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# Epidemic cholera in Ecuador: multidrug-resistance and transmission by water and seafood

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SUMMARY

To determine risk factors for cholera in an epidemic-disease area in South America, a case–control investigation was performed in Guayaquil, Ecuador, in July 1991. Residents > 5 years old who were hospitalized for treatment of acute, watery diarrhoea and two matched controls for each were interviewed regarding sources of water and food, and eating, drinking, and hygienic habits. Interviewers inspected homes of case-patients and controls to document water treatment, food-handling, and hygienic practices. Faecal specimens and shellfish were cultured for *Vibrio cholerae* O 1. Isolates were tested for susceptibility to a variety of antimicrobial agents. Drinking unboiled water (odds ratio [OR] = 4·0, confidence interval [CI] = 1·8–7·5), drinking a beverage from a street vendor (OR = 2·8, CI = 1·3–5·9), eating raw seafood (OR = 3·4, CI = 1·4–11·5), and eating cooked crab (OR = 5·1, CI = 1·4–19·2) were associated with illness. Always boiling drinking water at home (OR = 0·5, CI = 0·2–0·9) was protective against illness. The presence of soap in either the kitchen (OR = 0·3, CI = 0·2–0·8) or bathroom (OR = 0·4, CI = 0·2–0·9) at home was also protective. *V. cholerae* O 1 was recovered from a pooled sample of a bivalve mollusc and from 68% of stool samples from case-patients. Thirty-six percent of the isolates from stool specimens were resistant to multiple antimicrobial agents. Specific prevention measures may prevent transmission through these vehicles in the future. The appearance of antimicrobial resistance suggests the need for changes in current methods of prevention and treatment.


Fig. 1. Map of Ecuador with location of Guayaquil and Quito, the capital, indicated.

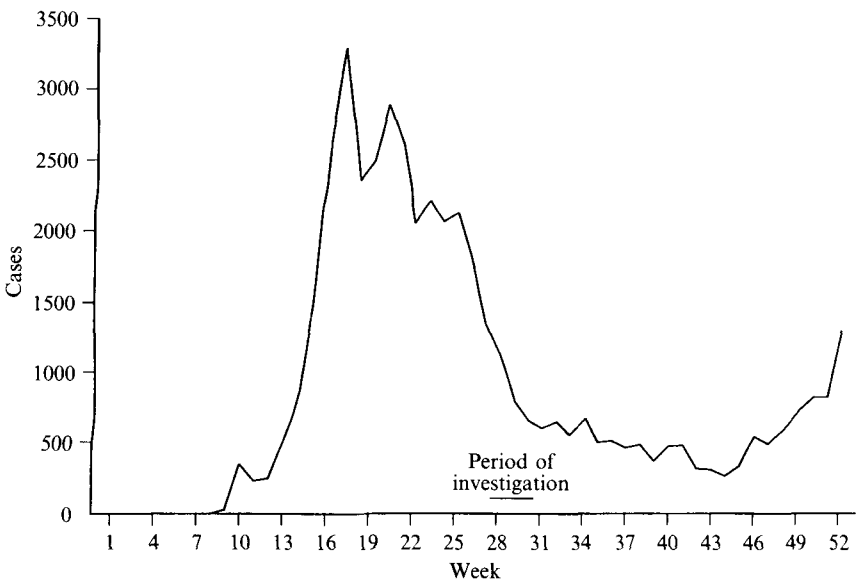


Fig. 2. Cases of cholera in Ecuador, 1991, by week of onset. —, Period of investigation.

## INTRODUCTION

Epidemic cholera caused by toxigenic *Vibrio cholerae* O 1, biotype El Tor, serotype Inaba, appeared in Peru in late January 1991. Cholera was first reported in Ecuador on 28 February 1991. Cases were detected in Guayaquil, the largest city in Ecuador, on 3 March (Fig. 1). Guayaquil is located in the province of Guayas at the mouth of the Guayas river, surrounded by an estuary. A large

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indigent population in Guayaquil has limited access to treated drinking water and sewage treatment facilities. Following the appearance of cholera in Guayaquil, the population was advised to boil their drinking water and to seek treatment for severe diarrhoea.

During 1991, 46320 cases of diarrhoeal illness and 697 deaths were attributed to cholera in Ecuador (Fig. 2). The province of Guayas reported 32% of the national case total (R. Sempértegui, unpublished data). Most of these cases were from Guayaquil and adjacent areas. Within the city, over 50% of reported cases occurred among residents of Suburbio, a neighbourhood of approximately 600000 people (R. Cañizares, unpublished data).

