بسم الله الرحمن الرحيم

# Bacterial Diarrhoea In Infants With Special Reference To The Genus Escherichia In Khartoum State

**A Thesis Submitted By**


**Mohammed Yagoub Mohammed Elamir**

*"B.Sc. Of Microbiology, Omdurman Islamic University"*


**For The Master Degree Of Science In Pharmaceutical Microbiology**

**Under The Supervision Of**

**Dr. Elsheikh Ali Elobeid**


Department Of Pharmaceutics

Faculty Of Pharmacy

University Of Khartoum


**Khartoum**

**2005**

بسم الله الرحمن الرحيم

[وَ وَصَّينَا الإِنْسَانَ بِوَالِدَيهِ حَمَلَتهُ أُمُّهُ وَهناً عَلَى وَهنٍ وَ فِصَالُهُ فِي عَامَينْ أَنِ اشكُرْ لِي وَ لِوَالِدَيكَ اِلَيَّ المَصِيرُ]*

الآية {14} من سورة لقمان


*TO THE SOUL OF MY MOTHER.*

*To MY FATHER,*

*TO MY BROTHERS AND SISTERS,*
FOR THEIR KIND HELP AND SUPPORT.

# CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ACKNOWLEDGEMENTS

I thank Dr.Elsheikh Ali Elobaid for keen supervision, and without whom this piece of work would have never seen light. I, also, wish to acknowledge Dr. Elamin Ibrahim Elnima for the encouragement and support all through the period of this work. I am, particularly, grateful to Professor Suleiman Mohammed Elsanousi, for the wealth of information he provided and his tremendous encouragement. To Dr. Eljabalabi, who generously supplemented with most of the chemicals and culture media which were used in this study, I feel deeply indebted.

My special acknowledgements and deepest thanks are extended to Dr. Tayseer Mandour Elmahdi for her unlimited support during the period which has circumstantially elaborated since the very beginning up to the last moment of its curriculum and without whose aid it could have been impossible to carry out this work, so I feel entirely incapable of finding the expression of gratitude that corresponds. I can't forget the great assistance of Dr. Ali Elwakeel, without whom this work could not have seen light, I thank him deeply.

My great acknowledgements are further extended to colleague Manhal for her great assistance and support. I, also, thank all of my colleagues: Mamoun, Ashraf, Sara, Muatasim, Haitham M. Khair, Azza, Hind, and Haoly for the pleasant and favourable atmosphere they have created to push the work forth.

Also, I am heavily indebted to colleague Yomna for her technical aid, and I owe plentiful thanks to my colleagues Mohammed Elfatih Ornasseir and Mohammed A. Sattar for their great help.

My thanks are conveyed to the staff of the Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum, especially, the staff of Microbiology Laboratory for providing facilities and technical assistance. No less, I am indebted to all the staff of the Faculty of Basic Medical Sciences, Omdurman Islamic University for their great support.

Also I am very grateful to all the staff of the Faculty of Pharmacy, Omdurman Islamic University, and all the staff of Tropical Medicine Research Institute.

My thanks are extended to the Directors and staff working in the laboratories of Omdurman Paediatric Teaching Hospital, Ahmed Gasim Paediatric Hospital, Ibrahim Malik Hospital and Aba-Yazeed Hospital for the continuous assistance during collection of samples.

Special thanks are extended to my brother Khalid for his great assistance during printing of thesis.

Special gratitude is due to all my friends for their patience during the period of work. I wish to express my deep gratitude for all those who offered me hands through the course of this work.

**بسم الله الرحمن الرحيم**

# موجز الأطروحة


دلّت الدراسات على انتشار الاسهالات الحادة بين الأطفال الرضع كما أثبتت تنامي معدل مقاومة البكتريا المسببة لها للمضادات الحيوية خصوصاً في البلدان الأقل نموءاً.

عادةً تحدث عدوى الاسهالات البكتيرية في الأطفال الرضع كنتيجة حتمية لسوء المسلك الصحي للأفراد و المجتمعات.

و بناء عليه فقد هدفت هذه الدراسة الى:

عزل و تحديد أنواع البكتريا المسببة للاسهالات بين الأطفال الرضع في ولاية الخرطوم، السودان، و تحديد مدى حساسيتها للمضادات الحيوية مع التركيز على جنس الاشريكية.

من أجل هذه الأهداف تم تزريع عينات الفسحة باستعمال الأوساط الزرعية التفريقية و الانتقائية المناسبة مثل وسط أجار الماكونكي و وسط أجار الايوسين مع أزرق الميثيلين بطريقة الزرع الخطي، و من ثم أخذت العزلات البكتيرية النقية و أُخضِعت للاختبارات الكيموحيوية لتحديد هويتها، كما استخدم المضاد المصلي لتنميط سلالات الاشريكية القولونية.

في هذه الدراسة تم جمع 234 عزلة بكتيرية من أصل 200 طفل رضيع مصاب بالاسهال الحاد. تم اجراء سلسلة من الاختبارات لتنقية و تحديد هوية هذه العزلات، حيث تم التعرف على 234 عزلة سالبة لصبغ جرام، 141 منها عرفت على أنها الاشريكية القولونية و صنِفت السلالات الممرضة منها كما يلي: 34 منها على أنها الاشريكية القولونية الممرضة، 16 اشريكية قولونية مسممة، 12 اشريكية قولونية مدممة.

تم عزل الأنواع الممرضة من البكتريا و التعرف عليها بناء على الفحوصات المجهرية و التفاعلات الصبغية و الخصائص الاستنباتية و الاختبارات الكيموحيوية. لقد وجدت هذه السلالات كالآتي: الاشريكية القولونية (141)، المتقلبة (23)، الشغيلا (14)، السالمونيلا (13)، الكليبسيلا (13)، الزائفة الزنجارية (13)، الستروباكتر (12)، المورقانيلا (4)، و واحدة من المعوية.

اشتمل هذا البحث على تحديد مستوى حساسية هذه السلالات تجاه عدة أنواع من المضادات الحيوية. أوضحت الدراسة وجود مقاومة عالية و متعددة للمضادات الحيوية المستعملة.

## ABSTRACT

The principal objective of this research was to determine the causative microorganisms of infantile diarrhoea, focusing mainly on *Escherichia* species, and making comparative study of rest of bacterial pathogens associated with diarrhoea, as far as their identification and their reaction to different antimicrobial agents of common use.

The present work methodology included the isolation, purification, identification and antibiotic sensitivity testing of bacteria which were isolated from infants suffering diarrhoea in different parts of Khartoum State, Sudan. The method adopted for native isolation and identification purposes was the culturing of faeces in differential and selective culture media such as MacConkey's agar and Eosin Methylene Blue agar using streaking method. The purified isolates were, then, subjected for biochemical activity tests and serotyping.

The results obtained revealed that, 234 isolates were collected from the 200 stool samples from infants, which were subjected to a series of purification and identification experiments. All the 234 isolates were Gram-negative rods in shape, and alone *E. coli* constituted 141 of all the identified isolates. Of these isolates 34 were classified, using serotyping, as enteropathogenic *E. coli*, 16 as Enterotoxigenic *E. coli* and 12 as Enterohaemorrhagic *E. coli*. The yield of other pathogens was, also, detected and subjected to identification tests. The tests revealed 23 isolates of *Proteus*, 14 isolates of *Shigella* spp., and 13 isolates of each of *Salmonella*, *Klebsiella*, *Pseudomonas*. Also *Citrobacter* was

represented in 12 samples in this study. *Morganella* was isolated from 4 samples.

