Introduction

Campylobacteriosis is a collective discipline for infectious diseases caused by members of the genus "Campylobacter". Campylobacter jejuni and coli1 have public health importance with Campylobacter jejuni accounting for about 80-90% of the cases.2 Campylobacteriosis is a zoonotic disease. In addition to dogs, cats and birds, animals such as poultry may act as a reservoir.3 Poor hygiene and inadequate sanitation along with close proximity to animals in developing countries contribute to its easy and frequent acquisition. In developing countries Campylobacter isolation rate (estimated mainly from laboratory based surveillance data) ranges from 5 to 17%.4,5 In addition, case-controlled community based studies from developing world estimates 40,000 to 60,000 cases per 100,000 children of <5 years of age.6,7 By contrast, the figure for developed countries is only 300 cases per 100,000 rendering campylobacteriosis a common pediatric disease in the developing world.

Indiscriminate use of antibiotics to treat a variety of infections as well as the introduction of fluoroquinolones in veterinary medicine have emerged as a major factor fostering the rapid emergence of resistance among Campylobacter species.

Rapid emergence of resistance against fluoroquinolones, tetracyclines and macrolides has been reported in several studies from North America and Europe. Several studies published from Asia, mainly from Thailand, China, India and Bangladesh5,9,10 reports significant rise in antimicrobial resistance against enteric pathogens including Campylobacter. Unfortunately such data is lacking from Pakistan. These data are not only important in the management of severe infections, in addition this information can also be used as an epidemiological tool. Trends of antimicrobial resistance can be monitored and guidelines can be developed to promote appropriate use of antimicrobial therapy both at local and national level. Therefore, the objectives of this study were to analyze frequency of isolation and trends in antimicrobial resistance among Campylobacter isolates over the last 11-year period.

Materials and Methods

This study was conducted a descriptive analysis of all stool samples submitted for bacterial culture in the laboratory from year 1992-2002. All submitted stool samples were plated directly on MacConkey, Xylose-lysine-deoxycholate agar, Tellurite Taurocholate Gelatin agar and incubated at 37°C for 24 hours. Samples were also inoculated in Selenite-F broth and alkaline peptone water for enrichment and after overnight incubation at 37°C these were subcultured on Salmonella-Shigella and Tellurite Taurocholate Gelatin agar respectively. The isolated organisms were identified by serological and conventional biochemical tests. API 20E and 20NE (Bio Murex, France) were used for identification if required. For isolation of Campylobacter, stool samples were inoculated on agar based Campylobacter media supplemented with 40% hemolyzed sheep blood with added antibiotics, namely vancomycin, polymixin B, trimethoprim and cefoperazone. Plates were incubated for 48 hours in microaerophillic environment at 420C. Oxidase and catalase positive curved seagull shaped gram-negative rods showing hippurate hydrolysis were further tested for antimicrobial susceptibility by Kirby Bauer disc diffusion method.11 Statistical Analysis was done by using descriptive statistics of SPSS version 10. Values are expressed as percentages, mean and rates.

Results

Total 52,777 stool specimens were processed during the study period (1992-2002). Bacterial pathogens were isolated from 8,483 specimens with an overall isolation rate of 16%. Figure 1 shows the yearly isolation rate of bacterial pathogens in stool samples from 1992-2002. In general the isolation rate
fluctuated between 14-18% although there was a peak of 22% in 1994. Further analysis of culture positive specimens showed that Vibrio species were the most common pathogen isolated followed by Salmonella species. Campylobacter remained third in frequency with isolation rate of 24.8% (Table 1). Among Campylobacter species, C. jejuni was the predominant pathogen isolated followed by C. coli (Table 2). Isolation rate of Campylobacter was higher (45.7%) in children under two years of age as compared to other age groups (Figure 2). Isolation rate was similarly high in elderly population, which could be related to the immune status in this subgroup. While analyzing the antimicrobial susceptibility data a steady rise in resistance against ampicillin, tetracycline and ofloxacin was observed (Figure 3). Quinolone resistance among Campylobacter isolates has increased significantly since 1995. Resistance against erythromycin declined to almost 1% during the study period.

