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Clindamycin Resistance Constitutive and Inducible Patterns in Erythromycin Resistant Clinical Isolates of *Staphylococcus* Species

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Abstract: Methicillin resistant *Staphylococcus aureus* (MRSA) is a well-recognised hospital pathogen. In the recent years, MRSA is increasingly being isolated from the community. Clindamycin is frequently the drug of choice in such isolates. However, use clindamycin in erythromycin resistant *Staphylococcus* isolates could result in treatment failure as a result of inducible clindamycin resistancein spite of showing*in vitro* sensitivity. Current study was conducted to detect the presence of inducible clindamycin resistance in erythromycin resistant *Staphylococcus* isolates by D-zone test,correlate clindamycin resistance phenotypes with minimum inhibitory concentrations (MICs) of clindamycin, erythromycin, oxacillin and vancomycin among the isolates and. correlate various resistance phenotypes with methicillin resistance. One-hundred and fifty non duplicate isolates of *Staphylococcus* species were identified and antibiotic susceptibility testing was done using Kirby Bauer's disc diffusion method. MICs were determined using E-test for oxacillin, vancomycin,clindamycin and erythromycin using E-test strips (Himedia).Out of150 *Staphylococcus*clinical isolates, 96 were *S.aureus* and 54 were coagulase negative*Staphylococci* (CONS).About 78 (81.2%) of the *S.aureus* isolates and 39 (72.2%) of the CONS were found to be methicillin resistant. Inducible clindamycin resistance was reportedin 59 (39.3%) of the isolates, constitutive resistance phenotype in 48% while 12.7% demonstrated MS phenotype.Out of inspected isolates 18 and 11.3% had MICs for clindamycin between 0.01-0.06 µg/mLand 0.06-0.1 respectively. However12.5% had MIC ranging from 4-8 µg/mLand more than half of the isolates(58%) had MIC > 8 µg/mL. Constitutive resistant phenotype (cMLS) was the predominant phenotype in methicillin resistant isolates. MS phenotype was the predominant among MSSA (methicillin sensitive *S. aureus*) while MSCNS (methicillin sensitive CONS) cMLS (46.7%) predominated.MIC of all erythromycin resistant isolateswere ≥240 µg/mL. Nearly16.7% of the cMLS and 57.9% of MS isolates were found to be oxacillin sensitive and 83% of iMLS and 83.3% of MS phenotype isolates were oxacillin resistant on MIC testing. 47.2% of cMLS and 73.6% of MS isolates had MIC ≤ 2 µg/mL for vancomycin and 52.7% of cMLS and 26.3% of MS isolates had MICs in intermediate range for vancomycin. D-testing might help clinicians to decide whether to use clindamycin in *Staphylococcal* infectionswhen erythromycin resistance is present. Determination of MICs helps to identify exact sensitivity profile of isolates in cases where clinical failure occurs due to misleading disk diffusion tests.

Key words: MRSA · Clindamycin · Inducible

INTRODUCTION

Macrolide-lincosamide-streptogramin (MLS) antibiotics are commonly used in treatment of staphylococcalinfections especially methicillin resistant *Staphylococci* [1]. Clindamycin (CLI) is a frequent choice for some staphy lococcalin fections, especially skin and soft-tissue infections. 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) [2,3] 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

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methylation of the 23S rRNA, reducing binding by MLS agents to the ribosome [4]. Rarely resistance could be due to inactivation of lincosamides by chemical alteration mediated by *lnuA* gene [5].

Erythromycin (ERY) is an effective inducer whereas CLI is a weak inducer [6]. In vitro *S.aureus* isolates with constitutive resistance are resistant to both ERY and CLI whereas those with inducible resistance are resistant to ERY and appear sensitive to CLI ($iMLS_B$) [7]. If clindamycin is used for treatment of infection with such an isolates ($iMLS_B$), selection for constitutive *erm* mutants occurs which may lead to treatmentfailure. This inducible MLS_B resistance can be detected by a simple disc approximation test, commonly referred to as D-test. For this test, an ERY (15µg) disc is placed 15-26 mm (edge to edge) from a CLI (2 µg) disc in a standard disc diffusion test. Following incubation, a flattening of the zone in the area between the discs where both drugs have diffused indicates that the organism has inducible clindamycin resistance [8].

Current study was undertaken to study the prevalence of inducible clindamycin resistance in erythromycin resistant *Staphylococcus* isolates using D- Test.To correlate various clindamycin resistance phenotypes with clindamycin, erythromycin, oxacillin and vancomycin minimum inhibitory concentrations (MICs) and to study these resistance phenotypes in relation to methicillin resistance.

