study indicates the importance of the D test in detecting inducible clindamycin resistance in Gram positive cocci and to use it as an aid in the optimal treatment of the patients.

Key Words: Gram positive cocci, inducible clindamycin resistance, D test

INTRODUCTION

Gram positive organisms are one of the leading pathogens which cause skin and soft tissue infections. [1] The emergence of resistance to antimicrobial agents among staphylococci is an increasing problem [2]. Methicillin resistance Staphylococcus aureus (MRSA) is a notorious nosocomial pathogen which is prevalent in many countries. [3]

The Macrolide- Lincosamide –StreptograminB (MLSB) family of antibiotics serves as an alternative to drug resistant Gram positive organisms, including staphylococci and streptococci [4]. The MLS antibiotics are structurally unrelated but are related microbiologically because of their similar modes of action. They inhibit protein synthesis by binding to the 23S r RNA [2],[3],[5].

Clindamycin is the preferred agent in this group because of its excellent tissue penetration except in CNS, because it accumulates in abscesses, it has good oral absorption and no dosage requirements are needed in the presence of renal disease [1],[2]. Clindamycin is also a useful alternative for penicillin in penicillin allergic patients [1],[5].

However, the widespread use of the MLSB antibiotics has led to an increase in the number of staphylococcal strains which develop resistance to these antibiotics.

Macrolide resistance may be due to the enzymes which are encoded for by a variety of erm genes- MLSB phenotype and may be constitutive (cMLSB) or inducible (iMLSB). A second mechanism of resistance is due to active efflux pump which is encoded for by the msrA gene (MS phenotype) [4],[5],[6]. Strains with inducible clindamycin resistance are not detectable by the routine antimicrobial susceptibility tests as they appear to be erythromycin resistant and clindamycin susceptible in vitro, when they are not placed adjacent to each other. In these cases, the treatment of an infection which is caused by a strain carrying an inducible erm gene by using clindamycin, can lead to clinical failures [5]. In the resistance which is mediated by the msrA genes, the clinical isolates appear to be erythromycin resistant and clindamycin sensitive, both in vivo and in vitro and do not result in clinical therapy failures. The Clinical and Laboratory Standards Institute (CLSI) [7] has recommended the erythromycin –clindamycin disc approximation test (D zone) to detect inducible clindamycin resistance. We studied the prevalence of erythromycin induced clindamycin resistance in Gram positive cocci including S.pyogenes and S.pneumoniae, as there are only a few studies which are available on this aspect.

MATERIAL AND METHODS

A total of 168 consecutive, non duplicate clinical isolates were recovered from pus, blood, urine, CSF, sputum, endotracheal tip, and other specimens which were received at the Department of Microbiology over the period from November 2009 to July 2010.

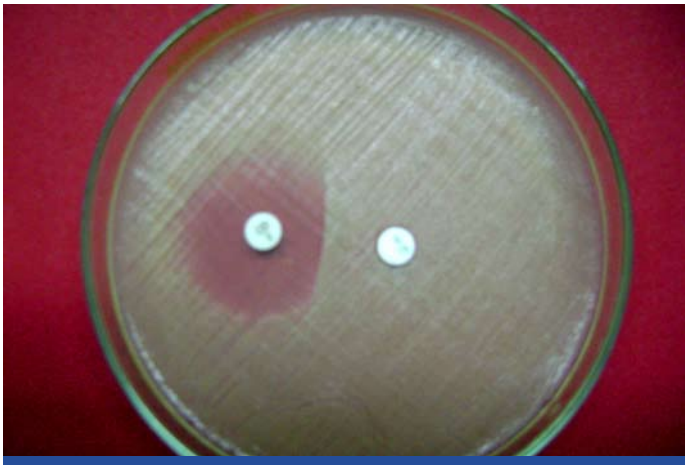
The isolates included Methicillin resistant S.aureus (n=47), Methicillin susceptible S.aureus (n=73), Coagulase negative Staphylococci (n=36), Streptococcus pyogenes (n=8) and Streptococcus pneumoniae (n=4) [Table/Fig 1]. All the isolates were identified by using conventional methods. The isolates that were found to be erythromycin resistant by the Kirby-Bauer disc diffusion method were subjected to the D zone test for inducible clindamycin resistance as per the CLSI guidelines. The clindamycin (2µg) and erythromycin (15µ g) discs were procured from HiMedia India, Private Limited.

The clindamycin (2µg) discs were placed at a distance of 15mm (edge to edge) from the erythromycin (15µ g) discs on the same plate and were incubated at 37°C overnight. A flattening of the zone (D shaped) around clindamycin in the area between the two discs indicated inducible clindamycin resistance.

Three different phenotypes were identified [4].

