A 5-year study of the bacterial pathogens associated with acute diarrhoea on the island of Crete, Greece, and their resistance to antibiotics

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Accepted in revised form 24 June 2002

Abstract. During a 5-year period (1995–1999) a total of 7090 stool samples obtained from patients with acute diarrhoea, mostly community-acquired, were examined for bacterial pathogens, in the Greek island of Crete. One or more enteric pathogens were isolated from 987 patients (14%). Salmonella enterica were the most commonly isolated bacteria (6%), followed by Campylobacter spp. (4.2%), and enteropathogenic Escherichia coli (EPEC) (1.8%). Yersinia enterocolitica (0.6%), Shigella spp. (0.3%), and Aeromonas hydrophila (0.04%), were less frequently isolated. Clostridium difficile was isolated from 65 out of 451 diarrhoeal specimens examined (14.4%). Toxin B was detected in all cases. No verotoxigenic E. coli strains were identified. Resistance to ampicillin was observed in 31.5% of the Salmonella, 58.3% of the Shigella and 31.5% of the EPEC isolates. Resistance to trimethoprim-sulfamethoxazole was observed in 4.4% of the Salmonella, 30.5% of the Shigella, and 18.5% of the EPEC isolates. High percentages of resistance to quinolones (44.5% to norfloxacin, and 40.5% to ciprofloxacin), were found among Campylobacter isolates, while resistance to erythromycin was observed in 14.9% of them. With the present study we continue the surveillance of bacterial pathogens associated with diarrhoeal disease on the island of Crete.

Key words: Bacterial pathogens, Crete, Diarrhoea, Resistance

Introduction

Diarrhoeal diseases are a common cause of morbidity and mortality in many countries, especially among children [1, 2]. In countries like Bangladesh, India, Guatemala, and Brazil, the attack rates reach 7–19 cases per person per year [3], while in parts of the USA the attack rate is 1.4 episodes per person per year, or lower [1, 4].

Crete, is the largest island of Greece. During the last 20 years the living standards of the population have improved dramatically. Prosperity brought immigrants seeking employment. Additionally, the island is a tourist resort. Despite the high living standards, diarrhoeal diseases remain a health problem due to the fact that the rapid changes did not permit effective organization of the public health services [5, 6].

In 1994, we reported an outbreak of Shigella sonnei in a local village and for the first time, in 1997, the bacterial pathogens associated with diarrhoea on the island [5, 6]. With the present study we continue the effort to determine the prevalence of enteropathogenic bacteria on the island, and their resistance to antibiotics.

The study has examined cases of diarrhoea in the area of Heraklion, a city with a population of 150,000 and a metropolitan area of 300,000. This area is served by two major hospitals of equal capacity. All acute cases are admitted randomly, regardless of insurance status and nationality. Hence, the patients are representative of the local population and thus, the study can describe the types of diarrhoeal diseases that occur in this area, accurately.

Materials and methods

From January 1995 to December 1999, 7090 stool samples were collected from patients presenting with acute diarrheal disease to the University Hospital of Heraklion. If diarrhoea developed at home, it was considered community-acquired, whereas if it developed while the patient was being hospitalized for at least 3 days, it was considered nosocomial. Acute diarrhoea was defined as the passage of three or more loose or watery stools within the previous 24 hours, for <15 days. One specimen from each patient was collected in plastic containers or on sterile cotton swabs with the Cary-Blair transport medium (BBL Microbiology Systems, Cockeysville, MD), and was examined in the laboratory, timely. Stools were cultured for Salmonella spp., Shigella spp., Campylobacter spp., Yersinia spp., enteropathogenic Escherichia coli (EPEC), and Aeromonas spp. MacConkey, Salmonella–Shigella, and Hektoen agar plates (all media products of bioMérieux, Marcy l’ Etoile, France), were used for the isolation of Salmonella spp. and
Shigella spp. They were incubated at 37 °C under aerobic conditions for 24 hours. For the enrichment of Salmonella spp., stool specimens were also inoculated into Müller–Kaufmann broth (Diagnostics Pasteur, Marnes-la-Coquette, France), and subcultured onto MacConkey, Salmonella–Shigella, and Hectoen agar plates after overnight incubation at 37 °C. For the detection of Yersinia enterocolitica, stool samples were plated onto Yersinia-selective agar plates with cefsulodin–irgasan–novobiocin supplement (bioMérieux) and incubated for 48 hours at 25 °C. For Yersinia enrichment, a portion of stool was inoculated into 10 ml of phosphate-buffered saline (Diagnostics Pasteur), incubated at 4 °C for 3 weeks and subcultured in cefsulodin–irgasan–novobiocin agar on a weekly basis. Identification of Salmonella spp., Shigella spp., and Yersinia spp. was performed by standard microbiological methods, the API 20E (bioMérieux) and commercial antisera (Diagnostics Pasteur) [7].