## METHODS

### *Patients and controls*

To determine risk factors for and protective behaviours against illness, a case–control investigation was conducted in Guayaquil.

Patients were selected between 8 and 24 July 1991, from Suburbio residents > 5 years old admitted for acute, watery diarrhoea to three major hospitals in Guayaquil: Hospital Luis Vernaza, Hospital Guayaquil, and Hospital de Infectología. Patients were eligible for inclusion if they had spent the week before the onset of illness in Suburbio and reported having five or more diarrhoeal stools in a 24-h period, without haematochezia or subjective fever. They were asked about symptoms, sources of water and food, and eating, drinking, and hygienic habits during the 3 days before illness onset.

Within 72 h after the patient interview, two age- and sex-matched neighbourhood controls who had had no diarrhoea since 31 March were identified by starting at the home of the patient and going door-to-door systematically. Controls were asked about the 3-day period before the matched patient's illness onset.

### *Home inspections*

The interviewers inspected the subjects' homes to document water and food handling practices and other household characteristics.

### *Microbiology*

*Culture.* Two rectal swabs from each patient were transported in Cary–Blair medium and plated on thiosulphate–citrate–bile salts–sucrose agar (TCBS). Colonies typical of *V. cholerae* on TCBS were subcultured and tested for agglutination with *V. cholerae* O 1 polyvalent and monovalent antisera. Duplicate specimens were cultured at the National Institute of Hygiene, Guayaquil and the Enteric Diseases Branch, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. All suspected *V. cholerae* O 1 isolates from the National Institute of Hygiene were transported to CDC. Isolates were serologically confirmed and tested for cholera toxin production by enzyme-linked immunosorbent assay [1] and representative isolates were biochemically identified and biotyped.

Samples of fresh shellfish (shrimp, concha [a bivalve mollusc], and oysters) purchased from a Guayaquil market on 1 August were transported on ice to CDC.

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Specimens were placed in alkaline peptone broth for 6 h at 37 °C for enrichment before plating on TCBS agar. Colonies typical of *V. cholerae* were subcultured and tested for agglutination with *V. cholerae* O 1 antisera.

*Antimicrobial susceptibility.* Selected *V. cholerae* O 1 isolates were tested for susceptibility to 12 antimicrobial agents as described by the National Committee for Clinical Laboratory Standards [2, 3]. Isolates were tested using ampicillin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, furazolidone, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole.

*Multilocus enzyme electrophoresis.* Multilocus enzyme electrophoresis (MEE) was used to characterize selected outbreak-related Ecuador *V. cholerae* O 1 isolates from patients and from shellfish [4].

*Plasmid profile.* Plasmid profiles were determined for 4 antimicrobial-resistant and 2 susceptible isolates of *V. cholerae* O 1 from Ecuador and 1 susceptible isolate from Peru. The method used was modified from that described by Kado and Liu [5]. Samples were lysed in a solution of 50 mM Tris and 3% sodium dodecyl sulphate, pH 12·55 at 55 °C for 1 h and then extracted with acid phenol (pH was not measured and solution was not buffered) and chloroform. Samples were run on vertical 1% agarose gels at 70 V for 2 h.

### *Vibriocidal antibody testing*

A 10-ml blood sample from each subject was tested at CDC for vibriocidal antibody titres by the microtitre technique [6]. Controls with a titre $> 40$ were excluded because of possible recent, asymptomatic infection.

### *Water chlorine measurement*

Municipal water samples from four sources were tested for free chlorine using a colorimetric chlorine test kit (Hach; Loveland, Colorado, USA).

### *Statistical methods*

Logistic regression analysis was performed to determine which risk factors were associated with illness. The multivariate analysis included risk factors that were statistically associated with illness, potential confounders, or of theoretical interest and excluded some statistically significant variables with too few persons exposed to be evaluated in a multivariate model. All reported $P$ values are 2-tailed.

## RESULTS

### *Patients*

We interviewed 63 patients and 126 controls; median age of all subjects was 33 years (range 10–84), and 48% were women. The maximum number of diarrhoeal stools in 24 h was 5–10 for 33% of patients, 11–15 for 18%, 16–20 for 9%, and $> 20$ for 40%; 78% had vomiting and 60% had leg cramps. Patients entered the hospital a median of 11 h (range 2–146) after the onset of symptoms. The mean time between onset of patients' symptoms and interviews, obtaining of rectal swabs, and phlebotomy was 1·6 days (range 0–7).