Using the disc diffusion technique the clinical strains of *E. coli* (141), *Proteus mirabilis* (23), *Shigella* (14), *Salmonella* (13), *Klebsiella pneumoniae* (13), *Pseudomonas* aeruginosa (13), *Citrobacter freundii* (12), *Morganella morganii* (4), *Enterobacter* (1) were subjected to susceptibility tests to different antibiotics. The results of these tests indicated that many strains exhibited multiple drug resistance.

On reviewing the contemporary and current literature in this domain of research, a massive widespread of acute infantile diarrhoea was observed especially in less developed countries, where a high resistance of the causative pathogens to antibiotics was evident. It was, also, shown that the bacterial infantile diarrhoea infections usually occur as one product of poor personal and community hygiene.

# 1. LITERATURE REVIEW

## 1.1. INFECTION:

### 1.1.1. DEFINITION:

The term infection is derived from the Latin *inficer*, meaning "to put or dip into, to stain." It implies an interaction between two living things, the host and the parasite, with a competition for superiority. If the parasite is successful, disease results; if the host is successful, disease does not occur and immunity or increased resistance may develop. In the most exact sense, infection implies the presence of microorganisms in or on the body of the host, (Pelczar *et al*., 1982).

Infection is the mechanism through which microbial agents reach their potential victims and elicit pathogenic reaction, (Dubos, 1958). The term means the multiplication of an infectious agent within the body. The multiplication of the bacteria that are part of the normal flora of the gastrointestinal tract, skin, etc, is generally not considered an infection. On the other hand, the multiplication of pathogenic bacteria, (e.g. *Salmonella* species) – even if the person is asymptomatic – is deemed an infection, (Brooks *et al.*, 1998).

The pathogenesis of bacterial infection includes the initiation of the infectious process and the mechanisms that lead to the development of signs and symptoms of disease. Characteristics of bacteria that are pathogens include transmissibility, adherence to host cell, invasion of host cells and tissues, toxigenicity, and ability to evade the host's immune system. Many infections

caused by bacteria, which are commonly considered to be pathogens, are unapparent or asymptomatic. Disease occurs if the bacteria or immunological reactions to their presence cause sufficient harm to the person, (Brooks *et al.*, 1998).

### 1.1.2. GASTROENTERITIS:

#### 1.1.2.1. INTRODUCTION:

Gastroenteritis is the inflammation of the stomach and intestine. It is used to refer to a group of diarrhoeal diseases characterized by vomiting, abdominal pain, diarrhoea and in severe cases prostration, (Bradley *et al*., 1980).

Acute diarrhoea is an extremely wide spread disorder, particularly serious in the young and old, affecting hundreds of millions of people every year. Among infants and children, it is responsible for more deaths than any other cause in the world, (W.H.O., 1982).

Acute diarrhoea is a symptom, not a disease in itself, of any condition that affects the integrity of the intestines. Certain chemical agents, foodstuffs and other diseases can also cause diarrhoea. Infection usually comes from contaminated food or water that may contain more than one type of diarrhoeagenic organisms. The contamination consists of excreta from human or animal already infected, though not necessarily suffering from diarrhoea. Where hygiene is poor, diarrhoeal infections, therefore, tend to circulate perpetually within the local population, having their most serious effects on those with least resistance: the elderly, the sick, the malnourished, visitors from other areas and, above all, infants and children, (W.H.O., 1982).

Acute diarrhoea is still a leading cause of infantile death in less-developed countries and the most important symptom associated with malnutrition and bad hygiene practice. The first study of worldwide morbidity and mortality from diarrhoeal diseases, based on population estimate in 1980, showed that there

were 744-1000 million episodes of diarrhoea and 4-6 million deaths each year in that decade from diarrhoeal disease in children younger than five years in Africa, Asia, and Latin America, (Isabel and Affonso, 2001).

Though undiagnosed, *Escherichia coli* still remains an important cause of diarrhoeal diseases especially in children in developing countries. On the base of distinct virulence properties and symptoms, diarrhoeagenic *Escherichia coli* have been classified in to five distinct types: Enteropathogenic *Escherichia coli*, (EPEC), Enterotoxigenic *Escherichia coli*, (ETEC), Enteroinvasive *Escherichia coli*, (EIEC), Enterohaemorrhagic *Escherichia coli*, (EHEC) or Verocyto-toxin-producing *Escherichia coli*, (VTEC), and Enteroaggregative *Escherichia coli*, (EAggEC), (Dutta S. *et al*., 2002 ).

A remarkable epidemiologic difference between typical and atypical (EPEC) serotypes is their geographic distribution. Typical (EPEC) serotypes have traditionally been associated with outbreaks of infantile diarrhoea, (Trabulsi, *et al.*, 2002).

In developing countries Enterotoxigenic *Escherichia coli* that produce heat stable toxin Shiga Toxin, ($ST_a$) may be responsible for 50% - 80% of the reported cases of diarrhoea. Heat stable toxins are also a major cause of diarrhoea in laboratory and domestic animals, (Chakrabarti, *et al*., 2002).

Some of *Escherichia* species other than *E. coli* are also suspected to be causative agents of diarrhoea such as *Escherichia blatae*, which was isolated from the intestinal tract of cockroaches, yet it was not reported in clinical material, (Barrow and Feltham, 1999). *Escherichia fergusonii* has been isolated from animals and humans clinical material, (Barrow and Feltham, 1999).

*Escherichia coli* that cause diarrhoea are extremely common worldwide. These *E. coli* are classified by the characteristics of their virulence properties, and each group causes disease by a different mechanism, (Brooks *et al* 1998).

### 1.1.2.2. ENDOGENOUS TRANSMISSION OF DIARRHOEA:

At birth the intestine is sterile, but organisms are soon introduced with food. In breast–fed infants, the intestine contains large numbers of lactic acid *Streptococci* and *lactobacilli*. These aerobic and anaerobic organisms, (e.g. *Bifidobacterium* species produce acid from carbohydrates and tolerate the pH value 0f 5.0. In bottle–fed infants, a more mixed flora exists in the bowel, and *lactobacilli* are less prominent, (Brooks *et al*., 1998).

The upper small intestine of the normal infant, as in the adult, may harbour some Gram–positive flora washed down from the oropharynx, (Donaldson, 1964; Gorbach, 1971).

Intestinal bacteria have traditionally been considered to induce disease when they invade and alter the intestinal epithelial cells or when they produce local toxins.

However, there may be no "pathogenic" microorganisms found in the stools of a considerable number of symptomatic patients, even in areas where diarrhoea is highly endemic, (Gordon *et al.*, 1964).

Diarrhoeal disease may follow contamination of the small intestine by common inhabitants of the gut, (Sharpe, 1962; Weil *et al.*, 1966; Brooks *et al.*, 1998).

### 1.1.2.3. IMMUNITY:

Normal and abnormal bacteria of the digestive tract have to be viewed in relation to immune mechanisms of the host in defence against enteric infections.