For the isolation of Campylobacter spp., stool samples were plated on campylosel agar (sheep blood agar with vancomycin, cefoperazone, amphotericin B, bioMérieux), and incubated at 42 °C for 48 hours, under microaerophilic conditions. Identification was performed by standard methods [8]. Sheep blood-ampicillin (30 μg) agar plates incubated for 24 hours at 37 °C were used for the isolation of Aeromonas spp. Identification of the oxidase positive colonies was performed by the API 20 NE system. For the investigation of EPEC, five colonies of E. coli were picked from MacConkey agar and were serotyped with commercial antisera by the slide-agglutination method (Diagnostics Pasteur). For the detection of shiga-like toxins (verotoxins) produced by verotoxigenic Escherichia coli (VTEC), a direct enzyme immunoassay (EIA) with a commercial kit (Meridian Diagnostics Europe, s.r.l., Milan, Italy), was performed in 342 stool specimens. For the isolation of Clostridium difficile (requested for 451 faecal samples) the specimen was plated onto cycloserin–cefoxitin–fructose agar (CCFA) and for the detection of C. difficile toxin B either in stools, or in culture isolates, an EIA was performed, with a commercial kit (provided by Meridian Diagnostics).

All bacterial isolates were tested by the disk diffusion method recommended by the National Committee for Clinical Laboratory Standards, for resistance to ampicillin, chloramphenicol, tetracycline, erythromycin, gentamicin, trimethoprim-sulfamethoxazole, norfloxacin and ciprofloxacin [9].

Results

During the study period 7090 specimens were examined, one from each patient. Among the patients tested, 6570 had community-acquired disease, while 520 nosocomial disease. Pathogens were isolated from 987 patients (14%). Of those, 538 (55%) were male and 45% female. Eighty-nine percent were local Greeks, while 11% foreigners. Among the foreigners, 39% were tourists, mainly from Central and Northern Europe, while 61% were aliens from Mediterranean countries (Table 1).

Salmonella enterica strains were isolated in 428 cases (6%). Among them, one was identified as Salmonella enterica serotype typhi, one as S. enterica serotype paratyphi B, 324 (70%) S. enterica serotype enteritidis, 70 (16%) S. enterica serotype typhimurium, and 8 (2%) S. enterica serotype virchow, and 8 (2%) S. enterica serotype newport. The remaining 16 strains (3.7%) belonged to 11 additional serotypes. Campylobacter spp. were isolated in 301 cases (4.2%). Of them, 244 (81%) were Campylobacter jejuni and 57 (19%) Campylobacter coli. EPEC was recovered from 130 patients (1.8%), who were all children under the age of two. These strains belonged to 11 different serotypes. C. difficile enteritis was diagnosed in 65 cases out of the 451 examined. Of them, 46 (70%) were positive by both culture and detection of toxin B, 12 (19%) only by detection of toxin B in feces, and 7 (11%) only by isolation of toxigenic strains. All patients tested for C. difficile were being hospitalized and were receiving antibiotics. Yersinia enterocolitica was isolated in 46 cases (0.6%). All strains belonged to serotype O:3. Shigella spp. were isolated in 36 cases (0.5%). Shigella flexneri was the predominant species (63.9%), followed by Shigella dysenteriae (25%) and

Table 1. Demographic characteristics for patients with acute diarrhoea

<table>
<thead>
<tr>
<th>Demographic information</th>
<th>Patients with positive stool cultures (N = 987)</th>
<th>Patients with negative stool cultures (N = 6103)</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>538 (55%)</td>
<td>3780 (62%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>449 (45%)</td>
<td>2323 (38%)</td>
<td></td>
</tr>
<tr>
<td>Greek</td>
<td>878 (89%)</td>
<td>4760 (78%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Foreigners</td>
<td>109 (11%)</td>
<td>1343 (22%)</td>
<td></td>
</tr>
<tr>
<td>Tourists</td>
<td>43 (39%)</td>
<td>230 (17%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aliens</td>
<td>66 (61%)</td>
<td>1113 (83%)</td>
<td></td>
</tr>
</tbody>
</table>

* Incidence of diarrhoea is compared between male and female patients, Greek and foreigners, tourists and aliens.