Walailak J Sci & Tech 2007; 4(2): 155-163.

Incidence of Constitutive and Inducible Clindamycin Resistance in Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*

Monthon LERTCANAWANICHAKUL, Kittisak CHAWAWISIT, Apinya CHOOPAN, Krisanawan NAKBUD and Kung DAWVEERAKUL

School of Allied Health Sciences and Public Health, Walailak University, Nakhon Si Thammarat 80161, Thailand

ABSTRACT

Without the double-disk test, all the *Staphylococcus aureus* isolates with inducible clindamycin resistance would have been misclassified as clindamycin susceptible, resulting in an underestimated clindamycin resistance rate. Clindamycin resistance rates may vary by geographic region and methicillin susceptibility. Hence it should be determined in individual settings. The high frequency of methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA) isolates with *in vitro* inducible clindamycin resistance at hospitals raises concern that clindamycin treatment failures may occur with MSSA as well as with MRSA infections. Clinical laboratories should report in vitro inducible clindamycin resistance in *S. aureus* isolates and clinicians should be aware of the potential of clindamycin treatment failure in patients with infections caused by inducible resistant strains. In this study, the percentage of inducible clindamycin resistance at two hospitals (Maharaj Nakhon Si Thammarat Hospital, Nakhon Si Thammarat, Thailand and Chumphon Khet Udomsak Hospital, Chumphon, Thailand) were 50 (25/50) and 8.3 (1/12) for methicillin resistant *Staphylococcus aureus*, respectively. Given the data of inducible resistance to clindamycin found in the two hospitals, we conclude that susceptibility testing of staphylococci should include the disk diffusion induction test (D-test) for usefulness of therapeutic treatment of staphylococci infections.

Keywords: Methicillin resistant *Staphylococcus aureus*, D-test, inducible clindamycin resistant

INTRODUCTION

Increasing frequency of methicillin resistant *Staphylococcus aureus* (MRSA) infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide-lincosamide-streptogramin group B (MLS_B) antibiotics to treat such infections [1]. These antibiotics are structurally distinct but functionally similar because they inhibit protein synthesis by targeting the 50S ribosomal subunit [2-4]. However, their widespread use has led to an increase in the number of staphylococcal strains resistant to MLS_B antibiotics. Target site modification is the most common mechanism of acquired resistance to MLS_B in staphylococci (the so-called MLS_B phenotype) [2]. MLS_B resistance can be either constitutive (MLS_Bc) or inducible (MLS_Bi). *In vitro*, MRSA isolates with constitutive resistance are resistant to erythromycin (ER) and clindamycin (CL), while isolates with inducible resistance are resistant to ER but appear susceptible to CL [1,4,6]. MLS_Bi is not recognized by using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests [7], including the Vitek system [6] and disc diffusion testing with ER and CL discs (double discs) in nonadjacent positions [1,7]. Failure to identify MLS_Bi may lead to clinical failure of CL therapy (a frequent choice, particularly, for staphylococcal skin and soft tissue infections) [1,8-9]. MLS_Bi can be detected by a disc induction test, a distorted "D-shaped" zone of inhibition is observed around CL if an ER disk is placed near by (15 mm to 20 mm) [10]. The aim of this study was to determine the incidence of constitutive and inducible CL resistance in clinical isolates of MRSA, in order to avoid poor clinical outcomes but retain the usefulness of CL.

MATERIALS AND METHODS

Bacterial strains

The clinical isolates of 62 MRSA were collected from patients with skin and soft tissue infections at Maharaj Nakhon Si Thammarat Hospital (n = 50), Nakhon Si Thammarat, Thailand and Chumphon Khet Udomsak Hospital (n = 12), Chumphon, Thailand. These isolates were MRSA, identified by using conventional laboratory methods [11-13].

Antibiotic susceptibility test

Antibiotic susceptibilities of MRSA isolates were determined by disk diffusion methods. Oxacillin susceptibility was performed as previously described by Huang et al. (2000) [12], and *S. aureus* ATCC 25923 was used as the control strain for the disk diffusion method. The following antibiotic disks from oxoid were tested at the following concentrations: oxacillin (1 μg), clindamycin (2 μg) and erythromycin (15 μg). Antibiotics susceptibilities were studied by disc diffusion methods based on the guidelines from the Clinical and Laboratory Standard Institute (CLSI, formerly National Committee for Clinical Laboratory Standards) criteria [13].

