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Evaluation of Disk Approximation and Single-Well Broth Tests for Detection of Inducible Clindamycin Resistance in *Streptococcus pneumoniae*▽

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This study evaluated an agar disk diffusion D-zone test and an erythromycin-clindamycin (ERY + CLI) single-well broth test for inducible CLI resistance in *Streptococcus pneumoniae*. The standard CLSI disk approximation test and a single-well combination test incorporating 1 plus 0.5 μg/ml ERY + CLI detected >96% of isolates containing the *ermB* determinant.

Constitutive or inducible clindamycin resistance can occur in *Streptococcus pneumoniae* due to an *ermB* gene that encodes a ribosomal methylase that alters the primary binding site of both macrolides and clindamycin (8, 9). Inducible clindamycin resistance is not detected by routine testing of clindamycin alone (1). The Clinical and Laboratory Standards Institute (CLSI) has recommended a disk approximation (D-zone test) with erythromycin and clindamycin disks and a single-well combined erythromycin-plus-clindamycin broth microdilution test for detection of inducible clindamcyin resistance in staphylococci and beta-hemolytic streptococci (5) but has not made specific recommendations for detecting similar resistance in pneumococci.

This three-laboratory collaborative study has assessed the disk approximation test and the single-well (1 μg/ml erythromycin plus 0.5 μg/ml clindamycin) induction test recommended by the CLSI for beta-hemolytic streptococci for applicability to pneumococci (5). The disk approximation tests employed standard erythromycin (15 μg) and clindamycin (2 μg) disks placed 12 mm from edge to edge on Mueller-Hinton 5% sheep blood agar plates incubated for 20 to 24 h at 35°C in a 5% CO_2 environment. A positive D-zone test was noted when flattening of the clindamcyin zone adjacent to the erythromycin disk with erythromycin-resistant isolates was observed (5). The single-well broth test was carried out using three different brands (BBL [BD Microbiology] Difco [BD Microbiology], and Oxoid) of Mueller-Hinton broth supplemented with 3% lysed horse blood prepared as frozen panels specifically for this study according to CLSI guidelines (4). The panels also included concentrations of erythromycin and clindamycin tested alone in order to determine MICs and to detect constitutive clindamycin resistance. The panels were inoculated with 5×10^5 CFU/ml and were incubated at 35°C for 20 to 24 h in ambient air (4). *S. pneumoniae* ATCC 49619 was used as the control strain on each day of testing, and *Staphylococcus aureus* ATCC BAA977 was used for quality assessment of the D-zone test and the erythromycin-plus-clindamycin combination well (5). The study isolates were tested for the *ermB* determinant by PCR as a reference method for comparison, and some strains were also tested for the *mefA* determinant (12).

A total of 102 nonduplicate pneumococcal isolates were tested for susceptibility to erythromycin and clindamycin separately, by the D-zone test, and with the single-well broth induction test (Table 1). The isolates had been selected for this study from among recent CDC Active Bacterial Core surveillance (ABCs) isolates because they were known to be erythromycin resistant and clindamycin susceptible and presumptively demonstrated inducible clindamycin resistance when exposed to erythromycin based upon prior testing (9). They were not consecutive clinical isolates. The disk approximation test revealed a total of 65 pneumococcal strains with D zones indicative of inducible clindamycin resistance. The *ermB* determinant was found to be present in all 65 strains, and one additional isolate contained the *ermB* gene but did not produce a D zone or grow in the single well containing the erythromycin-clindamycin combination (Table 1). With only one exception, the single-well broth test also detected the inducibly clindamycin-resistant (D-zone- and *ermB*-positive) isolates. There were slight medium differences that affected the quality of growth in the broth test wells but did not affect the sensitivity of the single-well test. When compared to PCR for *ermB* as the reference point, the agar-based D-zone test had a sensitivity of 98.5% (65 of 66). The sensitivity of the broth test with the combination of 1 μg/ml erythromycin plus 0.5 μg/ml clindamycin was 97% (64 of 66) when compared to the PCR for *ermB* and 98.5% when compared to the D-zone test. The specificity was 100% for both phenotypic induction tests. Thirty-seven erythromycin-resistant strains that were D-zone and broth single-well test negative did not contain the *ermB* determinant. A convenience sample of 16 of those strains was tested, and all

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TABLE 1. Overall agreement between PCR for *ermB*, separate erythromycin, and clindamycin MICs, D-zone tests, and single-well broth induction tests

Institution (n^a)	No. of strains PCR pos. for *ermB*[b]	MIC, range (μg/ml)		No. of strains positive by:	
		Erythromycin	Clindamycin	D-zone	Broth (1 + 0.5)[c]
UTHSCSA (58)	38	>32	0.06–0.25	38	38
MDH (25)	15	32–>32	0.06–0.25	15	14
CDC (19)	13[d]	32–>32	0.06–0.12	12	12

[a] *n*, no. of strains.
[b] All strains were tested for the *ermB* determinant; pos., positive.
[c] One microgram per milliliter erythromycin + 0.5 μg/ml clindamycin.
[d] One isolate was PCR positive for *ermB* but was negative for inducible resistance by both D-zone and broth combination well tests.

were found to contain only the *mefA* determinant (data are not further depicted).

Similar to the beta-hemolytic streptococci, there are two principal mechanisms of macrolide resistance in *S. pneumoniae* (7, 8). One is an active efflux mechanism encoded by the *mefA* genes that affects only macrolides (9). The second mechanism is methylation of the ribosomal binding site used by the macrolides, lincosamides, and streptogramin B drugs mediated by an *erm* gene (9). Resistance to macrolides and clindamycin has increased in the United States despite the overall effectiveness of the 7-valent protein conjugate pneumococcal vaccine (8). Expression of macrolide-lincosamide-streptogramin B (MLS_B) resistance in pneumococci due to *ermB* can either be constitutive or inducible (9). Inducible resistance is not detected by standard clindamycin MIC or disk testing (9).

Some evidence exists, especially with staphylococci, that there is a risk of spontaneous conversion from the inducible to the constitutive resistance phenotype during clindamycin therapy as the result of a single mutation in a promoter region that controls expression of the *erm* genes (9, 10). Thus, patients could be at risk of clinical failure if inducible clindamycin resistance was not detected in situations in which long-term clindamycin monotherapy was contemplated. Especially in pediatrics, clindamycin is recommended as a second- or third-line therapy (particularly in penicillin-allergic patients) for pneumococcal bone and joint infections (2), acute otitis media (11), and sinusitis (3). Clindamycin has been used in combination with rifampin for chemoprophylaxis during an outbreak of pneumococcal meningitis at a day care center (6). Because pneumococci contain the same *erm*-mediated inducible clindamycin resistance mechanism found in the beta-hemolytic streptococci and *S. aureus*, prudence would suggest that detection of inducible resistance in pneumococci should be considered when long-term therapy of septic arthritis, osteomyelitis, chronic sinusitis, or otitis with clindamycin is under consideration. This can be accomplished by use of either the agar-based disk D-zone test or the single-well broth microdilution test with 1 μg/ml erythromycin plus 0.5 μg/ml clindamycin now advocated by the CLSI for the beta-hemolytic streptococci (5).

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