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Survey of In Vitro Susceptibilities of *Vibrio cholerae* O1 and O139 to Antimicrobial Agents

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***Vibrio cholerae* O139 (173 strains) and O1 (221 strains) were tested for their in vitro susceptibilities to 39 antimicrobial agents. Both O139 and O1 strains were highly susceptible to azithromycin, cephems, minocycline, penems, and newer fluoroquinolones. O139 strains (94.8%), O1 Indian El Tor strains (97%), and Bangladeshi El Tor strains (50%) were highly resistant to streptomycin, sulfamethoxazole, and trimethoprim and moderately resistant to chloramphenicol and furazolidone, in sharp contrast to O1 Peruvian El Tor and O1 classical strains. Some Bangladeshi El Tor strains (43.3%) showed tetracycline resistance as well.**

Vibrio cholerae O1 biotype El Tor, the causative agent of the seventh cholera pandemic (6), was first recorded in 1961 in Indonesia and continues to possess the capacity of causing cholera endemics. For instance, in 1991, El Tor strains appeared in South and Central America, starting in Peru, where no large cholera endemics had been recorded in this century (31, 32). In 1982, the classical biotype of *V. cholerae* O1 (the causative agent of the previous cholera pandemics) reappeared in Bangladesh and continues to persist (16). Large explosive cholera endemics due to *V. cholerae* non-O1 strains that elaborate cholera toxin occurred in India and Bangladesh from October 1992 (1, 10, 24, 33). *V. cholerae* O139 Bengal, which is currently classified as the epidemic non-O1 strain, has spread to neighboring countries (e.g., Thailand [4], Pakistan [7], and Singapore [5]), and imported cases associated with *V. cholerae* O139 have now been reported from the United States (28), Japan (17), and Switzerland (3).

In addition to being resistant to streptomycin (24) and the vibriostatic agent O/129 (1), *V. cholerae* O139 strains are usually resistant to sulfamethoxazole-trimethoprim (co-trimoxazole) (1, 24, 28) and to furazolidone (24); the last two are often recommended for the treatment of cholera (9, 23). Moreover, most of the *V. cholerae* O1 El Tor strains in Bangladesh are resistant to tetracycline (1). In this study, we investigated the in vitro susceptibilities of *V. cholerae* O139 strains to 39 antimicrobial agents and compared them with those obtained for *V. cholerae* O1 strains belonging to the classical and El Tor biotypes.

All *V. cholerae* strains used in this study were of clinical origin. The 173 O139 strains examined were isolated during the period 1992 to 1993 and included 134 strains from India, 30 from Bangladesh, and 9 from Thailand. Of the 72 O1 classical strains examined, 66 were isolated from 1960 to 1965 in India while 6 were isolated in 1982 in Bangladesh. A total of 149 O1 El Tor strains examined in this study included 67 from India isolated from 1985 to 1990, 52 from Peru isolated in 1991, and 30 from Bangladesh isolated in 1994. Isolates were stored frozen at −80°C.

The antimicrobial agents were gifts from their manufacturers. Amoxicillin was used in combination with clavulanic acid (a β-lactamase inhibitor). Penems used were FCE22101 (8) and SY5555 (11a) (alternatively named SUN5555 [21], ALP201 [2a], and WY-49,605). Azithromycin is a 15-membered ring macrolide (25). Newer quinolones included BAYy3118 (2) and DU6859a (27). Furazolidone, 3-(5-nitrofurfurylideneamino)-2-oxazolidinone, was also used. Sulfamethoxazole and trimethoprim were used alone and in combination at ratios of 5:1 (as in the combination drug) and 20:1 (the expected ratio in the human body). O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate) was purchased from Sigma Chemical, St. Louis, Mo.

