Use of Moore Swabs for Isolating *Vibrio cholerae* from Sewage

TIMOTHY J. BARRETT,1,* PAUL A. BLAKE,1 GEORGE K. MORRIS,1 NANCY D. PUHR,1 HENRY B. BRADFORD,2 and JOY G. WELLS1

Bacterial Diseases Division, Bureau of Disease Control, Atlanta, Georgia 303331 and State Department of Health and Human Resources, Office of Health Services and Environmental Quality, New Orleans, Louisiana 70160

The Moore swab method was shown to be a practical and sensitive technique for the isolation of *Vibrio cholerae* from sewage. In each of three instances in which cholera patients lived in homes connected to municipal sewers, *V. cholerae* was isolated from the community sewage plant intake at the time of the patients' illness. Sewer systems became negative within 1 day after patients were treated with tetracycline. Sewer surveillance using the Moore swab also found evidence of infections occurring in areas where surveillance of diarrheal illness failed to detect cholera. Culturing community sewage by the Moore swab method proved to be an economical and effective way of determining areas where *V. cholerae* infections were occurring.

Moore in 1948 first introduced the use of cotton gauze swabs for isolating *Salmonella* from sewage-contaminated waters (7). He placed cotton gauze swabs in the sewage system of a small town in England where a paratyphoid epidemic had occurred. The swabs were collected after 48 hours and cultured for the presence of paratyphoid bacilli. By following the occurrence of paratyphoid organisms in the sewers, Moore was able to trace the source of the contamination to a single paratyphoid carrier (7). Moore later used the swab method to detect typhoid carriers in small towns (8) and, ultimately, typhoid and paratyphoid carriers in urban areas (9). Since that time, the Moore swab has been used on numerous occasions during epidemic investigations and environmental surveys for isolating *Salmonella*, and the swab has been shown to be quantitatively sensitive for isolating *Salmonella* from raw milk (12). Using a modified Moore swab method, Isaacson was able to isolate *Vibrio cholerae* from sewers in South African gold mines and, in some cases, to trace the source of contamination (4, 5).

The Center for Disease Control has participated in three investigations in which the Moore swab has been used to search for *V. cholerae* O1 in sewage and sewage-contaminated water. First, in 1973, in Texas, the swab method was successfully used to isolate the organism from a cholera patient's septic tank (11). Second, in 1974, in Guam, the swab proved to be useful in detecting *V. cholerae* O1 in sewers, storm drains, and surface water as well as in septic tanks, and it detected the organisms in sewage coming from areas in which surveillance of diarrheal disease had failed to detect cholera cases (6). This paper evaluates use of the Moore swab in the third field investigation, which occurred in 1978 in Louisiana. After a single unexplained case of cholera was detected in southwestern Louisiana, investigation ultimately revealed 10 additional *V. cholerae* O1 infections in 5 municipalities in the area, all caused by eating locally caught crabs (P. A. Blake, D. T. Allegra, J. D. Snyder, T. J. Barrett, L. McFarland, C. T. Caraway, J. C. Feeley, J. P. Craig, J. V. Lee, N. D. Puhr, and R. A. Feldman, N. Engl. J. Med., in press). This investigation determined the sensitivity of the swab method, the persistence of *V. cholerae* O1 in sewer lines, the efficacy of the swab in tracing the source of *V. cholerae* O1 in sewer lines, the efficacy of the swab in tracing the source of *V. cholerae* O1 isolates from sewage, and the relative value of 6- and 18-h enrichment in alkaline peptone broth.

**MATERIALS AND METHODS**

Moore swabs were made by cutting pieces of cotton gauze 2 to 4 feet long by 6 inches wide (60 to 120 cm by 15 cm), folding the gauze lengthwise several times to form a tight cylindrical roll, and tying the center with a strong wire. The swabs were wrapped in heavy paper and sterilized by autoclaving.

The ends of the wires holding the swabs were tied to nylon fishline, and the swabs were suspended by the lines in the water or sewage to be tested and left in place for 24 to 48 (usually 24) h. The swabs were then removed, the wires holding the swabs were cut aseptically, and the swabs were submerged in alkaline peptone broth with a pH of 8.6 (225 ml in a 0.5-liter jar). The jars were transported to the laboratory in an ice chest to prevent possible overheating. At the lab-
oratory the jars were incubated at 35°C and subcultured to thiosulfate-citrate-bile salts-sucrose (TCBS) agar (BBL Microbiology Systems) after 6 and 18 h. After 18 h of incubation at 35°C, the TCBS plates were examined for colonies resembling \textit{V. cholerae}. If present, up to 10 (usually 7 or 8) typical colonies per plate were picked to triple sugar iron (TSI) agar. Although \textit{V. cholerae} typically ferments sucrose and thus yields an acid slant on TSI, not all strains do so rapidly. Therefore, all isolates showing a TSI reaction of alkaline or acid slant, acid butt, no gas, and no \(H_2S\) were considered suspect \textit{V. cholerae} and were screened by agglutination in polyvalent \textit{V. cholerae} O1 antisera. Isolates agglutinating in polyvalent antiserum were further tested by agglutination in monospecific Inaba and Ogawa antiserum. Cultures agglutinating in either monospecific antiserum were considered to be \textit{V. cholerae} O1 and were subsequently confirmed biochemically (3).

