Original article

Antimicrobial sensitivity pattern of *Campylobacter* species among children in Jimma University Specialized Hospital, Southwest Ethiopia

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Abstract

Background: *Compylobacter* species are the major causes of diarrhoeal illness in children in Ethiopia. Thus, updated local information is very crucial in order to take effective control measures on this pathogen.

Objectives: To determine the prevalence and antibiotic susceptibility patterns of *Campylobacter* species in children younger than 15 years of age.

Method: A cross sectional study was conducted whereby stool specimens were collected from 430 children who had diarrhoea and were investigated for presence of common enteric bacterial pathogens and intestinal parasites. Stool specimens were inoculated on Salmonella-Shigella agar and Campylo agar plates, and then isolation, biochemical characterization, and antibiotic sensitivity testing were done in accordance with the standard methodology. Parasites were detected by direct stool microscopy.

Result: *Campylobacter* species were isolated from 11.6% of the total patients. The isolation rates of *Salmonella* and *Shigella* species were 5.8% and 4.9% respectively. Sixty five percent (283/430) of the children were found to be infected by one or more parasites. Close contact with cats or dogs, duration and consistency of diarrhoea were associated with the isolation of *Campylobacter* species. The antimicrobial sensitivity study findings showed that all tested isolates were sensitive to *chloramphenicol*, *gentamicin* and *kanamycin*. A majority of the strains of *Campylobacter* species were sensitive to *tetracycline* and *erythromycin*. The majority and half of the isolates were resistant for *trimethoprim-sulfamethoxazole* and *ampicillin*, respectively.

Conclusion: The findings of this research indicate that C*ampylobacter* species is an important etiological agent of childhood diarrhoea and therefore, it should be properly diagnosed in routine investigation and physicians should prescribe the appropriate drugs either after sensitivity testing or, in areas where there are no facilities for culturing, they have to refer updated information on local sensitivity pattern. [*Ethiop.J.Health Dev*. 2004;18(3):185-189]

Introduction

Diarrhoeal diseases have been recognized since the beginning of civilization, and remain one of the most prevalent public health problems of today. About two-thirds of the world population live in areas regarded as under developed and it is estimated that over 1.3 billion cases of diarrhoeal illness occur each year in these areas, resulting in five million deaths; of these over 2.7 million deaths occur in children below fifteen years of age (1). In Ethiopia, various studies have invariably concluded that diarrhoeal diseases are major causes of infant and child mortality and morbidity. About 39,000,000 episodes of diarrhoea per year were estimated to occur in Ethiopia; out of which 230,000 deaths occur in children below five years of age (2). The pediatric admission review at Jimma hospital showed that diarrhoea was the second leading cause of admission and hospital deaths, accounting for 11% and 15.8% respectively (3). Studies done in different parts of Ethiopia; former Ethio-Swedish children Hospital, Gondar teaching hospital, and Dembia district have shown that *Campylobacter* species were major causes of diarrhoeal illnesses (4-8). Diarrhoea caused by *Campylobacter* species is often a mild and self limited disease, but some of the more serious cases may require antibiotic treatment, like for instance, for patients with severe extra-intestinal manifestations such as septicemia and meningitis (9). The available Information on anti-microbial sensitivity of *Campylobacter* species differs somewhat in different countries (8,10,11). In Ethiopia, at present, there is only one published study on the anti-microbial sensitivity pattern of *Campylobacter* species (8). The purpose of this study is to determine the prevalence and anti-microbial susceptibility pattens of *Campylobacter* species in children younger than 15 years.

Methods

A cross sectional study was conducted to determine the prevalence and anti-microbial sensitivity pattern of *Campylobacter* species among children at the Jimma University Specialized Hospital, Pediatric out patient Department, from September 2002 to June 2003.

Jimma University Specialized Hospital has 300 beds and provides curative and preventive service for 300-400 patients per day at its outpatient department. The Pediatric and Child Health Department with its 65 bed capacity gives inpatient services to patients younger than 15 years of age and manages 80-100 children daily in the out patient section. The School of Medical Laboratory

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Technology and the hospital are in the same campus and are located at a distance of 200-300 meters apart.

All children younger than 15 years of age who came to the pediatric outpatient department complaing of diarrhoea or dysentery during the study period were taken as study subjects. In this study, diarrhoea was defined as the presence at least three loose stools or one watery stool per day and dysentery as the presence of blood in the stool. Patients under 15 years of age, for the purpose of this study, were considered as children.

