Inducible clindamycin resistance among *Staphylococcus aureus* isolates from skin and soft tissue infections: a study from Brunei Darussalam

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ABSTRACT

Introduction: Clindamycin is one of the important antibiotics in treating *Staphylococcal* soft tissue infections. Clinical failure of clindamycin therapy has been reported to be due to inducible clindamycin resistance which cannot be detected by routine in-vitro antibiotic susceptibility testing. This study was undertaken to detect the presence of inducible clindamycin resistance among *Staphylococcus aureus* (*S. aureus*) isolates and its association with methicillin resistance. **Materials and Methods:** D zone test was performed by incorporating it in the routine antibiotic susceptibility test for all *S. aureus* isolates and methicillin resistance was detected by doing cefoxitin disk diffusion test according to Clinical Laboratory Standard Institute (CLSI) guidelines 2014. **Results:** Forty four among 56 erythromycin resistant *S. aureus* isolates were inducible clindamycin resistant (78.6%). It was also observed that percentages of inducible resistance and constitutive clindamycin resistance were higher amongst MRSA as compared to MSSA (60.7%, 7.1% and 9.9%, 2.4% respectively). **Conclusion:** Clinical laboratories should perform D zone test routinely to guide the clinicians about the inducible clindamycin resistance in *S. aureus* isolates and to prevent therapeutic failure.

Keywords: Clindamycin resistance, D zone test, Methicillin resistant staphylococcus aureus

INTRODUCTION

Staphylococcus aureus (S. aureus) is one of the most common organisms causing skin and soft tissue infections. [1] According to 2014 Infectious Diseases Society of America updated guidelines, these infections can be treated by beta-lactams (dicloxacillin, cephalexin), macrolides (erythromycin), lincosamides (clindamycin)), doxycycline, trimethoprim-sulfamethoxazole or glycopeptides (vancomycin), depending upon the severity of infection and suspected or confirmed resistance to methicillin. [2] Clindamycin, a semi-synthetic derivative of lincomycin is a preferred and

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most efficient agent in treating *Staphylococcal* soft tissue and skin infections including osteomyelitis since it has excellent tissue penetration, rapid oral absorption, with no requirement of dosage adjustment in the presence of renal disease. [3] However, widespread use of macrolides, lincosamides, streptogramin B (MLS_B) antibiotics has led to an increase in the number of *Staphylococcal* strains acquiring resistance to MLS_B antibiotics. [4-7] This resistance can be due to three ways; a) through target site modification by methylation or mutation, b) through efflux of the antibiotic, c) by drug inactivation, which result in a variety of phenotypic resistance. The target site modification mediated by erythromycin ribosomal methylation (*erm*) genes predominantly *erm A* and *erm C* are the most common mechanism for such resistance among *Staphylococci*. [4, 5] The resistance results from decreased binding of the antibiotics to their overlapping targets on the ribosome, which are probably altered in conformation due to dimethylation by enzyme methylase encoded by the *erm* genes. This mechanism confers resistance to erythromycin and most other macrolides (M), lincosamides (L, lincomycin and clindamycin) and streptogramin type B(S_B) and this pattern is referred to as MLS_B phenotype which is expressed either as constitutive (MLS_B phenotype) or inducible ($iMLS_B$ phenotype). [5] In inducible type of resistance, the bacteria produce inactive mRNA that is unable to encode methylase. The mRNA becomes active only in the presence of a macrolide inducer. By contrast, in constitutive expression mRNA is produced even in the absence of an inducer. [4]

In isolates harbouring inducible clindamycin ($iMLS_B$ phenotype) resistance, exposure to clindamycin (which is not an inducer) in-vivo may result in clindamycin resistance due to selection of pre-existing constitutive *erm* mutants, especially when the organism is at high inoculum. This can also be due to sub-inhibitory concentrations of erythromycin or other macrolides that bring about induction of the methylating enzyme. [4, 5] But by in-vitro antibiotic susceptibility tests, only macrolide resistance manifests while clindamycin appears as sensitive leading to clindamycin susceptibility report which may lead to therapeutic failure. [4, 5]

Clindamycin resistance due to MLS_B resistance mechanism in Staphylococci manifests more frequently when the strain possesses the *erm A* gene as compared with the *erm C* gene. [5] The *erm A* gene is mostly carried in methicillin-resistant *S. aureus* (MRSA) and is located on transposons whereas the *erm C* gene is mostly responsible for erythromycin resistance in methicillin-sensitive strains of *S. aureus* (MSSA) and is located on plasmids .[4, 5]

In resistance due to efflux pump, constituted by *msr A* gene and chromosomal genes, which is specific for macrolide (M) and streptogramin (S), then the *Staphylococcal* isolates appear erythromycin resistant and clindamycin sensitive in vitro and do not typically become clindamycin resistant during therapy as clindamycin is neither an inducer nor a substrate for efflux pump (MS phenotype). [4]

The incidence of inducible clindamycin resistance is highly variable from region to region and from the country to country. [3, 6, 8] Hence, local statistics on this resistance is of

crucial value for empiric therapy. This snap-shot study aim to find out the incidence of *S. aureus* harbouring inducible clindamycin resistance ($iMLS_B$) in Brunei Darussalam and also to understand the relationship between MRSA and inducible clindamycin resistance.