Identification of MLS_Bi phenotype by D-test

To identify the MLS_Bi phenotype in CL-sensitive (CL^S) and ER-resistant (ER^r) isolates, we performed a D test. Isolates were evenly plated on a Mueller-Hinton (M-H) agar plate at a McFarland concentration of 0.5, and disks of CL (2 μg) and ER (15 μg) were placed at a distance of 18 mm apart from the edge as recommended by the CLSI [13]. The plates were incubated at 37 °C for 16 to 18 h and observed for blunting of the CL zone of inhibition or D zone. Presence of a D-shaped zone was reported as a positive test for the MLS_Bi phenotype, and absence of a D-shaped zone was considered as a negative test for the MLS_Bi phenotype.

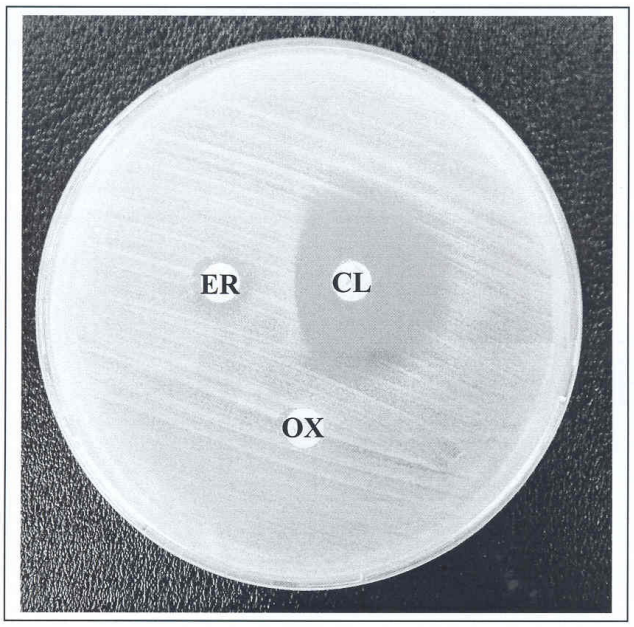
RESULTS

All 62 clinical isolates of *Staphylococcus aureus* used in this study were resistant to oxacillin (**Figure 1**), and identified as MRSA. Moreover, ER^r was detected in 100 % of the clinical isolates (62 of 62 isolates) in MRSA. The D-test was done to determine the MLS_Bi resistance of the CL^S and ER^r isolates. Among the total 62 MRSA isolates, 36 (58.06 %) were constitute macrolide, lincosamide, and group B streptogramin resistance (MLS_Bc), and the D-test showed that 26 (41.94 %) of these clinical isolates had the induce macrolide, lincosamide, and group B streptogramin resistance (MLS_Bi) phenotype (**Table 1**). The organism shown is a clinical isolate of MRSA that contains the *erm* gene and demonstrates the MLS_Bi phenotype (**Figure 1a**) or MLS_Bc phenotype (**Figure 1b**).

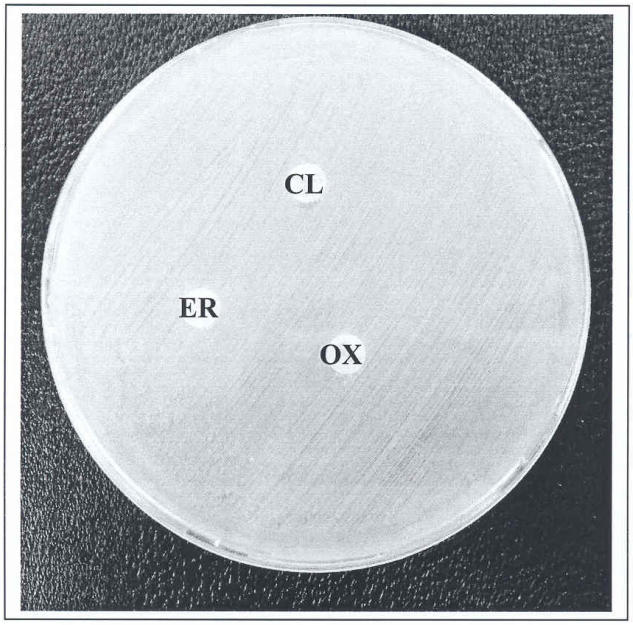
Table 1 Incidence of MLSBi and MLSBc among MRSA isolates

D-test	Resistance phenotype	Susceptibility		No. of isolates (%)
		ER	CL	
Positive	MLS_Bi	R	S	26 (41.94)
Negative	MLS_Bc	R	R	36 (58.06)
control	-	S	S	-

