

## Bacterial Etiology and Antimicrobials Susceptibility of Diarrhea among Displaced Communities during 2006-2008

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**Objective:** The aim of the study was to isolate and identify the common bacteria causing acute diarrhea among displaced persons and assess antimicrobial sensitivity of bacterial isolates.

**Setting:** Mandella (South Khartoum) and Wad Elbashir (West Omdurman) Displaced Camps, Health Centers.

**Design:** Descriptive Cross Sectional Study.

**Method:** Four hundred and twenty patients, age group ranging from 3 months to 55 years suffering from diarrhea and attending the health centers were included in the study. Stool specimens or rectal swabs were collected and investigated for enteropathogenic bacteria. Personal characteristic data were collected using direct interviewing questionnaire. The study was conducted from April 2006 to September 2007. Ethical clearance was obtained from Sudan Ministry of health, Research Ethical Committee.

**Result:** Bacterial pathogens were detected in 110 (26.2%) of the patients. Children under 5 years were mostly affected, 178 (42.4%), followed by children over 5 years (5-14 years), 142 (33.8%), and then adults (more than 14) 100 (23.8%). Different pathogens were isolated, however *Shigella spp* were frequently isolated (41.8%). Most isolates were sensitive to Cefuroxime, followed by Ciprofloxacin and Gentamicin.

Most patients depend on donkey cart as water source 222 (52.9%), 120 (28.6%) patients getting their water directly from water pump and 70 (16.7%) patients are using storage tanks as a water supply. Two hundred and thirty (54.8%) patients are consuming untreated drinking water. At the same time 76.1% of patients under study have no disposal latrines.

**Conclusion:** Most cases of bacterial diarrhea in displaced camps are caused by *Shigella spp*, especially among adults, and children over 5 years; in children under 5 years the main culprit was *Campylobacter jejuni*. High degree of resistance to antimicrobials was observed against several antimicrobials: Amoxicillin, Erythromycin, and Co-Trimoxazole, but low resistance to Cefuroxime.

Most patients consume water from donkey cart, drink untreated water, had no latrines, and live in a poor environmental condition; all of them are risk factors for acquiring diarrhea.

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Diarrheal diseases constitute a major public health problem, particularly in the developing world, where the rate of mortality and morbidity is very high<sup>1</sup>. The World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhea occur every year in developing countries, resulting in 3 million deaths<sup>2</sup>.

An inadequate water supply, both in quantity and quality, poor sanitation, overcrowding and malnutrition are the main factors implicated in the occurrence, spread and severity of diarrheal disease. Malnutrition and diarrheal disease are closely linked; malnutrition increases the severity and prolongs duration of diarrhea and it may cause malnutrition<sup>2</sup>.

Among refugees and displaced populations, diarrheal disease is a major cause of morbidity and one of the main causes of mortality. The mortality rates attributable to diarrheal diseases in the refugee camps of Somalia, Sudan, Ethiopia, Malawi and Goma was 28-85%<sup>3,4</sup>. However, this high mortality rate from diarrhea can be easily decreased by fluid replacement therapy.

Diarrheal disease would markedly impair the development of the country because of the cost it incurs on the economy. Acute and complicated cases of diarrhea may cause a loss of working hours and days. Many countries have recognized the importance of diarrheal diseases as priorities when designing national primary health care programs.

Displaced population around Khartoum state live in camps characterized by poor, unhealthy condition, suffering from diarrhea, the etiology of which remains obscure.

The aim of this study is to investigate the bacterial etiology and antimicrobials sensitivity associated with diarrhea in displaced camps.

## **METHOD**

Four hundred and twenty stool specimen were collected from patients suffering from diarrhea, using sterile clean dry containers or rectal swabs transported in Carry Blair transport medium (Oxoid-England).

Displaced persons who live in the camp and suffering from diarrhea (all age groups) were included in the study. Others who do not live in the camp and those who have no diarrhea were excluded. Ethical approval was obtained from Research Ethical Committee, Sudan Ministry of health.

