

Original Article

Inducible clindamycin resistance due to expression of erm genes in *Staphylococcus aureus*: Report from a Tertiary Care Hospital Karachi, Pakistan

Naima Fasih, Seema Irfan, Afia Zafar, Erum Khan, Rumina Hasan
Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi, Pakistan.

Abstract

Objective: To assess the frequency of phenotypic expression of inducible resistance of clindamycin due to expression of erm genes, in clinical isolates of *Staphylococcus aureus* (*S. aureus*), by double disk diffusion test (D-test).

Method: This was a cross sectional study conducted in the clinical laboratory of Aga Khan University Hospital, Karachi. A total of 2432, non duplicate clinical isolates of *S. aureus*, consisting of 1562 methicillin sensitive *S. aureus* (MSSA) and 870 methicillin resistant *S. aureus* (MRSA), were selected from February 2007 to January 2008. One hundred and thirty eight isolates of *S. aureus* were selected based on discordant resistance pattern (erythromycin resistant and clindamycin sensitive) on Kirby Bauer Disk Diffusion test and phenotypic expression of inducible resistance was assessed using D-test.

Result: Analysis of 2432 isolates showed that 64% (n=1553) were susceptible to both clindamycin and erythromycin by disc diffusion method, while 30% (n=741) showed constitutive resistance (in vitro resistance to both drugs). 6% (n=138) isolates showed clindamycin-erythromycin discordance on disc diffusion (in vitro sensitive to clindamycin and resistant to erythromycin). Among the discordant isolates 72% (n=99) had inducible resistance phenotype detected by D-test and of these 85 isolates (62%) were MRSA.

Conclusion: Inducible resistance is common in our clinical isolates; D-test (a simple phenotypic test) should be performed on all *S. aureus* isolates showing clindamycin-erythromycin discordance on disc diffusion, to avoid erroneous reporting resulting in treatment failure (JPMA 60:750; 2010).

Introduction

Staphylococcus aureus (*S. aureus*) is a common cause of both community and nosocomial acquired infections. Infections range from minor skin infections to life threatening conditions such as endocarditis, pneumonia and septicaemia. Increasing antimicrobial drug resistance in *S. aureus* is one of the major concerns.¹ Rate of methicillin resistant *S. aureus* (MRSA) has dramatically increased in recent years.^{1,2} In Pakistan exact prevalence of MRSA is not known, however it is a common hospital acquired pathogen and different hospitals have reported its prevalence to be around 22-39%.³⁻⁵ Traditionally MRSA has been considered a nosocomial pathogen and vancomycin considered as drug of choice. However vancomycin usage is associated with considerable side effects and cost. Moreover overuse of vancomycin has led to the emergence of staphylococcal strains with reduced susceptibility to it.⁶ Also in the past decade MRSA has emerged as pathogen for community acquired infections (CA-MRSA) Unlike hospital acquired MRSA, the CA-

MRSA are known to be sensitive to drugs other than vancomycin, such as, ciprofloxacin, trimethoprim-sulphamethoxazole and clindamycin (CL).⁷

CL is a good substitute to treat soft tissue infections by both MRSA and MSSA infections. Its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production are its advantages. CL, further more, is a useful choice in cases of penicillin allergy.⁸ Again development of resistance especially inducible resistance is a major barrier in its usage.

CL belongs to the macrolide, lincosamide and streptogramin (MLS) family that act through inhibition of protein synthesis. Bacterial resistance to this group may be expressed through different mechanisms including target site modification, macrolide efflux pump and enzymatic antibiotic inactivation.⁹ Modification of the ribosomal target is encoded by the erm genes that cause production of methylase enzymes which reduce binding of the drug to the

rRNA target. This resistance can be either constitutive or inducible. If the *erm* genes are consistently expressed, isolates show in vitro resistance to erythromycin (ER), CL, and to other members of MLS, known as constitutive resistance phenotype. In case of inducible resistance, the *erm* genes require an inducing agent to express resistance to CL. ER can act as a strong inducer of methylase synthesis. These isolates known as inducible resistance phenotype show in vitro resistance to ER and susceptibility to CL. CL therapy in this phenotype can lead to clinical failure.¹⁰⁻¹³ *S. aureus* can also develop isolated macrolide resistance based on presence of an efflux pump, encoded by the *msrA* gene which leads to resistance to macrolides and type B streptogramins but not to lincosamides. These isolates known as MS phenotype also show in vitro resistance to ER and susceptibility to CL same as in inducible resistance phenotype, but CL therapy can be safely given in infections with this phenotype and there is no risk of clinical failure. Therefore, it is important to differentiate these two mechanisms of resistance.

