The role of campylobacter species in diarrhea among children under five years of age in Ramadi city ,west of Iraq .

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Abstract:

Objective: The main objectives of the present study were to identify the incidence of *Campylobacter* species and their antibiotic susceptibility and risk factors among children with diarrhea under five years of age admitted to maternity and children hospital in Ramadi city.

Materials and methods: Two hundred eighty children under five years of age admitted to maternity and children hospital in Ramadi city for management of diarrhea over a period of one year from (1st of October 2005 to 30th of September 2006), were studied for identification of *Campylobacter* species as a cause of diarrhea. Each child was studied for the followings: age, sex, residency, source of water supply, associated symptoms, type of feeding and family history of chicken breeding.

Specific cultures of stool specimens for *Campylobacter* were done for all patients and also for ordinary bacterial agents. Isolates were identified and the percentage of the causative agents were discussed .

Results: *Campylobacter* species as a causative agent of acute diarrhea in Ramadi city was found to be 8.92%.The incidence of *Campylobacter species* among other bacterial causes of diarrhea was 21.2%. Ciprofloxacin , nalidixic acid and clindamycin demonstrated high level of susceptibility (96%, 92% and 96% respectively), while the remaining antibiotics showed either moderate susceptibility or resistance.

Conclusion: Campylobacter species is an imprtant cause for diarrhea among children under five years of age admitted to maternity and children hospital in Ramadi city. Bottle feeding and chicken breeding are important risk factors for getting Campylobacter infection. Ciprofloxacin, nalidixic acid and clindamycin are the most effective treatment for Campylobacter species, and recommended as drugs of choice.

Keywords: campylobacter , diarrhea ,under five ,Ramadi.

Introduction:

Diarrhea is a common cause of increased morbidity and mortality in children in developing countries. According to WHO fact diarrhea occurs worldwide and causes 4% of all death and 5% of health loss to disability. It kills around 2.2 million people globally each year, mostly children in developing countries.[1]

Bacteria are important agents in the long list of causes of diarrhea and among them *Campylobacter* which is now being recognized as one of the principal causes of gastroenteritis,[2] and often exceeding those of *Salmonella* and *Shigella.*[3]

The genus *Campylobacter is* thin, curved, gram negative, non-spore forming rods, includes more than 18 species which are considered pathogenic to humans.[4] *Campylobacter jejuni* and *Campylobacter coli* are the two main species involved in human infections.[5,6]

The human infection with *Campylobacter* gastroenteritis has seasonal variation, and this differs from one country to another. However, in hot areas it occurs in summer season.[7]

Clinically, patients with *Campylobacter* gastroenteritis develop fever, which may be the early manifestation, diarrhea, vomiting, malaise and tenesmus. Diarrhea may be bloody or watery. Older children also complain of abdominal pain, which is periumbilical and cramping in nature.[8] Serological studies suggest that 20-45% of patients with Gullian-Barre syndrome have evidence of recent *Campylobacter jejuni* infection .[9]

Campylobacter species gastroenteritis is usually self-limiting disease. However, antibiotics have a role in reducing the symptoms, shortening the span of illness and controlling the transmission in the community.[10_15]

Little information are available on the subject in our country, as most of the laboratories are not carrying out culture of *Campylobacter* routinely, therefore the present study aimed to identify the incidence of *Campylobacter* species among other enteropathogenic causes of diarrhea during a year and to assess the emerging resistance pattern against routinely used antimicrobials. Also, to identify the risk factors among children under 5 years of age addmitted to maternity and children hospital in Al-Ramadi city.

Material and Methods:

Patients under 5 years of age who were admitted to the maternity and children hospital in Ramadi city for management of diarrhea were included in the study to find out the role of *Campylobacter* species as a causative agent for diarrhea over a period of 1 year from (1st of October 2005 to 30th of September 2006). Each child was studied for age , sex , residency , source of water supply , associated symptoms { fevere , vomiting ,diarreah (watery or bloody) , tenesmus , convulsion }and family history of chicken breeding . Stool specimens were collected in wide mouth sterile containers to be delivered to the hospital laboratory as soon as possible.

