Enterobacteriaceae: Etiological Agents of Diarrhea

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To investigate the prevalence of Enterobacteriaceae among patients diagnosed with gastrointestinal infection and the risk factors associated with such infections, a total of 250 fecal samples were collected from the identified patients and their history and clinical examination findings recorded. Stool characteristics were noted and examinations performed, including direct microscopy of faeces, culturing and identification of causative agents. Confirmatory biochemical and serological tests were also carried out. Out of total samples, 84(33.6%) revealed enteropathogenic etiological agents. Among them 34 (13.6%) were bacterial pathogens, of which 27(10.8%) were from family Enterobacteriaceae and 7(2.8%) from Vibrionaceae, while the remaining 50 (20%) were of parasitic ova and or cysts. Among the Enterobacteriaceae positive cases, 9 (33.3%) were identified as Shigella species, 2 (7.4%) were Salmonella species, while 16 (59.3%) were strains of pathogenic E. coli. Of the 9 Shigella species 5 (55.5%) were Poly B serotypes, while 3 (33.3%) were Poly A serotypes and 1 (11.1%) was Poly D serotype. Of the 2 Salmonella species, one was Salmonella typhi and other as Salmonella paratyphi. Of the 16 E. coli strains isolated, 11 (68.7%) were Poly 2 and 5 (31.2%) were Poly 3 serotypes. Abdominal cramps - severe to mild - and pus cells in stool were the most prominent clinical features, followed by mucus in the stool (96.3%). Of the positive cases for Enterobacteriaceae isolates, 17 (62.3%) were from male samples and 10 (37.7%) from female samples. Among these positive cases, 12 (44.4%) were from children under the age of 6 years and of these 8 cases were from boys and 4 from girls. Of the positive cases reported in this study, 19 (70.4%) were from urban centers - 10 in Islamabad, 9 cases in Rawalpindi, while 8 (29.6%) of the cases were reported among people residing in the surrounding rural areas. Infection reported in the urban areas may be attributed to the overall poor quality of water supply and sanitation systems, high poverty rate in these areas and the lack of good hygiene practices among food vendors and users, as well as the overall lack of awareness and quality education. To combat such endemic diseases it is essential to prioritize political commitments for sustained socio-economic development, through the provision of resources, to address quality education, urban and rural planning and the commissioning of affordable, indigenous, cost-effective technologies for sanitation and wastewater treatment.

Key words: Enterobacteriaceae, diarrhea, etiology, Rawalpindi, Islamabad

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INTRODUCTION

Diarhea is one of the commonest diseases amongst children and accounts for the highest cause of infant mortality worldwide, resulting in a significant public health impact, particularly in developing countries (Karamat et al., 1993). Gastrointestinal diseases account for an estimated 12,600 child deaths each day in Asia, Africa and Latin America. Etiological agents associated with diarrhea include viruses, bacteria and parasites. As a result of the increase in extreme weather conditions, such as prolonged droughts followed by torrential downpours leading to flash floods, over the past decades, the prevalence of diarrheal diseases has increased even in developed countries. In addition, other natural disasters This has lead to the prioritization of funding research work leading to the better understanding of the pathophysiology of infectious diarrheal diseases (Zia ullah et al., 1997; Tariq, 1998).

Pathogens associated with diarrhea vary in their virulence, causing mild to frank bloody diarrhoea. Bacteria species of the Family Enterobacteriaceae are the most common bacterial agents associated with gastrointestinal infections - particularly diarrhoea. This bacterial family consists of a large heterogeneous group of gram-negative rods (Brooks et al., 2001) and includes species of E. coli, Salmonella and Shigella, the most common causative agents. These species may induce diarrhoea by either of two mechanisms: production of enterotoxins, such as in the case of E. coli and Salmonella species, while others are entero-invasive strains that penetrate the intestinal epithelium causing necrosis of the tissue resulting in bloody diarrhoea, as in the case of shigellosis (Aleamo, 1997).

E. coli, a normal resident of intestinal tract has numerous pathogenic serotypes based on their virulence mechanism. These include enterotoxigenic E. coli (ETEC) that are the etiological agents often associated with traveler’s diarrhoea, enteropathogenic E. coli (EPEC) the common causative agent of infantile diarrhoea, enterobacterae E. coli (EHEC) and entero-invasive E. coli (EIEC) both of which has a similar mode of action, but with varying intensity, to that of Shigellosis, resulting bloody diarrhoea (Cheesbrough, 1991; Aleamo, 1997; Brooks et al., 2001).

