SHORT ARTICLES

HAEMOPHILI IN FAECES

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SUMMARY. By plating faeces on a selective medium, haemophili were isolated from 26% of 612 samples from patients of all ages and 12% of 525 samples from apparently healthy meatworkers. Most isolates were Haemophilus parainfluenzae. Only specimens received for laboratory investigation for pathogens were examined in this study; the faecal carriage of haemophili in healthy persons is not known.

INTRODUCTION

This study was prompted by the finding of Haemophilus species on Skirrow's medium (Skirrow, 1977) during the examination of faeces for Campylobacter. Haemophili do not appear to have been reported in faeces previously. A more appropriate selective medium was therefore devised to determine their incidence in faeces.

MATERIALS AND METHODS

Faeces. Samples were from two sources: those designated as group 1 were from patients aged 1 week–92 years being investigated for a variety of gastrointestinal symptoms. Group-2 samples were from meatworkers aged 15–64 years being tested routinely for intestinal bacterial pathogens.

Isolation medium. Heated blood agar was used, to which the following antibiotics were added: bacitracin 5000 μg/L, vancomycin 10 mg/L, polymyxin B 2500 μg/L and nystatin 5000 μg/L. Plates were inoculated in the standard manner and incubated at 37° in air containing 10% CO2. Haemophilus colonies were subcultured on to heated blood agar for gram staining to determine microscopic morphology and to confirm purity.

Tests for dependence on X and V factors were done on Oxoid "Iso-sensitest" Agar and each isolate was also tested for its ability to grow, and for X and V factor dependence, on MacConkey Agar No. 2 (Gibco). X, V and XV-factor strips (B. B. L.) were placed on these media after inoculation by flooding with a heavy suspension of each isolate in saline.

Haemolysis was determined on blood agar with added V-factor strips.

Antibiotic-sensitivity tests were performed by disk diffusion on Oxoid "Isosensitest" Agar containing 7% lysed horse-blood.

Bile sensitivity was determined on heated blood agar containing the equivalent of 20–25% (20 g/L) or 40–50% (40 g/L) of fresh ox bile (Oxoid).

Urease production was detected on Christiansen's urea agar slopes (CSL) with added XV strips.

β-galactosidase production was detected in ONPG Broth (Bio-Science) containing an XV strip.

Ornithine decarboxylase (ODC) production was detected with Pathotec I.D. Strips.

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All biochemical test media, antibiotic-sensitivity test media and media in tests for growth were inoculated from the same heavy suspension of the organism in saline. All biochemical tests were incubated for 48 h. Tests for indole production and reduction of nitrate were used early in the study but were discontinued because they were of no help in biotyping. Strains of *H. parainfluenzae* were subsequently typed by the method of Kilian (1976) by urease, ODC and ONPG tests.

**RESULTS**

One hundred and fifty-nine (26%) of 612 samples of faeces from group-1 patients yielded haemophili. Of 525 samples from group 2, 63 (12%) yielded haemophili. In all age groups, the percentage of haemophili isolated from group 1 (see the table) was higher than from group 2.

Haemophili were found more often in samples from group 1 when bacterial pathogens or parasites were present. Overall, 31·9% of patients with pathogenic bacteria or parasites in their faeces were found to be excreting haemophili also. Of 115 samples of faeces containing parasites 38 (33%) contained haemophili. Microscopy for parasites was not done on samples from group 2.

The numbers of haemophili isolated ranged from a few colonies only to profuse growth and many samples contained more than one type. No difference was noted in these respects between groups 1 and 2.

Haemophili remained viable in faecal specimens refrigerated at 5–10°C for at least 3 days and were isolated from some specimens after refrigeration for 2 weeks.

In all, 289 strains of haemophili were isolated from 222 faecal samples. Seventeen strains were classified as *H. influenza*, 235 as *H. parainfluenzae* and 37 could not be classified because of variable results in tests for X-factor dependence. The porphyrin test, as described by Kilian (1974), for differentiating *Haemophilus* strains could not be done because the substrate could not be obtained.

During the second half of the study, strains of *H. parainfluenzae* were biotyped by the method of Kilian (1976). Of 115 strains, 49 (42·6%) were of biotype I, 48 (41·7%) of biotype II and 18 (15·7%) of biotype III.

Most of the strains of *H. parainfluenzae* isolated produced colonies varying in colour from grey to yellow with a butyrous consistency but not easily emulsifiable. Long thin bacilli predominated in gram-stained smears, often curved and filamentous and often in clumps. Some

