Surveillance of Bacterial Pathogens Associated with Acute Diarrhea in Lima, Peru

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ABSTRACT

Objectives: A study was conducted in Lima, Peru, from January to April 1995, to determine the bacterial pathogens associated with acute diarrhea in adults, their susceptibility to common antimicrobials, the risk factors involved in cholera transmission, and the best clinical predictors of cholera.

Methods: A random sample of adult patients with acute diarrhea was studied. Epidemiologic and clinical data and risk factors to acquire diarrheal diseases were evaluated. Identification of bacteria and susceptibility to antimicrobials were determined.

Results: The study included 336 patients. *Vibrio cholerae* O1 (52.7%), *Shigella* spp. (4.8%), and *Salmonella* spp (2.7%) were the pathogens most commonly isolated. No resistance to antimicrobials was observed. Patients with cholera had less access to municipal water (P = 0.0018) and were less likely to have homes connected to a sewage system (P = 0.0003) or to have indoor toilet facilities (P = 0.0001) than those without cholera. Liquid stools (odds ratio [OR] = 16.51; confidence interval [CI] = 13.71-19.02; P = 0.003), severe dehydration (OR = 2.48; CI = 1.57-3.38; P = 0.0083), generalized cramps (OR = 4.63; CI = 3.10-6.17, P < 0.0001), and washerwoman's hands (OR = 2.45; CI = 1.55-3.34; P = 0.017) were the best clinical predictors of cholera in this setting.

Conclusions: Cholera is still prevalent in Lima, and people living in environments with low sanitary conditions are especially at risk. Clinical signs of severe dehydration and liquid stools were the best predictors of cholera.

Key Words: cholera, diarrheal diseases, *Vibrio cholerae*


Diarrheal diseases caused by various enteropathogens are important causes of morbidity and mortality in developing countries. Knowing the most prevalent enteropathogens that exist in endemic areas, their susceptibility pattern to common antimicrobials used, and their changes in epidemiologic and clinical characteristics helps guide the planning of control and preventive measures. The recent discovery of a previously unrecognized epidemic non-O1 *Vibrio cholerae*, referred to as the O139 or Bengal strain, and the recognition of recent changes in the susceptibility pattern of *V. cholerae* O1 and *Shigella* spp demonstrate the importance of surveillance programs.

In January 1991, a diarrheal epidemic, caused by *V. cholerae* O1 biotype El Tor, struck Peru and extended to other Latin American countries. Since then, *V. cholerae* O1 has been the most common pathogen associated with acute diarrhea in adults in Peru. To evaluate the relative importance of *V. cholerae* and other bacteria as a cause of acute diarrhea in adults 4 years after the introduction of cholera, a surveillance study was conducted in a large university hospital in Lima, Peru. During this investigation, clinical and epidemiologic differences between diarrheas produced by *V. cholerae* O1 and those produced by other enteropathogens were studied in search of characteristics that may help to define a cholera case.

MATERIALS AND METHODS

The study was conducted at the emergency room and rehydration unit at the Cayetano Heredia National Hospital (CHNH), a hospital that offers medical care to people of northern Lima with poor financial resources. Serving a population of approximately 2 million people, CHNH offers medical attention to approximately 45,000 patients per year and has been the hospital that has received the highest burden of diarrheal diseases since the beginning of the cholera epidemic in Lima, the capital of Peru.
Patients included in the study were 13 years of age and older who arrived at the hospital with acute diarrhea, defined as three or more loose stools in a 24-hour period and a duration of less than 72 hours. Severe dehydration was defined as the presence of febrile or impalpable pulse, unrecordable blood pressure, plus one of the following signs: sunken eyes, hoarse voice, protracted skin pinch retraction, and washerwoman’s hands. Patients excluded were those who had taken antimicrobials active against enteropathogens in the previous 72 hours. Surveillance was conducted during a 24-hour period. Two days per week were randomly selected, with each day of the week having the same probability of being selected.

A study investigator interviewed eligible patients immediately after admission into the hospital, not knowing the agent responsible for the diarrheal episode, using a standard questionnaire that focused on demographic and epidemiologic data, including duration of illness, use of oral rehydration therapy (ORT), hygienic behavior, source and storage of drinking water, and disposal of feces. Clinical information relevant to a diarrheal episode also was obtained, and a complete physical examination was performed on admission. A stool specimen was obtained by rectal swab, from each eligible patient, on arrival at the hospital. Stool samples were sent to the microbiology laboratory in Cary-Blair medium; these samples were cultured in an appropriate media to isolate Vibrio cholerae O1, Shigella spp, Salmonella spp, Plesiomonas spp, and Aeromonas spp, following standard techniques.4 Differentiation among pathogenic strains of Escherichia coli was not performed. No attempt was made to isolate viruses or parasites. Vibrio cholerae O1 strains were evaluated for susceptibility to tetracycline, doxycycline, and ciprofloxacin by the disk diffusion method, using standard techniques.9

Data were collected and processed using Statistical Package for Social Sciences for Windows 3.0 (SPSS Inc., Chicago, IL) and Epi Info 5.0 (USD Universal, Inc., Stone Mountain, GA). Categoric variables were evaluated using chi-square with continuity correction or Fisher’s exact test when applicable. Continuous variables were analyzed using Student’s t-test or Mann-Whitney u test as needed. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. A logistic regression analysis was performed to identify independent predictors of cholera. Level of significance lower than 0.05 was used; all tests were two-tailed. The study was approved by the Institutional Review Board of Cayetano Heredia University. The patients gave oral consent to participate in the study.

