ANTIBIOTIC RESISTANCE IN CAMPYLOBACTER JEJUNI IN RAWALPINDI AND ISLAMABAD – A Preliminary Study

Arif Maqsood Ali 1, Ayaz Hussain Qureshi 2, Shahid Rafi 3, Iqbal Ahmad Khan 4 & Shujaat Hussain 5

ABSTRACT:
Objective: To determine the antimicrobial resistance in Campylobacter jejuni isolated from stools of children suffering from diarrhoea/dysentery in our setup against the antimicrobials commonly used as empirical therapy.
Study: A prospective cross sectional descriptive study.
Place and duration of study: Department of Microbiology, Army Medical College and Military Hospital, Rawalpindi from 29 August to 29 November 2002.
Patients and methods: The study was carried out on eighteen isolates recovered from one hundred stool samples of children up to the age of twelve years admitted with diarrhoea/dysentery in Military hospital, Rawalpindi. The samples were collected in clean polypropylene containers containing Cary Blair medium. These were transported to the Microbiology Department, Army Medical College, Rawalpindi within 1-2 hours. The samples were inoculated on Modified Preston (Oxoid) and Karmali media (Oxoid) beside other routine stool culture media. The cultures were incubated at 42°C under microaerophilic conditions. The growth after 48 hours was provisionally identified by colonial morphology, oxidase test, Gram staining and motility. The organisms were identified to species level by hippurate hydrolysis, urease test, nitrate reduction, catalase test, H2S production, resistance to cephalothin and sensitivity to nalidixic acid. Sensitivity testing was carried by Modified Kirby Bauer disc diffusion technique on lysed horse Blood Agar against ampicillin (10 ug), erythromycin (15ug), tetracycline (10ug), chloramphenicol (30ug), trimethoprim/sulphamethoxazole (1.25ug/23.75ug), nalidixic acid (30ug) and ciprofloxacin (5ug).
Results: One isolate (7.14%) was resistant to ciprofloxacin, three (16.66%) to chloramphenicol and four (22.22%) to nalidixic acid, five (27.77%) to erythromycin, seven (38.88%) to tetracycline, sixteen (88.88%) to trimethoprim/sulphamethoxazole and ampicillin respectively.
Conclusion: The susceptibility pattern reflects variable susceptibility with maximum resistance to ampicillin and trimethoprim/sulphamethoxazole. Four isolates were resistant to nalidixic acid.

KEY WORDS: Campylobacter jejuni, Diarrhoea/dysentery, Antimicrobial resistance, Children.

INTRODUCTION

Diarrhoea is a common cause of increased morbidity and mortality in children in developing countries and Pakistan is no exception. According to WHO fact sheet diarrhoea occurs worldwide and causes 4% of all deaths and 5% of health loss to disability. It kills around 2.2 million people globally each year, mostly children in developing countries1. Bacteria are important agents in the long list of the causes of diarrhoea and among them Campylobacter jejuni (C. jejuni) is now being recognized around the world as one of the principal causes of gastroenteritis2. Pakistan being a developing...
country has a high incidence of diarrhoeal diseases especially in children and C. jejuni may be one of the principal causes of diarrhoea. Little information is available on the subject in our setup, as most of the laboratories are not carrying out cultures for C. jejuni routinely. This may be due to lack of awareness of the subject or due to financial constraints. A study was designed to assess the existing sensitivity pattern against routinely used antimicrobials using disc diffusion method as empirical therapy in children admitted with diarrhoea /dysentery in Military Hospital, Rawalpindi.

PATIENTS AND METHODS

The study was performed on 18 clinical isolates recovered from one hundred stool samples of children up to the age of twelve years admitted with diarrhoea/dysentery in Military hospital, Rawalpindi. The samples were collected in clean polypropylene containers with screw caps, containing Cary Blair medium for their transport to the Pathology Laboratories, Army Medical College, Rawalpindi. The samples so collected were inoculated on Modified Preston and Karmali media (Oxoid) in parallel with cultures on Deoxycholate Citrate Agar and Thiosulphate Citrate Bile Salt Agar (Oxoid). The cultures were incubated at 42°C in Anaerobic Jar (Oxoid USA, Columbia, MD) under microaerophilic conditions using gas-generating kit CN 035 (Oxoid). The growth was identified after 48 hours by colony morphology, oxidase test, Gram staining and motility. The organisms were identified to species level by a positive catalase test, a negative urease test, failure to produce H2S, non-fermentation of sugars, resistance to cephalothin, nitrate reduction and hippurate hydrolysis. Sensitivity testing was carried out on lysed horse Blood Agar using Modified Kirby Bauer technique against ampicillin (10ug), erythromycin (15ug), tetracycline (10ug), chloramphenicol (30ug), trimethoprim/ sulphamethoxazole (1.25ug/23.75ug), nalidixic acid (30ug) and ciprofloxacin (5ug). The sensitivity plates were incubated at 37°C under microaerophilic conditions generated in a similar manner as for primary isolation. The results were read after 24 hrs. and interpreted in accordance with NCCLS criteria.