In the laboratory, stool specimens were inspected macroscopically for the presence of blood or mucus and examined microscopically for the presence of pus cells, RBCs, parasites and yeasts in addition to chemical examination including pH and reducing substances.

Cultures were done to all stool specimens as follows:

1- Ordinary culture media, including MacConkey agar (oxoid, England), blood agar (oxoid, England) and S.S. agar (oxoid, England). To isolate and identify the organism that can grow on these types of media.

2- Cultures for *Campylobacter* species was done on *Campylobacter* agar base media supplemented with *Campylobacter* supplement.

This type of culture was done on special atmosphere containing gas generating kits for *Campylobacter*.

Plates in the atmosphere were incubated for about a week at 40-42 ^{o}C to be identified at the end of the incubation period.

Isolates were identified according to their culture characteristics, microscopical appearance and their response to biochemical test.

Sensitivity testing was carried out by modified Kirby Bauer disk diffusion technique on Molar Hintan agar as described by the WHO, 1991[16] to identify the susceptibility pattern of *Campylobacter* species isolates against ciprofloxacin, nalidixic acid, clindamycin, cefotaxime, eryhtromycin, ampicillin, amikacin, gentamicin, co- trimoxazole and tetracycline.

Statistical analyses were done using SPSS version 11 computer software (statistical package for social sciences). The statistical significance of association between two categorical variables was assessed by Chi-square test. P value less than 0.05 was considered statistically significant.

Results:

Two hundred eighty children under five years of age complaining of diarrhea addmitted to maternity and children hospital in Al-Ramadi City over a period of 12 months were studied for isolation of *Campylobacter* species from stool culture. Out of 280 stool cultures, 25 samples (8.92%) yielded the growth of *Campylobacter* species. The majority of cases (60%) were under one year, while 36% were between 1-3 years of age and only 4% from 3-5 years, the difference was significant among the three age groups (P. value < 0.05).

Out of these culture positive cases, 52% were males and 48% were females; there is no significant difference between both genders (P. value 0.135). Table 1.

The percentage of *Campylobacter* species was found to be 21.2% among other types of bacterial agents isolated in our study. Table 2

Rural area, bottle and mixed feeding and chicken breeding were found to be significant factors for developing *Campylobacter* gastroenteritis (P. value < 0.05 for each one) whereas the source of water supply showed no significant difference (P. value 0.115). Table3

Depending on gross examination of stool samples, 12 patients (48%) of all positive cultures were bloody diarrhea, whereas 13 patients (52%) were non-bloody diarrhea. Table 4

Regarding the clinical status of patients, fever represented the most frequent symptom of *Campylobacter* acute gastroenteritis (96%) followed by vomiting (88%) and tenesmus (64%), other symptoms as convulsion was not reported. Figure 1

During the four seasons of the year we noticed that the majority of cases occurred from beginning of June to the end of August. Figure 2 .

In vitro susceptibility to antibiotics, the isolated campylobacter bacteria showed lower resistance to ciprofloxacin, clindamycin and nalidixic acid (4%, 4% and 8% respectively), while the resistance to amikacin, gentamicin and cefotaxime was moderate (20%, 28% and 36% respectively).

Higher resistance towards erythromycin (56%) , ampicillin (68%), tetracycline (72%), and co-trimoxazole (72%) was observed . Table 5

Table 1. Sex difference of Campylobacter +ve cases, intervals by age

Age groups (years)	Gender		Total (%)
	Male	Females	
<1	8	7	15 (60%)
1-3	4	5	9 (36%)
3-5	1	-	1 (4%)
Total	13 (52%)	12 (48%)	25 (100)%

P. value of sex difference 0.135

Table 2.Distribution of *Campylobacter* species among other types of bacterial agents according to different age groups.

