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In Vitro Activities of Five New Quinolones against 88 Genital and Neonatal *Haemophilus* Isolates

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In vitro activities of five new quinolones against 88 strains of *Haemophilus influenzae* and *H. parainfluenzae* isolated from genitourinary or neonatal infections were studied. All strains were susceptible, and MICs were similar to those for respiratory tract isolates. However, *H. influenzae* biotype IV appeared to be more susceptible to norfloxacin, enoxacin, and ciprofloxacin than the other biotypes were.

Haemophilus influenzae and *H. parainfluenzae* are common commensals of the oropharynx (1, 7, 13). *H. influenzae* is frequently isolated from cases of infection or superinfection of the respiratory tract; capsulated strains cause meningitis and sepsis. Susceptibility of these strains to antibiotics is regularly checked. *H. influenzae* and *H. parainfluenzae* are also responsible for genital infections and neonatal sepsis. Both bacteria have been isolated from the vaginas of 0.2 to 2% of asymptomatic women (14). Recently, a specific tropism of some strains for the genital tract has been suggested. Genital *H. influenzae* differs from oropharyngeal strains in biotype and serotype, with a high prevalence of biotype IV and nonagglutinable strains in maternofetal and neonatal systemic infections (1, 3, 9, 14). Susceptibility of genital and neonatal *Haemophilus* spp. to antibiotics is poorly documented. The new quinolones have been proposed to treat *Haemophilus* infections (8), although susceptibility of genital isolates of *Haemophilus* spp. to these drugs has not yet been assessed. To clarify this point, we have investigated the activity of five quinolones against 88 genital and neonatal *Haemophilus* isolates.

A total of 74 strains of *H. influenzae* and 14 strains of *H. parainfluenzae* were isolated from 1 January 1980 through 31 October 1986 in the Bretonneau University Hospital in Tours, France. Only one isolate per clinical case was analyzed. When the same strain was isolated from a mother and her newborn infant, only the isolate originating from gastric fluid was included in the study. Isolates were from (i) 25 lower-genital-tract infections (11 cervicovaginitis, 8 urethritis, and 6 Bartholin gland abscess cases), which yielded 18 *H. influenzae* and 7 *H. parainfluenzae* isolates; (ii) 30 pelvic infections or complicated genital infections (25 endometritis, 4 salpingitis, and 1 orchiepididymitis cases), which yielded 29 *H. influenzae* and 1 *H. parainfluenzae* isolates; (iii) three spontaneous abortions, which yielded three *H. influenzae* isolates; (iv) eight asymptomatic carriers (male partners of infected women), which yielded four *H. influenzae* and four *H. parainfluenzae* isolates; and (v) 22 neonatal infections, which yielded 20 *H. influenzae* and 2 *H. parainfluenzae* isolates from the gastric fluids of newborn infants. In one neonatal case of *H. influenzae* biotype II, serotype b, the cerebrospinal fluid also tested positive for *H. influenzae*. In none of the neonatal cases did blood cultures test positive. This contrasts sharply with the situation in the United States, where sepsis is not rare (3, 14); these differences are discussed elsewhere (9).

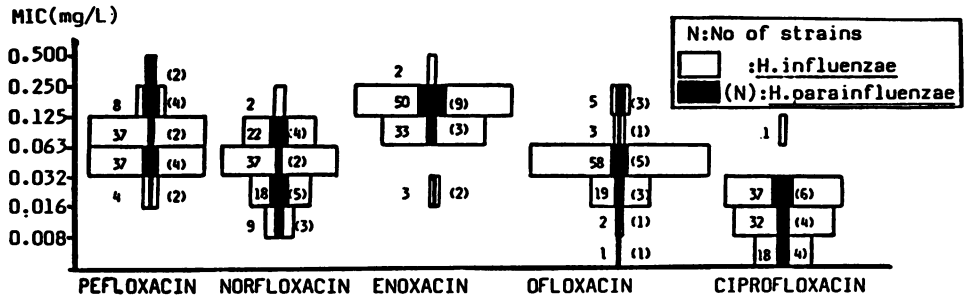

FIG. 1. Distribution of MICs of five new quinolones for 74 isolates of *H. influenzae* and 14 isolates of *H. parainfluenzae* originating from genitourinary or neonatal infections.