The infective dose is a most important factor in determining whether disease is induced. Gastrointestinal microorganisms must multiply before infection, and may be pathogenic only in large numbers. Even the classic enteropathogens may not infect if the dose is small. For example, the minimal infective dose for

*Shigella* is $10^1$; *Salmonella* $10^5$; E. *coli* $10^8$, and *Vibrio cholerae* $10^8$, (Gordon, 1971; Dupont, 1974).

Non-specific immune host factors such as age and nutritional status are of considerable importance. Newborn infants seem more susceptible to develop enteric infections. Diarrhoeal disease is a particularly important problem in malnourished children, (Gordon, 1971). They suffer an increased incidence and severity of diarrhoea and this disease may cause or contribute greatly to the aggravation of their nutritional status. The prevalent poor sanitary conditions in developing countries may expose the child to excess of bacteria, while the marginal nutritional status decreases the host capacity to respond to enteric infection.

Local gastrointestinal defenses are probably of greatest consequence in the induction of diarrhoea. Microorganisms must be swallowed before infection, but may never reach the small bowel as viable bacteria because of susceptibility to gastric acidity. There is an increased incidence and seriousness of Salmonellosis and cholera infections under conditions of reduced gastric acidity, (Waddell and Kunz, 1956; Gianella *et al.*, 1971). The increased rate of diarrhoeal disease in malnourished children may also be related to the altered gastric acidity.

In addition, the cleansing role of intestinal motility also plays a role in determining pathogenicity and in insuring the absence of bacteria in the upper segments of the bowel of normal infants, (Waddell and Kunz, 1956; Gianella *et al.*, 1971).

Diarrhoea, itself, may, thus, be a protective mechanism, limiting the time of contact between the infecting organism and the intestinal epithelium. Interference with diarrhoea by pharmacological agents, frequently employed in its treatment, may cause a more severe disease by increasing the time of

exposure of the intestinal epithelial cells to the infective microorganism, (Dupont and Hornick, 1973).

Other important factors are intestinal antibodies, (secretory IgA) that may prevent adherence of the organism to mucosa, (Freter *et al.*, 1963), or may interfere with the multiplication of the ingested organisms, (Dupont *et al.*, 1972) and the bacterial flora itself that seems to have a strong homeostatic influence on potential pathogenic organisms, either by competition for space and nutrients or by elaborating antibacterial catabolites, (Freter, 1974; Wolin, 1974).

**1.1.2.4. ROUTE OF INFECTION:**

In addition to the type and total concentration of microorganisms capable of inducing enteric disease, the flora must be considered in terms of localization. Microorganisms must contaminate the small bowel to induce disease, (Young *et al.*, 1960; Sharpe, 1962). This was confirmed by studies in which bacteria, instilled in the colon, did not produce disease but when the small intestine was infected, diarrhoea did occur, (Young *et al.*, 1960).

Enteric infection usually follows oral ingestion of the microorganism with rapid proliferation and colonization of the small intestine. However, it may also follow a retrograde spread proximally into the small intestine by established resident strains in the colon, or perhaps from systemic spread as seen in children with infection in distal areas. In *Shigella* infections, there is proliferation in the lumen of the small bowel but mucosal penetration by these bacteria occurs selectively in the colon, (Alekseen *et al.*, 1960; Takeuchi *et al.*, 1966).

Certain invasive strains of *E. coli* can cause a disease indistinguishable from shigellosis, (Dupont *et al.*, 1971), while others induce diarrhoea by proliferation in the lumen of small intestine with release of a toxin as in cholera.

**1.1.2.5. MECHANISM OF DIARRHOEA:**

Diarrhoea occurs when there is sudden increase in volume of stools primarily due to excessive water content of faeces. This results from alterations in the transport of fluid and electrolytes at the intestinal level. Therefore, enteric pathogenicity of microbial population should be defined by the capacity to induce such abnormalities. Microorganisms or their metabolic by-products must interfere with the net movement of water across intestinal cell membranes to induce water loss and diarrhoea. This may be achieved either by increasing secretion, or by inhibiting absorption of actively transported solutes principally sodium and glucose to which water transport is linked, (Curran and Schultz, 1968).

Under normal circumstances, the fluxes of water across the small intestine are very huge, (Davenport, 1971). However, the conservation of water by the intestine is so efficient that only a small amount is excreted in the faeces. Therefore, a slight change in either absorption or secretion of any solute can cause a very marked change in the net intestinal flow of water.

Consequently, even relatively minor alteration of solute fluxes induced by gastrointestinal flora may significantly alter the delicate balance of water movement in the intestine. Diarrhoea will result when there is excess in the volume of fluid delivered to the colon from the upper segments of the small intestine, which surpasses the reabsorptive ability of the large intestine, (Davenport, 1971).

Binding of enteropathogenic *E. coli* to the brush border mucosa triggers a cascade of trans-membrane and intracellular signals, causing cytoskeletal reorganization and formation of a specific lesion, termed the attaching and effacing lesion. Several enteropathogenic *E. coli* gene products have been implicated in formation of attaching and effacing lesions, (Loureiro *et al*., 1998).

**1.1.2.6. TYPS OF MICROBIAL DIARRHOEA:**

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

(a) The classic pathogenicity is by invasion, penetration and disruption of intestinal epithelium.

(b) A toxin produced by the microorganism stimulates the toxigenic mechanism where the mucosal integrity is preserved but water and electrolytes are secreted.

(c) The disease is caused by injury of the intestinal epithelium by a variety of substances. These are generated by the metabolic activity of bacteria on food stuffs and / or host secretions.

**1.1.2.6.1. INVASIVE DIARRHOEA:**

Bacillary dysentery is the classic model of pathogenicity resulting from invasion and penetration of intestinal mucosa leading to mucosal inflammation and epithelial cell disruption. Pathogens such as Shigella and invasive strains of *E. coli* have a similar mechanism, (Dupont *et al.*, 1971).

Although *Salmonella*, also, invades the epithelium, it does not induce an extensive destruction of the mucosa. In such cases, the epithelial lining is left intact and organisms reach the *laminapropria*, where an inflammatory response is elicited, (Alexeen *et al.*, 1960). The reverse can be seen in amoebiasis. The protozoan invades and destroys the intestinal mucosa but produces a minimal inflammatory reaction.

In invasive diarrhoea, the intestinal alterations in water transport may vary. Experimental infection with *Salmonella typhimurium* impaired the absorptive transport capacity of the jejunum–caecum and colon and when diarrhoea occurred, there was also a marked secretion of sodium and water by the ileum as is seen in toxigenic diarrhoea, (Powell *et al.*, 1971).

On the other hand, in experimental *Shigella flexneri* infection in monkeys, there was an intestinal transport defect with net jejunal and colonic secretion of water and electrolytes. Ileac electrolytes transport was normal. Unlike the toxigenic diarrhoeas, dysentery results from a colonial transport defect, and the diarrhoea is secondary to jejunal secretions, (Rout *et al.*, 1975).

However, viral infections were demonstrated to be the most important etiological agent in gastroenteritis in children, (Agus *et al.*, *1973*). Over 70% of diarrhoeal patients were shown to have a virus detected by electron microscopy. Several viruses that differ morphologically and antigenically have been described by Flewett *et al.*, (1974) and Davidson *et al.*, (1975). They have been referred to as Reo virus, Arbo virus, Corona virus and Rotavirus. They appear to have a world–wide distribution and principally affect infants.

