ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2019; 5(2): 123-126
www.allresearchjournal.com
Received: 19-12-2018
Accepted: 22-01-2019

Supare Sundaram
Senior Resident, Department of Microbiology, GMC, Nagpur, Maharashtra, India

Rahangdale Hina
Assistant Professor, Department of Microbiology, GMC, Nagpur, Maharashtra, India

Agrawal Vandana
Professor, Department of Microbiology, GMC, Nagpur, Maharashtra, India

Kokate Sandeep
Associate Professor, Department of Microbiology, GMC, Nagpur, Maharashtra, India

Shrikhande Sunanda
Professor and Head, Department of Microbiology, GMC, Nagpur, Maharashtra, India

Correspondence
Rahangdale Hina
Assistant Professor, Department of Microbiology, GMC, Nagpur, Maharashtra, India

Clindamycin as a plausible treatment option for methicillin resistant Staphylococci: Significance of inducible clindamycin resistance

Supare Sundaram, Rahangdale Hina, Agrawal Vandana, Kokate Sandeep and Shrikhande Sunanda

Abstract
Introduction: There has been renewed interest in use of Macrolide-Lincosamide-Streptogramin B (MLS_B) due to increasing resistance in methicillin resistant *Staphylococcus aureus* (MRSA). Clinical failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to MLS_B antibiotics. We report different resistant phenotypes of MLS_B in *Staphylococcus aureus* (*S. aureus*) and coagulase negative *Staphylococcus* species (CONS).
Material and Methods: A total of 277 Staphylococcal isolates were collected from invasive clinical samples over a period of one year. Isolates were identified by standard microbiological procedures and subjected to antimicrobial susceptibility testing as per CLSI guidelines. Staphylococcal isolates were screened for methicillin resistance and Macrolide-Lincosamide-Streptogramin B (MLS_B) phenotypes.
Results: Out of 277 isolates, 253 were *S. aureus* and 24 were CONS. Among 163(58.14%) MRSA isolates, 55(33.74%) and 34(20.86%) isolates showed inducible ($iMLS_B$) and constitutive ($cMLS_B$) clindamycin resistance respectively, while 19(11.11%) isolates presented with M/MS_B phenotype. Inducible and constitutive clindamycin resistance was found to be greater in MRSA isolates than in methicillin sensitive *Staphylococcus aureus* (MSSA) (11.11% $iMLS_B$, 13.33% $cMLS_B$, 17.78% M/MS_B).
Conclusion: Higher prevalence of $iMLS_B$ and $cMLS_B$ phenotypes specially among MRSA emphases the need of D-test to be performed while using clindamycin as an alternative to higher and parenteral antistaphylococcal antimicrobials.

Keywords: Clindamycin resistance, phenotype, MRSA, MLS_B

1. Introduction
Staphylococcus, in particular methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a major global problem both in the community and the hospital [1, 2, 3]. There is a need to enforce common and appropriate antimicrobials in the treatment policy of such infections as an alternative to expensive and parentral antimicrobials like vancomycin, telavancin, daptomycin, linezolid, ceftaroline or rifampicin [4].
Often referred to as a superbug MRSA infections may begin as a skin sore, pimple or boil before becoming serious and potentially harmful [5]. Medication options for MRSA infections that are of low cost and can be given orally include clindamycin, doxycycline or minocycline of tetracycline group, trimethoprim sulfamethoxazole and ciprofloxacin [6, 7].
This promoted us to identify invasive clinical isolates of MRSA in our hospital and to access clindamycin as a treatment option for them.

2. Material and Methods:
This observational study was conducted in the department of Microbiology in a 1400 bedded tertiary care hospital in central India from January 2018 to December 2018.

i) Isolate pool: A total of 277 nonreplicate clinical and invasive isolates of Staphylococci were obtained from blood, CSF, pleural fluid and deep seated abscess using standard bacteriological techniques and identified by conventional methods [8, 9].

ii) Antimicrobial susceptibility testing: In addition to the regular panel of antimicrobial discs, each isolate was screened for methicillin resistance by using cefoxitin (30 µg/disc). Strength of the inoculum was adjusted and matched with turbidity of 0.5 McFarland standard. Performance of the test and interpretation of results was as per CLSI guidelines [10].

iii) D test: Presence of inducible clindamycin resistance was detected by D-test [11].

Test: Erythromycin (15ug/ disc) was placed at a distance of 15mm (edge to edge) from clindamycin (2ug/disc) on Muller Hinton agar plate, previously innoculated with bacterial suspension equivalent to 0.5 McFarland standard. Following overnight incubation at 37 ^{0}C, flattening of zone (D-shaped) around clindamycin in the area between the two disc, indicate inducible clindamycin resistance.