MLS_Bi, inducible resistance; MLS_Bc, constitutive resistance
ER, erythromycin; CL, clindamycin; R, resistance; S, susceptibility
control, *Staphylococcus aureus* ATCC 25923



(a)



(b)

Figure 1 Example of double disk test (D-test) demonstrating erythromycin (ER) disk induction of clindamycin (CL) resistance; a blunting of the zone of inhibition around the CL disk is produced that forms a D-shape (a), result for detection of inducible CL resistance (MLS_Bi). In constitutive resistance (MLS_Bc), the organism is clearly resistant to both ER and CL (b). Both phenotypes are methicillin resistant *Staphylococcus aureus* (MRSA), had no inhibition zone around the oxacillin (OX) disk.

DISCUSSION AND CONCLUSIONS

Acquired staphylococcal resistance to lincosamides such as clindamycin is largely mediated by ribosomal methylases encoded by one of several *erm* genes. These enzymes methylate the bacterial ribosome at the binding site for macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics, thus inhibiting antibiotic activity. Such resistance may be inducible (MLS_Bi) or constitutive (MLS_Bc).

Also, MRSA strains that are susceptible to CL but resistant to ER, may have the phenotype of in vitro MLS_Bi due to the presence of ER *erm* genes. For these strains, there is a high rate of mutation to constitutive resistance, which would then be selected during CL therapy and treatment failure. However, CL may be an important therapeutic option for infections due to the same ER/CL susceptibility pattern, that harbor *msrA*, which encodes an ATP-dependent efflux pump. This resistance determinant confers resistance only to 14-and 15-membered ring macrolides and type B streptogramins and not to lincosamides, such as CL [14-16].

In vitro, MLS_Bi can be detected in ER resistant *S. aureus* through the use of a double disk diffusion assay (D-test) [14-16], resulting in a D-shaped blunting of a circular zone of inhibition around CL disk on the side facing the ER disk (**Figure 1a**). If there is no distortion of the zone of inhibition around the CL disk, then the ER^r can be attributed to macrolide-specific efflux mechanisms, such as the presence of *msrA*. Although there are also reports of successful use of CL in treating patients with D-test positive isolates, however, isolates appear susceptible to CL in the absence of an inducing agent (ER), there is widespread reluctance to prescribe CL for treatment of patients with infections caused by such organisms because of concerns that resistance to CL will develop during therapy. Without the double-disk test, all the *S. aureus* isolates with inducible CL resistance would have been misclassified as CL^S, resulting in an underestimated CL resistance rate [1,6].

There have been relatively few reports of CL treatment failure in infections due to MRSA with *in vitro* inducible CL resistance [8,17-19], furthermore, CL treatment failures may occur with methicillin susceptible *S. aureus* (MSSA) as well as with MRSA infections [6]. It is thus recommended by most experts that CL therapy be avoided for *Staphylococcus* sp. isolates that display MLS_Bi resistance, despite a low CL MIC. The proportion of *S. aureus* with *in vitro* inducible CL resistance may vary by hospital, graphic region, age group, bacterial species and methicillin susceptibility [6,19-25]. Failure to identify *in vitro* inducible CL resistance when the ER^r CL^S phenotype is detected may lead to clinical failure of CL therapy. Conversely, labeling all ER^r staphylococci as CL resistance or not reporting CL resistance when ER^r is present will likely prevent the use of CL in treating infections that would likely respond to CL therapy [19-26].

It may be risky to use CL when ER testing shows a resistant or intermediate phenotype. The risk might currently be lower with MRSA, but this disparity may not continue or not be true elsewhere. Routine D-tests might allow clinicians to retain confidence in CL when ER^r is present [19-26]. It believed that clinical laboratories

should report *in vitro* inducible CL resistance in *S. aureus* isolates and that clinicians should be aware of the potential for clinical failure when CL is used to treat serious infections due to *S. aureus* (MRSA or MSSA) with *in vitro* inducible CL resistance. However, the cost benefit of routinely performing the D-test must be evaluated in each laboratory setting after first determining the incidence of the MLS_Bi and MLS_Bc resistance phenotypes. Decisions about routine testing of staphylococci with the Er^r/intermediate CL^S phenotype should be made on an institution-by-institution basis after obtaining local prevalence data [2,4,6,19,26].

ACKNOWLEDGEMENTS

This work was partially supported by Walailak University. The help from the staff from the laboratories at Walailak University is gratefully acknowledged.