Age groups (years)	Culture positive				Culture negative
	Campylobacter	E.coli	Shigella	Salmonella	
<1	15	41	6	2	94
1-3	9	28	4	2	53
3-5	1	9	1	0	15
Total	25(21.2)	78(66.1)	11(9.3)	4(3.3)	162 (58%)
	118(42%)				

Table 3. the effect of residency , water supply , type of feeding and chicken breeding on campylobacter gastroentritis .

associated factor	Variables	No.	%	P. Value
	Rural	18	72	
Water supply	Urban	7	28	0.115
	Pipe	14	56	
Type of feeding	Other source	11	44	<0.05
	Breast	5	20	
Chicken breeding	Bottle and others	20	80	<0.05
	Breeding	19	76	
	No breeding	6	24	

Table 4. Type of campylobacter diarrhea according to clinical presentation

Type of diarrhea	No.	%
Bloody	12	48
Non-bloody	13	52

Table 5. Result of susceptibility of different antibiotics against isolated campylobacter

Antibiotic	Susceptibility	No.	%
Ciprofloxacin	S	24	96
	R	1	4
Nalidixic acid	S	23	92
	R	2	8
Clindamycin	S	24	96
	R	1	4
Erythromycin	S	11	44
	R	14	56
Amikacin	S	20	80
	R	5	20
Gentamicin	S	18	72
	R	7	28
Cefotaxime	S	16	64
	R	9	36
Ampicillin	S	8	32
	R	17	68
Co-trimoxazole	S	7	28
	R	18	72
Tetracycline	S	7	28
	R	18	72

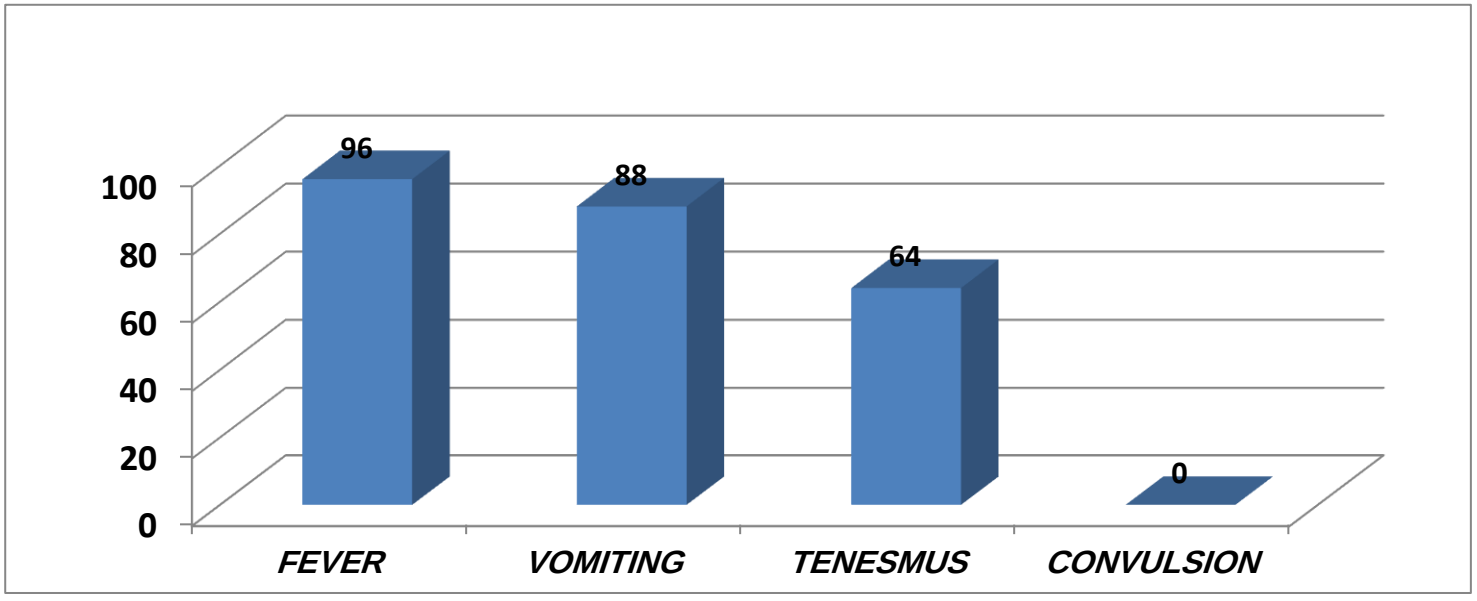

Figure 1. The frequency of clinical manifestatioins of Campylobacter Gastroenteritis

