Association of *Escherichia coli* with Diarrhea in Children Less Than Two Years of Age at Kassala Hospital

By
Magdi Babiker Omar

B.Sc., University of Shendi (2003)

Supervisor
Dr. Elhassan Mohammed Ali Saeed

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Department of Microbiology
Faculty of Veterinary Medicine
University of Khartoum

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بسم الله الرحمن الرحيم

والله وهم على وهم هنا أمها حملته بالديه الإنسان ووصيناه إلي ولولبديك لديني اشكرو

(الشي)...

۱۴ ماه
Dedication

To my parents, brothers and sisters

To all people in this country

To my friends

With love

Magdi
ACKNOWLEDGEMENTS

First of all, I render my gratitude and praise to the almighty "Allah" who offered me health, patience and ability to undertake this work.

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ABSTRACT

This study was conducted to investigate the role of *E. coli* in causing diarrhea in children less than two years of age at Kassala Children Hospital. A total of 50 stool specimens were collected from children with different ages (1-24 months) and sexes. These specimens were subjected to bacteriological examination, with attention to the result of parasitological examination.

The prevalence of protozoal infection (*Entamoeba histolytica* and *Giardia lamblia*) was found to be 24%. The bacterial isolation in MacConkey’s agar revealed the presence of 39 Gram-negative lactose-fermenting rod isolates. These isolates were further cultured in Brain Heart Infusion broth for biochemical identification using the Analytical Profile Index (API) kit. Twenty seven isolates out of total samples were identified as *E. coli* with a prevalence rate of 54% of the total specimens.

The detection rate of *E. coli* was found to vary between children age groups. Children aged 7-18 months were found most affected. However detection rate of *E. coli* in both sexes of children was found almost similar.

Effect of education of children’ mothers was found inversely proportional with *E. coli* infection. Children of graduate mothers were least affected compared to higher and elementary school education.

Using the disc diffusion method, the antimicrobial sensitivity test was performed for all of the 27 *E. coli* isolates against 12 antimicrobial agents. All of the isolates showed high sensitivity to Amikacin, Ofloxacin and Chloramphenicol, while Norfloxacin, Tetracycline and Pefloxacin were the least effective.
ملاحظة: الأطروحات

الملخص الثاني

دون الأطفال بأسلوب القولانية

الإشاريات علاقة المعرفة

doors تمت كسلة بمدن الأطفال بمستشفى

جمعية حيث 50 مرن أعمارهم تراوحوا أطفال مرن ببراز العينة 1-24 البكتيرية للفحص وتم الجنس كمساشر في الميقات االأخذه،

كانت بالطفيليات الإصابة نسبة 24% العينات مجمعة مرن

على التسجيل الأمية لامبيد والجارديا (.

علي الحصول ماتكوني في العينات زراعة وعند 39 للهـ إلازالة القولانية االإرشادية

ないこと الواعدة

الكيميائية الأخرى API.

علي تعرفه تتم العينات مجمعة مرن

على البتولة والقلب المخز

الكيميائية التحليل الشريطة

API.

علي تعرفه تتم العينات مجمعة مرن

على البتولة والقلب المخز

الكيميائية التحليل الشريطة

API.
INTRODUCTION

One of the most common causes of morbidity and mortality among infants and children of developing nations was claimed to be the diarrhea caused by bacteria (Nataro, Steiner and Guerrant, 1998).

Bacterial pathogens have evolved many ingenious ways to infect their hosts and cause disease, including the subversion and exploitation of target host cells. One of such subversive microbe is enteropathogenic *Escherichia (E.) coli* (EPEC), a major cause of infantile diarrhea in developing countries. It poses a significant health threat to children worldwide (Vallance and Finlay, 2000).

*Escherichia coli* was the most commonly identified pathogen, and at least five distinct pathotypes have been characterized, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E.coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC). EAEC is an emerging food-borne pathotype that can cause acute and persistent diarrhea in children, human immunodeficiency virus-infected persons, and international travelers (Adachi *et al*, 2002). EAEC has been associated with diarrhea in both developing and industrialized countries (Huppertz *et al*, 1997) including the United States of America (Okeke and Nataro, 2001) and sometimes causes large outbreaks of gastrointestinal illness (Itoh *et al*, 1997).

*Escherichia coli* is a straight Gram-negative and nonsporing rod occurring singly or in pairs. Most of the strains are motile with peritrichous flagella. It grows readily on simple nutrient media (Krieg and Holt, 1984).

El-Sheikh and El-Assouli (2001) found that 13% of children suffering from diarrhea in Kingdom of Saudi Arabia were infected by *E. coli*. Also, Torres *et al*. (2001) declared that co-infection with *E. coli* and other agents
were detected in more than one-third of positive samples. They found that EPEC was the most prevalent pathogen, *Rotavirus*, *Cryptosporidium*, *Campylobacter*, *Salmonella* and parasites like *Giardia lamblia* were also reported. *E. coli* is one of the enteric bacteria that are part of the normal flora and incidentally causes disease, while others like *Salmonella* and *Shigella* are regularly pathogenic for humans (Brooks Butel and Moves, 1998).

In the Sudan, bacterial diarrhea in children was shown to make a higher incidence, whereas enteropathogenic *E. coli* was the most dominant (Erwa, Yousif and Gumaa, 1971). *E. coli* was reported to be accounted for 72.8% of the isolates from stools (Erwa, 1975). Recently, Hussein (2007) found that bacteria were the main cause of diarrhea in infants and children below five years of age in Khartoum State. *E. coli* was the predominating species, it was isolated from 96% (48/50) of cases and it constituted 62.3% of the total isolates.

**Objectives of the study**

- To isolate *E. coli*–like organisms from diarrheal samples taken from children at Kassala hospital and to identify them using Analytical Profile Index (API) biochemical kit and to determine the incidence rate of *E. coli* in these samples.
- To determine the antimicrobial sensitivity of the *E. coli* isolates.
CHAPTER ONE
LITERATURE REVIEW

1.1 Definition of diarrhea

Abnormal fecal discharge characterized by frequent and/or fluid stool; usually resulting from disease of the small intestine and involving increased fluid and electrolyte loss (Mims et al., 1999).

Classification of diarrheal diseases of children is usually made on etiological grounds (Wilson and Miles, 1964). This classification was considered by these two authors as tentative owing to lack of sufficient convincing evidence for the existence of some of the causes. Moreover, descriptive terms are used, which are related to epidemiological circumstances, e.g. summer diarrhea and infantile gastroenteritis. The use of these terms usually overlooks the etiological factor. The etiological classification by Wilson and Miles (1964) seems to be useful in diagnostic work.

