

Prevalence of Intestinal Parasitic and Bacterial Pathogens in Diarrhoeal and Non-diarrhoeal School Children's at Hail, Saudi

*Mohamed A. Fareid¹, Ibrahim M. Alshankyty² and Omar, H. Amer²

¹ Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

² Clinical laboratory Science Dept., Faculty of Applied Medical Science, Hail University

*mohamedfareid73@yahoo.com

Abstract: Diarrhoeal diseases constitute one of the most important causes of illness and death all over the world. This study will assess and monitoring the presence of intestinal parasites as well as bacterial pathogens among school children's at Hail city. Stool samples were collected from 200 schoolchildren's and were analyzed by standard microbiological and parasitological techniques. The incidence of *Cryptosporidium* spp. (50%), *Giardia lamblia* (42%), and *Entamoeba histolytica* (30%) were the most common parasitic agents which are detected in diarrhoea stool samples, while the incidence of *Entamoeba coli* (8%) and *Ascaris lumbricoides* (6%) were unexpectedly low. *Entrobium vermicularis* was highly significant ($p = 0.003$). The isolation of about six different bacterial isolates, especially *Escherichia coli* confirms the fact that these organisms play an important role in the etiology of intestinal tract infection in Hail. We found that *E. coli* was the most frequently isolated bacteria in both diarrhoea and non-diarrhoea stool samples was (88%) and (85.3%), respectively. However, the incidence of all bacterial isolates in diarrhoeal stool samples were significant ($p=0.001$). This depicts these bacteria as veritable etiological pathogen of infectious childhood diarrhoea. The disk diffusion testing for the antibiotic susceptibility illustrates a generally increased resistance to Ampicillin, by all bacteria isolates tested. While most of bacterial isolates showed high susceptibility to Augmentin, Ciprofloxacin, Ofloxacin and Ceftazidime.

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1. Introduction

Gastrointestinal infections are major causes of morbidity and mortality throughout the world and particularly in developing countries where mortality rate due to infectious diarrhoea could be as high as 56% (WHO, 2004). Intestinal parasites and protozoan infections are amongst the most common infections worldwide. It is estimated that some 3.5 billion people are affected, and that 450 million are ill as a result of these infections, the majority being children (WHO, 1998; Steketee, 2003; Mehraj *et al.*, 2008). The morbidity of intestinal parasitic infections is greatest among children of school age and may have an adverse effect on growth (Nematian *et al.*, 2008; Aly and Mostafa, 2010). Previous studies in Saudi Arabia revealed high prevalence rates of infection with intestinal parasitic diseases among specific populations including food handlers (14%), Riyadh school children (14.2%), expatriates (55.7%), the Abha community (13.2%) and patients attending hospitals (31.3%) (Abdel Hafez *et al.*, 1987; Ahmed and Bolbol, 1989; Al-Fayez and Khogheer, 1989; Al-Madani *et al.*, 1989; Ali *et al.*, 1992).

The bacterial pathogens usually responsible include *Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*, *Aeromonas* etc. (Presterl *et*

al., 2003; El-Sheikh and El-Assouli, 2001; Seung-Hak, 2006). Although in developed countries and a few developing countries, the mortality rates have declined considerably in recent times due to improvement in general hygiene and advances in health care, the problem still persists in so many other countries where outbreaks of diarrheal diseases continue to affect millions of infants and young children (Presterl *et al.*, 2003; Hart, 1989). The epidemiological significance of each *E. coli* category in childhood diarrhoea varies with the geographical area. Several studies on the incidence of diarrhoeal illnesses caused by different classes of diarrhoeagenic *Escherichia coli* (DEC) and enteropathogenic *E. coli* (EPEC) have been conducted mainly in Latin America, Africa, South and Southeast Asia, and the Middle East (Nataro and Kaper, 1998; Nataro, 2002; Robins-Browne and Hartland, 2002; Presterl *et al.*, 2003; Sarantuya *et al.*, 2004; Alikhani *et al.*, 2006 and Samie, 2009). The aim of the present work was to study the occurrence and prevalence of parasitic as well as bacterial infection of intestinal tract among school children's in Hail.