Susceptibility testing of bacterial strains was done by the agar dilution method with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) according to standard procedures (14, 26). The final concentrations of antimicrobial agents were from 0.004 to 128 μg/ml. The test bacteria were grown for 18 h at 37°C with agitation in L broth (18) and diluted to approximately 10^6 CFU/ml. Aliquots of the bacterial suspension (approximately 10^4 CFU per spot) were inoculated on the surface of antimicrobial agent-containing agar plates. Incubation was for 20 h at 37°C. The MIC was determined as previously described (14, 26). *Escherichia coli* NIHJ JC-2 was used as a reference strain for quality control (12, 14, 19, 20, 22). When the susceptibility to sulfamethoxazole or trimethoprim was tested, Mueller-Hinton agar supplemented with 7.5% (vol/vol) defibrinated horse blood (frozen and thawed) was also used, in addition to Mueller-Hinton agar alone (13).

The MICs of the antimicrobial agents against clinical isolates of O139 are summarized in Table 1. Among antimicrobial agents tested, the newer quinolones (norfloxacin, ofloxacin, tosufloxacin, ciprofloxacin, sparfloxacin, BAYy3118, and DU6859a) showed the greatest activity (MICs, ≤0.06 μg/ml). Six (I-2, I-7, I-14, I-15, I-64, and I-72) of the 173 O139 strains (3.5%) were resistant to ampicillin (MIC, ≥256 μg/ml), tetracycline (MIC, 8 to 16 μg/ml), chloramphenicol (MIC, 32 μg/ml), kanamycin (MIC, ≥256 μg/ml), and gentamicin (MIC, 128 to ≥256 μg/ml). With the exception of one strain (B20), the remaining 172

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TABLE 1. MICs of antimicrobial agents for 173 clinical isolates of *V. cholerae* O139

Antimicrobial agent	MIC (μg/ml)		
	50%	90%	Range
Penicillins			
Ampicillin	2	4	2–≥256
Amoxicillin-clavulanic acid (2:1)	8	8	4–32
Penems			
FCE22101	2	2	1–8
SY5555	1	2	1–4
Broad-spectrum cephems			
Cefoperazone	0.06	0.12	0.03–16
Cefixime	0.06	0.06	0.03–1
Tetracyclines			
Tetracycline	0.25	0.5	0.25–16
Doxycycline	0.25	0.25	0.12–2
Minocycline	0.12	0.12	0.06–0.25
Chloramphenicol	8	8	1–32
Aminoglycosides			
Streptomycin	≥256	≥256	8–≥256
Kanamycin	8	8	4–≥256
Gentamicin	1	2	0.25–≥256
Macrolides			
Spiramycin	128	128	64–128
Oleandomycin	64	128	64–128
Midecamycin	32	32	16–64
Josamycin	16	16	8–32
Rokitamycin	16	16	8–32
Kitasamycin	16	16	8–32
Roxithromycin	8	16	4–32
Clarithromycin	8	8	4–16
Erythromycin	4	8	1–8
Azithromycin	0.5	0.5	0.12–1
Lincomycin	≥256	≥256	64–≥256
Clindamycin	64	64	16–64
Nalidixic acid (older class of quinolones)	0.25	0.5	0.12–16
Others			
Polymyxin B	128	128	8–≥256
Colistin	≥256	≥256	≥256
SMX[a]	≥256	≥256	8–≥256
TMP[b]	≥256	≥256	0.5–≥256
SMX-TMP (20:1)	≥256	≥256	1–≥256
SMX-TMP (5:1)	≥256	≥256	0.5–≥256
Furazolidone	4	8	0.12–8
O/129	≥256	≥256	4–≥256

[a] SMX, sulfamethoxazole.
[b] TMP, trimethoprim.

strains were all highly resistant to streptomycin (MIC, ≥64 μg/ml), sulfamethoxazole (MIC, ≥256 μg/ml), trimethoprim (MIC, ≥128 μg/ml), and O/129 (MIC, ≥256 μg/ml).

The MICs at which 50% of the isolates were inhibited (MIC_{50}s) and MIC_{90}s of the antimicrobial agents for O1 clinical isolates were very similar to the MIC_{50}s and MIC_{90}s for O139 (Table 1), except those of tetracycline, chloramphenicol, furazolidone, streptomycin, sulfamethoxazole, trimethoprim, and O/129. One strain (0.5%) belonging to the classical biotype was resistant to ampicillin (MIC, 64 μg/ml), tetracycline (MIC, 8 μg/ml), chloramphenicol (MIC, 32 μg/ml), kanamycin (MIC, ≥256 μg/ml), streptomycin (MIC, ≥256 μg/ml), sulfamethoxazole (MIC, ≥256 μg/ml), trimethoprim (MIC, ≥256 μg/ml), and O/129 (MIC, ≥256 μg/ml).