The intestinal epithelial cells were penetrated by the viral pathogens and induced mucosal inflammation, villus shortening, crypt hypertrophy, increased epithelial cell mitosis, dilatation of endoplasmic reticulum, and an increase in intracellular multivesiculate bodies, (Agus *et al.*, 1973; Davidson *et al.*, 1975). Brush border enzyme activities were also decreased. On the other hand, the colonic mucosa was spared in this syndrome. Viruses were also able to evoke intestinal water secretion by a mechanism which may be different from that induced by toxins, (McClung *et al.*, 1976).

However, it was postulated that lactase is the receptor and uncoated enzyme for infantile enteritis, (rota viruses), (Holmes *et al.*, 1976). This hypothesis is consistent with the observation that rota virus seems to infect only gut epithelium rich in lactase activity and may thus explain the high incidence of lactose intolerance in gastroenteritis, (Lifshitz *et al.*, 1971). Infants who have high lactase activity are more susceptible to the virus, and in lactose–deficient individuals, the incidence of infection with these viruses may be decreased.

### 1.1.2.6.2. TOXOGENIC DIARRHOEA:

The second group of pathogenic mechanisms inducing diarrhoea has been extensively reviewed, (Banwell and Sherr, 1973). It may be implicated in many diarrhoeal disorders in children in addition to classic prototype cholera. It should be pointed out that toxigenicity and invasiveness of bacteria are genetically transmissible factors, which may facilitate the penetration of the host epithelium, by the organisms. *E. coli* may express both enterotoxic and invasive properties in different strains and even in the same strain, (Dupont *et al.*, 1971).

The toxigenic strains of *E. coli* have been frequently implicated as a cause of traveller's diarrhoea. Up to 72% of Turista episodes in Mexico were associated with heat labile toxins of *E. coli* isolated from stools of American students with diarrhoea, (Gorbach *et al.*, 1975). However, healthy individuals who had no diarrhoea also had these strains detected in 15% of stool specimens.

*Clostridium perfringens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and even some *Shigella* and *Salmonella* strains have also been found to produce enterotoxins, (Banwell and Sherr, 1973). In toxigenic diarrhoea, the organism multiplies in the lumen or on the surface of the epithelium of the small intestine and exerts its pathogenic effects by producing a toxin. In Staphylococcal food poisoning, ingestion of preformed toxin may play a role, but this does not seem too important in cholera or *E. coli* diseases.

Enterotoxins cause the small intestinal epithelial cells to secrete electrolytes and water while the colon is usually impervious to its action. Diarrhoea results from small intestinal secretion of large quantities of water and electrolytes that are not compensated for by the colonial reabsorptive capacity. Enteric toxins may produce disease by a process of activation of intestinal secretary processes that have been associated with the activation of intracellular cyclic adenosine monophosphate system, (Banwell and Sherr, 1973).

### 1.1.2.6.3. DIARRHOEA CAUSED BY INJURIOUS FACTORS:

Such bacterial metabolic activity generates a variety of substances, which can be injurious to the intestine, i.e., deconjugated bile salts, (Midtvedt, 1974), hydroxy fatty acids, (Binder, 1973), or alcohol and short chain organic acids, (Klipstein *et al.*, 1973). These substances may induce ultra structural and functional abnormalities of the intestine, which may lead to diarrhoea.

### 1.1.2.6.4. DIARRHOEA CAUSED BY BACTERIAL OVERGROWTH:

A wide spectrum of enteric microorganisms may alter intestinal functions, when present, in excessive number, in the small intestine, (Gracey *et al.*, 1975).

Regardless of the mechanism inducing diarrhoea, there is a frequent proliferation of non-specific faecal and colonial bacteria in the upper segments of the small intestine of many patients, (Burke and Anderson, 1966; Lifshitz *et al.*, 1970). These increased enteric microbial populations may be directly responsible for the diarrhoea. However, in the majority of instances it appears to be a secondary alteration that may result from the disease process, (Coello-Ramirzer and Lifshitz, 1972).

Among the factors that may influence enteric bacterial dissemination and proliferation in the upper bowel segments, in diarrhoea, are the disturbed – motility, the presence of free carbohydrates in the lumen, (Coello-Ramirzer and Lifshitz, 1972) and / or presence of plasmids in bacteria conferring to them, pathogenic properties, (Wolin, 1974) and metabolic interactions among intestinal microorganisms, (Freter, 1974; Wolin, 1974).

### 1.1.2.7. THE CAUSATIVE AGENTS OF INFANTILE DIARRHOEA:

### 1.1.2.7.1. INTRODUCTION:

Enteropathogenic *E. coli*, (EPEC) is a leading cause of infantile diarrhoea in developing countries. In industrialized countries, the frequency of these

organisms has decreased, but they continue to be an important cause of diarrhoea, (Nataro *et al.*, 1998).

In a study carried out in Saudi Arabia among children who are suffering from diarrhoea *E. coli* was detected in 13% of the cases, (El – Sheikh *et al.*, 2001).

In a study about microorganisms associated with infantile diarrhoea in a group of 256 children admitted to a public paediatric hospital in Montevideo, Uruguay, diagnostic procedures were updated to optimize detection of potential pathogens, which were found in 63.8% of cases, and to be able to define their characteristics down to molecular or antigenic type. Co-infection with two or more agents was detected in more than one–third of positive studies. *Escherichia coli* enteric virotypes, especially enteropathogenic *E. coli*, (EPEC) were shown to be prevalent. *Rotavirus*, *Cryptosporidium*, *Campylobacter*, (mainly *Campylobacter jejuni*), and *Shigella flexneri* were also often identified. Enterotoxigenic *E. coli*, *Salmonella*, and *Giardia lamblia* were sporadically recognized. Unusual findings included two enteroinvasive *E. coli* strains, one *Shigella dysenteriae* 2 isolates, and a non–O:1 *Vibrio cholerae* culture., (Torres, *et al.*, 2001).

Some enteric organisms, e.g. *E. coli*, are part of normal flora and incidentally cause disease, while others, the *Salmonellae* and *Shigellae*, are regularly pathogenic for humans, (Farmer, 1995; Brooks *et al.*, 1998).

**1.1.2.7.2. ESCHERICHIA:**

According to Ismail, (1994) the attempts to implicate certain organisms in infantile enteritis were started by Escherich, who first isolated *E. coli* from the stool of infants with diarrhoea in 1885, (Wilson and Miles, 1964).

In late 1940 serious outbreaks of infantile diarrhoea occurred in London and Aberdeen. The study showed that certain serological types of *E. coli* were responsible, (Cruickshank *et al.*, 1975).

The incidence in developing countries of enterotoxigenic *E. coli* diarrhoeas was highest in the children below the age of two years, (infants). This declined rapidly by the age of four years, (WHO, 1981).

Enteropathogenic *E. coli* produce no toxins but they colonize the duodenum, jejunum and ileum as has been demonstrated in post mortem, (WHO,1980).

Enteroinvasive *E. coli* have an invasive character similar to that of *Shigella* species. Laboratory studies using tissue culture and experimental animals including the rabbit ileal loop showed that enteroinvasive *E. coli* produce no enterotoxin. They invade the epithelium and are able to produce kerato–conjunctivitis in the pig's eye, (Sereny's test), a test used to demonstrate enteroinvasive property, (WHO, 1980).