2. Material and Methods

Collection of samples

In total, 200 faecal samples were collected in sterile universal screw cap bottle from school children's of two public primary schools in Hail city, KSA. The bottles were brought home, and the samples were collected the next morning. The samples were then transported in cooler bags to the Microbiology Laboratory at the University of Hail.

Microscopic examination

A wet mount was prepared for each sample and observed under the light microscope for eggs and trophozoites of various parasites. Samples were then preserved in 10% formal saline to be re-examined later. Two different concentration methods, Formolether sedimentation technique and Zinc sulfate floatation technique, were applied to all specimens in order to separate protozoan cysts and helminthes eggs from the bulk of fecal matter through differences in specific gravity. All specimens were also examined by the modified Ziehl-Neelsen acid-fast stain to detect the oocysts of *Cryptosporidium parvum* according to WHO (1991) and Cheesbrough (2008).

Isolation and identification of bacteria

The faecal samples were cultured on differential and selective media for bacteria cultivation in order to isolate bacteria enteropathogens. Those were MacConkey, Blood agar medium, Xylose lysine desoxycholate agar (XLD) and Salmonella-Shigella agar medium (SSA). After aerobic incubation at 37°C for 24-48 hours, the plates were observed for bacterial growth. Identification of isolates was done using standard biochemical methods as described by Cowan and Steel (1974). Identification was confirmed with the API 20E system (BioMerieux).

Detection of enteropathogenic *Escherichia coli* (EPEC) Serogrouping

Strains biochemically identified as *E. coli* were selected and both lactose-positive and lactose-negative *E. coli* strains were serogrouped by using slide agglutination tests into the different EPEC serogroups (O₂₆, O₅₅, O₈₆, O₁₁₁, O₁₁₄, O₁₁₉, O₁₂₄, O₁₂₅, O₁₂₆, O₁₂₇, O₁₂₈, O₁₄₂) using O-specific antisera, according to the manufacturer's instructions (Bio-Rad). Strains that agglutinated with 4% saline were defined as rough.

Antibiotic susceptibility

Antibiotic susceptibility of the isolated species namely *Escherichia coli*, *Klebsiella species*,

Staphylococcus aureus, *Salmonella species*, *Shigella* spp. and *Proteus* spp. tested for their susceptibility to some antibiotics (Ampicillin, Augmentin, Erythromycin, Gentamycin, Nalidixic acid, Ciprofloxacin, Ofloxacin, Tetracycline, Ceftazidime, Imipenem and Piperacillin) by modified disc diffusion technique Kirby-Bauer according to the National Committee for Clinical Laboratory Standards (NCCLS, 2003).

Statistical analysis

Statistical analysis was performed by the chi-square test. The differences were considered significant when the p-value was less than 0.05.

3. Results

Demographic information of study population

The patients were aged 4-12 year(s), of the 200 samples collected from the school children's; 50 (25%) were diarrhoeal (unformed) and 150 (75%) non diarrhoeal (formed).

Prevalence of parasitic infections

Table 1 presents the prevalence of different intestinal parasitic profiles. *Cryptosporidium parvum* (50%) and *Giardia lamblia* (42%) were the most common organisms found in diarrhoeal stool samples, followed by *Entamoeba histolytica* (30%). Also the incidence of *Cryptosporidium parvum* (32.6%) and *Giardia lamblia* (29.3%) were the most common organisms found in non-diarrhoeal stool samples, followed by *Entamoeba histolytica* (14.6%). The incidence of *Entrobium vermicularis* in diarrhoeal stool samples was highly significant (p=0.003) in compared to the other parasitic agents. While in total all parasitic agents was highly significant (p=0.001).