**Table**

<table>
<thead>
<tr>
<th>Micro-organisms detected</th>
<th>Number (and percentage) of patients yielding indicated result in age group (years):</th>
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<tbody>
<tr>
<td></td>
<td>0–&lt;2</td>
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<tr>
<td><strong>Haemophilus</strong> only</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>80</td>
</tr>
<tr>
<td>Haemophilus and other</td>
<td></td>
</tr>
<tr>
<td>Other bacteria or parasites or both, only</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>176</td>
</tr>
<tr>
<td>Total with Haemophilus</td>
<td>43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>0–&lt;2</th>
<th>2–&lt;6</th>
<th>6–10</th>
<th>11–20</th>
<th>21–50</th>
<th>&gt;50</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>(45·5)</td>
<td>(27·9)</td>
<td>(41·8)</td>
<td>(61·7)</td>
<td>(52·5)</td>
<td>(75·9)</td>
<td>(46·1)</td>
</tr>
<tr>
<td>(11·4)</td>
<td>(8·2)</td>
<td>(19·8)</td>
<td>(15·0)</td>
<td>(15·0)</td>
<td>(13·8)</td>
<td>(12·9)</td>
</tr>
<tr>
<td>(13·1)</td>
<td>(24·5)</td>
<td>(15·4)</td>
<td>(6·7)</td>
<td>(3·8)</td>
<td>(0)</td>
<td>(13·1)</td>
</tr>
<tr>
<td>(30·1)</td>
<td>(39·5)</td>
<td>(23·1)</td>
<td>(16·7)</td>
<td>(28·7)</td>
<td>(10·3)</td>
<td>(27·9)</td>
</tr>
<tr>
<td>(24·4)</td>
<td>(32·7)</td>
<td>(35·2)</td>
<td>(21·7)</td>
<td>(18·8)</td>
<td>(13·8)</td>
<td>(26·0)</td>
</tr>
</tbody>
</table>
smooth strains consisting of cocccobacilli were isolated but these were rare and were not more common in either group. β-haemolytic strains were uncommon in both groups.

Of the 289 strains isolated 41 (14.2%) were resistant to ampicillin. Seventy-seven strains were resistant to one or more of the following: ampicillin, tetracycline, erythromycin, trimethoprim, compound sulphonamides, chloramphenicol or kanamycin. Seventeen (5.9%) of the strains were resistant to trimethoprim, this resistance accounting for their growth on Skirrow's medium.

Of the strains of *H. parainfluenzae* isolated, 97% grew well on MacConkey agar around V-factor strips. On heated blood agar containing the equivalent of 20% fresh ox bile, 95.7% grew, and 83.4% grew in the presence of 40% bile. Of the 17 strains of *H. influenzae*, 13 grew on MacConkey agar. Subsequently, some strains of *H. influenzae* isolated from the respiratory tract were tested. More than half of them grew on MacConkey agar when X and V factors were supplied. One strain, from a case of meningitis, did not grow on this medium but did grow on heated blood agar containing the equivalent of 20% fresh ox bile.

To determine whether bile contains enough X and V factors to support growth of haemophili, a few isolates were tested on nutrient agar and blood agar which were streaked, after inoculation, with a solution of ox bile. Results of these tests showed that bile does provide enough V factor to allow normal growth of *H. parainfluenzae*. However strains of *H. influenzae* grew weakly, suggesting that little haemin was present, because these strains grew well on chocolate agar in the presence of bile.

### DISCUSSION

Kilian (1976) biotyped 122 strains of *H. parainfluenzae* from various sources. He found 37.2% to be of biotype I, 37.2% of biotype II and 25.6% of biotype III. Kilian's biotypes I and II were predominantly from oral and upper respiratory sites while strains of biotype III came from a wide variety of sources. These figures suggest that faecal isolates are similar to the biotypes from oral and upper respiratory sites.

In their colony morphology on Skirrow's medium, some trimethoprim-resistant strains of haemophilus isolated during this study resembled *Campylobacter* species. They were usually oxidase and catalase positive; curved forms were common in gram-stained smears and they generally failed to grow on aerobic blood agar. The sensitivity patterns of these two unrelated genera of bacteria were remarkably similar. It is important, therefore, that laboratories perform motility and appropriate biochemical tests to differentiate between *Haemophilus* and *Campylobacter* species. A V-factor strip placed on an aerobic blood agar subculture allows for easy recognition of *Haemophilus*.

V factor was shown to be present in ox bile but haemin was lacking. Haemin may become available from the normal diet or from the presence of blood from damaged mucosa. NAD, NADP or their precursors could also be available in the intestine from other bacteria or mucosal secretions. However, the predominance of haemin-independent strains in faeces suggests that V factor is readily available while X-factor concentrations may be variable. Kilian *et al.* (1972) found haemin-dependent strains to be rare in the oral cavity but such strains predominated in the nasopharyngeal area.

The great numbers of haemophili recovered in this study suggest that these organisms do more than merely survive the adverse acid environment of the stomach when swallowed in saliva. The more compatible environment of the small intestine, with a regular supply of V factor from bile and possibly other sources, could allow their multiplication. Whether they multiply in the lumen or on mucosal surfaces, as in the respiratory tract and mouth, is not clear. The slightly higher isolation rate of haemophilus from patients with proven bacterial or parasitic infection may be due to the less acidic environment associated with gastric disturbances and an opportunistic invasion of damaged mucosa.

This study was limited to faeces received for routine investigation. Studies of normal infants and children could demonstrate whether haemophili are part of the normal bowel flora or act as pathogens in the gut. The pathogenicity of *H. parainfluenzae* has always been considered to be low and some biotypes of *H. influenzae* are part of the normal flora in the upper respiratory tract.
Isolation of these organisms from sites other than normally sterile areas has always posed a problem for the bacteriologist and the clinician as to their pathogenic significance. The same problem may now apply to their isolation from faeces.

REFERENCES