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DETECTION OF *SALMONELLA* IN SHELLFISH GROWN IN POLLUTED SEAWATER

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ABSTRACT

Three bays along the South African coast were studied for the presence of *Salmonella* spp in seawater, effluent and stormwater discharges into the bays and in shellfish harvested at the same sites. The microbial quality of water and shellfish samples was studied using faecal coliforms and enterococci as indicators of pollution. A combination of Rappaport-Vassiliadis enrichment broth and bismuth sulphite agar was found to provide the best enumeration method for *Salmonella* spp in samples from a wide range of origins. A poor relationship between levels of indicator organisms and the presence of *Salmonella* spp was observed at both low and high densities of indicators. A correlation between the presence of *Salmonella* spp in shellfish and surrounding seawater was frequently found and in most instances *Salmonella* contamination could be traced to discharges in the vicinity. The findings of this study question the suitability of faecal coliform limits as guidelines for microbial quality of shellfish and shellfish harvesting grounds. Most seawater sites did not comply with the recommended indicator limits. *Salmonella* spp were detected in seawater and shellfish samples which were free of faecal coliforms.

KEYWORDS

Salmonella, shellfish, seawater, indicators, marine pollution, guidelines.

INTRODUCTION

Waterborne salmonellosis remains a persistent threat to human health in many developing countries. Increase in pollution of natural water by discharge of sewage effluent intensifies the occurrence of pathogenic organisms, mainly *Salmonella* spp, thus increasing the risk to public health (Morinigo *et al.*, 1990). A wide range of bacterial, viral and protozoan parasite species may accumulate in shellfish grown in faecally polluted water and consumption of polluted shellfish has been associated with a range of infectious diseases, including salmonellosis (Halliday *et al.*, 1991).

Rapid growth in urbanisation, as observed along the South African coastal zone, is often coupled with limited sanitary facilities. This results in an increased discharge of polluted stormwater run-off and effluent into the marine environment and may subsequently pollute seawater and shellfish with pathogenic organisms, including *Salmonella.* Consumption of raw or only partially cooked shellfish magnifies the health risks associated with their pollution. It is, therefore, of great importance to devise standards or guidelines which will guard against such risks. Most guidelines available world-wide are based on limits for coliform or faecal coliform bacteria and shortcomings of such limits for shellfish quality have previously been pointed out (Gerba, 1988; Grabow *et al.*, 1989).

In this study, the microbial quality of mussels and oysters harvested in three bays along the South African coast were studied with particular reference to *Salmonella* spp. The microbial quality of seawater at shellfish harvesting ground was evaluated against South African guidelines.

MATERIALS AND METHODS

Three sites at Hout Bay, one site at False Bay and five sites in Algoa Bay were studied. Seawater, sewage effluent, stormwater, and black mussel and oyster samples were collected from these sites at monthly intervals (during 1988-1989). The presence of faecal coliforms and enterococci were studied in 100 mℓ or appropriate serial dilutions of seawater, effluent and stormwater samples and 50 g of homogenised shellfish flesh samples. Both indicators were analysed using membrane filtration in accordance with Grabow *et al.* (1989).

Analyses for *Salmonella* bacteria were carried out on 500-1000 mℓ seawater samples, 10-100 mℓ of highly polluted effluents and stormwater samples or 50 g homogenised shellfish flesh. Five enrichment (selenite brilliant green, selenite brilliant green mannitol, selenite enrichment broth, tetrathionate enrichment and Rappaport-Vassiliadis enrichment broth) and 3 selection (*Salmonella Shigella* agar, brilliant green agar and bismuth sulphite agar) media were evaluated for the enumeration of *Salmonella* spp in seawater, effluents and shellfish samples. All enrichment and selective media were obtained from Oxoid Ltd.,UK. The most efficient combination for the detection of *Salmonella* spp, in seawater, effluents and shellfish flesh samples was used during the remainder of the study. This included pre-enrichment with peptone water at pH 7.0 at 35°C, 16-20h; enrichment with Rappaport-Vassiliades enrichment broth at 42°C, 22-24h; selection on bismuth sulphite agar at 35°C, 24h; and confirmation of typical and suspected colonies using biochemical (TSI and Urea agar) and serological tests (APHA, AWWA and WPCF, 1989).

