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A Simplified Method for Testing *Bordetella pertussis* for Resistance to Erythromycin and Other Antimicrobial Agents

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Present methods of antimicrobial susceptibility testing of *Bordetella pertussis* are time consuming and require specialized media that are not commercially available. We tested 52 isolates of *B. pertussis* for resistance to erythromycin, trimethoprim-sulfamethoxazole, chloramphenicol, and rifampin by agar dilution with Bordet-Gengou agar (BGA) containing 20% horse blood (reference method), Etest using BGA and Regan-Lowe agar without cephalexin (RL−C), and disk diffusion using BGA and RL−C. The organisms tested included four erythromycin-resistant isolates of *B. pertussis* from a single patient, a second erythromycin-resistant strain of *B. pertussis* from an unrelated patient in another state, and 47 nasopharyngeal surveillance isolates of *B. pertussis* from children in the western United States. The results of agar dilution testing using direct inoculation of the organisms suspended in Mueller-Hinton broth were within ±1 dilution of those obtained after overnight passage of the inoculum in Stainer-Scholte medium, which is the traditional method of testing *B. pertussis*. The Etest method produced MICs similar to those of the agar dilution reference method for three of the four antimicrobial agents tested; the trimethoprim-sulfamethoxazole results were lower with Etest, particularly when the direct suspension method was used. Most of the Etest MICs, except for that of erythromycin, were on scale. Disk diffusion testing using RL−C medium was helpful in identifying the erythromycin-resistant strains, which produced no zone of inhibition around the disk; susceptible isolates produced zones of at least 42 mm. Thus, the antimicrobial susceptibility testing of *B. pertussis* can be simplified by using the Etest or disk diffusion on RL−C to screen for erythromycin-resistant isolates of *B. pertussis*.

Pertussis continues to be an important disease of infants, children, and adults (4, 9). Although the disease is preventable by vaccination, cases continue to be observed in the United States and elsewhere (4–6). The causative agent of pertussis, *Bordetella pertussis*, traditionally has been susceptible to most antimicrobial agents, including erythromycin, which is the drug of choice for both treatment and prophylaxis (3, 6, 7).

Antimicrobial susceptibility testing of *B. pertussis* has been undertaken only rarely since the late 1960s. In the pioneering studies of Bannatyne and Cheung (1, 2), the method used for determining MICs was a tedious, nonstandardized procedure that required preincubation of organisms in a special broth, followed by inoculation onto either Bordet-Gengou agar (BGA) or a charcoal-containing agar supplemented with as much as 33% animal blood (1, 8, 17). These studies indicated that this organism was universally susceptible to several antimicrobial agents, including erythromycin (1). Surveillance studies undertaken in the last decade revealed no changes in the effectiveness of erythromycin (6, 7, 13). Thus, routine susceptibility testing of *B. pertussis* was considered unnecessary. In 1994, a strain of *B. pertussis* was recovered from a patient in Arizona with whooping cough who did not respond to erythromycin therapy. The isolate was subsequently shown to be resistant to erythromycin (11). Since the onset of this study, another isolate, from Utah, was also reported to be erythromycin resistant (12). The initial erythromycin-resistant strain prompted us to screen for additional isolates of antimicrobial-resistant *B. pertussis*. However, the traditional method for testing pertussis isolates was found to be cumbersome and costly. Thus, the goals of this study were to simplify the method for antimicrobial susceptibility testing of *B. pertussis*, determine the effectiveness of the Etest (a susceptibility testing method that has proven to be useful for testing a variety of microorganisms [10]) for performing MIC tests on *B. pertussis*, and evaluate a disk screening method for erythromycin resistance.

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MATERIALS AND METHODS

Bacterial strains. Fifty-two clinical isolates of *B. pertussis* were tested. Four erythromycin-resistant isolates of *B. pertussis* were obtained from one patient on 4 separate days and were subsequently shown by pulsed-field gel electrophoresis to be identical. However, at the time of the study, we hypothesized that the four isolates may show different resistance profiles since they were isolated at different times during the course of the patient's illness and had been exposed to varying concentrations of erythromycin. Since the isolates could have shown increasing levels of resistance, they were treated as independent isolates. Testing the resistant isolates multiple times also ensured the reproducibility of the test methods. These resistant isolates were provided by Michael Saubolle, Good Samaritan Hospital, Phoenix, Ariz. A second erythromycin-resistant strain of *B. pertussis* was provided by Brian Lee, Children's Hospital, Oakland, Calif. (unpublished observations). The remaining nasopharyngeal isolates were provided by Gary Cage of the Arizona State Health Department, Phoenix, Ariz., and Christopher R. Peter of the Public Health Laboratory, San Diego, Calif. Identification of all isolates was reconfirmed at the Centers for Disease Control and Prevention (CDC), Atlanta, Ga., by standard biochemical methods (14), fluorescent antibody staining, and PCRs using primer sets developed at the CDC.

