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Emergence of Fluoroquinolone-Resistant Strains of *Vibrio cholerae* O1 Biotype El Tor among Hospitalized Patients with Cholera in Calcutta, India

Ciprofloxacin and norfloxacin are broad-spectrum fluoroquinolones and possess excellent activity against *Vibrio cholerae* O1 and O139 serogroups (6). Clinical studies have shown that these drugs are effective in treatment of cholera in adults and children (2–4). For the past 11 years, we have been monitoring the incidence of antibiotic susceptibility and genotypic changes in *V. cholerae* isolates from cholera patients admitted at the Infectious Diseases Hospital, Calcutta, India. Here, we report the emergence of fluoroquinolone-resistant strains of *V. cholerae* O1 Biotype El Tor among hospitalized patients with cholera.

Antimicrobial susceptibility analysis of *V. cholerae* strains was performed by disk diffusion (1) on Mueller-Hinton agar (Difco, Detroit, Mich.) with commercial disks (HiMedia, Mumbai, India). The following antibiotic disks were used: ciprofloxacin, 5 μg; nalidixic acid, 30 μg; norfloxacin, 10 μg; and tetracycline, 30 μg. Characterization of strains as susceptible, intermediately resistant, or resistant was based on the size of the inhibition zone according to the manufacturer's instructions. These zone size interpretive criteria for susceptibility corresponded to MICs of 0.25, 0.06, and 0.06 μg/ml for nalidixic acid, norfloxacin, and ciprofloxacin, respectively. Strains showing intermediate zones of growth inhibition were classified as resistant on the basis of previous MIC studies with *V. cholerae* (6).

We have reported considerable increases in fluoroquinolone resistance among *V. cholerae* strains belonging to non-O1, non-O139 serogroups during 1996 (5). All the *V. cholerae* strains of serogroup O1 isolated in or before 1994 are susceptible to ciprofloxacin. From 1995, we have recorded progressive increases in ciprofloxacin and norfloxacin resistance among *V. cholerae* O1 strains, with the highest occurrences of 38.8% in 1999 and 25% in 2000, respectively (Table 1). To our knowledge, this is the first report on such high incidence of fluoroquinolone resistance among toxigenic *V. cholerae* O1 strains. The MICs of ciprofloxacin and norfloxacin for ciprofloxacin-resistant *V. cholerae* strains ranged between 9 and >32 μg/ml and between 192 and >256 μg/ml, respectively, when tested with the E-test strips on Mueller-Hinton agar (AB Biodisk, Solna, Sweden). The incidence of nalidixic acid resistance among *V. cholerae* O1 strains was low (<10%) before 1993 and peaked during subsequent years (1999; 100%), as shown in the Table 1. Possibly, ciprofloxacin resistance might have emerged in direct response to the selective pressure exerted by nalidixic acid coupled with disproportionate use of fluoroquinolones in the clinical settings. It is worth to mention here that the increase in the incidence of nalidixic acid-resistant strains of *V. cholerae* O1 (probably with a single mutation in *gyrA* and/or other related genes) portended a further increase in the incidence of strains with clinically significant resistance to fluoroquinolones (with two or more mutations in the *gyrA* gene). We are in the process of identifying the mutational "hot spots" in the quinolone resistance-determining region.

An interesting observation in the present report is the low incidence of quinolone resistance in the O139 serogroup. One possible elucidation is the low frequency of nalidixic acid resistance among *V. cholerae* O139 strains (Table 1), and therefore the frequency of double mutations, a prerequisite for fluoroquinolone resistance, is low, as reflected by resistance O139 strains to fluoroquinolones.

In this study we encountered a higher incidence of *V. cholerae* O1 resistance to ciprofloxacin than to norfloxacin, which is generally less potent than ciprofloxacin. The possible explanation for this counterintuitive result is (i) since ciprofloxacin is in extensive use for all the bacterial infections in this part of the world, conditions of high selective pressure would have forced the mutant *V. cholerae* strains to multiply and establish themselves as the dominant population; continued selective pressure favored these progeny to have further mutations; (ii) ciprofloxacin and norfloxacin breakpoints are not comparable, at least for *V. cholerae* strains (the MIC for 50% of the strains [MIC_{50}], MIC_{90}, and MIC ranges need to be determined for both drugs to prove this hypothesis); or (iii) ciprofloxacin and norfloxacin accumulation kinetics might differ among *V. cholerae* O1 strains. Additional, extensive studies are needed to test these possibilities in order to determine the mechanisms re-

TABLE 1. Resistance to quinolones among *V. cholerae* O1 and O139 isolates from cholera patients

Yr	No. of isolates		No. of resistant strains (%)[a]					
			Nalidixic acid		Ciprofloxacin		Norfloxacin	
	O1	O139	O1	O139	O1	O139	O1	O139
1989	49		3 (6.1)		ND		ND	
1990	59		1 (1.7)		ND		ND	
1991	30				ND		ND	
1992	26	10	2 (7.7)					
1993	20	87	1 (5)	2 (2.3)				
1994	74	40	73 (98.6)					
1995	84	42	82 (97.6)	9 (21.4)	2 (2.4)	1 (2.4)	3 (3.6)	1 (2.4)
1996	69	64	68 (98.5)	5 (7.8)	4 (5.8)	3 (4.7)		
1997	53	71	50 (94.3)	5 (7)	10 (18.9)	1 (1.4)	4 (7.5)	
1998	201	55	197 (98)	7 (12.7)	20 (10)		1 (0.5)	
1999	49	60	49 (100)	1 (1.7)	19 (38.8)	1 (1.7)	8 (16.3)	
2000	16	2	12 (75)		3 (18.7)		4 (25)	

[a] Based on the MICs for *V. cholerae* O1 (6). ND, not done.

sponsible for heterogeneous fluoroquinolone resistance among *V. cholerae* O1 strains.

Emergence of fluoroquinolone resistance in *V. cholerae* will certainly complicate the therapeutic use of these drugs, and attention must be paid to this trend. Fortunately, *V. cholerae* O1 and O139 strains are susceptible to tetracycline, which is an effective drug for treatment of cholera at the Infectious Disease Hospital.

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