

Detection of Inducible Clindamycin Resistance among Staphylococcal Isolates from Different Clinical Specimens in Northwestern Nigeria

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Abstract

Background: Macrolide (MLS_B) resistance is the most widespread and clinically important mechanism of resistance encountered with Gram-positive organisms. Resistance may be constitutive (cMLS_B phenotype) or inducible (iMLS_B phenotype). The iMLS_B phenotypes are not differentiated by using standard susceptibility test methods, but can be distinguished by erythromycin-clindamycin disk approximation test (D-test) and demonstration of resistance genes by molecular methods. **Aims:** To demonstrate in vitro iMLS_B in erythromycin-resistant (ER) and clindamycin-susceptible (CLI-S) clinical isolates of *Staphylococcus aureus* and interpretation of susceptibility tests to guide therapy. **Materials and Methods:** One hundred isolates of *Staphylococcus aureus* were recovered from various clinical specimens. All the *Staphylococcus aureus* were identified by conventional microbiological methods including colony morphology, Gram stain, catalase, slide coagulase and tube coagulase. Polymerase chain reaction (PCR) was used to amplify both the *S. aureus* specific sequence gene and *mecA* gene of 100 isolates with the amplicon size of 107 and 532 bp respectively. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method. Erythromycin-resistant isolates were examined for iMLS_B by using double disk approximation test (D-test) at 15 mm disk separation. **Results:** All the isolates (n=100) expressed *S. aureus* specific sequence gene in their PCR products. Only 5 isolates (5.0%) were confirmed as Methicillin resistant *Staphylococcus aureus* (MRSA) based on the detection of *mecA* gene. Seven percent of *Staphylococcus aureus* were ER among which 4.0% were Methicillin susceptible *Staphylococcus aureus* (MSSA) and 3.0% were MRSA. The inducible MLS_B (iMLS_B) phenotype was detected in 3 MRSA isolates and all the 4 erythromycin-resistant MSSA strains were positive for the cMLS_B phenotypes. Sixty per cent of iMLS_B phenotypes were observed to be methicillin-resistant. All the isolates (MSSA and MRSA) were susceptible to vancomycin. **Conclusions:** The identification of multi-resistant MSSA with in vitro inducible clindamycin resistance at our health institutions raises concern of clindamycin treatment failures with methicillin-resistant infections. It is recommended that microbiology laboratories should include the D-test for inducible resistance to clindamycin in the routine antibiotic susceptibility testing.

Keywords

D-Test, Inducible Clindamycin Resistance, *Staphylococcus aureus*

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1. Introduction

Infections due to staphylococci, especially methicillin resistant *S. aureus* (MRSA) are increasing among in-patient clinics and treatment of these infections pose difficulties

(Manian *et al.*, 2003; Maltezou *et al.*, 2006). Macrolides, lincosamides and streptogramin (MLS) antibiotics are used in the treatment of staphylococcal infections. Macrolide (MLS_B) resistance is the most common and clinically important mechanism of resistance encountered with Gram-positive organisms. MLS antibiotics are structurally