*Case–control investigation*

*Univariate analysis.* Results are shown on Table 1.

*Water and other beverages.* Patients and controls had similar sources of household drinking water, with most having jury-rigged connections to the municipal supply (33% of patients and 41% of controls), delivery by tank trucks (22 and 18%), or running water directly into the home (16 and 19%).

Drinking any unboiled water was strongly associated with illness (OR = 3·6, CI = 1·8–7·5), while always boiling drinking water at home was protective (OR = 0·5, CI = 0·2–0·9). Other home water treatment, such as use of chlorine bleach, was rare.

Although drinking specific juices, ices, and other beverages were not associated with illness, patients were more likely than controls to have purchased a beverage from a street vendor during the 3 days before onset of illness (OR = 2·8, CI = 1·3–5·9). Orange juice was the most popular beverage and was the only beverage independently associated with illness (OR = 4·3, CI = 1·3–14·0).

*Seafood.* Eating raw fish was associated with illness (OR = 10·0, CI = 1·2–85·6); it is usually eaten in the form of ceviche, briefly marinated in lemon or lime juice. Eating raw shellfish (oysters, shrimp, mussels, or concha) was also associated with illness (OR = 3·2, CI = 0·9–11·0) but this association was not statistically significant. Shellfish are also eaten as ceviche. Concha was the most popular raw shellfish, eaten by five patients and four controls (OR = 2·5, CI = 0·7–9·3). Eating any raw seafood, either raw fish or raw shellfish, was significantly associated with illness (OR = 4·0, CI = 1·4–11·5). Eating cooked crab was associated with illness (OR = 5·1, CI = 1·4–19·2). No one ate raw crab.

*Other foods.* Raw fruits, raw and cooked vegetables, reheated rice, and reheated noodles and specific sources of food, such as street vendors and restaurants, were not associated with illness.

*Household characteristics.* Home visits revealed that water was stored in plastic or metal containers holding approximately 55 gallons (84% of case homes and 75% of control homes), buckets (67 and 45%), wide-mouth pots (41 and 26%), and/or tubs (29 and 21%). No storage container type was associated with illness. Having soap in the kitchen (OR = 0·3, CI = 0·2–0·8) or bathroom (OR = 0·4, CI = 0·2–0·9) at the time of the visit was protective, as was having a container filled with boiled water (OR = 0·4, CI = 0·2–0·9).

Homes of patients and controls had similar numbers of occupants, numbers of bedrooms, occupant density (number of persons per bedroom), and cooking fuel availability.

*Analysis excluding controls with elevated vibriocidal titres.* Fifty-three (42%) of 126 controls had a vibriocidal antibody titre > 40 (Geometric mean antibody titre = 389, range = 80–20480). The results of statistical analysis of risk factors was similar when repeated excluding controls with elevated vibriocidal antibody titres (Table 2).

*Multivariate analysis.* No significant interactions were found among the variables included in the analysis. Drinking unboiled water ($P = 0·02$), purchasing juice from a street vendor ($P = 0·02$), and eating raw seafood ($P = 0·06$) were independently associated with illness when simultaneously placed in the model.

Table 1. *Univariate analysis of risk factors for cholera among patients and all controls, Guayaquil, Ecuador, 1991*

| Risk factor | Patients* | Controls* | Odds ratio | Confidence interval | *P* |
|---|---|---|---|---|---|
| Unboiled water | 44/62 | 54/124 | 3·6 | 1·8–7·5 | 0·004 |
| Boiled water present in home | 39/62 | 100/124 | 0·4 | 0·2–0·9 | 0·015 |
| Always boiling drinking water at home | 38/62 | 92/120 | 0·5 | 0·2–0·9 | 0·031 |
| Always boiling water for refreshments at home | 17/31 | 50/67 | 0·4 | 0·1–1·4 | 0·16 |
| Juice | 57/63 | 119/126 | 0·6 | 0·2–1·8 | 0·06 |
| Street vendor beverage | 23/63 | 23/125 | 2·8 | 1·3–5·9 | 0·0078 |
| Orange juice | 41/62 | 90/126 | 0·8 | 0·4–1·5 | 0·44 |
| Street vendor orange juice | 10/62 | 6/116 | 4·3 | 1·3–14·0 | 0·015 |
| Raw fish | 5/63 | 1/126 | 10·0 | 1·2–85·6 | 0·036 |
| Concha or mussels | 6/63 | 8/126 | 1·6 | 0·5–4·7 | 0·44 |
| Raw concha | 5/63 | 4/126 | 2·5 | 0·7–9·3 | 0·17 |
| Crab† | 9/63 | 5/126 | 5·1 | 1·4–19·2 | 0·016 |
| Shellfish‡ | 23/63 | 33/126 | 1·6 | 0·8–3·0 | 0·16 |
| Raw shellfish | 7/63 | 5/126 | 3·2 | 0·9–11·0 | 0·069 |
| Seafood§ | 43/63 | 82/126 | 1·2 | 0·6–2·4 | 0·64 |
| Raw seafood | 11/62 | 6/124 | 4·0 | 1·4–11·5 | 0·011 |
| Soap in bathroom | 13/58 | 51/123 | 0·4 | 0·2–0·9 | 0·009 |
| Soap in kitchen | 44/61 | 111/124 | 0·3 | 0·2–0·8 | 0·008 |

