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), Stevenson
(1953), Moore (1959) and Shuval, Cohen and Purser (1968). Published reports on the
faecal contamination of Indian beaches are recent and restricted to Gore (1971), Gove and
Singhal (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 (250 ml) 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 Triple Sugar Iron Agar slants and incubated for 24 hrs at 37°C. Thereafter, slants showing (i) Acid butt, alkaline slant, formation of gas and H₂S, (ii) Acid butt, alkaline slant, no gas no H₂S were inoculated (A) Lactose broth, (B) Sucrose broth, (c) Saličin 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 H₂S.
(b) Acid butt, alkaline slant, no gas, no H₂S.
(v) Tryptone broth : No purple colour on addition of Kovac's reagent.
(vi) Urea Agar : Negative
(vii) Lysine Iron Agar slants : Purple slant, acid butt and H₂S.
(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>
<thead>
<tr>
<th>Month</th>
<th>Beach</th>
<th>Faecal matter</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1978</td>
<td>Vizhinjam</td>
<td>+ + +</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Kovalam</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Shankumugham</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Quilon</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Neendakara</td>
<td>+ + +</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Alleppey</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>August 1978</td>
<td>Calicut</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>West Hill</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td></td>
<td>Kappad</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mopla</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Payyambalam</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>(Cannanore)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 1978</td>
<td>Vizhinjam</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td></td>
<td>Kovalam</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Shankumugham</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Quilon</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Neendakara</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alleppey</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>October 1978</td>
<td>Calicut</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>West Hill</td>
<td>-</td>
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<td></td>
<td>Kappad</td>
<td>-</td>
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<td></td>
<td>Mopla</td>
<td>+ +</td>
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<td>Payyambalam</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>(Cannanore)</td>
<td></td>
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</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Month</th>
<th>Beach</th>
<th>Coliform* /100 gm (wet basis)</th>
<th>E. coli* /100 gm (wet basis)</th>
<th>S. faecalis* /100 gm (wet basis)</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1978</td>
<td>Vizhinjam</td>
<td>9000</td>
<td>1400</td>
<td>1500</td>
<td>Present</td>
</tr>
<tr>
<td>&quot;</td>
<td>Neendakara</td>
<td>3000</td>
<td>3800</td>
<td>2000</td>
<td>&quot;</td>
</tr>
<tr>
<td>August 1978</td>
<td>West Hill</td>
<td>700</td>
<td>100</td>
<td>100</td>
<td>&quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>Kappad</td>
<td>1700</td>
<td>200</td>
<td>1000</td>
<td>&quot;</td>
</tr>
<tr>
<td>October 1978</td>
<td>Mopla</td>
<td>1800</td>
<td>2000</td>
<td>1300</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* 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 faecal 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, Acllpey, Payyambalam and Calicut was low and registered low coliform, E. coli and S. faecalis counts corresponding 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 contended 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 0 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 investigation, 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
Isolation and significance of salmonella sp.

(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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REFERENCES


*Original paper not seen*