Bacterial Shedding in Household Contacts of Cholera Patients in Dhaka, Bangladesh

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INTRODUCTION

Vibrio cholerae is the Gram-negative bacterium that causes cholera, an acute dehydrating diarrheal disease. Annually there are more than three million cases of cholera and more than 100,000 deaths worldwide.1 V. cholerae strains can be differentiated by serogroup based on the O side chain of the lipopolysaccharide of the outer membrane. Serogroup O1 is further divided into two major serotypes (Inaba and Ogawa) and biotypes. V. cholerae O1 biotype El Tor is the current cause of epidemic cholera worldwide. Cholera occurs in epidemic patterns and also causes endemic disease in some areas, including Bangladesh, where the incidence of cholera is estimated at 200 cases/100,000 persons annually. In endemic cholera, most severe and fatal cases occur in children.2 In epidemic cholera, persons may lack pre-existing immunity and attack rates are more uniformly distributed across age groups.

Multiple V. cholerae infections within the same household are common.3,4 These infections may occur through shared sources of contaminated food or water, or through secondary transmission by the fecal-oral route from one household member to another. Among household contacts, known risk factors for infection include young age, blood group O, genetic factors, nutritional factors, and lack of pre-existing immunity to V. cholerae.5 Infected household contacts may or may not be symptomatic, and most commonly shed V. cholerae for one to several days.3 A longer duration of bacterial shedding has been observed in a few household contacts; however, risk factors contributing to prolonged shedding are not known.3,7,8 In a previous analysis of household contacts in Bangladesh, no relationship was found between bacterial shedding duration and age, blood group O status, or diarrhea.3

Our group has previously described characteristics associated with susceptibility to V. cholerae infection and clinical outcomes in a cohort of household contacts of patients with severe cholera in Bangladesh. In this study, we describe the duration of bacterial shedding and specifically analyze risk factors for prolonged bacterial shedding in a more recent cohort of cholera patients and their household contacts.9

METHODS

This study was conducted at the International Center for Diarrheal Disease Research, Bangladesh (icddr,b) Dhaka Hospital. This institution cares for more than 120,000 patients per year, including approximately 20,000 with cholera, most of whom live in urban high-risk cholera areas of Dhaka. Patients who came to the hospital during 2006–2011 with acute watery diarrhea were eligible for inclusion in this study if stool cultures were positive for V. cholerae as the sole pathogen, if they were between 2 and 60 years of age, resided in or around Dhaka, were without significant comorbid conditions, and consented for a study with a one-year follow-up period and intermittent blood draws. An index case-patient was defined as the first person from a household who had acute, watery diarrhea requiring hospitalization with a stool culture positive for V. cholerae. Only one index case-patient per household was enrolled.

Index case-patient stool was cultured on taurocholate-tellurite-gelatin agar (TTGA) overnight, and suspect colonies were confirmed by the slide agglutination method using specific monoclonal antibodies.10,11 Rectal swab samples obtained from contacts starting the day after index case-patient enrollment (beginning on study day 2) were transported in Cary-Blair media after collection at the participant households or at the icddr,b. Specimens were inoculated for enrichment in alkaline bile peptone broth and on TTGA and incubated overnight. Colony identification was performed after plating on TTGA. Serum was assayed for vibriocidal antibody responses of both serotypes at each time point of follow-up by using guinea pig complement and V. cholerae O1 Ogawa (X-25049) or Inaba (T-19479) as the target organism.12

Contacts were observed for nine days after household index case-patient enrollment, beginning on the date of index case-patient stool culture confirmation (study day 2). Rectal swabs and clinical data were collected during daily home visits on days 2 through 10. At each study visit, the level of dehydration was assessed according to the World Health Organization...
(WHO) dehydration scale. If dehydration was present, contacts were given oral rehydration solution and were referred to the icddr,b for treatment.

Antibiotic use, including type of antibiotic and duration of use, was self-reported by participants and answers were recorded by the study staff at each visit. On the first home visit, contacts were questioned about antibiotic use during the previous week. Some participants were not aware of the antibiotic type he or she had taken, and antibiotic doses were not recorded. Based on *V. cholerae* microbiobiologic data for the study period, doxycycline, ciprofloxacin, and azithromycin were considered active against *V. cholerae* in this study analysis.\(^{13-15}\) There is documented *V. cholerae* resistance to doxycycline and increasing minimum inhibitory concentrations to ciprofloxacin in Bangladesh during the study period, and these antibiotics were considered effective against *V. cholerae* in this analysis because isolates were still likely to be sensitive based on epidemiologic surveillance data from the year of use, and misclassification of these isolates would result in a less conservative estimate of the effect of antibiotic treatment on bacterial shedding.\(^{13-15}\) The duration of bacterial shedding was defined as the time between any two positive rectal swab cultures, including days between positive rectal swab cultures when a negative culture was obtained. Prolonged shedding was defined as shedding *V. cholerae* for four or more days.\(^{3}\) Contacts were not aware of rectal swab results.

