Detection of Inducible Clindamycin Resistance in Staphylococci by Disk Diffusion Induction Test

Sureerat Chelae MSc*,
Varaporn Laohaprertthisarn BSc*, Manthana Phengmak MSc*,
Urairat Kongmuang PhD*, Sineenart Kalnauwakul MD*

** Microbiology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand*

Objective: *To detect inducible clindamycin (CL) resistance in staphylococci by disk diffusion induction test (D-test).*
Material and Method: *One thousand one hundred eighty clinical isolates of staphylococci were tested for inducible CL resistance by placing erythromycin (E) disk and clindamycin disk 12 mm apart (edge to edge) on Mueller-Hinton agar plate inoculated with staphylococci. The flattening of CL zone (D-shaped zone) near E disk indicated an inducible CL resistance was observed after 18-24 h of incubation.*
Results: *Inducible CL resistance was detected in 9.9% of staphylococci isolates. It was found in methicillin-resistant Staphylococcus aureus (MRSA) more than methicillin-sensitive Staphylococcus aureus (MSSA) and coagulase-negative staphylococci (CoNS) 35.9%, 4.7%, and 5.5%, respectively.*
Conclusion: *To avoid misinterpretation of CL result, D-test is recommended for routine detecting of inducible CL resistance in staphylococci. It provides the confident laboratory report of CL as resistant (D-shaped zone positive) or as susceptible (D-shaped zone negative) particular for E resistant isolates.*

Keywords: *Clindamycin, Drug resistance, D-test, Methicillin-resistant Staphylococcus aureus, Microbial sensitivity tests, Staphylococcus aureus*

J Med Assoc Thai 2009; 92 (7): 947-51
Full text. e-Journal: *http://www.mat.or.th/journal*

Staphylococcus aureus and coagulase-negative staphylococci (CoNS) are recognized as causing nosocomial and community-acquired infections. Treatments of these infections are a growing problem because of the increasing methicillin resistance among staphylococci[1,2]. Macrolide (*e.g.*, erythromycin), lincosamide (*e.g.*, clindamycin), and streptogramin B (*e.g.*, quinupristin-dalfopristin) antimicrobial agents are widely used in the treatment of staphylococcal infections[3,4]. However, failures during therapy with macrolide, lincosamide, and streptogramin B (MLS_B) are more commonly reported[5,6]. Resistance to MLS_B can occur by two different mechanisms: an active efflux mechanism encoded by the *msrA* gene (macrolides streptogramins resistance) and ribosomal target modification encoded by the *erm* gene (MLS_B resistance)[7]. MLS_B resistance in staphylococci can be either constitutive or inducible. *In vitro*, staphylococci isolates with constitutive resistance are resistant to both erythromycin (E) and clindamycin (CL) while isolates with inducible resistance are resistant to E but appear susceptible to CL. Constitutive resistance to CL can be detected by standard susceptibility testing methods; however, an inducible CL resistance cannot be detected based on the size of the inhibition zone. Failure to identify inducible CL resistance leads to incorrect laboratory reports and treatment problems[6,8].

Erythromycin is an effective inducer of inducible MLS_B resistance. It will induce production of the methylase, which allows CL resistance to be expressed. To detect inducible CL resistance strains, the disk diffusion induction test (D-test) has been used by several authors[9-12]. The test is performed by

Correspondence to: Chelae S, Microbiology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat-Yai, Songkhla 90110, Thailand. Phone: 074-451-582, Fax: 074-212-908. E-mail: jsureera@medicine.psu.ac.th

placing an E disk in close proximity to a CL disk. As the E diffuses through the agar, the resistance to CL is induced, resulting in a flattening of CL zone of inhibition adjacent to the E disk, giving a D-shaped zone. Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if staphylococci are not tested for inducible CL resistance. Thus, the aim of the present study was to detect the inducible CL resistance in staphylococci by the D-test.

Material and Method

Clinical samples and bacterial isolates:

Various types of samples obtained from infected body sites of patients admitted in Songklanagarind Hospital were routinely cultured in the clinical microbiology laboratory. Staphylococci isolates were tested for coagulase by the plasma tube method and sugar fermentation to distinguish between *S. aureus* and CoNS. Then, *S. aureus* was subsequently identified as methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) using the oxacillin disk test. All of these staphylococci were further tested for inducible CL resistance by the D-test.

