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# Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci

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**Resistance to macrolides in staphylococci may be due to active efflux (encoded by *msrA*) or ribosomal target modification (macrolide-lincosamide-streptogramin B [$MLS_B$] resistance; usually encoded by *ermA* or *ermC*). $MLS_B$ resistance is either constitutive or inducible following exposure to a macrolide. Induction tests utilize closely approximated erythromycin and clindamycin disks; the flattening of the clindamycin zone adjacent to the erythromycin disk indicates inducible $MLS_B$ resistance. The present study reassessed the reliability of placing erythromycin and clindamycin disks in adjacent positions (26 to 28 mm apart) in a standard disk dispenser, compared to distances of 15 or 20 mm. A group of 130 clinical isolates of *Staphylococcus aureus* and 100 isolates of erythromycin-resistant coagulase-negative staphylococci (CNS) were examined by disk approximation; all CNS isolates and a subset of *S. aureus* isolates were examined by PCR for *ermA*, *ermC*, and *msrA*. Of 114 erythromycin-resistant *S. aureus* isolates, 39 demonstrated constitutive resistance to clindamycin, while 33 showed inducible resistance by disk approximation at all three distances. Only one isolate failed to clearly demonstrate induction at 26 mm. Of 82 erythromycin-resistant CNS isolates that contained *ermA* or *ermC*, 57 demonstrated constitutive clindamycin resistance, and 25 demonstrated inducible resistance, at 20 and 26 mm. None of the 42 *S. aureus* isolates or 18 CNS isolates containing only *msrA* and none of the erythromycin-susceptible isolates yielded positive disk approximation tests. Simple placement of erythromycin and clindamycin disks at a distance achieved with a standard disk dispenser allowed detection of 97% of *S. aureus* strains and 100% of CNS strains with inducible $MLS_B$ resistance in this study.**

Bacterial resistance to antimicrobial agents generally involves drug inactivation, target site modification, impermeability, or efflux mechanisms. Macrolide antibiotic resistance in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) may be due to an active efflux mechanism encoded by *msrA* (conferring resistance to macrolides and type B streptogramins only) (16, 17) or may be due to ribosomal target modification, affecting macrolides, lincosamides, and type B streptogramins ($MLS_B$ resistance). *erm* genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation of the 23S rRNA, reducing binding by MLS agents to the ribosome (15). Resistance is induced by the binding of a macrolide to upstream translational attenuator sequences, leading to changes in mRNA secondary structure, exposure of the ribosomal binding site, and translation of the *erm* methylase. Alterations in these 5′ upstream sequences, including deletions, duplications, and other mutations, lead to constitutive expression of the methylase gene and constitutive $MLS_B$ resistance (1, 15, 24). Strains with inducible $MLS_B$ resistance ($MLS_B$i) strains demonstrate in vitro resistance to 14- and 15-member macrolides (e.g., erythromycin), while appearing susceptible to 16-member macrolides, lincosamides, and type B streptogramins; strains with constitutive $MLS_B$ resistance ($MLS_B$c strains) show in vitro resistance to all of these agents (15).

MLS antibiotics are commonly used in treatment of staphylococcal infections. Clindamycin is a frequent choice for some staphylococcal infections, particularly skin and soft-tissue infections, and as an alternative in the penicillin-allergic patient. Inducible $MLS_B$ resistance is not recognized by using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests. Failure to identify inducible $MLS_B$ resistance may lead to clinical failure of clindamycin therapy (3). Conversely, labeling all erythromycin-resistant staphylococci as clindamycin resistant prevents the use of clindamycin in infections caused by truly clindamycin-susceptible staphylococcal isolates.

Low levels of erythromycin are the most effective inducer of inducible $MLS_B$ resistance (23). To detect $MLS_B$i strains, there are special disk approximation tests that incorporate erythromycin induction of clindamycin resistance (23). These tests involve the placement of an erythromycin disk in close proximity to a disk containing clindamycin or lincomycin. As the erythromycin diffuses through the agar, resistance to the lincosamide is induced, resulting in a flattening or blunting of the lincosamide zone of inhibition adjacent to the erythromycin disk, giving a D shape to the zone (D-zone effect). Jenssen and colleagues suggested that closer spacing than in a standard disk dispenser was necessary to discern inducible resistance, with optimal spacing of 10 to 15 mm (6); 20-mm spacing of disks and a higher concentration of erythromycin (30 μg) have also been suggested (18).