After informed consent was secured, intern medical doctors working in the out patient department interviewed parents for their clinical history using structured questionnaires. Variables included were exposure to domestic animals, use of antibiotics in the last 6 days, type of stool, duration of diarrhoea, presence of fever, abdominal pain, vomiting and tenesmus.

Fresh stool samples were collected from each study subject using sterile screw-capped containers without transport the media and delivered to the the laboratory of School of Medical Laboratory Technology within one hour of collection.

Culture and identification of Campylobactor species: Specimens were inoculated on Campylo agar plates (Oxoid, Ltd, England) and kept in gas jar containing campylobacter gas pack systems to maintain the microaerophilic condition. The jar was incubated at a temperature of 42^{o}C for 48 hrs. The identification was made by characteristic appearance on culture medium, gram stain, oxidase, and catalase reaction (12-14).

Culture and identification of Salmonella and Shigella species: Specimens were inoculated on Salmonella-Shigella agar (Oxoid, Ltd, England) plates and were incubated for 24-48 hours. The suspected colonies were inoculated on nutrient broth and confirmed by the pattern of biochemical reactions using the standard procedures (12-14).

Microscopic examination of stool for ova and parasites: Parasites were identified through direct microscopy using saline and iodine wet mount.

Antimicrobial sensitivity testing of Campylobacter species: Anti-microbial sensitivity test was performed using the standard agar disc diffusion method and turbidity of the inoculum was matched with the turbidity standard McFarland 0.5 (15). *Campylobacter* species were tested for the following antimicrobial agents (obtained from Becton, Dickinson, USA): *Ampicillin* (10µg), *Gentamicin* (10µg), *Chloramphenicol* (30µg), *Thrimthoprim-sulphamethoxazole* (25µg), *Kanamycin* (30µg) Tetracycline (30µg) and *Erythromycin* (15µg).

A standardized inoculum of each isolate was swabbed on to antibiotic sensitivity medium; discs were added after drying the plates for 3-5 minutes. The plates were incubated at 42^{o}C for 48 hours in anaerobic jars using CO_2 generating kits.

A standardized reference strain of *E. coli* (ATCC 25922), sensitive to all the antimicrobial drugs being tested was used as a control for the study. The diameter of the zone of inhibition around the discs was measured to the nearest millimetre using a metal calliper and the isolates were classified as sensitive and resistant according to the standardised table supplied by the manufacturer (16). Intermediate readings were very few and were considered as sensitive.

Ethical clearance was obtained from the Research and Publications Office of Jimma University. After getting informed consent data and stool samples were collected and, based on the result, appropriate treatment was given. Data were cleared and entered into a computer and statistical analysis was performed using SPSS for windows version 7.5 (17). Chi-square was used to test differences between proportions and P-values <0.05 was considered statistically significant.

Results

Out of the 430 children that had diarrhea, 257 (60%) were male and 173 (40%) were female making the male to female ratio 1.5:1. The age of the studied children ranges from 2 to 180 months: 66(15.3%) younger than 12 months, 69(16%) 12-23 months, 45(10.5) 24-35 months, 29(6.7%) 36-47 months and majority, 188 (43.7%), were 60 months and above (Fig 1).

Bacterial pathogens were isolated from 96 (22.3%) of the 430 children that were younger than 15 years of age. The isolation rates were 5.8% for Shigella species, 4.9% for Salmonella species, and 11.6% for Campylobacter species. Sixty-five percent (281/430) of the children were infected by one or more parasites (Table 1). Thirty-five (13.6%) of the male and 15(8.7%) of the female children were positive for Campylobacter species with a P-value of 0.16. Most Campylobacter isolates were from children who were below 5 years of age. The culture positivity for Campylobacter was higher for children below 48 months of age (Fig.1).

