Sarcina ventriculi in the Faeces of Healthy Children and Adults in Southern India

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ABSTRACT

Sarcina ventriculi in southern India. Fresh stool specimens were obtained from different groups of subjects and cultured for S. ventriculi. The organism was found in 67% of specimens from rural children aged 2-7 years, in 6% of specimens from urban children of the same age, and in 42% of specimens from adults. The prevalence of the organism in the stools appeared to be related to the type of diet and to the degree of exposure to a contaminated environment. Its significance in the human gut could not be established.

INTRODUCTION

Sarcina ventriculi is wide spread in nature(1). It was believed that normal faeces of man and animals did not contain S. ventriculi(2), though it had been found in the gastric and intestinal contents of man in the presence of gastro-intestinal disorders(1,3). However, Crowther (4) demonstrated the presence of this organism in the faeces of healthy adults who were subsisting on a largely vegetarian diet, and particularly those living in the tropics. There has been no previous report on the occurrence of S. ventriculi in the faeces of normal healthy children. This paper deals with the prevalence of this organism in various groups of people, including children, in and around Vellore, Tamil Nadu, India.

MATERIALS AND METHODS

Freshly voided stools samples were collected from both children and adults. None of the subjects included in the study had any diarrhoeal disorder at the time of collection of the stool samples. None of them were also on any antibiotic therapy. There were 27 rural and 18 urban children aged 2 to 7 years. The adult group included 31 healthy adults and 24 hospitalised adults.

The rural children were from a village 3 miles from the laboratory. They belonged to the lower socio-economic group and ate a typical vegetarian diet similar to that described by Rao and Rao(5). They were all adequately nourished according to the classifica-

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**Wellcome Trust in collaboration with the World Health Organization
tion of the Jamaica Conference(6). In the village, there were no facilities for disposal of faeces, and the drinking water supply was unprotected.

The urban children belonged to a higher socio-economic group and ate a balanced mixed diet, including both animal and vegetable proteins. They lived in the hospital residential campus with modern sanitary facilities for disposal of faeces and a well protected water supply. Thus the environment was totally different from that of the village.

The healthy adults were a mixed group from the staff of the research and diagnostic microbiology laboratories of the hospital. They lived in different areas in and around Vellore, some with facilities for disposal of faeces, others without such facilities. They included both strict vegetarians and non-vegetarians who generally ate a typical vegetarian diet(5).

Most of the hospitalised adults were from the rural area. They belonged to the poorer socio-economic group and were on a largely vegetarian diet(5). They were hospitalised for investigation of anaemia or other conditions but had no evidence of gastrointestinal disease.

Method of isolation. The method of Crowther(4) was followed. In brief, 0.5g of faeces was weighed and suspended in 4.5 ml of sterile normal saline to make a 1-10 dilution. For selective isolation, as S. ventriculi forms heat resistant spores (7, 8), the suspension was homogenised and heated in a water bath at 70°C for 10 min. Serial ten-fold dilutions from 10^1 to 10^4 were prepared. After each dilution 0.1 ml was spread on to half plate each of Willis and Hobbs medium (WH) (9) and tomato juice agar (TI) (Oxoid). The plates were incubated at 37°C for 48 hours in an anaerobic jar as described earlier(10).

Sarcinae were also isolated occasionally from dilutions of unheated stool specimens inoculated routinely on reinforced clostridial agar (Oxoid).

Plates were examined for typical colony morphology. They were sub-cultured both aerobically and anaerobically to confirm the anaerobic nature of the colonies, and were also inoculated into Robertson’s cooked meat broth (RCM) with 1% glucose (4) for further study. Results were expressed as the number of viable organisms per gram of faeces. Smears were made and stained by Gram’s method.

A limited number of biochemical tests and the antibiotic susceptibility tests were done as described by Holdeman and Moore(11).

1. Fermentation tests using peptone-yeast-extract (PY) sugar broths with 1% of the following carbohydrates, with pH adjusted to 7.2 - 7.4.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Lactose</th>
<th>Maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Xylose</td>
<td>Mannite</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Arabinose</td>
<td></td>
</tr>
</tbody>
</table>
The sugars were held for a week before reporting as negative. The fermentation of sugars was recorded by determining the terminal pH of the individual PY sugar broths using a Beckman pH meter. Before recording the results, smears were made from all test broths to ascertain the purity of the cultures.

2. Indole test using the Ehrlich's reagent, employing the supernatant of RCM broth culture 48 hours' old.

3. Catalase test by the slide method with isolated colonies of \textit{S. ventriculi} using 3% hydrogen peroxide (12).

4. Antibiotic susceptibility test using reinforced clostridial agar media (Oxoid) enriched with 1% glucose, by the single disk* sensitivity test method.

**RESULTS**

\textit{Colony characteristics: Willis and Hobbs medium.} When immediately taken out from the anaerobic jar the typical colonies of \textit{S. ventriculi} appeared raised, slightly rough and pale yellow in colour, 2-4 mm in size, and surrounded by a yellow halo of varying size. When exposed to oxygen, the colonies turned pink in colour and were surrounded by a pale pink halo due to lactose fermentation. Colonies were very typical and easily distinguishable from the colonies of \textit{Cl. welchii}.

\textit{Tomato juice agar.} The colonies appeared dirty yellow and raised with a rough surface and an irregular margin, 3-4 mm in size.