Discussion

During the last twenty-five years Campylobacter jejuni has been recognized as an important enteric pathogen. It is a common cause of diarrhea among international travellers, and appears to be associated with Guillain-Barre syndrome, a devastating illness.2 Like in many other developing countries, ecosystem of the heavily populated areas of Pakistan contains a high background level of fecal pollution associated with transmission of enteric pathogens through water, food, human and animal. Hence, gastroenteritis remains a major cause of diarrhea in pediatric population of our country.12 Analysis of our data shows that with an overall isolation rate of 16% Campylobacter remained third with the isolation rate of about 24.8%. This isolation rate is higher in comparison with other studies previously published from South East Asia as in a report from Bangladesh, isolation rate is documented to be 17.4%5 while from Thailand it is only 13%.13 Similar to various studies,12,14 analysis of data also reveals a higher frequency of isolation of Campylobacter jejuni followed by C. coli and C. lardi. In our study, age wise analysis of Campylobacter showed that the pathogen was most commonly isolated from stool samples of children less than two years of age. This is in accordance with earlier results from this region.12,15,16 A previously published study from Pakistan reports Campylobacter as the most frequent isolate from stool samples of children less than one year old with the isolation rate of 13.1%.12 One reason for high rate of acquisition in children could be the low IgA, IgG and IgM levels in response to Campylobacter infection in infancy especially during first six months of life resulting in repeated episodes of infection in early period of life.2 Decline in the frequency of infection with age is attributed to the immunological response due to repeated exposure of this pathogen from environment. This also explains the early appearance of antibodies against Campylobacter antigens in the serum of children in developing countries. The level of antibodies tends to be much higher in these children than their counterpart from the developed world.17,18 The second objective of our study was to evaluate trends in antibiotic resistance among Campylobacter isolates. Review of available data shows a global rise in antimicrobial resistance. Number of studies from around the world reported the alarming increase in resistance among Campylobacter against commonly used antimicrobials.9,19,20 We also observed a steady rise in antimicrobial resistance against three of the four commonly used antibiotics, i.e., ampicillin, tetracycline and fluoroquinolone. Acquisition of antibiotic resistance was high against tetracycline rising from 8% in 1992 to 30% in 2001. Similar trend was observed for ampicillin where resistance has been increased from 14% to 26% rendering these antibiotics relatively ineffective for empirical treatment of Campylobacter enteritis. Another striking feature observed in our study was a continuous rise in quinolone resistance during the study period. None of the isolates were quinolone resistant in year 1992 as compared to year 2002 when 23% of the isolates were resistant to ofloxacin. Similar phenomenon has been observed worldwide. Recently, trend of antimicrobial resistance in Campylobacter jejuni was published from Barcelona.19 Authors found a rapid increase in quinolones resistance, from 1% in 1985 to 82% in 1998. A 6-year follow-up study from Sweden documented the same trend.20 In addition, two different studies from Thailand conducted between

Table 1: Distribution of organisms isolated during the study period

<table>
<thead>
<tr>
<th>Organism Isolated</th>
<th>Total number of isolates (n=6485)</th>
<th>% Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio species</td>
<td>2666</td>
<td>37.4%</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>2146</td>
<td>26.4%</td>
</tr>
<tr>
<td>Campylobacter species</td>
<td>2108</td>
<td>24.8%</td>
</tr>
<tr>
<td>Shigella species</td>
<td>1066</td>
<td>12.5%</td>
</tr>
<tr>
<td>Others</td>
<td>397</td>
<td>4.90%</td>
</tr>
</tbody>
</table>

*Monospecies, Flexomonas species, Enteropathogenic E. Coli etc.

Table 2: Isolation rate (%) of Campylobacter species during 1992-2002

<table>
<thead>
<tr>
<th>Organism Isolated</th>
<th>% Isolation Rate (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>85.15% (1705)</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>8.58% (181)</td>
</tr>
<tr>
<td>Campylobacter lardi</td>
<td>5.12% (108)</td>
</tr>
<tr>
<td>Campylobacter species</td>
<td>1.15% (24)</td>
</tr>
</tbody>
</table>

