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# Emergence of Multiply Antibiotic-Resistant *Vibrio cholerae* in Bangladesh

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In December 1979, a *Vibrio cholerae* O1 resistant to tetracycline, ampicillin, kanamycin, streptomycin, and trimethoprim-sulfamethoxazole was obtained from a patient with cholera at the Matlab Hospital, Bangladesh. All 256 isolates of *V. cholerae* O1 stocked in the previous six months were tested for antibiotic sensitivity: 54 were resistant to tetracycline, and 44 of these were resistant to all five antibiotics. The clinical presentation and hospital course for 51 patients with resistant strains of *V. cholerae* O1 and 102 patients with sensitive strains were compared by their medical records. Patients with resistant strains were indistinguishable from controls by age, sex, or severity of symptoms at presentation. All were treated with tetracycline, and patients with the resistant strains purged longer (mean, 37 vs. 25 hr; $P < 0.01$) and in greater volume (mean, 4.3 vs. 2.3 liters; $P < 0.01$) than their controls with cholera due to susceptible strains. A resistance plasmid was identified. Based on these results, antibiotic use in areas with resistant vibrios must be reconsidered.

For more than 15 years, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has maintained a field station in Matlab, which treats patients from a defined surveillance area who have cholera and other diarrheal diseases. Fluid replacement is the most important component of therapy, but tetracycline is given routinely to patients with cholera because it decreases the duration and volume of purging, the duration of excretion of vibrios, and the amount of fluid replacement required [1]. Each year, selected isolates of *Vibrio cholerae* O1 have been tested for tetracycline sensitivity, and no resistant strains were found from the hundreds of isolates tested. In December 1979, strains of *V. cholerae* O1 resistant to tetracycline as well as to ampicillin, kanamycin, streptomycin, and trimethoprim-sulfamethoxazole were recovered from patients with cholera at the Matlab field hospital. These patients appeared to have a prolonged hospital course and continued to purge after receiving tetracycline for more than two days. Because this outbreak occurred in an area already under epidemiologic surveillance, we were able to reconstruct the temporal and geographic emergence of the resistant isolates and the clinical characteristics of the patients with resistant strains of *V. cholerae* O1.

The area under diarrheal disease surveillance includes 170,000 people who are served by a single field hospital in Matlab, 40 km southwest of Dacca, Bangladesh. Most mild cases of diarrhea in the area are treated with oral therapy at home, but moderate and severe cases are generally brought to the hospital for treatment. On the average, 15 patients from the surveillance area are admitted daily by trained paramedics or doctors, who examine the patients, obtain stool specimens for culture, and begin treatment with iv or oral electrolyte replacement fluid. The stool volumes, fluid inputs and outputs, vital signs, and clinical conditions of each patient are recorded at least every 8 hr. Tetracycline is given to patients with severe diarrhea or dehydration who appear clinically to have cholera or who are positive for vibrios on culture.

In the study area, cholera is endemic, with an annual incidence for hospitalized cases of three per 1,000 persons; it accounts for 12%–14% of hospitalized patients. Each month, about 30 representative isolates of *V. cholerae* O1 are stocked on trypticase slants in the field hospital laboratory and kept for reference.

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## Materials and Methods

*Microbiology.* Strains of multiply antibiotic-resistant *V. cholerae* (MARV) were first recognized in December 1979 [2]. All isolates of *V. cholerae* O1 stocked in the Matlab laboratory since June 1979 and all subsequent isolates of *V. cholerae* O1 were tested for antibiotic sensitivity using the method of Bauer et al. [3] with filter paper disks containing tetracycline (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), and trimethoprim-sulfamethoxazole (trimethoprim, 1.25 μg; sulfamethoxazole, 23.75 μg). A MARV was defined as an isolate resistant to five antibiotics—tetracycline, ampicillin, kanamycin, streptomycin, and trimethoprim-sulfamethoxazole, and a MARV variant was defined as an isolate resistant to tetracycline but sensitive to one or more of the other five antibiotics. All isolates were sensitive to chloramphenicol and gentamicin.

The MIC of tetracycline and ampicillin was determined for 29 isolates. Serial twofold dilutions of each antibiotic solution were made in Mueller-Hinton broth to give final concentrations of 160–0.16 μg/ml. An inoculum containing 0.05 ml of the test strain after incubation for 4–5 hr was added to each tube except the final dilution (0.16 μg/ml). This final tube and another tube with only broth and the test strain served as controls for sterility and growth, respectively. The antibiotic concentration of the last tube showing no growth of test organisms after overnight incubation was recorded as the MIC.