**1.1.2.7.2.1. DEFINITION:**

*Escherichia* originally described and named by Theodore Escherich in 1885 *bacterii commune*, later renamed *Escherichia coli*, (Poul, *et al.*, 2001).

*Escherichia* is the 'Type genus' for the family Enterobacteriaceae and *Escherichia coli* is the type species for the genus. *E. coli* is possibly the most studied as well as one of the more important bacterial pathogens. In addition, the genus includes the less important species: *E. adecarboxylata*, *E. blattae*, *E. fergusonii*, *E. hermanii* and *E .vulneris*, (Barrow & Feltham, 1999).

**1.1.2.7.2.2. CHARACTERISTICS OF THE FAMILY ENTEROBACTERIACEAE:**

This family has the following characters:

They are Gram–negative rods, either motile with peritrichous flagella or non motile, they grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on MacConkey's agar, grow aerobically and anaerobically, (are facultative anaerobes); ferment rather than oxidize glucose, often with gas production; are catalase–positive, oxidase–negative, and reduce nitrate to nitrite; and have a 39 – 59 G+C DNA content, (Brooks *et al.*, 1998).

**1.1.2.7.2.3. CLASSIFICATION:**

According to Bergey's manual, the genus Escherichia belongs to the family Enterobacteriaceae and this family includes also the following genera: *Buttiauxella*, *Cedecea*, *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, *Tatumella*, *Yersinia* and others, (Barrow & Feltham, 1999).

**1.1.2.7.2.3.1. ESCHERICHIA SPECIES:**

**(i) *E. blattae***:

This species was first described by Burgess, McDermott & Whiting, (1973); among bacteria isolated from the gut of the cockroach. It differs *from E. coli* both in oxidizing gluconate and fermenting malonate, as well as in failing to form indole, acidify mannitol or sorbitol, or produce β–galactosidase. It would probably be better placed in another genus. The species has not been reported from human clinical specimens, (Barrow & Feltham, 1999; David Green Wood, 2002).

**(ii) *E. fergusonii*:**

*E. fergusonii* has been isolated from animal and human clinical material, as proposed by Farmer *et al.*, (1985), to include the group of motile, non–lactose fermenting strains which are indole and lysine decarboxylase–positive. They differ from *E. coli* in their ability to ferment cellobiose, (Farmer *et al.*, 1985).

**(iii) *E. hermanii***:

***E. hermanii*** isolated particularly from wounds, were described by Brenner *et al.*, (1982) to include motile Indole-positive strains which ferment cellobiose, grow in KCN and may produce a yellow pigment on suitable media; lactose fermentation and lysine decarboxylase production vary from strain to strain, (Barrow & Feltham, 1999).

**(iv) *E. vulneris***:

It was also described by Brenner *et al.*, (1982) as a new species to include a group of strains many of which were isolated from human wounds. They are motile, indole–negative, lysine decarboxylase–positive and ferment cellobiose; lactose fermentation is either delayed or absent.

**(v) *E. adecarboxylata***:

The species was described by Leclerc, (1962). Strains are indole–positive, lysine decarboxylase–negative and usually produce a yellow pigment. The species has received little attention but strains are occasionally isolated from clinical specimens. Ewing and Fife in 1972 included *E.adecarboxylata* in *Enterobacter agglomerans*, (= *Erwinia herbicola*); the species is certainly misplaced in *Escherichia* and a new genus *Leclercia* has been proposed to accommodate it, (Tamura *et al.*, 1986).

**(vi) *E. coli***:

It is found in the intestine of man and animals also can be found in the intestines of birds and fish, and in soil, which is contaminated by animal faeces, (Omer, 1990).

Strains of *E. coli* are usually motile by means of peritrichous flagella, (Omer, 1990) and some especially those from extra–intestinal infections, may

produce a polysaccharide capsule, (Green Wood, 2002). They grow well on non–selective media, aerobic and facultative anaerobic, catalase – positive, oxidase–negative, attacks sugars fermentatively; gas normally produced and citrate–negative; KCN–negative. It ferments mannitol, usually with the production of gas. Most strains acidify lactose promptly and form gas from it at 37˚c and at 44˚c. Adonitol and inositol are seldom fermented. Indole usually produced from tryptophan at 37˚c and at 44˚c gives M.R reaction, and negative V-P reaction. Most strains are urease–negative and none deaminate phenylalanine. They do not produce $H_2S$ and do not oxidize Gluconate, (Omer, 1990).

Certain strains are haemolytic when grown on media containing suitable erythrocytes, (Green Wood, 2002).

*E. coli* has possessed lysine and glutamic acid decarboxylase. It does not liquefy gelatine. It is an intestinal parasite of man and animals, causing both suppurative and diarrhoeal diseases. GC content of DNA ranges between 50 – 52 moles %, (Omer, 1990).

**1.1.2.7.2.3.2. SEROTYPING OF *E. coli*:**

Serotyping is based on the distribution of Lipopolysaccharide, (LPS) or somatic, (O) antigens, and flagellar, (H) and capsular, (K) antigens, as detected in agglutination assays with specific rabbit antibodies, (Green Wood, 2002).

**1.1.2.7.2.3.2.1. (O) ANTIGENS:**

Over 170 different O antigens have been recognized, (Green Wood, 2002).

O antigens are the most external part of the cell wall lipopolysaccharide and consist of repeating units of polysaccharide. Some O–specific polysaccharides contain unique sugars. O antigens are resistant to heat and

alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM, (Brooks *et al.*, 1998).

Serotyping may detect cross reaction, because of shared epitopes on the LPS expressed by strains of *E. coli* and organisms belonging to the genera: *Brucella*, *Citrobacter*, *Providencia*, *Salmonella*, *Shigella and Yersinia*, (Green Wood, 2002).

Occasionally, O antigens may be associated with specific human diseases, e.g. specific O types of *E. coli* are found in diarrhoea and in urinary tract infections, (Brooks *et al.*, 1998).

**1.1.2.7.2.3.2.2. (H) ANTIGENS:**

H antigens are located on flagella and are denatured or removed by heat or alcohol. Treating motile bacteria with formalin preserves them. Such H antigens agglutinate with anti–H antibodies, mainly IgG. The determinants in H antigens are a function of the amino acid sequence in flagellar protein, (flagellin), (Brooks *et al.*, 1998).Within a single serotype, flagellar antigens may be present in either or both of two forms, called phase 1, (conventionally designated by lowercase letters), and phase 2, (conventionally designated by Arabic numerals), (Brooks *et al.*, 1998). More than 50 H antigens have been identified. Most are monophasic but rare diphasic strains have been reported, (Green Wood, 2002). There are only a few significant cross–reactions between them and with the H antigens of other members of the *Enterobacteriaceae*, (Green Wood, 2002). The organism tends to change from one phase to the other; this is called variation. H antigens on the bacterial surface may interfere with agglutination by anti–O antibodies.

**1.1.2.7.2.3.2.3. (K) ANTIGENS:**

The term ‘K antigen’ was first used collectively for surface or capsular antigens that prevent flagellar – specific antibodies from binding to the somatic

antigens. In the past these antigens were divided into three classes, (L, A and B) according to the effect of heat on the agglutinability, antigenicity and antibody–binding power of bacterial strains that expressed them.