Biotypes were studied with the API 20E biochemical test (API System, Montalieu Vercieu, France) by the method of Holmes et al. (5) and were controlled with a specific *Haemophilus* identification system (HNID panel; American MicroScan, Campbell, Calif.). Capsular serotypes were determined upon isolation by slide agglutination with specific antisera (Difco Laboratories, Detroit, Mich.) and coagglu-

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TABLE 1. Susceptibilities of 88 genital and neonatal isolates of *H. influenzae* and *H. parainfluenzae* to five new quinolones

Organism (no. of isolates)	Antimicrobial agent	MIC (mg/liter)[a]			
		50%	90%	Range	Geometric mean (95% confidence interval)
H. influenzae biotype I (11)	Pefloxacin	0.125	0.125	0.032–0.125	0.086 (0.062–0.118)
	Norfloxacin	0.063	0.125	0.032–0.125	0.063 (0.047–0.084)
	Enoxacin	0.125	0.25	0.125–0.25	0.171 (0.134–0.218)
	Ofloxacin	0.063	0.063	0.032–0.063	0.049 (0.039–0.061)
	Ciprofloxacin	0.016	0.032	<0.008–0.032	0.017 (0.012–0.023)
H. influenzae biotype II (34)	Pefloxacin	0.125	0.125	0.063–0.25	0.102 (0.089–0.116)
	Norfloxacin	0.063	0.125	0.032–0.125	0.071 (0.059–0.084)
	Enoxacin	0.25	0.25	0.125–0.25	0.208 (0.187–0.230)
	Ofloxacin	0.063	0.063	0.032–0.125	0.057 (0.051–0.063)
	Ciprofloxacin	0.032	0.032	<0.008–0.032	0.021 (0.018–0.025)
H. influenzae biotype III (13)	Pefloxacin	0.063	0.125	0.063–0.125	0.073 (0.061–0.088)
	Norfloxacin	0.063	0.063	0.032–0.125	0.053 (0.042–0.068)
	Enoxacin	0.25	0.25	0.125–0.25	0.172 (0.138–0.213)
	Ofloxacin	0.063	0.063	0.032–0.063	0.053 (0.045–0.063)
	Ciprofloxacin	0.016	0.032	<0.008–0.032	0.018 (0.013–0.025)
H. influenzae biotype IV (7)	Pefloxacin		0.125	0.032–0.125	0.063 (0.043–0.090)
	Norfloxacin		0.063	0.016–0.063	0.019 (0.012–0.031)
	Enoxacin		0.125	0.032–0.125	0.102 (0.063–0.165)
	Ofloxacin		0.063	0.016–0.063	0.042 (0.025–0.070)
	Ciprofloxacin		0.016	<0.008–0.016	0.009 (0.007–0.013)
H. influenzae biotype V (4)	Pefloxacin			0.063–0.25	
	Norfloxacin			0.063–0.25	
	Enoxacin			0.125–0.5	
	Ofloxacin			0.032–0.25	
	Ciprofloxacin			<0.008–0.032	
H. influenzae biotype VI (4)	Pefloxacin			0.125–0.25	
	Norfloxacin			0.063–0.25	
	Enoxacin			0.125–0.5	
	Ofloxacin			0.063–0.25	
	Ciprofloxacin			0.016–0.125	
H. influenzae biotype VII (1)	Pefloxacin			0.125	
	Norfloxacin			0.125	
	Enoxacin			0.25	
	Ofloxacin			0.125	
	Ciprofloxacin			0.032	
H. parainfluenzae (14)	Pefloxacin	0.063	0.5	0.032–0.5	0.113 (0.06–0.215)
	Norfloxacin	0.032	0.125	0.016–0.125	0.044 (0.028–0.070)
	Enoxacin	0.25	0.25	0.032–0.25	0.160 (0.104–0.246)
	Ofloxacin	0.063	0.25	<0.008–0.25	0.060 (0.033–0.108)
	Ciprofloxacin	0.016	0.032	<0.008–0.032	0.017 (0.012–0.025)

[a] 50% and 90%, MIC for 50 and 90% of the isolates, respectively.

tination reagents (Phadebact-*Haemophilus* test; Pharmacia Diagnostics, Uppsala, Sweden). β-Lactamase activity was screened with Cefinase chromogenic cephalosporin (bio-Mérieux, Charbonnières-les-Bains, France).