Antimicrobial agents. Antimicrobial agents were obtained from several companies: erythromycin from Eli Lilly (Indianapolis, Ind.), rifampin from Marion Merrell Dow, Inc. (Cincinnati, Ohio), chloramphenicol from Parke-Davis (Ann Arbor, Mich.); and trimethoprim-sulfamethoxazole from Hoffman-La Roche, Inc. (Nutley, N.J.). Antimicrobial stock solutions were prepared following National Committee for Clinical Laboratory Standards (NCCLS) guidelines (15), at 10× the desired concentration. Three milliliters of the 10× stock solutions were dispensed into 50-ml centrifuge screw-cap bottles for preparation of each agar dilution plate to obtain the final concentrations of 0.06 to 256 μg of erythromycin per ml, 0.5 to 4.0 μg of rifampin per ml, 1.0 to 8.0 μg of chloramphenicol per ml, and 0.06/1.2 to 4/76 μg of trimethoprim-sulfamethoxazole per ml.

Agar dilution plates. Plates were prepared at CDC with BGA (Difco Laboratories, Detroit, Mich.) containing 0.1% glycerol (Baxter Healthcare Corporation, McGraw Park, Ill.) and 20% (200 ml/liter) defibrinated horse blood (HB)

TABLE 1. Results of MIC testing of *B. pertussis* isolates with the agar dilution reference method and Etest method on BGA and RL−C[a]

Antimicrobial agent	Growth method		Direct suspension method		
	Agar MICs on BGA (*n*)[b]	Etest MICs on BGA (*n*)[c]	Agar MICs on BGA (*n*)	Etest MICs on BGA (*n*)	Etest MICs on RL−C (*n*)
Erythromycin	≤0.06 (47)	0.016 (1)	≤0.06 (44)	≤0.06 (47)	<0.016 (2)
	>256 (4)	0.03 (15)	0.12 (3)	>256 (5)	0.03 (40)
		0.06 (30)	64 (1)		0.06 (5)
		0.12 (1)	>256 (4)		>256 (5)
		>256 (4)			
Rifampin	≤0.5 (51)	0.25 (3)	≤0.5 (52)	0.125 (17)	0.5 (29)
		0.5 (44)		0.25 (19)	1.0 (22)
		1.0 (4)		0.5 (22)	2.0 (1)
Chloramphenicol	≤1.0 (51)	0.5 (12)	≤1.0 (52)	0.25 (19)	0.5 (26)
		1.0 (39)		0.5 (30)	1.0 (21)
				1.0 (3)	2.0 (5)
Trimethoprim-sulfamethoxazole[d]	≤0.06 (4)	0.12 (4)	≤0.06 (4)	0.004 (2)	0.004 (4)
	0.12 (47)	0.25 (40)	0.12 (47)	0.015 (16)	0.06 (5)
		0.5 (7)	0.25 (1)	0.03 (3)	0.12 (28)
				0.06 (16)	0.25 (14)
				0.125 (15)	0.5 (1)

[a] MIC values given in micrograms per milliliter.
[b] *n*, number of organisms tested in each category. The California isolate was tested by using the direct suspension method only.
[c] Etest MIC results that were between standard doubling dilutions were rounded up to the next highest doubling dilution for analysis.
[d] Number refers to the trimethoprim concentration in micrograms per milliliter.

(15). Thirty grams of BGA were dissolved with heating in 500 ml of distilled H_2O and combined with an additional 300 ml of warm distilled H_2O containing 10 g of glycerol. The agar was autoclaved and placed in a 53°C water bath for approximately 25 to 30 min. Because BGA solidifies rapidly when cooled, the 200 ml of HB was also allowed to equilibrate in the 53°C water bath before adding it to the agar. Twenty-seven milliliters of BGA with HB was added to each tube containing the 10× solutions of antimicrobial agents. The tubes were inverted once, then the contents were quickly poured into square petri dishes (100 mm in diameter) and were allowed to solidify. Plates were stored in plastic bags at 4°C and were used within 1 week. The final pH of the medium was not confirmed.

