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Antimicrobial susceptibility studies

Investigation of inducible clindamycin and telithromycin resistance in isolates of β-hemolytic streptococci

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Abstract

We evaluated the accuracy of an erythromycin–clindamycin double-disk test (D-zone test) and an erythromycin–telithromycin D-zone test for detection of inducible resistance in isolates of β-hemolytic streptococci with erythromycin resistance. The results of these tests were compared to results of a broth microdilution (BMD) induction test using combinations of erythromycin and either clindamycin or telithromycin. Of 29 erythromycin-resistant, clindamycin-susceptible isolates, 16 were positive by the erythromycin–clindamycin D-zone test; all of these demonstrated inducible clindamycin resistance by BMD. Twelve isolates were D-zone test–negative, did not demonstrate inducible resistance by BMD, and were positive for a *mef* determinant. Of 39 erythromycin-resistant, telithromycin-susceptible isolates, 13 were erythromycin–telithromycin D-zone test–positive, 19 questionably positive (unclear blunting of the zone), and 7 were D-zone test–negative. The erythromycin–telithromycin D-zone test result did not correlate with inducible resistance by BMD or the presence of an *erm* or *mef* gene. These results demonstrate that the erythromycin–clindamycin D-zone and BMD induction tests accurately detect inducible clindamycin resistance, but the erythromycin–telithromycin D-zone test is not reliable for detecting inducible telithromycin resistance.

Keywords: Erythromycin; D-zone test; Streptococci

1. Introduction

β-Hemolytic streptococci cause a variety of infections. These organisms are typically susceptible to penicillin and other β-lactam agents. In patients with penicillin allergies, alternative therapies, such as macrolides (e.g., erythromycin), lincosamides (e.g., clindamycin), and ketolides (e.g., telithromycin), are often considered for treatment of infections. Because resistance to erythromycin and clindamycin is becoming more common in β-hemolytic streptococci, including inducible resistance to clindamycin, accurate susceptibility methods for testing are required to guide therapy (Leclercq, 2002).

Streptococci become resistant to erythromycin either by acquiring a *mef* gene, which encodes an efflux pump, or by acquiring an *erm* gene, which encodes a methylase that modifies the erythromycin binding site on ribosomal RNA (Leclercq, 2002). Since the *mef*-encoded efflux pump cannot export clindamycin or telithromycin, isolates containing *mef* remain susceptible to these agents, expressing the M (macrolide only) phenotype. Constitutive expression of an *erm*-encoded methylase results in resistance to erythromycin, clindamycin, and streptogramin B drugs (the $cMLS_B$ phenotype). Induction of the *erm*-encoded methylase results in resistance to erythromycin and clindamycin ($iMLS_B$ phenotype). Clindamycin is a poor inducer of *erm*. A conversion from inducible to constitutive expression occurs by a single base substitution in the leader sequence of *erm* genes (Fines et al., 2001). Telithromycin, such as erythromycin and clindamycin, acts by binding to rRNA. Constitutive *erm*-mediated resistance can also confer telithromycin resistance in some streptococcus isolates, but whether induction of the *erm*-encoded methylase results in telithromycin resistance remains to be established (Jalava et al., 2001). Inducible telithromycin resistance was recently described for *Staphylococcus* spp. with *erm*-mediated resistance (Davis et al., 2005).

The $iMLS_B$ phenotype can also be found in erythromycin-resistant, clindamycin-susceptible isolates of *Staphylococcus*

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aureus. Several cases of clindamycin treatment failures for *S. aureus* infections have been reported (Lewis and Jorgensen, 2005). For this reason, a test to detect isolates with inducible clindamycin resistance, the clindamycin–erythromycin D-zone test, was included in the most recent documents of the Clinical and Laboratory Standards Institute (CLSI, 2005; formerly National Committee for Clinical laboratory Standards). This D-zone test can be performed using a standard disk diffusion procedure (Fiebelkorn et al., 2003; Steward et al., 2005) or by disk testing on the agar plate used to check the purity of inocula (Jorgensen et al., 2004).

Although the erythromycin–clindamycin D-zone test has been used to detect $iMLS_B$ resistance in isolates of group A, group B, and group G β-hemolytic streptococci (GAS, GBS, and GGS, respectively) (Hasenbein et al., 2004; Heelan et al., 2004; Jalava et al., 2001; Tang et al., 2004), the test has not been evaluated for routine use in the clinical laboratory. Thus, we evaluated the sensitivity and specificity of the clindamycin–erythromycin D-zone test compared to broth microdilution (BMD) for detection of the $iMLS_B$ phenotype in GAS, GBS, GGS, and group C streptococci (GCS). Because the occurrence of inducible telithromycin resistance and optimal methods for detection of such resistance are unclear, we used BMD for the detection of inducible resistance and compared this to a telithromycin–erythromycin D-zone test, which could be used for routine detection of inducible telithromycin resistance in the clinical laboratory.