* Number exposed/total for whom information was noted.
† Crab was never eaten raw.
‡ Includes shrimp, oyster, mussel and concha.
§ Includes shellfish and fish.

### *Microbiology*

*Culture.* Toxigenic *V. cholerae* O 1, biotype El Tor, serotype Inaba was recovered from 42 (68%) of 62 stool samples and from a pooled sample of concha.

*Antimicrobial susceptibility.* Antimicrobial susceptibility profiles were determined for 40 isolates: 33 from samples collected from patients in the investigation, 6 collected from patients from Guayaquil from earlier in the epidemic, and the single isolate from concha. Two minimum inhibitory concentration (MIC) phenotypes were apparent (Table 3). One was generally susceptible to the antimicrobial agents tested and the other was multiply resistant. Resistance was noted to chloramphenicol, doxycycline, kanamycin, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Fifteen of 33 isolates from the investigation and 1 of 6 isolates from earlier in the epidemic were of the resistant phenotype. The concha isolate was of the susceptible phenotype. The remaining 9 isolates from the investigation and 5 other isolates from Guayaquil from earlier in the epidemic were also susceptible when tested by the disk diffusion method. Thus, 15 (36%) of 42 isolates from the investigation and 1 (9%) of 11 isolates from earlier in the epidemic were multiply resistant.

The 25 patients who recalled taking an antimicrobial agent before entry into the hospital were not less likely to have *V. cholerae* O 1 recovered or more likely to have a resistant strain (data not shown).

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Table 2. *Univariate analysis of risk factors for cholera among patients and controls (excluding controls with vibriocidal antibody titre > 40), Guayaquil, Ecuador, 1991*

| Risk factor | Patients* | Controls* | Odds ratio | Confidence interval | *P* |
|---|---|---|---|---|---|
| Unboiled water | 34/51 | 42/102 | 3·3 | 1·4–7·8 | 0·004 |
| Boiled water present in home | 34/51 | 84/102 | 0·7 | 0·3–1·4 | 0·26 |
| Always boiling drinking water at home | 32/51 | 77/98 | 0·4 | 0·2–0·9 | 0·04 |
| Always boiling water for refreshments at home | 13/25 | 41/54 | 0·3 | 0·1–1·3 | 0·09 |
| Juice | 46/52 | 98/104 | 0·5 | 0·2–1·9 | 0·17 |
| Street vendor beverage | 18/52 | 18/103 | 4·5 | 1·5–14·1 | 0·009 |
| Orange juice | 34/51 | 73/104 | 0·6 | 0·3–1·4 | 0·13 |
| Street vendor orange juice | 7/51 | 6/98 | 2·0 | 0·5–8·5 | 0·17 |
| Raw fish | 5/52 | 1/104 | 7·4 | 0·9–64·1 | 0·07 |
| Concha or mussels | 6/52 | 6/104 | 3·7 | 0·7–18·4 | 0·12 |
| Raw concha | 5/52 | 2/104 | 6·5 | 0·8–56·9 | 0·09 |
| Crab† | 8/52 | 5/104 | 4·7 | 1·0–23·1 | 0·06 |
| Shellfish‡ | 19/52 | 28/104 | 1·5 | 0·7–3·1 | 0·16 |
| Raw shellfish | 6/52 | 3/104 | 7·4 | 0·9–64·1 | 0·07 |
| Seafood§ | 36/52 | 71/104 | 0·9 | 0·4–2·0 | 0·7 |
| Raw seafood | 10/51 | 4/102 | 6·8 | 1·5–32·0 | 0·02 |
| Soap in bathroom | 10/47 | 45/101 | 0·4 | 0·2–0·9 | 0·03 |
| Soap in kitchen | 36/50 | 93/103 | 0·3 | 0·09–0·7 | 0·009 |

* Number exposed/total for whom information was noted.
† Crab was never eaten raw.
‡ Includes shrimp, oyster, mussel and concha.
§ Includes shellfish and fish.