Initially, a Moore swab was placed in the intake line of the sewage plant in the index cholera patient's town to determine if other infections were occurring. When \textit{V. cholerae} O1 was isolated from the swab, an effort was made to trace the source of the organism by placing Moore swabs in main sewerage lines and backtracking through the tributary lines. Subsequently, sewer swab surveillance of cities and towns in southwestern Louisiana was extended to include 28 municipal sewerage systems in 22 municipalities in an area approximately 50 by 110 miles (80 by 177 km). The septic tanks of four households in which cholera infections occurred were also sampled by using the Moore swab.

The sensitivity of the method was studied by examining the results from swabs taken from sewer lines running between untreated persons known to be infected and the sewage treatment plant, including the proportion of swabs positive as the sewage became more dilute and the volume increased, i.e., as it moved from the patients' neighborhoods to pumping stations and on to the sewage treatment plant. The persistence of \textit{V. cholerae} O1 in sewerage lines was determined by examining the results of Moore swabs taken from sewers serving infected persons 1 to 7 days after they began to take tetracycline. Finally, we compared the results of subculturing Moore swabs after 6 and 18 h of enrichment in alkaline peptone broth.

\section*{RESULTS}

Isolation of \textit{V. cholerae} O1 from the index patient's town sewerage system provided the first evidence that other cases were occurring. During September and October 1978, \textit{V. cholerae} O1 was isolated from sewerage systems in six municipalities; investigation of persons with diarrheal illness revealed cholera cases in three of these towns, but 11 of 21 isolates from sewerage systems had no likely human source identified. Surveillance and investigation of persons with diarrheal illness found infected persons in two other towns without municipal sewerage systems. All isolates of \textit{V. cholerae} O1 from sewers, patients, and the environment were biotype El Tor, serotype Inaba.

\textit{V. cholerae} O1 was isolated from 7 (70\%) of the 10 Moore swabs taken from sewer lines running between untreated infected persons and the sewage treatment plant (Table 1). Although the numbers are small, distance from the infected person (and thus the dilution of the stool) had no apparent effect on the proportion of positive swabs. In contrast, \textit{V. cholerae} O1 was isolated from only 1 (6\%) of 16 24-h swabs removed from sewers serving infected persons 1 to 7 days after they began to take tetracycline (Table 1). The only positive swab came from a manhole that served a 3-block area and was less than 1 block from a patient who had begun tetracycline therapy 3 days before the swab was removed.

Two of the four septic tanks of households in which cholera infections occurred were positive. One tank was positive 2 days after the two infected household members began taking tetracycline; it was not retested. A second tank was positive 2 days and negative 7 days after the infected person left the household. The other two tanks were culture-negative, but one was first tested 25 days after the patient was hospitalized, and the other had special treatment equipment.

The usefulness of the swab in tracing the course of \textit{V. cholerae} O1 in sewage under ideal circumstances was demonstrated in one town after the organism was isolated from a Moore swab taken on 9 September 1978 from sewage entering the treatment plant. On 13 September, a second Moore swab was put into the sewage treatment plant, and swabs were also placed in 17 pumping stations, each of which drained a specific part of the town. \textit{V. cholerae} O1 was isolated from two sites: the treatment plant and a pumping station serving an area of approxi-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Sewage sampled} & \textbf{Serving untreated infected person} & \textbf{Serving infected person 1–7 days posttreatment} \\
\hline
Neighborhood sewers & 5 (3) & 7 (1) \\
Pumping stations & 3 (2) & 5 (0) \\
Treatment plants & 2 (2) & 4 (0) \\
\hline
\textbf{Proportion positive} & \textbf{70\%} & \textbf{6\%} \\
\hline
\end{tabular}
\caption{Moore swab sampling of sewage from tetracycline-treated and untreated persons with \textit{V. cholerae} O1 infections, by distance from source, in southwestern Louisiana, September 1978}
\end{table}

\textsuperscript{*} Twenty-four-hour Moore swab removed from sewage 1 to 7 days after treatment with tetracycline was initiated.
mately 50 blocks. The investigators placed Moore swabs in the tributary lines, and within 4 days the source was traced to a 2-block area in which a house-to-house search could have been implemented. During this time, however, 1 individual from this area was hospitalized in shock and was found to have culture-confirmed cholera. The patient's daughter, who was also culture-positive but not ill, remained at home and was probably the source of V. cholerae 01 in their neighborhood sewage after the patient was hospitalized. Ironically, these patients were apparently infected 13 September 1978, too late to have been the source of the initial sewage isolate which prompted the search.