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In this study, we reassessed the reliability of simply placing erythromycin and clindamycin disks in adjacent positions in a standard disk dispenser and compared it to that of special disk approximation tests that employ closer disk spacing. The ability of disk induction tests to predict the resistance genotype was determined by performing PCR for the *ermA*, *ermC*, and *msrA* genes with a selected group of *S. aureus* and CNS clinical isolates.

## MATERIALS AND METHODS

**Selection of test strains.** A group of 130 isolates of *S. aureus* and 100 isolates of CNS were selected from recent (1998 to 2003) clinical isolates recovered in our laboratory; duplicate isolates were not included. One hundred fourteen *S. aureus* isolates were selected based on erythromycin resistance by standard NCCLS disk diffusion testing (12); approximately one-third of these were methicillin-resistant *S. aureus* isolates. Strains that appeared susceptible to clindamycin and those that appeared resistant by standard disk testing were also included. An additional 16 erythromycin- and clindamycin-susceptible isolates of *S. aureus* were randomly selected. Similarly, 100 CNS isolates were selected based on erythromycin resistance by standard disk testing or by testing with the Vitek 2 instrument (bioMerieux, Inc., Durham, N.C.). CNS isolates were identified to the species level with the Vitek 2 GPC ID card (9).

**Quality control strains.** Control strains for disk diffusion tests included *S. aureus* ATCC 25923 (macrolide and clindamycin susceptible, negative for *ermA*, *ermC*, and *msrA*) and *S. aureus* 58-424, which contained *ermA* and demonstrated inducible $MLS_B$ resistance (F. C. Tenover, Centers for Disease Control and Prevention). Positive controls for PCR included reference strains *S. aureus* RN1551 (containing *ermA*; F. C. Tenover), *S. aureus* RN4220 (with plasmid pE194 containing *ermC*; J. Sutcliffe, Pfizer, Inc.), and *S. aureus* RN4220 (with plasmid pAT10 containing *msrA*; J. Sutcliffe).

**Disk diffusion tests.** The standard NCCLS disk diffusion test was performed on each isolate using unsupplemented Mueller-Hinton agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and standard 15-μg erythromycin disks and 2-μg clindamycin disks (Becton Dickinson). For the first part of the experiment, a standard disk diffusion dispenser (Becton Dickinson) was used to dispense the two test disks. This resulted in a distance of 28 mm from disk edge to disk edge if the disks were placed in peripheral positions in the dispenser or 26 mm if the disks were placed in interior positions. For *S. aureus* isolates, on the same agar plates, two additional pairs of disks were placed by hand to provide distances of 15 and 20 mm between the respective erythromycin-clindamycin disk pairs. For CNS isolates, only 20- and 26-mm placements were used. Following incubation for 16 to 18 h at 35°C, zone diameters were measured in the usual manner; significant ingrowth within a zone up to the edge of the disk was considered constitutive resistance. In addition, each clindamycin zone was examined carefully by using both incident light to examine the plate against a dark background and transmitted light to detect any flattening or blunting of the shape of the clindamycin zone, indicating inducible resistance.

**Preparation of whole-cell DNA.** Whole-cell DNA from *S. aureus* and CNS isolates was extracted with the QIAamp DNA minikit (Qiagen, Inc., Valencia, Calif.), with the following modifications. Approximately 25 colonies from an overnight growth on a sheep blood agar plate were suspended in 1 ml of 10 mM $NaPO_4$ buffer, pH 7.0, and centrifuged at 10,000 × *g* for 5 min. The cell pellet was resuspended in 100 μl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) with 20 μl of lysostaphin (1 mg/ml) (Sigma Chemical Company, St. Louis, Mo.), and the mixture was incubated at 37°C for 60 min. Proteinase K and buffer AL were added according to the QIAamp DNA minikit protocol D for bacteria. Lysates were then applied to QIAamp spin columns, and total DNA was extracted according to the manufacturer's protocol. Extracted DNA was stored at −20°C until PCR was performed.

