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ISOLATION AND SIGNIFICANCE OF *SALMONELLA* SP. FROM SOME BEACHES OF KERALA

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ABSTRACT

Salmonella sp obtained from the sand samples of some beaches of Kerala during July-October 1978 have been reported. Its percentage occurrence was 22·7. However, water samples failed to give positive *Salmonella* isolations and there appears to be some relation between the coliforms, *E. coli* counts and the presence of *Salmonella*. The occurrence and significance of *Salmonella* in bathing beaches in relation to public health hazard have been highlighted.

INTRODUCTION

In recent years increasing attention has been drawn on the discharge of sewage and land drainage into coastal waters including the beaches. Sewage pollution of beaches is a health hazard to bathers apart from aesthetic considerations and hence needs proper precautions based on scientific investigations. Faecal pollution of beaches by 'Indicator bacteria' like coliforms, *E. coli* and *S. faecalis* has been identified and studied by Regnier and Park (1972), Tinker (1976), Berger, Jensen, Ludwig and Romer (1963), Moore (1954), Bonde (1967), Grunnet, Gundstrep and Bonde (1970), Bonde (1968), Stevensen (1953), Moore (1959) and Shuval, Cohen and Purer (1968). Published reports on the faecal contamination of Indian beaches are recent and restricted to Gore (1971), Gore and Singbal (1973), Dwivedi, Gore, Bhargava and Rajguru (1974) and Raveendran, Gore and Unnithan (1978).

Pollution of coastal waters by ever increasing sewage discharge and other sources has intensified the occurrence of pathogen particularly *Salmonella* in estuarine and marine environments. Published report on *Salmonella* isolations from estuarine and marine environments are by Buthiaux and Lewrs (1953), Steiniger (1955, 1956), Grunnet and Niesten (1969), Paoletti (1964), Johannsen (1967), Jorgensen (1962), Slanetz, Bartley and Stanley (1968), Brezenski and Russomanno (1969), Brezenski (1971) and Yosphe-Purer and Shuval (1972).

Although *Salmonella* isolation has been reported from seafoods of India (Arul James and Iyer, 1972; Joseph, Mathen and Iyer, 1976; Nerkar, Lewis and Kumta, 1975; and Iyer, Joseph and Mathen, 1975) no published record is available on *Salmonella* isolations from beaches of India. Therefore, the present attempt suggests the importance of pathogen like *Salmonella* on bathing beaches and the possible health risks to bathers.

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MATERIALS AND METHODS

Beaches were divided into three transects for convenience and the distance between the transects was kept approximately 8–9m. The zone of active sea bathing and 'seaside resort' was prominently studied. Surface sand samples were collected using sterile glass slides in sterile glass petri dishes from High Tide (HT), Mid Tide (MT) and Low Tide (LT) levels of each of the transect and taken to the laboratory in iced condition. Seawater samples (250ml) were collected directly from the surf zone in sterile glass bottles by wading some distance into the sea. The sand samples from HT, MT and LT levels of all the three transect were pooled together and used for isolation of *Salmonella*. Sand and water samples from some of the beaches viz. Vizhinjam, Shankumugham, Kovalam, Quilon, Neendakara, Alleppey, Calicut, West Hill, Kappad, Mopla and Payyambalam, were collected from July to October 1978 (Monsoon and postmonsoon periods) along with the samples for routine observations on the faecal pollution of these beaches by 'indicator bacteria'; continuous isolations of *Salmonella* in the ensuing months could not be done. Therefore, the present communication includes only a limited yet useful data on *Salmonella* from the above beaches of Kerala.

A total of 66 sand samples (22 pooled samples) and 22 water samples were subjected for *Salmonella* isolation.

Attempts were neither made to quantify *Salmonella* nor differentiate the various serotypes. AOAC methods were employed for the detection of *Salmonella*. Since this is the first report on the isolation of *Salmonella* from beaches of India, it is imperative to give detailed methodology of isolation including media used and the various biochemical reactions involved.