1.2 Association of E. coli with diarrhea in children

Enteropathogenic E. coli (EPEC) is a leading cause of infantile diarrhea in developing countries. In industrial countries, the frequency of these organisms has decreased, but they continue to be an important cause of diarrhea (Nataro et al., 1998). El-Sheikh and El-Assouli (2001) reported a prevalence rate of 13% for E. coli in children suffering from diarrhea. Also, co-infections with two or more agents, including E. coli, were detected in more than one-third of children positive cases (Torres et al., 2001). Enteropathogenic E. coli was found more prevalent than Rotavirus, Cryptosporidium, Campylobacter, Salmonella and parasites like Giardia lamblia. Some enteric organisms e.g. E. coli are part of the normal flora and
incidentally cause disease, while others like *Salmonella* and *Shigella* are regularly pathogenic for humans (Brooks *et al*., 1998)

### 1.3 Types of diarrhea

Once a microorganism contaminates the intestine, it may produce diarrhea by any one or combination of the following mechanisms:

#### 1.3.1 Invasive diarrhea

Invasive diarrhea is considered as the classical form resulting from invasion and penetration of intestinal mucosa leading to mucosal inflammation and epithelial cell disruption.

#### 1.3.2 Toxigenic diarrhea

This mechanism of diarrhea is implicated in many diarrheal disorders in children. Toxogenic and invasive forms of bacteria are transmissible factors, which may facilitate the penetration of the host epithelium by organisms. *E. coli* may express both enterotoxigenic and invasive properties in different strains and even in the same strain.

The toxigenic strains of *E. coli* have been frequently implicated as a cause of traveler’s diarrhea. Up to 72% Turista episodes in Mexico were associated with heat labile toxins of *E. coli* isolated from stools of American students with diarrhea. However, healthy individuals who had no diarrhea also had these strains detected in 15% of stool specimens.

#### 1.3.3 Injurious diarrhea

Bacterial metabolites may induce ultra structural and functional abnormalities of the intestine, which may lead to diarrhea (Gorbach, 1975).

#### 1.3.4 Diarrhea caused by bacterial overgrowth
A wide spectrum of enterobacteria may alter intestinal functions when present in excessive numbers in the small intestine. Increase in microbial populations may be directly responsible for the diarrhea (Lifshitz et al., 1971)

1.4. Epidemiology of diarrhea due to *E. coli*

An important landmark on the understanding of epidemiology and etiology of diarrheal disease and the cause of intestinal infection dates back as far as 1854 when John Snow had shown that an outbreak of cholera in London could be traced to single source of polluted water, the broad street pump (Cruickshank et al., 1973). The attempts to implicate certain organisms in infantile enteritis were started by Escherich who first isolated *E. coli* from stool of infants with diarrhea in 1885 (Wilson and Miles, 1964). Diarrheal disease itself is one of the commonest pathological conditions all over the world especially in the developing countries. Infantile diarrhea and different forms of dysentery prevail in many areas in the world (Cruickshank et al., 1973).

To describe the epidemiology and etiology of acute diarrhea among children treated exclusively in an outpatient setting in Greece, 132 children (median age two years) were included in a study conducted by Multezou et al. (2001). Enteropathogens were detected in 63 (48%) of them. Isolates included *Rota* virus (19), *Salmonella* spp. (12), *Campylobacter* spp. (10), *Aeromonas* spp. (9), enteropathogenic *E. coli* (6), *Adeno* virus (6), *Giardia lamblia* (4), *Yersinia enterocolitica* (2), and *Shigella* sp. (1).

Stool cultures (561) from Yemeni children presenting diarrhea were identified (Banajeh, Ba-Oum and Al-Sanabani, 2001); a total of 190 (33.9%) were positive for bacteria which were shown to be most prevalent agents. *Rota* virus, *Cryptosporidium*, *Campylobacter* and *Shigella flexneri* were also
identified. Identical enterotoxigenic \textit{E. coli}, \textit{Salmonella} and \textit{Giardia lamblia} were sporadically recognized. Unusual findings included two enteroinvasive \textit{E. coli} strains, two \textit{Shigella dysyntriae} isolates and one \textit{Vibrio cholerae} culture (Torres \textit{et al.}, 2001).

Fecal samples (576) were collected from children (0-5 years old) suffering from acute diarrhea in Kingdom of Saudi Arabia; one or more types of enteropathogens were identified in 45.6% of these stool specimens (El-Sheikh and El-Assouli, 2001). Mixed infections were detected in 12.2% of the cases. \textit{E. coli} was detected in 13.0%, of which 3.8% were enteropathogenic \textit{E. coli}.

Marc \textit{et al.} (1978) reported an isolation rate of 13% for enteropathogenic \textit{E. coli} from 220 children (<12 months) and a rate of 6% of 143 children (12-35 months) suffering from diarrhea in Canada. Enteropathogenic \textit{E. coli} strains were the most prevalent enteropathogens isolated from stools of hospitalized infants with severe acute diarrhea in Sao Paulo (Brazil), and these strains were able to induce moderate to severe fecal fluid losses in infants and the duration of diarrhea was below 15 days (Oliva \textit{et al.}, 1997).

In the summer of 1998, a large outbreak of \textit{E. coli} serotype O157:H7 infection was occurred in Alpine Wyoming. One hundred fifty seven ill persons had been identified; stool from 71 (45%) yielded \textit{E. coli} O157:H7 in two cohort studies. Illness was significantly associated with drinking municipal water. Among persons exposed to water the attack rate was significantly lower in town residents than in visitors (23% and 50%, respectively) (Olsen \textit{et al.}, 2002).

1.5. Epidemiology of diarrhea in the Sudan
In the Sudan, 5.4 million episodes of diarrhea were reported in 1977 (Kamal, 1988); 50% of them were in children under five years. Data from Khartoum Emergency Hospital (1977) revealed that 46% of cases of death in children who were hospitalized in that year were due to gastroenteritis. The statistical data from Juba Teaching Hospital in 1982 indicated that 95% of the total deaths were due to diarrheal diseases in children in Juba district. These deaths were distributed as such 40% under one year and 55% from 1–5 years. Also, according to Taha (1981), in a series of 2000 deaths of children below the age of five years, 1200 (60%) were reported to have died of gastroenteritis followed by respiratory tract infection showing that diarrheal diseases and respiratory tract infections are the major killing diseases (Erwa et al., 1971).

