First Do No Harm: 
Making Oral Rehydration Solution Safer 
in a Cholera Epidemic 

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Abstract 

Oral rehydration solution (ORS) is lifesaving therapy for cholera and pediatric diarrhea. During a cholera epidemic in Guinea-Bissau, we evaluated the microbiologic quality of ORS prepared at a hospital and tested a simple intervention using special vessels for disinfecting tap water with bleach and for preparing, storing, and dispensing ORS. Few coliform bacteria and Escherichia coli were recovered from tap water; however, pre-intervention ORS contained numerous bacteria including E. coli and toxigenic Vibrio cholerae O1. In contrast, ORS samples from intervention vessels had few or no coliform bacteria, no E. coli, and no V. cholerae. Mean pre-intervention counts of coliform bacteria (3.4 X 10^7 colony-forming units [cfu]/100 ml) and E. coli (6.2 X 10^3 cfu) decreased significantly during the intervention period to 3.6 X 10^2 cfu and 0 cfu, respectively (P < 0.001). This simple system using bleach disinfectant and special storage vessels prevents bacterial contamination of ORS and reduces the risk of nosocomial transmission of cholera and other enteric pathogens.
Since its discovery in the mid-1960s, oral rehydration solution (ORS), hailed as "the greatest medical discovery of the 20th century,"\(^1\) has become the cornerstone of modern therapy for cholera and pediatric diarrheal disease and has saved countless lives around the world.\(^2\) Despite evidence that enteropathogenic bacteria survive and multiply in ORS,\(^3\)-\(^5\) and that ORS prepared in the developing world is frequently contaminated with these pathogens,\(^6\)-\(^10\) the benefits of administering ORS to dehydrated patients with diarrhea have always been perceived as outweighing the risks.

In many clinics in the developing world, where access to potable water is limited, ORS is prepared from water that has not been chlorinated or boiled. Even in clinics that use safe water, ORS may easily become contaminated when it is stored in open buckets or extracted by patients or staff dipping cups and hands into large open containers (Figure A).

A simple system for point-of-use disinfection and safe storage\(^11\) has proven to be highly effective in improving the microbiologic quality of household drinking water\(^12,13\) and of street-vended beverages.\(^14\) We reasoned that the elements of this system (point-of-use water treatment with sodium hypochlorite disinfectant and the use of closed, narrow-mouth storage vessels with spigots) could easily be adapted to preparation and storage of ORS.

**METHODS**

**Study site and procedures.**

Guinea-Bissau has experienced recurrent epidemic cholera since 1987. During the most recent epidemic from October 6, 1996 through November 15, 1997, more than 25,000 cases of cholera and nearly 1,000 associated deaths were reported. Bissau, the capital city, reported more than 18,000 cases and 225 cholera deaths. Many oral rehydration treatment centers were established in response to the epidemic. In Bissau, approximately 80% of cholera patients seeking treatment were referred to the cholera ward of Simão-Mendes National Hospital.

At Simão-Mendes Hospital, ORS was prepared at 8:00 AM every morning in two 10-liter plastic buckets and one 50-liter plastic barrel. Every morning, the clinic staff person responsible for preparing ORS would discard any remaining from the previous day and rinse the buckets and barrel with soap and untreated municipal tap water.
After rinsing, the containers were used to prepare 50–70 liters of ORS in 10-liter batches by adding one packet of oral rehydration salts for every liter of tap water. Once the ORS was prepared, two drops of commercial bleach (5% sodium hypochlorite) (Lavax ®; Brandesco Company, Lisbon, Portugal) per liter of ORS was added to the containers.

The two full 10-liter buckets were then placed on the cholera ward, where patients could obtain ORS either by dipping their own cup into the bucket or by asking another patient or a nurse to do so. Each patient received a makeshift 8- or 16-ounce cup, often made from an empty intravenous fluid bottle, on admission to the cholera ward. Nursing staff seldom spontaneously offered ORS to patients. When the 10-liter buckets were empty or nearly empty, clinic staff would refill them by either dipping the 10-liter bucket inside the 50-liter ORS storage barrel or by using a cup to scoop ORS out of the 50-liter barrel and into the 10-liter bucket.

We conducted an intervention trial to determine whether disinfecting water with bleach before ORS preparation and using closed, narrow-mouth vessels for ORS preparation and storage would improve the microbiologic quality of ORS prepared at Simão Mendes Hospital.