D-test:

The test was performed as previously described[12]. Briefly, a 0.5 McFarland suspension of staphylococci in brain heart infusion broth was inoculated on a 100-mm diameter Mueller-Hinton agar plate. A 2-µg CL disk and a 15-µg E disk were placed at an edge-to-edge distance of 12 mm using an eight-disk dispenser. After incubation for 18 to 24 h at 35°C, the diameter of the inhibition zone was measured. In addition, each CL zone was examined carefully to detect a flattening or blunting of the shape (D-shaped zone) near the E disk. The interpretation as susceptible (S), intermediate (I) or resistant (R) was based on National Committee for Clinical Laboratory Standards (NCCLS) guidelines: E-S $\geq$ 23 mm, E-I = 14-22 mm, E-R $\leq$ 13 mm; CL-S $\geq$ 21 mm, CL-I = 15-20 mm and CL-R $\leq$ 14 mm[13]. If the E zone is $\leq$ 13 mm and the CL zone is $\geq$ 21 mm and they both have a circular shape, the staphylococci is negative for inducible CL resistance (negative D-test) and susceptible to CL. If the E zone is $\leq$ 13 mm and the CL zone is $\geq$ 21 mm with a D-shaped zone around the CL disk, the staphylococci are positive for inducible CL resistance (positive D-test) and it indicates an inducible CL resistance. Control strains with known positive D-test and negative D-test were included for quality assessment purposes.

Results

Staphylococci isolated from clinical samples

Between June 2007 and March 2008, 1180 isolates of staphylococci were collected from various types of clinical samples. The isolates were identified as 636 CoNS and 544 *S. aureus*, and of the latter 181 (33%) were MRSA and 363 (67%) were MSSA, as shown in Table 1. The majority of staphylococci were found from pus samples. *S. aureus* was isolated more frequently from pus samples (208 of 544, 38%), followed by sputum samples (179 of 544, 33%).

D-test

All staphylococci isolated from clinical samples were tested for inducible CL resistance using the D-test. A positive D-test, D-shaped zone around CL disk indicating an inducible CL resistance, was detected in 117 of 1,180 isolates (9.9%). Concerning the *S. aureus*, an inducible CL resistance was found in MRSA more than in MSSA, 65 of 181 (35.9%) and 17 of 363 (4.7%) respectively, whereas, 35 of 636 isolates of CoNS (5.5%) were inducible CL resistance as shown in Table 2.

Table 1. *S. aureus* and coagulase-negative staphylococci isolated from various sources of clinical samples

Source of sample	*S. aureus*		Coagulase-negative staphylococci
	MRSA	MSSA	
Body fluid	11	11	38
CSF	0	0	14
Blood	25	25	54
Pus	39	169	209
Sputum	72	107	74
Tissue	22	34	66
Throat	1	2	9
Urine	11	15	172
Total	181	363	636

Table 2. Detection of inducible clindamycin resistance of *S. aureus* and coagulase-negative staphylococci by D-test

	No. of positive induction tests (%) (D-shaped zone)	No. of tested isolates
MRSA	65 (35.9)	181
MSSA	17 (4.7)	363
CoNS	35 (5.5)	636

Table 3. Clindamycin induction test phenotypes of *S. aureus* and coagulase-negative Staphylococci

Isolates	Phenotype				
	D-positive No. (%)	D-negative No. (%)	R No. (%)	S No. (%)	No. of isolates
MRSA	65 (35.9)	2 (1.1)	111 (61.3)	3 (1.6)	181
MSSA	17 (4.7)	0	16 (4.4)	330 (90.9)	363
CoNS	35 (5.5)	113 (17.7)	215 (33.8)	273 (42.9)	636

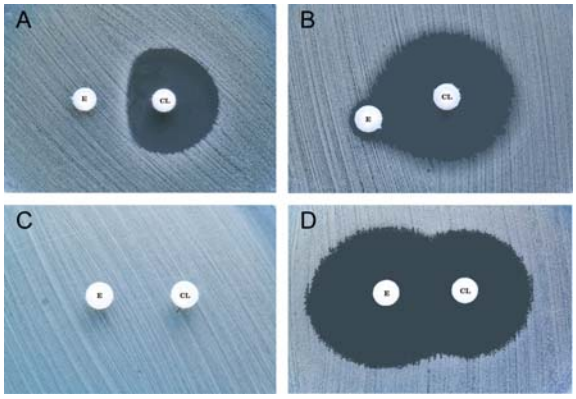

Fig. 1 This figure shows four phenotypes observed during the D- test on Staphylococci
A) D-positive phenotype, B) D-negative phenotype, C) R-phenotype, D) S-phenotype, E = erythromycin disk; CL = clindamycin disk

Phenotypes

The disk diffusion based on the D-test produced four phenotypes of staphylococci, designated as D-positive, D-negative, resistant (R) and susceptible (S) (Fig. 1, Table 3). A D-shaped zone around the CL disk (D-positive phenotype), indicating an inducible CL resistance, was found in 35.9% of MRSA, 4.7% of MSSA, and 5.5% of CoNS. On the other hand, 1.1% of MRSA, none of MSSA, and 17.7% of CoNS were CL susceptible showing a circular shape zone around the CL disk (D-negative phenotype). The isolates of 61.3% of MRSA, 4.4% of MSSA, and 33.8% of CoNS were both CL and E resistant (R phenotype), whereas 1.6% of MRSA, 90.9% of MSSA, and 42.9% of CoNS were both CL and E susceptible (S phenotype).

