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Detection and prevalence of inducible clindamycin resistance in staphylococci

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Staphylococcus aureus and coagulase-negative staphylococci (CNS) are recognized as causing nosocomial and community-acquired infections in every region of the world. The resistance to antimicrobial agents among staphylococci is an increasing problem. Clindamycin (CL) is considered to be one of the alternative agents in these infections. This study demonstrates a simple, reliable method (double-disc diffusion test) for detecting inducible resistance to CL in erythromycin-resistance (ER-R) isolates of *S. aureus* and CNS. A total of 883 (52.3 %) isolates of *S. aureus* and 804 (47.7 %) isolates of CNS were selected from recent (2003–2005) clinical isolates recovered in the laboratory of the authors; duplicate isolates were not included. A total of 214 (12.6 %) *S. aureus* and 308 (18.3 %) CNS isolates were selected based on ER-R and CL sensitivity using standard National Committee for Clinical Laboratory Standards disc diffusion testing. A total of 1687 staphylococcal isolates were included, consisting of 27.5 % meticillin-resistant *S. aureus*, 24.8 % meticillin-sensitive *S. aureus*, 36.1 % meticillin-resistant CNS and 11.6 % meticillin-sensitive CNS isolates: 30.9 % of staphylococcal isolates (214 *S. aureus* and 308 CNS) that were erythromycin resistant and CL sensitive were tested for inducible resistance using the D-test. A D-shaped zone around the CL was observed for 70.9 % of staphylococcal isolates (81.8 % of *S. aureus* isolates and 63.3 % of CNS isolates) with an ER-R and a clindamycin-sensitive (CL-S) phenotype. The organism was positive for inducible clindamycin resistance (CL-R). There was a 21.9 % level of inducible macrolide-lincosamide-streptogramin B resistance phenotype among all the staphylococcal isolates. When the *S. aureus* and CNS strains among all the staphylococcal isolates were compared statistically, inducible CL-R in CNS strains was determined to be 23 % more positive ($P=0.028$, odds ratio 0.77, 95 % confidence interval 0.61–0.98). When a statistical comparison was performed among ER-R but CL-S staphylococcal isolates inducible CL-R in *S. aureus* strains was determined to be 2.6 times more positive ($P=0.000$, odds ratio 2.6, 95 % confidence interval 1.68–4.04). A simple, reliable method of detecting inducible resistance to CL in ER-R isolates of *S. aureus* and CNS is described. Clinical microbiology laboratories should use the double-disc diffusion test as standard practice with all ER-R staphylococci. CL should not be used in patients with infections caused by inducibly resistant staphylococcal isolates. Therapeutic failures may thus be avoided.

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INTRODUCTION

Staphylococcus aureus and coagulase-negative staphylococci (CNS) are recognized to be causing nosocomial and community-acquired infections in every region of world. The increasing prevalence of meticillin resistance among staphylococci is an increasing problem (Fokas *et al.*, 2005). Once these organisms are recognized to be causing infections in a region, it is of interest to determine which of the alternatives to vancomycin are suitable for therapy. *In vitro* susceptibility to clindamycin (CL), trimethoprimsulfamethoxazole, erythromycin (ER), quinolone antibiotics and tetracyclines has frequently been reported (Hussain *et al.*, 2000; Groom *et al.*, 2001; Naimi *et al.*, 2001). The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics is commonly used in the treatment of staphylococcal infections (Fiebelkorn *et al.*, 2003). However, one important issue in CL treatment is the risk of clinical failure during therapy. Therapeutic failures caused by MLS_B inducible resistance are being more commonly reported.

Abbreviations: CI, confidence interval; CL, clindamycin; CNS, coagulase-negative staphylococci; ER, erythromycin; MRCNS, meticillin-resistant coagulase-negative staphylococci; MRSA, meticillin-resistant *S. aureus*; MSCNS, meticillin-sensitive coagulase-negative staphylococci; MSSA, meticillin-sensitive *S. aureus*; OR, odds ratio.

The MLS family of antibiotics has three different mechanisms of resistance: target site modification, enzymic antibiotic inactivation and macrolide efflux pumps (Goldman & Capobianco, 1990; Leclercq & Courvalin, 1991a, b). Inducible MLS_B resistance cannot be determined using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests (Fiebelkorn *et al.*, 2003). Low levels of ER are the most effective inducer of inducible MLS_B resistance (Weisblum & Demohn, 1969). Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if isolates are not tested for inducible CL resistance (CL-R) (Fiebelkorn *et al.*, 2003; Drinkovic *et al.*, 2001).