Interpretation: Three different phenotypes were appreciated after testing and then interpreted

a) **iMLS$_B$ phenotype :** *Staphylococcus* isolates showing resistance to erythomycin (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 was labeled as D-test positive.
b) **cMLS$_B$ phenotype:** *Staphylococcus* isolates which showed resistance to both erythromycin (≤13mm zone size) and clindamycin (≤14mm zone size) with circular shape zone of inhibition if any around clindamycin was labelled as cMLS$_B$ phenotype.
c) **M/MS$_B$ phenotype:** *Staphylococcus* isolates exhibiting resistance to erythomycin (≤13mm zone size) while sensitive to clindamycin (≥21mm zone size) and giving circular zone of inhibition around clindamycin was labeled as having M/MS$_B$ phenotype.

iv) Quality control

a) Quality of Muller Hinton agar was checked for sterility and by its ability to support the growth of ATCC 25923 S.aureus.
b) Quality of discs of erythromycin (15ug) and clindamycin (2ug) disc was checked by an appropriate zone of inhibition using ATCC 25923 *S. aureus*

3. Results

Out of total 277 invasive clinical isolates of *Staphylococcus* spp, 253(91.34%) were *Staphylococcus aureus* and the remaining 24(8.66%) were coagulase negative Staphylococci. Of these 114 (41.16%) isolates were sensitive to both erythomycin (E) and clindamycin (CD).

Table 1: Phenotypes of Macrolide-Lincosamide-Streptogramin B resistance in Staphylococci

Phenotype	*Staphylococcus aureus* (n =253)		Coagulase negative *Staphylococcus* spp.(n=24)		Total (%)
	MRSA (%)	MSSA (%)	MRCONS (%)	MSCONS (%)	
E-S; CD-S	55(33.74)	52(57.78)	1(10)	6(42.88)	114(41.16)
iMLS$_B$	55(33.74)	10(11.11)	1(10)	0	66(23.83)
cMLS$_B$	34(20.86)	12(13.33)	7(70)	7(50)	60(21.66)
M/MS$_B$	19(11.66)	16(17.78)	1(10)	1(7.14)	37(13.36)
Total (%)	163(58.84)	90(32.29)	10(3.61)	14(5.05)	277(100%)

S- Sensitive; E-erythromycin; CD– clindamycin; MSCONS- methicillin sensitive coagulase negative Staphylococci; MRCONS- methicillin resistant coagulase negative Staphylococci

Inducible clindamycin resistance was found in almost one third 55(33.74%) of 163 MRSA and in 10(11.11%) out of 90 MSSA with a phenotype iMLS$_B$.

Constitutional clindamycin resistance was found in 34(20.86%) MRSA isolates & 12(13.33%) of MSSA isolates with phenotype cMLS$_B$

Resistance to macrolide with or without streptogramins B with phenotype M/MS$_B$ was found in 19(11.66%) of MRSA & 16(17.78%) of MSSA.

Of the 24 coagulase negative Staphylococci (CONS), 10(3.61%) were MRCONS. Of these 1(10%) isolates showed inducible clindamycin resistance & 7(70%) isolates showed constitutional clindamycin resistance.

4. Discussion and Conclusion

The frequency of Staphylococcal infections and its resistance to different antimicrobials is increasing worldwide. It has led to decrease in options available for treatment of drug resistant Staphylococcal infections. Hence, there is a renewed interest in use of clindamycin for treatment of drug resistant Staphylococcal infections [11].

Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral administration, good tissue penetration, accumulation at the site of infections and no renal dosing adjustments are needed [12]. Good oral absorption makes it an acceptable option in out-patient therapy or as follow up after parenteral therapy [13, 14]. Clindamycin is a good alternative for the treatment of both methicillin resistant and sensitive Staphylococcal infections and also in penicillin allergic patients [15, 16, 17].

In the present study, out of 253 S.aureus, methicillin resistant isolates (58.84%) were more than methicillin sensitive isolates (32.29%) (Table 1)

In light of the restricted range of antibiotics available for the treatment of MRSA with known limitations of glycopeptides and high cost of polypeptides, clindamycin may be considered for the management of serious soft tissue infections [18, 19].

It has been reported that in-vitro clindamycin susceptibile isolates have often failed to give in-vivo response to clindamycin [20]. The resistance to clindamycin besides being constitutive is also inducible. The resistance is due to ‘erm gene’ that codes for enzyme methylase that inactivates clindamycin. At times the ‘erm gene’ may fail to express itself leading to sensitive phenotypes in the in-vitro tests. However, their expression can be induced in vivo leading to treatment failure. Erythromycin is one such inducer which can induce the expression of ‘erm gene’ in-vitro. This forms the basis of the D-test which is of immense clinical utility.

