

Use of Vaginal Tampons in Sewer Surveys for Non-O1 *Vibrio cholerae*

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Vaginal tampons were shown to be a practical alternative to conventional Moore swabs for isolating *Vibrio cholerae* from sewage. Associated laboratory investigations demonstrated improved isolation of *V. cholerae* by using 12- or 18-h enrichments in alkaline peptone water, in comparison with 6-h enrichments, when cultures were incubated at ambient temperatures.

Moore introduced the use of cotton gauze swabs to assist in the isolation of salmonellae from sewage-contaminated water in 1948 (5). The technique was later found useful for isolating salmonellae from raw milk (9), and since 1974 it has been used to assist in surveillance or outbreak investigations of *Vibrio cholerae* (1-4, 7, 8). Vaginal tampons offer the possibility of a practical alternative to Moore swabs because time-consuming assembly is not required and tampons are uniformly manufactured, relatively inexpensive, aseptic (if not sterile), and similar in size and shape to Moore swabs. Our principal objective was to perform a *V. cholerae* sewer survey and compare recoveries of *V. cholerae* from tampons to recoveries from conventional Moore swabs. We also evaluated *V. cholerae* isolation by using small volumes (50 ml) of alkaline peptone water (APW; pH 8.6) and 6-, 12-, and 18-h enrichments at ambient temperatures.

Survey procedures. The study area was a lower-economic-class neighborhood in northern Jakarta, Indonesia, encompassing an area of approximately 50 km². Although cholera is endemic in Jakarta, there were no known cases in the neighborhood during our surveillance testing. The sewers were open cement or masonry ditches (approximately 75 cm wide by 75 cm deep), and there was usually one on each side of a narrow street.

Moore swabs were made under aseptic conditions as described by Barrett et al. (1) and consisted of cotton gauze strips which had been tightly rolled into a cylindrical shape (approximately 2 by 6 cm when dry) and tied in the center with nylon fishing line. Vaginal tampons (size "super") were a commercial variety made of rayon fibers radiating from a central axial core of cotton string (Tambrands, Lake Success, N.Y.). Although vaginal tampons are not widely used by the indigenous populations of Indonesia and most other developing countries, they can usually be obtained in larger cities at pharmacies or hotels frequented by foreigners. Prices vary from region to region because of importation duties and demand but are generally the equivalent of \$0.15 to \$0.30 per tampon.

The first survey was performed during the rainy season. At each test site, a swab-tampon pair was suspended in the open sewer by fishing lines, about 1 m apart, and left there for 36 to 48 h. Upon recovery, swabs and tampons were

separately sealed in aseptic plastic containers and transported to the laboratory within 3 h for processing.

Containers holding the swabs or tampons were drained of excess liquid, and 250 ml of APW was added to each. APW enrichments were incubated for 18 h at 36 ± 1°C and then subcultured by streaking them onto a single plate of thiosulfate-citrate-bile salts-sucrose agar (TCBS). After overnight incubation of TCBS subcultures at 36 ± 1°C, a maximum of 10 *V. cholerae*-like colonies from each plate were individually subcultured onto Kligler iron agar, sucrose semisolid agar, and motility-indole-ornithine medium and then incubated overnight at 36 ± 1°C. Motile, oxidase-positive, sucrose-positive, indole-positive, ornithine-positive isolates showing an alkaline slant and acid butt, no gas, and no H₂S in Kligler iron agar were considered *V. cholerae* and were not subjected to further biochemical tests. *V. cholerae* isolates were screened by agglutination in polyvalent antiserum to *V. cholerae* serogroup O1.

In a second sewer survey during the dry season, only tampons were used to determine the optimum enrichment time at ambient temperatures. Tampons were suspended in each of 12 different locations, recovered after 2 days, and enriched with 250 ml of APW for each tampon. Enrichment cultures were incubated on a laboratory countertop at room temperature (24 to 30°C). Enrichment broths were subcultured onto TCBS agar after 6, 12, and 18 h and then processed as described above.

In a third survey, also performed during the dry season, one of 21 pairs of tampons which had been placed in different locations for 2 days was enriched with 250 ml of APW as described above, and the other tampon was transferred from the collection container to a sterile 50-ml centrifuge tube. After transfer to the smaller container, excess liquid was removed, and the tube was filled to the top with 30 to 40 ml of APW. Enrichment broths were incubated at ambient temperature, subcultured after 6, 12, and 18 h of incubation, and processed as described above.

Although *V. cholerae* O1 isolates are common clinical isolates in our laboratory, no *V. cholerae* O1 isolates were recovered during our investigations with tampons. Therefore, the data presented are based on isolations of non-O1 *V. cholerae* (nonagglutinable), which we used as an indicator for each method's usefulness as an isolation technique for *V. cholerae* O1.

Statistical tests for differences in proportions were the chi-square test with Yates' correction and Fisher's exact

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TABLE 1. Test results for 37 Moore swab-vaginal tampon pairs cultured for *V. cholerae*^a

Culture results ^b for test pair		No. of pairs	% of total
Moore swab	Tampon		
+	+	8	21.6
+	-	8	21.6
-	+	7	18.9
-	-	14	37.8
Total		37	99.9

^a The swabs and tampons were suspended in sewers in Jakarta for 2 days before culture.

^b +, positive for non-O1 *V. cholerae* (nonagglutinable); -, negative for *V. cholerae*.

test; the latter test was used when an expected cell size was <5 (6).