\textit{Reinforced clostridial agar (RCA).} From unheated stool specimens, inoculated routinely on RCA, colonies of sarcinae were observed at times. They appeared as greyish raised colonies with a rough surface and an irregular margin, 2-4 mm in size.

\textit{Microscopic morphology.} When stained by Gram's method, sarcinae were seen as large gram positive cocci, often mixed with decolourised forms, in packets of 8's and 4's. There was no suggestion of spore formation. However, no special staining to demonstrate spores was done.

\textit{Other features.} Aerobic subculture showed no growth, confirming the anaerobic nature of the organism. It was found extremely difficult to maintain the isolated strains of \textit{S. ventriculi} viable and hence the limited biochemical studies were confined to only 4 isolates.

The 4 strains tested produced acid and gas from glucose, sucrose, salicin, xylose and lactose at the end of 48 hours of anaerobic incubation. The pH of the sugar broths that were fermented ranged between 5.0 to 5.4 the original pH being 7.2 to 7.4. Maltose, arabinose and mannite were not fermented. All were catalase negative; two were indole negative and two were indole positive.

*Purchased as dry disks from Difco Laboratories, Detroit, Michigan, U.S.A.
The antibiotic sensitivity test showed all the four isolates resistant to erythromycin, penicillin, kanamycin, neomycin and colistin. But they were all susceptible to tetracycline, chloramphenicol and ampicillin.

Prevalence. The prevalence of *S. ventriculi* in the different groups is shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No.</th>
<th>Number positive</th>
<th>% positive</th>
<th>Range of count/g faeces</th>
<th>Mean count/g faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural children</td>
<td>27</td>
<td>18</td>
<td>67</td>
<td>$10^2$-$10^7$</td>
<td>$10^{4.7}$</td>
</tr>
<tr>
<td>Urban children</td>
<td>18</td>
<td>1</td>
<td>6</td>
<td>$10^3$</td>
<td>$10^{3.2}$</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>31</td>
<td>13</td>
<td>42</td>
<td>$10^2$-$10^7$</td>
<td>$10^{5.1}$</td>
</tr>
<tr>
<td>Hospitalised adults</td>
<td>24</td>
<td>10</td>
<td>42</td>
<td>$10^5$-$10^7$</td>
<td>$10^{4.8}$</td>
</tr>
</tbody>
</table>

The viable counts of sarcinæae ranged between $10^2$-$10^7$ per gram of faeces but in most cases, the counts were in the vicinity of $10^5$ organisms per gram of faeces.

Isolation rates on different media. The isolation rates of *S. ventriculi* on tomato juice agar and Willis and Hobbs media are shown in table 2. Though the number of isolates on WH was slightly more, the difference between this and TJ was not statistically significant.

<table>
<thead>
<tr>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (TJ+RCA+WH)</td>
<td>42</td>
</tr>
<tr>
<td>TJ</td>
<td>32</td>
</tr>
<tr>
<td>WH</td>
<td>36</td>
</tr>
</tbody>
</table>

TJ = Tomato juice agar  
RCA = Reinforced clostridial agar  
WH = Willis and Hobbs’s medium

DISCUSSION

Isolation and characterization. As sarcinæae were most often present in counts of $10^5$ organisms per gram of stool, unless special selective methods were introduced, it would not have been possible to detect the organism, as the other organisms, forming the predominant fecal flora (10), would mask its presence. Sarcinæae occurred in the faeces in numbers similar to *Cl. welchii* (10), and formed a minor component of the fecal flora.

The colonial morphology, particularly on Willis and Hobbs medium, the microscopic appearance of the organisms and their anaerobic nature were typical of sarcinæae. Though
gas-liquid chromatography could not be done to distinguish it from *S. maximum* which produces butyric acid from glucose, the fact that the pH, following fermentation of sugar, was between pH 5.0 to 5.4 was taken as indicative of *S. ventriculi* (2).

Though *S. ventriculi* is described as indole negative (11), of the 4 strains tested in this report, two produced indole. In the report of Crowther (4) its ability to produce indole was not mentioned.

The organism differed from other gram positive spore formers and anaerobic gram positive cocci, in being resistant to erythromycin and penicillin (13). All the four isolates were all susceptible to tetracycline, chloramphenicol and ampicillin. However, like the gram positive anaerobes, they were resistant to neomycin, kanamycin and colistin (13).

*Prevalence in the population.* *S. ventriculi* was isolated from 42 of the 100 stool samples studied. The organism was common in the stools of adults and rural children, but was very rare in the stools of the urban children in the study.

In a study of adults, Crowther (4) found that those who were living in the tropics and eating a vegetarian diet had the organism more frequently in their stools. The prevalence among the three groups of adult vegetarians, including a group of adults living in and around Vellore, studied by him, ranged from 33% for Vegans in England to 84% for adults in Uganda. Among Caucasians living on a mixed diet Crowther (4) found a prevalence of about 7%, which is similar to that seen in urban children in the present study. Crowther (4) suggested that diet was the major determinant for the prevalence of *S. ventriculi*.

It is possible that the biochemical environment in the large gut of vegetarians may favour colonization by *sarcinae* as shown by the occurrence of *sarcinae* in the stools of most of the rural children (67%) and the adults (42%) who more or less ate a largely vegetarian diet. In contrast only one (7%) among the 18 urban children had *sarcinae*. Faecal pollution of the environment also seemed a factor in the case of the rural children.

However the significance of this finding in relation to the microbial flora of the human gut could not be established.

**ACKNOWLEDGEMENTS**

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