Table 3. *Minimum inhibitory concentrations (MICs) of antimicrobial agents for selected isolates of* Vibrio cholerae *O 1 from Guayaquil, Ecuador, 1991*

| Antimicrobial agent | Susceptible phenotype MIC (μg/ml) range | Resistant phenotype MIC (μg/ml) range |
|---|---|---|
| Chloramphenicol | 1·0 | 32·0–> 32·0 |
| Doxycycline | < 0·25 | 4·0 |
| Kanamycin | 2·0–8·0 | > 64·0 |
| Streptomycin | 8·0–32·0 | 128·0–512·0 |
| Sulfisoxazole | < 4·0–128·0 | > 512·0 |
| Tetracycline | < 0·25 | 32·0 |
| Trimethoprim/sulfamethoxazole | < 0·06 | > 8·0 |
| Ampicillin | 2·0–4·0 | 2·0–8·0 |
| Ciprofloxacin | < 0·03 | < 0·03 |
| Erythromycin | 2·0–8·0 | 8·0–16·0 |
| Furazolidone | < 0·25–2·0 | 0·5–2·0 |
| Nalidixic acid | < 0·5 | < 0·5 |

*Multilocus enzyme electrophoresis.* MEE was performed on 3 outbreak-related isolates of the susceptible phenotype, 3 outbreak-related isolates of the resistant phenotype and the isolate from the concha. MEE indicated that both the susceptible and resistant outbreak-related isolates and the concha isolate were indistinguishable from each other and of the same electrophoretic type (ET4) as the strain associated with the epidemic in Latin America [4].

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*Plasmid profile.* A single 110 MDa plasmid was found in all of four multidrug-resistant Ecuador isolates tested. No plasmids were found in the two susceptible isolates from Ecuador and the one susceptible isolate from Peru.

### *Water chlorine levels*

Two sources for tank trucks that delivered water to Suburbio had 1·5 parts per million (p.p.m.) and 1·0 p.p.m. free chlorine. Tap water in the city centre had 0·1 p.p.m. of free chlorine. No chlorine was detected in water from a jury-rigged connection at the periphery of the water system.

## DISCUSSION

When the cholera epidemic struck Peru in 1991 and spread into Ecuador, the modes and vehicles of transmission were unknown. Public health officials had to decide on preventive measures based on knowledge of how cholera spread in previous epidemics and on anecdotal information. This investigation and parallel investigations in Peru provided the first information from controlled studies about the most important modes and vehicles of transmission for cholera in Latin America [7–9]. Identification of specific modes of cholera transmission allows control measures and education to be more focused and, presumably, more effective. Specific, simple interventions to prevent cholera transmission can be designed using these results.

This is the first investigation to implicate fish and shellfish as vehicles of transmission in South America. In addition, *V. cholerae* O 1 was isolated from concha during this investigation. The isolate was of the same electrophoretic type as isolates from patients. Although seafood eaten raw may have played an important role in the early part of the epidemic in Peru, it was almost immediately suspect, and the public was warned by the Minister of Health against consuming seafood raw. The public complied, and subsequent case–control studies were unable to evaluate the role of seafood because few patients or controls were eating raw seafood when the studies were conducted [7, 8].

In Ecuador, public health warnings gave more emphasis to other potential vehicles, and our study associated cholera with eating raw fish, raw seafood, and cooked crab. We presume the raw seafood was consumed in the form of ceviche, but we did not collect information on preparation methods. In experimentally contaminated ceviche, *V. cholerae* O 1 has been reported to be eliminated when the fish is marinated long enough in sufficiently acidic juice [10]. However, contamination of internal fish or shellfish parts that are not exposed to acid, exposed to less acid, or marinated for a shorter time may allow *V. cholerae* O 1 to survive and infect the consumer.