Stool specimens were plated when possible after arrival in the laboratory using different types of selective media including xylose lysine deoxy cholate agar (XLD), selenite F broth (Lab M-UK) and MacConkey agar, with, and without Sorbitol, Micro Master-India, incubated aerobically at 37°C for 18 hours. Butzlers selective media, Oxoid-England, incubated in the

presence of Co<sub>2</sub> and hydrogen gas-pack for 48 hours at 42°C, thiosulphate citrate bile salt Sucrose agar (TCBS), and alkaline peptone water (Himedia-India) incubated at 37°C for 48 hours.

The colonial morphology on all media inoculated was studied and identification was carried out using biochemical reactions according to the Analytical Profile Index (API) (Himedia-India). Serological typing was performed using specific antisera, Sifin-Germany, to determine the serotypes of *Salmonella spp.*, *Shigella spp.*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enterohemorrhagic *E. coli* by using slide agglutination technique.

In vitro antimicrobial sensitivity of different isolates against wide range of antimicrobials was assessed using Kirby Baur technique on Muller Hinton agar (Himedia-India). Using *E. coli* ATCC 25922 as control strain, the zone of Inhibition was interpreted according to Bauer et al<sup>5</sup>. Antimicrobials tested were: Ciprofloxacin (5mcg), Norfloxacin (30mcg), Chloramphenicol (30mcg), Erythromycin (15mcg), Gentamicin (10mcg), Co-trimoxazole (15mcg), Amoxicillin (20mcg), Doxycycline (30mcg), Ceftazidime (30mcg) and Cefuroxime (30mcg). Data were analyzed using SPSS (version 11.5) program.

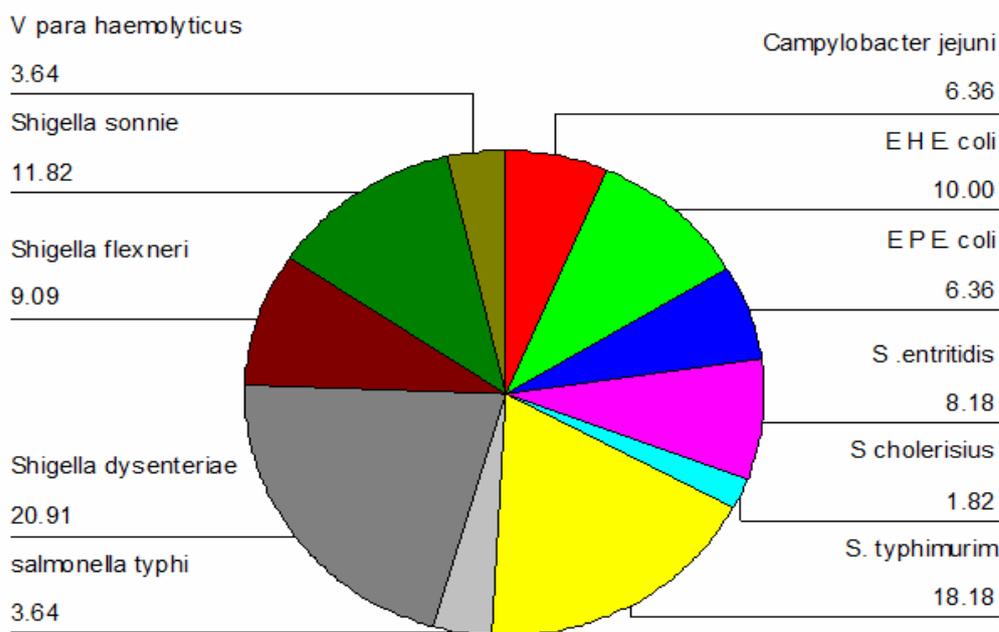
## RESULT

Four hundred and twenty stool specimens were collected and analyzed for bacterial pathogens causing diarrhea during the 17 month study-period, from April 2006 to September 2007. The patients studied included adults and children. Most infection (42.4%) was among children under 5 years, see Table 1.