Disk diffusion plates. BGA plates for disk diffusion were prepared at CDC by using the same method as used for agar dilution plates. Seventy-two milliliters of agar was dispensed aseptically into 15 150-mm-diameter round Petri dishes and was allowed to solidify. Plates were stored at 4°C and used within 1 week. Regan-Lowe agar without cephalexin (RL−C) plates were prepared with Oxoid charcoal agar (Unipath, Ltd., Baskingstoke, Hampshire, England) following the manufacturer's direction. Fifty grams of charcoal agar was dissolved in 800 ml of distilled water, autoclaved for 25 min, and placed in a 50°C water bath for approximately 25 to 30 min. Two hundred milliliters of prewarmed defibrinated HB was added to the agar media and mixed. Seventy-two milliliters of agar was dispensed aseptically into 15 150-mm-diameter round Petri dishes and was allowed to solidify. Plates were stored at 4°C and used within 4 weeks.

Antimicrobial agent disks and strips. Standard antimicrobial disks were obtained from Becton Dickinson Microbiology Systems (Cockeysville, Md.) and contained the following amounts of drugs: erythromycin, 15 μg; rifampin, 5 μg; chloramphenicol, 30 μg; and trimethoprim-sulfamethoxazole, 1.25/23.75 μg. Etest strips were obtained from AB Biodisk (Piscataway, N.J.). The strips used in this study contained the following concentrations of drugs: erythromycin, 0.016 to 256 μg/ml; rifampin, 0.002 to 32 μg/ml; chloramphenicol, 0.016 to 256 μg/ml; and trimethoprim-sulfamethoxazole, 0.002 to 32 μg/ml (the MIC scale refers to the trimethoprim component).

SS broth. Components for Stainer-Scholte (SS) broth were obtained from Sigma Chemical Co., St. Louis, Mo. SS broth (17) was prepared at CDC and contained the following components per liter: glutamic acid (sodium glutamate), 10.71 g; proline, 0.24 g; NaCl, 2.50 g; KH_2PO_4, 0.50 g; KCl, 0.20 g; $MgCl_2$, 0.10 g; $CaCl_2$, 0.02 g; and Tris base, 1.52 g. All components were dissolved in 1 liter of distilled water and filter sterilized. The sterile broth was stored in a screw-cap container. Unsupplemented broth is stable for 4 weeks at 4°C. The SS supplement was prepared by diluting 12 ml of 1N HCl in 88 ml of distilled water. One-tenth gram of ferrous sulfate was added with stirring, followed by the addition of 0.4 g of L-cysteine, 0.2 g of ascorbic acid, 0.04 g of niacin, and 1.0 g of reduced glutathione. The supplement required 20 to 30 min of stirring to dissolve completely. The solution was filter sterilized and stored at 4°C. Ten milliliters of supplement were added per liter of medium. When added to SS medium, the solution turned a pale pink, then cleared. Supplemented SS medium was kept at 4°C and was discarded after 1 week.

Inoculum suspension. Two inoculum suspension methods, growth in defined media and direct suspension, were used in this study. Inoculum for the growth method was prepared by making a dense suspension of the organism, equivalent to a 2 McFarland standard, from a 36- to 48-h RL−C culture plate in 10 ml of supplemented SS medium. The suspension was incubated at 36°C, with shaking, for 24 h. Turbidity of the 24-h culture was adjusted with supplemented SS medium to equal a 0.5 McFarland standard. Inoculum for the direct suspension method was prepared by selecting several colonies from a 36- to 48-h RL−C culture plate and suspending them in 5 ml of Mueller-Hinton broth (MH) (Difco). This inoculum was also adjusted to equal a 0.5 McFarland standard. Portions of the adjusted inocula were further diluted 1:10 for use on BGA dilution plates.

Agar dilution method. The agar dilution test was performed according to the traditional susceptibility test method for *B. pertussis*, using only the BGA plates, by both the growth method and the direct suspension method. The agar plates were inoculated with a Steers replicator (Melrose Machine Shop, Woodlyn, Pa.). The final concentration per spot was 10^4 CFU. Inoculated plates were allowed to dry for approximately 5 to 10 min, then incubated at 35°C in a high-humidity incubator. MICs were read at both 24 and 36 h.