Tampon-swab comparisons. In the first survey, tampon-swab pairs were recovered from 37 different locations (Table 1). With 250-ml APW enrichments incubated at 36 ± 1°C for 18 h, there was no significant difference between frequencies of isolation of nonagglutinable *V. cholerae* on swabs and tampons; of 37 culture pairs, 15 (40.5%) tampons were positive and 16 (43.2%) Moore swabs were positive ($P = 1.00$). However, it was clear that collecting two samples in each location, instead of one, greatly improved isolation frequencies for *V. cholerae*; of 23 sample pairs that were culture positive for nonagglutinable vibrios by either method, 15 (65.2%) were positive with only one of the two samples.

Enrichment times. The results of the second survey (Table 2) suggested that enrichment for 12 or 18 h may provide improved recovery of *V. cholerae* in comparison with enrichment for 6 h, although differences in isolation frequencies were not statistically significant ($P = 0.055$). The higher frequencies of isolation after 12 or 18 h of enrichment were accompanied by a larger number of suspect *V. cholerae* colonies identified on the TCBS subcultures.

Small- and large-volume enrichment. In the third survey, there were fewer isolations of *V. cholerae* when 50-ml APW enrichments were used than when 250-ml enrichments were used (Table 3). This difference was significant for the 12- and 18-h cultures ($P = 0.032$), but when the 6-h enrichments were included, the difference was less apparent ($P = 0.069$ overall). As in the previous survey, the 12- and 18-h enrichments provided higher isolation frequencies than 6-h enrichment at ambient temperatures of incubation; this difference was most pronounced within the 250-ml volume group ($P_{50 \text{ ml}} = 0.239$; $P_{250 \text{ ml}} = 0.005$).

TABLE 2. Test results for 12 vaginal tampons cultured for *V. cholerae* after enrichment in APW^a

Time of enrichment (h)	No. (%) of tampons positive	Avg no. of suspect colonies tested/plate ^b
6	9 (75.0)	6.9
12	12 (100.0)	8.7
18	12 (100.0)	10.0

^a The tampons were suspended in sewers in Jakarta for 2 days before enrichment.

^b The maximum number of colonies per plate selected for further testing was 10.

TABLE 3. Comparison of 50- and 250-ml enrichment volumes of APW for detection of *V. cholerae*^a

Time of enrichment (h)	Vol of APW (ml)	No. of tampons tested	No. (%) positive
6	50	21	14 (66.7)
6	250	21	16 (76.2)
12	50	21	19 (90.5)
12	250	21	21 (100.0)
18	50	20 ^b	16 (80.0)
18	250	21	21 (100.0)

^a Pairs of vaginal tampons were suspended in sewers in Jakarta for 2 days, enriched in APW for the indicated times, and subcultured.

^b One sample was not subcultured at 18 h.

Limitations and recommendations. These data, which have been derived from three Jakarta sewer surveys, have shown that vaginal tampons may be useful substitutes for conventional Moore swabs for isolation of *V. cholerae* from sewers or other aquatic environments. Tampons are convenient and inexpensive, and survey results showed no difference between Moore swab and tampon recovery of *V. cholerae*. We were unable to isolate *V. cholerae* O1 during our test surveys, most likely because there was no evidence that *V. cholerae* O1 shedding was occurring in the study area population. We believe that if the surveys were repeated in an area with active cases of cholera, vaginal tampons would be as effective as Moore swabs for isolation of *V. cholerae* O1 and that both would yield improved results if duplicate sampling were employed at each test site and if each sample were processed separately.

We investigated the use of ambient temperatures of incubation during the enrichment process because cholera is a tropical disease and the use of Moore swabs in *V. cholerae* sewage surveys has been limited to warmer climates. Because of limited incubator space in most laboratories, the use of 250-ml or larger containers for swab enrichment makes incubations at 37°C impractical in surveys of more than a few sites. Although we did not directly test for differences between recoveries at 37°C and at ambient temperatures, there was no indication that one temperature range was better than the other for *V. cholerae* recovery. Ambient-temperature incubations allow an unlimited number of survey sampling sites.

Using ambient temperatures for enrichment incubations, we found evidence that 12- to 18-h enrichments were superior to 6-h enrichments. This was in contrast to the results of Barrett et al. (1), who concluded that 6-h enrichments were as effective as 18-h enrichments, but was in agreement with those of Spira and Ahmed (7), who found that 18-h enrichments were superior to 6-h enrichments. However, it should be borne in mind that in both of the aforementioned investigations, enrichment broths were incubated at 37°C, in contrast to our use of ambient-temperature incubation.

Because of the large volumes of APW required for conventional Moore swab enrichments, we investigated *V. cholerae* recovery with much smaller volumes of broth. Our selection of 50-ml containers for enrichment of swabs was largely arbitrary and was based on what we perceived as a practical container size and a readily available container which offered significant reductions in medium consumption. Although 50-ml containers yield fewer positive cultures than 250-ml containers, future testing of intermediate vol-

umes (e.g., 100 ml) may provide results similar to large-volume enrichment results, with significant savings in medium costs and bench and incubator space.

In summary, both Moore swabs and vaginal tampons are useful implements for isolating *V. cholerae* from sewage, but substantially higher isolation frequencies may be obtained by using duplicate samples at each test site. For enrichment, swabs should be immersed in a large volume (250 ml) of APW, but incubation of enrichment broths at ambient temperatures for 12 to 18 h can lessen the required incubator space while still providing good recoveries.

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