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STUDY OF BACTERIAL AGENTS OF INTESTINE IN INFANTS UNDER ONE YEAR OF AGE SUFFERING GASTROENTERITIS OBSERVED AMONG THE IRAQI REFUGEES RESIDING AT IRAN'S WESTERN CAMPS

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ABSTRACT

One hundred and thirty specimens of faeces of infants under one year old suffering form gastroenteritis and residing at the Camp of Iraqi Kurdish refugees (Paveh and Sar Pol Zahab cities) were collected and evaluated from the point of some bacterial agents. At the same time, some important clinical findings and environmental conditions were also collected for the study. The results showed that gastroenteritis mostly had a rapid incidence accompanied by fever, pain, straining, tenesmus and mucus-containing faeces. From a total number of 130 specimens collected, 10% *Proteus*, 18% *Pseudomonas*, 10% *Aeromonas*, 1% *Serratia*, 1% enteropathogenic *E. coli*, 4% *Klebsiella*, 2% *Shigella*, and 3% *Yersinia* were isolated. Antibiotic sensitivity test indicated a severe resistance of opportunistic pathogens against two antibiotics, i.e., chloramphenicol and co-trimoxazole which were mostly isolated in Sar-Pol-Zahab Camp. We can link this to prophylaxis with these two antibiotics the result of which is the augmentation of normal flora and its change to pathogens. The studies have shown that there is a significant relation between the camp's living conditions, nutrition, public health and isolated microbes.
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INTRODUCTION

The indications prove that intestinal infections are among the most common diseases leading to infant death in 85% of the population.[3,5,7-9] Diarrhea is the cause of roughly five to 10 million deaths per year in Asia, Africa, and Latin America and is considered as the prime cause of death in the first five years of life (15-25%).[10-14]

In recent decades, lack of sufficient attention to public health has endangered the life of a majority of the world's children and subjected them to lethal diarrhea, thus creating a situation that existed thirty years ago.[13,15-18,20]

It is estimated that annually around 4.5 million children die of this disease in Asia, Africa and Latin America and it has become known as the main cause of infant death in the poor tropical regions since long ago.[9] According to World Health Organization, the annual children's mortality is 12 million.[19,20]

Economic, social and cultural conditions play a very important role in the extent, intensity and the kind of the infectious agent. With the increase in the number of children in the developing countries, there have been more cases of gastrointestinal disease eventually resulting in more infant and child mortality.[18,20-23]

Different factors participate in the etiology of diarrhea. Reports indicate that moderate to severe diarrhea is caused

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by viruses like rotaviruses in up to 80%, bacteria in up to 15% and the remaining 5% by parasites like *Giardia* and less than 1% by fungi.[4] As to adults in developed countries, the major pathogenic agents causing diarrhea are mainly enterotoxigenic *E. coli* (ETEC) and *Shigella* among passengers and *Campylobacter* among the aboriginal inhabitants, followed by *Yersinia*, *Vibrio* and *Salmonella* and *Shigella*.[11] As to the children of the developed countries, *Salmonella*, *Shigella*, *Campylobacter* and less frequently *E. Coli*, *Aeromonas*, *Yersinia*, *Vibrio* and *Clostridium* are considered as the cause of the gastrointestinal disease. Recent studies show that enteropathogenic *E. coli* (EPEC), *Salmonella*, *Campylobacter*, *Shigella*, and *Yersinia* are considered in the said order as the cause of the gastrointestinal diseases.[11-13,17-19,22,23]

It is necessary to make an etiologic study of gastrointestinal diseases among children in unsettled and mobile societies like emigrants, refugees, and afflicted people for quick settlement of their problems. In view of the repetition of these cases, past experiences will be our guide

TABLE I

TEST \ Case no.	25 a	25 b	25 c	59
KIA				
MOT 25°C	+	+	+	+
MOT 37°C	-	-	-	-
ONPG	+	+	+	+
ADH	-	-	-	-
LDC	+	+	+	+
ODC	-	-	-	-
CIT	-	-	-	-
H_2S	-	-	-	-
U	+	+	+	+
TDA	-	-	-	-
IND	+	-	+	-
VP25°C	+	+	+	+
VP37°C	-	-	-	-
MR	+	+	+	+
GEL	-	-	-	-