Recently 'K antigen' refers to the acidic polysaccharide capsular antigen, and those of *E. coli* may divided into two groups, (group I and II) that largely correspond to the former A and L antigens, (Green Wood, 2002).

K antigens are external to O antigens on some but not all Enterobacteriaceae. Some are polysaccharides, including the K antigens of *E. coli*; others are proteins. K antigens may interfere with agglutination by O antisera. Also, they may be associated with virulence, (e.g. *E. coli* strains producing K1 antigen are prominent in neonatal meningitis, and K antigens of *E. coli* cause attachment of the bacteria to epithelial cells prior to gastrointestinal or urinary tract invasion, ( Brooks *et al.*, 1998).

**1.1.2.7.2.3.2.4. FIMBERIAL ANTIGENS:**

Strains of *E. coli* exhibit fimbriae and strains may occur in both sex pili and more than one type of fimbrial structure. With a given culture, there may exist individual cells fimbriae and others with none, and there is reversible variation between the fimbriated and the non–fimbriated phase, (Green Wood, 2002).

Type 1 fimbriae can mediate adhesion to a wide range of human and animal cells that contain the sugar mannose. Such adhesion might be involved in pathogenicity.

Filamentous protein structure resembling fimbriae cause mannose–resistant haemagglutination, and there is good evidence to suggest that they play an important part in the pathogenesis of diarrhoeal disease and in urinary tract infection. They include the K88 antigen found in strains causing enteritis of pigs, the K99 antigen found in strains causing enteritis of calves and lambs,

and the Colonization Factor Antigens, (CFAs) expressed by enterotoxigenic *E. coli*, (ETEC) causing diarrhoeal disease in human, (Green Wood, 2002).

**1.1.2.7.2.3.3. TOXINS AND ENZYMES:**

Like most Gram–negative bacteria, *E. coli* possess complex lipopolysaccharides in their cell walls. These substances, endotoxins, have a variety of pathophysiologic effects, (Brooks *et al.*, 1998).

As general the lipopolysaccharides , (LPS, endotoxin) of Gram–negative bacteria are divided from cell walls and are often liberated when the bacteria lyses. The substances are heat–stable, have molecular weights between 3000 and 5000 (Lipooligosaccharides, LOS) and several million, (Lipopolysaccharides, LPS), and can be extracted, (e.g. with phenol–water ), (Brooks *et al.*, 1998).

**1.1.2.7.2.3.3.1. COLICIN, (BACTERIOCIN):**

Many Gram–negative organisms produce bacteriocins. These virus–like bactericidal substances are produced by certain strains of bacteria active against some other strains of the same or closely related species. Their production is controlled by plasmid. Colicins are produced by *E. coli*, marcescins by *Serratia*, and pyocins by *Pseudomonas*. Bacteriocin producing strains are resistant to their own bacteriocin, thus bacteriocins can be used for "typing" of organisms, (Brooks *et al.*, 1998).

**1.1.2.7.3. OTHER CAUSATIVE AGENTS OF DIARRHOEA:**

**1.1.2.7.3.1. *Vibrio* species:**

*Vibrio* species and related organisms are responsible for diarrhoeal diseases particularly *Vibrio cholerae* O–group 1(epidemic strain), (WHO, 1980).

Non-O1 *Vibrio cholerae* strain has been isolated from children suffering from diarrhoea in Uruguay, (Torres *et al.*, 2001).

Eight new countries were affected in 1978, and these with several previously affected countries in Asia are causing major problems for the national health authorities, (WHO, 1980).

Cholera mortality was reduced to less than 1% in well-equipped countries, (WHO, 1980). Sporadic cases of *Vibrio eltor* were reported in Alabama, (USA) in April 1977. In May 1977 an epidemic occurred in Bangladesh. Cholera vibrios are reported in sewage water in Brazil in 1978, and later in Louisiana, (USA) in 1979. Eleven other sporadic cases were reported due to consumption of shellfish. Finally workers in USA have reported 270 strains of *Vibrio cholerae* – O 1 Eltor isolated over eleven years from various sources, (WHO, 1980).

In endemic areas, the severe clinical picture occurs with greatest frequencies amongst children, (Bradley *et al.*, 1980).

**1.1.2.7.3.2. *Campylobacter* species*:***

*Campylobacter fetus* subspecies *jejuni* has emerged in the last few years as an important causative agent of acute diarrhoea in children. According to Skirrow and Rogers, (1971) *Campylobacter* species had been shown to be a relatively common cause of diarrhoea in Belgium. In the Netherlands, North America, Canada, Sweden and United Kingdom, this organism was isolated in 5 – 14 % of diarrhoea cases and 1% of asymptomatic cases, (WHO, 1982).

*Campylobacter jejuni* has been isolated in 1.5% of cases studied in Jordan to detect bacterial, viral, and parasitic enteric pathogens associated with diarrhoea in children, (Youssef *et al*., 2000).

**1.1.2.7.3.3.*Yersinia enterocolitica*:**

One of the latest recognized causes of enteric infection in many parts of the world is *Yersinia enterocolitica*. According to WHO, (1978), in a large study in Sweden, the organism was isolated from two percent of cases studied. Similar

results, (1 – 3 %) were reported from Belgium, Canada and Federal Republic of Germany, (WHO, 1978).

*Yersinia enterocolitica* was detected in 0.4% of positive diarrhoeal cases studied in Jordan among hospitalized children, (Youssef *et al*., 2000).

**1.1.2.7.3.4. *Salmonella* species:**

The genus *Salmonella* now comprises about more than 2000 serotypes that can infect a wide range of cold–blooded animals as well as warm–blooded animals, (Cruickshank *et al.*, 1975).

These infections may be asymptomatic but in case of disease two patterns are recognized, one is manifested as septicaemia caused by the *S. typhi* group and the other as a form of enteritis caused by a wide range of serotypes.

Two million infections with *Salmonella* occurred in the United States yearly, of which 500,000 required hospitalization, (Giannella, 1986). In England and Wales, a similar extrapolation of laboratory confirmed data would give an estimated 200,000 human infections yearly, (WHO, 1981).

Studies carried out by the WHO advisory team, in seven developing countries in 1960 – 1965, had confirmed that salmonellosis has a world – wide distribution, (WHO, 1981). Its highest incidence is in the first year of life, and more particularly in the early months.

The relative frequency of salmonellosis varies from one country to the other according to such factors as community habits, hygienic standards of food preparation, service establishments, animal husbandry and intensive veering system. In USA, 40% of food poisoning was due to salmonellosis while this figure goes to 80% in UK. The source of infection was contaminated animal foods, (WHO, 1980).

*Salmonella* species was presented in stool of 4.5% of children suffering from diarrhoea in a study carried out in Jordan, (Youssef *et al*., 2000).

**1.1.2.7.3.5. *Shigella* species:**

The genus *Shigella* is subdivided into four subgenera according to biochemical reactions, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnie.* The first three are further subdivided by serotyping into ten serotypes of *Shigella dysenteriae*, eight serotypes of *Shigella flexneri*, and fifteen serotypes of *Shigella boydii*, while four *Shigella sonnie* colicin typing and rarely phage typing is used, (Cruickshank *et al.*, 1975).

Shigellosis has a global distribution, with a highest incidence in countries where the hygiene is poor as it is a faeco–oral infection.

In developed countries *Shigella sonnie* predominates in these conditions, while in developing countries *Shigella flexneri* incidence is described and vice versa.