**PCR for *ermA*, *ermC*, and *msrA* genes.** Multiplex PCR was performed with oligonucleotide primers specific for the *ermA* and *ermC* genes as described by Khan and colleagues, amplifying 610- (*ermA*) and 520-bp (*ermC*) gene fragments (8). Each reaction was carried out in a final volume of 50 μl and included 5 μl of 10× PCR buffer ($MgCl_2$ free; 200 mM Tris-HCl [pH 8.4], 500 mM KCl) (Invitrogen, Inc., Carlsbad, Calif.), 4 μl of 50 mM $MgCl_2$ (Invitrogen), 1 μl each of dATP, dTTP, dCTP, and dGTP (10 mM; Applied Biosystems, Inc., Foster City, Calif.), 1 μl each of the *ermA* forward primer, the *ermA* reverse primer, the *ermC* forward primer, and the *ermC* reverse primer (50 μM; Sigma Genosys, The Woodlands, Tex.), 31.75 μl of molecular grade water, 0.25 μl of AmpliTaq Gold *Taq* polymerase (Applied Biosystems, Inc.), and 1 μl of extracted template DNA.

TABLE 1. Detection of inducible lincosamide resistance by disk induction testing with erythromycin and clindamycin disks at different distances

| Organism and phenotype[d] (no. of isolates) | No. of isolates with positive disk induction test[a] at a separation[b] (mm) of: | | |
|---|---|---|---|
| | 15 | 20 | 26–28 |
| *S. aureus* (130) | | | |
| $Ery^s$ $Cli^s$ (16) | 0 | 0 | 0 |
| $Ery^r$ $Cli^s$ (75) | 34 | 34 | 33[c] |
| $Ery^r$ $Cli^r$ (39) | NA[e] | NA | NA |
| CNS (100) | | | |
| $Ery^r$ $Cli^s$ (43) | ND[f] | 25 | 25 |
| $Ery^r$ $Cli^r$ (57) | ND | NA | NA |

[a] Positive test indicated by flattening of the clindamycin zone.
[b] Distance between disks from edge to edge.
[c] One *S. aureus* isolate showed no blunting or flattening of the clindamycin zone at a disk spacing of 26 mm. This isolate contained the *ermA* gene by PCR.
[d] Ery, erythomycin; Cli, clindamycin; r, resistant; s, susceptible.
[e] NA, not applicable.
[f] ND, not done.

Amplification was performed using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Inc.). Reaction mixtures were initially heated for 10 min at 95°C (hot start) and then underwent 35 cycles of amplification. Each cycle consisted of a 60-s denaturation step at 95°C, a 55-s annealing step at 53°C, and a 60-s extension step at 72°C. The extension step of the final cycle was extended by 5 min. PCR for the *msrA* efflux gene was performed with primers described by Lina and colleagues that amplified a 940-bp product (10). Each reaction was carried out in a final volume of 50 μl and was identical to those for *ermA*/*ermC* multiplex PCR, with the substitution of 1 μl each of the *msrA* forward primer and the *msrA* reverse primer (50 μM) and 33.75 μl of molecular-grade water. Thermocycling conditions included a 10-min hot start at 95°C and 35 cycles of amplification, with each cycle consisting of a 45-s denaturation step at 94°C, a 60-s annealing step at 52°C, and a 60-s extension step at 72°C. The extension step of the final cycle was extended by 5 min. PCR products were stored at 4°C until they were analyzed by gel electrophoresis. Positive and negative controls were included in each run. Positive controls for *ermA* and *ermC* included reference strains *S. aureus* RN1551 and *S. aureus* RN4220 (with *ermC*); *S. aureus* RN4220 (with *msrA*) served as a positive control for *msrA*. *S. aureus* ATCC 29213 (negative for *ermA*, *ermC*, and *msrA*) was tested multiple times with each primer set. Molecular-grade water (1 μl) was substituted for template DNA as the negative control included in every run.

**Gel electrophoresis.** Amplified DNA was detected by gel electrophoresis through 1.8% agarose gels containing 0.5 μg of ethidium bromide/ml for 90 min at 100 V. The sizes of the PCR products were estimated with standard molecular weight markers (Hi-Lo DNA marker; Minnesota Molecular, Inc., Minneapolis, Minn.). Isolates were considered positive for *ermA*, *ermC*, or *msrA* in the presence of the respective PCR products of the expected sizes.