Phenotypic detection of inducible resistance can be done by double disk diffusion test (D-test). D-test is simple, reliable, inexpensive and easy to interpret with high sensitivity and specificity.¹⁴ Molecular markers for the *erm* genes are available, but they are costly and inconvenient for everyday use. In a developing country like Pakistan with high burden of MRSA, where health associated finances is borne by the patient, alternatives to vancomycin are in need. Clindamycin is a good option but prevalence of inducible resistance should be known, as it varies by geographical location and bacterial species. So the aim of this study was to assess the frequency of phenotypic expression of inducible *erm* gene expression in clinical isolates of *S. aureus* by D-test.

Material and Methods

This was a cross sectional study conducted in the clinical laboratory of Aga Khan University Hospital, Karachi from February 2007 to January 2008. 2432 consecutive isolates of *S. aureus* were collected from clinical samples, by convenient sampling. These comprised of 1562 MSSA and 870 MRSA isolates. Duplicate isolates were excluded. Sources were wound, pus, tissue, respiratory secretions, bone, joint fluid, ascitic fluid, pleural fluid and blood. *S. aureus* was identified using conventional methodology (colony morphology, gram staining, catalase, coagulase test and DNase test). Oxacillin resistance was detected by taking cefoxitin as a surrogate marker.

Susceptibility testing of CL (2 µg), ER (15µg) and cefoxitin (30µg) were performed by Kirby Bauer disk diffusion method and interpreted according to Clinical Laboratory Standard Institute (CLSI) break points.¹⁵

Interpretation of the diameters of zones of inhibition was as follows:

ER-S $\geq$ 23 mm; ER intermediate 14-22 mm; ER-R $\leq$ 13 mm
CL-S $\geq$ 21 mm; CL intermediate 15-20 mm; CL-R $\leq$ 14 mm
Cefoxitin-S $\geq$ 22 mm; Cefoxitin-R $\leq$ 21 mm

138 isolates of *S. aureus* were selected based on discordant resistance pattern for ER and CL (E-R or I and CL-S) on Kirby Bauer Disk Diffusion test. These isolates were then tested for detection of phenotypic expression of inducible *erm* genes, by double disk diffusion test (D-test).

Detection of inducible resistance *erm* gene expression:

Phenotypic expression of *erm* gene activation was detected by performing D-test using CLSI guideline.¹⁵ A 0.5 McFarland equivalent suspension of organisms was inoculated onto a Mueller - Hinton agar (MHA) plate, the ER (15µg) disk was placed 15-26 mm (edge to edge) apart from CL (2 µg) disk on MHA. Plates were analyzed after 18 hours of incubation at 35°C.

Principles of D-test:

When tested in close proximity, ER (inducing agent) diffuses into the media and induces the *erm* gene expression. This effect extends up to the sensitivity zone on one side of the CL disk leading to a D-shaped zone of inhibition (Figure).

Interpretation of the D-test:

IsolateS showing circular zones of inhibition with diameter of $\leq$ 13 mm for ER and $\geq$ 21 mm for CL were interpreted as negative for inducible resistance (D-test negative). Isolates with same inhibitory diameters as above but a D-shaped zone around the CL, were interpreted as positive for inducible resistance (D-test positive) (Figure).

Data Management and statistical analysis:

The data was coded and entered into SPSS 16.0 software for statistical analysis. The frequencies of inducible CL-R in *S. aureus*, MSSA and MRSA were calculated and expressed in percentage.

Result

A total of 2432 *S. aureus* isolates were analyzed during the study period, 1562 (64%) were MSSA and 870 (36 %) were MRSA. Of total isolates 1553 (64%) were susceptible to both clindamycin and erythromycin (CL-S, ER-S). 741 (30%) strains showed constitutive resistance; in vitro resistance to both drugs (CL-R, ER-R). The remaining 138 (6%) isolates expressed clindamycin-erythromycin discordant (CL-S, ER-R) result.

Among the discordant *S. aureus* isolates, 99 (72%) had inducible resistance phenotype and of these 85 (62%) isolates were MRSA. Frequency of inducible resistance

Table: Frequency of inducible resistance phenotype in ER-CL discordant *S. aureus*, MRSA and MSSA.