This study demonstrates a simple, reliable and significant method (double-disc diffusion test) of detecting inducible resistance to CL in ER-resistance (ER-R) isolates of *S. aureus* and CNS.

METHODS

Staphylococcal isolates were selected from clinical isolates recovered in our laboratory from 2003 to 2005; duplicate isolates were not included. A total of 214 *S. aureus* and 308 CNS isolates were selected based on ER-R and CL sensitivity using standard National Committee for Clinical Laboratory Standards (NCCLS) disc diffusion testing. These isolates were tested for inducible resistance using the D-test. A 0.5 McFarland equivalent suspension of organisms was inoculated onto a Mueller–Hinton agar (MHA) plate as described in the NCCLS recommendations (National Committee for Clinical Laboratory Standards, 2003). CL (2 μg) and ER (15 μg) discs were placed 15 mm apart from the centre on the MHA. Plates were analyzed after 18 h of incubation at 35 °C. Interpretation of the diameters of zones of inhibition was as follows: ER sensitive (ER-S) $\geqslant$23 mm, ER intermediate resistance 14–22 mm, ER-R $\leqslant$13 mm; clindamycin sensitive (CL-S) $\geqslant$21 mm, CL intermediate resistance 15–20 mm and CL-R $\leqslant$14 mm. If the ER zone is $\leqslant$13 mm and the CL zone is $\geqslant$21 mm and both have a circular shape, the organism is negative for inducible resistance (D-test negative). If the ER zone is $\leqslant$13 mm and the CL zone is $\geqslant$21 mm with a D-shaped zone around the CL, the organism is positive for inducible resistance (D-test positive) (Fig. 1).

RESULTS

A total of 1687 staphylococcal isolates (883 *S. aureus* and 804 CNS) obtained from consecutive clinical specimens were included, consisting of 27.5 % (464) meticillin-resistant *S. aureus* (MRSA), 24.8 % (419) meticillin-sensitive *S. aureus* (MSSA), 36.1 % (608) meticillin-resistant CNS (MRCNS) and 11.6 % (196) meticillin-sensitive CNS (MSCNS) isolates. Of the staphylococcal isolates, 49.8 % had the CL-S phenotype, 28.3 % the constitutive resistance phenotype and 21.9 % the inducible resistance phenotype (Table 1). The constitutive resistance phenotype predominated over the susceptible phenotype and inducible resistance phenotype (44.2, 31.5 and 24.4 %, respectively) among the MRSA isolates. The CL-S phenotype predominated over the inducible resistance phenotype and constitutive resistance phenotype (80.7, 14.8 and 4.5 %, respectively) among MSSA isolates. The constitutive resistance phenotype predominated over the susceptible phenotype and inducible resistance phenotype (38.3, 36.0 and 25.7 %, respectively) among the MRCNS isolates. The CL-S phenotype predominated over the inducible resistance phenotype and constitutive resistance phenotype (69.9, 19.9 and 10.2 %, respectively) among the MSCNS isolates (Table 1).

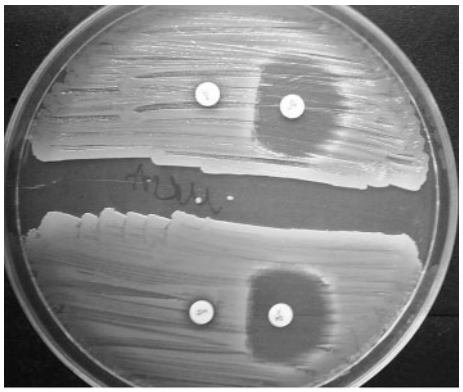

Fig. 1. Double-disc diffusion test (D-test) demonstrating ER disc induction of CL-R; a blunting of the zone of inhibition around the CL disk is produced that forms a D shape. The chi-square test was used to determine significant differences between categorical variables. $P<0.05$ was considered significant.