Man is the only reservoir and the natural host of *Shigella* species in contrast to *Salmonella* species, infants below six months, especially neonates, are rarely infected with *Shigella* species in contrast with *Salmonella* species, (Chrise, 1980).

One major problem of *Shigella* spp. infections is its antibiotic resistance. The most common pattern of such resistance according to WHO, (1980) was that of *sulphonamide* reported globally. Multiple plasmid–mediated resistance was noted involving tetracycline, ampicillin, and chloramphenicol, and it is now known to be common.

*Shigella* spp. was found in 4.9% of children with diarrhoea, (Youssef *et al*., 2000).

**1.1.2.7.3.6. *Klebsiella pneumoniae*:**

It is also not surprising that there has been some reports linking this genus to epidemics of diarrhoea, because some strains of *Klebsiella* appear to

have acquired the plasmids from *E.coli* that code for the heat – labile or heat – stable entero toxins. Another important virulence factor of *Klebsiella pneumoniae* is the production of aerobactin, a siderophore that can obtain iron from transferring (Lippincoh *et al*., 1991).

K. pneumoniae strains recovered as pure or predominant isolate from stool specimens of 50 children below three years of age, presenting with acute watery diarrhoea, were studied for heat-labile enterotoxin production. Twenty three (46%) of the 50 crude toxin concentrates showed positivity in rabbit ileal loops and skin permeability tests. Antigenically 17 (34%) and 20 (40%) of the toxin extracts reacted with immuno-purified anti H-LT antibody in latex particle agglutination and immuno-dot blot assays respectively. Polyacrylamide-gel electrophoresis, Western-blotting and enzyme-immunoassay revealed heat-labile enterotoxin and its subunits in 19 (68%) of 28 extracts tested. In 5 of 10 strains tested the toxigenicity could be transferred to recipient *Escherichia coli* J-35 in plasmid transfer experiments. *Klebsiella* induced enterotoxigenic diarrhoea and produced a heat-labile toxin which seems to be biologically, antigenically and possibly genetically related to the heat-labile toxin of the cholera-coli family (Panigrahi *et al*., 1991).

### 1.1.2.8. HOW TO CONTROL DIARRHOEA:

Protection from diarrhoea is still a problem to be considered in this most mortal disease. Improvement in economic levels, nutrition, and sanitation are of paramount importance. Hand washing, refrigeration, proper cooking, safe water supply and control of insects can be achieved by improvement in socioeconomic and environmental standards.

According to the, (WHO)'s "Golden Rules" for safe food preparation, one should: cook food thoroughly, eat cooked food immediately, store cooked foods carefully, reheat cooked food thoroughly, avoid contact between raw

foods and cooked foods, wash hands repeatedly, keep all kitchen surfaces meticulously clean, protect foods from insects; rodents; and other animals, use pure water, (WHO, 1996).

Food for infants should preferably not be stored at all.

For certain groups at high risk of developing specific enteric infection, it may be feasible to control diarrhoea through use of an oral vaccine, (Dupont *et al.*, 1972).

It is unlikely, however, that immunological approaches will offer the solution in the wide-spread control of diarrhoeal diseases among the general population due to a myriad of bacterial, viral and protozoal etiologies. To those who are sick a variety of antibacterial or pharmacological agents, that may break the cycle of events and / or modify the host reaction, may be offered on rational basis. However, replacement of fluid and electrolytes losses and proper dietary intake still remain the most important means to treat the disease and many of its complications, (Lifshitz, 1973).

A study was carried out in Myanmar to gather more information, on the types of feedings and hands–washing practices of mothers, as the determinant of severe dehydration, in infants with acute diarrhoea, due to *E. coli*. The results suggested that there was a need for appropriate intervention programs to promote exclusive breast–feeding and hand–washing practices with soap and water after cleansing children's defecation, and before and after feeding of infants, (Oo *et al.*, 2000).

A study was carried out in Brazil; to study the reactivity of human colostrums containing IgA antibodies against EPEC, where Enteropathogenic *E. coli* diarrhoea was endemic among infants. The study suggested that maternal antibodies may protect infants from EPEC infection by interfering with the adherence process, (Loureiro *et al*., 1998).

In 1994 results were published from two randomized, double-blind, placebo-controlled trials of vitamin A supplementation in Brazil and India. They indicated that, in addition to its effect on mortality from diarrhoea, vitamin A supplementation could play a significant role in reducing the severity of diarrhoeal morbidity, (WHO, 1995).

**1.1.2.9. EPIDEMIOLOGY OF DIARRHOEA:**

To describe the epidemiology and etiology of acute diarrhoea among children, treated exclusively in an outpatient setting in Greece, one hundred and thirty–two children, (median age: 2 years) were included in the study, (Maltezou, *et al.*, 2001). An enteropathogen was detected in 63, (48%) of them. Isolates included *Rota* virus, (19 patients), *Salmonella sp.*, (12), *Campylobacter* sp., (10), *Aeromonas* sp., (9), enteropathogenic *E. coli*, (6), adenovirus, (6), *Giardia lamblia*, (4), *Yersinia enterocolitica*, (2) and *Shigella* sp., (1).

Stool cultures, (561) from Yemeni children presenting diarrhoea, were analyzed, (Banajeh *et al.*, 2001). A total of 190, (33.9%) were positive for bacterial culture. Most of the positive cultures, (58%) were from children aged 1 – 12 months. The majority of the positive cultures were enteropathogenic *E. coli*, (58.4%). *Salmonella* spp., and *Shigella* spp., (20% each). *Campylobacter* were found to be an extremely uncommon agent of childhood diarrhoea making only 1.6% of the positive cultures, (Banajeh *et al.*, 2001).

A study about microorganisms associated with infant diarrhoea in a group of 256 children admitted to public hospital in Montevideo, Uruguay was carried out, (Torres *et al.*, 2001). The potential pathogens were found in 63.8% of cases. Co infection with two or more agents was detected in more than one – third of positive studies. *Escherichia coli* enteric virotypes, especially enteropathogenic *E. coli*, (EPEC), were shown to be prevalent. *Rotavirus*,

*Cryptosporidium*, *Campylobacter*, (mainly *C. jejuni)*, and *Shigella flexneri* were also often identified. Enterotoxigenic *E. coli*, *Salmonella* and *Giardia lamblia* were sporadically recognized. Unusual findings included two enteroinvasive *E. coli* strains, one *Shigella dysentriae* 2 isolates, and a non – O: 1 *Vibrio cholerae* culture, ( Torres *et al.*,2001).

Faecal samples, (576) were collected from children, (0 – 5 year(s) old) suffering from acute diarrhoea in Saudi Arabia. One or more enteropathogen(s) were identified in 45.6% of the stool specimens. Mixed infections were detected in 12.2% of the diarrhoeal cases. *E. coli* were detected in 13%, of which 3.8% were enteropathogenic *E. coli*, (El – Sheikh *et al.*, 2001).

A total of 421 strains of *Escherichia coli* isolated between 1992 and 1993 from paediatric patients with diarrhoea, (n=345) and controls, (n=76) less than two years of age at Addis Ababa. Of the 345 *E. coli* isolates from patients, 72, (20.8%) and of the 76 *E. coli* isolates from controls 13, (7.1%) were positive for EPEC. Of the 345 *E. coli* strains, 21(6.1%) and of the 76 *E. coli* strains, 5(6.6%) were positive for more than one pool of antisera, (Asrat, 2001).