Bacterial etiology of diarrhea in children was shown to make a higher incidence with enteropathogenic *E. coli* as the most dominant pathogen (Erwa et al., 1971).

A study carried out in Khartoum North in 1975 among 654 cases of children suffering from diarrhea, *E. coli* was shown to be the most prevalent (Erwa, 1975). Enteropathogenic *E. coli* was isolated from children with diarrhea below five years of age in Juba town (Sharaf, 1986). According to Ismail (1994), 320 isolates were obtained from children suffering from gastroentritis, 76.3% of them were *E. coli*.

**1.6 Route of infection**

Enteric infection usually follows oral ingestion of the organism with rapid proliferation and colonization of the small intestine. However, it may also follow retrograde spread proximally into the small intestine by established resident strains in the colon, or from systemic spread as infection in children (Takeuchi, Formal and Sprinz, 1966).
Infection of certain invasive strains of *E. coli* is indistinguishable from shigellosis, while other organisms induce diarrhea by proliferation in the lumen of small intestine with release of toxin as cholera (Dubos, 1958).

### 1.7 Mechanisms of infection

Enteropathogenic organisms cause diarrhea either by their invasiveness or by the ability to excrete an enterotoxin. Invasive organisms such as *Shigella*, *Salmonella* and certain strains of *E. coli* penetrate the mucosa of the distal small intestine and colon and multiply within the mucosa causing acute inflammatory reactions and mucosal damage; leading to a disturbance in the colonic salt and water transport. Thus, diarrhea is caused by the inability of damaged colon to absorb the increased fluid and salt coming from small intestine. The non–invasive organisms such as *V. cholerae* and some strains of *E. coli* elaborate an enterotoxin, which induces secretion of water and electrolytes from the small gut without affecting the mucosal structure (Soad, 1980). Also, there is evidence that invasive organisms stimulate prostaglandin synthesis at the site of inflammation and that the prostaglandins induce fluid secretion.

### 1.8 *Escherichia coli*

#### 1.8.1 Definition

*Escherichia coli* is an emerging enteric pathogen that causes acute and chronic diarrhea among children, human immunodeficiency virus-infected patients, and travelers to developing regions of the world (Jamal *et al.*, 2007).

#### 1.8.2. Historical background

The first isolation of *E. coli* was made by an Austrian Pediatrician, Dr. Theodor Escherich in Munich in 1885. At that time it was named *Bacterium coli*. 
The organism was isolated along with other bacteria from the feces of newborn babies. It was found to be concomitant with breast feeding. Escherich described it as a short plump rod, growing readily on gelatin or agar. On potato and coagulated milk, it grew as a slimy mass with the production of acid. In the early days different names have been applied to the organism such as *Bacillus escherichii* in 1889, *Bacillus coli* in 1895 and *Aerobacter coli*. The genus *Escherichia* first was proposed by Migula in 1895 and became firmly established in 1919 by Castellani and Charmers in the third edition of the Manual of Tropical Medicine (Tortura, Funke and Case, 1986). Its pathogenicity was firstly suggested by Laurelle in 1889.

### 1.8.3. Classification

According to the eighth edition of Bergy’s Manual of Determinative Bacteriology (1984), the genus *Escherichia* belongs to the family enterobacteriaceae which includes the following:

- **Tribe I**: *Eschericheae*
- **Tribe II**: *Klebsielleae*
- **Tribe III**: *Proteusae*
- **Tribe IV**: *Yersinieae*
- **Tribe V**: *Erwinieae*

The tribe Eschericheae includes five genra:

- **Genus I**: *Escherichia*
- **Genus II**: *Edwardsiella*
- **Genus III**: *Citrobacter*
- **Genus IV**: *Salmonella*
- **Genus V**: *Shigella*

The genus *Escherichia* includes six species (Barrow and Feltham, 1993):

1. *Escherichia coli*
II. *E. adecarboxylata*

III. *E. hirmanii*

IV. *E. fergusonii*

V. *E. vulneris*

VI. *E. blattae*

### 1.8.4. Principal characteristics

#### 1.8.4.1. Cellular characteristics

*Escherichia coli* is a straight Gram-negative and nonsporing rods, often motile, aerobic and facultative anaerobic (Barrow and Feltham, 1993)

#### 1.8.4.2. Habitat

Members of *E. coli* are normal inhabitants of the intestinal tracts of vertebrates including man (Buxton and Frazer, 1977). The organism becomes established in the intestine shortly after birth when the sterile intestine of the fetus is seeded with bacteria derived from the mother and the environment. *E. coli* continues throughout adult life as the major facultative anaerobic species of bacteria in the intestine and is usually the dominant isolate on aerobic culture of feces or intestinal contents. Most strains of *E. coli* are harmless saprophytes but others are virulent pathogens that affect intestine or extra intestinal sites (Gyles and Thoen, 1993).

#### 1.8.4.5. Resistance

The organism is fairly resistant to drying and chemical disinfectants. Pasteurization usually destroys the organism although some heat resistant strains may withstand such treatment (Gillespie and Timonery, 1981). In the majority of instances a temperature of 55 °C for one hour or 60 °C for 20 minutes is lethal to the organism. It is killed rapidly by autoclaving at 120 °C. Freezing in liquid air for 2 hours destroys 95% of the cells. Some individuals resist freezing in ice for six months. The organism may survive
for weeks or months in water, feces and dust in animal houses. Their high susceptibility to the efficacy of these disinfectants is reduced if it presents in mucus and feces (Buxton and Frazer, 1977).

The biochemical localization of phenylmercuric borate (PMB) on *E. coli* shows that this disinfectant associates essentially with proteins. Four important metabolic activities have been tested in the presence of PMB, namely: respiration, protein-synthesis, RNA and DNA synthesis. These four metabolic functions are rapidly and totally inhibited at low concentration of PMB (Cortat, 1978).