Raw fish have been implicated previously in outbreaks in Guam and Kiribati (formerly the Gilbert Islands) [11, 12], and contaminated crab, oysters, and shrimp harvested from the Gulf of Mexico have been the principal sources of cholera infections in the United States [13, 14]. During 1991, cholera outbreaks affecting 11 persons in New York and New Jersey were associated with crab brought from Ecuador in travellers' luggage [15, 16]. In laboratory experiments using *V. cholerae* O 1-contaminated live crabs, crabs boiled for less than 10 min or

steamed for less than 30 min still harboured viable *V. cholerae* O 1 [13]. In Ecuador and other areas where cholera is present, seafood, particularly crab, should be eaten well cooked and while still hot.

Drinking unboiled water appeared to be the single most important risk for illness in Guayaquil. There were multiple opportunities for contamination of drinking water. Water collected from jury-rigged connections may have been contaminated at the source. Although the water source for tank trucks had adequate chlorine, handling and storage of water in households could allow contamination. Most water containers we observed had wide openings and were uncovered or had easily opened covers that would allow chlorine to evaporate rapidly and water to be contaminated by hands and utensils. Such contamination could easily overwhelm the chlorine. Studies in Peru and Calcutta have suggested that boiling water before drinking and storing water in a narrow-mouthed, capped container decreases the risk of cholera transmission [7, 8, 17–19]. Our investigation did not document the type of container used to store boiled water, but having boiled water in the house at the time of inspection was protective. Boiling is sometimes impractical because of the high cost or unavailability of fuel, and in these situations other methods of home water disinfection, such as use of chlorine or iodine, are possible. However, maintaining a supply of the chemical and proper storage and measurement may be problems.

Consumption of juices, especially orange juice, purchased from street vendors was associated with illness. Orange juice is normally sufficiently acidic to kill vibrios, therefore this association may be a marker for another street vendor item. This risk was not found in Trujillo, Peru, because street vendors had been banned before the investigation began [8]. In Piura, Peru, however, consuming beverages from street vendors also was associated with cholera [7]. Street vendors and their customers can be taught methods to reduce contamination with *V. cholerae* O 1, such as making beverages from boiled or treated water, serving beverages hot, cooling drinks with ice placed outside rather than in the beverage container, using lemon juice to decrease the pH of beverages to 4·5 or less, and washing glasses and utensils with soap and boiled or treated water.

This is the first report of substantial antimicrobial resistance in *V. cholerae* O 1 from South America. Although the reasons why resistance emerged at this time are not clear, the resistance occurred in a setting that provided substantial environmental pressure. First, beginning in March 1991, the Subsecretariat for Health used antimicrobial prophylaxis as one means for controlling cholera. Within 72 h after diagnosis of a case, adult family members were contacted and treated with 500 mg of tetracycline every 4 h for 5 days. Pregnant women and children were treated with erythromycin or trimethoprim/sulfamethoxazole. Shortly before the investigation, the length of prophylaxis changed to 3 days. This practice stopped altogether just before the investigation. Second, antimicrobial agents are available without prescription in Ecuador, and people may have taken self-prescribed antimicrobial agents as prophylaxis for cholera or treatment for suspected cholera. Third, antimicrobial agents are used to control non-cholera *Vibrio* infections in hatching shrimp in Guayaquil which may have exerted additional environmental pressure leading to resistance in *V. cholerae* O 1.

The new appearance of resistance in the epidemic of cholera in Latin America

suggests that resistance may have been transferred to *V. cholerae* O 1 via a plasmid from other vibrios or from other bacteria. Further studies are needed to determine if resistance genes are carried on the plasmid we found in our isolates. Resistant *V. cholerae* O 1 has been noted previously in Asia and Africa [20, 21]. Resistance emerged in Tanzania in 1977 after massive use of tetracycline prophylaxis. Although the effect of prophylaxis on the epidemic in Guayaquil is hard to measure, the emergence of antimicrobial resistance suggests that this practice is now of limited utility and should be avoided.

The protective effect of soap in the home may indicate a specific protective effect, or it may be a marker for families with better hygienic practices. Regardless, without soap, hands and utensils cannot be washed effectively and may contaminate food and water in the home. Promoting the distribution and use of soap for washing hands and utensils may be a useful control strategy.

Construction and maintenance of central facilities for treatment and distribution of drinking water and proper disposal of human waste could replace many interim prevention measures. The Pan American Health Organization has proposed a $200 billion plan to provide these facilities for all of Latin America [22]. This late twentieth-century sanitation revolution would provide the best prevention against illness and death from cholera as well as from other diarrhoeal diseases.

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