**Cholera ward intervention.**

During the study period, a patient census on the cholera ward was obtained from the medical staff and clinic log book. Cholera patients were confirmed by collecting rectal swabs for culture from selected patients. Patient and medical staff satisfaction with the intervention was assessed through open-ended interviews during the post-intervention period.

For five consecutive days, samples of tap water were collected at 8:00 AM, and samples of ORS were collected from each container at 8:00 AM, 4:00 PM, and 8:00 AM the following morning. We then instructed hospital staff to use four 20-liter, closed, narrow-mouth vessels with spigots for preparing, storing, and dispensing ORS each morning, instead of their usual containers (Figure B).
The ORS preparer was instructed to rinse the inside of each vessel thoroughly with 2 drops of the commercial bleach solution in 1 liter of water each morning before making new ORS. Once the rinsed vessels were filled with tap water, the ORS preparer added two drops of bleach per liter of water to each vessel and allowed disinfection to occur for 30 min before adding packaged oral rehydration salts. The vessels were then placed on the wards to be used as needed (Figure B). After a two-day adaptation period, we resumed microbiologic testing according to the previous schedule for five additional days. Follow-up visits for sample collection and testing were conducted one and two weeks after the intervention period ended. Human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this study.

**Laboratory investigation.**

At collection, we measured residual total and free chlorine levels in all samples using the N,N-diethyl-phenylenediamine colorimetric method (chlorine kit; Hach Company, Loveland, CO). All samples for microbiologic analysis were collected in thiosulfate-containing whirlpacks for chlorine inactivation and were transported in a cooler to the National Public Health Laboratory of Guinea-Bissau. Samples were refrigerated and filtered within 24 hr of collection using a standardized membrane filtration technique for enumerating bacterial contamination. Samples were processed individually with autoclaved or disposable equipment. Serial dilutions were performed with sterile distilled water to obtain sample volumes of 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 ml. Sterile, distilled water was used as a control sample for all specimens. A total of 100 ml of each dilution was filtered. Membrane filters were transferred with sterile forceps to labeled Petri plates containing m- ColiBlue 24 broth medium (Hach Company) on an absorbent pad to distinguish coliforms from *Escherichia coli*. Plates remained at room temperature for 1–2 hr inverted, and were incubated at 35 ± 0.5°C for 24 ± 4 hr before coliform bacteria and *E. coli* colonies were counted. If colonies were too numerous to count, the concentration was estimated to be twice the upper limit of the countable range of the highest dilution.

To test for the presence of *Vibrio cholerae* O1 in ORS samples, bacteria colonies were extracted only from membrane filters containing *E. coli*. All bacterial colonies on selected absorbent pads were placed in alkaline peptone water and incubated at 37°C for 6–8 hr, then inoculated into thio-sulfate-citrate-bile salts-sucrose medium. After 10 hr, all suspicious bacterial colonies were tested for agglutination in diagnostic antisera. Isolates that agglutinated in *V. cholerae* O1 antisera were transported to the Centers for Disease Control and Prevention (CDC) for antimicrobial susceptibility testing and cholera toxin gene confirmation by the polymerase chain reaction.

To test the microbiologic quality of packaged oral rehydration salts (World Health Organization; KB Company, Berlin, Germany), three randomly selected packets were each mixed with one liter of distilled water and were processed as described earlier for enumeration of coliform bacteria and *E. coli* colony counts.

**Statistical analysis.**

Microbiologic contamination was analyzed as the geometric mean of colony-forming units (cfu) of coliform bacteria and *E. coli*. Generalized estimating equations were used to control for the repeated days of observation. A *P* value ≤ 0.05 was considered significant.
RESULTS

Daily patient census on the cholera ward was similar in the pre-intervention and intervention periods, ranging from 20 to 25 patients pre-intervention (mean = 22 patients) and from 15 to 20 patients (mean = 17 patients) during the intervention period. Rectal swabs were collected from 34 (10%) of 352 patients presenting to the clinic during the study period; toxigenic *V. cholerae* 01 biotype El Tor was recovered from 14 (41%) of these 34 swabs.

