Occurrence of enteropathogenic bacteria in children under 5 years with diarrhoea in south Tehran

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ABSTRACT

This study was carried out on 1600 rectal swabs from children under 5 years of age admitted at the health centre in Islamshahr, Tehran province, Islamic Republic of Iran, during 1998–99. The specimens were examined for various bacterial pathogens. Isolation rates were: enteropathogenic Escherichia coli 6.8%, Shigella spp. 3.4%, Salmonella spp. 2.9%, Campylobacter spp. 0.9%, Yersinia spp. 0.7%. The isolation rate was highest in the summer, except for Yersinia spp., which was predominantly isolated in spring. The results of this study demonstrate the significance of Yersinia spp. and Campylobacter spp. in patients with diarrhoea.

Présence de bactéries entéropathogènes chez des enfants de moins de 5 ans souffrant de diarrhée dans le sud de Téhéran

RÉSUMÉ La présente étude a été réalisée sur 1600 écouvillonnages rectaux effectués chez des enfants de moins de 5 ans admis au centre de santé d’Islamshahr, province de Téhéran (République islamique d’Iran) en 1998-1999. Les prélèvements ont été examinés à la recherche de divers agents pathogènes bactériens. Les taux d’isolement étaient les suivants : Escherichia coli entéropathogène 6.8 %, Shigella spp. 3.4 %, Salmonella spp. 2.9 %, Campylobacter spp. 0.9 %, Yersinia spp. 0.7 %. Le taux d’isolement était plus élevé en été, sauf pour Yersinia spp. qui était isolé principalement au printemps. Les résultats de cette étude montrent l’importance de Yersinia spp. et de Campylobacter spp. chez les patients souffrant de diarrhée.
Introduction

Diarrhoeal diseases constitute a major cause of morbidity in children. These diseases account for approximately 5–10 million deaths each year in Asia, Africa and Latin America, and are the major cause of death among 15%–20% of children under 5 years old. The annual child mortality rate reported by the World Health Organization is 120 million, of which 5 million are associated with diarrhoeal disease. Most deaths, however, occur in developing countries [1–3].

The infectious agents causing diarrhoea are usually transmitted by the faecal–oral route, i.e. through digestion of contaminated food, drinking contaminated water or direct contact with contaminated stool [4,5]. Diarrhoea is caused by a variety of infectious agents. Among the bacterial agents, 5 are important. These are *Shigella* spp., *Salmonella* spp., *Escherichia coli*, *Yersinia* spp. and *Campylobacter* spp. [6–10].

This is the first study from a poor suburban area of Tehran to evaluate the frequency of the major pathogens of childhood diarrhoea.

Methods

The study was conducted collaboratively with the health administration network of Islamshahr, a suburb with a population of about 300 000 in the south of Tehran, Islamic Republic of Iran. Rectal samples were taken from all children with diarrhoea under 5 years of age (1600 children) referring to the Health Centre of Islamshahr City in the one-year period June 1998 to June 1999. The samples were inoculated into Cary–Blair transport medium and were taken to the microbiology laboratory at the School of Public Health, Tehran University of Medical Sciences. Rectal swabs were used since the study was carried out in a relatively large geographic area where collection and transportation of fresh stool specimens were not feasible [11]. The specimens were checked microscopically (direct smear, Gram staining), then each specimen was cultured according to the standard method [11], with slight modifications as described.

In order to evaluate the role of the 5 major bacterial pathogens mentioned in the introduction, all specimens were cultured using Endo-agar (Merck 104044, Darmstadt, Germany), *Salmonella–Shigella* agar (Merck 107667), *Yersinia* selective agar (CIN) (Merck 116434), *Yersinia* selective supplement (Merck 116466), *Campylobacter* selective agar (Merck 102248) and *Campylobacter* selective supplement (Merck 102249). The cultures were incubated at an appropriate temperature.