Subtypes of O139 and O1 strains showing different susceptibility patterns (with respect to tetracycline, chloramphenicol, furazolidone, streptomycin, sulfamethoxazole, trimethoprim, and O/129) are summarized in Table 2. The major susceptibility patterns of O139 strains (94.8%) and O1 Indian El Tor strains (97%) and major susceptibility pattern A of O1 Bangladeshi El Tor strains (50%) were indistinguishable from each other. Those resistance phenotypes (MICs [in micrograms per milliliter] of 4 to 8 [chloramphenicol], 2 to 8 [furazolidone], 64 to ≥256 [streptomycin], 128 to ≥256 [sulfamethoxazole], 128 to ≥256 [trimethoprim], and ≥256 [O/129]) were designated VC MAR1 (for *V. cholerae* multiple-antimicrobial-agent resistance). Major susceptibility pattern B of O1 Bangladeshi El Tor strains (43.3%) added tetracycline resistance (MIC, 8 μg/ml) to the VC MAR1 phenotype; this resistance phenotype was designated VC MAR2.

The major susceptibility pattern of O1 Peruvian El Tor strains (98.1%) was distinctly different from the major susceptibility pattern of O1 Indian El Tor strains (97%) (VC MAR1) but was quite similar to minor pattern B of O1 Indian El Tor strains (1.5%). The major susceptibility pattern of O1 Peruvian El Tor strains (98.1%) also resembled the major susceptibility pattern of O1 classical strains (94.4%).

Plasmids of *V. cholerae* strains were isolated and electrophoresed in 0.3 or 0.7% agarose with reference plasmid DNAs of known molecular size (including the 94.5-kb NR1 plasmid [30]) as previously described (15, 34). Six strains of *V. cholerae* O139 (I-2, I-7, I-14, I-15, I-64, and I-72) had plasmids with a molecular size of ca. 200 kb. These 200-kb plasmids encoded resistance to ampicillin, tetracycline, chloramphenicol, kanamycin, gentamicin, sulfamethoxazole, trimethoprim, and O/129 (34a). In contrast, two O139 strains and two O1 Indian El Tor strains with the VC MAR1 phenotype (susceptible to tetracycline, ampicillin, and gentamicin) and six O1 Bangladeshi El Tor strains with the VC MAR2 phenotype tested had no detectable plasmids. Thus far, no correlation has been found between plasmids and the VC MAR phenotypes.

The incidence of tetracycline-resistant O1 El Tor strains in Bangladesh was 1.9% (number of strains tested, 317) in 1990, 7.6% (number of strains tested, 1,377) in 1991, 61.1% (number of strains tested, 1,221) in 1992, and 85.4% (number of strains tested, 669) in 1993; the data were obtained with antibiotic discs. In contrast, in India, tetracycline resistance is not frequently found in O1 El Tor strains (data not shown). Thus, there are apparent differences in tetracycline susceptibility which are related to the geographic origin within the Asian subcontinent. The tetracycline-resistant strains found in this study were all susceptible to minocycline (MIC, 0.06 to 0.25 μg/ml).

Cefoperazone resistance was observed for some strains of *V. cholerae* O139 (2.3%) (MIC, 8 to 16 μg/ml [Table 1]) and *V. cholerae* O1 (1.4%) (MIC, 8 to 128 μg/ml). *V. cholerae* O139 and O1 also tended to be resistant to nalidixic acid, with resistance rates of 4% (MIC, 4 to 16 μg/ml) for O139 (Table 1) and 1.4% (MIC, 4 to 8 μg/ml) for O1. Such resistant strains may have been selected by use of the related antimicrobial agents.