Other important pathogens in the Enterobacteriaceae family are Salmonella and Shigella species. All members of Salmonella genus are pathogenic causing gastrointestinal infection commonly known as salmonellosis (Fernando et al., 1995). There are two main species of Salmonella identified as etiological agents of salmonellosis in humans: Salmonella typhi and Salmonella paratyphi. The disease symptoms are typically characterized by high fever, unformed or watery diarrhea, sometimes with blood, mucus and pus (Brooks et al., 2001). Shigellosis caused by Shigella species is characterized by blood and pus in the stool associated with abdominal cramps along with dysentery, watery diarrhea and bacteremia. Four main species of Shigella have been characterized as etiological agents of shigellosis and they are: S. flexneri, S. boydii, S. dysenteriae, S. sonnei (Cheesbrough, 1991; Fernando et al., 1995).

All three of these species have a similar mode of transmission via the fecal-oral route, through contaminated food, water and fingers, with flies and fomites often acting as vectors of the spread of contamination. The infective dose is extremely low, less than 200 organisms (Barren et al., 1994; Brooks et al., 2001).

Different workers have conducted survey studies on the epidemiology of etiological agents of diarrhoea (Qadi et al., 1991; Lima et al., 1997; Yousef et al., 2000). However, in Pakistan, only a few studies have collectively investigated the role of bacteria of the Enterobacteriaceae family in diarrhoea (Masoumi et al., 1995; Sultana and Mir, 1998). Separate studies have been carried on E. coli (Khan et al., 2002), Salmonella and Shigella sp. (Asghar et al., 2002) and their different epidemiological aspects, yet very few reports have collectively documented these etiological agents. This study was designed to do this very thing, to investigate the prevalence of Enterobacteriaceae among patients diagnosed with gastrointestinal infection and the risk factors associated with such infections, with the intention to devise improved control measures and minimize the infection rate in concerned human population.

MATERIALS AND METHODS

The study was conducted at the University of Arid Agriculture, Rawalpindi and the National Institute of Health, from August 200 to July 2001, among patients, diagnosed as having symptoms consistent with gastroenteritis and diarrhoea, visiting the NIH and the associated Pakistan Institute of Medical Sciences Hospital.

A total of 250 faecal samples were collected during this period from the identified patients and their history and clinical examination findings recorded on pre-designed questionnaires. Stool characteristics were also noted and stool examination and culture were performed.
Processing of samples included collection, direct microscopy of faeces, culturing of faeces and identification of causative agents, following the proven and accepted method as described by other researchers (Khan et al., 2002; Asghar et al., 2002). Fecal suspensions were cultured on MacConkey, Salmonella - Shigella (SS) agars, enriched with Selenite F Broth to facilitate the growth and isolation of the targeted pathogens. Biochemical and serological tests were carried out to confirm the identification of the isolates in accordance to methods described by Khan et al. (2002) and Asghar et al. (2002).

RESULTS AND DISCUSSION

Out of total 250 fecal samples, 84 (33.6%) revealed entero-pathogenic etiological agents. Among them 34 (13.6%) were bacterial pathogens, of which 27 (10.8%) were from family Enterobacteriaceae and 7 (2.8%) from Vibrionaceae, while the remaining 50 (20%) were of parasitic ova and/or cysts (Table 1). Other diarrhea causing bacteria were not included in the study.

The results of present study revealed that the bacterial infection caused by members of family Enterobacteriaceae were 27 (10.8%). These findings conform to those reported by other researchers who documented a low incidence of Enterobacteriaceae etiological agents in association with gastroenteritis (Gomes et al., 1991; Gandapur et al., 1993; Masoomi et al., 1995; Asghar et al., 2002). However, other researchers have reported varied incidence of Enterobacteriaceae pathogens (Karamat et al., 1993; Zia ullah et al., 1997). These differences in incidences may be attributed to many factors such as the use of different bacteriological techniques, geographical locations from where samples were collected (urban verses rural; developed verses remote areas), personal hygiene and socio-economic conditions of population under study.

Another important reason may be linked to that fact that most of diarrhea cases reported in this study were not of bacterial origin and that some cases may have been missed due to multiple factors including indiscriminate antibiotic use by the patients, the common practice of self-medication as well as the limited available diagnostic facilities (Khan et al., 2002; Asghar et al., 2002). Moreover the study under discussion was only confined to the isolation of Shigella Salmonella and E. coli. Infection by Shigella and Salmonella are more common in the rainy season and often occurs as outbreaks, that is why infection exists though at low levels, as reported by researchers (Admoni et al., 1995; Yamashiro et al., 1999).