The isolates were identified by biochemical and serological tests. For isolation of *Salmonella* spp., the specimens were inoculated in Selenite-F for 12 hours before primary culture. For isolation of *Yersinia* spp., the enrichment method (phosphate buffer saline, pH 7.0) with incubation at 4 °C was used (cold enrichment). After inoculation, the enrichment medium was incubated at 4 °C for 2 weeks, and then a loop of this was inoculated in CIN medium with supplement, and incubated for 2 further days at 25 °C. For isolation of *Campylobacter* spp., which has microaerophilic growth requirements, the gas pack system and anaerobic jar were used. After inoculation, the plates were incubated at 42 °C for 48 hours. Identification of suspicious colonies was then carried out according to standard criteria. Identification of enteropathogenic *E. coli* (EPEC) was done by slide agglutination with commercial antisera (bioMérieux, Marcy l’Etoile, France).
**Results**

Examination of the rectal swabs for 1600 children under 5 years of age showed that 235 (14.7%) children were harbouring 1 of the 5 major bacterial pathogens, i.e. enteropathogenic *E. coli* (EPEC) 109 (6.8%), *Shigella* spp. 54 (3.4%), *Salmonella* spp. 46 (2.9%), *Campylobacter* spp. 15 (0.9%), and *Yersinia* spp. 11 (0.7%) (Figure 1).

The isolation rate was much greater in males than in females, and frequency of the bacteria species isolated varied according to age group. For example, 67.0% of EPEC isolates were from children under 2 years old (serotypes: O26B6 42.2%, O55B5 24.8%, O119B14 13.8%, O127B8 9.2%, O111B4 6.4%, others 3.6%), whereas 25.9% of *Shigella* isolates and 21.7% of *Salmonella* were from children under 2 years (Table 1).

Three species of *Shigella* were identified in our study, *Sh. sonnei*, *Sh. flexneri* and *Sh. dysenteriae*, *Sh. sonnei* being the major isolate.

There was a high prevalence of *S. typhi* (group D), which contributed to 50.0% of total salmonellosis, and *S. paratyphi* B (group B) with a prevalence of 43.5%. Prevalence of *S. paratyphi* C (group C) was 6.5%.

Seasonal distribution showed that most of the cases of diarrhoea caused by *E. coli* and *Shigella*, *Salmonella* and *Campylobacter* species occurred in summer, probably owing to the increase in temperature, whereas most of the cases of diarrhoea caused by *Y. enterocolitica* occurred in spring (Table 2).

**Discussion**

Acute diarrhoea is a serious health problem for children under 5 years of age and also...
for adults [1,12]. The etiology of diarrhoea varies according to the geographic and climatic conditions. For example, in the cold areas of Europe, in contrast to the warm and tropical areas, *Vibrio cholera*, *Aeromonas* spp. and *Plesiomonas shigelloides* are seldom isolated, [3,6,12]. However, the prevalence of such psychrophilic bacteria as *Yersinia* in intestinal infections is higher in cold areas than in warm areas [13–15]. In the Islamic Republic of Iran, random studies have shown the contribution of bacterial etiologic agents to intestinal infections [16,17]. Our studies in Islamshahr showed that the 5 bacterial agents *E. coli*, *Shigella* spp., *Salmonella* spp., *Campylobacter* spp, and *Yersinia* spp. play an important role in gastroenteritis of children in this area, thus necessitating vigilance in maintaining environmental health indices.

From the diarrhoea specimens we examined, 14.7% were contaminated with one of the 5 causative bacterial agents. In a previous study done in 1993 in America, out of 4305 children with diarrhoea, 8.6% (372) were contaminated with one of the bacterial agents causing diarrhoea [14]. In another study carried out in New Caledonia in 1994, out of 2088 patients with diarrhoea, 5 bacterial pathogens were recovered in 587 (28%) cases [18].

The highest isolation rate (6.8%) was for EPEC. In a previous report made by Katouli et al. in which the frequency of EPEC serogroups was studied in 2 cities of the Islamic Republic of Iran, Tehran and Sanandaj [16].

