JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1997, p. 2989–2991 Vol. 35, No. 11
0095-1137/97/$04.00+0

Surveillance and Detection of Erythromycin Resistance in *Bordetella pertussis* Isolates Recovered from a Pediatric Population in the Intermountain West Region of the United States

E. KENT KORGENSKI[1] AND JUDY A. DALY[1,2]*

Primary Children's Medical Center[1] and the Department of Pathology, University of Utah,[2] Salt Lake City, Utah 84113

Received 17 March 1997/Returned for modification 13 May 1997/Accepted 27 August 1997

Forty-seven *Bordetella pertussis* isolates recovered from January 1985 to June 1997 at Primary Children's Medical Center were tested for erythromycin resistance. Agar dilution MICs were determined on Regan-Lowe agar. Forty-six isolates were found to be erythromycin susceptible (all MICs were less than or equal to 0.12 μg/ml). One isolate was found to be erythromycin resistant (MIC, 32 μg/ml). In addition, we compared Etest MIC results and disk diffusion zone diameter measurements, performed on commercially prepared Regan-Lowe agar, to the agar dilution MIC result. Etest MIC and/or disk diffusion testing on commercial Regan-Lowe agar appears to be an adequate method for erythromycin resistance screening of *B. pertussis* isolates.

Erythromycin is the antimicrobial agent of choice for treatment and postexposure prophylaxis of *Bordetella pertussis* infection, probably due to a higher achievable level in serum than other possible agents and the ability to penetrate the respiratory tract (2–4). The MIC of erythromycin against *B. pertussis* isolates usually ranges from 0.02 to 0.12 μg/ml. Resistance had not previously been reported until a *B. pertussis* isolate resistant to erythromycin was found in Yuma County, Ariz. (5). The resistance mechanism is not known at this time; however, it does not involve rRNA methylation. Studies are ongoing at the Centers for Disease Control and Prevention (CDC) to elucidate this mechanism and establish criteria for susceptibility testing. Currently, a standardized procedure and guidelines for in vitro testing are not reported in the literature. The difficulties of in vitro testing of *B. pertussis* isolates include its slow growth and fastidious nutritional requirements.

Primary Children's Medical Center (PCMC), located in Salt Lake City, Utah, is a pediatric tertiary care hospital serving the Intermountain West. The close proximity of Utah to Arizona suggested that erythromycin-resistant *B. pertussis* isolates could be present in our population. The purpose of this study was twofold: first, the evaluation of *B. pertussis* strains isolated at PCMC for erythromycin resistance and, second, the analysis of methods for in vitro susceptibility testing of these isolates. Personal correspondence with Fred C. Tenover (CDC) and James H. Jorgensen (National Committee on Clinical Laboratory Standards [NCCLS] and University of Texas Health Science Center) indicated that agar dilution MIC, Etest MIC, and disk diffusion testing on Regan-Lowe agar might be appropriate methods for testing *B. pertussis* isolates.

A total of 47 *B. pertussis* isolates recovered from January 1985 to June 1997 were included in this study. Thirty-three of the 47 isolates were recovered in the past 5 years (January 1992 to June 1997). Isolates had been saved in defibrinated rabbit blood and stored at −70°C. All isolates were subcultured three consecutive times on Regan-Lowe agar without cephalexin before susceptibility testing was performed.

The erythromycin agar dilution MIC was determined with Regan-Lowe agar (31.2 g of charcoal agar [Difco, Detroit, Mich.], 500 ml of deionized water, and 50 ml of defibrinated horse blood) according to NCCLS-recommended procedures (6). Plates (erythromycin doubling dilutions ranging from 0.06 to 64 μg/ml) were inoculated by a Steers replicator with a standardized inoculum giving a final concentration on the agar of approximately 10^4 CFU. Plates were incubated at 35°C in ambient air. Tests were run in duplicate and included controls.