In seven sewer swabs from which V. cholerae 01 was ultimately isolated that were subcultured to TCBS agar at both 6 and 18 h, there was no evidence that subculturing at 18 h offered any increased sensitivity over subculturing at 6 h. V. cholerae 01 was detected on all seven plates inoculated at 6 h and represented 71% (40/56) of colonies picked. The organism was detected in six of seven plates inoculated at 18 h and represented 70% (31/44) of colonies picked.

**DISCUSSION**

Although its sensitivity in comparison to other methods is undemonstrated, the Moore swab has now been shown to be useful in isolating V. cholerae 01 from septic tanks and sewerage systems in multiple investigations (4–6, 11). The sensitivity of the Moore swab in sewage is demonstrated by the isolation of V. cholerae 01 from 70% of swabs from sewage in which the organism was known to be at least intermittently present, and the absence of a drop in the proportion of positive swabs as the distance from the source and the volume of sewage increased. Thus, if swabs from sewage systems are found to be consistently negative, it is unlikely that the area served harbors large numbers of unrecognized V. cholerae 01 infections.

Sewer surveillance by using the Moore swab offers an effective way to determine whether V. cholerae 01 infections are occurring in an area with sewage systems. Sewer swab surveillance is totally under the control of the health department and does not depend upon seeking the cooperation of private individuals. It may lead to detection of asymptomatic infections and mild disease which would not lead infected persons to seek medical assistance or have stool cultures performed. Both in Guam (6) and in Louisiana, Moore swabs detected toxigenic V. cholerae 01 in sewers coming from areas in which no cases of cholera had been detected by surveillance of persons seeking medical attention because of diarrheal illness. Finally, it is relatively inexpensive, since the swabs can be made from locally available materials and a modest program should not require additional manpower. The major disadvantages of sewer swab surveillance are that infections in persons not served by a sewage system will be missed, and it is difficult to find the person who was the source of contamination of the sewage. Tracing the source of V. cholerae 01 excretion through sewage systems by using a series of sets of Moore swabs is laborious and too slow to make success likely. Waiting for laboratory results means that a minimum of 48 h is required between each successive set of swabs, and infected persons usually excrete V. cholerae 01 for only about 1 week (2). Infected persons must be found so that epidemiological investigations can be performed to determine the vehicle of transmission of the disease. Thus, an effective surveillance system for cholera could include periodic use of Moore swabs in the influents of municipal sewage plants to determine whether V. cholerae 01 infections are occurring and subsequent culture of stools from persons with diarrheal illnesses if the organism is found in sewage.

The results from Louisiana show that V. cholerae 01 usually persisted in sewage systems less than 1 day after the infected person was treated with tetracycline. The rarity of isolates from these sewage systems after treatment of the infected persons suggests that most or all sewage isolates are from humans, rather than from non-human sources such as washings from seafoods. Since long-term carriers of V. cholerae 01 are uncommon (1, 10), sewage isolates are also unlikely to have come from chronic carriers. Thus, isolation of the organism from sewage probably means that someone on the sewage system is currently or was very recently infected with V. cholerae 01. In the few septic tanks examined, however, V. cholerae 01 survived at least 2 days, perhaps because of low flow rates and less contact with toxic substances.

Since subculturing from alkaline peptone broth enrichment medium to TCBS at both 6 and 18 h almost doubled the laboratory work involved in culturing Moore swabs, the finding that subculturing at 6 h alone was equally effective offers a way to reduce the expense of using the method. These results may not apply to Moore swabs taken from environmental sources such as marshes, since these areas may have halophilic vibrios and other bacteria not commonly found in sewage.

In summary, this study (i) showed that Moore swabs detected V. cholerae 01 in sewage, (ii)
found evidence of infections occurring in areas in which surveillance of diarrheal illnesses had not detected cholera, (iii) found that sewerage systems became negative within 1 day after cases were treated with tetracycline, (iv) showed that subculturing at 6 and 18 h from alkaline peptone broth enrichment medium offered no advantage over subculturing at 6 h alone, and (v) demonstrated that sewage surveillance by using the Moore swab offers a practical, sensitive, and economical method to determine whether V. cholerae O1 infections are occurring in municipalities with sewerage systems.

LITERATURE CITED