**Table 1: Distribution of Bacterial Pathogens According to Age**

Entero pathogens	Children		Adults n = 100	Total = 420
	< 5 years n= 178	> 5 years n= 142		
<i>Shigella SPP</i>	5 (21.7%)	17 (53.1%)	24 (43.6%)	46
<i>Salmonella SPP</i>	4 (17.4%)	10 (31.3%)	21 (38.2%)	35
<i>Entero pathogenic E. coli</i>	5 (21.7%)	1 (3.1%)	1 (1.8%)	7
<i>Entero hemorrhagic E. coli</i>	3 (13%)	3 (9.4%)	5 (9.1%)	11
<i>Vibrio parahemolyticus</i>	0 (0%)	0	4 (7.3%)	4
<i>Campylobacter jejuni</i>	6 (26.1%)	1 (3.1%)	0	7
<b>Total</b>	<b>23 (100%)</b>	<b>32 (100%)</b>	<b>55 (100)</b>	<b>110</b>

Enteropathogens (positive culture) had been detected among 110 (26.2%) patients investigated. *Shigella SPP* found to be the major isolate, 46 (41.8%), as shown in Figure 1. Other isolates, include *Sh. dysenteriae* 23 (20.9%), *Sh. flexneri* 13 (11.8%) and *Sh. Boydii* 10 (9.1%), followed by *Salmonella spp* 35 (31.8%), which include *salmonella typhimurium* 20 (18.2%), *Salmonella Enteritidis* 9 (8.2%), *Salmonella typhi* 4 (3.6%), *Salmonella choleraesuis* 2 (1.8%). Next was Enterohemorrhagic *E. coli* 11 (10%), Enteropathogen *E. coli* and *Campylobacter jejuni* 7 (6.4%) each, *Vibrio parahemolyticus* 4 (3.6%).



**Figure 1: Percentage of Different Bacterial Pathogens Isolated from 110 Patients**

The main bacterial causative agent of diarrhea among children under 5 years was *campylobacter jejuni* (26.1%), while in children over 5 years and adults the major causative agents were *Shigella SPP* giving frequency rates of (53.1%), (43.6%) respectively, see Table 1.

Among the 110 bacterial pathogens, high resistance observed against Amoxicillin 90 (81.8%) then Erythromycin 64 (58.2%), and Co-Trimoxazole 61 (55.5%) and the lowest resistance was to Cefuroxime 36 (32.7%).

Most of the *shigella spp* isolates were sensitive to Ciprofloxacin (58.7%), Cefuroxime (56.6%), but high degree of resistance was observed against Amoxicillin (73.9%), Chloramphenicol (69.5%), Erythromycin (65.8%), Co-Trimoxazole (63%), and Doxycycline (60.8%).

Among *salmonella isolates*, 74.3% were sensitive to Cefuroxime and Norfloxacin. Most of them (88.5%) were resistant to Amoxicillin (57.1%), to Erythromycin and Co-Trimoxazole. All *E. P. E. coli* isolates were resistant to Amoxicillin, and 85.7% of *campylobacter jejuni* were resistant to Amoxicillin, and Gentamicin. Antimicrobials sensitivity results are summarized in Table 2 and 3.