Standard genetic techniques were used to test the ability of six MARV isolates to transfer their plasmid-borne antibiotic resistances to a receptor strain of *Escherichia coli* K12 (K12F⁻lac⁺Nx^r, Colindale strain) [4]. The donor and the recipient strains were mixed in a 28-ml screw-capped bottle and incubated for 18 hr at 37 C without shaking. Serial dilutions of the mating mixture were plated onto McConkey's agar (Difco, Detroit, Mich.) containing 30 μg of nalidixic acid/ml plus one of the following antibiotics in appropriate concentrations: tetracycline, ampicillin, kanamycin, streptomycin, or trimethoprim-sulfamethoxazole. Ten colonies for each plate were then tested for resistance to the other four antibiotics to see whether the resistances were transferable separately or always together.

*Record review.* Hospital records of all patients with MARV (or MARV variants) seen between August 1979 and January 1980 were retrieved and reviewed. For each patient, the next two patients with *V. cholerae* O1 sensitive to all of the antibiotics tested were chosen as controls from the bacteriology logbook. Information on the patient's age, sex, village, presenting complaints, physical findings, and hospital course was compared for the patients with resistant strains and controls with susceptible strains using a $\chi^2$ test.

**Table 1.** Emergence of multiply antibiotic-resistant *Vibrio cholerae* O1 (MARV) in Matlab, Bangladesh, June 1979–February 1980.

| Month | No. of strains of *V. cholerae* O1 | | Serotyping and antibiotic resistance of stocked isolates | | | | Percentage MARV |
|---|---|---|---|---|---|---|---|
| | | | Ogawa | | Inaba | | |
| | Isolated | Stocked | Total | MARV | Total | MARV | |
| June | 20 | 20 | 2 | 0 | 18 | 0 | 0 |
| July | 19 | 19 | 4 | 0 | 15 | 0 | 0 |
| August | 20 | 20 | 4 | 0 | 16 | 1 | 5 |
| September | 71 | 38 | 2 | 0 | 36 | 5 | 13 |
| October | 79 | 32 | 2 | 2 | 30 | 7 | 28 |
| November | 91 | 33 | 9 | 7 | 24 | 5 | 36 |
| December* | 100 | 33 | 5 | 0 | 28 | 5 | 15 |
| January | 67 | 67 | 8 | 1 | 59 | 21 | 32 |
| February | 43 | 43 | 0 | 0 | 43 | 0 | 0 |
| March | 25 | 25 | 2 | 0 | 23 | 0 | 0 |
| April | 9 | 9 | 0 | 0 | 9 | 0 | 0 |
| May | 41 | 41 | 10 | 0 | 31 | 0 | 0 |
| June | 24 | 24 | 12 | 0 | 12 | 0 | 0 |

* Outbreak identified.

Cases were plotted on a map of the area to identify any geographic clustering.

## Results

*Microbiology.* Between June 1979 and February 1980, the laboratory stocked 262 of the 510 isolates of *V. cholerae* O1 (table 1). Fifty-four (21%) of the 262 isolates were MARVs (44) or MARV variants (10). The first MARV identified was serotype Inaba, isolated on August 26, 1979; subsequently, MARVs of both serotypes Ogawa and Inaba were found. All 29 MARV isolates tested for MICs grew in ampicillin at a concentration of >160 μg/ml and in tetracycline at >80 μg/ml, levels demonstrating significant resistance.

Five of the six MARV strains used as donors successively transferred their resistance to all five antibiotics to the recipient strain of *E. coli* K12 by simple conjugation. Resistance to these antibiotics was therefore presumed to be encoded on a transmissible plasmid called RVC1. Each of the K12-RVC1 isolates was resistant to all five antibiotics tested regardless of the original antibiotic selection medium used. The sixth strain transferred resistance to only tetracycline and ampicillin to the K12 recipient. The MICs of tetracycline and ampicillin for the K12-RVC1 isolates were identical to those of the *V. cholerae* O1 RVC1 strains.

Ten isolates were sent to Colindale, England, where these findings were confirmed and the plasmid was characterized [5]. Three distinct R types were identified from these 10 isolates. In each strain, the resistances were encoded by a single C plasmid.