Inclusion Criteria:
Stool samples of either sex children up to the age of 12 years suffering from diarrhoea/dysentery were included in the study. The samples containing mucus, pus and/or blood were preferred.

Exclusion Criteria:
Children on antibiotics, three days prior to sample collection were excluded.

RESULTS

Out of 100 stool samples studied eighteen samples (18%) yielded the growth of C. jejuni (Table-I). Sixteen out of 18 isolates were resistant to trimethoprim sulphamethoxazole (88.88%), four were resistant to nalidixic acid (22.22%) and one was resistant to ciprofloxacin (5.55%) (Table-II).

Table-I: Frequency of Faecal Isolates
(n = 100)

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>18 (18%)</td>
</tr>
<tr>
<td>Vibrio cholerae biotype El Tor</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>serotype Ogawa</td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Table-II: Antibiotic Resistance of Campylobacter Jejuni Isolates
(n = 18)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>11 (61.11%)</td>
<td>7 (38.89%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2 (11.11%)</td>
<td>16 (88.89%)</td>
</tr>
<tr>
<td>Sulphamethoxazole-</td>
<td>2 (11.11%)</td>
<td>16 (88.89%)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>15 (83.33%)</td>
<td>3 (16.67%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>13 (72.22%)</td>
<td>5 (27.78%)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>14 (77.78%)</td>
<td>4 (22.22%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>17 (94.45%)</td>
<td>1 (5.55%)</td>
</tr>
</tbody>
</table>
DISCUSSION

*Campylobacter jejuni* (C. jejuni) enteritis is usually self-limiting. However, antibiotics have a role in reducing the symptoms, shortening the span of illness and controlling the transmission in the community. Erythromycin, tetracycline and quinolones have all been recommended in different clinical settings to treat campylobacter gastroenteritis\(^\text{11-16}\). Fluoroquinolones are frequently prescribed empirically for diarrhoeal illness including travelers diarrhoea because of their effectiveness against a wide range of enteric bacteria\(^\text{14-18}\). Fluoroquinolones also shorten the diarrhoea and severity of symptoms caused by *C. jejuni*\(^\text{19,20}\). Since the late 1980s resistance in campylobacter isolates to fluoroquinolones has been increasing especially in Europe\(^\text{13,21}\). A causal relationship has been found between the use of fluoroquinolones in animals and an increase in fluoroquinolone resistant campylobacter infection in humans\(^\text{21,22}\).

In our study one isolate (5.55%) was resistant to ciprofloxacin whereas four isolates (22.22%) were resistant to nalidixic acid. Ciprofloxacin resistant isolate was also resistant to nalidixic acid. Ciprofloxacin and nalidixic acid resistance reported from Tokyo, Japan was 7.3% and 10.3% respectively\(^\text{23}\). In Montreal, Canada these were 12.7% and 13.9% respectively\(^\text{24}\). In a study from Mallorca, Spain 27.8% was resistant to ciprofloxacin and 24.2% were resistant to nalidixic acid\(^\text{25}\). In Chennai, India while all the isolates (100%) were resistant to nalidixic acid, none was resistant to ciprofloxacin\(^\text{26}\). Nalidixic acid resistant *C. jejuni* isolates recovered from diarrhoeal stools were 15% and 40% in Germany and in Egypt respectively\(^\text{27,28}\). The highest reported resistance to quinolones is from Villarroel, Spain where nalidixic acid and ciprofloxacin resistance has almost doubled from 47.5% in 1990 to 88% in 1994\(^\text{29}\). Quinolone resistance reported from Lugo, Spain and Norway were 34% and 23.3% respectively\(^\text{30,31}\). However, other studies report minimal resistance to ciprofloxacin. In a study from Lukhnov, India only 2.7% were resistant to ciprofloxacin\(^\text{32}\). In a study carried out in Sweden from 1978 to 1988 no general increase in vitro resistance to antibiotics commonly used for human gastroenteritis caused by *C. jejuni* was observed. The number of fluoroquinolone resistant strains from 1988 to 1989 was 0.7% and 1.4% respectively\(^\text{33}\). The resistance to ciprofloxacin in our study is higher than that reported from Lukhnov, India but is lower than the resistance reported from Tokyo, Japan, Chennai, India, Spain, Germany and Egypt. However, the resistance to nalidixic acid in our isolates was similar to that reported from Spain. It was higher than that reported from Japan, Canada, Germany and Lukhnov, India but was lower than Chennai, India.