Disk diffusion and Etest methods. Antimicrobial agent disks and Etest strips were tested on BGA by both the growth method and the direct suspension method. Disk diffusion plates were inoculated according to NCCLS recommendations (16); Etest was performed according to the manufacturer's instructions. Etest MIC results that were between standard doubling dilutions were rounded up to the next highest doubling dilution for analysis. The comparison of results for the two media (BGA and RL−C) was done only with the direct suspension method since this is the method most likely to be used by a clinical laboratory. Plates were incubated and results were read as described for the agar dilution method.

Quality control and reproducibility testing. The quality control strains used in this study included *B. pertussis* ATCC 9797, *B. pertussis* CDC B100 (erythromycin-resistant strain), and *Staphylococcus aureus* strains ATCC 29213 and ATCC 25923. These strains were used for the initial agar dilution studies with BGA by using inoculum prepared by both the growth and direct suspension methods. Control strains were tested by disk diffusion and Etest for the remainder of the study on both BGA and RL−C by using the direct inoculum suspension method. Quality control strains were tested for an additional 10 working days on both BGA and RL−C against four antimicrobial agents, and additionally for 17 days against erythromycin only on RL−C media.

RESULTS

MIC determination methods. Fifty-two isolates of *B. pertussis* were tested against four antimicrobial agents by the reference agar dilution method by using BGA containing 20% de-

TABLE 2. Comparison of disk diffusion results on BGA and RL−C by using the direct suspension method

Antimicrobial agent	BGA		RL−C	
	No. of isolates	Zone diameters (mm)	No. of isolates	Zone diameters (mm)
Erythromycin	5	6	5	6
	47	42–46	47	43–46
Rifampin	52	26–31	52	16–24
Chloramphenicol	52	33–40	52	30–42
Trimethoprim-sulfamethoxazole	52	21–31	52	23–30

fibrinated HB. Prior testing with BGA plates prepared with 33% HB proved unsatisfactory because the agar was too soft, making it difficult to inoculate and read. The MIC results for erythromycin, rifampin, chloramphenicol, and trimethoprim-sulfamethoxazole on BGA for 51 of the isolates were similar whether the inoculum was prepared using the overnight growth suspension method in supplemented SS medium or by the direct suspension method in MH (Table 1). A second recently obtained erythromycin-resistant isolate from California was tested only by the direct suspension method. On BGA, the erythromycin MIC was 64 μg/ml; the MICs of chloramphenicol, rifampin, and trimethoprim-sulfamethoxazole were similar to those of the other *B. pertussis* isolates.

MICs were also determined by using the Etest method. The Etest results for the original 51 isolates on BGA media using the growth method were similar to those achieved by agar dilution on BGA. Agar dilution and Etest MIC results for erythromycin were >256 μg/ml for the four resistant isolates and ≤0.12 μg/ml for the remaining 47 susceptible isolates for all methods. However, when all 52 isolates were tested by using the Etest with the direct suspension method on BGA, the trimethoprim-sulfamethoxazole results were considerably lower than those observed for the growth method (Table 1). Etest results with the direct suspension method on RL−C agar were similar to those on BGA. Interestingly, the California isolate demonstrated erythromycin MICs of >256 μg/ml by Etest on both BGA and RL−C media. Although all of the results for chloramphenicol and rifampin would be considered susceptible, because the agar dilution results on BGA were below the lowest dilution tested, it is difficult to determine if the results are comparable to those produced by the Etest.

Disk diffusion. Disk diffusion results on BGA and RL−C using the direct inoculum method for erythromycin, rifampin, chloramphenicol, and trimethoprim-sulfamethoxazole are shown in Table 2. The results for erythromycin on both media showed no differences among the five resistant isolates, producing no zones of inhibition (indicated as 6 mm) around disks on either BGA or RL−C medium. Zone diameters for the 47 erythromycin-susceptible isolates ranged from 42 to 46 mm on BGA and 43 to 46 mm on RL−C. Disk diffusion zone diameters for rifampin and chloramphenicol were generally larger and easier to read on BGA than on RL−C. In fact, many of the zones for rifampin on RL−C would fall within the intermediate range if the interpretive criteria for staphylococci (intermediate range, 17 to 19 mm) or pneumococci (intermediate range, 17 to 18 mm) were used, even though the strains were considered susceptible to rifampin by MIC testing. The trimethoprim-sulfamethoxazole zones of inhibition, on the other hand, were more difficult to read on BGA due to indistinct edges. Even though the isolates grew better with additional incubation, there were no major differences among the results when read at 24 versus 36 h.