Discussion

Clindamycin is a useful drug in soft-tissue infections and serious infections caused by staphylococcal species, as well as anaerobes. It has excellent tissue penetration, accumulates in abscesses, and no renal dosing adjustments are needed[14]. Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. CL is also a good alternative antibiotic for the penicillin-allergic patient and infections due to MRSA[14-16]. However, recent reports indicate that treatment failure may occur in the case of inducible MLS_B resistance, in spite of *in vitro* susceptibility to CL[5,17].

Accurate susceptibility data are important for appropriate therapy decisions. In staphylococci, *in vitro* susceptibility testing for CL by disk diffusion testing with E and CL disks in nonadjacent positions may indicate false susceptibility[6,8]. Failure to identify inducible CL resistance may lead to clinical failure when CL is used therapeutically. On the other hand, if inducible CL resistance can be reliably detected on a routine basis in clinically significant isolates, CL can be safely and effectively used in those patients with true CL-susceptible isolates. There is an increasing interest in assessing the incidence or prevalence of inducible CL resistance in hospitals and the community[18-20]. However, the occurrence of inducible CL resistance varies widely by hospital and geographic region. Thus, it is necessary to provide the test that can detect inducible CL resistance in an individual hospital area.

Herein, the inducible CL resistance was detected by the D-test as previously described[12]. This test can separate strains that have the genetic potential (the presence of the *erm* gene) to become resistant during therapy from strains that are fully susceptible to CL. The D-shape of the CL zone adjacent to an E disk can serve to detect *S. aureus* and CoNS isolates with inducible resistance to CL. In reference to our staphylococci isolates, 35.9% of MRSA, 4.7% of MSSA, and 5.5% of CoNS exhibited an inducible CL resistance. The result suggested that a higher rate of

inducible CL resistance was found in MRSA than in MSSA and CoNS. The D-test results of staphylococci isolates showed four phenotypes including D-positive, D-negative, R and S. Most of MRSA (61.3%) showed R-phenotype while most of MSSA (90.9%) showed S-phenotype.

NCCLS recommends placing the CL and E disks anywhere from 15 to 26 mm apart for the D-test[13]. However, O' Sullivan reported that a 15-mm distance, in an edge to edge position, had a 100% sensitivity and specificity, while the 22-mm distance, in an edge to edge position, had a sensitivity of 87% and a specificity of 100% when compared with the presence of the *erm* gene as the gold standard for the detection of inducible CL resistance[21]. The same author also recommends that a distance of $\leq$ 15 mm between the CL and E disks, in an edge to edge position, should be used for the D-test. From the authors' experience, it was found that the D-shaped zone was more difficult to read as the disks were placed further apart (data not shown). In the present study, an automated disk dispenser was used to place the CL and E disks providing 12 mm apart for the authors' routine work. The distance of 12 mm between the CL and E disks for D-test was described by Steward[12]. Furthermore, our laboratory performs the D-test as a routine testing, whereas most published D-test studies select only the isolates that are E resistant, but CL susceptible for testing[8,12]. The authors were concerned that if the D-test was delayed until the E resistance testing was complete, the results might not be available for maximal clinical utility.

In conclusion, the D-test is easy to perform and inexpensive for practical work. The authors recommended this test to detect an inducible CL resistance in staphylococci as a routine work in clinical microbiology laboratories. This test provides confident laboratory reports for CL resistant staphylococcus strains (D-shaped zone positive) or CL susceptible strains (D-shaped zone negative), and particularly for E resistant isolates. Consequently, treatment using CL can be omitted in patients with infections caused by inducible CL resistance staphylococci, and therapeutic failures may thus be avoided.