Azithromycin was eightfold more active against both O139 and O1 strains than was erythromycin. Since erythromycin has

TABLE 2. Subtypes of *V. cholerae* O139 and O1 showing different MIC patterns

V. cholerae strain	Susceptibility pattern (%)	MIC (μg/ml)[a] of:						
		TC	CM	FZ	SM	SMX	TMP	O/129
O139[b]	Major (94.8)	0.25–0.5	**4–8**	**2–8**	**64–≥256**	**≥256**	**128–≥256**	**≥256**
	Minor A (1.2)	0.25	**8**	0.12–0.25	**128–≥256**	**≥256**	**≥256**	**≥256**
	Minor B (0.6)	0.25	1	**4**	8	8	0.5	4
El Tor, Bangladesh	Major A (50)	0.25–0.5	**8**	**8**	**128–≥256**	**≥256**	**≥256**	**≥256**
	Major B (43.3)	**8**	**8**	**8**	**128–≥256**	**≥256**	**≥256**	**≥256**
	Minor A (3.3)	0.25	1	0.25	16	**≥256**	0.5	4
	Minor B (3.3)	0.25	1	**8**	**128**	**≥256**	0.5	4
El Tor, India	Major (97)	0.25	**4–8**	**4–8**	**128–≥256**	**≥256**	**≥256**	**≥256**
	Minor A (1.5)	0.25	**8**	**8**	8	**≥256**	**≥256**	**≥256**
	Minor B (1.5)	0.25	1	0.25	16	8	0.5	4
El Tor, Peru	Major (98.1)	0.25	1	0.12–0.5	8–16	4–8	0.25–0.5	2–4
	Minor A (1.9)	0.25	1	0.25	8	**≥256**	0.25	2
Classical[c]	Major (94.4)	0.12–0.5	0.5–1	0.06–0.25	4–32	0.25–16	0.12–0.5	0.5–4
	Minor A (1.4)	0.25	1	**4**	16	8	0.5	4
	Minor B (1.4)	0.25	1	0.25	**≥256**	1	0.25	2
	Minor C (1.4)	0.25	0.5	0.12	8	**128**	0.5	2

[a] Abbreviations for antimicrobial agents: TC, tetracycline; CM, chloramphenicol; FZ, furazolidone, SM, streptomycin; SMX, sulfamethoxazole; TMP, trimethoprim. Higher MICs with each antimicrobial agent are shown in boldface type; higher versus lower MIC ranges are 8 versus 0.12 to 0.5 (tetracycline), 4 to 8 versus 0.5 to 1 (chloramphenicol), 2 to 8 versus 0.06 to 0.5 (furazolidone), 64 to ≥256 versus 4 to 32 (streptomycin), 128 to ≥256 versus 0.25 to 16 (sulfamethoxazole), 128 to ≥256 versus 0.12 to 0.5 (trimethoprim), and ≥256 versus 0.5 to 4 (O/129)..

[b] Six multiple-drug-resistant strains (strains I-2, I-7, I-14, I-15, I-64, and I-72 [3.5%]; described in the text) were omitted. The MICs (in micrograms per milliliter) of chloramphenicol, furazolidone, streptomycin, sulfamethoxazole, trimethoprim, and O/129 for the six strains were 32, 4 to 8, ≥256, ≥256, ≥256, and ≥256, respectively.

[c] One multiple-drug-resistant strain (1.4%; described in the text) was omitted. The MICs (in micrograms per milliliter) of chloramphenicol, furazolidone, streptomycin, sulfamethoxazole, trimethoprim, and O/129 for this strain were 32, 0.25, ≥256, ≥256, ≥256, and ≥256, respectively; the MIC of furazolidone was similar to the MIC of furazolidone for the major type (or minor types B and C).

a good safety record (29) and azithromycin has also been shown to be safe in children (11), azithromycin would be a potential, attractive chemotherapeutic agent of choice in treatment of *V. cholerae* O139 and O1 infections. Newer quinolones were extremely active against both O139 and O1 strains. However, newer quinolones have not usually been recommended in the treatment of cholera in the pediatric age group.

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