TABLE 3. Range of Etest MIC results for three quality control organisms

Strain	MICs of[a]:							
	Erythromycin		Rifampin		Chloramphenicol		Trimethoprim-sulfamethoxazole	
	BGA	RL−C	BGA	RL−C	BGA	RL−C	BGA	RL−C
S. aureus ATCC 29213[b]	0.5–1.0 (1.0)	0.5–1.0 (1.0)	0.12–0.25 (0.12)	0.12–0.25 (0.12)	8	8–16 (8)	0.06–0.125 (0.125)	0.125 (0.125)
B. pertussis ATCC 9797	0.06–0.12 (0.125)	0.06–0.12 (0.12)	2.0–4.0 (2)	2.0–4.0 (2)	0.12–0.5 (0.5)	0.12–2.0 (1)	0.008–0.06 (0.06)	0.016–0.06 (0.06)
B. pertussis CDC-B100	>256	>256	2.0–8.0 (2)	2.0–8.0 (2)	0.5–1.0 (0.5)	1.0–2.0 (1)	0.008–0.12 (0.06)	0.004–0.12 (0.06)

[a] Results are given in micrograms per milliliter. Modes are shown in parentheses.

[b] NCCLS quality control ranges for *S. aureus* ATCC 29213 are as follows: erythromycin, 0.25 to 1.0 μg/ml; rifampin, 0.004 to 0.015 μg/ml; chloramphenicol, 2 to 8 μg/ml; and trimethoprim-sulfamethoxazole, ≤0.5/9.5 μg/ml.

TABLE 4. Range of disk diffusion results for three quality control organisms[a]

Strain	Erythromycin		Rifampin		Chloramphenicol		Trimethoprim-sulfamethoxazole	
	BGA	RL−C	BGA	RL−C	BGA	RL−C	BGA	RL−C
S. aureus ATCC 25923[b]	23–28 (25)	21–25 (22)	23–27 (24)	14–21 (16)	18–20 (18)	16–19 (19)	22–27 (24)	21–23 (23)
B. pertussis ATCC 9797	39–42 (40)	37–43 (41)	20–27 (24)	12–14 (14)	38–42 (38)	28–38 (31)	27–34 (28)	24–34 (29)
B. pertussis CDC-B100	6	6	23–26 (24)	11–16 (14)	33–40 (38)	30–37 (30)	26–29 (26)	25–28 (26)

[a] Results are given in millimeters. Modes are shown in parentheses.
[b] NCCLS quality control ranges for *S. aureus* ATCC 25923 are as follows: erythromycin, 22 to 30 mm; rifampin, 26 to 34 mm; chloramphenicol, 19 to 26 mm; and trimethoprim-sulfamethoxazole, 24 to 32 mm.

Quality control test results. Quality control strains were tested on at least 10 consecutive working days. The results (ranges and modes) for the Etest are shown in Table 3. Using the NCCLS control ranges defined for *S. aureus* ATCC 29213, the erythromycin results determined by Etest on both BGA and RL−C were within the specified range of 0.25 to 1.0 μg/ml. All values for trimethoprim-sulfamethoxazole were also below the defined limit of ≤0.5/9.5 and would tentatively be considered in control. The chloramphenicol results were within range on BGA but not on RL−C, while the results for rifampin were consistently high on both media (quality control range for *S. aureus*, 0.004 to 0.015 μg/ml). The results for the two *B. pertussis* strains were consistent over the testing period.

The quality control disk diffusion results are presented in Table 4. For *S. aureus* ATCC 25923, the erythromycin results were always within the specified range (22 to 30 mm) on BGA, but clustered at the low end of the range on RL−C. Nonetheless, the RL−C results tended to be within range. The chloramphenicol results for *S. aureus* ATCC 25923 were mostly in control on BGA (defined range, 19 to 26 mm) but tended to be too small on RL−C. The rifampin results tended to be out of control on both media (defined range, 26 to 34 mm) as did the trimethoprim-sulfamethoxazole results with modes for BGA and RL−C that were at the low end of the range. The disk diffusion results for the *B. pertussis* strains showed considerable variation for chloramphenicol, although the other ranges were relatively narrow.