RESULTS

Between January 16 and April 30, 1995, the summer time in Peru, 336 patients (185 [55%] males; 151 [45%] females) were enrolled in the study. Mean age was 38 years (range, 13–90 y). The majority of the patients came from districts of Lima of low socioeconomic status within close proximity to the hospital. Of the 336 rectal samples collected, 208 (61.9%) samples had positive cultures for one or more enteropathogens. A total of 213 strains were isolated. One pathogen was isolated in 203 patients, and two pathogens were isolated in five patients. Table 1 lists all bacterial pathogens isolated. Vibrio cholerae O1 biotype El Tor was isolated in 177 patients (52.7%), being the most common bacteria isolated. In 172 patients, V. cholerae was the only agent isolated, and in five patients it was isolated with another bacteria. Of these 177 V. cholerae isolates, 167 (94%) were serotype Ogawa and 10 (6%) were serotype Inaba. Shigella spp (4.8%), Salmonella spp (2.7%), and other common enteric pathogens detected. All V. cholerae O1 strains were susceptible to the antimicrobials tested by the disk diffusion method, including tetracycline, doxycycline, and ciprofloxacin.

The total number of diarrheal and microbiologically confirmed cholera cases in the study period is shown graphically in Figure 1. Surveillance was conducted for 1 day only during weeks 8, 9, and 11 of the study. Isolation rates for V. cholerae O1 in the first 7 weeks of the study were higher than in the remaining 8 weeks (76.7% vs. 23.3%). The highest isolation rate for V. cholerae occurred during week 5 (Feb. 13–19) of the study.

Comparisons between patients with cholera and those without, according to epidemiologic and clinical
Acute diarrheal diseases contribute to the major public health problems that exist in developing countries. Patients with cholera were older than noncholera patients (mean age ± SD: 40 ± 17 y vs. 35 ± 15 y, P = 0.002); no difference according to gender was observed. Patients with cholera had lower sanitary conditions compared to noncholera patients. Cholera patients had less access to municipal water and were less likely to have homes connected to a sewage system or with an indoor toilet. No significant difference was found between the two groups with respect to a history of eating and drinking at street vendors. Patients with cholera had a longer duration of illness before arriving at the hospital (mean, 16 vs. 13 h, P = 0.003), higher number of stools the day before admission (mean, 9 vs. 6, P = 0.0004), and complained more frequently of having had limb cramps compared to noncholera patients (85% vs. 36%; P < 0.00001). The duration of hospitalization (mean, 25.1 vs. 5.8 h; P < 0.00001) and the total number of stools (mean, 15 vs. 11, P = 0.0001) also were higher in the cholera group than in noncholera patients.

Clinical signs of severe dehydration, such as sunken eyes, hoarse voice, washerwoman's hands, and protracted skin pinch retraction, were more frequently encountered among cholera patients than noncholera patients, thus requiring more often the use of intravenous fluids. No difference between cholera and noncholera patients was observed regarding the use of ORT before coming to the hospital. No mortality was observed during the study. Independent predictors of cholera identified through logistic regression analysis were liquid stools (OR = 16.51; 95% CI = 13.71-19.02; P = 0.0003), severe dehydration (OR = 2.48; 95% CI = 1.57-3.38; P = 0.0083), generalized cramps (OR = 4.65; 95% CI = 3.10-6.17; P < 0.0001), and washerwoman's hands (OR = 2.45; 95% CI = 1.55-3.34; P = 0.017).

**DISCUSSION**

These problems were further aggravated by the introduction of cholera in 1991 in Latin America. In many areas of Latin America, cholera proved to be fatal and necessitated the development of rigorous programs for its control. Surveillance programs proved to be of utmost importance to detect early cases of cholera, to detect changes in the susceptibility pattern of the bacterium to antimicrobials, and to establish clinical and epidemiologic criteria to define a case, to determine its actual prevalence, and to provide adequate treatment.

Results from this study raise several important issues. First, data from this surveillance study demonstrate that cholera is endemic in Peru. Second, *V. cholerae* O1 remains susceptible to first-line antimicrobials recommended for its treatment, such as tetracycline and doxycycline. Third, patients who acquired cholera were those with poor sanitary conditions. And finally, the best predictors of cholera in this population were the presence of watery diarrhea and signs of severe dehydration. No inclusion of children and selection of patients in a hospital setting preclude generalization of these results to the community settings, where cases with milder dehydration are likely to occur.

