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Antibiotic Resistance of Degraded Strains of *Bordetella pertussis*

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The susceptibilities to erythromycin, rifampin, polymyxin B, ampicillin, tetracycline, gentamicin, fusidic acid, trimethoprim, and spectinomycin of five virulent phase I strains of *Bordetella pertussis* and their degraded phase IV descendants were compared. Increases in MICs of 2- to 16-fold were observed for erythromycin, rifampin, tetracycline, fusidic acid, trimethoprim, and spectinomycin for four of the five degraded strains.

Phase change in *Bordetella pertussis* from the virulent phase I to the avirulent phase IV is associated with the loss of several functional activities and structural antigens (3). There is also limited information to suggest that such degraded strains are more antibiotic resistant than their wild-type parent strains (2).

Five degraded phase IV descendants of *B. pertussis* were obtained by serial weekly subculture of fresh clinical isolates on Bordet-Gengou medium after 40 to 60 passages. Their identity as phase IV variants was confirmed by their ability to grow on Stainer-Scholte medium containing 1% Casamino Acids (2, 4). The antibiotic susceptibility of the degraded phase IV and phase I parent strains was tested in parallel by an agar dilution procedure. All strains were grown on Bordet-Gengou medium for 3 days before inoculation into Stainer-Scholte broth (5). 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 B, rifampin, spectinomycin, and tetracycline were performed on Bordet-Gengou blood agar. For trimethoprim, Bordet-Gengou agar supplemented with 1% lysed horse blood was used.

Ampicillin (Ayerst Laboratories, Montreal, Quebec, Canada) and gentamicin (Schering Corp., Point Claire, Quebec, Canada) were dissolved in 0.02 M phosphate buffer (pH 7.2). Erythromycin (Abbott Laboratories, Montreal, Quebec, Canada) was dissolved in 30% ethanol and then further diluted in sterile distilled water. Rifampin (Dow Chemical Co., Indianapolis, Ind.) was dissolved in methanol and diluted further in sterile distilled water. Tetracycline (Pfizer Co., Ltd., Point Claire, Quebec, Canada), polymyxin B (Burroughs Wellcome, Ltd., La Salle, Quebec, Canada), 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 (1). *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, absence-of-growth control (phase I strains), Stainer-Scholte with 1% Casamino Acids-growth controls (phase IV strains), and control strains of *Staphylococcus aureus* and *Haemophilus influenzae* with known mean MICs on Bordet-Gengou medium were included in the assessments. Results were expressed as the fold increase in MIC of degraded over virulent strains.

The MICs for the phase I isolates were all in the expected range (1). Four of the five phase IV strains showed increases in MIC of 2- to 16-fold for several of the antibiotics tested (Table 1). All phase IV strains had an unaltered susceptibility to ampicillin, gentamicin, and polymyxin B, and one had an identical susceptibility pattern to its parent for all drugs. No phase IV strain was more sensitive than its parent phase I strain to any of the antibiotics tested. With the exception of ampicillin, these results are in broad agreement with those previously reported (2).

Phase change in *B. pertussis* was for many years regarded simply as a laboratory curiosity. Its significance in vaccine production and in defining the functional activities and structural antigens of *B. pertussis* is now fully recognized (2, 3). If there is a role for degraded phase IV strains in clinical whooping cough—and there is no evidence one way or the other, only anecdotal speculation because of the difficulty in identifying such nondescript, inert organisms from among the indigenous respiratory flora—then it could be reasoned that the relative antibiotic resistance of these strains might be a factor in either their generation, selection, or establishment in the pediatric host. The more we know about these

TABLE 1. Fold increase in MIC of phase IV over phase I strains of *B. pertussis*

Antibiotic	Phase IV strain and fold increase				
	1	2	3	4	5
Erythromycin	4	16	2	0	4
Rifampin	2	2	2	0	2
Tetracycline	2	2	2	0	2
Fusidic acid	4	8	4	0	0
Trimethoprim	0	4	0	0	0
Spectinomycin	4	8	4	0	0
Ampicillin	0	0	0	0	0
Polymyxin B	0	0	0	0	0
Gentamicin	0	0	0	0	0

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descendants of *B. pertussis*, the better we will be able to formulate and test hypotheses concerning the significance and possible involvement of these neglected organisms in human disease.

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