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unrelated, but microbiologically related antibiotics because of their similar mode of action. Resistance may be constitutive (cMLS_B phenotype) or inducible (iMLS_B phenotype). The iMLS_B phenotypes are not differentiated by using standard susceptibility test methods, but can be distinguished by erythromycin-clindamycin disc approximation test (D-test) and demonstration of resistance genes by molecular methods (Sesli *et al.*, 2008; Colakoglu, 2008; Dinç *et al.*, 2009; Pal *et al.*, 2010; Gatermann *et al.*, 2007). Resistance to MLS antibiotics occurs either through target site modification, efflux of antibiotics or drug modification (Leclercq, 2002). MLS antibiotics inhibit bacterial protein synthesis by binding to 23srRNA, which is a part of the large ribosomal subunit. In target site modification methylation of the A2058 residue, located in the conserved domain V of 23srRNA. The development of resistance in Staphylococcus species to macrolide, lincosamide and streptogramin B has limited the use of these antibiotics. Macrolide resistance may be due to enzymes encoded by a variety of erm genes-MLS_B phenotype and may be constitutive (cMLS_B phenotype) or inducible (iMLS_B phenotype). Another mechanism is active efflux pump encoded by the mrsA gene (MS phenotype). The MS and iMLS_B phenotypes are indistinguishable by using standard susceptibility test methods, but can be distinguished by erythromycin-clindamycin disk approximation test (D-test) and demonstration of resistance genes by molecular methods (Drinkovic *et al.*, 2001 and Steward *et al.*, 2005). The aim of this study was to determine the rate of inducible clindamycin resistance in both methicillin-resistant and susceptible strains of Staphylococcus in our hospital as data describing iMLS_B prevalence among Staphylococcus isolates in this region is unknown.

2. Materials and Methods

A total of 100 consecutive non – duplicated *Staphylococcus aureus* isolates were obtained from clinical samples in 8 health institutions (Microbiology department) across Northwestern Nigeria. The isolates were collected for duration of ten months from July 2009 to April 2010. The quality control and rejection criteria of specimen were followed (Muhammad *et al.*, 2010). All plates were examined for Staphylococcus by colonial morphology on nutrient agar (Cheesbrough, 2000). Catalase, coagulase, DNase tests and test for hemolysin were performed on all the isolates. *Staphylococcus aureus* ATCC 25923 was used as a reference control organism. All confirmed *Staphylococcus aureus* isolates were stored in 16% v/v glycerol broth at -80°C.

Antibiotic susceptibility testing was performed by Kirby

Bauer disc diffusion method according to the National Committee for Clinical Laboratory Standards (now Clinical Laboratory Standards institute) guidelines (NCCLS, 2006). The antibiotics (Mast Diagnostics, UK) tested includes penicillin (10µg), fusidic acid (10µg), ciprofloxacin (5µg), gentamycin (10µg), erythromycin (15µg), chloramphenicol (30µg), tetracycline (30µg), trimethoprim (2.5µg), augumentin (30µg), ofloxacin (10µg), peflacin (5µg), streptomycin (30µg), cefuroxime (30µg), cotrimoxole (25µg) and clindamycin (2µg). *Staphylococcus aureus* (ATCC25923) was the control strain in every test run. Methicillin resistance was detected by Polymerase chain reaction (PCR). Amplification of both the *S. aureus* specific sequence gene and mecA gene of 100 isolates with the amplicon size of 107 and 532 bp was done by PCR test. One MSSA control strain (ATCC 25923) was included in each batch of PCR run. (Wielders *et al.*, 2001; Meshref and Omer, 2011).

Disk approximation testing (D-test) was performed for each isolate according to Clinical and Laboratory Standards Institute (CLSI) method (CLSI, 2005). A 0.5 McFarland suspension was prepared in normal saline for each isolate and inoculated on Mueller-Hinton agar plate. Clindamycin (CLI)-2 µg and erythromycin (ER)-15µg disks were placed 15 mm apart edge to edge manually. Plates were incubated at 35 0 C for 24 h and zone diameters were recorded. Induction test categories were interpreted (Steward *et al.*, 2005).

3. Results

A total of 100 *Staphylococcus aureus* isolates were included, of which 5.0% were MRSA and 95.0% MSSA. Categorization of the isolates along with sources is depicted in (Table 1).