In both the pre-intervention and the intervention periods, few coliform bacteria and *E. coli* were recovered from samples of tap water, and there was no significant difference in the microbiologic quality of tap water as determined by the measures described below (Table 1).
Table 1.
Coliform Bacteria and *Escherichia coli* Colony Counts (per 100 ml) in Tap Water and Oral Rehydration Solutions (ORS) at 8 AM, 4 PM, and 24 hr at Simão-Mendes National Hospital, Bissau, Guinea-Bissau, November-December 1997 [* _ = not tested*]

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Pre-Intervention</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coliforms</td>
<td>E. Coli</td>
</tr>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap Water</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>7.4 x 10^3</td>
<td>1.0 x 10^2</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>4.8 x 10^6</td>
<td>4.0 x 10^2</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>2.1 x 10^6</td>
<td>1.0 x 10^2</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>1.0 x 10^5</td>
<td>2.0 x 10^1</td>
</tr>
<tr>
<td>24 hr ORS</td>
<td>4.0 x 10^6</td>
<td>2.0 x 10^1</td>
</tr>
<tr>
<td>24 hr ORS</td>
<td>4.0 x 10^6</td>
<td>6.0 x 10^1</td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap Water</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>1.8 x 10^2</td>
<td>-</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>4.0 x 10^6</td>
<td>9.0 x 10^1</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>4.0 x 10^6</td>
<td>4.0 x 10^2</td>
</tr>
<tr>
<td>24 hr ORS</td>
<td>4.0 x 10^8</td>
<td>1.2 x 10^4</td>
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<tr>
<td><strong>Day 3</strong></td>
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<td></td>
</tr>
<tr>
<td>Tap Water</td>
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<td>0</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>4.2 x 10^2</td>
<td>-</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>5.2 x 10^6</td>
<td>8.0 x 10^2</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>5.9 x 10^5</td>
<td>4.0 x 10^3</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>7.4 x 10^4</td>
<td>4.0 x 10^1</td>
</tr>
<tr>
<td>24 hr ORS</td>
<td>4.0 x 10^7</td>
<td>1.0 x 10^4</td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap Water</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>2.8 x 10^3</td>
<td>-</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>5.1 x 10^5</td>
<td>6.4 x 10^2</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>7.1 x 10^6</td>
<td>2.2 x 10^2</td>
</tr>
<tr>
<td>24 hr ORS</td>
<td>5.6 x 10^7</td>
<td>2.1 x 10^3</td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap Water</td>
<td>1.1 x 10^1</td>
<td>0</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>6.4 x 10^2</td>
<td>6</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8 am ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>6.4 x 10^4</td>
<td>2.1 x 10^2</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>1.3 x 10^6</td>
<td>7.2 x 10^2</td>
</tr>
<tr>
<td>4 pm ORS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24 hr ORS</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
However, these same measures showed dramatic differences in the microbiologic quality of ORS between the pre-intervention and intervention periods. In the pre-intervention period, both coliform bacteria and *E. coli* were routinely isolated in large quantities from ORS samples collected from the open ORS containers. Coliform bacteria, ranging from $1.8 \times 10^2$ to $4.1 \times 10^8$ cfu/100 ml (mean = $3.4 \times 10^7$ cfu/100 ml) were isolated from all 30 (100%) pre-intervention ORS samples. *Escherichia coli*, ranging from 4 to $5.3 \times 10^4$ cfu/100 ml (mean = $6.2 \times 10^3$ cfu/100 ml), was isolated from 29 (97%) of 30 pre-intervention ORS samples.

In contrast, during the intervention period, no *E. coli* and few coliform bacteria were detected in ORS samples. Coliform bacteria, ranging from 1 to $7.0 \times 10^3$ cfu/100 ml (mean = $3.6 \times 10^2$ cfu/100 ml) were isolated from 24 (65%) of 37 intervention ORS samples. When compared with the pre-intervention period, a significant decrease was noted in the proportion of intervention ORS samples contaminated with coliform bacteria ($P < 0.001$) and with *E. coli* ($P < 0.001$) (Table 1). Overall, there was a five-log reduction in mean coliform bacteria counts and total elimination of *E. coli* from intervention ORS samples.

In the pre-intervention period, mean coliform bacterial colony counts in ORS samples collected at 8:00 AM (mean = $2.7 \times 10^3$) were lower than mean counts in samples collected at 4:00 PM (mean = $2.1 \times 10^6$), which were, in turn, lower than mean colony counts in 24-hour-old samples collected at 8:00 AM the following morning (mean = $8.2 \times 10^7$) ($P < 0.001$) (Figure 2).