The erythromycin Etest (AB Biodisk, Piscataway, N.J.) MIC was determined on commercially purchased Regan-Lowe charcoal agar without cephalexin (BBL 4398326; Becton Dickinson Microbiology Systems, Cockeysville, Md.). A 0.5 McFarland standard equivalent inoculum was placed on the agar surface according to instructions from the manufacturer (1). Plates were incubated in ambient air.

Disk diffusion testing was performed according to NCCLS guidelines (7). A 15-μg erythromycin disk was placed on commercially purchased Regan-Lowe charcoal agar without cephalexin (BBL 4398326). The inoculum was standardized by using the NCCLS direct colony suspension method (7) and adjusted to match a 0.5 McFarland turbidity standard. Plates were incubated at 35°C in ambient air.

Agar dilution MICs were interpreted as the lowest concentration of antimicrobial agent that completely inhibited growth (one colony or less). Etest MIC results were read at the doubling dilution MIC or the next highest doubling dilution MIC above the intersection of growth and Etest strip. MICs less than or equal to 0.12 μg/ml were considered to indicate susceptibility (5). Disk diffusion zone diameters greater than 45 mm were considered to indicate susceptibility.

Susceptibility results were read and interpreted on days 2, 3, 4, and 5 of incubation. The results for agar dilution are shown (interpreted on days 2 and 5) in Table 1. Forty-six isolates showed a MIC less than or equal to 0.12 μg/ml and were considered susceptible. One isolate (1 of 47 [2.1%]) showed a MIC equal to 32 μg/ml and was considered resistant. Adequate growth on the control plate occurred at day 2 for 45 of 47 (95.7%) isolates, and the MICs were interpreted. The MICs on

* Corresponding author. Mailing address: Department of Pathology, University of Utah School of Medicine, Primary Children's Medical Center, 100 North Medical Dr., Salt Lake City, UT 84113-1100. Phone: (801) 588-3166. Fax: (801) 588-2435. E-mail: PCJDALY@IHC.com.

TABLE 1. Susceptibility results by method

Isolate no.	Agar dilution MIC (μg/ml)		Etest MIC (μg/ml)		Disk diffusion zone (mm)	
	Day 2	Day 5	Day 2	Day 5	Day 2	Day 5
1	≤0.06	≤0.06	0.06	0.06	52	58
2	≤0.06	≤0.06	0.06	0.06	48	55
3	≤0.06	≤0.06	0.06	0.06	46	52
4	≤0.06	≤0.06	0.06	0.06	52	56
5	≤0.06	≤0.06	0.06	0.06	53	54
6	≤0.06	≤0.06	0.06	0.06	50	56
7	≤0.06	≤0.06	0.06	0.06	48	58
8	≤0.06	≤0.06	0.06	0.06	52	58
9	≤0.06	≤0.06	0.12	0.06	52	58
10	≤0.06	≤0.06	0.06	0.06	50	58
11	≤0.06	≤0.06	0.06	0.06	50	56
12	≤0.06	≤0.06	0.06	0.06	53	57
13	≤0.06	≤0.06	0.06	0.06	54	58
14	NG[a]	≤0.06	NG	NG	NG	NG
15	≤0.06	≤0.06	0.12	0.06	50	54
16	≤0.06	≤0.06	0.06	0.06	50	56
17	≤0.06	≤0.06	0.06	0.06	48	52
18	≤0.06	≤0.06	0.12	0.06	48	54
19	≤0.06	≤0.06	0.12	0.06	47	50
20	≤0.06	≤0.06	0.12	0.06	47	53
21	≤0.06	≤0.06	0.12	0.06	51	56
22	≤0.06	≤0.06	0.12	0.06	50	56
23	≤0.06	≤0.06	0.12	0.06	49	56
24	≤0.06	≤0.06	0.12	0.06	47	54
25	≤0.06	≤0.06	0.06	0.06	49	54
26	≤0.06	≤0.06	0.12	0.06	47	50
27	≤0.06	≤0.06	0.06	0.06	49	57
28	NG	≤0.06	0.06	0.06	49	52
29	≤0.06	≤0.06	0.12	0.12	48	56
30	≤0.06	≤0.06	0.06	0.06	50	56
31	≤0.06	≤0.06	0.12	0.06	48	54
32	≤0.06	≤0.06	0.06	0.06	47	52
33	≤0.06	≤0.06	0.12	0.06	46	52
34	≤0.06	≤0.06	0.12	0.06	46	53
35	≤0.06	≤0.06	0.12	0.06	50	58
36	≤0.06	≤0.06	0.06	0.06	48	54
37	≤0.06	≤0.06	0.12	0.12	45	50
38	≤0.06	≤0.06	0.12	0.12	47	50
39	≤0.06	≤0.06	0.12	0.12	46	52
40	≤0.06	≤0.06	0.12	0.06	46	55
41	≤0.06	≤0.06	0.12	0.06	48	54
42	≤0.06	≤0.06	0.12	0.06	48	57
43	0.12	0.12	0.12	0.06	48	56
44	16	32	64	64	6	6
45	≤0.06	≤0.06	0.12	0.06	46	52
46	≤0.06	≤0.06	0.06	0.06	47	54
47	≤0.06	≤0.06	0.12	0.12	47	54