TEST \ Case no.	25 a	25 b	25 c	59
GLU	+	+	+	+
MAN	+	+	+	+
INO	+	+	-	-
SOR	+	+	+	+
RHA	-	-	+	+
SAC	+	+	+	+
MEL	-	-	+	+
AMY	+	+	+	+
ARA	+	+	+	+
OXI	-	-	-	-
SAL	-	-	-	-
ESC	-	-	-	-
CRMOX	+	+	+	+
LEC	+	+	+	+
LIP	-	-	-	-
DNASE	-	-	-	-

Table I. The result of lab studies regarding Yersinia is as follows:
25a: *Yersinia enterocolitica*, biovare 2, pathogen
25b: *Yersinia enterocolitica* biovare 3, pathogen
25c: *Yersinia enterocolitica*, biovare 3, pathogen
(It is different from 25b in some biologic specifications.)
59: *Yersinia intermedia* (pathogen)

KIA, Kiligler Iron Agar; MOT 25°C, motility at 25°C; Mot 37°C, motility at 37°C; ONPG, β-galactosidase; ADH, arginine dehydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; CIT, citrate; H_2S, Hydrogen sulfide; U, urease; TDA, tryptophan deaminase; IND, indole; VP 25°C, Voges-Proskauer at 25°C; VP 37°C, Voges-Proskauer at 37°C; MR, methyl-red; GEL, gelatin; GLU, glucose; MAN, mannose; INO, inositol; SOR, sorbitol; RHA, rhamnose; SAC, sucrose; MEL, melebiose; AMY, amylase; ARA, arabinose; OXI, Oxidase; SAL, Salicin; ESC, esculin; CRMOX, Congo-red-magnesium oxalate agar medium test; LEC, lecithin; LIP, lipase; DNase, deoxyribonuclease.

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in the future. Although some restrictions result in the existence of some bacteria on the basis of prevailing condition, we can never neglect the role of the other infectious agents.

MATERIAL AND METHOD

One hundred and thirty specimens of the faeces of the Iraqi refugees' infants under one year old residing at two camps of Bakhtaran Province were collected during Spring and Summer 1991, and transferred to the Bacteriology Department of the Health Faculty through the following transfer medium cultures: (1) Alkaline Peptone Water-Difco, Alkaline Phosphate-Buffered, and (3) Cary-Blair-Difco.

Specimen collection was made hy referring to the tents of the camp and filling out questionnaires indicating personal particulars, the characteristics of the disease and other environmental specifications. Specimens were collected through rectal swab and transferred to Tehran in transfer medium cultures in a temperature of around 4°C. Then the microbes were identified by special and differential medium cultures and by biochemical tests in accordance with standards; when necessary serotyping was carried out.[14,19-21]

In addition, special tests for identification of Vibrio were carried out. For this purpose, they were cultured on TCBS (Difco) in three stages instead of Alkaline Peptone Water (Difco), and plates were examined for suspectible colonies.[2]

In order to study *Yersinia*, cold-enrichment method (through 2 to 3-week incubation in 7°C transfer medium cultures with inoculated alkaline phosphate buffered) and CIN and YM cultures (CIN- Difco,YM-Merck) were used to make a better distinction of suspectible colonies. After that, suspectible colonies were tested for identification of biotypes through further biochemical examinations.[1,2]

In the last stage, strains found were serotyped using antibiogram disc. For supplementary study, some of them were serotyped by existing antiserum.[10] (Disks were provided by Bio Mérieux and experiments were carried out in accordance with factory standards).

RESULTS

One hundred and thirty children under one year of age suffering from diarrhea in seven Iraqi kurdish refugee camps in western Iran were examined for *Salmonella*, *Shigellae*, *Vibrio* and *Yersinia* gastroenteritis, and diarrhea resulting from EPEC. Then, a separate questionnaire was provided for each patient on the basis of clinical findings, duration of the disease, and personal specifications.

Out of this total number, 51 cases were from Heiravi Camp (Paveh City-Bakhtaran Province) and 79 from Jegiran consisting of 77 boys (59%) and 53 girls (41%). In 98 cases (68%) fever, in 37 cases (28.6%) pain, in 67 cases (51%) straining, and in 18 cases (15%) chills were reported. In 71 cases (55%) diarrhea was mainly lienteric and in the form of mucusal faeces. This was followed by 47 children (36%) who had watery diarrhea and 12 children (9%) with bloody flux.

In 20% of these cases, diarrhea had a gradual incidence and in 80% an abrupt incidence rapidly leading to an acute stage.

In 80% of the cases, the number of daily bowel

TABLE II. Specifications of different kinds of isolated microbes in acute gasteroenteritis cases.

