

## Antimicrobial Susceptibility Testing of Clinical Isolates of *Bordetella pertussis* from Northern California: Report from the SENTRY Antimicrobial Surveillance Program

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**Reports of an increased clinical incidence of pertussis and the development of resistance by *Bordetella pertussis* to erythromycin prompted the collection and testing of recent clinical isolates from patients in northern California against a range of antimicrobial agents by the Etest (AB BIODISK, Solna, Sweden) method. All isolates were fully susceptible to all eight agents tested (MIC,  $\leq 0.38$   $\mu\text{g/ml}$ ), including newer fluoroquinolones, such as gatifloxacin (MIC of which 90% of the isolates tested are inhibited, 0.006  $\mu\text{g/ml}$ ), which may be used in cases of adolescent or adult pertussis. Continued surveillance of *B. pertussis* isolates appears to be a prudent practice.**

The first clear description of pertussis (whooping cough) was made by Baillou in 1640 (6). Pertussis remains a highly contagious respiratory disease causing significant morbidity in children and now more recently in adults. The incidence of pertussis decreased dramatically in the late 1940s, with the introduction of whole-cell pertussis vaccines. However, even in countries with high vaccination rates, there have been recent reports of increases in cases in The Netherlands (1) and in the United States (2). Experience in the United States has shown the incidence of pertussis to be relatively stable in infants and young children, but it has increased markedly in adolescents and young adults (2). The cause of this increase is not clearly known, but may be due to increased accuracy of diagnosis and reporting of the disease or a decline in immunity over time. The incidence of pertussis as a cause of chronic cough syndromes in adults and adolescents was 19.9% in Canada (11).

Although erythromycin or macrolides remain the antimicrobials of choice for the treatment of *Bordetella pertussis* infections, there have been reports of the emergence of resistance to these agents in clinical isolates from the United States (3, 4). This study reports the susceptibility of 45 clinical isolates of *B. pertussis* collected from December 1998 to August 1999 in Northern California as part of the Special Objectives Phase of the SENTRY Antimicrobial Surveillance Program (10).

Thirty-six of the 45 *B. pertussis* isolates were viable, grew well on test media, and were available for susceptibility testing. The demographic data of all 45 patients were as follows: (i) 57.8% of patients were female; (ii) the age distributions of patients were  $\leq 6$  months (20 patients), 7 months to 1 year (4 patients), 2 to 10 years (9 patients), 11 to 16 years (7 patients),  $>16$  years (4 patients), and unknown (1 patient), and (iii) the site of acquisition in the community was 82.2%. The *B. pertussis* isolates were defined by biochemical reactions (catalase positive,

urease negative, and oxidase positive) after the finding of small, gram-negative coccobacilli on Bordet-Gengou potato infusion or Regan-Lowe agar. Direct fluorescent antibody tests were performed by the participating site to confirm identification prior to shipment of the isolates to the SENTRY monitor. Upon arrival, isolates were subcultured twice onto Bordet-Gengou agar (Remel, Kansas City, Mo.) and incubated in a moist, closed ambient air environment at 35°C for 72 h. Storage cultures were made with defibrinated rabbit blood (Remel) and kept at  $-80^{\circ}\text{C}$  until tested.

MICs of eight antimicrobial agents (azithromycin, erythromycin, clarithromycin, clindamycin, ciprofloxacin, gatifloxacin, trovafloxacin, trimethoprim-sulfamethoxazole) were determined by using the Etest (AB BIODISK, Solna, Sweden) methodology, a method validated elsewhere (3, 4; J. E. Hoppe and T. H. Tschirner, Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D10, 1994). Two large Mueller-Hinton agar plates, each containing 5% sheep blood, were inoculated with a swab taken from a colony suspension equal to that of a 0.5 McFarland standard (8, 9). The quality control strains used were *Haemophilus influenzae* ATCC 49247, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212. Each isolate was also sent to the University of Iowa Hygienic Laboratory (Oakdale Campus), Iowa City, for confirmation of identification by utilizing the direct fluorescent antibody test and other molecular techniques (5).

The MIC results are summarized in Table 1. All isolates appeared to be susceptible to all of the antimicrobial agents tested, erythromycin (“drug-of-choice”) having a MIC range of  $\leq 0.016$  to 0.19  $\mu\text{g/ml}$ , with a MIC at which 90% of the isolates tested are inhibited (MIC<sub>90</sub>) of 0.125  $\mu\text{g/ml}$ . The other macrolides had MIC<sub>90</sub> results slightly lower than those of erythromycin. The highest MIC for one isolate was that of clindamycin (0.38  $\mu\text{g/ml}$ ). The other four antimicrobial agents used (ciprofloxacin, gatifloxacin, trovafloxacin, and trimethoprim-sulfamethoxazole) all had potent activity against *B. pertussis*, with

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TABLE 1. Antimicrobial activity of eight antimicrobial agents tested by the Etest method against 36 strains of *B. pertussis*<sup>a</sup>

Antimicrobial agent	MIC ( $\mu\text{g/ml}$ ) <sup>b</sup>		
	50%	90%	Range
Azithromycin	$\leq 0.016$	0.064	$\leq 0.016$ –0.094
Clarithromycin	$\leq 0.016$	0.094	$\leq 0.016$ –0.125
Erythromycin	0.023	0.125	$\leq 0.016$ –0.19
Clindamycin	0.032	0.19	$\leq 0.016$ –0.38
Ciprofloxacin	0.008	0.032	$\leq 0.002$ –0.064
Gatifloxacin	$\leq 0.002$	0.006	$\leq 0.002$ –0.012
Trovaflaxacin	0.016	0.032	0.012–0.032
Trimethoprim-sulfamethoxazole	$\leq 0.002$	0.004	$\leq 0.002$ –0.012

<sup>a</sup> Thirty-six viable strains among 45 total isolates from a regional laboratory in northern California (1998).

<sup>b</sup> 50% and 90%, MIC<sub>50</sub> and MIC<sub>90</sub>, respectively.

MIC<sub>90</sub>s ranging from 0.004 to 0.032  $\mu\text{g/ml}$ . All isolates were inhibited by concentrations of fluoroquinolones and trimethoprim-sulfamethoxazole of 0.064  $\mu\text{g/ml}$  or less. Gatifloxacin (MIC<sub>90</sub>, 0.006  $\mu\text{g/ml}$ ) and trimethoprim-sulfamethoxazole (MIC<sub>90</sub>, 0.004  $\mu\text{g/ml}$ ) were the most active drugs tested.

The MIC range for erythromycin against *B. pertussis* has been generally described as 0.02 to 0.125  $\mu\text{g/ml}$ , and, until recently, no resistant isolate had been well documented (3, 4). None of the 36 viable isolates in this study was resistant to any of the antimicrobial agents tested. The highest MIC was only 0.38  $\mu\text{g/ml}$ . A strain of *B. pertussis* recently isolated in Arizona and resistant to erythromycin (32  $\mu\text{g/ml}$ ) was, however, cause for concern and for continued clinical laboratory vigilance (3). The development of newer and more reliable testing methods, such as PCR, will help to accurately and quickly identify *B. pertussis* (5, 7). Until these tests are available, direct fluorescent antibody testing provides a rapid confirmation of the presence of *Bordetella* (7), and tests involving the oxidase, catalase, and urease reactions will initially distinguish between *B. pertussis* and other *Bordetella* species, thus allowing suscep-

tibility testing by a reliable and practical dilution method (Etest) (3, 4, 7; Hoppe and Tschirner, 34th ICAAC).

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