**1.8.4.6 Cultural characteristics**

*Escherichia coli* is aerobic and facultative anaerobic. It is readily grown on simple laboratory media without the addition of blood serum, ascitic fluid or glucose. The optimum temperature for cultivation is 36-37°C but growth will occur over a temperature range of approximately 18-44 °C (Collee *et al.*, 1996)

The colonies usually develop to a size of 2-3 mm in diameter on agar media but there may be considerable variation in colony sizes of different strains. Organisms with well developed capsules give rise to relatively large opaque mucoid colonies (Buxton and Frazer, 1977). Uniform clouding is produced in broth after 12-13 hours incubation. Friable pellicles are formed in older culture, while considerable viscid sediment occurs in very old broth culture. Colonies on nutrient agar have slightly raised surfaces. They are smooth and glistening with circular outlines. Deep colonies are brownish and lenticular in shape. Thin, bluish-white translucent and glistening colonies are produced on gelatin. Their surfaces show radial ridges and the margins are irregular, giving them the shape of a grape leaf. Brownish and rather dry growth occurs on potato. Merchant and Packer (1977) stated that
colonies with blackish centers and a metallic sheen occur on eosin methylene blue agar. This criterion is of value for identification of the organism. Red colonies with a red zone in the medium surrounding the colonies occur in litmus lactose agar. Pink colonies are produced on MacConkey’s bile salt agar. (Buxton and Frazer, 1977).

### 1.8.4.7 Biochemical properties

The majority of strains form indole but does not produce H₂S, do not grow on citrate medium, and do not produce gelatinase. Most strains do not develop urease, give a negative Voges-Proskauer reaction and are positive to methyl red test (Barrow and Feltham, 1993).

All strains of *E. coli* ferment glucose and lactose with production of acid and gas, but a few strains are late lactose fermenters or may often fail to ferment this sugar (Barrow and Feltham, 1993).

Morris and Maurice (1960) stated that the organism ferments arabinose, rhamnose, xylose, fructose, galactose and maltose with the production of acid and gas. Sucrose may be fermented but cellobiose is not fermented. Starch, dextrin and glycogen are not fermented.

Generally, there is no single biochemical feature which is particularly characteristic of Escherichia group. A combination of various reactions is required for its classification (Robertson and Maclowry 1973).

### 1.8.4.8 Serological characteristics

The complex O, H and K antigenic structure of *E. coli* have been studied in details because it forms the basis by which the serotypes can be differentiated from one another (Buxton and Frazer, 1977).

Several schemes have been developed to characterize isolates of *E. coli* and to aid in the identification of pathogenic strains. Serotyping is the most widely used of these schemes, and approximately 137 O, 80 K and 56
H antigens have been identified and numbered in this scheme (Gyles and Thoen, 1993).

**1.8.4.8.1 Somatic (O) antigens**

These O antigens are somatic antigens occurring as part of the bacterial body and are composed of a polysaccharide phospholipid protein complex (Buxton and Frazer, 1977). They are obtained by autolysis of cells which is facilitated by simple physico-chemical treatment by solvents, heating, rapid freezing and thawing (Ewing and Edwards, 1986).

**1.8.4.8.2 Capsular (K) antigens**

These K antigens which occur as envelopes or capsules on most strains of *E. coli* are composed of polysaccharides (Buxton and Frazer, 1977). These antigens are associated with virulence, interfere with O agglutination and can be removed by heating to 100 °C for 1 hour, however, some strains require heating for 2.5 hour at 121 C° (Hofstad *et al.*, 1978).

Three types of K antigens, L, A and B have been described. The differentiation between these types is based on the varying thermostability of their agglutinability, on their capacity to provoke antibody formation and their antibody binding capacity (Jann and Jann, 1982).

**1.8.4.8.3 Flagellar (H) antigens**

Flagellar antigens are associated with flagella of motile *E. coli* strains. They are composed of proteins and are inactivated by heating at 100 °C (Ewing and Edwards, 1986). They are not often used in the antigenic identification of *E. coli* isolates and are not correlated with pathogenicity (Hofstad *et al.*, 1978).

**1.8.4.8.4 Fimbrial antigens**
Fimbriae (pilli) are slender, proteinaceous filaments that project from the surface of the bacteria and confer adhesive properties on organism (Gaastra and De Graaf, 1993). Fimbriae are antigenic and their antigens are not specific for bacterial serotypes or for groups. Repeated subculturing of fimbriated strains on solid media may result in the loss of fimbriae. Growth in fluid encourages their development.

Bacteria possessing fimbriae agglutinate red blood cells of various animal species and man. This form of haemagglutination reaction is due to the adherence of fimbriae to the surface of red blood cells (Buxton and Frazer, 1977).

1.8.4.6.5. Common antigen (CA)

Some serotypes develop another somatic antigen in addition to the O antigen. It appears to be common to many members of different bacterial groups; it composed of polysaccharide (Buxton and Frazer, 1977).

1.8.5. Virulence factors

Several structures and products of *E. coli* have either a demonstrated or a potential role in virulence in gut and other tissues. The structures include capsule, cell wall and pilli; the products include enterotoxins, cytotoxins, hemolysins and aerobactin (Gyles and Thoen, 1993).

1.8.5.1. Capsule

Certain strains of *E. coli* that cause diarrhea in calves and a subset of strains that cause diarrhea in pigs produce abundant capsule of polysaccharide that may aid in colonization of the intestine (Hadad and Gyles, 1982).

Studies on the ultrastructure of the capsulated *E. coli* in association with the intestine of calves suggest that the capsular material contributes to
the formation of microcolonies attached to the intestinal epithelium (Acres, 1985).

The capsule may contribute to virulence of invasive strains by being antiphagocytic and poor antigenic (Gyles and Thoen, 1993).

Most strains of *E. coli* are harmless saprophytes but others are virulent pathogens that affect the intestine or extra intestinal sites (Gyles and Thoen, 1993)

### 1.9. Diagnosis of diarrheal diseases

Routine culture and identification of *E. coli* from relevant samples are required for the laboratory diagnosis of septicaemia and infection of the intestinal tract due to *E. coli* and quantitative culture is necessary in the case of urinary tract infection. For enteric disease it is necessary to use methods that distinguish the pathogenic from non-pathogenic *E. coli*. Three approaches have been taken. The first involves initial culture followed by a method to determine the surface antigen or the production of enterotoxins that may be detected by a biological test or immunological methods such as ELISA. A second approach applicable (only when well preserved intestine is available), i.e demonstrate adherent *E. coli* attached to the intestinal epithelium. A third method involves demonstration of the isolate that contains DNA sequences found in *E. coli* genes that code for enterotoxin. The development of the polymerase chain reaction (PCR) amplification procedure involving oligonucleotide primers that can readily be purchased or synthesized has made it easier to detect *E. coli* with virulence factors (Woodward *et al.*, 1992).