## RESULTS

Results of disk induction testing for *S. aureus* and CNS are shown in Table 1. Examples of disk induction test phenotypes are shown in Fig. 1. The sensitivities of disk induction testing for detection of $MLS_B$i strains containing *ermA* or *ermC* were 100% at 15 and 20 mm and 97% (33 of 34) at 26 to 28 mm for *S. aureus*. Sensitivity was 100% at both 20 and 26 mm for CNS. Induction testing was also specific; none of the 16 erythromycin-susceptible *S. aureus* isolates demonstrated any flattening or blunting of the clindamycin or erythromycin zones. For *S. aureus* isolates with an inducible $MLS_B$ phenotype, the mean clindamycin zone size was 24.9 mm (range, 23 to 27 mm); for clindamycin-susceptible isolates, mean zone size was 25.4 mm (range, 21 to 27 mm). For CNS, inducible isolates had a mean

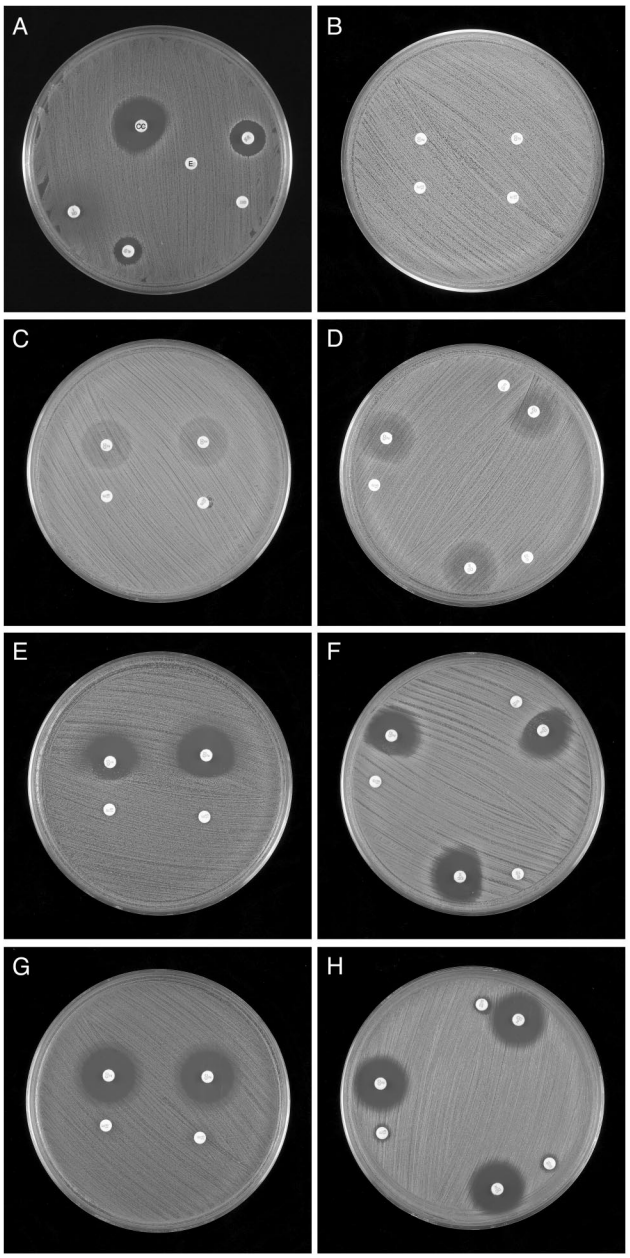


FIG. 1. Disk induction testing. (A) Positive disk induction test within a routine disk diffusion format (CNS; University Hospital). Erythromycin and clindamycin disks were placed in adjacent central positions in a standard disk dispenser. (B to D) Constitutive lincosamide resistance by disk induction testing. Growth extends to the edge of the clindamycin disk. (B) *S. haemolyticus* (*ermA ermC msrA*); (C) *S. epidermidis* (*ermA*), demonstrating a "double zone" of growth around the clindamycin disk; (D) *S. aureus* (*ermA*) showing a double-zone of growth and blunting of the outer clindamycin zone (discernible at 15 and 20 mm). (E and F) Positive disk induction test indicating inducible lincosamide resistance. Flattening of the clindamycin inhibition zone adjacent to the erythromycin disk is easily discernible at 15 and 20 mm (disks placed by hand), as well as at 26 mm. (E) *S. hominis* (*ermC*); (F) *S. aureus* (*ermC*). (G and H) Negative disk induction test indicating the absence of inducible lincosamide resistance. No flattening or distortion of the clindamycin zone adjacent to the erythromycin disk can be detected at a disk spacing of 15, 20, or 26 mm. (G) *S. epidermidis* (*msrA*); (H) *S. aureus* (*msrA*).

TABLE 2. Resistance genotypes of erythromycin-resistant isolates of *S. aureus* and CNS

| Organism and phenotype[a] | No. of isolates available for PCR/total no. of isolates | No. of isolates with genotype: | | | | | |
|---|---|---|---|---|---|---|---|