[a] NG, no growth.

day 2 for 44 of 45 (97.8%) isolates did not change on additional evaluation for each consecutive day through day 5. One of 45 isolates (2.2%) increased the MIC by 1 doubling dilution on day 5 versus day 2 evaluation. Adequate growth did not occur for 2 of 47 (4.3%) isolates on the control plate until day 3. The day 3 MIC results did not change on day 5.

The results for Etest MIC are shown (interpreted on days 2 and 5) in Table 1. Forty-five of 46 (97.8%) isolates showed a MIC less than or equal to 0.12 μg/ml and were considered susceptible. One isolate (1 of 46 [2.2%]) showed a MIC equal to 64 μg/ml and was considered resistant. One isolate failed to grow within 5 days, and results were not obtained. Forty-six isolates grew to a confluent growth on the commercial Regan-Lowe agar by day 2, and the MICs were determined. The MIC was interpreted on day 2 for 27 of 46 (58.7%) isolates, and that value remained the same through day 5. Nineteen of 46 (41.3%) isolates had a MIC of 0.12 μg/ml on day 2, which had changed to a MIC of 0.06 μg/ml on day 5.

TABLE 2. Distribution of differences in MICs: Etest versus agar dilution

MIC difference[a]	No. of isolates	% of isolates
<2	0	0
−2	0	0
−1	1	2.2
0	0	0
+1	40	87.0
+2	5	10.8
>2	0	0
Agreement[b]	41 of 46	89.1

[a] 0, percentage of isolates for which MICs are identical. −1 and +1 indicate ±1 $\log_2$ dilution.
[b] Number and percentage of isolates within the accuracy limits of the test (±1 $\log_2$ dilution).

The results of disk diffusion are shown (interpreted on days 2 and 5) in Table 1. Forty-five of 46 (97.8%) isolates showed a zone of inhibition that was larger than 45 mm and were considered susceptible. One isolate (1 of 46 [2.2%]) showed no zone of inhibition and was considered resistant. One isolate failed to grow within 5 days, and results were not obtained. Forty-six isolates had grown to a confluent lawn of growth by day 2 and could be interpreted at that time. Variation in zone diameters from day to day in evaluation occurred (mean range, 5.7 mm); however, 45 of 46 (97.8%) isolates had a zone size that remained above 45 mm. Forty-five of 46 (97.8%) isolates had a zone measurement that was greater on day 5 than on day 2; 1 of 46 (2.2%) isolates had the same measurement on day 5 and day 2.