Isolation and Identification of Bacteria

In this study, we isolated a total number of 203 bacterial isolates from 200 stool samples collected from the school children's (diarrhoeal and non-diarrhoeal stool samples) on the basis of morphological characters. These isolates were characterized with physiological and conventional biochemical tests. Their identifications were confirmed on the basis of the results of the following tests: Gram stain test, oxidase test, the oxidation/fermentation test. According to the test results, only 6 bacterial isolates were determined and all were Gram negative, rod shaped and facultative anaerobes. The isolates were oxidase and arginine dihydrolase negative except *Aeromonas* was positive, and glucose fermentation was positive for all bacterial isolates (Table 2).

Table 1. Prevalence of intestinal parasitic infections among school children stool samples in Hail

Parasitic organism	Diarrhoeal (n=50)		Non-diarrhoeal (n=150)		Significance (p-value)
	No.	(%)	No.	(%)	
<i>Cryptosporidium parvum</i>	25	50	49	32.6	0.08
<i>Giardia lamblia</i>	21	42	44	29.3	0.17
<i>Entamoeba histolytica</i>	15	30	22	14.6	0.03
<i>Entrobilus vermicularis</i>	11	22	10	6.6	0.003
<i>Entamoeba coli</i>	4	8	12	8	1.0
<i>Hymenolepis nana</i>	0	0	4	2.6	0.24
<i>Ascaris lumbricoides</i>	3	6	19	12.6	0.21
Total	82	164	157	106.3	0.001

Table 2. Biochemical characterization of bacterial isolates

Test	Reaction tested	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Klebsiella</i> spp.	<i>Shigella</i> spp.	<i>Proteus</i> spp.	<i>Aeromonas</i> spp.
ONPG	Beta-galactosidase	+ve	-ve	+ve	-ve	-ve	+ve
ADH	Arginine dihydrolase	-ve	-ve	-ve	-ve	-ve	+ve
LDC	Lysine decarboxylase	+ve	+ve	+ve	-ve	-ve	+ve
ODC	Ornithine decarboxylase	+ve	+ve	-ve	+ve	-ve	-ve
CIT	Citrate utilization	-ve	-ve	+ve	-ve	-ve	+ve
H ₂ S	H ₂ S production	-ve	+ve	-ve	-ve	+ve	+ve
URE	Urea hydrolysis	-ve	-ve	+ve	-ve	+ve	-ve
TDA	Deaminase	-ve	-ve	-ve	-ve	+ve	-ve
IND	Indole production	+ve	-ve	-ve	-ve	+ve	+ve
VP	Acetoin production	-ve	-ve	+ve	-ve	-ve	-ve
GEL	Gelatinase	-ve	-ve	-ve	-ve	+ve	+ve
GLU	Glucose fermentation	+ve	+ve	+ve	+ve	+ve	+ve
MAN	Mannitol fermentation	+ve	+ve	+ve	+ve	-ve	+ve
INO	Inositol fermentation	-ve	-ve	+ve	-ve	-ve	-ve
SOR	Sorbitol fermentation	+ve	+ve	+ve	-ve	-ve	-ve
RHA	Rhamnose fermentation	+ve	+ve	+ve	+ve	-ve	-ve
SAC	Sucrose fermentation	+ve	-ve	+ve	-ve	+ve	+ve
MEL	Melibiose fermentation	+ve	+ve	+ve	-ve	-ve	-ve
AMY	Amygdalin fermentation	-ve	-ve	+ve	-ve	+ve	-ve
ARA	Arabinose fermentation	+ve	+ve	+ve	+ve	-ve	+ve
OXI	Oxidase	-ve	-ve	-ve	-ve	-ve	+ve

Occurrence of bacterial pathogens in stool samples

Figure 1 illustrated the occurrence of the different bacterial organisms in diarrhoeal and non diarrhoeal samples. *Escherichia coli* were the most commonly-isolated organisms from diarrhoeal and non-diarrhoeal stools (88%) and (85.3%), respectively, followed by *Klebsiella* spp. and *Shigella* spp. (28 %), (18%) and (22%), (14.6%), respectively. *Proteus* spp. was less commonly isolated from diarrhoeal and non diarrhoeal samples. All the bacterial isolates in total were significant (p=0.001).