**Table 3. Comparison of Clinical Characteristics on Admission to Hospital between Cholera and Noncholera Patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cholera Cases</th>
<th>Noncholera Cases</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of vomiting</td>
<td>153 (86)</td>
<td>126 (79)</td>
<td>1.67</td>
<td>0.94–2.97</td>
</tr>
<tr>
<td>Generalized cramps</td>
<td>150 (85)</td>
<td>58 (36)</td>
<td>9.67</td>
<td>5.74–16.30</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>121 (68)</td>
<td>143 (90)</td>
<td>0.24</td>
<td>0.13–0.44</td>
</tr>
<tr>
<td>Sunken eyes*</td>
<td>168 (95)</td>
<td>109 (70)</td>
<td>7.88</td>
<td>3.71–16.74</td>
</tr>
<tr>
<td>Hoarse voice*</td>
<td>121 (68)</td>
<td>51 (33)</td>
<td>4.41</td>
<td>2.73–6.99</td>
</tr>
<tr>
<td>Washerwoman’s hands*</td>
<td>61 (40)</td>
<td>18 (12)</td>
<td>9.67</td>
<td>5.74–16.30</td>
</tr>
<tr>
<td>Protracted skin pinch retraction*</td>
<td>112 (63)</td>
<td>52 (34)</td>
<td>3.41</td>
<td>2.17–5.36</td>
</tr>
<tr>
<td>Severe dehydration*</td>
<td>113 (64)</td>
<td>24 (15)</td>
<td>9.93</td>
<td>5.64–16.90</td>
</tr>
<tr>
<td>Liquid stools</td>
<td>172 (97)</td>
<td>116 (70)</td>
<td>14.44</td>
<td>8.57–37.41</td>
</tr>
<tr>
<td>Requirement of intravenous fluids for rehydration</td>
<td>173 (97)</td>
<td>138 (88)</td>
<td>6.58</td>
<td>2.21–19.62</td>
</tr>
</tbody>
</table>

* For these physical findings, 150 noncholera patients were examined.

**Figure 1.** Cases of acute diarrhea and cholera from January 16 to April 30, 1995. Solid line = total number of acute diarrheal cases; dotted line = microbiologically confirmed cholera cases.
other countries. Studies conducted from the beginning of the epidemic in Peru show that *V. cholerae* O1 was responsible for the majority of acute cases of diarrhea in health centers. Begue et al showed that 2 years after the introduction of cholera in Peru, the epidemic still existed, with higher isolation rates in adults than in children, a typical finding observed with epidemic cholera. Even 4 years after the beginning of the cholera epidemic in Peru, a great number of cholera cases still are presenting to hospital centers, particularly with severe dehydration. Of the 336 patients included in this study, 177 (52.7%) had cholera, of whom 113 (63.8%) had severe dehydration. The best predictors of cholera in this hospital setting were the combination of severe dehydration and liquid stools. The absence of mortality associated with cholera observed in this study also is noteworthy, emphasizing the high standard of care and management provided by Peruvian physicians.

Recent studies in Asia and Africa have shown dramatic changes in the susceptibility pattern of enteropathogens such as *Vibrio* spp, *Shigella* spp, and *Salmonella* spp. Antimicrobials, once considered adequate for the treatment of these infections like cotrimoxazole, ampicillin, chloramphenicol, and tetracycline, no longer are useful. Improper use of antimicrobials for the treatment and prophylaxis of these diseases is responsible for this phenomenon. The situation in Peru is somewhat unique; this study and that of Begue et al show that common antimicrobials still are useful for treating cholera and other enteric diseases, and emphasize the usefulness of well-coordinated national efforts to promote the proper use of antimicrobials, restricting them to treatment of only severely dehydrated patients and discouraging the massive use of chemoprophylaxis.

People affected by cholera are those with the lowest financial resources and poorest hygienic facilities. This is of public health importance. Education and technical assistance are necessary for control and prevention. As a short-term solution, public education must continue to target the entire family regarding proper selection and preparation of foods; the need for cooking foods, especially seafood, thoroughly; and boiling of water. There also is a need to coordinate programs to ensure that citizens have access to a water supply adequate in quality and quantity. Ideally, there would be cooperation of the government, private sector, health officials, and the community in these efforts as well as in the treatment of sewage water and its adequate disposal.

Cholera still is prevalent in Lima; the bacterium remains sensitive to common antimicrobials, and poor sanitary conditions are responsible for its acquisition. Patients with watery diarrhea and severe dehydration should be considered to have the disease and be treated accordingly. Vaccines have not proved to be an effective measure of control against cholera; therefore, preventive measures should focus on education and proper infrastructure of sanitation facilities.

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**REFERENCES**