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

Sureerat Chelae MSc*, Varaporn Laohaprertthiarn 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

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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 (MLSₜ) are more commonly reported[5,6]. Resistance to MLSₜ can occur by two different mechanisms: an active efflux mechanism encoded by the msrA gene (macrolides streptogramins resistance) and ribosomal target modifi-

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

Erythromycin is an effective inducer of inducible MLSₜ 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
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. 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 ≥ 23 mm, E-I = 14-22 mm, E-R ≤ 13 mm; CL-S ≥ 21 mm, CL-I = 15-20 mm and CL-R ≤ 14 mm. If the E zone is ≤ 13 mm and the CL zone is ≥ 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 ≤ 13 mm and the CL zone is ≥ 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 ≤ 13 mm and the CL zone is ≥ 21 mm and they both have a circular shape, 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

<table>
<thead>
<tr>
<th>Source of sample</th>
<th><em>S. aureus</em></th>
<th>Coagulase-negative staphylococci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>MSSA</td>
</tr>
<tr>
<td>Body fluid</td>
<td>11</td>
<td>38</td>
</tr>
<tr>
<td>CSF</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Blood</td>
<td>25</td>
<td>54</td>
</tr>
<tr>
<td>Pus</td>
<td>39</td>
<td>209</td>
</tr>
<tr>
<td>Sputum</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>Tissue</td>
<td>22</td>
<td>66</td>
</tr>
<tr>
<td>Throat</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Urine</td>
<td>11</td>
<td>172</td>
</tr>
<tr>
<td>Total</td>
<td>181</td>
<td>636</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>No. of positive induction tests (%)</th>
<th>No. of tested isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(D-shaped zone)</td>
<td></td>
</tr>
<tr>
<td>MRSA</td>
<td>65 (35.9)</td>
<td>181</td>
</tr>
<tr>
<td>MSSA</td>
<td>17 (4.7)</td>
<td>363</td>
</tr>
<tr>
<td>CoNS</td>
<td>35 (5.5)</td>
<td>636</td>
</tr>
</tbody>
</table>
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 MLSB 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

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

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

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
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 \(d \leq 15\) 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**


การตรวจหาการคดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำในเชื้อสแต็ปไฟโรคอคไซโดยวิธี disk diffusion induction test

สุริรัตน์ เจาละ, ราทรี เลาหพฤฒิสาร, มัณฑนา เพ็งมาก, อุไรรัตน์ คงเมือง, สินีนาฏ กาลเนาวกุล

วัตถุประสงค์: เพื่อตรวจหาการคดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำในเชื้อสแต็ปไฟโรคอคไซโดยวิธี disk diffusion induction test (D-test)

วัสดุและวิธีการ: โดยทำการทดสอบเชื้อใน medio ที่ลาน 1,180 สายพันธุ์ เพื่อตรวจหาการคดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำโดยใช้ชุด Mueller Hinton agar และวางแผ่นยาคลินดามัยซินและอิริโธอมัยซินห่างกัน 12 มิลลิเมตร (ขอบถึงขอบ) รอบเชื้อ 18-24 ชั่วโมง ตรวจดู zone รูปลักษณ์ D โดยผ่านแผ่นยาคลินดามัยซินใกล้กับแผ่นยาอิริโธอมัยซิน เป็นตัวบ่งชี้ว่ามีการคดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำ

ผลการศึกษา: จากการทดสอบพบเชื้อคดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำอยู่ที่ 9.9 และพบในชุดแปลงได้ตลอดคีด ข้อสงสัย ที่ต่ำที่ยิ่งมีผลต่อการคดื้อยาคลินดามัยซินและยิ่งสูงไปในชุด ciclohexylamine คือ 35.9, 4.7 และ 5.5 ตามลำดับ

สรุป: เพื่อสรุปเกี่ยวกับการรายงานผลการคดื้อยาคลินดามัยซินชนิดที่เกิดจากการชักนำในเชื้อสแต็ปไฟโรคอคไซโดยวิธี D-test เป็นการประจุดที่ให้มีความน่าเชื่อถือในการรายงานผลการคดื้อยาคลินดามัยซินชนิดที่มี zone รูปลักษณ์ D และไม่คดื้อยาคลินดามัยซินชนิดที่ไม่มี zone รูปลักษณ์ D