Isolation and Antibiotic Susceptibility of *Salmonella, Shigella,* and *Campylobacter* from Acute Enteric Infections in Egypt

Momtaz O. Wasfy1, Buhari A. Oyofo1†, John C. David1, Tharwat F. Ismail1, Atef M. El-Gendy1, Zaynab S. Mohran1, Yehia Sultan2, and Leonard F. Peruski, Jr.1*

1U.S. Naval Medical Research Unit No. 3, Cairo; and 2Abbassia Fever Hospital, Cairo, Egypt

†Present address: US NAMRU No. 2, Jakarta, Indonesia

ABSTRACT

While *Campylobacter, Salmonella,* and *Shigella* remain major contributors to acute enteric infections, few studies on these pathogens have been conducted in Egypt. From January 1986 to December 1993, 869 *Salmonella,* *Shigella* and *Campylobacter* strains were isolated from stool specimens from 6,278 patients, presenting to the Abbassia Fever Hospital, Cairo, Egypt, with acute enteric infections. *Salmonella* predominated, totalling 465 isolates, followed by *Shigella* with 258 isolates, and *Campylobacter* with 146 isolates. Of the *Shigella* isolates, 124 were *Shigella* *flexneri,* 49 were *S. sonnei,* 47 were *S. dysenteriae* (mainly serotype 1, 2, and 3), and 38 were *S. boydii.* *Campylobacter* spp. comprised 92 *Campylobacter jejuni* and 54 *C. coli* isolates. Isolation of *Salmonella* was highest during the months of February-March, June-July, and October-November, while that of *Shigella* was maximal from July to October. Isolation of *Campylobacter* increased during May-June and again during August-October. Although *Salmonella* was sensitive to amikacin, aztreonam, ceftriaxone, and nalidixic acid, it was, however, resistant to erythromycin, streptomycin, ampicillin, chloramphenicol, and tetracycline. *Shigella (>80%)* was sensitive to amikacin, ceftriaxone, cefalothin, sulphamethoxazole-trimethoprim (except *S. sonnei*), aztreonam, and nalidixic acid. Resistance (>50%) was noted only for ampicillin, chloramphenicol, and tetracycline. *C. jejuni* and *C. coli* were resistant to cephalothin, aztreonam, and streptomycin. Some of the above antibiotics were employed to characterize the Egyptian isolates, but did not have any clinical utility in the treatment of diarrhoea. Significant differences (p<0.05) were observed in the resistance profiles of *Shigella* and *Salmonella* between late 1980s and early 1990s. The results suggest the use of fluoroquinolones or a third-generation cephalosporin as an empirical treatment of enteric diseases. However, alternative control strategies, including the aggressive development of broadly protective vaccines, may be more effective approaches to curbing morbidity and mortality due to acute enteric infections.

Key words: *Salmonella; Shigella; Campylobacter; Antibiotic resistance; Diarrhoea; Drug resistance, Microbial; Microbial sensitivity tests; Dysentery, Bacillary; Salmonella infections*

INTRODUCTION

Diarrhoeal disease and enteric infections are major causes of morbidity and mortality in the developing world, resulting in over a quarter of all childhood deaths (1). Globally, *Salmonella* and *Shigella* remain major contributors to acute enteric infections (2,3) with non-typhoidal *Salmonella* isolated in increasing numbers in diverse geographic regions (3,4). *Campylobacter* has...
emerged as a significant cause of gastroenteritis (5). When clinical laboratories include a screen for Campylobacter in routine enteric culture procedures, the percent-recovery for this organism often exceeds that of Salmonella and Shigella (6). However, screening for Campylobacter in acute enteric infections is often not a routine matter due to its relatively recent link to human disease and the complexity of procedures for its isolation and identification (7,8). As a result, there is little information available specifically on Campylobacter in acute enteric infections or antimicrobial resistance from the developing world (8). In 1984, the Egyptian Ministry of Health estimated infant mortality as 72 cases per 1,000 (7.2%), of which 43 cases (4.3%) were due to acute enteric infections (9). In a more recent report (10), mortality decreased in the early 1990s to 34 cases per 1,000, of which 9 cases were due to diarrhoea. Descriptive characterization of bacterial pathogens involved, including antibiotic susceptibility, remains poor, as few such studies have been conducted in Egypt on the causative bacterial agents and their antibiotic resistance profiles in the indigenous population (9,11). Because of these limitations in the available regional data, we analyzed the results of an ongoing hospital-based study to evaluate the percent-recovery, seasonality, and antibiotic resistance of Campylobacter, Shigella, and Salmonella isolated from patients with acute enteric infections reported to a major regional hospital in Egypt from 1986 to 1993.