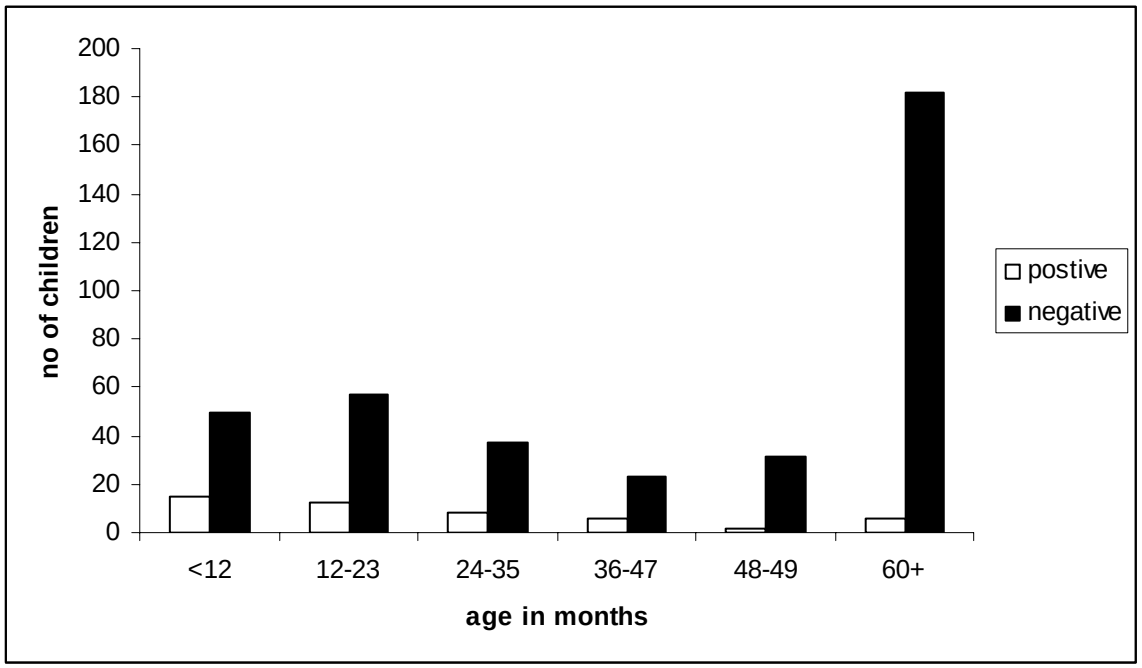

Figure 1: **Age distribution of children who tested positive for *Campylobacter* Spp in Jimma Hospital, Southwest Ethiopia, and 2002-2003**

Table 1: **Bacterial pathogens and parasites detected from 430 children with diarrhoea in Jimma University Specialized Hospital, Jimma South west Ethiopia, 2002/2003**

Etiological agents	No.	%
Campylabacter species positive	50	11.6
Negative	380	88.4
Shigella species positive	25	5.8
Negative	405	94.2
Salmonella species positive	21	4.9
Negative	409	95.1
Parasite positive	283	65.8
Negative	147	34.2
Types of Parasite		
*G. lamblia**	70	24.7
*E. histolytica**	69	24.4
A lumbricoides	66	23.3
T. trichuria	39	13.8
Hookworm	16	5.7
H. nana	11	3.9
S. mansoni	6	2.1
Str. stercoralis	4	1.4
E.vemicularis	2	0.7

* Includes both cyst and trophozite stages

Of the 262 children who had contacts with dogs or cats, 38 (13.5%) were found to be positive for *Campylobacter* species with a p-value of 0.03. Out of 150 children who had contact with other animals, 17(11.3%) were found to be positive for *Campylobacter* species. On the other hand, out of 280 children who had no history of animal contact, 33(11.8%) were positive for *Campylobacter* species with a P-value of 0.99.

Even though it was not statistically significant, most *Campylobacter* species positive children who complainted of abdominal pain and other symptoms like fever, tenesmus and vomiting, were not different from those who were culture positive and negative (Table 2). As is indicated on Table 2, strong association was observed between *Campylobacter* species positivity and the duration of diarrhea. Most positive cases were noted within 1 to 5 days duration. Fifty isolated *Campylobacter* species were tested against seven antibiotics. All isolated *Campylobacter* species were sensitive to *chlora-mphenicol, gentamicin and kana-mycin.* A*mpicillin* and *trimethoprim-sulfamethoxazole* were found to have resistance against *Campylobacter* species of 50% and 60%, respectively. The majority of the strains were sensitive to tetracycline and erythromycin (Table 3).