Vibriocidal antibodies were measured on days 2, 7, and 30 of the follow-up period. The fold-increase of vibriocidal titer between days 2 and 7, days 2 and 30, and days 7 and 30 was calculated. In areas with endemic cholera, there is frequently a background rate of vibriocidal antibodies in local populations and there is no threshold cutoff diagnostic of infection. A four-fold or greater increase between paired acute-phase and convalescent-phase measurements of the serogroup-specific vibriocidal titer was used to determine recent *V. cholerae* exposure.\(^{6,16}\)

In children and adolescents (defined by the WHO as \(< 19.5\) years of age), malnutrition was defined as a height-for-age z score \(\leq 2\) (for those \(< 5\) years old) or age-and-sex-adjusted–body mass index z score \(\leq 2\) (for those \(5-19\) years old) in accordance with WHO anthropometric measurements for different age thresholds.\(^{17}\) For children and adolescents \(\geq 5\) years old, Z scores were calculated by using AnthroPlus software (WHO, Geneva 2009; http://www.who.int/growththertools/en/). In adults > 19.5 years old, malnutrition was defined as a body mass index \(< 18.5\) in accordance with WHO standards.\(^{18}\)

Baseline characteristics of different groups were analyzed by using the Fisher’s exact test for categorical variables and the Mann-Whitney U test for continuous variables, including age. A multivariate analysis of risk factors for prolonged shedding was performed with a logistic regression model by using generalized estimating equations, with *P* values adjusted for clustering based on household. The final model was based on forward selection with predetermined cutoff criteria of *P* \(\leq 0.2\) for inclusion in the model. Statistical analyses were performed using Stata version 9.0 (Stata Corporation, Inc., College Station, TX). Informed consent was obtained from all participants or their guardians. The study was approved by the Ethical Review Committee of the icddr,b and the Institutional Review Board of Massachusetts General Hospital.

### RESULTS

During 2006–2011, we enrolled 295 household contacts of 153 patients with cholera. An average of 1.9 contacts were enrolled per index case-patient (range = 1–5 contacts). The median age of contacts was 20 years (range = 2–60 years), and men (52%) and women participated equally in the study. A total of 294 contacts completed 9 days of observation with 9 consecutive days of rectal swab sampling. The acquisition rate for daily rectal swab collection was 99.7% (2,638 of 2,646 possible follow-up swabs). Among 294 contacts studied, 71 had at least one positive rectal swab for *V. cholerae* O1 during the follow-up period (24%). No cases of O139 *V. cholerae* were detected. Contacts with multiple positive rectal swab specimen results demonstrated infection with only one serotype (either Ogawa or Inaba). In all contacts, the *V. cholerae* serotype detected was the same serotype as the index case-patient in that household. Among contacts with bacterial shedding, the median duration of shedding was two days. Sixteen infected household contacts had prolonged shedding (\(\geq 4\) days). Demographic and clinical findings of these patients compared with contacts without prolonged shedding are compared in Table 1. Contacts in households with another contact demonstrating prolonged shedding were more likely to become infected than were contacts that lived with persons who shed bacteria for \(\leq 3\) days (odds ratio = 3.1, 95% confidence interval = 1.2–8.3, *P* = 0.02). One contact with a positive rectal swab specimen that shed *V. cholerae* for one day was excluded from the analysis because of a lack of anthropometric information.

**Antibiotic use.** Nearly one-fourth of household contacts used antibiotics during the 10-day follow-up period (67 of 294, 23%). Most contacts using antibiotics reported symptoms of watery diarrhea (38 of 67, 57%). Contacts reporting no diarrheal symptoms also took antibiotics during the follow-up period (19 of 67, 28%). Among the 70 household contacts with positive rectal swab specimen results and available anthropometric information, approximately half took antibiotics (35 of 70, 50%), and most who took antibiotics used a drug effective against *V. cholerae* (32 of 35, 91%). The majority of infected contacts that took antibiotics reported taking azithromycin (30 of 35, 86%), and other antibiotics were used by less than 10% of contacts (amoxicillin [3 of 37, 8%] and one each ciprofloxacin and doxycycline [each 1 of 37, 3%]). All tested contacts were not aware of rectal swab results.