### Table 1 Age distribution of 235 culture-positive children with gastrointestinal infection

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>EPEC</th>
<th>Shigella</th>
<th>Salmonella</th>
<th>Campylobacter</th>
<th>Yersinia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (% )</td>
<td>No.</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>30 (27.5)</td>
<td>4 (7.4)</td>
<td>4 (8.7)</td>
<td>11 (73.3)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>1 to &lt; 2</td>
<td>43 (39.4)</td>
<td>10 (18.5)</td>
<td>6 (13.0)</td>
<td>4 (26.7)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>2 to &lt; 3</td>
<td>20 (18.3)</td>
<td>10 (18.5)</td>
<td>8 (17.4)</td>
<td>0 (0)</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>3 to &lt; 4</td>
<td>14 (12.8)</td>
<td>18 (33.3)</td>
<td>10 (21.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4–5</td>
<td>2 (1.8)</td>
<td>12 (22.2)</td>
<td>18 (39.1)</td>
<td>0 (0)</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>Total</td>
<td>109 (100)</td>
<td>54 (100)</td>
<td>46 (100)</td>
<td>15 (100)</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

*EPEC = enteropathogenic Escherichia coli.*

### Table 2 Seasonal distribution of pathogenic bacteria in stool cultures from 1600 children

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of cultures</th>
<th>EPEC</th>
<th>No. of cultures positive for:</th>
<th>Shigella</th>
<th>Salmonella</th>
<th>Campylobacter</th>
<th>Yersinia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Summer</td>
<td>300 (19.7)</td>
<td>59 (19.7)</td>
<td>28 (9.3)</td>
<td>22 (7.3)</td>
<td>8 (2.7)</td>
<td>2 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>547 (24.2)</td>
<td>13 (2.4)</td>
<td>11 (2.0)</td>
<td>12 (2.2)</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>353 (19.3)</td>
<td>13 (3.7)</td>
<td>6 (1.7)</td>
<td>5 (1.4)</td>
<td>1 (0.3)</td>
<td>3 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>400 (26.0)</td>
<td>24 (6.0)</td>
<td>9 (2.3)</td>
<td>7 (1.8)</td>
<td>5 (1.3)</td>
<td>5 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1600 (100)</td>
<td>109 (6.8)</td>
<td>54 (3.4)</td>
<td>46 (2.9)</td>
<td>15 (0.9)</td>
<td>11 (0.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses show the number of isolates/100 stool cultures. EPEC = enteropathogenic Escherichia coli.*
The highest isolated serogroup in patients was O20ac (13.6%) in Tehran and O18ac (19.1%) in Sanandaj. More than 27% of EPEC isolates in our study were from children 1 year old or under, 67.0% from those under 2 years and more than 82% were from children under 3 years. The results show the significance of age in the etiology of gastroenteritis in children under 5 years.

Of the 3 species of Shigella identified in our study (Sh. dysenteriae, Sh. flexneri and Sh. sonnei, which belong to groups A, B, and D respectively), Sh. sonnei was the major isolate. No Sh. boydii (group C) was isolated. It has been reported that in recent years Sh. flexneri has been the dominating species in the Islamic Republic of Iran [16,19] but in our study, this species was less prevalent than Sh. sonnei.

Salmonella was the next most prevalent organism. The Salmonella strains we isolated belonged to 3 groups, B, C and D. There was a significantly high prevalence of S. typhi (group D).

The fourth most prevalent etiologic agent found in children’s diarrhoea in this study was Campylobacter. The morphology of this bacterium is quite different from the 4 others. They are all Gram-negative bacilli belonging to the Enterobacteriaceae, whereas Campylobacter belongs to the Spirillaceae. The prevalence of Campylobacter was a little higher (0.9%) than Yersinia (0.7%). All Campylobacter strains (15) isolated were from children under 2 years of age. We identified 86.7% as C. jejuni and 13.3% as C. coli.

Eleven specimens (0.7%) were diagnosed as Yersinia spp., Y. enterocolitica, Y. intermedia, and Y. fredriksenii. Two species, Y. intermedia and Y. fredriksenii, are usually isolated from contaminated waters. Thus, controlling the sanitary and hygiene conditions of water is very important. Out of the total positive cases for Yersinia spp. (11), 73% (8) were seen in winter and spring seasons. This finding shows agreement with previous studies [13,20].

Finally, further studies on the prevalence of such rare intestinal pathogens as Aeromonas hydrophila, Clostridium difficile, and various E. coli strains should be performed in other geographic areas to obtain more comparable epidemiological data.

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References