Comparisons of MICs determined by agar dilution and by Etest are shown in Table 1. Agar dilution MICs were less than Etest MICs for 45 of 46 (97.8%) isolates (40 of 45 [88.9%] had a difference of 1 doubling dilution, and 5 of 45 [11.1%] had a difference of −2 doubling dilutions). One of 46 (2.2%) isolates had an Etest MIC that was less than the agar dilution MIC by a difference of 1 doubling dilution. There was essential agreement for 41 of 46 (89.1%) isolates (an Etest MIC that was within ±1 doubling dilution of the agar dilution MIC). Five of 46 (10.9%) isolates had an Etest MIC that was more than 1 doubling dilution. Table 2 contains the distribution of differences between the agar dilution and Etest methods.

Evaluation of methods showed essentially equivalent category interpretation results (susceptible) with agar dilution, Etest MIC, and disk diffusion. A limitation to this study was that erythromycin-resistant strains of *B. pertussis* were not detected, nor was a resistant strain located to be used in this evaluation. Therefore, the ability of each method to detect resistance remains in question. However, personal communication with M. Saubolle (Good Samaritan Regional Medical Center, Phoenix, Ariz.) revealed that the erythromycin-resistant strain of *B. pertussis* isolated in Arizona showed no zone of inhibition by either disk diffusion or Etest MIC testing (MIC, >64 μg/ml).

We recommend that a *B. pertussis* isolate be tested for erythromycin resistance only if there is therapeutic failure or for surveillance purposes. Routine testing, at this time, is not indicated. However, this data indicates that *B. pertussis* isolates could be screened by either disk diffusion or Etest MIC, on commercially available Regan-Lowe charcoal agar without

cephalexin. The inoculum should be standardized by using the NCCLS direct colony suspension method (7) and adjusted to a 0.5 McFarland turbidity standard. Incubate plates at 35°C in ambient atmosphere. Read daily until a confluent lawn of growth is present on the agar surface. Large zones (>45 mm) or high MICs (≤0.12 μg/ml) can be assumed to indicate susceptibility. Isolates with small zones or higher MICs should be suspected of being erythromycin resistant and sent to the CDC or a referral laboratory for further testing.

In conclusion, the results of this study show that one *B. pertussis* isolate recovered within the past 12 years at PCMC was resistant to erythromycin. This isolate was recently recovered (January 1997) and, with the recently recovered erythromycin-resistant isolate in Arizona, may indicate the emergence of erythromycin resistance in *B. pertussis.*

We thank the laboratory microbiologists for excellent assistance, especially Daniel Allen. We thank Debbie Hoffman for valuable assistance in the preparation of the manuscript.

REFERENCES

1. **AB Biodisk.** Etest package insert. AB Biodisk, North America, Inc., Piscataway, N.J.
2. **American Academy of Pediatrics.** 1994. Pertussis, p. 355–367. *In* G. Peter (ed.), 1994 red book: report of the Committee on Infectious Diseases, 23rd ed. American Academy of Pediatrics, Elk Grove Village, Ill.
3. **Bartlett, J. G.** 1994. Pocket book of infectious disease therapy, 5th ed., p. 17. Williams & Wilkins, Baltimore, Md.
4. **Hewlett, E. L.** 1995. *Bordetella* species, p. 2078–2084. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 4th ed. Churchill Livingstone, New York, N.Y.
5. **Lewis, S., B. Erickson, G. Cage, G. Harter, C. Kioski, S. Barefoot, L. Carmody, H. Houser, L. Sands, M. Saubolle, K. Lewis, and S. Barbour.** 1994. Erythromycin-resistant *Bordetella pertussis*—Yuma County, Arizona, May-October, 1994. Morbid. Mortal. Weekly Rep. **43:**807–810.
6. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard. NCCLS document M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **National Committee for Clinical Laboratory Standards.** 1993. Performance standards for antimicrobial disk susceptibility tests, 5th ed. Approved standard. NCCLS document M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.