MATERIALS AND METHODS

This prospective study was conducted at microbiology laboratory, RIPAS Hospital. A total of 280 *S. aureus* were isolated during this study period from all pus specimens and tested for inducible clindamycin resistance. The isolates were identified by Gram staining, colony morphology and slide coagulase test and then subjected to routine antibiotic susceptibility testing by modified Kirby Bauer s disk diffusion method on Muller Hinton agar plates as per Clinical Laboratory Standard Institute (CLSI) guidelines 2014. [9] The isolates with cefoxitin inhibition zone size <19mm were considered as MRSA.

Inducible resistance to clindamycin was tested by 'D zone test' as per CLSI guidelines 2014. [9] Erythromycin (15µg) and clindamycin (2µg) discs were placed in the routine disc dispenser i.e. the distance between them was 26mm. Following overnight incubation at 37°C, circular zone of inhibition with a zone size ≥21mm around the clindamycin with flattening on the side facing erythromycin disc (D zone), indicated inducible clindamycin resistance (Figure 1).

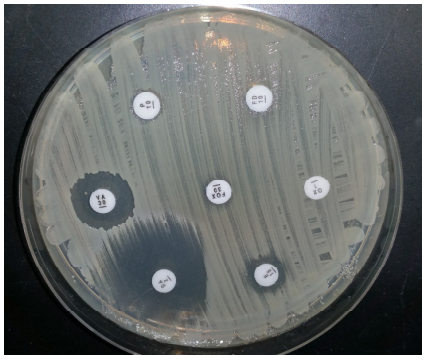

Fig. 1: Inducible clindamycin resistance indicated by a circular zone of inhibition (size ≥21 mm) around the clindamycin with flattening on the side facing erythromycin disc (D zone).

In the routine antibiotic susceptibility test, three different phenotypes were appreciated among erythromycin resistant *S. aureus* isolates. [8, 9]

1: Constitutive MLS_B phenotype - Staphylococcal isolates that showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition around clindamycin.

2: Inducible MLS_B ($iMLS_B$) phenotype - Staphylococcal isolates showing resistance to erythromycin (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.

3: MS phenotype - Staphylococcal isolate exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin disc.

Quality control (QC) of the erythromycin and clindamycin discs was performed in triplicates using *S. aureus* ATCC25923, according to the standard disc diffusion QC procedure. [9] Additional QC was performed with separate in-house selected *S. aureus* strains that demonstrated positive and negative D-test reactions. Quality control of commercially obtained media used in the study, was done with each shipment/batch of media. [10]

Results were tabulated and analysed statistically.

Table 1: Susceptibility to erythromycin and clindamycin among all *S. aureus* isolates.

	Clindamycin sensitive		Clindamycin resistant
Erythromycin Resistant (n=56)	D test negative, MS	D test positive, $IMLS_b$	Constitutive MLS_B
	4 (1.4%)	44 (15.7%)	8 (2.9%)

RESULTS

Two hundred and eighty *S. aureus* strains were tested for susceptibility by routine disc diffusion testing; 56 (22.2%) of them were erythromycin resistant.

D zone test analysis revealed 78.6% of isolates were inducible clindamycin resistant among erythromycin resistant *S. aureus* (n=44/56) (Table 1). Percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA (Table 2).

DISCUSSION

Clindamycin is an excellent drug for *Staphylococcal* infections, particularly skin and soft tissue infections and an alternative in penicillin allergic patients. [11] Since it has good oral bioavailability it is a good option as a stepping down antibiotic after intravenous antibiotics and for outpatient therapy. [4] 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. [12] Reporting *S. aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic option. Since the $iMLS_B$ resistance mechanism is not recognised by standard antibiotic susceptibility test methods and its prevalence varies according to geographical location, D zone test described in CLSI guidelines has become an imperative part of routine antimicrobial susceptibility test for all clinical isolates of *S. aureus* and should be included in the routine antimicrobial susceptibility method. [9, 13]

In our four months study, we found

Table 2: Association of clindamycin resistance with methicillin resistance among *S. aureus* isolates.

	MRSA (n=28)			MSSA (n=252)		
	CL-S		CL-R	CL-S		CL-R
ERY-S	5 (17.9%)		NIL	219 (86.9%)		NIL
ERY-R (n=56)	D-TEST negative, MS.	D-TEST positive, $iMLS_B$	Constitutive MLS_B	D-TEST negative, MS.	D-TEST positive, $iMLS_B$	Constitutive MLS_B
	2 (7.1%)	17 (60.7%)	2 (7.1%)	2 (0.8%)	25 (9.9%)	8 (2.9%)

ERY=Erythromycin, CL=Clindamycin, S=Sensitive, R=Resistant, Constitutive MLS_B=Constitutive MLS_B phenotype, $iMLS_B$ =Inducible MLS_B phenotype, MS=MS phenotype. MRSA=Methicillin resistant *Staphylococcus aureus*, MSSA=Methicillin Sensitive *Staphylococcus aureus*.