RESULTS

The results of the comparison study showed that Rappaport-Vassiliadis enrichment medium provided the best enrichment medium as indicated by the high number of samples in which *Salmonella* spp were enumerated (Fig 1; 70%). The lowest recoveries were obtained using selenite medium (Fig 1; 34%). Bismuth sulphite agar proved to be the most efficient selective medium (Fig 2; 73%). The combination of Rappaport-Vassiliadis enrichment broth and the selective bismuth sulphite agar was therefore used for the environmental studies.

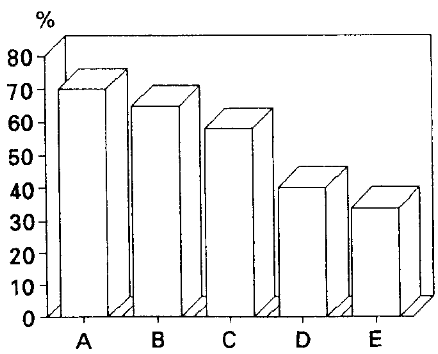

Fig. 1. Percentage of salmonellae recovered using five enrichment broths
A - Rappaport-Vassiliadis
B - Selenite-Brilliant green
C - Tetrathionate - (Müller-Kauffman)
D - Selenite - Brilliant-Green mannitol
E - Selenite - (Leifson)

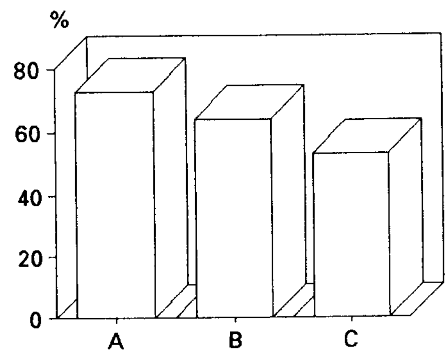

Fig. 2. Percentage of salmonellae using three selective agar media
A - Bismuth Sulphite agar
B - Brilliant green agar
C - *Salmonella Shigella* Agar

No clear relationship was found between the presence of *Salmonella* spp and levels of faecal coliforms in all environmental waters and shellfish samples studied (Table 1). A geometric mean of 12 cfu of faecal coliforms/100 mℓ (maximum counts 1 610) in Hout Bay seawater coincided with the presence of *Salmonella* spp, while no *Salmonella* were detected in seawater at the same site when a geometric mean of 286 (maximum counts 47 500) was recorded. Opposite patterns were also observed, in which higher counts of faecal coliforms did relate to *Salmonella* presence.

Table 1: Levels of Faecal Coliform Bacteria in Samples with Reference to *Salmonella* Bacteria

Location Bay area	Sample type	Number of samples	*Salmonella* Presence*	Faecal Coliforms****		
				G.M.***	Minimum	Maximum
Hout Bay	Seawater	5	+	12	0	1 610
	Shellfish	5	+	206	0	150 000
	Seawater	23	-	286	0	47 500
	Shellfish	26	-	52	0	48 000
False Bay	Stormwater	7	+	24 527	7 700	134 000
	Seawater	4	+	2 332	212	14 000
	Shellfish	4	+	52	4	139
	Stormwater	10	-	15 541	2500	168 000
	Seawater	9	-	95	0	1 800
	Shellfish	5	-	205	12	5 718
Algoa Bay **	Effluent	5	+	24 648	10	1 600 000
	Seawater	5	+	19 165	1080	150 000
	Shellfish	4	+	4 638	558	1 500 000
	Effluent	4	-	333 720	24 600	1 040 000
	Seawater	5	-	841	37	21 000
	Shellfish	5	-	9 187	368	82 233

* + Positive; - Negative for *Salmonella*
** Each sample is a composite sample of the 3-4 subsites
*** Geometric mean
**** Counts per 100 mℓ of water or 1g of shellfish flesh

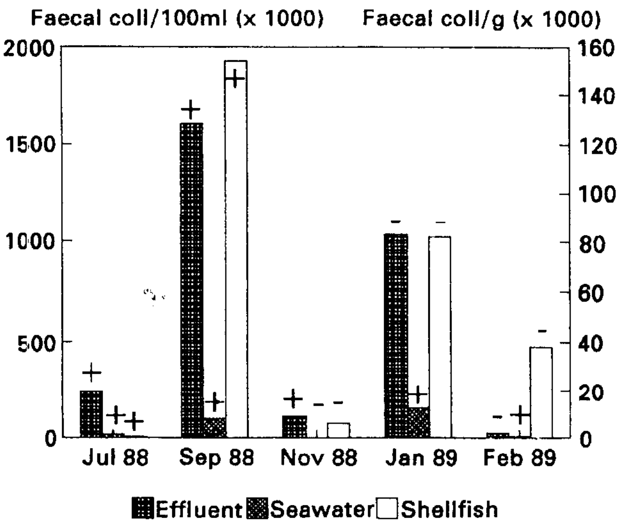

Fig. 3. Faecal coliforms and *Salmonella* in effluent, seawater and shellfish at Algoa Bay (Site 3)
+ Positive; - Negative for *Salmonella*