### Table 1

Demographic and clinical characteristics of 70 *V. cholerae*-infected contacts of cholera patients, Dhaka, Bangladesh*<sup>a</sup>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Contacts shedding (\leq 3) days, <em>n</em> = 54</th>
<th>Contacts shedding (\geq 4) days, <em>n</em> = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric mean age, years (range)</td>
<td>20 (3–56)</td>
<td>11 (3–40)</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>27/54 (50)</td>
<td>6/16 (38)</td>
</tr>
<tr>
<td>Blood group O</td>
<td>27/54 (50)</td>
<td>7/16 (44)</td>
</tr>
<tr>
<td>Serotype Ogawa</td>
<td>50/54 (93)</td>
<td>16/16 (100)</td>
</tr>
<tr>
<td>Fourfold increase in vibriocidal antibody</td>
<td>40/54 (74)</td>
<td>16/16 (100)</td>
</tr>
<tr>
<td>Watery diarrhea during follow-up</td>
<td>29/54 (54)</td>
<td>8/16 (50)</td>
</tr>
<tr>
<td>Required intravenous fluids during follow-up</td>
<td>2/54 (4)</td>
<td>2/16 (13)</td>
</tr>
<tr>
<td>Malnourishment†</td>
<td>19/54 (35)</td>
<td>12/16 (75)</td>
</tr>
</tbody>
</table>

*Values are no. (%) unless otherwise indicated.

†Definition of malnourishment: For adults \(\geq 19.5\) years of age, body mass index \(< 18.5\).

For persons \(< 5\) years of age, height-for-age z score \(\leq 2\). For persons 5–19 years of age, age-and-sex-adjusted body mass index z score \(\leq 2\).
surveillance isolates of *V. cholerae* at the icddr,b were sensitive to ciprofloxacin through 2011, although increasing minimum inhibitory concentrations were noted. 15 There is reported resistance of 10% of *V. cholerae* isolates to doxycycline in 2006, one year before the time the contact took this antibiotic; therefore, it is unknown if this treatment was effective. 14 *V. cholerae* is known to be resistant to amoxicillin. 15 Among contacts infected with *V. cholerae* who took antibiotics effective against this organism, most began taking antibiotics after bacterial shedding began (30 of 32, 94%). In most cases, contacts did not initiate antibiotics until after several days of bacterial shedding (median = 2 days, range = 1–7 days). Two contacts took antibiotics one and seven days before the bacterial shedding follow-up period. There was no relationship between status as a malnourished contact and whether antibiotics were taken (14 of 37 or 38% of contacts with malnutrition took antibiotics, and 21 of 33 or 64% without malnutrition took antibiotics; *P* = 0.38).

### Risk factors for prolonged shedding.

The median age of contacts with prolonged shedding was 11 years, compared with 20 years for contacts shedding ≤ 3 days (*P* = 0.005). The duration of shedding in different age groups stratified by malnutrition is shown in Table 2. Malnutrition was more prevalent among contacts with prolonged shedding (12 of 16, 75%) compared with those shedding ≤ 3 days (19 of 54, 35%; *P* = 0.009). There was no significant relationship between use of antibiotics effective against *V. cholerae* during the follow-up period and duration of bacterial shedding (22 of 54 [41%] with shedding ≤ 3 days versus 10 of 16 [63%] with prolonged shedding, respectively; *P* = 0.09). Contacts with prolonged shedding were more likely to have a four-fold increase in vibriocidal antibody. Among contacts who shed ≤ 3 days, 40 of 54 (74%) had a four-fold increase vibriocidal antibody response, and among contacts with prolonged shedding, 16 of 16 (100%) had a four-fold increase in vibriocidal antibody (*P* = 0.002).

To investigate possible interactions between risk factors, we performed a stepwise multivariate logistic regression in contacts with prolonged shedding, as shown in Table 3. Malnutrition was an independent predictor of prolonged shedding, and young age approached significance as an independent predictor of prolonged shedding. As noted above, many household contacts infected with *V. cholerae* initiated antibiotics after bacterial shedding began, which did not significantly influence the ultimate shedding duration in the multivariate model. No significant relationship was found between duration of bacterial shedding and symptom occurrence, symptom duration, symptom severity, or blood group O status.

### DISCUSSION

We report the results of a recent prospective evaluation of risk factors for prolonged *V. cholerae* shedding among household contacts of cholera patients in Bangladesh. We identified for the first time that malnutrition is a risk factor for prolonged shedding of *V. cholerae* in household contacts of cholera patients. We also found that contacts in a household with a person with prolonged shedding were more likely to become infected than were contacts who lived with persons who shed bacteria for ≤ 3 days, and this finding is consistent with previous results in similar cohorts.