Table 2: **Clinical findings and their association with positivity of *Campylobacter* species among 430 children with diarrhoea in Jimma University Specialized Hospital, Jimma South west Ethiopia, 2002/2003**

Clinical findings	Positive (%)	Negative (%)	p-values
Fever			
Yes	30 (10.3)	26 (89.7)	
No	20 (14.4)	119 (85.6)	.28
Vomiting			
Yes	20 (14.20	121 (85.8)	
No	30 (10.3)	259 (89.6)	.31
Tenesmus			
Yes	31 (11.5)	239(88.5)	
No	19 (11.9)	141 (88.1)	.97
Abdominal pain			
Yes	45 (11.1)	360 (88.9)	
No	5 (20)	20 (80)	.19
Duration of diarrhoea			
1-5 days	25 (9.1)	250 (90.9)	
6-10 days	13 (11.4)	101 (88.6)	
11-15 days	9 (34.6)	17 (65.4)	
≥16 days	3 (20)	12 (80)	.001
Consistency			
Watery	18 (8.7)	190 (91.3)	
Mucoid	11 (9.2)	109 (90.9)	
Bloody	11 (19.3)	46 (80.7)	
Mixed (blood & mucus)	10 (22.2)	35 (77.8	.01

Table 3: **Antimicrobial susceptibility patterns of 50 *Campylobacter* isolates in Jimma University Specialized hospital, Jimma south west Ethiopia, 2002/2003**

Anti-microbial tested	Sensitive strains No. (%)
Tetracycline	43 (86)
Chloramphenicol	50 (100)
Gentamicin	50 (100)
Erythromycin	45 (90)
Ampicillin	25 (50)
Kanamycin	50 (100)
Trimethoprim sulfamethoxazole	20 (40)

Discussion

This hospital-based study showed that 11.6% of all cases of diarrhoea were due to *Campylobacter* species, which is slightly lower than other findings in at Gondar and Addis Ababa with isolation rates of 13.8% and 15.3%, respectively (4,6). This could be due to differences in geographical location and study period. In tropical countries like Zaire and the Central African Republic, a higher incidence of campylobacteriosis has been noted during the rainy season (18). The finding of this study is more or less consistent with the isolation rate of 10.5%, studied at Dembia district, North West of Ethiopia (7). Most *Campylobacter* species associated diarrhoea (44/50) was seen in children below 5 years of age, which is similar with another study conducted at Dembia, Ethiopia (7). The distribution of *Campylobacter* species between females and males was different but was not statistically significant, which agrees with the study results reported in different parts of Ethiopia (4, 6, 7).

High infection rates were seen in children who have close association with cats and dogs, which indicates the direct connection of *Campylobacter* species infection with pets like cats and dogs, as it is already pointed out that direct contact with these animals is a frequent mode of transmission to humans (19). Compared with other studies done else where in the world, the isolation rate of this study is higher than the reports from Singapore (1.2%) and Italy (2.27%) and lower than the isolation rates reported from Egypt and Bangladesh, which were 16.8% and 26%, respectively (10,20,21,22).

In this study, watery diarrhoea was the most reported consistency among children who tested positive for *Campylobacter* species. There was significant association between the duration of diarrhoea and culture positivity as the majority were from 1 to 5 days duration, which is similar with study findings done at Gondar teaching hospital, and different from a study conducted at Dembia (4, 7).

Gastroenteritis caused by *Campylobacter* species is often a mild and self-limiting disease, but many of the more serious cases require antibiotic treatment. In this study, seven antibiotics were tested against 50 isolates of *Campylobacter* species. All tested isolates were sensitive for *chloramphenicol, gentamicin* and *kanamycin*. This could be because of the fact frequently that these antibiotics are prescribed less frequently in treating diarrhoeal cases either due to lesser availability or cost. Half of the isolates were resistant to *ampicillin* while the majority were resistant to *trimethoprim sulfameth-oxazole.* As is indicated in another similar study, this counld be either because they are commonly prescribed or are sold on the open market and private pharmacies without prescription (8). Compared with other similar research findings conducted in Addis Ababa, this study showed increased resistance to most tested drugs. Next to *chloramphenicol, gentamicin* and *kanamycin*, *erythromycin* is used for the treatment of *Campylobacter* species infection in most countries, but in some countries emerging drug resistant isolates present a particular problem in Thailand, for example, *erythromycin* resistance was 11% and 46% for *C. jejuni* and *C. coli* respectively and in Singapore it was 51%(23,10).

In summary, the findings of this research indicated that *Campylobacter* species is an important entero-pathogen prevalent in children younger than 15 years of age, and therefore, should be looked for in cases of childhood diarrhea. As it can be seen from this and previous studies, the resistance rate of *Campylobacter* species increased through time, which reflects either scarcity of diagnostic

laboratory facilities or inappropriate usage of the commonly available drugs. Therefore, providing updated information on the local sensitivity pattern of *Campylobacter* species is very helpful for health personnel who are primarily in charge of treating childhood diarrhoea.

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