We have determined the MICs of five quinolones: pefloxacin (Roger Bellon, Neuilly/Seine, France), norfloxacin (Merck Sharp & Dohme, Italian subsidiary of Merck & Co., Inc., Rahway, N.J.), enoxacin (Roger Bellon), ofloxacin (Roussel Uclaf, Compiegne, France), and ciprofloxacin (Bayer Pharma, Sens, France). MIC tests were carried out by agar dilution methods (12). Titered antibiotics were suspended according to the recommendations of the manufacturers. Constant volumes of increasing concentrations, from 0.008 to 8 mg/liter, were incorporated in Mueller-Hinton agar to which 5% Fildes enrichment medium (Difco Laboratories) was added. Approximately 10^4 bacteria were applied to the agar surface of the plates with an inoculum-replicating apparatus like that described by Steers. The last concentration inhibiting the culture was determined after a 48-h incubation at 37°C in conventional atmosphere. *Staphylococcus aureus* NCTC 6571 was used as a technical control.

Characterization of *H. influenzae* isolates showed that 11 were biotype I, 34 were biotype II, 13 were biotype III, 7 were biotype IV, 4 were biotype V, 4 were biotype VI, and 1 was biotype VII. Only four isolates were capsulated (three were serotype b and one was serotype a). Seven strains showed β-lactamase activity (one was biotype I, five were biotype II, and one was biotype IV). There were 3 biotype I and 11 biotype II isolates of *H. parainfluenzae*.

All 88 isolates of *H. influenzae* and *H. parainfluenzae* were susceptible to the five quinolones tested (Fig. 1). MICs for 90% of the *H. influenzae* isolates were, per liter, 0.125 mg for pefloxacin and norfloxacin, 0.25 mg for enoxacin, 0.063 mg for ofloxacin, and 0.032 mg for ciprofloxacin. These results do not differ from those obtained with strains isolated mostly from nongenital infections (ear, throat, and respiratory tract infections; 2, 4, 6, 8, 10). MICs for 90% of the *H. parainfluenzae* isolates were, per liter, 0.5 mg for pefloxacin, 0.125 mg for norfloxacin, 0.25 mg for enoxacin and ofloxacin, and 0.032 mg for ciprofloxacin. There was no statistical difference between geometric mean MICs for *H. influenzae* and *H. parainfluenzae*.

Susceptibility of *H. influenzae* to the five quinolones was also studied according to biotype (Table 1). Geometric mean MICs for the four prevalent biotypes (I, II, III, and IV) were compared using the Student *t* test. Biotypes I, II, and III showed no difference except for a greater susceptibility to pefloxacin for biotype III as compared with biotype II. Biotype IV appeared more susceptible to norfloxacin, enoxacin, and ciprofloxacin than biotypes I, II, and III and appeared more susceptible to pefloxacin than biotype II. Although differences were statistically significant, the small number of isolates belonging to biotype IV raises the question of how much these differences may be generalized. If valid, the differences indicate that strains of biotype IV are unique in *H. influenzae*. Only ofloxacin had an equal activity against *H. influenzae* biotypes I, II, III, and IV. A better knowledge of the mechanism of action of the quinolones could eventually shed light on this problem. Some data suggest that ofloxacin, aside from its action on bacterial DNA gyrase, might have another antibacterial action not inhibited by rifampin (11; J. T. Smith and N. T. Ratcliff, Abstr. 4th Mediterranean Congr. Chemother., abstr. no. 732, 1984). However, a similar observation was made for ciprofloxacin and is not supported by our MIC results.

The seven β-lactamase-producing *H. influenzae* isolates were not more resistant to quinolones than were penicillin-susceptible isolates. New quinolones, because of their activity on β-lactamase-producing gram-negative bacteria, might represent an alternative to broad-spectrum cephalosporin therapy for treatment of severe *Haemophilus* genital infections.

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