**Table 2: Antimicrobials Sensitivity Results of *Shigella*, and *Salmonella Spp* Isolates (Resistance Pattern)**

Antimicrobials	<i>Sh. dysenteriae</i> n = 23	<i>Sh. Sonnei</i> n = 13	<i>Sh. flexneri</i> n = 10	<i>Shigella SPP</i> n = 46 (100%)	<i>Salmonella spp</i> n = 35 (100%)
Ciprofloxacin	12	3	4	19 (41.3%)	11 (31.4%)
Norfloxacin	10	6	7	23 (50%)	10 (25.6%)
Chloramphenicol	18	12	2	32 (69.5%)	11 (31.4%)
Erythromycin	17	7	6	30 (65.8%)	20 (57.1%)
Gentamicin	13	7	6	26 (56.5%)	11 (31.4%)
Co-trimoxazole	19	12	8	29 (63%)	20 (57.1%)
Amoxicillin	17	10	7	34 (73.9%)	31 (88.5%)
Doxycycline	10	8	10	28 (60.9%)	18 (51.4%)
Ceftazidime	8	8	8	24 (52.2%)	16 (45.7%)
Cefuroxime	6	9	5	20 (43.4%)	10 (25.6%)

**Table 3: Antimicrobial Sensitivity Test Results of Different Isolates (Resistance Pattern)**

Isolates	No	Cf 5mcg	Nx 30mcg	C 30mcg	E 15mcg	G 10mcg	Co 15mcg	Am 20mcg	Do 30mcg	Ca 30mcg	Cu 30mcg
<i>Shigella spp</i>	46	19	23	32	30	26	29	34	28	24	20
<i>Salmonella typhimurium</i>	20	4	3	1	9	4	13	18	6	8	5
<i>Salmonella enteritidis</i>	9	4	4	7	7	4	5	8	5	6	2
<i>Salmonella choleraesuis</i>	2	1	2	1	2	0	0	2	2	1	1
<i>Salmonella typhi</i>	4	2	1	2	2	3	2	3	5	1	2
<i>E. P. E. coli</i>	7	4	4	5	5	4	0	7	5	4	2
<i>E.H. E. coli</i>	11	7	6	7	5	5	6	9	6	6	2
<i>V. para hemolyticus</i>	4	2	2	0	1	2	3	3	2	1	0
<i>C. Jejuni</i>	7	1	3	3	3	6	3	6	1	1	2
<b>Total</b>	110	44	48	58	64	54	61	90	60	52	36
	100%	40%	43.6%	52.7 %	58.2%	49.1%	55.5%	81.8%	54.5%	47.3%	32.7%

Cf: Ciprofloxacin      Nx: Norfloxacin      C: Chloramphenicol  
 E: Erythromycin      G: Gentamicin      Co: Co-trimoxazole  
 Am: Amoxicillin      DO: Doxycycline      Ca: Ceftazidime      Cu: Cefuroxime

## DISCUSSION

Diarrheal diseases are major causes of morbidity, the attack rates ranging from 2 to 12 or more per person per year in developed and developing countries. In addition, diarrheal illnesses account for an estimated 12,600 deaths each day in children in Asia, Africa, and Latin America. The causes of diarrhea include a wide variety of viruses, bacteria, and parasites, many of which have been recognized only in the last decade or two<sup>6</sup>.

There are more than 30 million refugees and internally displaced population in developing countries; they are dependent on international relief assistance for their survival. Displaced populations in northern Ethiopia (1985) and southern Sudan (1988) have suffered the highest crude mortality rates. Although mortality rates have risen in all age groups, excess mortality has been the greatest in children 1-14 year old<sup>1</sup>. The major causes of death have been measles, diarrheal diseases, acute respiratory tract infections, and malaria<sup>7</sup>.

In Khartoum state, in the rehabilitation camps (Mandella and Wad Elbashir), which accommodate more than 110,000 Sudanese displaced persons, there are poor nutrition, overcrowding, lack of water, and inadequate sanitation, that population is vulnerable to diarrheal diseases.

In this study, bacterial pathogens were detected in 26.2% of displaced patients studied. It is approximately similar to the results of Desenclos et al, in which two hundred patients with diarrhea in a rehabilitation camp in Ethiopia were studied in October 1985 to determine the presence of pathogens in the stool and their susceptibility to antibiotics; 42 (21.1%) patients of those had a positive culture<sup>8</sup>.