Discussion

During the last twenty-five years Campylobacter jejuni has been recognized as an important enteric pathogen. It is a common cause of diarrhea among international travellers, and appears to be associated with Guillain-Barre syndrome, a devastating illness.2 Like in many other developing countries, ecosystem of the heavily populated areas of Pakistan contains a high background level of fecal pollution associated with transmission of enteric pathogens through water, food, human and animal. Hence, gastroenteritis remains a major cause of diarrhea in pediatric population of our country.12 Analysis of our data shows that with an overall isolation rate of 16% Campylobacter remained third with the isolation rate of about 24.8%. This isolation rate is higher in comparison with other studies previously published from South East Asia as in a report from Bangladesh, isolation rate is documented to be 17.4%5 while from Thailand it is only 13%.13 Similar to various studies,12,14 analysis of data also reveals a higher frequency of isolation of Campylobacter jejuni followed by C. coli and C. lardi. In our study, age wise analysis of Campylobacter showed that the pathogen was most commonly isolated from stool samples of children less than two years of age. This is in accordance with earlier results from this region.12,15,16 A previously published study from Pakistan reports Campylobacter as the most frequent isolate from stool samples of children less than one year old with the isolation rate of 13.1%.12 One reason for high rate of acquisition in children could be the low IgA, IgG and IgM levels in response to Campylobacter infection in infancy especially during first six months of life resulting in repeated episodes of infection in early period of life.2 Decline in the frequency of infection with age is attributed to the immunological response due to repeated exposure of this pathogen from environment. This also explains the early appearance of antibodies against Campylobacter antigens in the serum of children in developing countries. The level of antibodies tends to be much higher in these children than their counterpart from the developed world.17,18 The second objective of our study was to evaluate trends in antibiotic resistance among Campylobacter isolates. Review of available data shows a global rise in antimicrobial resistance. Number of studies from around the world reported the alarming increase in resistance among Campylobacter against commonly used antimicrobials.9,19,20 We also observed a steady rise in antimicrobial resistance against three of the four commonly used antibiotics, i.e., ampicillin, tetracycline and fluoroquinolone. Acquisition of antibiotic resistance was high against tetracycline rising from 8% in 1992 to 30% in 2001. Similar trend was observed for ampicillin where resistance has been increased from 14% to 26% rendering these antibiotics relatively ineffective for empirical treatment of Campylobacter enteritis. Another striking feature observed in our study was a continuous rise in quinolone resistance during the study period. None of the isolates were quinolone resistant in year 1992 as compared to year 2002 when 23% of the isolates were resistant to ofloxacin. Similar phenomenon has been observed worldwide. Recently, trend of antimicrobial resistance in Campylobacter jejuni was published from Barcelona.19 Authors found a rapid increase in quinolones resistance, from 1% in 1985 to 82% in 1998. A 6-year follow-up study from Sweden documented the same trend.20 In addition, two different studies from Thailand conducted between
year 1995-2001 report 77%9 and 84%10 of resistant Campylobacter strains against quinolones. Frequency of Campylobacter strains resistant to quinolone reported in our study is low in comparison to other studies from our region; it is significant in terms of being the index report from Pakistan.

Another interesting finding of our study was the decline in resistance against erythromycin falling from 7% to 0%. Some of the published studies report a higher resistance rate against erythromycin.10,21 However, our result conflicts with the reports indicating that erythromycin resistance is on the rise in developing countries.22,23

The only limitation of our study was unavailability of the clinical information regarding patients, therefore, we were not able to comment that whether all these patients were diseased or this isolation rate merely reflect carrier state. Further studies are required to relate clinical findings of the patient with the isolation of Campylobacter species from the stool samples. In summary, in this paper we report the isolation rate of Campylobacter species from stool specimens received in our clinical laboratory and the antimicrobial susceptibility pattern of these isolates from Pakistan. Isolation of Campylobacter is higher from stool specimens of children of less than two years of age rendering Campylobacteriosis to be an important cause of gastroenteritis in our pediatric population. This study also demonstrates a steady rise in antibiotic resistance in Campylobacter isolates especially against quinolones with fall in resistance against erythromycin throughout the study period.

References
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Abstract

Objective:
To analyze frequency of isolation and trends in antibiotic resistance among Campylobacter isolates over 11 year period in Microbiology Laboratory, Aga Khan University from the year 1992-2002.

Methods:
Total 52,777 stool specimens were processed during the study period. Enteric pathogens isolated from 8,483 stool samples were further analyzed for frequency of isolation and antimicrobial resistance. Statistical Analysis was done by using descriptive statistics of SPSS version 10. Values were expressed as percentages, mean and rates.

Results:
Campylobacter species were third in frequency of isolation with an isolation rate of 24.8%. C. jejuni was the predominant pathogen followed by C.coli. Isolation rate of Campylobacter was higher (45.7%) among children under 2 years of age as compared to other age groups. A steady rise in resistance among Campylobacter isolates against ampicillin; tetracycline and ofloxacin has been noted whereas resistance against erythromycin remained fairly low.

Conclusion:
The isolation of Campylobacter is higher from stool specimens of children of less than two years of age rendering Campylobacteriosis to be an important cause of gastroenteritis in pediatric population. This study also demonstrates a steady rise in antibiotic resistance in Campylobacter isolates especially against quinolones with fall in resistance against erythromycin throughout the study period (JPMA 54:291;2004).