22.2% (n=56/280) of *S. aureus* isolates with erythromycin resistance. Among them 44 (78.6%) isolates were tested positive for inducible clindamycin resistance by D zone test, while 8 (2.9%) were constitutive clindamycin resistant and only 4 (1.4%) were truly sensitive to clindamycin (MS phenotype). These observations suggest that had D zone test not been performed, more than two third of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure.

The prevalence of inducible clindamycin varies from one geographical area to another. In this study we found a very high percentage (78.6%) of inducible clindamycin resistance. Comparatively other studies have shown less prevalence like in Delialioglu *et al*. from Turkey (7.8%) [6] and in a study done in Thailand by Chelae *et al.* (9.9%) [14] but in some other studies showed a slightly higher incidence as in Fokas *et al*. from Greece, it was 35% [15] and Urmi *et al*. from India it was 43%. [16] Hence our study showed a very high incidence of inducible clindamycin resistance in this geographic region.

It was also observed that percentages of inducible resistance and constitutive clindamycin resistance were higher amongst MRSA as compared to MSSA (60.7%, 7.1% and 9.9%, 2.4%). This is in concordance with few of the studies reported before. [12] A study conducted by Neela *et al*. from Malaysia showed 96.1% of erythromycin resistant MRSA were inducible clindamycin resistant. [17] Some studies have shown a high or equal frequency of inducible resistance in MSSA compared to MRSA. [18, 19]

In the context of the restricted range of available antibiotics for the treatment of methicillin resistant staphylococcal infections and with the known limitations of glycopeptides, clindamycin could be considered for the management of serious soft tissue infections due to its excellent penetration into skin and soft tissues. An accurate susceptibility report is paramount to avoid therapeutic failure due to inducible clindamycin resistance. This emphasises the need to incorporate D zone testing in the standard antimicrobial susceptibility tests. It is a simple, auxiliary and reliable method to detect inducible clindamycin resistance in routine clinical laboratories without any additional cost or human resources but contributing much to therapeutic management.

REFERENCES

1: Ki V, Rostein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Can J Infect Dis Med Microbiol 2008; 19:173-84.

2: Dennis LS, Alan LB, Henry FC, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the infectious diseases society of America. CID 2014; 59:147-59.

3: Mallick SK, Basak S, Bose S. Inducible clindamycin resistance in Staphylococcus aureus-a therapeutic challenge. J Clin Diag Research 2009; 3:1513-8.

4: Leclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. CID.2002; 34: 482-92.

5: Sivapalasingam S, Steigbigel NH. Macrolides, Clindamycin, Ketolides. In Mandell GL, Douglas JE, Bennets, editors, Principle and Practice of Infectious Diseases. Philadelphia: Churchill Livingstone; 2014:358-76.

6: Delialioglu N, Aslam G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in

Staphylococci isolated from clinical specimen. Jpn J Dis 2005; 58:104-6.

7: Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin resistant Staphylococcus expressing inducible clindamycin resistance in vitro. CID. 2003; 37:1257-60.

8: 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.

9: Clinical Laboratory Standard Institute (CLSI) 2014. Performance standards for antimicrobial susceptibility testing: twenty fourth information supplement 2014:68-170.

10: Clinical Laboratory Standard Institute (CLSI) 2012. Quality control for commercially prepared microbiological culture media, approved standard-third edition. 2012; 24:13-21.

11: Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance. J Antimicrob Chemother 2001; 48: 315–6.

12: 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.

13: 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.

14: Chelae S, Laohaprertthisam V, Phengmak M, Kongmuang U, Kalnauwakul S. Detection of inducible clindamycin resistance in Staphylococci by disk diffusion induction test. J Med Assoc Thai 2009; 92:947-51.

15: Fokas S, Fokas S, Tsironi M, Kalkani M, Dionysopouloy M. Prevalence of inducible clindamycin resistance in macrolide resistant Staphylococcus spp. Clin Microbiol Infect 2005; 11: 337–340.

16: Jethwani UN, Mulla SA, Shah LN, Panwala TR. Detecion of inducible clindamycin resistance by an automated system in a tertiary care hospital. Afr J Microbiol Res 2011; 5:2870-2.

17: Neela V, Sasikumar M, Ghaznavi GR, Zamberi S, Mariana S. In vitro activities of 28 antimicrobial agents against methicillin resistant Staphylococcus aureus (MRSA) from a clinical setting in Malaysia. Southeast Asian J Trop Med Public Health 2008; 39:885-92.

18: Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in Staphylococcus aureus and coagulase negative staphylococci in a community and a tertiary care hospital. J Clin Microbiol 2004; 42:2777–9.

19: Levin TP, Suh B, Axelrod P, Truant AL, Fekete T. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: Report of a clinical failure. Antimicrob Agents Chemother 2005; 49:1222–4.