REFERENCES

[1] R Gadepalli, B Dhawan, S Mohanty, A Kapil, BK Das and R Chaudhry. Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus*. *Indian J. Med. Res.* 2006; **123**, 571-3.

[2] R Leclercq. Mechanism of resistance to macrolide and lincosamides: nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.* 2002; **34**, 482-92.

[3] JS II Lewis and JH Jorgensen. Inducible clindamycin resistance in Staphylococci: should clinicians and microbiologists be concerned? *Clin. Infect. Dis.* 2005; **40**, 280-5.

[4] J Retsema and W Fu. Macrolides: structures and microbial targets. *Int. J. Antimicrob. Agents* 2001; **18 (Suppl 1)**, S3-S10.

[5] E Perez-Roth, F Claverie-Martin, N Batista, A Moreno and S Mendez-Alvarez. Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* clinical isolates in a Spanish hospital. Co-application method of multiplex PCR assay and conventional microbiology methods. *Diag. Microbiol. Infect. Dis.* 2002; **43**, 123-8.

[6] PC Schreckenberger, E Ilendo and KL Ristow. Incidence of constitutive and inducible resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J. Clin. Microbiol.* 2004; **42**, 2777-79.

[7] KR Fiebelkorn, SA Crawford, ML McElmeel and JH Jorgensen. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J. Clin. Microbiol.* 2003; **41**, 4740-44.

[8] D Drinkovic, ER Fuller, KP Shore, DJ Holland and R Ellis-Pegler. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J. Antimicrob. Chemother.* 2001; **48**, 315-6.
[9] JR Bradley. Newer antistaphylococcal agents. *Curr. Opin. Pediatr.* 2005; **17**, 71-7.
[10] CD Steward, PM Reney, AK Morrell, PP Williams, LK McDougal, L Jevitt, JE McGowan, Jr and FC Tenover. Testing for inducible of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. *J. Clin. Microbiol.* 2005; **43**, 1716-21.
[11] WE Kloos and TL Bannerman. *Staphylococcus* and *Micrococcus* spp. *In*: PR Murrey, EJ Baron, MA Pfaller, FC Tenover and RH Yolken (eds). Manual of Clinical Microbiology. 5th ed. Washington DC: American Society for Microbiology Press, 1995, p. 282-98.
[12] AH Huang, JJ Yan and JJ Wu. Rapid dissemination of *Staphylococcus aureus* with classic oxacillin resistance phenotype at a new university hospital. *J. Hosp. Infect.* 2000; **44**, 309-15.
[13] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing 2006; 16th Informational Supplement M100-S16. Wayne, PA: CLSI.
[14] B Weisblum. Insights into erythromycin action from studies of its activity as inducer of resistance. *Antimicrob. Agents Chemother.* 1995; **39**, 797-805.
[15] B Weisblum and V Demohn. Erythromycin-inducible resistance in *Staphylococcus aureus*: survey of antibiotic classes involved. *J. Bacteriol.* 1969; **98**, 447-52.
[16] F-J Schmitz, J Petridou, AC Fluit, U Hadding, G Peters and C von Eiff. Distribution of macrolide-resistant genes in *Staphylococcus aureus* blood-culture isolates from fifteen German university hospitals. M.R.S.A Study Group. multicenter study on antibiotic resistance in staphylococci. *Eur. J. Clin. Microbiol. Infect. Dis.* 2000; **19**, 385-7.
[17] AL Frank, JF Marcinak, PD Mangat, JT Tjhio, S Kelkar, PC Schreckenberger and JP Quinn. Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. *Pediatr. Infect. Dis. J.* 2002; **21**, 530-4.
[18] G Martinez-Aquilar, WA Hammerman, EO Mason Jr. and SL Kaplan. 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.
[19] GK Siberry, T Tekle, K Carroll and J Dick. Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. *Clin. Infect. Dis.* 2003; **37**, 1257-60.
[20] JMT Hamilton-Miller and S Shah. Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci. *J. Antimicrob. Chemother.* 2000; **46**, 941-9.
[21] WD Jenssen, S Thakker-Varie DT Dubin and MP Weinstein. Prevalence of macrolides-lincosamides-streptogramin B resistance and *erm* gene classes among

clinical strains of staphylococci and streptococci. *Antimicrob. Agents Chemother.* 1987; **31**, 883-8.

[22] FG Nicola, LK McDougal, JW Biddle and FC Tenover. Characterization of erythromycin-resistant isolates of *Staphylococcus aureus* in the United State from 1958 through 1969. *Antimicrob. Agents Chemother.* 1998; **42**, 3024-27.