While in meticillin-resistant staphylococcal isolates the constitutive CL-R phenotype level was 40.9 % and the inducible resistance phenotype level 25.1 %, in meticillin-sensitive staphylococcal isolates the constitutive CL-R phenotype level was 6.3 % and the inducible resistance phenotype level 16.4 %. When the results were statistically compared in meticillin-resistant staphylococcal isolates the

Table 1. Susceptibility to ER and CL among all staphylococcal isolates

Phenotype	MRSA (%)	MSSA (%)	MRCNS (%)	MSCNS (%)	Total no. (%)
ER-S, CL-S	122 (26.3)	323 (77.1)	127 (20.9)	116 (59.2)	688 (40.8)
ER-R, CL-R	205 (44.2)	19 (4.5)	233 (38.3)	20 (10.2)	477 (28.3)
ER-R, CL-S, D^-	24 (5.2)	15 (3.6)	92 (15.1)	21 (10.7)	152 (9.0)
ER-R, CL-S, D^+	113 (24.4)	62 (14.8)	156 (25.7)	39 (19.9)	370 (21.9)
Total	464 (27.5)	419 (24.8)	608 (36.1)	196 (11.6)	1687

constitutive CL-R phenotype was determined to be 10.2 times greater [$P=0.000$, odds ratio (OR) 10.2, 95 % confidence interval (CI) 7.13–14.66] and the inducible resistance phenotype 1.7 times greater ($P=0.000$, OR 1.7; 95 % CI 1.31–2.22) than that in meticillin-sensitive staphylococcal isolates.

There was a 21.9 % inducible MLS_B resistance phenotype level among all the staphylococcal isolates (19.8 % among *S. aureus* and 24.3 % among CNS isolates). When the *S. aureus* and CNS strains among all staphylococcal isolates were statistically compared, inducible CL-R in CNS strains was determined to be 23 % more positive ($P=0.028$, OR 0.77, 95 % CI 0.61–0.98) (Table 2). MRSA and MSSA strains among *S. aureus* isolates were also statistically compared, and inducible CL-R in MRSA strains was determined to be 1.85 times more positive ($P=0.000$, OR 1.85, 95 % CI 1.30–2.65).

A total of 522 (30.9 %) staphylococcal isolates (214 *S. aureus* and 308 CNS) that showed ER-R but were CL-S were tested for inducible resistance using the D-test. Three hundred and seventy (70.9 %) staphylococcal isolates (81.8 % of *S. aureus* and 63.3 % of CNS) with the ER-R and CL-S phenotype were observed to have a D-shaped zone around the CL. The organism was positive for inducible CL-R. When a statistical comparison was performed among the ER-R but CL-S staphylococcal isolates, inducible CL-R in *S. aureus* strains was determined to be 2.6 times more positive ($P=0.000$, OR 2.6, 95 % CI 1.68–4.04).

DISCUSSION

The increasing frequency of staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of CL therapy to treat such infections (Frank *et al.*, 2002). CL is frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration, and the fact that it accumulates in abscesses and no renal dosing adjustments are needed (Kasten, 1999). Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. CL is a good alternative for the treatment of both meticillin-resistant and -susceptible staphylococcal infections (Fiebelkorn *et al.*, 2003). CL-R can develop in staphylococcal isolates with the inducible phenotype, and spontaneous constitutively resistant mutants have been selected from such isolates both *in vitro* and *in vivo* during CL therapy (Drinkovic *et al.*, 2001; Panagea *et al.*, 1999; Siberry *et al.*, 2003). In 1969, McGehee *et al.* demonstrated the development of CL-R *in vivo* and *in vitro* in ER-resistant staphylococci (McGehee *et al.*, 1969). Other authors have confirmed the rapid *in vitro* conversion of inducible to constitutive MLS_B resistance in staphylococci (Panagea *et al.*, 1999; Werckenthin *et al.*, 1999). The 2004 NCCLS guidelines recommend use of the double-disc test and suggest that isolates with the inducible resistance phenotype should be reported as CL resistant (National Committee for Clinical Laboratory Standards, 2004). However, the clinical efficacy of CL treatment for infections caused by inducibly resistant staphylococci remains unclear. The few cases reported so far have presented conflicting results, and the elimination of a potentially useful drug such as CL is undesirable, especially for the treatment of MRSA infections (Rao, 2000).