In the present study 33.74% Staphylococcal infections with MRSA would not have responded to clindamycin had

inducible clindamycin resistance not been detected by D-test *in vitro*. Treatment using clindamycin can be omitted in patients with infections caused by inducibly resistant strains and therapeutic failures may thus be avoided.

Table 2: A three year review (2015-2018) of Phenotypes of MLS_B in MRSA& MSSA

STUDY	*Staphylococcus aureus*					
	MRSA			MSSA		
	$iMLS_B$ (%)	$cMLS_B$(%)	M/MS_B(%)	$iMLS_B$(%)	$cMLS_B$(%)	M/MS_B(%)
Kalbhor P *et al.* 2018 [21]	16(24.62)	12(18.46)	17(26.15)	48(18.97)	31(12.25)	50(19.76)
Jarajreh D *et al.* 2018 [22]	33(76.7)	8(18.6)	2(4.7)	-	-	-
Shetty J *et al.* 2017 [1]	13(27.1)	25(52.1)	8(16.7)	9(11)	18(22)	12(14.6)
Nikam A *et al.* 2017 [23]	23(29.87)	33(42.85)	13(16.83)	3(3)	15(15)	14(14)
More S *et al.* 2017 [24]	28(22.58)	31(25)	12(9.68)	13(8.9)	11(7.5)	21(14.38)
Adhikari R *et al.* 2017 [25]	19(27.9)	37(54.4)	7(10.3)	12(5.9)	42(20.8)	30(14.8)
Majhi S *et al.* 2016 [26]	32(24.8)	30(23.3)	29(22.5)	14(7.5)	7(8.7)	12(15)
Regha I *et al.* 2016 [27]	32(34.78)	10(10.87)	18(19.6)	4(3.1)	0	28(21.9)
Mokta K *et al.*2015 [28]	23(28.04)	24(29.26)	11(13.41)	25(9.32)	36(13.43)	18(6.71)
Banik A *et al.* 2015 [29]	20(15.38)	39(30)	11(8.47)	6(5.31)	2(1.77)	17(15.5)
Sande S *et al.* 2015 [30]	30(33.3)	42(46.6)	18(20)	16(25)	12(19.3)	34(54.8)
Supriyarajvi *et al.* 2015 [31]	23(30.66)	13(17.33)	15(20)	22(15.82)	7(5.03)	20(15.32)
Present study	55(33.74)	34(20.86)	19(11.66)	10(11.11)	12(13.33)	16(17.78)

Note: There was only one report on MLS_B phenotypes in CONS available i.e. Supriyarajvi *et al.* which has shown 21.56% of $iMLS_B$, 13.07% $cMLS_B$, 19.60% M/MS_B in MRCONS isolates and 11.21% $iMLS_B$, 15.03% $cMLS_B$ & 26.31% of M/MS_B in MSCONS isolates.

We found that the percentage of both $iMLS_B$ phenotypes and $cMLS_B$ phenotypes were higher among MRSA as compared to MSSA. Similar observations have been reported by all authors as shown in Table 2.

$iMLS_B$ phenotypes was found to be higher than $cMLS_B$ phenotype amongst MRSA in our study which is in agreement with some reports [21, 27, 31] and not in agreement with others [1, 23, 24, 25, 29, 30]. The differences could be due to their distribution in different geographical regions or variations in antimicrobial prescription pattern in different regions [32, 33].

Though clindamycin has been recommended for use in treatment of MRSA infections, it must be used with caution. Indiscriminate use can led to pseudomembranous colitis by Clostridium difficile. Its use should be avoided if the MRSA isolates are sensitive to tetracycline, ciprofloxacin or co-trimoxazole, which are also treatment options for MRSA.

It is a known fact that judicious use of antimicrobials should be promoted to treat common infections. Oral & cost effective antimicrobials should be preferred. Use of glycopeptides or polypeptide group of drugs should be avoided for treating MRSA as they are a reserve group to be used as a last resort in serious life threatening infections.

Lastly, usefulness of D-test for guiding the clinical use of clindamycin for treating MRSA infections is beyond doubt. Clindamycin should be used clinically only after the laboratory report indicates absence of both constitutive and inducible resistance in Staphylococcal isolates.