Table 1. Sources and categorization of *Staphylococcus aureus* isolates

Specimen	MRSA	MSSA	TOTAL
Wound swabs	3(9.7)	28 (90.3)	31 (31.0)
Ear swabs	0(0.0)	9 (100)	9 (9.0)
Blood culture	0(0.0)	8 (100)	8 (8.0)
Urine	2(7.7)	24 (92.3)	26 (26.0)
High vaginal swabs	12 (100)	12(100)	12 (12.0)
Sputum	0(0.0)	11 (11.0)	11 (11.0)
Semen	0(0.0)	1 (100)	1 (100)
Urethral swabs	0(0.0)	2 (100)	2 (100)
Total	5 (5.0)	95 (95.0)	100 (100)

Seven (7.0%) clinical isolates which showed erythromycin resistance were tested for inducible resistance by double disk approximation test. Out of 7 erythromycin-resistant strains 3 (3.0%) were iMLS_B phenotypes [60.0% MRSA (3/5)]. All the iMLS_B phenotypes were observed to be methicillin-resistant isolates. D-test yielded two distinct induction phenotypes, D-zone phenotype [Figure 1] was observed in 78

(18.13%) and D + phenotype [Figure 2] in 22 (5.34%) isolates. Both D and D + results were considered positive for CLI induction- iMLS_B phenotypes. One hundred and twenty-seven (29.53%) isolates showed ER resistant and CLI-susceptible zone diameters with no blunting of the zones (MS phenotype). Two hundred and two (46.97%) isolates showed ER and CLI resistance (cMLS_B phenotype), of which 98 were MRCNS, 85 MRSA and the rest were methicillin-sensitive [Table 2]. No hazy D zone (HD) phenotype was observed.

Susceptibility of iMLS_B phenotypes isolated were ampicillin 37.5%, amoxiclav 39.13%, cefepime 12.5%, ceftriaxone 50.0%, cephalexin 62.5%, cefoperazone-sulbactam 60.86%, cephalexin 23.07%, ciprofloxacin 78.78%, doxycycline 69.56%, linezolid 100%, netilmicin 46.60%, piperacillin-tazobactam 69.56% and vancomycin 100%.

Table 2. MLS_B resistance phenotypes in *S. aureus* isolates

Strains	MLS _B phenotypes (%)	
	Constitutive MLS _B Phenotypes (%)	Inducible MLS _B phenotypes (%)
MSSA	4 (100)	0 (0)
MRSA	0 (0)	84 (100)



Figure 1. Double disc test (D test) showing flattening of the zone of inhibition around clindamycin disc proximal to erythromycin disc (D shaped zone of inhibition)



Figure 2. Inducible MLS_B isolate- D phenotype

4. Discussion

In our study, 851 Staphylococcal isolates were obtained over a period of nine months (2008-09) in which 50.52% were ER-resistant. Among the ER-resistant *S. aureus* iMLS_B resistance was observed in 24.63% (51/207) similar to that reported by Fiebelkorn *et al.*, 2003; Jorgensen *et al.*, 2004 and Gadepalli *et al.*, 2006. Some investigators have reported a higher incidence of iMLS_B resistance (Goya *et al.*, 2004; Merino *et al.*, 2005; O'Sullivan *et al.*, 2006; Patel *et al.*, 2006; Angel *et al.*, 2008; Ciraj *et al.*, 2009) while others have indicated lower incidence. (Jenssen *et al.*, 1987; Hamilton-Miller and Shah, 2000; Delialioglu *et al.*, 2005; Huang *et al.*, 2006; Rahbar and Hajia, 2007). We observed almost a similar rate of iMLS_B resistance among *S. aureus* (24.63%) and CNS (22.42%), while others have reported variable results. The different patterns of resistance observed in various studies are because iMLS_B resistance varies by geographical region, age group, methicillin susceptibility and even from hospital to hospital. As observed in studies by Steward *et al.*, and Schreckenberger *et al.*, our CLI induction results also showed two phenotypes, D (18.13%) and D + (5.34%) phenotypes and both are considered to be positive D-zone test (Steward *et al.*, 2001 and Schreckenberger *et al.*, 2004). All the iMLS_B isolates were susceptible to vancomycin while 78.78% to ciprofloxacin. Rahbar and Hajia also found all iMLS_B isolates susceptible to vancomycin (Rahbar and Hajia, 2007).