### 1.10 Treatment and control of diarrheal diseases

In the past, diarrheal diseases of all etiologies and in all age groups were treated with antibiotics and antidiarrheal drugs, but still a large number of
deaths occur because of severe dehydration (Cash et al., 1970). Since then dehydration from all causes and in all ages could be effectively treated by simple oral rehydration. The most important treatment of diarrhea is prevention of dehydration. Rehydration and maintenance of proper fluid and electrolyte balance are the most important aspects of treatment, while intravenous rehydration is the best form of treatment for children who are in shock or unable to drink (Leung and Robson, 1989). Antimicrobial therapy should be used in severe cases of diarrheal diseases to reduce the duration of illness and may be used to prevent traveler's diarrhea (Nataro et al., 1998). For 162 diarrheagenic Escherichia coli strains and 28 Shigella strains, more than 75% were resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. Multi-resistance was detected in 89.5% of E. coli strains and 78.6% of Shigella strains. However, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern (Hoge et al., 1998). In addition, the overdose and misuse of antibiotics in the treatment of diarrhea could lead to an increase of antibiotic resistance (Hoge and Bodhidatta, 1995). Ismail (1994) studied microbiological identification and antibiotic resistance pattern of clinical isolates from children with gastroenteritis. The result demonstrated high levels of multi–drug resistance among the isolated enteric bacteria. In a study conducted by Elsadik (1998), 4% of the isolates were resistant to co-trimoxazole, 55% to ampicillin, 69% to erythromycin and 22% to chloramphenicol, while 100% of the isolates were sensitive to nalidixic acid, ciprofloxacin and gentamicin. The possibility of appearance of resistance
should always be put in mind, because resistance is increasing in most of enteric bacteria in Sudan (Alawia et al., 1994).

To control diarrheal diseases, attention should be focused on prevention of occurrence of the disease by: i. immunization, which is a crucial intervention for diarrhea control (Park, 1997); ii. breast feeding, newborn infant with diarrhea with no signs of dehydration can be treated with breast-feeding alone. It helps the infant to recover from an attack of diarrhea both in terms of nutrient supplies and in terms of its rehydration effect. In addition, it has protective properties that prevent further infection (Park, 1997).

In general, early symptomatic treatment and effective usage of antibiotics can substantially reduce the risk of severe morbidity or death (WHO/CDC, 1994).
CHAPTER TWO
MATERIALS AND METHODS

2.1 Collection of samples

Fifty stool specimens were collected from children aged 1-24 months, who suffered from diarrhea and were admitted to Kassala Children Hospital. Specimens were collected in sterile plastic containers with specific labels. A part of each specimen was put into another sterile plastic container for parasitological examination. The specimens were examined by the technicians in the hospital laboratory for presence of parasites. Their results were recorded for interpretation with bacteriological results. After parasitological examination the specimens were carried to the Laboratory of Veterinary Research Center (Kassala) for bacteriological examination.

The age of children was classified into four categories, 1-6 months, 7-12 months, 13-18 months and 19-24 months for a possible variance in susceptibility to *E. coli* infection (Table I).

Table I. Distribution of specimens according to age categories of children

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Number of specimens</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>7-12</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>13-18</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>19-24</td>
<td>13</td>
<td>26</td>
</tr>
</tbody>
</table>
The education level of the children mothers was also considered in this study to see if it has effect on infection rate (Table II).

### Table II. Distribution of children according to education level of their mothers

<table>
<thead>
<tr>
<th>Education level</th>
<th>Number of children</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elementary</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td>Higher school</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Graduate</td>
<td>14</td>
<td>28</td>
</tr>
</tbody>
</table>

2.2 Asepsis and sterilization

2.2.1 Flaming

Flaming was used to sterilize slides, cover slips and mouth of tubes and bottles.

2.2.2 Red heat

It was used to sterilize loop wires, needles and spatulas by holding them over Bunsen burner flame until became red.

2.2.3 Hot air oven

It was used to sterilize metals, glassware such as test tubes, graduate pipettes, flasks, Petri dishes and cotton swabs. The holding period was one hour and temperature was 160°C.

2.2.4 Moist heat (autoclaving)

Media, solutions and plastic ware were sterilized by autoclaving at 121°C (15 lb/inch²) for 15 minutes.

2.2.5 Irradiation

Ultraviolet irradiation for 20 minutes was used to sterilize the surface
of benches and walls of media pouring room.

2.2.6 Disinfection
Phenol disinfectant and 70% alcohol were used for disinfecting the floor and working room in the laboratory.

2.3 Reagents and indicators
2.3.1 Reagents
2.3.1.1 Normal saline
Normal, physiological, or isotonic saline was prepared as described in Oxiod Manual by dissolving 8.5 grams of sodium chloride to one liter of distilled water to obtain 0.85% concentration. The solution was sterilized by autoclaving at 121°C (15 lb/inch²) for 15 minutes.

2.3.1.2 Gram’s stain reagents
2.3.1.2.1 Lugol’s iodine
Formula: g/l

Potassium iodide  20 g
Iodine  10 g
Distilled water  1 litre

Potassium iodide was weighed and dissolved in about a quarter of water. Iodine was added to potassium iodide solution and mixed well; the solution was made up to one litre with distilled water, mixed well and then stored in dark place at room temperature.

2.3.1.2.2 Crystal violet
Formula:

Crystal violet  20.0 g
Ammonium oxalate 9.0 g
Ethanol Up to 95 ml
Alcohol was added to the crystal violet and mixed well until the dye was completely dissolved. Ammonium oxalate was weighed and dissolved in about 200 ml of distilled water and then it was added to the stain and made up to one litre with distilled water and mixed well; then stored at room temperature.

2.3.1.2.3 Acetone–alcohol decolorizer
Formula:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>500 ml</td>
</tr>
<tr>
<td>Ethanol or methanol, absolute</td>
<td>475 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

Distilled water was mixed well with alcohol; acetone was measured and added immediately to alcohol solution, mixed well and stored at room temperature.

2.3.1.2.4 Diluted carbol fuchsin
One volume of strong carbol fuchsin was added to 10 volumes of distilled water.

Strong carbol fuchsin consists of two solutions:
1. Solution A: Ten grams of basic fuchsin mixed with 10 ml of ethanol (95%) and dissolved in stoppered bottle and kept at 37 °C overnight.
2. Solution B: Five grams of phenol were mixed with 100 ml of distilled water and shacked to dissolve.