5. References

1. Shetty J, Afroz Z. Prevalence of constitutive and inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care institute in North India. Int J Res Med Sci. 2017; 5(7):3120-5.
2. Lall M, Sahni AK. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Med J Armed Forces India. 2014; 70:43-7.
3. Fokas S, Tsironi M, Kalkani M, Dionysopouloy M. Prevalence of inducible clindamycin resistance in macrolide resistant *Staphylococcus* spp. Clin Microbiol Infect 2005; 11:337-40.
4. Choo E, Chambers H. Treatment of Methicillin-Resistant *Staphylococcus aureus* Bacteremia. Iinfection & chemotherapy. 2016; 14(4):267-73.
5. Tiwari H, Das A, Sapkota D, Sivrajan K, Pahwa V. Methicillin resistant *Staphylococcus aureus*: prevalence and antibiogram in a tertiary care hospital in western Nepal. J Infect Dev Ctries. 2009; 3(9):681-4.
6. Charlebois E, Perdreau-Remington F, Kreiswirth B, Bangsberg D, Ciccarone D, Diep B *et al*. Origins of community strains of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 2004; 39:47-54.
7. Almer L, Shortridge V, Nilius A, Beyer J, Soni N, Bui M *et al*. Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis. 2002; 43:225-32.
8. Koneman E, Allen S, Janda W *et al*. Charts. In: Koneman's colour atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins. 2006, 1443-535.
9. Colle JG, Mikes RS, Watt B. Tests for identification of bacteria. In: Collee J G, Marmion BP, Fraser AG, Simmons A, editors. Mackie and MacCartney Practical Medical Microbiology. 14th ed. London: Churchil Livingston, 1996, 131 -49.
10. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100, Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
11. Frank A, Marcinak J, Mangat P, Tjhio J, Kelkar S, Schreckenberger P *et al.* Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J. 2002; 21:530-4.
12. Kasten M. Clindamycin, metronidazole, and chloramphenicol. Mayo Clin Proc. 1999; 74:825-33.
13. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. J Labn Physicians. 2011; 3:25-7.
14. Lyall K, Gupta V, Chhina D. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus*. J Mahatma Gandhi Inst Med Sci. 2013; 18(2):112-5.

15. 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
16. Lall M, Sahni A. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Med J Armed Forces India. 2014; 70:43-7.
17. Drinkovic D, Fuller E, Shore K, Holland D, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrob Chemother. 2001; 48:315-6.
18. Rao G. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? J Antimicrob Chemother 2000; 45:715.
19. 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.
20. Patel M, Waites K, Moser S, Cloud G, Hoesley C. Prevalence of inducible clindamycin resistance among community and hospital associated *Staphylococcus aureus* isolates. J Clin Microbiol. 2006; 44:2481-4.
21. Kalbhor P, Wanjare V, Shrikhande S. Detection of inducible and constitutive clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care hospital. 2018; 8(9):70-2.
22. Jarajreh D, Aqel A, Alzoubi H, Al-Zereini W. Prevalence of inducible clindamycin Resistance in methicillin resistant *Staphylococcus aureus*: the first study in Jordan. J Infect Dev Ctries. 2017:11:350-4.
23. Nikam A, Bhise P, Deshmukh M. Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates. Int J Res Med Sci. 2017; 5(2):543-7.
24. More S, Kasturi, Rathod V, Sinha R, Khan S. Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus aureus* in a Tertiary Care Hospital, Nanded, Maharashtra. Int. J Current Microbiol. App. Sci. 2017: 6(4):1232-9.
25. Adhikari R, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant *Staphylococcus aureus* in a tertiary care hospital, Kathmandu, Nepal. BMC Infect Dis. 2017: 17(1):483.
26. Majhi S, Dash M, Mohapatra D, Mohapatra A, Chayani N. Detection of inducible and constitutive clindamycin resistance among *Staphylococcus aureus* isolates in a tertiary care hospital, Eastern India. Avicenna J Med. 2016; 6(3):75-80.
27. Regha I, Deepa Harichandran and Sulekha B. Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus aureus* in a Tertiary Care Centre, Kerala, India. Int. J Curr. Microbiol. App. Sci. 2016; 5(4):929-934.
28. Mokta KK, Verma S, Chauhan D, Ganju SA, Singh D, Kanga A *et al.* J Clin Diagn Res. 2015; 9(8):20-3.
29. Banik A, Khyriem A, Gurung J, Lyngdoh V. Inducible and constitutive clindamycin resistance in *Staphylococcus aureus* in a north eastern Indian tertiary care hospital. J Infect Dev Ctries. 2015; 9:725-31.
30. Sande SV. Phenotypic detection and incidence of inducible clindamycin resistance among *Staphylococcus aureus* from tertiary care hospital. Int J Adv Med. 2015; 2(3):264-8.
31. Supriyarajvi, Gupta A, Tina G, Sharma BP. Detection of inducible Clindamycin Resistance among Staphylococcal isolates from various clinical specimens in a tertiary care institute in North west region of Rajasthan, India. Int J Curr Microbiol App Sci. 2015; 4(10):741-9.
32. Zorgani A, Shawerf O, Tawil K, El-Turki E, Ghenghesh K. Inducible Clindamycin Resistance among Staphylococci Isolated from Burn Patients. The Libyan journal of medicine. 2009; 4(3):104-6.
33. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and clindamycin resistance in *c* and coagulase negative staphylococci in a community and tertiary care hospital. J Clin Microbiol. 2004; 42:2777-9.