Out of total 27 bacterial isolates, 9 (33.3%) were Shigella species, 2 (7.4%) were Salmonella species, while 16 (59.3%) were strains of pathogenic E. coli. Other researchers have also reported on the significant role of E. coli as the etiological agent of gastroenteritis (Karamat et al., 1993). In case of Shigella and Salmonella low prevalence rate has also been reported by other researchers (Yousef et al., 2000), which once again may be attributed to the overwhelming use of antibiotics, through self-medication, which may eliminate the pathogens at an early stage, making it difficult to clinically isolate these microbes.

Despite of poor sanitary conditions prevalent in the Islamabad and Rawalpindi regions, as well as the reports of contaminated water supply (Chaudhry et al., 1994; Mubshir, 2003) the incidence rate of salmonellosis and shigellosis may also be low because of the cooking methods used at homes to cook vegetables and meats. As traditionally practiced, people tend to over cook the food to the point of being 'well done' thereby reducing the chances of transmission of disease. However, ready-to-eat foods in the market and even cooked food that is stored out in the open and allowed to cool may be still lead to food poisoning through production of heat stable toxins (Cheesbrough, 1991; Fernando et al., 1995; Brooks et al., 2001).

Of the 9 Shigella species isolated in this study, 5 (55.5%) were serologically identified as Poly B serotypes, while 3 (33.3%) were identified as Poly A serotypes and 1 (11.1%) as Poly D serotype. Of the 2 Salmonella species, one was identified as Salmonella typhi and other as Salmonella paratyphi, through the appropriate serology tests. As for the 16 E. coli strains isolated, 11 (68.7%) were serologically identified as Poly 2, whereas 5 (31.25%) were Poly 3 serotypes (Table 2).

The most prominent clinical features among the 27 cases of gastroenteritis, as reported in this study under discussion, were the abdominal cramps (100%), ranging from mild to severe and pus cells in stool (100%), followed by mucus in the stool (96.3%). Other symptoms included fever (55.6%), ranging from 100-102°F and blood in stool (37%). The pus cells, mucus and blood in stool were determined through microscopy.

This aspect of study was found to be in accordance with reports of other researchers (Hakeem et al., 2003; Hydrie et al., 2005), who reported greater incidence of pus and mucus cells as compared to blood cells in the stool samples that they studied. Furthermore, this study also revealed that the patients studied displayed an overall weakness and lethargy, which may be attributed to lack of
Table 2: Serotypes of *Shigella*, *Salmonella* and *E. coli* strains and clinical manifestations of infection among patients of gastroenteritis (n = 27)

<table>
<thead>
<tr>
<th>Pathogens/Genus/Serotypes/Species</th>
<th>Infected cases</th>
<th>Fever</th>
<th>Cramps</th>
<th>Blood in Stool</th>
<th>Mucus in Stool</th>
<th>Pus cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella</em> Poly A</td>
<td>3</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Poly B</td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Poly D</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>9 (33.3)</td>
<td>4 (14.8)</td>
<td>9 (33.3)</td>
<td>6 (22.2)</td>
<td>6 (22.2)</td>
<td>9 (33.3)</td>
</tr>
<tr>
<td><em>Salmonella</em> Typhi</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Paratyphi</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>2 (7.4)</td>
<td>2 (7.4)</td>
<td>2 (7.4)</td>
<td>-</td>
<td>2 (7.4)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td><em>E. coli</em> Poly 2</td>
<td>11</td>
<td>6</td>
<td>10</td>
<td>11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Poly 3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>16 (59.2)</td>
<td>15 (55.6%)</td>
<td>16 (59.2)</td>
<td>4 (14.8)</td>
<td>15 (51.8)</td>
<td>16 (59.2)</td>
</tr>
<tr>
<td>Grand Total (%)</td>
<td>27 (100%)</td>
<td>15 (55.6%)</td>
<td>27 (100%)</td>
<td>10 (37.0%)</td>
<td>26 (96.3%)</td>
<td>27 (100%)</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of infection by *Enterobacteriaceae* according to age and sex

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Suspected cases (n = 250)</th>
<th>Enterobacteriaceae positive cases (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>72</td>
<td>43</td>
</tr>
<tr>
<td>6-15</td>
<td>39</td>
<td>24</td>
</tr>
<tr>
<td>16 - 25</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>26 - 35</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>36 - 45</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total (%)</td>
<td>148 (59.2)</td>
<td>102 (40.8)</td>
</tr>
</tbody>
</table>

The study under discussion also revealed a greater prevalence of *Enterobacteriaceae* associated gastroenteritis among males than females, in that overall 62.3% of the positive cases were among boys and men (Table 3). Other researchers have also reported and documented higher cases among males than females, both within Pakistan and in other developing countries as well (Gomes et al., 1991; Karamat et al., 1993; Admoni et al., 1995; Iqbal and Ali, 1999; Asghar et al., 2002).