References

1. Asensio A, Canton R, Vaque J, Rossello J, Calbo F, Garcia-Caballero J, et al. Nosocomial and community-acquired meticillin-resistant Staphylococcus aureus infections in hospitalized patients (Spain, 1993-2003). J Hosp Infect 2006; 63: 465-71.
2. Frank AL, Marcinak JF, Mangat PD, Tjhio JT, Kelkar S, Schreckenberger PC, et al. Clindamycin treatment of methicillin-resistant Staphylococcus aureus infections in children. Pediatr Infect Dis J 2002; 21: 530-4.
3. 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.
4. 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.
5. Lewis JS, Jorgensen JH. Inducible clindamycin resistance in Staphylococci: should clinicians and microbiologists be concerned? Clin Infect Dis 2005; 40: 280-5.
6. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. Clin Infect Dis 2003; 37: 1257-60.
7. 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.
8. 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.
9. Perez LR, Caierao J, Antunes AL, d'Azevedo PA. Use of the D test method to detect inducible clindamycin resistance in coagulase negative staphylococci (CoNS). Braz J Infect Dis 2007; 11: 186-8.
10. 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.
11. 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.
12. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, et al. Testing for induction of clindamycin resistance in erythromycin-resistant isolates of Staphylococcus aureus. J Clin Microbiol 2005; 43: 1716-21.

13. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; 12th informational supplement. NCCLS document M100-S14. Wayne, PA: NCCLS; 2004.
14. Kasten MJ. Clindamycin, metronidazole, and chloramphenicol. Mayo Clin Proc 1999; 74: 825-33.
15. Martinez-Aguilar G, Hammerman WA, Mason EO Jr, Kaplan SL. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible Staphylococcus aureus in children. Pediatr Infect Dis J 2003; 22: 593-8.
16. Roberts S, Chambers S. Diagnosis and management of Staphylococcus aureus infections of the skin and soft tissue. Intern Med J 2005; 35 (Suppl 2): S97-105.
17. Rao GG. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? J Antimicrob Chemother 2000; 45: 715.
18. Azap OK, Arslan H, Timurkaynak F, Yapar G, Oruc E, Gagir U. Incidence of inducible clindamycin resistance in staphylococci: first results from Turkey. Clin Microbiol Infect 2005; 11: 582-4.
19. 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.
20. 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: 337-40.
21. O'Sullivan MV, Cai Y, Kong F, Zeng X, Gilbert GL. Influence of disk separation distance on accuracy of the disk approximation test for detection of inducible clindamycin resistance in Staphylococcus spp. J Clin Microbiol 2006; 44: 4072-6.

การตรวจหาการดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำในเชื้อสแต็ปไฟโรคอคไซโดยวิธี disk diffusion induction test

สุรีรัตน์ เจะและ, วราพร เลาหพฤฒิสาร, มัณฑนา เพ็งมาก, อุไรรัตน์ คงเมือง, สินีนาฏ กาลเนาวกุล

วัตถุประสงค์*: เพื่อตรวจหาการดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำในเชื้อสแต็ปไฟโรคอคไซ โดยวิธี disk diffusion induction test (D-test)*

วัสดุและวิธีการ*: ได้ทำการทดสอบเชื้อสแต็ปไฟโรคอคไซ จำนวน 1,180 สายพันธุ์ เพื่อตรวจหาการดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำโดยป้ายเชื้อบน Mueller Hinton agar แล้ววางแผ่นยาคลินดามัยซินและอิริโธรมัยซินห่างกัน 12 มิลลิเมตร (ขอบถึงขอบ) อบเชื้อ 18-24 ชั่วโมง ตรวจดู zone รูปอักษร D รอบแผ่นยาคลินดามัยซินใกล้กับแผ่นยาอิริโธมัยซิน เป็นตัวบ่งชี้ว่ามีการดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำ*

ผลการศึกษา*: จากการทดสอบพบเชื้อสแต็ปไฟโรคอคไซดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำร้อยละ 9.9 และพบในเชื้อสแตปฟิโลคอกคัส ออเรียส ที่ดื้อต่อยาเมธิซิลลิน มากกว่าเชื้อสแตปฟิโลคอกคัส ออเรียส ที่ไวต่อยาเมธิซิลลิน และเชื้อสแต็ปไฟโรคอคไซ โคแอกกูเลสลบ คิดเป็นร้อยละ 35.9, 4.7 และ 5.5 ตามลำดับ*

สรุป*: เพื่อป้องกันการรายงานผลยาคลินดามัยซินผิดพลาด ควรตรวจหาการดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำในเชื้อสแต็ปไฟโรคอคไซด้วยวิธี D-test เป็นงานประจำทำให้มีความมั่นใจในการรายงานผลเชื้อดื้อยาคลินดามัยซินเมื่อพบ zone รูปอักษร D และไวต่อยาคลินดามัยซินเมื่อไม่พบ zone รูปอักษร D*