[23] S Panagea, JD Perry and FK Gould. Should clindamycin be used as treatment of patients with infections caused by erythromycin-resistant staphylococci? *J. Antimicrob. Chemother.* 1999; **44**, 81-2.

[24] ML Sanchez, KK Flint and RN Jones. Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Is false susceptibility to new macrolides and clindamycin a contemporary clinical and in vitro testing problem? *Diagn. Microbiol. Infect. Dis.* 1993; **16**, 205-13.

[25] CA Sattler, EO Mason Jr and SL Kaplan. Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible *Staphylococcus aureus* infection in children. *Pediatr. Infect. Dis. J.* 2002; **21**, 910-6.

[26] TP Levin, B Suh, P Axelrod, AL Truant and T Fekete. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant *Staphylococcus aureus*: report of a clinical failure. *Antimicrob. Agents Chemother.* 2005; **49**, 1222-24.

บทคัดย่อ

มณฑล เลิศคณาวนิชกุล กิจติศักดิ์ ชววิสิฐ อภิญญา ชูพันธุ์ กฤษณวรรณ นาคบุตร และ กุ้ง ดาววีระกุล

อุบัติการณ์การดื้อยาคลินดามัยซินแบบเกิดขึ้นเองและแบบชักนำในสแตปฟิลโลคอคคัสออเรียสสายพันธุ์ดื้อยาเมธิซิลลินที่แยกได้จากผู้ป่วย

สแตปฟิลโลคอคคัสออเรียสที่แยกได้จากผู้ป่วยมักถูกรายงานผิดพลาดเกี่ยวกับอัตราการดื้อยาคลินดามัยซิน ถ้าหากไม่มีการทดสอบความไวของเชื้อต่อคู่ยาทดสอบที่เหมาะสม และพบว่าอัตราการดื้อยาคลินดามัยซินของเชื้อจะแตกต่างกันออกไปตามลักษณะทางภูมิศาสตร์และความไวของเชื้อต่อยาเมธิซิลลิน ซึ่งควรมีการตรวจสอบกลุ่มเชื้อดื้อยาลักษณะดังกล่าวในแต่ละสถานที่อย่างเหมาะสม โดยพบมีรายงานเกี่ยวกับกลุ่มเชื้อสแตปฟิลโลคอคคัสออเรียสทั้งที่ดื้อและไวต่อยาเมธิซิลลินสามารถถูกชักนำให้ดื้อต่อยาคลินดามัยซินเพิ่มสูงขึ้นในโรงพยาบาลโดยสัมพันธ์กับการใช้ยาคลินดามัยซินรักษาผู้ป่วยที่ติดเชื้อดื้อยาลักษณะดังกล่าว ดังนั้นทางห้องปฏิบัติการทางคลินิกควรมีรายงานการตรวจพบกลุ่มเชื้อสแตปฟิลโลคอคคัสออเรียสที่ดื้อยาคลินดามัยซินแบบถูกชักนำ เพื่อให้แพทย์มีความระมัดระวังในการใช้ยาคลินดามัยซินในการรักษาโรคติดเชื้อจากเชื้อดื้อยาลักษณะดังกล่าว โดยในรายงานการศึกษาครั้งนี้พบว่ามีอุบัติการณ์ของสแตปฟิลโลคอคคัสออเรียสสายพันธุ์ดื้อยาเมธิซิลลินที่ดื้อยาคลินดามัยซินแบบชักนำที่แยกได้โรงพยาบาล 2 แห่ง ได้แก่ โรงพยาบาลมหาราชนครศรีธรรมราช จังหวัดนครศรีธรรมราชและโรงพยาบาลชุมพรเขตรอุดมศักดิ์ จังหวัดชุมพร คิดเป็นร้อยละ 50 (25/50) และร้อยละ 8.3 (1/12) ตามลำดับ โดยจากข้อมูลที่ได้จึงควรมีการทดสอบความไวของเชื้อกลุ่มสแตปฟิลโลคอคคัสต่อยาปฏิชีวนะด้วยวิธีดิสดิฟฟิวชั่นร่วมด้วย (D-test) เพื่อประโยชน์ในการรักษาโรคติดเชื้อสแตปฟิลโลคอคคัส

สำนักวิชาสหเวชศาสตร์และสาธารณสุขศาสตร์ มหาวิทยาลัยวลัยลักษณ์ อำเภอท่าศาลา จังหวัดนครศรีธรรมราช 80161