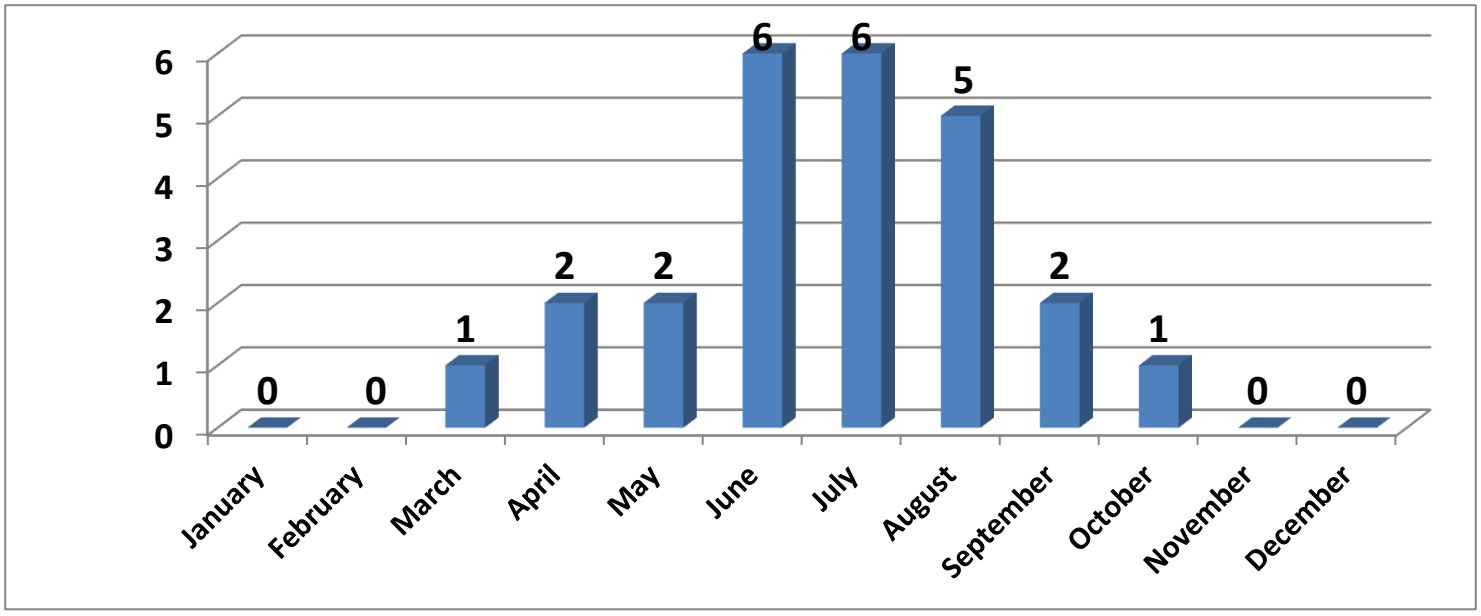

Figure 2. Seasonal variations of Campylobacter Gastroenteritis

Discussion:

Campylobacter species is one of the commonest causes of human bacterial diarrhea in an industrialized and developing countries[4,17,18], and is the first bacterial pathogen in developed countries[19,20].

The isolation rate of *Campylobacter* in our study was 8.92%, this result is within the range of isolation rate of *Campylobacter* in developing countries which is from 5-20%[12]. Poor hygien and sanitation and

close proximity to animals in developing countries all contribute to easy and frequent acqusition of any enteric pathogen including *Campylobacter*[7,21].

Males and females were found to be almost equally affected with gender variation of no significance, similar results were reported in England and Wales[11] and developing countries[21].