GENUS/ SPECIES	number of isolated species	relative frequency of species %	relative frequency of isolated microbes %	relative frequency of species %
Proteus mirabilis	8	57.1	15.38	6.15
Proteus vulgaris	5	35.7	9.61	3.84
Proteus stuartii	1	7.1	1.92	0.77
Pseudomonas aerugi	23	100	44.23	17.69
Aeromonas hydrophila	1	100	1.92	0.77
E.P.E. coli	1	100	1.92	0.77
Serratia marcescens	1	100	1.92	0.77
Klebsiella pneumoniae	5	100	9.61	3.84
Vibrio	0	0	0	0
Salmonella	0	0	0	0
Shigellae flexneri type 3	3	100	5.76	2.3
Yersinia enterocolitica	3	75	5.76	2.3
Yersinia intermedia	1	25	1.92	0.77
total	52		100	39.2

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TABLE III: Lab findings for different sexes and camps (where specimens were collected)

	Jegiran		Heiravi		
GENUS/SPECIES	F	M	F	M	Total
Proteus mirabilis	3	5	0	0	8
Proteus vulgaris	0	4	1	0	5
Proteus stuartii	1	0	0	0	1
Pseudomonas aeruginosa	13	10	0	0	23
Aeromonas hydrophila	1	0	0	0	1
E.P.E. coli	0	0	1	0	1
Serratia marcescens	1	0	0	0	1
Klebsiella pneumoniae	4	1	0	0	5
Vibrio	0	0	0	0	0
Salmonella	0	0	0	0	0
Shigellae flexneri type 3	0	0	3	0	3
Yersinia enterocolitica	0	0	0	1	1
Yersinia intermedia	0	0	1	0	1
Total	23	20	6	1	50

movements was 5-6 times; 8 cases (6.5%) had more than ten bowel movements a day and two died of sustained severe diarrhea and dehydration.

The results showed 3 cases of *Shigellae flexneri* serotype 3 (2.3%) but no *Salmonella* or *Vibrio* were isolated from samples. Three cases of *Yersinia enterocolitica* and one case of *Yersinia intermedia* (1.5%) were isolated from two patients. In addition to this finding, lab studies had interesting and unexpectable results. The microbes found consisted of 14 cases of pure *Proteus* (10.7%), 5 cases of *Klebsiella* (3.8%) and 23 cases of pure *Pseudomonas*. Lab findings are shown in Tables II, and III.

Patients suffering from yersiniosis consisted of a girl and a boy from Heiravi camp. Results of the chemical tests concerning these two cases are given in Table III.

Studies showed that a boy who had yersiniosis was infected by a series of yersinias whose biochemical differences are given in Table I.

Results of the sensitivity studies of the opportunistic pathogens mainly isolated in Jegrian camp in a pure form that are important from paraclinical point of view have been shown in Tables IV-VIII.

TABLE IV: Resistance of isolated *Shigellae* to drugs.

Antibiotic	S	I	R
NA	100	0	0
PB	100	0	0
GM	100	0	0
N	33	66	0
AMP	100	0	0
CXT	100	0	0
POL	33	66	0
CL	0	100	0
E	66	0	33
K	66	0	33
NET	66	0	33
TET	66	0	33
CRX	0	0	100
CTN	0	0	100
PEN	0	0	100

DISCUSSION

The community under study entered Iran steadily and in a short period of time, the officials of the related provinces were not prepared to receive them.

Volatile and demographic structure, physical fatigue, malnutrition, and the stress resulting from war affect the human immunity system seriously, to which should be added lack of heating facilities and equipment in cold and mountainous areas, and hygienic shortages; all made us believe that infectious diseases especially diarrhea should have a vast incidence especially among infants.

In the course of bacterial studies of the causative agent of gastroenteritis among infants and children, three major objectives were pursued: first, making contributions to the removal of the biggest hygienic problem of the camps, i.e., typhoid fever and other infant diarrhea in accordance with preliminary studies and available information; second, determining the role of some of the bacterial agents in causing diarrhea and its relative comparison with the results of other studies in advanced, normal, and underdev- eloped societies; finally answering this question that to what extent

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TABLE V: Resistance of isolated *Yersinia* to drugs.

Antibiotic	S	I	R
NA	100	0	0
SMT	100	0	0
GM	100	0	0
K	100	0	0
S	100	0	0
C	100	0	0
AMP	100	0	0
PB	100	0	0
CL	75	25	0
CTN	0	75	25
AMX	0	0	100
CTX	0	0	100
E	0	0	100
P	0	0	100
TET	75	0	25

TABLE VI: Resistance of isolated *Klebsiellas* to drugs.