MATERIALS AND METHODS

The prospective study was conducted in the Department of Microbiology, J.N Medical College. One hundred and fifty non duplicate clinical isolates of erythromycin resistant*Staphylococcus* species isolated from samples received from various outpatient and inpatient departments of the hospital were included in the study. The isolates were identified using standard biochemical tests according to standard techniques [9] and antibiotic susceptibility testing was done using Kirby Bauer's disc diffusion method on Mueller Hinton agar using erythromycin (15 µg), norfloxacin (5 µg), vancomycin (30 µg), clindamycin (2 µg), oxacillin (1 µg) and cefoxitin (30 µg) as described byClinical and Laboratory Standards Institute (CLSI) guidelines [10]. Erythromycin and clindamycin disks were placed adjacent to each other at a distance of 15mm (edge to edge) to detect inducible resistance. Isolate was labelled as erythromycin resistant if zone size was ≤13 mm and resistant to clindamycin if zone size was≤14. Sensitive resistant isolates were further tested for minimum inhibitory concentrations (MICs) of erythromycin, clindamycin, oxacillin and vancomycin using E- test strips (HiMedia). All the erythromycin-sensitive strains were excluded from the study.

Following phenotypes could beobserved after disk diffusion testing.

- Inducible MLS(iMLS) phenotype - Staphylococcal isolates showing resistance to erythromycin while being sensitive to clindamycin and giving D-shaped zone of inhibition around clindamycin with the flattening facing erythromycin disc.
- Constitutive MLS(cMLS)phenotype - Those *Staphylococcus* isolates, which showed resistance to both erythromycin and clindamycin with circular shape of zone of inhibition, if any around clindamycin.
- MS phenotype - Isolates exhibiting resistance to erythromycin and sensitivity to clindamycin and giving circular zone of inhibition around clindamycin.

Determination of minimum inhibitory concentration (MIC) using E-test:MICs were determined using E-test for oxacillin, vancomycin,clindamycin and erythromycin in all isolates. Test was done using E-test strips (Himedia) with the following graded concentrations of antibiotics according to manufacturer's instructions.

Oxacillin: OxacillinEzy MIC™ Strip (OXA) (0.016-256 µg/mL). MIC ≤ 2µg/mL was taken as sensitive and ≤ 4µg/mL as resistant for *S. aureus*. In CONS, MIC ≤ 0.25µg/mL was regarded as sensitive and ≤ 0.5µg/mL as resistant.

Vancomycin: VancomycinEzy MIC™ Strips (VAN)(0.016-256 µg/mL). MIC ≤ 4µg/mL was taken as sensitive, 8-16 µg/mL as intermediate and ≤ 32 µg/mLas resistant.

Clindamycin: Clindamycin HiComb™ MIC Strip having antibiotic concentration gradient from 0.001-8 µg/mL. MIC ≤ 0.5µg/mL was taken as sensitive, 1-2 µg/mL as intermediate and ≤ 4µg/mL as resistant.

Erythromycin: Erythromycin HiComb™ MIC Strip (0.01-240 µg/mL). MIC ≤ 0.5µg/mLwas taken as sensitive, 1-4 µg/mL as intermediate and ≤ 8µg/mL as resistant.

RESULTS

Of the 150 erythromycin resistant *Staphylococcus* isolates, 96 were of *S. aureus* and 54 were coagulase negative*Staphylococci*(CONS).Seventy nine of the 150 samples were recoveredfrom outpatient department while 71 were from inpatient department. Among 96 erythromycin resistant isolates of *S. aureus* 78 (81.2%) were found to be methicillin resistant while 39 (72.2%) of the CONS were resistant to methicillin. Inducible clindamycin resistance was found in 39.3% of the isolates, constitutive resistance phenotype in 48% while 12.7% demonstrated MS phenotype. Constitutive resistant phenotype was the predominant phenotype in methicillin resistant isolates (*S. aureus*and CONS). MS phenotype was the predominant among MSSA while MRCNS isolates were equally distributed amongiMLS and MS phenotypes (26.7%) which predominated over cMLS (4.7%) (Table 1).

MIC for erythromycin was found to be ≤240 µg/mL in all the resistant isolates. Among 59 iMLS isolates majority (83%) were resistant to methicillin as well while most (72.8%) of them were sensitive to vancomycin. 27.1% isolates showed intermediate sensitivity to vancomycin (MICs ranging between 4-8 µg/mL),however these isolates were interpreted as sensitive on disk diffusion testing (zone size >15 mm). 16.7% of the cMLS and 57.9% of MS isolates were found to be oxacillin sensitive and 83% of iMLS and 83.3% of MS phenotype isolates were oxacillin resistant on MIC testing. 47.2% of cMLS and 73.6% of MS isolates had MIC ≤ 2 µg/mL (sensitive) for vancomycin and 52.7% of cMLS and 26.3% of MS isolates had MICs in intermediate ranege for vancomycin (Table 2).

About 18% of all the isolates had MICs ranging from 0.01-0.06 µg/mL and 11.3% had MICs between 0.06-0.1.12.5% had MIC ranging from 4-8 µg/mL while 58% had MIC > 8 µg/mL. Majority of the iMLS (47.4%) and cMLS (81.9%) isolates had MIC >8 µg/mL. All the isolates with MS phenotype had MIC between 0.01-0.06 µg/mL (Table 3).

Table 1: Distribution of isolates according to clindamycin resistance phenotypes.