**Figure 2.** Mean coliform bacteria and *Escherichia coli* colony counts in samples of oral rehydration solution on a logarithmic scale by time of sample collection: 8:00 AM, 4:00 PM, and 8:00 AM the following morning.

cfu = colony forming units
The same pattern was observed for mean *E. coli* colony counts (2.8 X 10¹ versus 3.5 X 10³ versus 1.1 X 10⁴) (*P* < 0.01). These data suggest that either bacterial multiplication or repeated introductions of bacteria, or both, occurred in ORS containers on the wards during the day. In the intervention period, we also found a significant difference between mean coliform bacterial colony counts in ORS samples collected at 8:00 AM, 4:00 PM, and 8:00 AM the following day (3.0 X 10¹ versus 2.9 X 10² versus 9.7 X 10²) (*P* < 0.001), but no *E. coli* were found in any of the samples.

Toxigenic *V. cholerae* O1, biotype El Tor, serotype Ogawa was recovered from four (80%) of five pre-intervention ORS samples tested. All *V. cholerae* isolates were resistant to tetracycline, doxycycline, trimethoprim-sulfamethoxazole, ampicillin, and furazolidone; the same serotype and antibiogram were observed in more than 90% of the clinical isolates. No *V. cholerae* was found in five randomly selected intervention ORS samples.

No free or total chlorine was detected in tap water samples in either the pre-intervention or intervention periods. During the pre-intervention period, no chlorine was detected in any of the ORS samples 30 min after bleach had been added to prepared ORS. During the intervention period, free chlorine levels in water samples from the vessels 30 min after bleach treatment and immediately before the addition of ORS ranged from 2.0 to 2.7 mg/L (mean = 2.5 mg/L), consistently above the World Health Organization (WHO) recommended level of 0.5 mg/L for point-of-use water consumption. Interestingly, 30 min after packaged oral rehydration salts were added to the chlorine-treated water, no total or residual free chlorine was detected. Neither coliform bacteria nor *E. coli* were detected in any of the packaged oral rehydration salts mixed with distilled water.

At one- and two-week follow-up visits, the intervention storage vessels were still in use, and samples of ORS from these vessels remained clean and free of contamination with *E. coli*. Mean coliform bacteria colony counts in ORS samples from 8:00 AM and 4:00 PM on these days were 1.7 X 10² cfu/100 ml at one-week and 1.0 X 10⁴ cfu/100 ml at two-weeks follow-up. Post-intervention interviews revealed that clinic staff and patients preferred the closed, narrow-mouth ORS storage vessels to the old method of ORS storage and delivery from open buckets.

The plastic storage vessels used in this study were manufactured in the United States (Tolco Company, Toledo, OH), where they are sold for $6.00 each. Through an arrangement with Rotary International and United States Agency for International Development, similar vessels are now produced and marketed in Bolivia for approximately $5.00 each. The cost of local commercial bleach in Guinea Bissau was $1.34 for a two-liter bottle. Since the clinic already had bleach and ORS, the cost of the intervention was that of the plastic vessels, a total of $20.00. Thus, most oral rehydration clinics could easily afford this intervention.

**DISCUSSION**

Oral rehydration solution is a life-saving medication for dehydrating diarrheal disease; however, it is easily contaminated when prepared in the field, and supports the growth and survival of many types of enteropathogenic bacteria. Our study documented that highly contaminated ORS was routinely ingested by patients on a hospital cholera ward in Guinea-Bissau and that a simple inexpensive system for ORS preparation and storage of ORS greatly reduced bacterial contamination.

Although the hazards of ingesting contaminated ORS have never been well documented, we believe they could be appreciable. *Vibrio cholerae* was isolated from
only 14 (41%) of 34 patients tested on this ward; many other patients who were admitted for treatment of acute, watery diarrhea were likely infected with other bacterial pathogens, such as enterotoxigenic and other diarrheogenic *E. coli*, *Salmonella*, *Shigella*, and *Campylobacter*. We demonstrated that ORS on the ward was contaminated with toxigenic *V. cholerae* that matched patient isolates, and we suspect that it also harbored other bacterial pathogens, although we did not attempt to identify them. By protecting ORS from contamination on the cholera ward, the risk of nosocomial transmission of cholera and other enteric pathogens would be greatly diminished.