Malnutrition is common in areas where cholera occurs. Historically, nutritional factors and malnutrition are known to influence susceptibility to cholera and duration of symptoms. 6, 19 The influence of malnutrition on the course of bacterial shedding of *V. cholerae* has not been previously studied. Mechanisms for prolonged shedding in malnourished persons may be caused by differences in intestinal flora between malnourished and healthy persons. This theory is supported by a study demonstrating that malnourished Bangladeshi children had lower bacterial diversity in stool during the recovery period after cholera when compared with healthy children. 20

There are few known factors that influence the duration of *V. cholerae* shedding. We found that all 16 contacts with long term shedding had a ≥ 4-fold vibriocidal titer increase, demonstrating that patients with prolonged shedding did not have a less robust immune response to *V. cholerae* infection by this measurement. Duration of shedding may be determined in part by other immunologic responses to *V. cholerae* independent of the vibriocidal antibody response, such as innate immune responses or local mucosal immunity. These factors may vary depending on previous antigenic exposure and host factors such as co-infections, genetic susceptibility, and blood group. 21

One small study in Nigeria previously reported months of *V. cholerae* shedding in several persons infected with human immunodeficiency virus (HIV). 22 Cholera continues to affect high HIV prevalence populations in Africa and the Caribbean, and further research on HIV and cholera co-infection is needed. Our results also demonstrate that antibiotic use is common among household contacts of cholera patients. We suspect that this finding is common in our population because of the

### Table 2

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Malnourished</th>
<th>Duration of shedding, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10, n = 26</td>
<td>17/26 (68%)</td>
<td>9/17 (53%) ≥ 4</td>
</tr>
<tr>
<td>10.5–19, n = 6</td>
<td>5/6 (83%)</td>
<td>1/5 (20%) ≥ 4</td>
</tr>
<tr>
<td>≥ 19.5, n = 38</td>
<td>9/38 (24%)</td>
<td>4/5 (80%) ≤ 3</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. contacts</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnourishment</td>
<td>31/70 (44%)</td>
<td>5.6 (1.8–18)</td>
<td>0.005</td>
<td>1.4 (1.3–13)</td>
<td>0.020</td>
</tr>
<tr>
<td>Age ≤ 10 years</td>
<td>26/70 (37%)</td>
<td>4.1 (1.3–12)</td>
<td>0.014</td>
<td>1.1 (0.99–8.3)</td>
<td>0.052</td>
</tr>
<tr>
<td>Report of watery diarrhea</td>
<td>37/70 (53%)</td>
<td>0.97 (0.29–3.3)</td>
<td>0.96</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Use of antibiotics effective against <em>V. cholerae</em></td>
<td>32/70 (46%)</td>
<td>2.5 (0.85–7.3)</td>
<td>0.09</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Blood group O</td>
<td>34/70 (49%)</td>
<td>0.78 (0.25–2.4)</td>
<td>0.66</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dehydration requiring intravenous fluids</td>
<td>4/70 (6%)</td>
<td>3.5 (0.20–62)</td>
<td>0.38</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*OR = odds ratio, CI = confidence interval.*
heavily used informal drug sector. This study was not designed to measure the effect of prophylactic antibiotic use on the risk of infection or duration of shedding because most infected participants initiated antibiotics after shedding began. Antibiotic prophylaxis to prevent cholera for persons at risk of cholera at a community or household level is not recommended by the WHO and is not the current standard of care. Initiation of effective antibiotics in the early stages of symptomatic *V. cholerae* infection is known to decrease the duration of bacterial shedding. Surprisingly, in our study, the initiation of antibiotics after bacterial shedding began appears to have had little effect on the duration of shedding. This finding could be caused by a less pronounced effect of antibiotics on duration of shedding after shedding has begun; use of counterfeit, adulterated, or non-potent products; or because many of the household contacts in this study were malnourished, which may have led to differences in antibiotic absorption or activity. Alternatively, the effectiveness of antibiotics on shedding may be different in the setting of asymptomatic or mildly symptomatic individuals with low organism burdens, compared with patients with severe disease requiring hospitalization for treatment of cholera. It is possible that targeted prophylaxis of household contacts with appropriate antibiotics before infection might lead to more judicious antibiotic use, provide a direct benefit to those who take prophylaxis, and reduce transmission among household contacts of cholera patients and potentially in the wider community. This suggestion is supported by earlier trials of targeted chemoprophylaxis in household contacts of cholera patients that demonstrated significant efficacy in reducing the incidence of *V. cholerae* infection and symptomatic disease. For example, in a 1968 trial in Bangladesh, a five-day course of tetracycline given at the time of or after shedding began, as in this study. Overall, our study identifies malnutrition as a novel risk factor associated with prolonged shedding of *V. cholerae* among household contacts of cholera patients and suggests that prolonged shedding may contribute to disease transmission, although further studies are needed to measure this contribution. In addition, antibiotic use is common in this population, but is often initiated after bacterial shedding begins in symptomatic cases. Additional trials are needed to evaluate the possible role of antibiotic prophylaxis in household contacts of cholera patients prior to infection.

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