Of the total 250 fecal specimens analyzed in this study, 148 (59.2%) were from male patients diagnosed to be afflicted with illnesses consistent with gastroenteritis, while 102 (40.8%) were from females showing similar signs and symptoms. Of all these cases, 27 (10.8%) were found to be positive for *Enterobacteriaceae* isolates, of which 17 (62.3%) were from male samples and 10 (37.7%) from female samples. Among the positive cases, 12 (44.4%) were from children under the age of 6 years and of these 8 cases were from boys and 4 from girls (Table 3).

Previous studies carried out in the Rawalpindi and Islamabad regions have reflected similar findings, indicating a higher prevalence rate of *Enterobacteriaceae* among children under 6 years of age (Chaudhry et al., 1994; Tariq, 1998; Munib, 2003). The findings of this study are also in accordance to other reports as well (Quadri et al., 1991; Karamat et al., 1993). The high incidence in children is attributed to the eating habits of children, particularly those who start attending school, away from the supervision of parents, where unhygienic may prevail as well as the sale and consumption of ready-to-eat food is quite rampant. In addition, children may acquire an infection from infected children, or care-givers working at schools (Gandahar et al., 1993).
Vectors contaminating their food or drink (Sultana and Mir, 1998). Researchers have also reported that poor hygienic and sanitary status and over crowding appear to be the major causes of the outbreaks of gastroenteritis and related illnesses (Karamat et al., 1994), as lack of potable tap water, improper sanitation facilities and poverty (low income groups) have been found to be associated with diarrhea (Hafiz et al., 1991; Karamat et al., 1994; Rafi et al., 2001; Hakeem et al., 2003; Hydrie et al., 2005).

As for the education level of the patients positive for Enterobacteriaceae, this study under discussion revealed that the vast majority (66.7%) of the confirmed cases were among the population that had received no formal education, followed by those who has received 12 years of schooling (22.2%) and only single cases were reported among those patients who had under-graduate and post-graduate degrees. These findings are in agreement with those reported by other researchers who effectively argue that quality education is essential to holistically address the poverty and environmental issues (Hafiz et al., 1991; Karamat et al., 1994; Anonymous, 2002; Haider, 2003; Shehzad, 2003).

There is no doubt that infections caused by Enterobacteriaceae pathogens will remain endemic as long as there is inadequate socio-economic development, poor infrastructure maintenance and a rapid population growth. Poverty, lack of quality education and awareness and a lack of the general sense of responsibility for personal and environmental cleanliness, will allow such pathogens to flourish and persist (Pugh, 1989).

This study also revealed a strong correlation between prevalence of diarrheal diseases and poverty, as the higher incidence of such ailments has been reported amongst the low-income class may be attributed to contaminated water supply, poor sanitation and unhygienic food practices: aspects prevalent in poorer slum areas of the twin cities of Rawalpindi and Islamabad (Brooks et al., 2001; Shehzad, 2002).

Infection reported in developed areas in these twin cities, as reported in this study, may be attributed to the overall poor quality of water supplied by the municipal government. Constant commuting of household workers and care-givers, from the under-developed areas of the cities, to the affluent regions of the cities, may be another source of infection transmission, particularly among the children and elderly, whom they look after (Chaudhry et al., 1994; Mubashir, 2003).

Causes of Enterobacteriaceae associated diarrhea typhoid in Rawalpindi and Islamabad may be associated with the densely populated urban centers with adjoining suburbs, coupled with poverty and poor infrastructure development, like sanitation and waste disposal, lack of quality education, inadequate primary health care facilities, cumulatively resulting in the contamination of food produce and water. The lack of adequate health care facilities, affordable diagnostic laboratories and proper counseling of parents on methods of good hygiene practices, in particular, has lead to an increase of such illnesses in urban centers throughout Pakistan (Hafiz et al., 1991; Karamat et al., 1994).

Without a doubt, countries that have inadequate infrastructure to support a growing population, coupled with poor wastewater treatment facilities and improper sanitation mechanisms or systems, will always be prone to gastrointestinal infections, particularly those caused by enteric gram-negative bacilli. To combat such endemic diseases it is essential to prioritize political commitments for sustained socio-economic development, through the provision of resources, to address quality education, urban and rural planning and the commissioning of affordable, indigenous, cost-effective technologies for sanitation and wastewater treatment.

REFERENCES


