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Antimicrobial Susceptibilities of *Bordetella* Species Isolated in a Multicenter Pertussis Surveillance Project

TERRENCE A. KURZYNSKI,[1]* DIANE M. BOEHM,[1] JEAN A. ROTT-PETRI,[1] RONALD F. SCHELL,[1,2] AND PATRICIA E. ALLISON[3]

State Laboratory of Hygiene[1] and Department of Medical Microbiology,[2] University of Wisconsin, Madison, Wisconsin 53706, and Colorado Department of Health, Denver, Colorado 80220[3]

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MICs for 90% (MIC_{90}s) of 75 *Bordetella pertussis* strains for amoxicillin, erythromycin, rifampin, and sulfamethoxazole-trimethoprim were 1, ≤0.12, 1, and 4 μg/ml, respectively. Susceptibility rates were all ≥93%. Only 17% of the strains were susceptible to tetracycline. The MIC_{90}s of ciprofloxacin, enoxacin, norfloxacin, ofloxacin, and roxithromycin were ≤0.06, 0.5, 0.25, 0.12, and 0.5 μg/ml, respectively. For *B. parapertussis*, the MIC_{90}s were 16-fold higher with amoxicillin and rifampin and 2- to 4-fold higher with the fluoroquinolones and roxithromycin.

Determination of the incidence of infection caused by *Bordetella pertussis* and *Bordetella parapertussis* was the primary objective of the recently completed Multicenter Pertussis Surveillance Project. The 2-year study was conducted from 1984 to 1986 by the State Public Health Laboratories in Baltimore, Md.; Denver, Colo.; Milwaukee, Wis.; and Oklahoma City, Okla. We report the susceptibilities of *Bordetella* species isolated during this study to a variety of antimicrobial agents. Susceptibilities of *B. pertussis* were previously reported (2–5, 22, 23), but our isolates were from separate households from a wider geographical area and included *B. parapertussis* strains.

All project isolates were recovered from nasopharyngeal specimens taken from children, adolescents, and adults. Fifty-percent Regan-Lowe agar (Oxoid, Columbia, Md.) was used for transportation of nasopharyngeal swabs and enrichment cultures. Regan-Lowe agar and Bordet Gengou agar (Difco Laboratories, Detroit, Mich.) with 40 μg of cephalexin per ml were used as isolation media. Isolates were selected from separate households to increase the chance of variability in susceptibilities to the antimicrobial agents tested. Subculturing was restricted to avoid phase changes to resistant forms (3). Standard criteria were used for identification (9). Inocula from primary or subculture plates (3 to 4 days old) were transferred to heart infusion broth containing 0.1% agar or to sheep blood and then were stored at −70°C.

B. pertussis isolates were obtained from the following states: Colorado, 22 strains; Maryland, 11 strains; Oklahoma, 13 strains; and Wisconsin, 29 strains. *B. parapertussis* isolates were from Colorado (14 strains) and Wisconsin (10 strains). An additional 22 strains of *B. parapertussis* were isolated from specimens received outside the project area in Wisconsin. These strains were all isolated from October through February 1986. Eleven strains of *B. bronchiseptica* were also tested, including four from Wisconsin and seven from the Centers for Disease Control. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were included as controls.

Antimicrobial powders of known potency were provided as follows: amoxicillin and tetracycline, Bristol Laboratories, Syracuse, N.Y.; erythromycin, Eli Lilly & Co., Indianapolis, Ind.; rifampin, Merrel Dow Pharmaceuticals, Inc., Cincinnati, Ohio; sulfamethoxazole-trimethoprim (SXT), Burroughs Wellcome Co., Research Triangle Park, N.C.; ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; enoxacin, Warner Lambert, Ann Arbor, Mich.; norfloxacin, Merck & Co., Inc., Rahway, N.J.; and roxithromycin, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. Stock solutions of most antimicrobial agents were prepared according to the manufacturers instructions at 1,280 μg/ml and kept frozen at −70°C until use. Enoxacin was prepared at 640 μg/ml, sulfamethoxazole was prepared at 19,000 μg/ml, and trimethoprim was prepared at 1,000 μg/ml.

An agar dilution procedure patterned after the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS) (16) was used. Suspensions of test strains were thawed, subcultured to Bordet Gengou agar, and incubated for 3 days at 35°C under high humidity. Inocula were prepared in Mueller-Hinton broth by making a suspension of cells picked from multiple colonies and adjusting the turbidity to a McFarland 0.5 standard. Based on quantitative cell counts using six strains of *B. pertussis*, the resulting suspensions ranged from 2.6×10^8 to 6.2×10^8 cells per ml, with an average count of 4.4×10^8 cells per ml. Serial twofold concentrations of antibiotics were then incorporated into Bordet Gengou agar containing 20% defibrinated sheep blood. Thymidine phosphorylase (Burroughs Wellcome) was added to plates containing SXT before solidification at a final concentration of 0.3 IU/ml. Inocula were applied with a Steers replicator, resulting in a final inoculum range of 2.6×10^5 to 6.2×10^5 cells per spot. Plates were incubated at 35°C under high humidity for 2 days, and endpoints were read as recommended by the NCCLS (16).

