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Antimicrobial Susceptibility of *Bordetella pertussis* Strains Isolated from 1960 to 1981

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The susceptibilities to erythromycin, rifampin, polymyxin B, ampicillin, tetracycline, gentamicin, fusidic acid, trimethoprim, and spectinomycin of 100 strains of *Bordetella pertussis* isolated between 1960 and 1981 were compared. No change in susceptibility to any of these drugs was noted.

There is little information on the antibiotic susceptibility of *Bordetella pertussis* (2, 6). The last study recorded in over a decade covered observations on only five strains (4). It has tacitly been assumed that the susceptibility pattern of strains is stable, although periodic surveys have not been performed. Also, there are few laboratory guidelines to indicate alternatives to erythromycin for the prophylaxis or treatment of pertussis.

We tested 50 separate clinical isolates of *B. pertussis* that were collected at The Hospital for Sick Children between 1960 and 1970 and 50 between 1970 and 1981. Most strains had been passaged only three times between isolation or lyophilization and testing and none more than five times. All strains were grown on Bordet-Gengou medium for 3 days before inoculation into Stainer-Scholte broth (7). The cultures were incubated at 37°C for 1 day in a shaker water bath to produce a suspension with an absorbance of 0.3 to 0.4 at 560 nm on a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). This was equivalent to a density of 3.5×10^8 to 4.0×10^8 cells per ml as determined by viable count. The suspension was further diluted 1:50 with Stainer-Scholte broth so that each prong of the replicator head delivered a final inoculum of 5×10^4 to 5×10^5 cells. Susceptibility tests for ampicillin, erythromycin, fusidic acid, gentamicin, polymyxin, rifampin, spectinomycin, and tetracycline were performed on Bordet-Gengou blood agar. For trimethoprim, Bordet-Gengou supplemented with 1% lysed horse blood was used.

Ampicillin (Ayerst Laboratories, Montreal, Province of Quebec) and gentamicin (Schering Corp., Pointe Claire, Province of Quebec) were dissolved in 0.02 M phosphate buffer (pH 7.2). Erythromycin (Abbott Laboratories, Montreal, Province of Quebec) was dissolved in 30% ethanol then further diluted in sterile distilled water. Rifampin (Dow Chemical, Indianapolis, Ind.) was dissolved in methanol and diluted further in sterile distilled water. Tetracycline (Pfizer Co., Ltd., Pointe Claire, Province of Quebec), polymyxin B (Burroughs Wellcome Ltd., La Salle, Province of Quebec), fusidic acid (Mast Laboratories, Ltd., Liverpool, United Kingdom) and spectinomycin (The Upjohn Co., Kalamazoo, Mich.) were dissolved in sterile distilled water. Trimethoprim (Burroughs Wellcome Ltd.) was dissolved in 0.1 N lactic acid and further diluted with sterile distilled water. The agar-dilution method of susceptibility testing was used. *B. pertussis* strains were inoculated onto Bordet-Gengou blood agar plates by using a multiple replicator apparatus. Plates were incubated at 37°C in the dark for 42 to 45 h. Susceptibility was indicated by the absence of visible growth at the point of inoculation. All strains were tested in duplicate. Sterility controls, drug-free solvent controls, nutrient agar absence-of-growth control, and control strains of *Staphylococcus aureus* and *Haemophilus influenzae* with known mean inhibitory concentrations (MICs) on Bordet-Gengou medium were included in the assessments. MICs that inhibited 50 and 90% of the strains (MIC_{50} and MIC_{90}) were calculated by the Reed and Muench method (6).

There was no evidence of changes in the susceptibility of strains during the 1960 to 1981 period in *B. pertussis* (Table 1). Some of the MICs are lower than ones previously reported (2, 4, 6). The methodological differences in the present study are partly responsible (e.g., the use of shaker-cultured log-phase *B. pertussis* organisms, which permits the early reading of test results and minimizes the possibilities of antibiotic deterioration). Of the nine drugs, erythromycin was the most active with an MIC_{90} of 0.025 μg/ml, consolidating its position as the drug of choice for the prophylaxis and treatment of pertussis. Rifampin and polymyxin were the next most active with MIC_{90} in the 0.125-μg/ml range. These values are in agreement with those

TABLE 1. Comparison of susceptibility values for *B. pertussis* isolates in 1960 to 1969 and 1970 to 1981

Drug	MICs (μg/ml) for 1960–1969 isolates			MICs for 1970–1981 isolates		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Erythromycin	0.012–0.1	0.016	0.024	0.012–0.1	0.015	0.035
Polymyxin B	0.03–0.12	0.049	0.099	0.03–0.12	0.041	0.076
Rifampin	0.12–0.25	0.092	0.125	0.12–0.25	0.092	0.125
Fusidic acid	0.03–0.5	0.078	0.21	0.03–0.25	0.13	0.22
Tetracycline	0.06–0.5	0.091	0.213	0.06–0.5	0.15	0.25
Ampicillin	0.125–0.5	0.18	0.24	0.125–0.5	0.18	0.24
Gentamicin	0.06–0.5	0.18	0.365	0.06–0.5	0.25	0.44
Spectinomycin	0.6–5.0	0.79	1.25	0.3–2.5	0.84	1.4
Trimethoprim	1.0–2.0	1.39	1.86	0.25–2.0	1.37	1.85

of other investigators (4). Ampicillin, tetracycline, gentamicin, and fusidic acid had MIC_{90} between 0.21 and 0.44 μg/ml. Spectinomycin inhibited 90% of the *B. pertussis* isolates at a concentration of 1.25 μg/ml, which is similar to the level at which some *Neisseria gonorrhoeae* are inhibited. Of the drugs tested, trimethoprim was the least active with an MIC_{90} of 1.86 μg/ml. In limited trials, trimethoprim has appeared to be clinically effective (1) despite the poor in vitro activity reported here and elsewhere (3).

As with other microorganisms, a demonstration of stable susceptibility patterns in *B. pertussis* isolates over 20 years, particularly from one location, does not guarantee immutability. Periodic surveys of susceptibility are needed. Should resistance arise, data from studies such as this may assist in identifying alternative agents for clinical use.

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