METHODS AND MATERIALS

Sample collection
Single stool samples from each of 6,278 patients with acute enteric infections were examined for the presence of Salmonella, Shigella, and Campylobacter over the course of this study from 1986 to 1993. All patients were Egyptian nationals with a mean age of 14.1 years (ranging from 4.8 to 23.4 years) admitted to the Abbassia Fever Hospital, Cairo, Egypt, based on clinical signs of acute diarrhoea or enteric fever. This hospital is one of two major fever hospitals in the greater Cairo metropolitan area which provide low-cost medical care to a regional population in excess of 15 million. Stool specimens were transferred within one hour of their collection from patients to the adjacent bacteriology laboratory at the U.S. Naval Medical Research Unit No. 3 for analysis.

Bacterial analysis
Stool samples were streaked onto selective media (Difco Laboratories, Detroit, Michigan, USA) for the isolation and identification of Campylobacter, Salmonella, and Shigella. Briefly, stools were streaked onto MacConkey, Salmonella-Shigella (SS), Xylose-Lysine-Deoxycholate (XLD), Hektoen-Enteric agars (HE), and were incubated at 35-37 °C for primary isolation. Inoculation into Selenite-F enrichment broth and subculturing on SS agar were performed to improve the recovery of Salmonella (12). Suspect isolates were identified, using the API-20E rapid identification kits (Bio-Merieux Vetek Inc, Hazelwood, Missouri, USA) and commercial antisera (Difco Laboratories) as appropriate. For the isolation of Campylobacter, stools were streaked onto modified Skirrow’s agar and incubated at 42 °C in an anaerobic atmosphere. Suspect colonies were identified as Campylobacter isolates on the basis of morphology, Gram stain, motility, as well as oxidase and catalase tests. C. jejuni and C. coli (vs C. lari) were differentiated on the basis of hippurate and indoxyl acetate hydrolysis. Typically, C. jejuni is positive for the two tests, while C. coli is positive for indoxyl acetate hydrolysis only (12). Antibiograms for Salmonella and Shigella isolates were determined by the disk-diffusion method (13) on Mueller-Hinton agar, using 12 antibiotics: amikacin (30 µg), ampicillin (10 µg), aztreonam (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg), and sulphamethoxazole-trimethoprim (25 µg). Antibiograms for Campylobacter were also determined by the disk-diffusion method, except that 5% sheep blood was added to the Mueller-Hinton agar and susceptibility to sulphamethoxazole-trimethoprim was not determined because of the potential for erroneous results on blood-based media.

RESULTS

In total, 869 Salmonella, Shigella, and Campylobacter strains were isolated from single stool specimens collected from each of 6,278 patients diagnosed as having acute enteric infections (Table 1). Culture-positive specimens constituted 13.9% of the total, with the identification of 869 pathogens. Salmonella predominated, totalling 465 isolates (54%), followed by Shigella with 558 isolates (39%), and Campylobacter with 146 isolates (17%). Of the 465 Salmonella isolates, S. typhi comprised the majority with 245 isolates (53%). Salmonella group B had 94 isolates (10%), group D 41 isolates (9%), group C2 40 isolates (9%), and group C1 36 isolates (8%). Of the 258 Shigella isolates, S. flexneri was most frequently identified, comprising 124 isolates (48%), followed by S. sonnei with 49 isolates (19%), S. dysenteriae with 47 isolates (18%, mainly serotype 1, 2, and 3), and S. boydii with 38 isolates (15%). All 146 Campylobacter strains were identified as either C. jejuni (92 isolates, 63%) or C. coli (54 isolates, 37%).

The figure shows the percent-recovery of each of the three genera on a monthly basis. Salmonella isolation was highest in the month of February-March, June-July, and October-November, and was lowest in April, September, and December. Isolation of Shigella was generally lower during November-March and maximal from July to October. Campylobacter isolation was more uniform over the course of the year, but increased during
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May-June and again during August-October, which roughly corresponded to the periods of the lowest recovery of *Salmonella*.

Antibiotic resistance for those isolates available for testing is summarized in Table 2. Most *Salmonella* isolates, irrespective of species, were sensitive to amikacin, aztreonam, ceftriaxone, and nalidixic acid. In contrast, more than 70% of all isolates were resistant to streptomycin, erythromycin, ampicillin, chloramphenicol, sulphamethoxazole-trimethoprim, and tetracycline.

The four *Shigella* species shared similar susceptibility profiles for most antibiotics. They were resistant to ampicillin (except *S. sonnei*), cephalosporins, chloramphenicol (except *S. boydii* and *S. sonnei*), streptomycin, and erythromycin. Susceptibility profiles of both *C. jejuni* and *C. coli* were similar during the study period (Table 2). They were resistant to cefalexin, aztreonam, cephalosporins, chloramphenicol, erythromycin, and gentamicin. *C. coli* was more resistant than *C. jejuni* to both ampicillin (69% vs 48%) and tetracycline (24% vs 6%), while *C. jejuni* was more resistant than *C. coli* to nalidixic acid (40% vs 24%).