Interpretive MIC criteria indicating susceptibility (S), moderate susceptibility (MS), and resistance (R) for the following antibiotics were taken from the NCCLS recommendations (16) and were as follows: amoxicillin: S, ≤1 μg/ml; MS, 2 to 16 μg/ml; and R, >16 μg/ml; erythromycin: S, ≤0.5 μg/ml; MS, 1 to 4 μg/ml; and R, >4 μg/ml; SXT: S, ≤9.5/0.5 μg/ml; and R, ≥16 μg/ml; and tetracycline: S, ≤1 μg/ml; MS, 2 to 8 μg/ml; and R, >8 μg/ml. For rifampin, the criteria of Thornsberry et al. (20) were used: S, ≤2 μg/ml; MS, 4 μg/ml; and R, ≥8 μg/ml. In the absence of NCCLS recommendations for the newer antimicrobials agents, the following susceptibility breakpoints were used: ciproflox-

* Corresponding author.

acin, ≤1 μg/ml (10); enoxacin, ≤4 μg/ml (18); norfloxacin, ≤1 μg/ml (6); ofloxacin, ≤2 μg/ml (12); and roxithromycin, ≤1 μg/ml (7).

The amoxicillin, erythromycin, rifampin, and SXT MICs for 90% (MIC_{90}s) of *B. pertussis* strains were within the susceptible breakpoint values (Table 1). The tetracycline MIC for 50% of strains (MIC_{50}) of 2 μg/ml exceeded the 1-μg/ml susceptibility breakpoint, while the MIC_{90} of 4 μg/ml was within the moderately susceptible category. The incidences of susceptibility were as follows: amoxicillin, 93%; erythromycin and SXT, 100%; rifampin, 95%; and tetracycline, 17%. All of the isolates were moderately susceptible to amoxicillin and tetracycline, and 97% were moderately susceptible to rifampin. Results for the conventional antimicrobial agents against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 control strains were within the NCCLS acceptable ranges (16).

Although the amoxicillin MIC_{90} for *B. pertussis* was 1 μg/ml and 93% of the strains were susceptible, amoxicillin and ampicillin have been reported to be clinically ineffective because of low bronchopulmonary concentrations (21). The low MIC_{90} of erythromycin confirms other reports (2, 5, 23) and supports the continued role of erythromycin as the antibiotic of choice for treating *B. pertussis* infections. SXT was also very effective, which supports its role in prophylaxis (1, 8, 11). Although our results confirmed the in vitro effectiveness of rifampin (2, 22), its role in treating *B. pertussis* infections has been questioned (22).

It should be noted that the MIC_{90}s of rifampin and tetracycline were 8- to 19-fold higher than those reported by Bannatyne and Cheung (2). In addition, our amoxicillin MIC_{90} was fourfold higher than their ampicillin value. Although there is a definite need for standardization of susceptibility testing for *Bordetella* species, the higher resistance we observed is probably real rather than the result of methodological differences that have been shown to affect results for *B. pertussis* (G. Zackrisson, J. E. Brorson, and B. Trollfors, Letter, Eur. J. Clin. Microbiol. 3:566–567, 1984).

Ciprofloxacin was the most active of the four fluoroquinolones against *B. pertussis*, with an MIC_{90} of ≤0.06 μg/ml. For three isolates (4%), the MIC was above 0.06 μg/ml. Ofloxacin was also very active, with an MIC_{90} of 0.12 μg/ml. For four isolates (6%), the MIC was above 0.12 μg/ml. All isolates were susceptible to ciprofloxacin and ofloxacin.

TABLE 1. Antimicrobial susceptibilities of *Bordetella* species

Organism (no. of isolates tested)	Antimicrobial agents	MIC (μg/ml)			% Susceptible[a]
		Range	50%	90%	
Bordetella pertussis (75[b])	Conventional				
	Amoxicillin	0.5–4	1	1	93
	Erythromycin	≤0.12–0.5	≤0.12	≤0.12	100
	Rifampin	0.12–8	0.5	1	95
	SXT[c]	1–8	4	4	100
	Tetracycline	1–8	2	4	17
	Newer				
	Ciprofloxacin	≤0.06–1	≤0.06	≤0.06	100
	Enoxacin	0.25–8	0.5	0.5	98
	Norfloxacin	0.12–8	0.25	0.25	97
	Ofloxacin	≤0.06–2	0.12	0.12	100
	Roxithromycin	≤0.06–4	0.25	0.5	98
Bordetella parapertussis (46[d])	Conventional				
	Amoxicillin	1–32	8	16	2
	Erythromycin	≤0.12–4	0.25	0.25	98
	Rifampin	4–64	8	16	0
	SXT[c]	2–4	2	4	100
	Tetracycline	0.5–4	1	4	0
	Newer				
	Ciprofloxacin	≤0.06–0.5	≤0.06	0.12	100
	Enoxacin	0.5–4	0.5	1	100
	Norfloxacin	0.25–2	0.5	0.5	98
	Ofloxacin	0.12–0.5	0.12	0.5	100
	Roxithromycin	0.5–4	0.5	2	76
Bordetella bronchiseptica (11)	Conventional				
	Amoxicillin	4–>128	64	128	0
	Erythromycin	4–32	8	32	0
	Rifampin	64–>128	>128	>128	0
	SXT[c]	2–>128	4	128	82
	Tetracycline	4–128	64	64	0
	Newer				
	Ciprofloxacin	1–4	4	4	27
	Enoxacin	4–32	8	16	27
	Norfloxacin	4–64	16	32	0
	Ofloxacin	2–8	4	8	9
	Roxithromycin	2–128	16	32	0