In developing countries *Campylobacter* diarrhea is most commonly isolated below one year of age[21] . The same result was found in our study, this may be attributed to decline of

maternal immunity transferred to infants in the next six months of age, lack of active immunity and the introduction of food that may be contaminated.[22]

The source of transmission route of human *Campylobacter* infection is not fully understood, but handling and eating of poultry meat have been shown to be important risk factors.[23,24,25] Consumption of undercooked poultry meat is reported to be the major cause of sporadic *Campylobacter* infection ; *Campylobacter* species was isolated from 73.7% of poultry carcasses in Turkey[28], 46% in Germany[29], 46% in Japan[30] and 73% in USA[31].

Other risk factors are unpasteurized milk and milk products, unchlorinated or inadequately chlorinated water supply, bottle and mixed feeding. [26] Our finding of no significant diffrence between pipe water and other sources is propably due to poor chlorniation during the study period related to bad security state in Ramadi.

Among associated factors, type of feeding and chicken breeding were found to be the most important factors. Several studies have shown the benefit of breast milk in protection from enteric *Campylobacter* species[19,21].Circulating antibodies against flagellin and possibly maternal antibodies seem to protect against intestinal colonization of *Campylobacter*. Results showed that most French and all African breast milk possessed anti-flagellin IgA.[27]

Seasonal variation of *Campylobacter* infection varied according to geographical location.[32] Results showed that increase incidence of *Campylobacter* infection is associated with increase temperature[33,19].In tropical countries there is no noticeable seasonal variation[12]. In our study, the peak incidence was observed in June, July and August.

Regarding antibiotic susceptibility, the isolated microorganism from studied cases showed lower resistance to ciprofloxacin, clindamycin and nalidixic acid (4%, 4% and 8% respectively). Ciprofloxacin and nalidixic acid resistance reported from Japan was(7.3% ,10.3% [34]),Canada (12.7% ,13.9%[35]), Spain (27.8% , 24.2%[36]),and Pakistan (5.55% , 22.22%[37]) respectively. However, lower resistance to ciprofloxacin in India 2.7%,[38] and Egypt 4%.[39]

Clindamycin resistance reported in Germany was 2%[40], in Spain 2.8%[36] and in USA 2%[41]. This result may be due to a very little use of these drugs in our society.

Erythromycin resistance in our study was relatively high 56%. Similar results were obtained in Pakistan 62%[37], Singapore 63%[42], and Spain 34.5%[36]. However, most of other studies showed low level of resistance to erythromycin, in India 1.3%[38], 1% in London[43],and 4% in Malaysia[44]. The high rate of erythromycin resistance in our country may be due to erythromycin being frequently prescribed.

The *ampylobacter* resistance to co-trimoxazole, ampicillin and tetracycline was very high in our study (72%, 68% and 72% respectively). Almost similar results were obtained in Spain[36], Egypt[39], Germany[40] and Pakistan[37].

Amikacin, gentamicin and cefotaxime showed moderate resistance to *Campylobacter* (20%, 28% and 36% respectively). The result of this study is nearly similar to other studies. In Spain, amikacin 21.6%, gentamicin 22.2% and cefotaxime 31.6%[36]. Similar observation was reported in some developing countries[45].

This variation of antibiotic susceptibility may be related to over-use of antibiotics which became wolrd-wide problem especially in developing countries.

Conclusion and Recommendation:

Campylobacter species is an important bacterial cause of gastroenteritis in children under five years old in Ramadi city , so physician should be aware for this problem and laboratories should be facilated for isolation 0f this bacteria.

Bottle feeding and chicken breeding are risk factors for *Campylobacter* gastroenteritis,so families should be educated about handling the chichen inside houses and during food preparation to prevent cross contamination from raw poultry to other food items. Educative programs for mothers should be done to encourage breast feeding as a preventive measure for *Campylobacter* gastroenteritis.

Ciprofloxacin,naldixic acid, and clindamycin are the most effective in vitro against *Campylobacter* species . we advice physicians to be aware of these findings when prescribing drugs against Campylobacter .

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