Preliminary enrichment was made by adding 50 gm of sand samples to 450 ml of Lactose broth and incubated for 24 hrs at 37°C. In the case of water samples, 200 ml was mixed with 400 ml Selinite broth (double strength). After 24 hrs, 1 ml each of these was added to 9 ml each of Selinite cystine broth and Tetrathionate broth and incubated for 24 hrs at 37°C. Following this, streaking was made on (a) Brilliant Green Agar, (b) S.S. Agar and (c) Bismuth Sulphite Agar and incubated again for 24 hrs at 37°C. Presumptive colonies from each of these media were streaked and stabbed on to Tripple Sugar Iron Agar slants and incubated for 24 hrs at 37°C. Thereafter, slants showing (i) Acid butt, alkaline slant, formation of gas and H_2S, (ii) Acid butt, alkaline slant, no gas no H_2S were inoculated (A) Lactose broth, (B) Sucrose broth, (c) Salicin broth, (D) Dulcitol broth, (E) Tryptone broth, (F) Streaked on to Urea Agar slants, (G) Streaked on and stabbed to Lysine Iron Agar slants; the following reactions and growth were noted:

(i)	Brilliant green Agar	: Transparent light pink/colourless colonies turning the background to reddish purple.
(ii)	S.S. Agar	: Colourless colonies.
(iii)	Bismuth Sulphite Agar	: Black, colonies with silvery metallic sheen.
(iv)	Triple Sugar Iron Agar	(a) Acid butt, alkaline slant, gas and H_2S.
		(b) Acid butt, alkaline slant, no gas, no H_2S.
(v)	Tryptone broth	: No purple colour on addition of Kovac's reagent.
(iv)	Urea Agar	: Negative

(vii)	Lysine Iron Agar slants	: Purple slant, acid butt and H_2S.
(viii)	Lactose broth	: No acid, no gas.
(ix)	Sucrose broth	: No acid, no gas
(x)	Salicin broth	: No acid, no gas.
(ix)	Dulcitol broth	: Acid and gas.

RESULTS AND DISCUSSION

Salmonella occurred in a few beaches in certain months (Tables I and II). No attempts were made to quantify the *Salmonella* population so also the serotypes were not differentiated. Considerable difficulties were encountered to make *Salmonella* counts in the present environment which receives continuous discharges from various sources. Grunnet, Gundstrep and Bonde (1970) have stated that in receiving waters, it is only possible to detect *Salmonella* qualitatively. The presence of *Salmonella* only in the beach sand and not in water as found in the present investigation is interesting. It is possible that sand acts as a filter and offers a better substratum and, therefore, the presence of *Salmonella* is restricted to sand samples only. This presumption could be supported by the findings of Moore (1954), who, after considerable observation on certain beaches in U. K. reported that sand is a better index in general of the degree of faecal pollution in a given beach. Even, in the heavily polluted beaches like Vizhinjam, Neendakara and Mopla, the water samples failed to yield any positive isolations of *Salmonella*

Table I. Occurrence of *Salmonella* in some beaches of Kerala.

Month	Beach	Faecal matter			*Salmonella*
		HT	MT	LT	
July 1978	Vizhinjam	+++	++	++	*
	Kovalam	—	—	—	—
	Shankumugham	—	—	—	—
	Quilon	—	—	+	—
	Neendakara	+++	++	++	*
	Alleppey	++	+	++	—
August 1978	Calicut	+	—	++	—
	West Hill	+	+	+++	*
	Kappad	+	—	++	*
	Mopla	++	+	++	—
	Payyambalam (Cannanore)	—	—	+	—
September 1978	Vizhinjam	+++	++	+++	—
	Kovalam	—	—	—	—
	Shankumugham	—	—	—	—
	Quilon	—	+	—	—
	Neendakara	++	+	++	—
	Alleppey	++	+	+	—
October 1978	Calicut	+	—	++	—
	West Hill	—	—	+	—
	Kappad	—	—	+	—
	Mopla	++	—	+	*
	Payyambalam (Cannanore)	—	—	+	—

HT=High Tide; MT=Mid Tide; LT=Low Tide; +=Minimum present; ++=Medium; +++=Abundant; *=Present; −=Absent.

Table II. Quantitative abundance of coliform, *E. coli* and *S. faecalis* in some beaches of Kerala.