| | | *ermA* | *ermC* | *msrA* | *ermA msrA* | *ermC msrA* | *ermA ermC msrA* |
| *S. aureus* | 86/114 | | | | | | |
| $C^{s}$ | 31/41 | 0 | 0 | 31 | 0 | 0 | 0 |
| $C^{r\text{-}ind}$ | 28/34 | 8 | 20 | 0 | 0 | 0 | 0 |
| $C^{r\text{-}const}$ | 27/39 | 22 | 0 | 0 | 0 | 5 | 0 |
| *CNS* | 100/100 | | | | | | |
| $C^{s}$ | 18/18 | 0 | 0 | 18 | 0 | 0 | 0 |
| $C^{r\text{-}ind}$ | 25/25 | 5 | 18 | 0 | 1[c] | 1[c] | 0 |
| $C^{r\text{-}const}$ | 57/57 | 13 | 33 | 1[b] | 3[d] | 3[e] | 4[f] |

[a] Determined by standard disk diffusion test and disk induction test C, clindamycin; S, susceptible; r-ind, inducible $MLS_B$ resistance; r-const, constitutive $MLS_B$ resistance.
[b] One isolate of *S. stimulans* (see text).
[c] One *S. epidermidis* isolate.
[d] Three *S. epidermidis* isolates.
[e] Three *S. haemolyticus* isolates.
[f] Four *S. haemolyticus* isolates.

clindamycin zone size of 28.5 mm (range, 21 to 32 mm) and susceptible isolates had a mean zone size of 28.5 mm (range, 21 to 35 mm). An inducible $MLS_B$ phenotype could not be predicted based on the size of the clindamycin zone of inhibition.

Thirty *S. aureus* isolates (23%) showed significant ingrowth within a larger clindamycin zone; this represented 77% of *S. aureus* isolates with a constitutive $MLS_B$ phenotype. Interestingly, a subset of 20 of these isolates showed blunting of the outer zone at 15 or 20 mm (Fig. 1D), including one that showed blunting at 26 to 28 mm. Disk testing was repeated for isolates showing significant ingrowth by selecting colonies in both confluent and light-growth areas to rule out mixed cultures; repeat testing from single colonies revealed the same results. Of 19 *S. aureus* isolates with significant ingrowth that were available for genetic analysis, 18 contained *ermA* (including all 12 isolates with blunting of the outer zone that were available for analysis) and 1 contained *ermC*. Four CNS isolates showed this effect; three contained *ermC* (two *S. epidermidis* isolates and one *S. simulans* isolate), and one contained *ermA* (*S. epidermidis*) (Fig. 1C). Growth within the clindamycin zone of inhibition was considered indicative of the constitutive $MLS_B$ phenotype and was easily discernible on examination with reflected light in all *S. aureus* and CNS isolates showing significant clindamycin zone ingrowth.