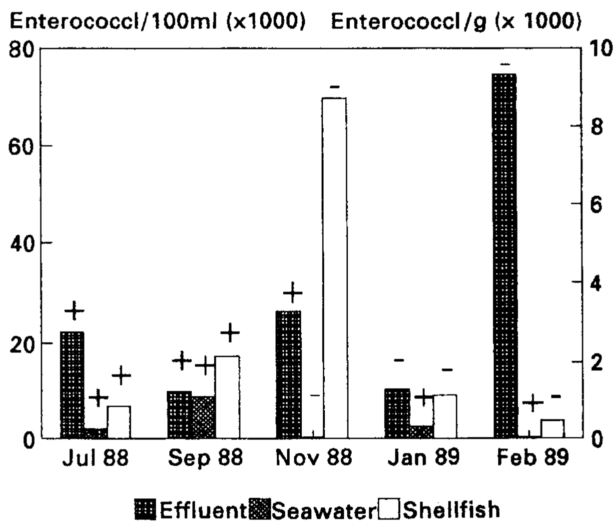

Fig. 4. Enterococci and *Salmonella* in effluent, seawater and shellfish ar Algoa Bay (Site 3)
+ Positive; - Negative for *Salmonella*

The percentage of samples which tested positive for *Salmonella* did not differ greatly between water and effluent samples and shellfish samples. At False bay 41% of stormwaters, 31% of seawaters, and 44% of shellfish samples contained *Salmonella*. The three sites tested in Hout Bay indicated 18% and 16% of seawater and shellfish samples as *Salmonella* positive while in Algoa Bay 56% of the effluent, 50% of seawater samples and 44% of the shellfish samples were also found to contain *Salmonella*. No *Salmonella* spp were detected in shellfish harvested from sites 1, 2 and 5 in Algoa Bay (Table 1) although the seawater at site 5 was found to be polluted with *Salmonella* on one occasion.

Comparison of the presence of *Salmonella* spp and levels of faecal coliforms and enterococci indicated no clear correlation between *Salmonella* presence and indicator levels (Figs 3 and 4). Although a poor relationship was found between the presence of *Salmonella* in effluent and surrounding seawater, and the presence of the bacteria in shellfish and related harvesting ground, all contamination of shellfish samples could be traced to either polluted harvesting ground or polluted discharges in the vicinity.

DISCUSSION

The use of Rappaport-Vassiliadis enrichment broth and the selective bismuth sulphite agar yielded the best results for detection of *Salmonella* spp from a wide range of environmental waters and shellfish.

The poor relationship between levels of faecal coliforms and enterococci and the presence of *Salmonella* has been reported in other studies (Morinigo *et al.*, 1990 and Faghri *et al.*, 1984). The detection of *Salmonella* spp at low levels of indicator organisms or in the absence of indicators has been attributed to the pre-enrichment and enrichment steps used for the detection of *Salmonella* spp (Morinigo *et al.*, 1990).

Evaluation of the water quality of the shellfish harvesting grounds at the studied bay areas against South African guidelines (Lusher, 1984) indicated that a large number of seawater samples exceeded the 50 and 90 percentile limits. *Salmonella* spp were detected predominantly in seawater or shellfish grown in seawater which exceeded the maximum faecal coliform limit. However, *Salmonella* spp were detected in shellfish grown in seawater free of faecal coliforms on a number of occasions, indicating possible shortcomings of the faecal coliform limit. This indicates that *Salmonella* spp may be present in shellfish which are of an acceptable microbial quality when evaluated only against faecal coliform limits. Recent technological developments allow for accurate and relatively simple direct detection methods for *Salmonella* spp using either immuno-assays or genetically based techniques (APHA, AWWA and WPCF, 1989; Tsen *et al.*, 1991). The use of such techniques offers a tool for the direct evaluation, control and management of *Salmonella* spp pollution in the marine environment.

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