Strong carbol fuchsin was prepared by pouring 10 ml of solution A into 100 ml of solution B.
2.3.2 Broth media

2.3.3.1 Brain Heart Infusion broth

- **Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf brain heart infusion solids</td>
<td>12.5</td>
</tr>
<tr>
<td>Beef heart infusion solid</td>
<td>5.0</td>
</tr>
<tr>
<td>Protease peptone (Oxoid L 46)</td>
<td>10.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Preparation**

Thirty-seven grams of dehydrated medium were added to 1 litre of distilled water mixed well and the pH was adjusted to 7.4. Then it was distributed into final containers and sterilized by autoclaving at 121 °C for 15 min.

2.3.3 Solid media

2.3.3.1 MacConkey’s agar

This medium contained peptic digest of animal tissue (20 g), lactose (10 g), bile salt (1.5 g), sodium chloride (5 g), crystal violet (0.001 g), neutral red (0.05 g) and agar (15 g).

Fifty two grams of dehydrated powder were added to 1 litre of distilled water, mixed and then boiled in water bath to dissolve the ingredients completely. After dissolution and adjustment of the pH to 7.2 the medium was sterilized by autoclaving at 121°C for 15 minutes and then poured into sterile Petri dishes in 20 ml amounts and the plates were left to solidify at room temperature on flat surface.

2.3.3.2 Mueller-Hinton agar

*Ingredients*
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef infusion</td>
<td>6.0</td>
</tr>
<tr>
<td>Casein hydrolysate (Oxoid L41)</td>
<td>17.5</td>
</tr>
<tr>
<td>Starch</td>
<td>1.5</td>
</tr>
<tr>
<td>Agar No.1 (Oxoid L 11)</td>
<td>10.0</td>
</tr>
</tbody>
</table>

An amount of 35 g was suspended in 1 litre of distilled water and brought to boiling to dissolve completely. The pH was adjusted to 7.2. Then, it was sterilized by autoclaving at 121 °C for 15 min.

### 2.4 Cultivation, isolation and purification of bacteria

After specimens were brought to the laboratory, a part of the faecal specimen was taken by a sterile loop and streaked onto MacConkey’s agar by four quadrant fashion under aseptic conditions to differentiate between lactose-fermenting and non-lactose-fermenting colonies. Plates were incubated aerobically at 37°C for 24 hours. After incubation period, the plates were examined for different types of colonies which were differentiated by cultural characteristics and microscopic examination of Gram's stained smears. Gram negative lactose fermenting colonies were subcultured on MacConkey’s agar and incubated aerobically at 37°C for 24 hours. Pure cultures were then preserved in the refrigerator for further testing.

### 2.5. Microscopic examination of the isolates

All colony types were subjected to Gram's staining technique. Lactose-fermenting colonies which showed Gram negative short plump rods which arranged singly and rarely in pairs were especially suspected to be *E. coli*.

#### 2.5.1. Preparation of smears

Using a sterile loop, a drop of a sterile normal saline was put in the centre of a clean microscope slide. A part of a colony was picked up by
sterile loop and emulsified in the drop of the normal saline and spreaded in an oval shape in the centre of the slide. The smears were then allowed to dry in the air and fixed by gentle flaming.

2.5.2 Staining method

Staining method was done as described by Barrow and Feltham (1993):

1. Fixed smears were flooded with crystal violet for 90 seconds.
2. Slides were declined by forceps and gently washed with distilled water.
3. Slides were flooded with Lugol's iodine for 60 seconds.
4. Slides were washed with distilled water.
5. Slides were decolorized with acetone-alcohol for 5 seconds.
6. Slides were washed with distilled water.
7. Counter staining with diluted carbol fuchsin for 30 seconds.
8. Slides were washed with distilled water.
9. Slides were dried with filter paper and a drop of oil immersion was put on each slide and slides were examined with oil immersion objective lens for presence of bacteria.

2.6. Biochemical testing

2.6.1. Analytical Profile Index (API) kits (KB 0003)

The API was purchased from HiMedia Laboratories Pvt. Limited, Mumabia, India. The test was performed according to the manufacturer's instructions. The kit was described to identify enterobacteria.

2.6.2.1 Materials provided with the kit

1) Kit strips of Part I tests which contained 12 tests (Fig. 5) (ONPG, lysine decarboxylase, ornithine decarboxylase, urease, phenylalanine deamination,
nitrate reduction, H₂S production, citrate utilization, Voges-Proskauer’s test, methyle red test, indole production, malonate utilization).

2) Kit strips of Part II tests which contained 12 tests (Fig. 5) (esculin, arabinose, xylose, adointol, rhamnose, cellobiose, melibiose, saccharose, raffinose, trehalose, glucose, lactose and oxidase).

3) Kits were supplied with sufficient materials for all the biochemical tests.

4) Oxidase reagent discs (DD 018).

5) Result interpretation chart and entry data sheet.

6) TDA reagent (R036) for phenylalanine deaminase test.

7) Baritt reagent A (R029) and B (R030) for Voges-Proskauer’s test.

8) Methyl red reagent (1007) for methyl red test

9) Kovac’s reagent (R008) for indole test.

10) Sulphanilic acid (R015).

11) N, N- dimethyl-1-napthylamine reagent (R009).

2.6.2.2 Storage and stability of the Kits

The kits were stored at 2-8 °C as recommended by the manufacturer and used before the stated expiry date.

2.6.2.3 Preparation of the inoculum

The KB0003 kit cannot be used directly for clinical specimens, but pure culture from isolated organisms on agar plate are used. A single isolated colony was inoculated in 5ml brain heart infusion broth and incubated at 37 °C for 4 hours or until an obvious turbidity was reached. Oxidase test was done by using the oxidase discs (00018) provided separately with the kit.

2.6.2.4 Inoculation of the strips
The kit was opened aseptically by peeling of the sealing foil and then each well was inoculated with 50 µl of broth culture by using surface inoculation method and then incubated at 37 °C for 24 hours.

2.6.3 Interpretation of result

Interpretation of results was done as per the standards given in the identification index.

Addition of reagents wherever required was done at the end of incubation period as follows:

**Part I**: Phenylalanine deamination test (Well No. 5): 2–3 drops of TDA reagent (R036) were added. Development of dark green colour within one minute indicates a positive reaction. No change in colour denoted a negative reaction.

**Nitrate reduction test** (Well No. 6): 1-2 drops of sulphanilic acid (R015) and 12 drops of N, N-dimethyl-1-naphthylamine reagent (R009) were added. Immediate development of pinkish red colour on addition of reagent indicated positive reaction. No change in colour considered as a negative reaction.