Antibiotic	S	I	R
K	100	0	0
GM	100	0	0
NA	100	0	0
SMT	80	0	20
CTX	60	0	40
TET	60	0	40
COL	0	80	20
AMX	0	0	100
PEN	0	0	100
C	0	0	100

TABLE VII: Resistance of *Pseudomonas aeruginosa* to drugs.

Antibiotic	S	I	R
CAR	8/7	17.4	74
CXT	95/6	4.3	0
AMK	78/3	4.3	17.4
FOX	0	4.3	95.7
GM	95/7	4.3	0
COL	87/0	8.7	4.3
NF	91/4	4.3	4.3
C	78/3	17.4	4.3
CTX	0	0	100
C	34/7	8.7	56.6
CMX	34/7	3.4	61

the environmental factors play a role in causing diarrhea.

Considering the specifications and conditions of the region under study, it is clear that there is a possibility for epidemic diseases like typhoid fever and shigellosis in Jegiran Camp, because Sar-Pol-Zahab camp was densely crowded and with less hygienic facilities compared to Paveh camp. So its tropical climate led us to use antibiotics as prophylaxis for preventing the spread of contagious diseases which has been confirmed by lab results.

The growth of 23 cases of *Pseudomonas aeruginosa* with the frequency of 18% in a pure form in cultures which in itself consists of the 50% of the isolated microbes could indicate an epidemic of *Pseudomonas* infection. This fact is confirmed by resistance of more than 50% of the isolated *Pseudomonas* species to co-trimoxazole and chloramphenicol (the two effective drugs for prophylaxis and treatment of typhoid fever). This indicates change of natural flora because of the unsystematic use of antibiotics, creating problems in infants and children.

In view of the resistance pattern of microbes, the existence of *Serratia marcescens*, *Klebsiella pneumoniae* and *Proteus* further proves this idea. It means that for instance the extent of the resistance of the isolated *Proteus* in Sar-Pol-Zahab to co-trimoxazole and chloramphenicol was 100%. These opportunistic microbes were mainly isolated from Sar-Pol-Zahab.

In Paveh camp where the climatic and hygienic conditions were more appropriate (a mountainous area with relatively cool weather, where water from natural springs was available

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TABLE VIII: ***Proteus*** **and** ***Providencia*** **resistance to drugs (isolated in children with gastroenteritis)**

Antibiotic	S	I	R
GM	100	0	0
SMT	0	0	100
E	0	0	100
P	0	0	100
TET	0	0	100
CTN	0	0	100
C	0	0	100
K	78.5	0	21.5
NF	64	22	14
CTX	28.5	21.4	50
B	14.2	14.2	71.4
AMX	7	7	85
CL	0	7	93

GM, Gentamicin; SMT, Sulphamethoxazole; E, Erythromycin; P, Penicillin; TET, Tetracycline; CTN, Cephalothin; CL, Chloramphenicol; K, Kanamycin; NA, Nalidixic acid; CTX, Cefotaxime; B, Bacitracin; AMX, Amoxycillin; C, Cefsulodin; CAR, Carbenicillin; CXT, Cefoxitin; AMK, Amikacin; NF, Nitrofurantoin; CTZ: Ceftazidim; CMX, Cotrimoxazale, PB, Benzylpenicillin; S, Streptomycin; CRX, Cefuroxime; NET, Netilmicin. AMP, Ampicillin; POL= polymyxin; COL, Colistin; FOX, Furoxane; N, Neomycin.

for drinking), the microbes isolated from gastroenteritis were more natural and normal. The climatic condition of this camp (Paveh) corresponded with the reproduced *Yersinia*. Studies on *Yersinia* made some points clear: first, employment of cold-enrichment is more effective than direct culture; second, experiences showed that utilizing CIN environment is more reliable than environments like YM for distinguishing *Yersinia*; third, with the main examination of virulence factors like congo-red absorption and the incapability of using Esculin and Salicin, it is assumed that reproduced microbes might be pathogenic.

The isolated *Shigellas* of Paveh camp were all of type 3 and from the statistical point of view corresponded with the normal conditions of Iran's society.

In this case, it is proposed to refrain from the unsystematic use of antibiotics and to employ the possibilities like fully lactobacilli yogurt aimed at strengthening the natural flora and to make more extensive microbiologic research in the other fields of bacterial agents like *Campylobacter* and also viral agents like rotaviruses. Meanwhile, we cannot neglect the role of intestinal *Proteus* causing gastroenteritis.

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