To improve the microbiologic safety of ORS requires that it be prepared in a clean container from uncontaminated ingredients and that it be protected from contamination during storage and administration to patients. The point-of-use disinfection and safe storage system evaluated in this study address these critical control points. Rinsing the vessels with chlorinated water before preparing ORS helps eliminate any bacteria present from the previous day. Adding adequate hypochlorite disinfectant to water used to prepare ORS, and waiting at least 30 min before adding the packaged salts, assures that any bacterial contaminants remaining in the vessel or present in the water will be inactivated before ORS is added. Finally, storing the prepared ORS in a narrow-mouth, closed container with a spigot through which it can be dispensed provides an effective barrier to the introduction of bacteria from contaminated hands, cups, other implements, or insects. In our study both bleach and a special storage container were necessary to achieve our microbiologic results. The bleach worked to avoid initial contamination of ORS with dirty water. However, once the ORS salts were added, the bleach no longer worked, so the effect over time of keeping bacterial contamination low may be attributed solely to the closed container.

Recognizing the risks of contamination, WHO recommends that boiled or chlorinated water be used to prepare ORS, and that prepared ORS be discarded after 24 hr. However, boiling the amount of water recommended for oral rehydration, as much as 10–20 liters per patient per day, is time-consuming, expensive in many developing countries, and often impractical when fuel is scarce. 20-22 Many oral rehydration clinics have limited or no access to centrally chlorinated water, making point-of-use chlorination the only practical strategy. An alternate to hypochlorite solutions, potash alum, has been used to reduce bacterial contamination of water and ORS;4,23 however, it is a less effective disinfectant and may interfere with the physiologic properties of ORS by lowering the pH.

The current WHO recommendations do not adequately address the problem of preventing ORS contamination during storage and service to patients.20 When ORS for more than one patient is prepared and stored in a bulk container, the risk of contamination is high. Where feasible, clinic staff may choose to wait until a patient presents to the clinic to prepare a fresh batch of ORS in an individual container for that patient’s use only. Fresh ORS must be prepared again in that container each time the patient needs more. Although this approach would limit ORS contamination from sources exterior to the patient, and thereby reduce the risk of nosocomial transmission of enteric diseases, it requires a sufficient supply of individual containers, and far more staff time devoted to ORS preparation than is often available. In response to epidemic cholera, oral rehydration treatment centers are often hastily established with minimal facilities and staff, whose capacity to prepare or serve individual containers of ORS to each patient can quickly be overwhelmed by an influx of acutely ill persons. Even in more permanently established rehydration centers, the bulk preparation of ORS is often the only practical means to assure that supply meets demand.
The point-of-use disinfection and safe storage intervention we evaluated enabled safe preparation and storage of ORS in bulk. Sodium hypochlorite disinfectant is widely available as commercial bleach or can be generated locally through electrolysis of salt and water.\textsuperscript{11} A standard concentration must be used to ensure that adequate free chlorine levels are achieved in the water used for rinsing the vessels and for preparing ORS. The hypochlorite disinfectant must be added to water at least 30 min before the addition of packaged oral rehydration salts to ensure a bactericidal effect. The sugars in ORS, which promote the growth of bacteria, also react with and inactivate chlorine.\textsuperscript{24} This interaction between ORS and hypochlorite is not mentioned in most standard texts on ORS preparation. Adding chlorine bleach to prepared ORS, as done at this clinic before the intervention, is likely to have little effect, as the chlorine is rapidly consumed. In our field experiment, we found that once ORS was added to bleach-treated water, residual total and free chlorine levels rapidly decreased below detectable levels. Since there is no residual chlorine activity in the ORS, the ORS should still be discarded after 24 hr, even if made from chlorinated water.

In areas of both endemic and epidemic diarrheal disease, closed narrow-mouth vessels, such as those used here, and hypochlorite bleach can easily be used for preparation, storage, and administration of ORS to reduce potential transmission of enteric pathogens. Their application in refugee camps, where devastating outbreaks of diarrheal diseases are commonplace, may prove particularly helpful.

In any diarrheal disease treatment center that uses ORS mixed in bulk, health-care providers should apply this intervention to prepare and provide ORS without doing harm to the patient.

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