In our study, *Shigella spp* were the most prevalent pathogens isolated among adults (43.6%), and children over 5-years old (53.1%), see Table 1. This result was expected, because the frequency of shigellosis had increased with overcrowding, poor sanitation, and inadequate water supply<sup>9</sup>.

Adkins et al studied the prevalence of bacterial pathogens among 2,908 patients of all age groups with diarrhea who were admitted to San Lazaro Hospital in Manila in 1983 and 1984, and he reported one or more enteric pathogens isolated in the samples of 1,698 (58.4%) patients<sup>10</sup>.

In the present study, campylobacter *jejuni* (26.1%), *Enteropathogenic E. coli* and *Shigella spp* (21.7%) were the most isolated pathogens in children under 5 years, see Table 1. This result is almost similar to the results obtained by Stewien et al who studied cases of diarrhea among infants and young children admitted to the pediatric clinic of the University Hospital of São Paulo during the period of 13 months<sup>11</sup>. Vergara et al revealed the results of isolation, frequency and distribution of enteropathogens in children under five years old suffering from diarrhea for less than seven days and without previous antibiotic treatment<sup>12</sup>.

In the present study, *Campylobacter jejuni* was the main bacterial pathogen isolated (26.1%) among children under 5 years, see Table 1. On the other hand, Varavithya et al studied diarrhea in children less than 5 years of age in a low-income community and found *Campylobacter jejuni* to have 12% frequency rate<sup>13</sup>.

In the present study, *vibrio parahemolyticus* was isolated from adults only, and the frequency rate was 7.3%, Table 1, 75% of them were resistant to Amoxicillin, and Co-Trimoxazole; no *vibrio cholerae* were detected. This rate is lower if compared with another study by Echeverria et al, who found 19% of their patients infected with *vibrio parahemolyticus*<sup>14</sup>.

In present study, the antimicrobials sensitivity was similar to what was found by Temu et al who reported that all *Shigella* strains showed high resistance to Ampicillin, Tetracycline, Trimethoprim-Sulphamethoxazole and Chloramphenicol, However all their isolates were fully susceptible to Ciprofloxacin, Nalidixic acid, Erythromycin, Cefuroxime and Gentamicin<sup>15</sup>.

In the present study, most of the isolates (81.8%) were resistant to Amoxicillin (table3); the same result was reported by Battikhi<sup>16</sup>.

Salem et al reported that most of the *Shigella* isolates were susceptible to Cefotaxime, and resistant to the other antibiotics. All the tested enteropathogenic *E. coli* isolates were resistant to Amoxicillin, which is similar to our study<sup>17</sup>.

Antibiotic treatment of diarrhea is rarely indicated as there is little evidence that it will improve symptoms, and indeed, it may prolong carriage of some organisms or results in antibiotic associated diarrhea. However, antibiotic treatment of diarrhea may be helpful in the following cases<sup>18</sup>:

1. Salmonella infection which has invaded the blood stream, meninges, or bone.
2. Salmonella in the elderly accompanied with severe gastrointestinal symptoms.
3. Severe shigellosis.
4. Campylobacter infection with colitis or bloody stools.
5. Salmonella, shigella or campylobacter infection in an immuno-compromised patient or a patient with relevant underlying disease e.g. ulcerative colitis with moderate to severe symptoms<sup>18</sup>.

## CONCLUSION

**This study revealed that 26.2% of the patients were infected by bacterial enteropathogens. Children under 5 years were mostly affected; the major bacterial pathogen causing diarrhea in displaced camps of Khartoum was *Shigella spp*, which shows high resistance to most of the tested antimicrobials. Most isolates were sensitive to Cefuroxime.**

**The study emphasizes the need for continuous monitoring of the occurrence of *Shigella* organisms and their antimicrobial susceptibility pattern for the successful treatment and control of *Shigella* dysentery, and for the development of public health policy for populations in displaced camps who are at risk for shigellosis and other infections.**

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