Specimen	MRSA	MSSA	CONS	S.pneumoniae	S.pyogenes	Total
Pus	18	31	7	0	2	58
Blood	2	14	5	0	0	21
Urine	8	17	17	0	0	42
CSF	0	0	2	1	0	
Sputum	3	0	0	3	6	12
Tracheal swab	7	9	4	0	0	20
Ear swab	5	0	1	0	0	6
Others	4	2	0	0	0	6
Total	47	73	36	4	8	168

[Table/Fig 1]: Sources and categorization of clinical isolates.



[Table/Fig: 2] Isolate with inducible Clindamycin resistance (D test positive)

The Inducible MLS$_B$ phenotype:

Isolates which were resistant to erythromycin and sensitive to clindamycin with a D zone of inhibition around the clindamycin disc. [Table/Fig 2]

The Constitutive MLS$_B$ phenotype:

Isolates which were resistant to both erythromycin and clindamycin.

The MS phenotype:

Isolates which were resistant to erythromycin and susceptible to clindamycin.

RESULTS

One hundred and sixty eight isolates of Gram positive cocci from various specimens were tested for susceptibility to erythromycin and other antibiotics by the Kirby-Bauer disc diffusion test. 73(43%) isolates were resistant to erythromycin. The results which were observed, are depicted in [Table/Fig 3]

Organism	Total no of isolates	iMLSB phenotype (%)	cMLSB phenotype (%)	MS phenotype (%)	No.of isolates Susceptible to E and C
MSSA	73	8(11)	7(10)	9(12)	49(67)
MRSA	47	13(28)	9(19)	14(30)	11(23)
CONS	36	6(17)	-	5(14)	25(53)
S.pyogenes	8	1(13)	-	1(13)	6(75)
S.pneumoniae	4	-	-	-	4(100)
Total	168	28	16	29	95

[Table/Fig 3]: The observed results among the various isolates of Gram positive cocci

DISCUSSION

73 (43%) out of 168 Gram positive isolates including MRSA, MSSA, CONS and Group A streptococci were erythromycin resistant. Among these, 28 (16%) of the isolates showed inducible clindamicin resistance by the D test, 16(10%) showed constitutive resistance and 29 (18%) showed the MS phenotype. Some investigators have reported a higher incidence of iMLSB resistance, while others have indicated a lower incidence [1],[5]. In our study, we also observed a similar rate of iMLSB resistance among S.aureus (28%) and CONS (17%), while a few others have reported variable results .This variability could be due to the differences in the geographical area, age group, or methicillin susceptibility. All the 28% iMLSB resistant S.aureus isolates were susceptible to Linezolid and Vancomycin, while only 67% of the isolates were susceptible to ciprofloxacin It was also observed that the percentages of inducible clindamycin resistance were higher in the MRSA isolates (28%) as compared to the MSSA (11%) and the CONS (17%) isolates. This is in concordance with various studies which reported the prevalence of erythromycin induced clindamycin resistance. We also observed that the MS phenotype was also higher in the MRSA (30%), MSSA (12%) and the CONS (14%) isolates, which was contrary to the findings of the studies performed by Fiebelkorn et al [8], Fokas et el [9] and Jenssen and Schimitz [10].

Constitutive resistance was seen in 19% of the MRSA and 10% of the MSSA, which was contrary to the findings of the study which was done by Angel et al, which did not find constitutive resistance.

Though there are a number of reports on the pattern of macrolides resistance in Gram positive organisms, each report from different regions has shown a different pattern of resistance. *S.pneumoniae* and S.pyogenes did not have constitutive resistance, which was in agreement with the reports by Angel et al [1]. Macrolide resistance in *S.pneumoniae* is increasing since 1990 and this could be due to target modification by the ermB genes or due to a non inducible, macrolides specific efflux mechanism which is encoded for by the mefE genes [11].

MLSB resistance is the most widespread and clinically important mechanism of resistance which has been encountered in Gram positive organisms due to the production of methylases and the efflux pump mechanism.

Clinically, bacterial strains exhibiting iMLSB have a high rate of mutation to constitutive resistance and the use of non inducer antibiotics such as clindamycin can lead to the selection of constitutive mutants and may result in clindamycin treatment failure [3],[12].

The emergence of resistance to multiple antibiotics among the Gram positive cocci has left very few therapeutic options for clinicians. A therapeutic decision is not possible without the relevant clinical data.

The increasing frequency of MRSA with in vitro inducible clindamycin resistance raises a concern of clindamycin treatment failures and this is where the D test becomes significant.

To conclude, due to the emergence of resistance to antimicrobial agents, the accurate drug susceptibility data of the infecting microbe is essential for deciding the therapeutic option. The Erythromycin-Clindamycin disc approximation test or the D test is a simple, reliable method to detect Clindamycin resistance in erythromycin resistant isolates .The D test is an easy to perform test which can enable us in guiding the clinicians regarding the judicious use of clindamycin in skin and soft tissue infections.

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