A subset of 86 *S. aureus* isolates and all 100 CNS isolates were available for genetic analysis (Table 2). All clindamycin-susceptible isolates with a negative disk approximation test contained *msrA*, but not *ermA* or *ermC*. The majority of *S. aureus* isolates tested possessed only one resistance mechanism; five (6%) possessed an *erm* gene plus *msrA*. A total of 12 (12%) CNS isolates (including 5 *S. epidermidis* isolates and 7 *S. haemolyticus* isolates) possessed two or more resistance genes. Four isolates of *S. haemolyticus* contained all three resistance determinants and showed a constitutive $MLS_B$ resistance phenotype (Fig. 1B). One *S. simulans* isolate with obvious constitutive $MLS_B$ resistance was repeatedly positive only for the

TABLE 3. Phenotype and genotype distribution of CNS by species

| Species (no. of isolates) and phenotype | No. of isolates of with: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Phenotype[a] | | | Genotype | | | | | |
| | $C^s$ | $C^{r\text{-}ind}$ | $C^{r\text{-}const}$ | *ermA* | *ermC* | *msrA* | *ermA msrA* | *ermC msrA* | *ermA ermC msrA* |
| *S. epidermidis* (68) | 9 | 16 | 43 | | | | | | |
| $C^s$ | | | | 0 | 0 | 9 | 0 | 0 | 0 |
| $C^{r\text{-}ind}$ | | | | 3 | 11 | 0 | 1 | 1 | 0 |
| $C^{r\text{-}const}$ | | | | 10 | 30 | 0 | 3 | 0 | 0 |
| *S. haemolyticus* (20) | 7 | 4 | 9 | | | | | | |
| $C^s$ | | | | 0 | 0 | 7 | 0 | 0 | 0 |
| $C^{r\text{-}ind}$ | | | | 1 | 3 | 0 | 0 | 0 | 0 |
| $C^{r\text{-}const}$ | | | | 1 | 1 | 0 | 0 | 3 | 4 |
| *S. simulans* (3) | 0 | 1 | 2 | | | | | | |
| $C^{r\text{-}ind}$ | | | | 1 | 0 | 1 | 0 | 0 | 0 |
| $C^{r\text{-}const}$ | | | | 0 | 1 | 0 | 0 | 0 | 0 |
| *S. auricularis* (3) | 0 | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 0 |
| *S. hominis* (3) | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| *S. warneri* (2) | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| *S. capitis* (1) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |

[a] Determined by standard disk diffusion test and disk induction test. Abbreviations are as defined for Table 2.

efflux determinant *msrA*, in the absence of both *ermA* and *ermC* (Table 3).

## DISCUSSION

This study has demonstrated that careful examination of the shape of the clindamycin zone adjacent to a standard 15-μg erythromycin disk in a conventional disk diffusion test can serve to detect *S. aureus* or CNS strains with inducible resistance to clindamycin. Flattening of the clindamycin disk diffusion zone in an erythromycin-resistant isolate (D-zone effect) appears to be a reliable indicator of $MLS_B$i strains that harbor either the *ermA* or *ermC* gene. Constitutively $MLS_B$-resistant strains are easily recognized by a clindamycin zone diameter of ≤14 mm with or without significant ingrowth. Erythromycin-susceptible strains do not possess inducible clindamycin resistance.

In our study, the majority of *S. aureus* isolates with constitutive $MLS_B$ resistance showed significant ingrowth in the clindamycin zone (an outer zone of confluent growth and an inner zone of lighter growth extending to the edge of the disk); all but one isolate available for genotypic analysis contained *ermA*. Di Modugno and colleagues described this phenomenon for staphylococcal isolates with *ermA*; they described two end points obtained by broth microdilution, with a transition first from confluent to light growth and then from light growth to no growth (2). Single methylation of the ribosomal target by the ErmA methylase versus dimethylation by the ErmC methylase may be related to this phenomenon, or other factors may be involved. The explanation for significant ingrowth in one *S. aureus* isolate and three CNS isolates containing *ermC* is unclear. However, this phenomenon was easily recognized in disk tests and did not lead to reporting false clindamycin susceptibility.