*Chart review.* The records of 51 of 54 patients from whom a MARV or MARV variant was isolated between August 1979 and February 1980 and 102 charts of controls infected with susceptible *V. cholerae* O1 were reviewed. There was no clustering of early cases by geographic location, and the first five cases occurred in individuals who lived more than 15 miles apart and who had no history of recent travel in the area. The age and sex distribution and clinical presentation (the percentage with fever, dehydration, vomiting, and diarrhea with blood and mucus) were not significantly different between patients with MARVs and those with sensitive strains. Although all patients received tetracycline therapy, patients with resistant strains had more severe purging (mean, 4.3 vs. 2.3 liters; $P < 0.01$), and even in the first 24

**Table 2.** Hospital course of patients with multiply antibiotic-resistant strains of *Vibrio cholerae* compared with patients with sensitive strains in Matlab, Bangladesh, August 1979–January 1980.

| Hospital course | Antibiotic pattern: No. resistant (%) ($n$ = 51) | Antibiotic pattern: No. sensitive (%) ($n$ = 102) | $P$* |
|---|---|---|---|
| Stool volume [l] in first 24 hr | | | |
| <1 | 11 (21) | 53 (52) | |
| 1–2 | 20 (40) | 29 (28) | |
| 3–4 | 9 (18) | 10 (10) | <0.01 |
| ≥5 | 11 (21) | 10 (10) | |
| Total stool volume [l] | | | |
| ≤1 | 11 (22) | 57 (56) | |
| 2–4 | 17 (33) | 26 (25) | |
| ≥5 | 23 (45) | 19 (19) | <0.01 |
| Duration of diarrhea in 8-hr periods | | | |
| ≤1 | 11 (22) | 39 (38) | |
| 2–4 | 12 (23) | 34 (33) | |
| ≥5 | 28 (55) | 29 (29) | <0.01 |
| No. of tetracycline doses before diarrhea stopped | | | |
| 0–3 | 9 (18) | 43 (42) | |
| 4–6 | 14 (27) | 28 (28) | |
| ≥7 | 28 (55) | 31 (30) | <0.01 |

* For comparison of values from patients with resistant vs. sensitive strains at all measurements.

hr, there was a significant decrease in stool volume among patients with a tetracycline-sensitive isolate (table 2).

## Discussion

Although individual isolates of MARV serogroup O1 have been identified before [6], this outbreak is only the second that has been reported and traced to a plasmid [7, 8]. All previous R-plasmids in *V. cholerae* have demonstrated resistance to chloramphenicol, which differentiates them from the R-plasmid described in this epidemic. The first outbreak in Tanzania was widespread and long-lasting, characteristics that have been attributed to extensive use of tetracycline for prophylaxis. In Bangladesh, prophylaxis is not common, and the antibiotic-resistant strain disappeared after five months. This disappearance is consistent with laboratory observations of Yokota et al. [9], who found R-plasmids in strains of *V. cholerae* O1 to be unstable and easily eliminated in drug-free conditions. Other outbreaks of MARV serogroup O1 should be expected, and laboratories identifying individual isolates should look further and more intensively for their antibiotic resistance.

The description of the outbreak reported from Tanzania [7] included only laboratory findings. The outbreak reported here occurred in our surveillance area, so the clinical presentation and hospital course of patients with resistant and susceptible strains of *V. cholerae* O1 could be compared. These patients were indistinguishable at presentation, but patients with a resistant strain who were treated with tetracycline purged longer and in greater volume than treated patients infected with a sensitive strain.

Epidemiologic assessment of this outbreak is incomplete. We could not adequately assess whether this was a point-source outbreak because patients infected with *V. cholerae* O1 but without symptoms are not identified through hospital surveillance. Furthermore, because the first cases occurred four months before the resistant isolates were found, it was difficult to identify any common exposure in the past. Either a geographic clustering of cases or the occurrence of a single serotype would have suggested a point source for this outbreak, but neither was observed. The lack of an identifiable point source raised the possibility that a more complicated mechanism involving plasmid transfer in the environment might be occurring.

In December, once surveillance for antibiotic-resistant isolates became routine, two MARV cases were identified at the Dacca Hospital of the ICDDR,B. Although one was traced back to the Matlab surveillance area, a second case had just come from an area 70 km in the opposite direction. If MARVs should reappear, a full investigation could further our understanding of the transmission of *V. cholerae* O1 between families and the environment and within a country where cholera is endemic.

Tetracycline has long been the antibiotic of choice for treatment and prophylaxis of cholera, and consideration of alternative drugs has been confined to the treatment of pregnant women and children in whom tetracycline was contraindicated. In areas where MARVs have emerged, an alternative treatment regimen must now be considered, and the prophylactic use of tetracycline should be discontinued.

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