2. Materials and methods

2.1. Bacterial isolates

Thirty-nine clinical isolates of β-hemolytic streptococci, including 6 GAS, 22 GBS, 3 GCS, and 8 GGS isolates, were selected for this study from the culture collections of the Centers for Disease Control and Prevention, Project ICARE (Fridkin et al., 1999) and Loyola University Medical Center, Maywood, IL. All isolates were resistant to erythromycin (MIC ≥8 μg/mL) and susceptible to either clindamycin (MIC ≤0.5 μg/mL) or telithromycin (MIC ≤1 μg/mL). The Lancefield group for each isolate was confirmed using the Streptex kit (Remel, Lenexa, KS).

2.2. Antimicrobial susceptibility testing

The MICs of erythromycin, clindamycin, and telithromycin were determined using the CLSI BMD method using panels prepared in-house with Mueller–Hinton broth (Difco Brand, BD Diagnostics, Sparks, MD) supplemented with 5% lysed horse blood (NCCLS, 2003a). The BMD induction test was performed using a limited checkerboard of erythromycin and clindamycin with wells containing erythromycin (0.5–8 μg/mL) with clindamycin (0.25–2 μg/mL) or a limited checkerboard of erythromycin and telithromycin with wells containing erythromycin (0.5–8 μg/mL) with telithromycin (0.25–8 μg/mL). Erythromycin was obtained from Eli Lilly and Company (Indianapolis, IN), clindamycin was obtained from Sigma-Aldrich (St. Louis, MO), and telithromycin was obtained from Aventis Pharmaceuticals (Bridgewater, NJ).

The D-zone test was performed using Mueller–Hinton agar plates with 5% sheep blood (BBL brand, BD Diagnostics). The inoculum was prepared in saline to the density of a 0.5-McFarland standard according to the CLSI disk diffusion procedure (NCCLS, 2003b). An erythromycin disk (15 μg) was placed 12 mm (edge-to-edge) from either a clindamycin disk (2 μg) or a telithromycin disk (15 μg) on the plate. Plates were incubated at 35 °C for 20 h in 5% CO_2. Blunting of the clindamycin zone or telithromycin zone proximal to the erythromycin disk was classified as D-zone test–positive (inducible resistance), whereas no blunting of the zone was classified as D-zone test–negative (no induction).

The purity plate D-zone test was done by inoculating a trypticase soy agar plate containing 5% sheep blood (BBL brand, BD Diagnostics) with either a 1:20 or a 1:200 dilution of a bacterial suspension equivalent to a 0.5-McFarland standard. The dilutions were prepared in saline, and the agar plates were inoculated using a 10-μL disposable loop. The first third of the plate was streaked for confluent growth and the other two thirds were streaked for isolated colonies. Disks for the D-zone test were placed 12 mm apart in the first third of the plate and incubated at 35 °C for 20 h in 5% CO_2. The D-zone test results were read as described above.

S. aureus ATCC BAA-977, *S. aureus* BAA-976, and *S. aureus* ATCC 25923 were tested daily for quality control of the D-zone test. *Streptococcus pneumoniae* ATCC 49619 were tested daily for quality control of the BMD tests.

2.3. Detection of macrolide and clindamycin resistance genes

Bacterial lysates were prepared by suspending a loopful of bacteria in 100 μl of MEQ H_2O and heating to 95 °C for 10 min. The suspension was briefly centrifuged and the supernatant was used for DNA amplification.

A multiplex polymerase chain reaction (PCR) assay was used to screen for genes known to confer macrolide resistance in streptococci, *erm*A(TR), *erm*B, *erm*C, and *mef*E/A. The primers and conditions of this multiplex assay were a modification of those previously reported (Turng et al., 2003) (Table 1). Briefly, the primers for all of the resistance determinants and for the 16S rRNA gene (internal control) were combined in a single tube. The 50-μL PCR consisted of 1× buffer, 3 mmol/L $MgCl_2$, 10 mmol/L dNTP mix, 0.5 μmol/L of each primer (except 16S rRNA primers at 0.05 μmol/L), 1.25 U Taq polymerase (Applied Biosystems, Foster City, CA), and 2 μL of bacterial lysate. Cycling parameters were 94 °C for 4 min followed by 30 cycles of 94 °C for 15 s, 50 °C for 15 s, 72 °C for 30 s, followed by an elongation step at 72 °C for 2 min. Isolates that produced a PCR product of approximately 640 bp were considered positive for *erm* and a 348-bp PCR product was