Phenotype	MRSA (n=78)	MSSA (n=18)	MRCNS (n=39)	MSCNS (n=15)	Total (n=150) (%)
iMLS	33	6	16	4	59 (39.3)
cMLS	38	5	22	7	72 (48)
MS	7	7	1	4	19 (12.7)

Table 2: Correlation of MICs for oxacillin and vancomycin with clindamycin resistance phenotype.

Antibiotic	MIC (µg/mL)	iMLS (n=59)	cMLS (n=72)	MS (n=19)
Oxacillin	≤ 2	10	12	11
	≥4	49	60	8
Vancomycin	≤ 2	43	34	14
	4-8	16	38	5
	≥ 16	-	-	-

Table 3: Clindamycin MIC ranges in different phenotypes.

MIC	iMLS (n=59)	cMLS (n=72)	MS (n=19)
0.01-0.06	8	-	19
0.06-0.1	13	4	-
4-8	10	9	-
>8	28	59	-

DISCUSSION

In recent times, clindamycin has become an excellent drug for some Staphylococcal infections and as an alternative to vancomycin in (Community Acquired MRSA)CAMRSA strains. It has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics [11]. However there has also been a considerable increase in resistance to clindamycin among clinical isolates including inducible resistance.

The differentiation of inducible MLS_B ($iMLS_B$ phenotype) isolates from isolates with (MS phenotype) resistance is a critical issue because of the therapeutic implications of using clindamycin to treat a patient with an inducible clindamycin-resistant *S.aureus* isolate.

Also from such isolates, spontaneous constitutively resistant mutants have arisen both *in vitro* testing and *in vivo* during clindamycin therapy [12]. Moreover negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option [13].

In our study from among 150 erythromycin resistant isolates, 39.3% had inducible clindamycin resistance. Further, this inducible resistance was higher in MRSA (42.3%) isolates as compared to MSSA (33.3%) and higher in MRCNS (41%) compared to MSCNS (4.7%). Similar pattern has been observed in earlier studies also. Gadepalli *et al*. reported 30% inducible clindamycin resistance in MRSA and 10% in MSSA [14]. Study conducted by Ajantha*et al*.showed inducible clindamycin resistance of 74% in MRSA and 45% in MSSA [15]. But there are a few studies which have reported higher proportion of inducible resistance in MSSA (68%) as compared to MRSA (12.5%) [16]. Hence the true sensitivity to clindamycin may vary from hospital to

hospital, geographic location, patient age, bacterial species and bacterial susceptibility profile [17-19].

On disk diffusion testing, constitutive resistance (48%) was found to be higher than inducible (39.3%) and MS (12.7%) phenotypes. Similar results were found in study by Fiebelkorn *et al.* [8] in 2003 in which out of 114 erythromycin-resistant *S.aureus* isolates, 39 demonstrated constitutive resistance pattern to clindamycin while 33 showed inducible resistance. We found 8.9 and 2.6% of MS phenotype in MRSA and MRCNS respectively. Though MS phenotype is not usually seen in methicillin resistant isolates,a study conducted by Gupta *et al.* [6] in 2009 demonstrated 16% MS phenotype were MRSA. These differences highlight the variations and importance of inducible clindamycin resistance investigation in different geographical settings.

MICs were determined for all isolates using E-test. Unlike disk diffusion test, E test did not differentiate among inducible and constitutive phenotypes. However we observed that all cMLSisolates with MICs for clindamycin in the sensitive range were lying between 0.06-0.1 µg/mL while among those with iMLS phenotype 8 isolates had MIC ranging from 0.01-0.06 µg/mL and 13 isolates had MIC between 0.06-0.1 µg/mL.

There were 21 isolates of *Staphylococci*which had MICs in sensitive range but they revealed inducible resistance on disk diffusion testing. These patients would suffer treatment failure in case isolate is not specifically tested for induction. However, MIC determination helps to detect intermediate susceptibility to clindamycin which could not be detected in case only disk diffusion methods are employed. Also it is useful to correlate the MICs of antibiotics with resistance phenotypes. In our study we found 12.5% of cMLS and 16.9% of iMLS phenotype had MICs in intermediate range. In our study all the isolates with MS phenotype had MIC in sensitive range (0.01-0.06 µg/mL) indicating these isolates can be used fortreatment.However, a study by Sireesha and Setty [1] in 2012demonstrated MIC of clindamycin to be >128 µg/mL in all the MS phenotypes which they attributed to hetero-resistance or some other unknown mechanism. Moreover, there are also reports of successful use of clindamycin in treating patients with D-test-positive isolates [20,21]. Studies have also revealed that it may be risky to use clindamycin when erythromycin testing shows a resistant or intermediate phenotype [14]. Hence, MIC determination is an important tool to determine the use of antibiotics in patients where simple disk diffusion testcharacteristics could not differentiate sensitive from resistant isolates. Molecular markers for the *erm* gene are also available, but they are costly and inconvenient for everyday use [9, 22].

Hence implementation of disc induction test provides an inexpensive, reproducible and reliable method during routine antimicrobial susceptibility testing to distinguish inducible from constitutive clindamycin resistance among isolates. E-test is also a simple laboratory method to determine MIC values and to identify isolates whose resistance pattern and hence clinical outcome cannot be ascertained by simple disk diffusion method.

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