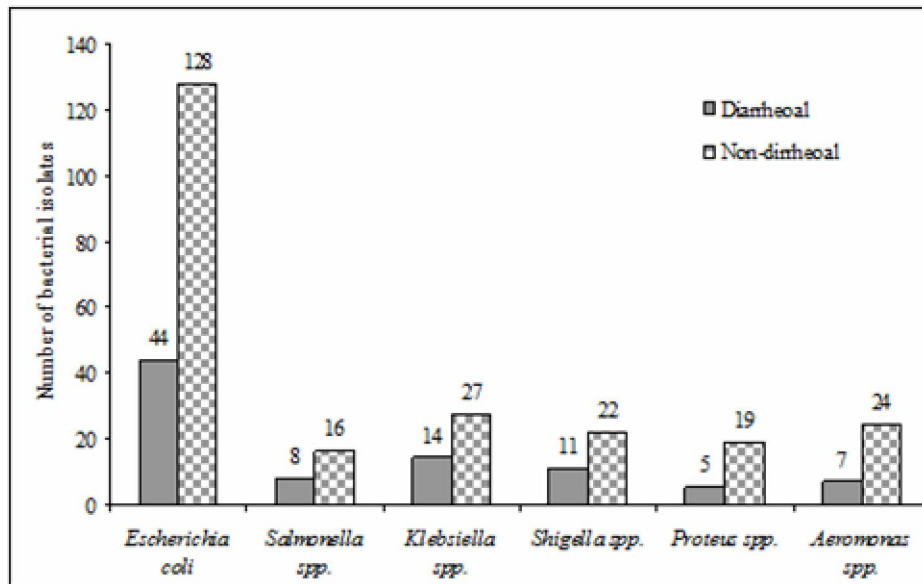


Figure 1. Prevalence of intestinal parasitic infections among school children stool samples in Hail

Enteropathogenic *Escherichia coli* (EPEC) Serogroups

Table 3 indicated that EPEC serogroups isolated from 77.27% (34 of 44) of children with diarrhoea, compared with 6.25% (8 of 128) of those without diarrhoea ($P = 0.001$). EPEC strains isolated in this study belonged to 12 EPEC serogroups (O₂₆, O₅₅, O₈₆, O₁₁₁, O₁₁₄, O₁₁₉, O₁₂₄, O₁₂₅, O₁₂₆, O₁₂₇, O₁₂₈, O₁₄₂). In children with diarrhoea, O₁₁₁ serogroup EPEC (27.27%) was the most prevalent, followed by O₁₄₂ (20.45%) and O₁₂₇ (6.81 %).

Antimicrobial susceptibility profiles of six bacterial species

Table 4 represent the antimicrobial susceptibility profiles of six different bacterial species tested: *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Shigella* spp., *Proteus* spp. and *Aeromonas* spp. against 11 antibiotics. Isolates of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *proteus* spp. *Aeromonas* spp. and *Klebsiella species* showed a generally increasing resistance to Ampicillin, while Augmentin, Ciprofloxacin, Ofloxacin and Nalidixic acid were more effective on all the bacterial pathogens tested. On the other hand, all the bacterial pathogens tested were moderate sensitive to Gentamycin and Erythromycin.

4. Discussion

In Saudi Arabia and many developing countries, diarrhea caused by bacterial pathogens especially *E. coli* remains one of the major causes of morbidity and mortality in infants and young children

(Al-Jurayyan, 1994; Akinyem *et al.*, 1998; Okeke *et al.*, 2003; and Al-Braiken, 2008). Although in developed and a few developing countries, recent improvements in biological techniques have drastically increased the rate of diagnosis, isolation of bacterial pathogens and consequently reduced the global death rate due to bacterial diarrhea diseases (Sarantuya *et al.*, 2004). The objectives of this study were to determine the prevalence of intestinal parasitic and bacterial infections in relation to diarrhoeal symptoms and to determine the antimicrobial susceptibility profiles of the bacterial isolates in schoolchildren's at Hail city.