Month	Beach	Coliform* /100 gm (wet basis)	*E. coli** / 100 gm (wet basis)	*S. faecalis** / 100 gm (wet basis)	*Salmonella*
July 1978	Vizhinjam	9000	1400	1500	Present
,, ,,	Neendakara	30000	3800	2000	,,
August 1978	West Hill	700	100	100	,,
,, ,,	Kappad	1700	200	1000	,,
October 1978	Mopla	1800	2000	1300	,,

* The counts are average of all the three transects pooled together.

which may readily be explained in the light of Moore's (1954) findings from South Devon Beach studies.

Beaches like Vizhinjam, Neendakara and Mopla had abundant faecal deposits throughout the investigation while in West Hill and Kappad beaches faeces was predominant in August only and this accounts for the occurrence of *Salmonella* even in these two less polluted beaches. However, failure to detect *Salmonella* in September even in heavily polluted beaches like Vizhinjam, Mopla and Neendakara is rather strange but may be explained in the words of Geldreich (1974-75), who remarked "inability to detect *Salmonella* in a place where faecal pollution is well-marked, does show that occurrence of *Salmonella* is highly variable". Macrofaeces in Kovalam, Shankumugham, Quilon, Aellppey, Payyambalam and Calicut was low and registered low coliform, *E. coli* and *S. faecalis* counts correspondingly no positive *Salmonella* isolations. In general, *Salmonella* incidence occurred in association with coliforms, and *E. coli* in all the beaches.

Grunnet, Gundstrep and Bonde (1970) reported that *Salmonella* were increasing with increasing *E. coli* number in Gulf of Aarhas; noticed a rectilinear relationship between *Salmonella* and MPN *E. coli*. They contented that at hygienic levels 1000 *E. coli*/100 cc of Gulf water, *Salmonella* isolations were mostly positive. This standard can be conveniently compared to the counts obtained from sand samples in the present beaches. Geldreich (1974) also reported a correlation between coliform densities and *Salmonella* occurrence in estuarine environment. According to his observations in cases where faecal coliforms exceed 200/100 ml, *Salmonella* occurrence ranged from 6.5 to 31% It was double when the density of coliforms is 1000/100ml in estuarine water. In grossly polluted waters *Salmonella* occurrence was 44 to 72%. Paoletti (1964) reported a frequency of 13% positive *Salmonella* isolations when the coliform counts ranged between O and 1000/100ml, 29% when the same was 1000 and 10000/100ml, 40% when the same was over 10000/100ml. Johannsen (1967) and Jorgensen (1962) working from Scandinavian coast reported that *Salmonella* was scarce when *E. coli* counts were less than 10000/100ml in 1/3 of the samples tested; 90% positive *Salmonella* isolations were registered when the *E. coli* counts were above 100000/100ml. These findings are comparable with the present data although in a limited sense. In the present ivestigation, the percentage occurrence of *Salmonella* was 22.71, and the average coliform and *E. coli* per 100 gm of sand was 7200 and 1250 respectively. Comparison could be made with Paoletti's observation who reported 29% *Salmonella* isolation when the coliform count was 1000-10000/100ml. The presence of *Salmonella* in Kappad and West Hill beaches where the *E. coli* counts are comparatively low

(100 and 200/100gm), may be explained by the fact that pathogens like *Salmonella* can survive for long periods in the sea and due to rapid destruction of coliforms, *Salmonella* can be present even in coastal waters of relatively low coliform densities (< 2400/100ml) (Moore, 1954).

Beaches showing acute faecal pollution like Vizhinjam, Neendakara and Mopla registered coliform, *E. coli* and *S. faecalis* counts above the accepted limits (Raveendran, Gore and Unnithan, 1978). The presence of pathogen like *Salmonella* has further aggravated the situation. This organism can survive for long periods particularly in the beach environment and may come into direct contact with people and therefore poses a health risk. Certainly, then, these beaches along with those less polluted ones like Kappad and West Hill should be viewed with alarm from public health stand point apart from aesthetic considerations. The public health risk associated with bathing and recreation on these beaches has to be correlated with epidemiological findings. Since, no evidence of this nature has so far come to the notice of the authors, it is not advisable to comment on the unsuitability of these beaches for recreation and associated human activities.

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* Original paper not seen