ER-R,CL-S (Discordant Isolate)-n	Inducible resistance phenotype- % (n)
<i>S. aureus</i> (138)	72% (99)
MRSA (85)	70% (60)
MSSA (53)	73% (39)

CL; Clindamycin, ER; Erythromycin, S; Sensitive, R; Resistance, MRSA; Methicillin resistant *S. aureus*, MSSA; Methicillin resistant *S. aureus*.

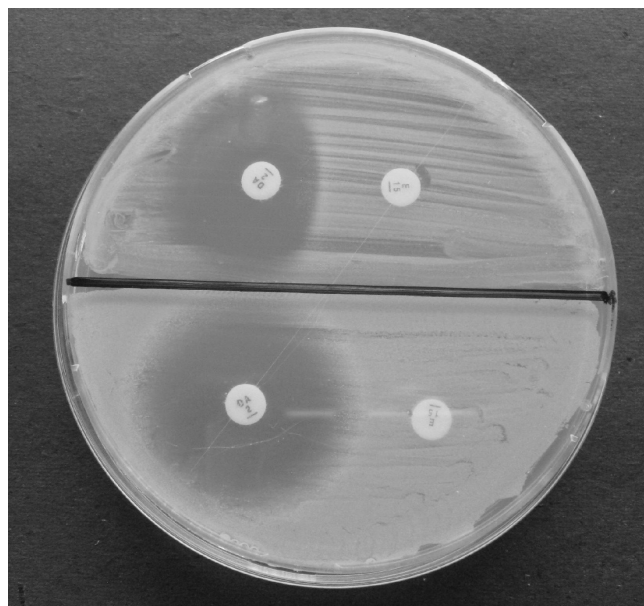


Figure: Double disk diffusion test. A; positive test showing blunting of zone of inhibition around CL disk and formation of D shape. B; negative test showing no blunting of zone of inhibition around CL disk. CL; Clindamycin, E; Erythromycin.

phenotypes in ER-CL discordant *S. aureus*, MRSA and MSSA is shown in Table. Our results show that the frequency of inducible resistance amongst MRSA and MSSA was similar.

Overall 99 (4%) out of 2432 isolates of *S. aureus* included in this study were reported as being resistant to clindamycin based on D-test finding. Such resistance would otherwise have been missed on routine Kirby-Bauer disc diffusion method.

Discussion

The increased frequency of staphylococcal infections along with augmented problem of antimicrobial resistance has led to renewed interest in CL usage. The new guidelines for treatment of skin and soft tissue infections,¹⁶ as well as for diabetic foot infections¹⁷ recommend CL as good choice, for empirical and therapeutic treatment for mild to moderate infections and a good alternative to penicillin allergic patients; however, the guidelines also highlight the risk of

therapeutic failure of using CL in inducible resistant phenotype. In 1969, McGehee et al demonstrated the development of CL-R in vivo and in vitro in ER-R staphylococci.¹⁸ Other investigators have confirmed the rapid in vitro conversion of inducible to constitutive resistance in staphylococcus.¹⁹ Till date, no case-control clinical studies investigating the relationship between inducible resistance and CL therapy have been published. So clinical efficacy of CL usage in infections, caused by inducibly resistant staphylococci, remains unclear. But there have been a number of reported CL therapy failures in staphylococcus infections due to inducible resistance phenotype.¹⁰⁻¹³ Therefore questioning the safety of CL in ER-R staphylococcus.

Inducible clindamycin resistance by D-test method is reported in the present study. The phenotypic erm genes expression in clinical *S. aureus* isolates yielded at Clinical Laboratory of Aga Khan University Hospital. Inducible resistance pattern on disc diffusion was notable in both MRSA and MSSA isolates, Majority of discordant isolates showed inducible expression of erm genes by D-test. This expression was more pronounced in MRSA isolates.

Our findings are consistent with studies published by other authors. A study conducted in Turkey by Yilmaz G et al showed 81.8% inducible resistance in ER-R/CL-S *S. aureus* isolates with predominance in MRSA.²⁰ Levin et al conducted a study from Pennsylvania identified inducible resistance in 54.2% of *S. aureus* isolates of which 72.2% were MRSA.¹¹ Similarly, a study from Alabama reported discordant resistance in 70% of MRSA and 30% MSSA isolates, inducible resistance was noticeable by D-test in 60% of these isolates.²¹ Contrary to our study Schreckenberger et al from Chicago studied 83 % inducible resistance in discordant isolates but majority were MSSA.²² A study conducted at the University of Texas Health Science Center, demonstrated that 29% of their discordant *S. aureus* showed inducible resistance.¹⁴ Gadepalli R et al from India showed 21% inducible resistance.²³ Another study from Turkey revealed 24.3% inducible resistance higher incidence 10.2% in MSSA.²⁴ Lim et al in a Korean hospital found 14.6% inducible resistance.²⁵ These findings are suggesting that inducible resistance phenotype varies widely by hospitals and geographical region and also among MSSA and MRSA.