The prevalence and the profiles of intestinal infections vary among continents and even within countries or sub regions. We found that *Cryptosporidium parvum*, *Giardia lamblia*, and *E. histolytica* were the most common parasitic agents in diarrhoeal stool samples with infection rates (50%), (42%) and (30%), respectively. They can be transmitted orally by drinking water and both are environmental contaminants of the water supply (Omar *et al.*, 1991); they are also the most common in Saudi Arabia. This observation contrasts the observation of Samie *et al.* (2009).

In Cape Town, South Africa, higher rates of infection were described for *Ascaris* (24.8%), *Trichuris* (50.6%), *Hymenolepis nana* (2.2%), *Enterobius* (0.6%) *Giardia* (17.3%), hookworm (0.08%), and *Trichostrongylus* (0.1%) among children attending nine schools in a low-income but well-served community independent of diarrhoeal symptoms (Adams *et al.*, 2005).

Table 3. The incidence of enteropathogenic *Escherichia coli* (EPEC) Serogroups

<i>Escherichia coli</i> Serogroup	Diarrhoeal (n=44)		Non-diarrhoeal (n=128)		Significance (p value)
	No.	(%)	No.	(%)	
O ₂₆	1	2.27	0	0	0.08
O ₅₅	2	4.54	0	0	0.01
O ₈₆	1	2.27	0	0	0.08
O ₁₁₁	12	27.27	3	2.34	0.001
O ₁₁₄	0	0	1	0.78	0.08
O ₁₁₉	1	2.27	0	0	0.08
O ₁₂₅	2	4.54	1	0.78	0.10
O ₁₂₆	2	4.54	0	0	0.01
O ₁₂₇	3	6.81	1	0.78	0.02
O ₁₂₈	1	2.27	1	0.78	0.41
O ₁₄₂	9	20.45	1	0.78	0.001
Total	35	77.27	8	6.25	0.001

Table 4. Susceptibility profiles of isolates to 11 different antibiotics.

Bacteria	Aug	G	T	E	Cip	Ofx	NA	Ap	CAZ	IMI	PRL
<i>Escherichia coli</i>	3+ve	3+ve	2+ve	2+ve	3+ve	3+ve	3+ve	-ve	3+ve	3+ve	3+ve
<i>Salmonella</i> spp.	3+ve	3+ve	3+ve	2+ve	3+ve	3+ve	3+ve	-ve	3+ve	2+ve	3+ve
<i>Klebsiella</i> spp.	3+ve	2+ve	3+ve	2+ve	3+ve	3+ve	2+ve	-ve	3+ve	3+ve	2+ve
<i>Shigella</i> spp.	3+ve	3+ve	2+ve	2+ve	3+ve	3+ve	3+ve	-ve	3+ve	3+ve	2+ve
<i>Proteus</i> spp.	3+ve	2+ve	3+ve	2+ve	3+ve	3+ve	3+ve	-ve	3+ve	3+ve	3+ve
<i>Aeromonas</i> spp.	3+ve	3+ve	2+ve	2+ve	3+ve	3+ve	2+ve	-ve	-ve	2+ve	3+ve

Ap=Ampicillin (10 µg); Aug=Augmentin (30 µg); Cip=Ciprofloxacin (5 µg); E=Erythromycin (15 µg); G=Gentamycin (120 µg); NA=Nalidixic acid (30 µg); Ofx=Ofloxacin (5 µg); T=Tetracycline (30 µg); CAZ=Ceftazidime (30 µg); IMI= Imipenem (10 µg); PRL=Piperacillin (100 µg); 3+ve= Highly sensitive; 2+ve=Moderate sensitive; -ve= Resistance to antibiotics.

Similarly, *Giardia lamblia* (42%) was found in association with diarrhoeal cases in our study. The only intestinal helminthes detected were *Ascaris lumbricoides* and *Hymenolepis nana* in a very low incidence of (18.6%) and (2.4%), respectively in both diarrhoeal and non diarrhoeal stool samples. These data are in agreement with many previous results as El-Sheikh and El-Assouli (2001), Al-Shammari (2001), Al-Harathi and Jamjoom (2007) and Aly and Mostafa (2010).