DISCUSSION

The traditional antimicrobial susceptibility testing method for *B. pertussis* involves stabilizing the organisms in complex medium (SS broth) overnight prior to inoculating a test medium that contains as much as 33% animal blood (1). Bannatyne and Cheung (1, 2) used the overnight growth method to keep organisms in phase I (virulent phase) because avirulent forms of *B. pertussis* (phase IV) were thought to become more resistant to antimicrobial agents. The simplified approach of suspending the organisms directly in MH and inoculating BGA containing 20% lysed HB appears to give MIC results comparable to those of the traditional method in which the organisms are grown overnight in SS media. Although we initially attempted to compare the MIC results with BGA containing 33% lysed HB to those achieved with only 20% HB, the former media proved to be too soft, and these efforts were abandoned. Nonetheless, the results with erythromycin, in particular, were encouraging. Reducing the concentration of HB to 20% not only makes preparation of the medium easier but less costly as well. Although we did not observe significant differences in MIC results for the other three drugs tested, since the results with BGA for rifampin and chloramphenicol were off scale, it is difficult to ascertain their accuracy (Table 1). However, with the exception of the five erythromycin-resistant isolates, the MICs of the isolates we tested were similar to those reported in previous studies (1, 8, 13).

Our results also suggest that RL−C media, which is available commercially in powdered form, can be used in place of BGA to screen for erythromycin-resistant isolates but may not be appropriate for testing other antimicrobial agents. The RL−C formulation (i.e., without cephalexin) was chosen so that we could use *S. aureus* strains ATCC 25923 and ATCC 29213 for quality control; both strains are susceptible to cephalexin. The quality control results for the *S. aureus* strains suggest that the rifampin results, particularly those generated by disk diffusion, were unreliable on both BGA and RL−C because they were so disparate from the established ranges. The quality control results for MIC and disk diffusion testing of chloramphenicol, particularly on BGA, were close to the published ranges for *S. aureus*, although those determined on RL−C were more frequently outside the established ranges, making the results of questionable utility. Many of the results with trimethoprim-sulfamethoxazole disks, even on BGA, were also outside the designated range for *S. aureus* ATCC 25923, although the MIC results (all ≤0.5/9.5 μg/ml) would tentatively be considered in control. While the NCCLS quality control ranges were not developed by using BGA or RL−C media, the *S. aureus* results can be used as a guide to assess the quality of the disks and media. Our data suggest that erythromycin MIC results can be reported with confidence; however, the results of the chloramphenicol and trimethoprim-sulfamethoxazole tests generated with the direct suspension method with either BGA or RL−C should be viewed with caution. Rifampin MIC results appear to be unreliable. While the quality control ranges presented here for the *B. pertussis* strains do not satisfy the requirements of NCCLS for inclusion in their tables (since they were not performed in multiple laboratories with multiple lots of media), these data can be used as a guide for other laboratories that would like to perform susceptibility testing of *B. pertussis*.

Since an erythromycin-resistant strain of *B. pertussis* had been recognized (11), it was important to establish an alternate antimicrobial susceptibility testing method that would be easier for nonreference laboratories to perform than the traditional BGA method using SS medium. The Etest erythromycin MIC results on both BGA and RL−C for the five resistant isolates were >256 μg/ml, although the erythromycin MIC on BGA for the California isolate was only 64 μg/ml. This suggests that the Etest can be used as a screening method for detection of resistance but that actual MICs for various drugs may need to be performed on BGA for accuracy. Since RL−C media is readily available, the Etest screening approach may be possible even for smaller laboratories. However, it should be noted that the Etest has not yet been approved in the United States for *B. pertussis* testing, thus, this remains a research method. Disk diffusion testing using RL−C also showed promise as a screening test for the erythromycin-resistant strain. This test is inex-

pensive and easy to perform, especially when large numbers of strains require screening.

The mechanism of erythromycin resistance in both *B. pertussis* strains remains unknown (11). Thus, we cannot be sure that other erythromycin-resistant strains will be as easy to detect as these strains. However, given the large zone diameters that are typical of most erythromycin-susceptible strains, we are confident that strains with decreased susceptibility to this drug would be detected. Although another isolate of *B. pertussis* from Utah has been reported as erythromycin resistant in vitro (there was no mention of whether this strain resulted in a treatment failure in the report) (12), this isolate, which had an erythromycin MIC reported to be lower than that of the Phoenix isolate, proved to be fully susceptible in our laboratory by the Etest method and failed to show a reduced zone size when tested by disk diffusion. It is possible that the organism lost its resistance phenotype in transit or upon multiple subcultures. Nonetheless, the possibility of encountering additional erythromycin-resistant strains of pertussis, particularly from therapeutic failures, should not be discounted.