The flattening of the clindamycin zone of inhibition by an adjacent erythromycin disk is more obvious if the two disks are placed closer together than the standard 26- to 28-mm spacing of a standard disk dispenser. A distance of 15 or 20 mm between disks provided the most obvious flattening of the clindamycin zones among the strains examined in this study. However, simply placing the erythromycin and clindamycin disks in adjacent positions in a normal disk pattern (especially in the central positions, resulting in 26-mm disk separation) appears to provide a reliable means of detection of the $MLS_B$i strains in a clinical laboratory setting without specialized testing or nonstandard disk placement. Jenssen and colleagues were able to predict the *erm* genotype by disk induction testing using a disk spacing of 10 to 15 mm (6). Using disk spacing achieved with a standard disk dispenser (26 to 28 mm), we also correlated disk induction testing with the presence of *erm* genes and were able to detect an $MLS_B$i phenotype in all but one of the clinical *S. aureus* isolates tested and in all CNS isolates. The *S. simulans* isolate with obvious constitutive clindamycin resistance and positive only for the efflux determinant *msrA* may contain another lincosamide resistance mechanism not detected in this study, such as one encoded by *ermB*, which has rarely been reported in staphylococcal isolates primarily of animal origin (4, 10, 20). Other lincomycin resistance elements in staphylococci previously described, such as lincosamide-modifying enzymes, could also be involved (15).

In 1969, McGehee and colleagues demonstrated the development of clindamycin resistance in vivo and in vitro in erythromycin-resistant staphylococci (11). Other investigators have confirmed the rapid in vitro conversion of inducible to constitutive $MLS_B$ resistance in staphylococci (14, 24). There have also been a number of reported clindamycin or lincomycin therapy failures in serious infections due to staphylococci with inducible $MLS_B$ resistance, indicating that it is not uncommon (3, 5, 11, 21, 22; G. G. Rao, Letter, J. Antimicrob. Chemother. **45:**715). This has led to questioning the safety of clindamycin use against any erythromycin-resistant staphylococci. Because of the high reported incidence of inducible $MLS_B$ resistance, particularly in *S. aureus* (5), it has been suggested that in vitro erythromycin resistance could serve as a surrogate for all MLS agents, regardless of susceptibility test results, and that disk induction testing be performed on isolates from serious CNS infections (18).

Some published studies have indicated that approximately 45% of erythromycin-resistant *S. aureus* isolates have inducible $MLS_B$ resistance (6, 19); that is, for almost every isolate showing constitutive clindamycin resistance (easily discernible by standard methods), there is an isolate with inducible resistance that would go unrecognized without disk induction testing. However, the incidence of constitutive and inducible $MLS_B$ resistance varies by geographic region and even from hospital to hospital, with some studies showing high local incidence of either constitutive or inducible $MLS_B$ resistance in staphylococcal isolates (2, 5, 6, 13, 15). In addition, other mechanisms conferring resistance to macrolides and not lincosamides, such as efflux mechanisms encoded by *msrA*, are not uncommon (4) and may be increasing in frequency (15, 18). A significant number of erythromycin-resistant staphylococcal isolates may show true clindamycin susceptibility. In our clinical laboratory, of 617 erythromycin-resistant *S. aureus* isolates obtained over a

12-month period (in 2002), 310 (50%) were clindamycin susceptible, with no indication of inducible resistance by disk induction testing as described in this report; of 624 erythromycin-resistant CNS isolates, 206 (33%) were clindamycin susceptible. Therefore, the broad assumption of clindamycin resistance based on erythromycin resistance and the elimination of clindamycin as a potential therapeutic agent for up to 50% of erythromycin-resistant staphylococcal infections are problematic.

Clindamycin is a useful drug in the treatment of skin and soft-tissue infections and serious infections caused by staphylococcal species, as well as anaerobes. It has excellent tissue penetration (except for the central nervous system) and accumulates in abscesses, and no renal dosing adjustments are needed (7). Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. Clindamycin is also of particular importance as an alternative antibiotic in the penicillin-allergic patient.

Accurate susceptibility data are important for appropriate therapy decisions. In staphylococci, in vitro susceptibility testing for clindamycin may indicate false susceptibility by the broth microdilution method and by disk diffusion testing with erythromycin and clindamycin disks in nonadjacent positions. However, if inducible resistance can be reliably detected on a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in those patients with true clindamycin-susceptible strains. In this study, we have described a simple, reliable method to detect inducible resistance to clindamycin in erythromycin-resistant isolates of *S. aureus* and CNS.

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