The presence and frequent of bacterial pathogen causes intestinal tract infections among school children's was detected. Mostly encountered bacterial pathogens include *Escherichia coli*, *Klebsiella* spp., *Shigella* spp., *Salmonella* spp. *Aeromonas* spp. and *Proteus* spp. The current study was the first report recorded in Hail, implicated *E. coli* with the highest incidence in diarrhoeal and non diarrhoeal samples of (88%) and (85.3%),

respectively. A recent study in Gaza, also, identified *E. histolytica/dispar* and *Campylobacter coli/jejuni* among other organisms as major cause of acute diarrhoea in Palestinian children aged less than five years (Waku *et al.*, 2005 and Abu-Elamreen *et al.*, 2008). On the other hand, our results revealed that *Cryptosporidium parvurn* and *Escherichia coli* were the most agents associated with diarrhoeal and non diarrhoeal cases in Hail city. In children with diarrhoea, O₁₁₁ serogroup EPEC (27.27%) was the most prevalent followed by O₁₄₂ (20.45%) and O₁₂₇ (6.81 %). Also, the incidence of O₅₅, O₁₁₁, O₁₂₆, O₁₂₇ and O₁₄₂ serogroups EPEC in diarrhoeal stool samples were highly significant (P<0.05). This observation contrasts with the findings of previous studies from Bangladesh (Albert *et al.*, 1995), Saudi Arabia (El-Sheikh and El-Assouli, 2001) and Uruguay (Torres *et al.*, 2001).

Aeromonas species principally associated with gastroenteritis are *A. caviae*, *A. hydrophila*, and *A. veronii* biovar *sobria*. *A. caviae* is particularly associated with young children especially under 3 years of age. Many studies reported the isolation of several species of *Aeromonas* from patients with gastroenteritis which has been extensively reviewed (Janda, 1991; Joseph, 1996; and Kivanc *et al.*, 2011). Similarly, our work indicated that *Aeromonas* species represent the mostly encountered bacterial pathogen causes intestinal tract infections among school children's with the incidence in both diarrhoeal and non diarrhoeal cases (14%) and (16%), respectively.

We evaluated antimicrobial susceptibility profiles of six different bacterial species: *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Shigella* spp., *Proteus* spp. and *Aeromonas* spp. against 11 antibiotics. Data depicts a prevalence of antimicrobial resistance by these strains to Ampicillin. However, all the strains had varying percentage susceptibility to Augmentin, Nalidixic acid, Ceftazidime, Imipenem, Ofloxacin and Ciprofloxacin. This means that these antibiotics are fairly effective in the treatment of these pathogens but the use of Ciprofloxacin in young children has grave risks. In Ethiopia, the susceptibility pattern of diarrhoea-causing bacteria, including *Yersinia enterocolitica*, *Shigella*, *E. coli*, and *Salmonella* to Gentamicin, Nalidixic acid, Norfloxacin, and Polymyxin B was greater than 90% for the tested strains while resistance to Cephalothin, Ampicillin, and Tetracycline was greater than 50% (Andualem and Geyid, 2005). Similarly our results indicated that Ampicillin was not active against most organisms tested while Gentamicin was effective against most organisms tested.

In conclusion, the present study has demonstrated that protozoan organisms, such as *Cryptosporidium parvum*, *E. histolytica*, *Giardia lamblia*, and *Entrobium vermicularis*, are common parasitic causes of diarrhoea in Hail city while *Escherichia coli*, *Klebsiella specie*, *Shigella* spp., *Salmonella* spp. and *Aeromonas* spp. and are the most common bacterial causes of diarrhoea. Our results put the light on drinking water in Hail city and preventive measures should be taken for the eradication of these parasitic and bacterial infections include public health education, clean water supply, and periodic examination of schoolchildren for parasitic infections.

Corresponding Author:

Dr. Mohamed A. Fareid
Botany and Microbiology Department
Faculty of Science (Boys)
Al-Azhar University, Cairo, Egypt.
E-mail: mohamedfareid73@yahoo.com

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