[a] Susceptibility rates for the conventional antimicrobial agents are based on NCCLS-recommended MIC equivalents. Rates for the newer antimicrobial agents are tentative and are based on documented achievable levels.
[b] Sixty-eight *B. pertussis* strains were tested against ciprofloxacin, enoxacin, norfloxacin, ofloxacin, and roxithromycin.
[c] The SXT ratio was 19:1.
[d] Forty-five *B. parapertussis* strains were tested against ciprofloxacin, enoxacin, norfloxacin, ofloxacin, and roxithromycin.

Enoxacin and norfloxacin were less active. Roxithromycin was also active against *B. pertussis*. The MIC_{90} was fourfold higher than the erythromycin MIC_{90}, which may negate the advantage of improved pharmacokinetics of roxithromycin (7). Results for the *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 control strains for all fluoroquinolones and roxithromycin were in agreement with those of Clyde Thornsberry at the Centers for Disease Control, Atlanta, Ga. (personal communication).

Erythromycin and SXT were the only conventional antimicrobial agents with MIC_{90}s within the susceptibility category for *B. parapertussis* (Table 1). One strain (2%) was relatively resistant to erythromycin (MIC, 4 μg/ml), and none was resistant to SXT. Amoxicillin and rifampin MIC_{90}s were 16-fold higher than those observed for *B. pertussis*. The incidences of susceptibility were 2% for amoxicillin and 0% for rifampin and tetracycline. Applying the NCCLS moderately susceptible category breakpoints, the incidences of susceptibility were 91% for amoxicillin and 100% for tetracycline but only 2% for rifampin. Presumably, amoxicillin would be as ineffective against *B. parapertussis* as it is against *B. pertussis* (21). These results are important because mixed infections of *B. pertussis* and *B. parapertussis* and epidemics caused by *B. parapertussis* have been reported (13–15). Members of the Multicenter Pertussis Surveillance Project noticed an increased incidence of *B. parapertussis* isolation during the surveillance project. For example, in Wisconsin 24 *B. parapertussis* strains were isolated from throughout the state during 1985 while only 1 isolate had been found in the previous 4 years.

Ciprofloxacin and ofloxacin were active against *B. parapertussis*. All strains were susceptible to these antimicrobial agents. Enoxacin and norfloxacin MIC_{90}s were 1 and 0.5 μg/ml, respectively. All strains were susceptible to enoxacin, and for one strain (2%) the norfloxacin MIC was 2 μg/ml (resistant). The roxithromycin MIC_{90} was 2 μg/ml, which was fourfold higher than that observed for *B. pertussis*. The incidence of susceptibility was 76%, which was significantly lower than the 98% observed for *B. pertussis* isolates. The roxithromycin MIC_{90} was eightfold higher than the MIC_{90} observed with erythromycin. Overall, the MIC_{90}s of the fluoroquinolones and roxithromycin for *B. parapertussis* were two- to fourfold higher than those observed for *B. pertussis*.

Although no *B. bronchiseptica* isolates were recovered in the Multicenter Pertussis Surveillance Project, they can occasionally be of significance in compromised patients (17) and animals and can carry R factors (19). Only SXT showed relatively good activity against *B. bronchiseptica*, with 82% of the strains being susceptible (Table 1). Even ciprofloxacin and enoxacin showed marginal activity. None of the isolates was susceptible to amoxicillin, erythromycin, rifampin, tetracycline, norfloxacin, and roxithromycin. The rates of moderate susceptibility to amoxicillin, erthromycin, rifampin, SXT, and erythromycin were 27, 27, 0, 87, and 18%, respectively. This pattern of resistance was extremely different from that of the other *Bordetella* species.

In summary, *B. pertussis* may be becoming more resistant to rifampin, tetracycline, and, to a lesser extent, amoxicillin. Tetracycline inhibited only 17% of the strains, while the remaining antimicrobial agents inhibited 93% or more of the strains. *B. parapertussis* strains were susceptible to erythromycin, SXT, and the fluoroquinolones. Only a small percentage (≤2%) were susceptible to amoxicillin, rifampin, and tetracycline. Although the fluoroquinolones are very effective against *B. pertussis* and *B. parapertussis*, it is unlikely that they will be used in treating these infections in children. In vitro, roxithromycin does not appear to offer any advantage over erythromycin for either *B. pertussis* or *B. parapertussis*. *B. bronchiseptica* strains were distinct in that only SXT inhibited most (82%) of the strains.

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