**Voges-Proskauer test** (Well No. 9): 2-3 drops of Baritt reagent A (R029) and 1 drop of Baritt reagent B (R030) were added. Pinkish red colour developed within 5-10 minutes indicated a positive test. No change in colour or a slight change in colour (due to reaction of Baritt reagent A with Baritt reagent B) denoted a negative reaction.

**Methyl red test** (Well No. 10): 1-2 drops of methyl red reagent (1007) were added. Reagent remained red in colour if the test is positive. If the reagent decolorized and became yellow the test was considered negative.
**Indole test** (Well No. 11): 1-2 drops of Kovac’s reagent (R008) were added. Development of pinkish red colour within 10 seconds indicated positive reaction. If the reagent remained pale coloured the test was considered negative.

2.7. **Antimicrobial sensitivity test**

Disc diffusion method was performed using multidisks from Axiom Laboratory, New Delhi. The antimicrobial agents used are shown in Table III.

2.7.1. **Preparation of inoculums and inoculation**

Two to three colonies from MacConkey’s agar plate (overnight culture) of the test organism were emulsified in 5 ml of sterile normal saline, and 1 ml was added on the surface of plate of Mueller-Hinlton agar and then evenly distributed by rotating the plate to cover all the surface. The excess broth culture was aspirated by a sterile Pasteur pipette and the plates were left to dry.

2.7.2. **Application of antimicrobial discs**

The antimicrobial discs were removed from their respective containers and carefully placed on the surface of the inoculated medium with the sterile forceps. Finally, the disc pressed lightly with a sterile forceps to make complete contact with the surface of the medium. The plates were incubated overnight at 37 °C and then examined for inhibition zones.
Table III. Antimicrobial agents used for susceptibility testing of obtained fecal isolates

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Code</th>
<th>Concentration (mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicilin</td>
<td>AM</td>
<td>10</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>BA</td>
<td>25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GM</td>
<td>10</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>CH</td>
<td>30</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>FR</td>
<td>30</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE</td>
<td>30</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CP</td>
<td>5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AK</td>
<td>30</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>NA</td>
<td>30</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>PF</td>
<td>10</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>NX</td>
<td>10</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>OF</td>
<td>5</td>
</tr>
</tbody>
</table>

2.7.3. Interpretation of the diameter readings of inhibition zones

The diameter of inhibition zones was recorded and results were interpreted as stated in Table IV.
Table IV. Interpretative chart for diameter of inhibition zones of antimicrobial discs

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Concentration (mcg)</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicilin</td>
<td>10</td>
<td>22 or less</td>
<td>23 - 30</td>
<td>31 or more</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>25</td>
<td>11 or less</td>
<td>12 - 16</td>
<td>17 or more</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>12 or less</td>
<td>13 - 14</td>
<td>15 or more</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>12 or less</td>
<td>13 - 17</td>
<td>18 or more</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30</td>
<td>14 or less</td>
<td>15 - 17</td>
<td>18 or more</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>14 or less</td>
<td>15 - 18</td>
<td>19 or more</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
<td>15 or less</td>
<td>16 - 20</td>
<td>21 or more</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>14 or less</td>
<td>15 - 17</td>
<td>18 or more</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>30</td>
<td>15 or less</td>
<td>16 - 20</td>
<td>21 or more</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>10</td>
<td>15 or less</td>
<td>16 - 20</td>
<td>21 or more</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>15 or less</td>
<td>16 - 20</td>
<td>21 or more</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5</td>
<td>15 or less</td>
<td>16 - 20</td>
<td>21 or more</td>
</tr>
</tbody>
</table>
Figure 1. Blank API strip
CHAPTER THREE
RESULTS

3.1 Level of parasitic infection
Out of 50 stool specimens examined under microscope, twelve samples showed protozoal parasites which were *Entamoeba histolytica* and *Giardia lamblia* (Table V)

Table V. Frequency of protozoal parasites in the stool specimens

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Frequency</th>
<th>% from total specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. histolytica</em></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>5</td>
<td>10</td>
</tr>
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<td><em>E. histolytica</em> and</td>
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<td>6</td>
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<tr>
<td><em>G. lamblia</em></td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>24</strong></td>
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3.2 Bacterial types according to microscopic and cultural characteristics
Forty-two out of 50 stool specimens were found having bacteria. Different types of both Gram-positive and Gram-negative bacteria (bacilli and cocci) were encountered. Thirty-nine specimens contained Gram-negative lactose-fermenting bacilli, which were suspected for *E. coli*.

3.2.1. *E. coli*–like isolates
All Gram-negative rods with single and pair arrangements and lactose-fermenting colonies on MacConkey’s agar (Figure 5) were considered *E. coli*-like organisms. Isolates having such characteristics amounted for a prevalence rate of 78 % of the total specimens.
Further, properties of the colonies were large, either mucoid or non-mucoid, glistening, convex and with entire margin. The microscopic features showed that rods were slender or stout, short or medium in length.

3.3 Biochemical identification of the *E. coli*-like organisms

All the 39 Gram-negative *E. coli*-like isolates were identified biochemically using the Analytical Profile Index (API). The API included 25 tests which were mentioned before (2.6.2.1).

Out of the 39 *E. coli*-like isolates, 27 were confirmed to be *E. coli* isolate (Table VI). An example of the API kit result is shown by Fig 6.

3.4 Distribution of the *E. coli* isolates according to age

The association of *E. coli* with age was found varied between age groups (Table VII and Fig 2). The most affected age was 7–18 months.

3.5 *E. coli* isolates related to sex

The isolation rate of *E. coli* for both sexes of cases showed no difference (Table VIII and Fig 3).

3.6 Effect of level of education of mothers on infection rate

As education is expected to be important in application of hygiene measures, its effect has been considered in this study. Table IX and Fig 4 show clearly that the infection rate was inversely proportional with education.

3.7. Antimicrobial sensitivity

The antimicrobial susceptibility testing was conducted to all *E. coli* isolates. All of the 27 *E. coli* isolates showed high sensitivity to Ofloxacin, Amikacin and Chloramphenicol (Table XI). Norfloxacin, Tetracycline and Pefloxacin were the least effective.
Table VI. Biochemical characteristics of 27 isolates of *E. coli*

<table>
<thead>
<tr>
<th>Biochemical characters</th>
<th>%+ve</th>
<th>Biochemical characters</th>
<th>%+ve</th>
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<td>ONPG</td>
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<td>Aesculin hydrolysis</td>
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<td>Arabinose</td>
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<td>H₂S production</td>
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<td>Oxidase</td>
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+%ve=percent of positive isolates.
Table VII. Prevalence of *E. coli* infection according to age

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<th>Age group (month)</th>
<th>Number of examined cases</th>
<th>Number of positive cases</th>
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<td>13 – 18</td>
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Table VIII. Prevalence of *E. coli* infection according to sex

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<th>Sex</th>
<th>Number of patients</th>
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Table IX. Prevalence of *E. coli* infection according to education level of children’s mother’s

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<th>Education level</th>
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Table X. Result of antimicrobial sensitivity of 27 isolates of E.coli from diarrheal ch

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S = High sensitivity  M = Moderate sensitivity  R = Resistance

Note: The antibiotic abbreviations found in Table III.