Table 2. Susceptibility to ER and CL in *S. aureus* and CNS strains

Phenotype	*S. aureus* (%)	CNS (%)	*P*	OR	95 % CI
ER-S, CL-S	445 (50.4)	243 (30.2)	0.000	2.35	1.91–2.88
ER-R, CL-R	224 (25.4)	253 (31.5)	0.005	0.74	0.60–0.98
ER-R, CL-S, D^-	39 (4.4)	113 (14.1)	0.000	0.28	0.19–0.42
ER-R, CL-S, D^+	175 (19.8)	195 (24.3)	0.028	0.77	0.61–0.98
Total	883	804	–	–	–

This study demonstrates that the D shape of the CL zone adjacent to an ER disc in a conventional disc diffusion test can serve to detect *S. aureus* or CNS strains with inducible resistance to CL. A total of 81.8 % of *S. aureus* and 63.3 % of CNS isolates with the ER-R and CL-S phenotype demonstrated inducible resistance. Overall, the results indicate a high incidence (21.9 %) of the inducible MLS_B resistance phenotype among all the staphylococcal isolates (19.8 % of *S. aureus* and 24.3 % of CNS isolates). Our results demonstrate that in the case of a staphylococcal infection inducible MLS_B resistance in CNS strains is greater than that in *S. aureus* strains. When the clinician is faced with a staphylococcal isolate, the probability that this strain may be inducible MLS_B resistant is 21.9 %. If this strain is CNS, then the probability that this strain may be inducible MLS_B resistant will be 20.3 % greater. Provided that this strain is meticillin resistant, the possibility that the strain may be inducible MLS_B resistant is 1.7 times higher compared to a meticillin-sensitive strain. Provided that the clinician is informed of ER-R and CL-S phenotype staphylococcal isolates, the probability of inducible MLS_B resistance is high. If it is an *S. aureus* strain, its inducible MLS_B resistance is 2.6 times higher than that of CNS strains. In such a case, the doctor is advised to provide treatment considering inducible MLS_B resistance.

In a study conducted in Brazil, 11.3 % of *S. aureus* and 13.7 % of CNS isolates were determined to have the inducible MLS_B resistance phenotype (Van der Heijden *et al.*, 2001). It was reported that 80 % of *S. aureus* and 63 % of CNS isolates with the ER-R and CL-S phenotype demonstrated inducible resistance in a New York City medical centre (Van Horn & Toth, 2003). Fiebelkorn concluded that 29 % of 114 ER-R *S. aureus* isolates demonstrated inducible CL-R while 30 % of CNS isolates demonstrated inducible CL-R (Fiebelkorn *et al.*, 2003).

In our study, the inducible CL-R phenotype level was 24.4 % among MRSA isolates, 14.8 % among MSSA isolates, 25.7 %

among MRCNS isolates and 19.9 % among MSCNS isolates. In another study conducted in Turkey, 5.7 % among MRSA isolates, 3.6 % of MSSA isolates, 30.8 % of MRCNS isolates and 15.3 % of MSCNS isolates were determined to have the inducible CL-R phenotype (Azap *et al.*, 2005). Schreckenberger *et al.* (2004) reported incidences of inducible CL-R of 7–12 % for MRSA, 19–20 % for MSSA and 14–35 % for CNS in two hospitals.

In the light of the restricted range of antibiotics available for the treatment of meticillin-resistant staphylococcal infections and the known limitations of vancomycin, CL should be considered for the management of serious soft tissue infections with meticillin-resistant staphylococci that either are sensitive to CL or exhibit inducible CL-R (Rao, 2000).

The double-disc diffusion test is necessary to correctly discriminate between inducible CL-R and susceptibility to CL. However, if inducible 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-S strains. ER-R staphylococci should not be assumed to have CL-R.

In this study, we describe a simple, reliable method of detecting inducible resistance to CL in ER-R isolates of *S. aureus* and CNS. Clinical microbiology laboratories should use the double-disc diffusion test as standard practice with all ER-R staphylococci. Furthermore, in applying the susceptibility test to staphylococcal isolates, clinical microbiology laboratories should place the ER disc 15 mm apart from the CL disc. Thanks to the early detection of inducible MLS_B resistance, such a measure will enable the clinician to save time. Consequently, treatment using CL can be omitted in patients with infections caused by inducibly resistant strains, and therapeutic failures may thus be avoided.

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