Semisolid Media for Isolation of Salmonella spp. from Coastal Waters

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The use of two semisolid media (semisolid Rappaport and semisolid Rappaport-Vassiliadis) for the isolation of Salmonella spp. from coastal waters was compared with the use of conventional media. Two hundred and fifty six samples were studied; Salmonella spp. were detected in 83. The semisolid media were the most sensitive, detecting 73 samples instead of the 53 detected by the conventional media (P < 0.001). The rate of isolation of Salmonella spp. showed an increase of 56.6% when the semisolid media were added to the conventional media, it being possible to detect 54.2% of the total organisms 1 day earlier.

Fecal pollution of coastal waters is usually monitored by enumeration of indicator microorganisms, such as fecal coliforms and fecal streptococci (1). However, there is sometimes no close relationship between the presence of indicator organisms and of enteric pathogens (9, 10, 18), and Salmonella spp. have been found to survive better than Escherichia coli in seawater and estuary water (17, 19). These observations have led some authors to recommend that the pathogen itself be sought (2) as an index (3, 15) and to suggest that the detection of Salmonella spp. supplement the determination of indicators (5, 7, 9). This situation would make it necessary to have sensitive techniques available to detect Salmonella spp. in polluted waters. At present, most of the methods which are used include preenrichment followed by selective enrichment in a liquid medium and plating on a selective and differential solid medium (6, 12, 14, 21).

Recently, semisolid plating media were used to isolate Salmonella spp. from feces (8, 11) and foods (4, 16), with better results being achieved in all cases than with the use of conventional media. In this work we evaluated semisolid media for the isolation of Salmonella spp. from coastal recreational waters by comparing them with conventional media.

During the months of June, July, August, and October of 1986, 256 samples of water were collected at 34 stations located in 21 bathing beaches of the Vizcaya coast area (northern Spain). Sterile 1-liter polyethylene bottles were used to collect the samples, and analysis was performed in the laboratory within 4 h.

The samples (500 ml) were filtered through sterile 0.45-

μm-pore membrane filters (HAWG 047; Millipore Corp.). The filters were rolled, placed in 10-ml tubes of buffered peptone water (Oxoid Ltd.), gently shaken, and incubated at 35°C for 20 h. For the conventional method, 0.1 ml was transferred to 10 ml of Rappaport-Vassiliadis broth (RV) (20) and 1 ml was transferred to 10 ml of selenite F broth (SF; Difco Laboratories). The RV was incubated at 43°C and the SF was incubated at 35°C for 24 h. After incubation, each of the broths was streaked on brilliant green agar (BGA; Difco) and bismuth sulfite agar (BSA; Difco) and incubated at 35°C for 24 and 48 h, respectively.

For the semisolid method, two semisolid agars were used: semisolid Rappaport (SR) prepared as described by Goossens et al. (8) and semisolid Rappaport-Vassiliadis (SRV) prepared similarly but with RV instead of Rappaport broth. In this method one loopful of the preenrichment broth (buffered peptone water) was used to inoculate the edge of the SR and SRV plates. The semisolid media were also inoculated similarly with the enrichment broths (RV and SF). Because incubation at 43°C produced an unacceptable loss of sensitivity (16), the semisolid media were incubated at 35°C for 24 h.

Typical colonies in BGA and BSA and organisms that migrated in SR and SRV were identified biochemically and serologically (16). Confirmation of the strains was carried out at the National Salmonella Reference Centre, Majadahonda, Madrid, Spain. Statistical evaluation of the data was done with the MacNemar test for paired samples (13).

Of the 256 samples of coastal recreational waters tested, 83 were found to be positive with at least one of the media used. A total of 86 strains belonging to 18 serotypes were isolated. The most frequently isolated serotype was enteriditis (42 strains), followed by derby (7 strains), mikawasimia (6 strains), and paratyphi B (5 strains); other serotypes isolated on more than one occasion were panama, infantis, typhimurium, virchow, and brandenburg. Of these 9 serotypes, 7 were among the 10 most frequently isolated in Spain in 1986 (Anonymous, Bol. Microbiol. Semanal. 29–30:1–2, 1987). Also, the enteriditis serotype is the one most commonly isolated from human beings, in terms of both number of cases and number of outbreaks (Anonymous, Bol. Microbiol. Semanal. 29–30:1–2, 1987; I. Perales and A. Audicana, Letter, Lancet ii:1133, 1988). These facts suggest that the Salmonella serotypes detected in this study reflect those that prevail in the terrestrial habitat.

Results of seeding semisolid media after enrichment in broth were similar to those from meat products (16), except that SF-SR was less sensitive, probably because this combination is more toxic for stressed Salmonella spp. present in seawater. In general, seeding semisolid media after enrichment in broth did not show great advantages over conventionally plating media with coastal waters (Table 1), especially after enrichment in RV. However, semisolid media seeded directly from the preenrichment broth performed well, detecting the greatest number of samples that were negative on other media. This result suggests that the semisolid media are highly selective, as direct isolation from the preenrichment broth on other plating media is almost impossible because of the greater numbers of background floras that overgrow Salmonella spp. In addition, Salmo-
nella spp. only have to grow on one selective medium, whereas with the conventional method the chance for isolation might be reduced by the Salmonella spp. having to grow on two different selective media (enrichment and plating media). Furthermore, direct seeding of the semisolid media from the preenrichment broth yielded 45 positive samples (54.2% of the total) 1 day earlier than did the conventional method. SRV proved to be more productive than SR after enrichment but had a lower specificity (Table 1).

A comparison of the results of the two methods revealed the following. When conventional media were used, 53 of 83 samples (63.9%) were detected (10 Salmonella spp. were identified exclusively by this method); the specificity (as defined in Table 1, footnote b) was 34.2%. When semisolid media were used, 73 of 83 samples (87.5%) were detected (30 Salmonella spp. were identified exclusively by this method); the specificity was 22.2%. The difference between the two methods was statistically different ($P < 0.001$). The use of semisolid media along with conventional media for the detection of Salmonella spp. from coastal waters increased the number of positive samples from 53 to 83, an increase in detection of 56.6%. Therefore, we conclude that although semisolid media have some limitations (16) and lower specificities than conventional media, their use significantly increases the number of Salmonella-positive samples detected. Moreover, in our opinion the inoculation of semisolid media with greater volumes of broth (0.1 ml), in accordance with the methods followed by other authors (4; E. Erkiaga and I. Perales, Abstr. VI Reunión Nac. Microbiol. Alimentos, Madrid, Spain, p. 112–113, 1988), might additionally increase the sensitivity of those media.

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LITERATURE CITED