In summary, the reference agar dilution method for testing *B. pertussis* can be simplified by reducing the amount of animal blood used and by preparing a direct inoculum suspension for inoculation of the agar without the need for overnight growth in SS broth. The disk diffusion and Etest methods can also be used to screen isolates for suspected erythromycin resistance. While routine MIC testing of *B. pertussis* is still not warranted, isolates from patients who appear to have failed an appropriate course of erythromycin therapy should be screened for resistance, and those that appear resistant should be tested by the agar dilution reference MIC method on BGA.

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REFERENCES

1. **Bannatyne, R. M., and R. Cheung.** 1982. Antimicrobial susceptibility of *Bordetella pertussis* strains isolated from 1960 to 1981. Antimicrob. Agents Chemother. **21:**666–667.
2. **Bannatyne, R. M., and R. Cheung.** 1984. Antibiotic resistance of degraded strains of *Bordetella pertussis*. Antimicrob. Agents Chemother. **25:**537–538.
3. **Bass, J. W.** 1986. Erythromycin for treatment and prevention of pertussis. Pediatr. Infect. Dis. **5:**154–157.
4. **Centers for Disease Control and Prevention.** 1995. Pertussis: United States, January 1992–June 1995. Morbid. Mortal. Weekly Rep. **44:**525–529.
5. **Cherry, J. D.** 1995. Nosocomial pertussis in the nineties. Ped. Infect. Dis. J. **16:**553–555.
6. **Christie, C. D. C., A. M. Glover, M. J. Willke, M. L. Marx, S. F. Reising, and N. M. Hutchinson.** 1995. Containment of pertussis in the regional pediatric hospital during the greater Cincinnati epidemic of 1993. Ped. Infect. Dis. J. **16:**556–563.
7. **de Seres, G., N. Boulianne, and B. Duval.** 1995. Field effectiveness of erythromycin prophylaxis to prevent pertussis within families. Pediatr. Infect. Dis. J. **14:**969–975.
8. **Field, L. H., and C. D. Parker.** 1980. Antibiotic susceptibility testing of *Bordetella pertussis*. Am. J. Clin. Pathol. **74:**312–316.
9. **Herwaldt, L. A.** 1991. Pertussis in adults: what physicians need to know. Ann. Intern. Med. **151:**1510–1512.
10. **Huang, M. B., C. N. Baker, S. Banerjee, and F. C. Tenover.** 1992. Accuracy of the E Test for determining antimicrobial susceptibilities of staphylococci, enterococci, *Campylobacter jejuni*, and gram-negative bacteria resistant to antimicrobial agents. J. Clin. Microbiol. **30:**3243–3248.
11. **Lewis, K., M. A. Saubolle, F. C. Tenover, M. F. Rudinsky, S. D. Barbour, and J. D. Cherry.** 1995. Pertussis caused by an erythromycin-resistant strain of *Bordetella pertussis*. Ped. Infect. Dis. J. **14:**388–391.
12. **Korgenski, E. K., and J. A. Daly.** 1997. Surveillance and detection of erythromycin resistance in *Bordetella pertussis* isolates recovered from a pediatric population in the intermountain west region of the United States. J. Clin. Microbiol. **35:**2989–2991.
13. **Kurzynski, T. A., D. M. Boehm, J. A. Rott-Petri, R. F. Schell, and P. E. Allison.** 1988. Antimicrobial susceptibilities of *Bordetella* species isolated in a multicenter pertussis surveillance project. Antimicrob. Agents Chemother. **32:**137–140.
14. **Marcon, M. J.** 1995. *Bordetella*, p. 566–573. *In* E. J. Baron, P. Murray, F. C. Tenover, M. A. Pfaller, and R. A. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
15. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
16. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests, 6th ed. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
17. **Stanier, D. W., and M. J. Scholte.** 1970. A simple chemically defined medium for the production of phase I *Bordetella pertussis*. J. Clin. Pathol. **25:**732–733.