When susceptibility of all the isolates was compared between late 1980s and early 1990s, a significant increase (p<0.05) in the resistance profiles of some *Salmonellae* to chloramphenicol and cephalothin was observed (Table 3). Similarly, in recent years, resistance of *Shigella* to sulphamethoxazole-trimethoprim had significantly increased, but that of tetracycline had decreased (p<0.05).

**DISCUSSION**

Hospital patients are generally assumed to reflect the relative importance and seriousness of prevalent illnesses within a region (14). Our investigation took advantage of the large patient population suffering from acute enteric diseases admitted to the Abbassia Fever Hospital in Cairo to determine the percent isolation and antibiotic susceptibility of *Salmonella*, *Shigella*, and *Campylobacter* over an 8-year period from 1986 to 1993. The predominant bacterial pathogen isolated was *Salmonella*, followed by *Shigella* and *Campylobacter*, indicating that they remain significant threats to health in this region.

Apart from *S. typhi*, which was the most common pathogen in this study, isolates of other *Salmonella* serogroups were found in approximately 1% or less of specimens. Other reports from neighbouring countries found total *Salmonella* isolation ranging from 2% to 18% of specimens (15-18). We found a greater percentage of specimens positive for *S. typhi* or *S. paratyphi*. This could reflect the source of our faecal specimens that were obtained from a hospital ward that receives a large...
Table 2. Percent antibiotic resistance by pathogen

<table>
<thead>
<tr>
<th>Species</th>
<th>Antibiotic</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>C. hollisae</th>
<th>C. jejuni var. C. jejuni</th>
<th>C. jejuni var. C. jejuni</th>
<th>C. jejuni var. C. jejuni</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. flexneri</td>
<td>Amoxicillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>69</td>
<td>48</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
<td>100</td>
<td>96</td>
<td>96</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>60</td>
<td>90</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Nitrofurantoin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole-trimethoprim</td>
<td>94</td>
<td>90</td>
<td>90</td>
<td>94</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

The value in parenthesis is number of tested specimens.

The emergence of resistance to quinolones in Campylobacter may be an ominous trend that has not been previously noted in Egypt. However, quinolones are now emerging as causative agents of pseudotumor cerebri in infants and young children (30).
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Table 3. Significant changes in the resistance profiles of some enteric pathogens isolated from Egyptian diarrhoea patients during an interval of 4 years before and after 1990

<table>
<thead>
<tr>
<th>Species*</th>
<th>Antibiotic*</th>
<th>1986-1989</th>
<th>1990-1993</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of resistant isolates</td>
<td>% of total</td>
<td>No. of resistant isolates</td>
<td>% of total</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Am</td>
<td>0</td>
<td>0.0</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0</td>
<td>0.0</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Te</td>
<td>0</td>
<td>0.0</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Cb</td>
<td>0</td>
<td>0.0</td>
<td>103</td>
</tr>
<tr>
<td><em>Salmonella</em> Group B</td>
<td>C</td>
<td>19</td>
<td>73.0</td>
<td>12</td>
</tr>
<tr>
<td>Group C2</td>
<td>C</td>
<td>4</td>
<td>50.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Cr</td>
<td>0</td>
<td>0.0</td>
<td>6</td>
</tr>
<tr>
<td><em>S. dysenteriae</em></td>
<td>Te</td>
<td>8</td>
<td>50.0</td>
<td>2</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>SxT</td>
<td>4</td>
<td>28.6</td>
<td>6</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>Te</td>
<td>5</td>
<td>55.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Key to antibiotic names: Am=ampicillin, Cr=cephalothin, C=chloramphenicol, Cb=carbenicillin, Te=tetracycline, and SxT=trimethoprim-sulfamethoxazole. P values for differences in susceptibility of other species or serogroups were high at 95% confidence level.

We have observed significant differences (p<0.05) in the resistance profiles of some serogroups of *Salmonella* between late 1980s and early 1990s (Table 3). A similar observation was seen in the Indian subcontinent, Asia, and Africa since 1989 (20,31). Strains of *S. typhi*, resistant to chloramphenicol, ampicillin, and trimethoprim, have been responsible for numerous outbreaks in these areas (20,22,31). It appears that ciprofloxacin may be the *de facto* antibiotic of choice for enteric infections. Unfortunately, chromosomally-encoded resistance to ciprofloxacin has now been observed in a small number of strains from the Indian subcontinent, making it of paramount importance to limit its unnecessary use (20,25).

This study reports the rates, seasonality, antimicrobial susceptibility, virulence, and regional importance of the pathogens associated with acute enteric infections in Egypt. Data indicate that there is a little difference between our findings and those of the neighbouring countries. Because of the health threat posed by these pathogens, their antibiotic resistance should be continuously monitored (32). Control strategies, such as improving public hygiene, preventing judicious use of existing enteric vaccines and antibiotics, or the development of newer, more broadly protective vaccines, may be more effective in curbing the morbidity and mortality associated with these agents.

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REFERENCES