Table XI. Overall sensitivity percentage of 27 isolates of *E. coli*
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Figure 2. Prevalence of *E. coli* infection according to age group
Figure (3). Prevalence of *E. coli* infection according to sex

![Bar chart showing prevalence of E. coli infection by sex.](image)

Figure 4. Prevalence of *E. coli* infection according to education level of children’s mothers

![Image of a Petri dish with bacterial colonies.](image)
Figure 5. A plate showing lactose-fermenting *E. coli* on MacConkey's agar

Figure 6. An example of API result of biochemical properties of isolated *E. coli*.
CHAPTER FOUR
DISCUSSION

Diarrhea, a condition that has a major impact on global health due not only to worldwide prevalence and health costs, but also it is regarded as a major cause of death in much of the world especially in developing nations, where the effect is greatest among the young people (WHO, 1984).

In the present study attempt was made to investigate the prevalence of *Escherichia coli* in diarrhea stools of children under two years of age at Kassala Hospital, Eastern Sudan. The infant patients suffering from diarrhea were brought to the hospital from Kassala town, outskirts and surrounding villages. This study showed isolation of *E. coli* from 27 (54%) out of fifty specimens. The specimens were cultured on MacConkey's lactose bile salt agar (Buxton and Frazer, 1977, Barrow and Feltham, 1993) and the *E. coli*-like isolates (lactose-fermenting Gram-negative rods) were identified by their microscopic, cultural and biochemical (using API biochemical kit) properties.

In the present study different types of bacteria (both Gram-negative and Gram-positive) were encountered, but *E. coli* was the most prevalent organism (54% of specimens and 78% of isolates) and with prevailing colonies on agar plates, so the association of *E. coli* with these cases is highly suspected to be causal agent of diarrhea. Other microorganisms are also suspected for contribution to this infant diarrhea especially those isolates of relatively significant counts. Also, parasitic, especially protozoal infection is suspected to be the cause of diarrhea in some cases. Twelve (24%) of the specimens in this study demonstrated either *Entamebea histolytica* (4 specimens), *Giardia lamblia* (5 specimens) or both *E.
*histolytica* and *G. lamblia* (3 specimens). Detection rate of *E. coli* in the present study is in partial agreement with that of Erwa (1975) and Elmuzzamal (2004), who reported that *E. coli* was the predominant bacteria associated with child diarrhea in Khartoum State and constituted 27% and 37%, respectively. The present result is also considered comparable to that of Ismail (1994), Mohammed (2005) who found a prevalence of 76.3%, 60%, respectively, for *E. coli* in diarrheal specimens taken from children. The high prevalence (54%) of *E. coli* in the current study and similar previous results put this species as the most important diarrhea-causing bacterial agent in children. Further, Hussein (2007) also found *E. coli* as the most dominant organism, isolated from 96 % of specimens investigated for aerobic bacterial association.

On the other hand, Sharaf (1986) and Elsadik (1998) reported that *Shigella* spp. were found as the most predominant bacteria associated with child diarrhea in Juba district and Khartoum State, respectively.

As the questionnaire used in this study revealed that the majority of infants were breast-fed, so it was unlikely to put the blame of infection on bottle feeding or bad use of utensils, yet it remains untested assumption that the high rates of diarrhea infection in these societies are attributed to poor community status and personal hygiene. Mothers were not educated enough to realize the dangers of not bothering to wash their hands and breasts properly prior to feeding their children. In this study the high prevalence of *E. coli* infection was inversely proportional with education level of mothers. Twenty one (42%) of the 50 children, their mothers were of just elementary education, while 15 (30%) their mothers were of higher school education and 14 (28%) their mother's were graduates. *E. coli* was isolated from 71% of cases belong to mothers of elementary education, 60% of cases belong to
mothers of higher school education and 21.4% of cases belong to graduate mothers. Bad food storage and handling and poor hygiene of water supply could also contribute unfavorably. This situation, if far from being uncommon, was reported by the WHO in 1984.

The current study found that the children aged 1 – 6 months were less affected by *E. coli* than children aged 7 – 18 months, while the infection is very low in the age of 19 – 24 months. The relatively lower infection rate in the youngest group of children may be because they only breast-fed, unlike other older groups which may also exposed to possible intake of contaminated food. Similar observations were reported by Hussein (2007), Park (1997), Elhassan (2002) and Elmuzzamil (2004).

Isolation rate of *E. coli* according to sex of children showed no difference.

Antibiotic sensitivity testing of the *E. coli* isolates showed that the isolates were 100% sensitive to Chloramphenicol, Amikacin and Ofloxacin. This finding was also found by Torres *et al.* (2001) and Banajeh *et al.* (2001). Norfloxacin and Tetracycline were intensively used for treatment of *E. coli* (Adach *et al.*, 2001) but here were found not effective. This resistance may be attributed to the misuse of these agents.
Conclusion and Recommendations

Conclusion
The prevalence of *E. coli* in diarrhea stool of children less than two years of age at Kassala Hospital was found to be 54% and it was the most dominant bacterial species.

Amikacin, Ofloxacin and Chloramphenicol were the most effective antimicrobial agents for *E. coli* isolates, while Tetracycline and Norfloxacin were found the least effective agents.

Recommendations

1. Due to the high incidence of child diarrhea due to *E. coli* found in the current and previous studies, appropriate control measures are critical to be implemented at all levels.
2. As susceptibility of *E. coli* to antimicrobial agents is expected to be unstable, antibiotic sensitivity test is recommended to be a routine practice, especially for cases unresponsive to treatment.
3. Further studies are recommended to examine a much larger numbers of children with diarrhea covering different populations.
4. Recommendation to use commercially available biochemical kits such as Analytical Profile Index (API) in clinical diagnosis which considered to be easy to perform, rapid, sensitive and cheap.
References


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