Twenty seven percent of *C. jejuni* isolates in our study were resistant to erythromycin. In studies reported from Harare, Zimbabwe and Wroclow, Poland 15% of *C. jejuni* isolates were resistant to erythromycin while in Brazil 18.2% were resistant to erythromycin\(^\text{34,35}\). A very high degree of resistance to erythromycin (65%) in *C. jejuni* was observed in a study from Thailand where the antibiotic was found to have no effect on the duration of diarrhoea\(^\text{36}\). Similarly, a high level resistance to erythromycin was seen in a study from Singapore General hospital\(^\text{37}\). However, most of the other studies had shown low level of resistance to erythromycin\(^\text{23,29,30}\). In Lukhnov, India resistance in *C. jejuni* to erythromycin was 1.3%. It was 1% in London, UK 4%, in Kuala Lumpur, Malaysia while in Saudi Arabia 7.3%\(^\text{31,38,36,39}\). In a study carried out in Mallorca, Spain the rate of erythromycin resistance remained low (0.9% - 03.5%) during the period 1987-1991\(^\text{25}\). The high rate of erythromycin resistance in our study may be due to erythromycin being frequently prescribed in our set up.

The resistance of *C. jejuni* to trimethoprim/sulphamethoxazole and ampicillin was very high among our isolates (88.89%), as compared to the resistance reported from Brazil where 56.8% out of 22 isolates of *C. jejuni* were resistant to both the drugs\(^\text{35}\). All the strains were resistant to trimethoprim/sulphamethoxazole.
in the study carried out in Mallorca, Spain during the period 1987-1991. Ampicillin and trimethoprim/sulphamethoxazole are frequently prescribed to treat diarrhoea/dysentery and are readily available in our clinical setting. This clinical practice in our setting might be a contributing factor in increased resistance to trimethoprim/sulphamethoxazole and ampicillin in our study.

In our study 16.67% isolates were resistant to chloramphenicol. Resistance to chloramphenicol was common in studies from Vietnam and Thailand. All isolates in studies reported from Brazil and Zimbabwe were susceptible to chloramphenicol. Only 2.6% resistance was seen in isolates in Mallorca, Spain. Fifty percent of the isolates were found resistant in a study carried out in Egypt. Our figures remain less than those reported from Egypt but are higher than those reported from Spain, Brazil and Zimbabwe.

Tetracycline resistance (38.89%) in our setup is similar to the studies from Quebec, Canada, Lugo, Spain, Tokyo, Japan and Saudi Arabia where it was 40.7%, 43.1%, 43.2% and 32.7% respectively. Our results are higher than those reported from Lucknow, India and Wroclaw, Poland where 10% and 9.3% were found resistant to tetracycline respectively.

The C. jejuni revealed significant resistance to commonly used antimicrobials like ampicillin, trimethoprim/sulphamethoxazole, tetracycline, erythromycin and nalidixic acid in our study. In contrast chloramphenicol and fluoroquinolones had adequate activity against most of the C. jejuni. However, because of the adverse effects of chloramphenicol on bone marrow and fluoroquinolones on cartilage both the drugs may have to be reserved as a last option for C. jejuni.

ACKNOWLEDGEMENT

The author(s) are grateful to Pak-US Laboratory for Sero-epidemiology (PULSE) Army Medical College, Rawalpindi for providing funding, lab facilities and technical assistance without which this project could not be completed.

REFERENCES

Arif Maqsood Ali et al.