Due to the emergence of resistance to antimicrobial agents accurate drug susceptibility data of the infecting microbe is an essential factor in making appropriate therapeutic decisions. MLS_B resistance is the most widespread and clinically important mechanism of resistance encountered with Gram-positive organisms due to the production of methylases and efflux proteins. In vitro susceptibility testing for clindamycin may indicate false susceptibility by the broth microdilution method and by disk diffusion testing with erythromycin and clindamycin disks in nonadjacent positions. Erythromycin-clindamycin disc approximation test or D-test is a simple, reliable method to detect inducible resistance to clindamycin in erythromycin-resistant isolates of Staphylococci (Steward *et al.*, 2001 and Fiebelkorn *et al.*, 2003). Sensitivity of D-test performed at 15-20 mm disk spacing was 100% when correlated with detection of *erm* and *msr* genes by polymerase chain reaction (PCR) (Steward *et al.*, 2001; Fiebelkorn *et al.*, 2003; Jorgensen *et al.*, 2004; Merino *et al.*, 2005 and O'Sullivan *et al.*, 2006).

Clinically, bacterial strains exhibiting iMLS_B have a high rate of spontaneous mutation to constitutive resistance and use of non-inducer antibiotics such as clindamycin can lead to selection of constitutive mutants at frequencies of 10⁻⁷ cfu

(McGehee *et al.*, 1968; Goyal *et al.*, 2004; Schreckenberger *et al.*, 2004; O'Sullivan *et al.*, 2006). McGehee and other investigators have confirmed this rapid in vitro conversion of inducible to constitutive MLS B resistance in Staphylococci (McGehee *et al.*, 1968 and Panagea *et al.*, 1999; O'Sullivan *et al.*, 2006). There have also been a number of reported clindamycin or lincomycin therapy failures in serious infections due to Staphylococci with iMLSB resistance, indicating that it is not uncommon (Drinkovic *et al.*, 2001 and Panagea *et al.*, 1999). Clindamycin has long been an attractive option in the treatment of skin and soft tissue infections (SSTI) and serious infections because of its efficacy against MRSA and MSSA, as well as anaerobes. This has led to questioning the efficacy of clindamycin use against any erythromycin-resistant Staphylococci spp. However, if inducible resistance can be reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in patients with true clindamycin-susceptible strains.

In the present study, 29.53% of erythromycin-resistant Staphylococcal isolates showed true clindamycin susceptibility (MS phenotype). Patients with infections caused by such isolates can be treated with clindamycin without emergence of resistance during therapy.

The high frequency of methicillin-resistance isolates (87.12%) with in vitro inducible clindamycin resistance at our institute raises concern of clindamycin treatment failures with methicillin-resistant infections.

5. Conclusion

It can be concluded that it is important for laboratories to be aware of the local prevalence of iMLSB isolates. On the basis of their data they can choose whether or not to perform the D-test routinely. The D-test is an easy, sensitive, and reliable means for detection of iMLSB strains in a clinical laboratory setting without specialized testing facilities. The prevalence of iMLSB may change over time with the emergence of strains with different sensitivity patterns, so periodic surveys should be performed if testing is not routine.

Recommendations

Currently, some authors recommend avoidance of clindamycin for treatment of complicated infections which may have a high bacterial burden, such as abscesses or osteomyelitis. Conversely labelling all erythromycin-resistant staphylococci as clindamycin resistant would prevent the use of clindamycin in infections caused by truly clindamycin susceptible isolates. Clindamycin may be useful for non-MLSBI infections especially less severe *S. aureus* infections.

This study reflects the prevalence of MLSBI at a tertiary care centre; however prevalence may differ from institute to institute. Microbiological laboratories should adopt testing for MLSBI among *S. aureus* isolates and report isolates exhibiting MLSBI as clindamycin resistant.

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