Clinical laboratory can easily miss inducible resistance by using in vitro susceptibility tests such as broth and agar dilution, and disc diffusion testing, when ER and CL discs are placed in nonadjacent positions. Accurate results of antibiotics susceptibility tests are important for appropriate and effective therapy. This is important to apply D-test on a routine basis to detect inducible resistance, so CL can be used effectively in staphylococcal infections. Therefore, CLSI also recommends this test for clinical microbiology laboratory.

Conclusion

High inducible resistance due to *erm* genes expression was detectable by D-test in discordant *S. aureus* isolates. In view of therapeutic implication, D-test was found to be a simple effective test that should be performed on all *S. aureus* isolates showing clindamycin-erythromycin discordance on disc diffusion.

References

- Schito GC. The importance of the development of antibiotic resistance in *Staphylococcus aureus*. *Clin Microbiol Infect* 2006; 12 Suppl 1: 3-8.
- Rajadurai K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian J Med Microbiol* 2006; 24: 34-8.
- Siddiquei GM, Karamat KA, Hannan A. Prevalence of Methicillin Resistant *Staphylococcus Aureus*: A study at PAF Hospital Sargodha. *Pak J Pathol* 1999; 10: 26-8.
- Qureshi AH, Rafi S, Qureshi SM, Ali AM. The current susceptibility patterns of methicillin resistant *Staphylococcus aureus* to conventional anti *Staphylococcus* antimicrobials at Rawalpindi. *Pak J Med Sci* 2004; 20: 361-4.
- Hussain S, Shams R, Ahmad K, Perveen R, Riaz B. Prevalence of Methicillin Resistant *Staphylococcus Aureus* (MRSA) in surgical site infections in a tertiary care hospital. *Int J Pathol* 2005; 3: 81-5.
- Smith TL, Pearson ML, Wilcox KL, Cruz C, Lancaster MV, Robinson-Dunn B, et al. Emergence of Vancomycin resistance in *Staphylococcus aureus*. Glycopeptide Intermediate *Staphylococcus Aureus* Working Group. *N Eng J Med* 1999; 340: 493-501.
- Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and health-care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003; 290: 2976-84.
- Kasten MJ. Clindamycin, metronidazole, and chloramphenicol. *Mayo Clin Proc* 1999; 74: 825-33.
- Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* 2002; 34: 482-92.
- Lewis JS 2nd, Jorgensen JH. Inducible clindamycin resistance in *Staphylococci*: should clinicians and microbiologists be concerned? *Clin Infect Dis* 2005; 40: 280-5.
- 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.
- Rao GG. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? *J Antimicrob Chemother* 2000; 45: 715.
- 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.
- Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol* 2003; 41: 4740-4.
- National committee for clinical laboratory standard. Performance standards for antimicrobial susceptibility testing. Sixteen informational supplement 2006.M100-S16 (M7-A7).
- Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin Infect Dis* 2005; 41: 1373-406.
- Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2004; 39: 885-910.
- McGehee RFR, Barre FF, Finland F. Resistance of *Staphylococcus aureus* to lincomycin, clindamycin and erythromycin. *Antimicrob Agents Chemother* 1968; 8: 392-7.
- Panagea S, Perry JD, Gould FK. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? *J Antimicrob Chemother* 1999; 44: 581-2.
- Yilmaz G, Aydin K, Iskender S, Caylan R, Koksali I. Detection and prevalence of inducible clindamycin resistance in staphylococci. *J Med Microbiol* 2007; 56: 342-5.
- Patel M, Waites KB, Moser SA, Cloud GA, Hoesley CJ. Prevalence of inducible clindamycin resistance among community- and hospital-associated *Staphylococcus aureus* isolates. *J Clin Microbiol* 2006; 44: 2481-4.
- 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.
- 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.
- Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. *Jpn J Infect Dis* 2005; 58: 104-6.
- Lim JA, Kwon AR, Kim SK, Chong Y, Lee K, Choi EC. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. *J Antimicrob Chemother* 2002; 49: 489-95.