*E. coli* strains were isolated from faecal samples of 50 infants, having acute diarrhoea, during the period of May – August, 1979, (Panhotra, 1983). These infants attended the Paediatric Emergency Department of the Postgraduate Institute of Medical Education and Research, Chandijarh, India, (Panhotra, 1983).

The isolation rate of *E. coli* belonging to the traditional serotypes enteropathogenic from infants was studied in Canada. Enteropathogenic *E. coli* were found in 13, (6%) of 220 children younger than 12 months of age, and in 9, (6%) of 143 children 12 – 35 months of age, all of whom had diarrhoea, (Marc *et al.*, 1978).

Enteropathogenic *E. coli* strains are the most prevalent enteropathogenic agents isolated in the stools of hospitalized infants with severe acute diarrhoea in Sao Paulo, Brazil, and these strains are able to induce moderate to severe faecal fluid losses in infants and the duration of diarrhoea is usually below 15 days, (Oliva *et al*., 1997).

In the summer of 1998, a large outbreak of *Escherichia coli*, O157:H7, infections occurred in Alpine, Wyoming. 157 ill persons have been identified; stool from 71, (45%) yielded *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% vs. 50%), (Olsen *et al*., 2002).

**1.1.2.10. EPIDEMIOLOGY OF DIARRHOEA IN SUDAN:**

Bacterial etiology of diarrhoea in children was shown to make a higher incidence, whereas enteropathogenic *E. coli* were the most dominant pathogens, (Erwa *et al.*, 1971).

A study was carried out in Khartoum North in 1975 among 654 cases of children suffering from diarrhoea. Enteropathogenic *E. coli* enteritis was shown to be the prevalent. Of 128,(72.8%) of the isolates were enteropathogenic *E. coli*, 83, (12.7%) strains were untypable *E. coli*, (Erwa, 1975).

Enteropathogenic *E. coli* were isolated from children with diarrhoea below five years of age in Juba town, (Sharaf, 1986).

According to Ismail, (1994), 320 clinical strains isolated from children suffering from gastroenteritis. The isolates of *E. coli* were, (76.3%), (Ismail, 1994).

**1.2. ANTIBIOTICS:**

**1.2.1. DEFINITION:**

The term “Antibiotic” was first used by Waksman in 1942, (Waksman and Lechevalier, 1962).

Antibiotic was originally defined as a substance produced by one microorganism, (Bacteria, Fungi, Actinomycetes), which inhibited the growth of other microorganisms, (Gilman *et al.*, 1980).

The advent of synthetic methods has, however, resulted in a modification of this definition and an antibiotic now refers to a substance produced by a microorganism, or to a similar substance, (produced wholly or partially by chemical synthesis), which in low concentrations inhibits the growth of other microorganisms, (Hugo and Russell, 2002).

Antibiotics differ in their chemical, physical and pharmacological properties and mechanisms of action and antibacterial spectra, (Pratt, 1977; Gilman *et al.*, 1980; Garrod *et al.*, 1981).

**1.2.2. ANTIBIOTIC ACTION:**

According to their effect antibiotics are either bactericidal drugs; have a rapid lethal action and they cause microbial cell death, e.g. penicillins, cephalosporins, amino glycosides and polymyxin, or bacteriostatic drugs; merely inhibit the growth of organisms or inhibit bacterial cell replication but do not kill the organisms, e.g. sulphonamides, tetracyclines, and chloramphenicol, (Pratt, 1977; Garrod *et al.*, 1981; Thomas *et al.*, 1988; Reynolds, 1989).

The differences are not clear–cut and most drugs are to, varying extents, both bactericidal and bacteriostatic, (Thomas *et al.*, 1988). A few compounds, e.g. sulphonamides, are either cidal or static according to the composition of the environment, (Blood, Pus, Urine, etc.) in which the infecting organisms are growing, (Pratt, 1977).

### 1.2.3. CLASSIFICATION OF ANTIBIOTICS:

There are several methods used to classify and group antimicrobial agents, and all are chambered by exceptions and overlaps. The most common classification has been based on chemical structure and proposed mechanism of action as follows, (Gilman *et al.*, 1980):

1- Agents that inhibit synthesis of, or activate enzymes that disrupt bacterial cell walls to cause loss of viability. These include the penicillins and cephalosporins, which are structurally similar and dissimilar agents such as cycloserine, vancomycin, restocetin and bacitracin.

**2-** Agents that act directly on the cell membrane, affecting permeability and leading to leakage of intracellular compounds, or become firmly bound to the cytoplasmic membrane and act by damaging this structure; these include the detergents, polymyxin and colistimethate.

**3-** Agents that affect the function of bacterial ribosomes to cause a reversible inhibition of protein synthesis. These drugs include chloramphenicol, the tetracyclines, and the macrolides antibiotics such as erythromycin, lincomycin and its congener clindamycin.

**4-** Agents that bind to 30 ribosomal subunit and cause the accumulation of protein synthesis initiation complexes, misreading of the mRNA code, and the production of abnormal polypeptides. These include the amino glycosides group of antibiotics, which are bactericidal.

**5-** Agents that affect nucleic acid metabolism, such as rifampicin, which inhibits DNA dependent RNA polymerase.

6- The antimetabolites including trimethoprim and the sulphonamides, which block specific metabolic steps that are essential to the microorganism.

Another classification with functional utility is based on the general antimicrobial activity of the various groups of drugs:

1- Active mainly against Gram–positive cocci and bacilli, which tend to have a relatively narrow spectrum of activity. These include penicillin G, the semi synthetic penicillinase–resistant penicillin, the macrolides, the lincomycins, vancomycin and bacitracin.
2- Active mainly against aerobic Gram–negative bacilli include the amino glycosides, and polymyxin.
3- Relatively broad–spectrum drugs that affect both the Gram–positive cocci and Gram–negative bacilli include the broad–spectrum penicillins, (ampicillin and carbencillin) the cephalosporins, the tetracyclines, chloramphenicol, trimethoprim and the sulphonamides, (Gilman *et al.*, 1980; Reynolds, 1989).

Antibiotics can be classified according to the organisms they act on:, (Reynolds, 1989):

1- **Antibacterials**, which act on bacteria and are used in the treatment and prophylaxis of bacterial infections, e.g. β–lactam antibiotics, (penicillins and cephalosporins), amino glycosides, macrolides, polymyxins.
2- **Antifungals,** which act on fungi and are used in prevention or treatment of fungal infections. These include polyene and azole derivatives, (triazoles,

imidazoles) and fatty acid antifungal agents, (propionic acid).

3- **Antiprotozoals,** which act on protozoa and are used primarily in the treatment of protozoal infections.
4- **Antivirals,** which act on viruses and are used in the treatment of viral infection or for providing protection, usually for a brief period only, against viral infections.

### 1.2.3. ANTIBIOTIC GROUPS:

#### 1.2.3.1. INHIBITORS OF CELL WALL SYNTHESIS:

##### 1.2.3.1.1. PENICILLINS:

Penicillin was obtained from the mould *Penicillium notatum* or *P. chrysogenum*, and penicillin was the first antibiotic to be isolated and used therapeutically in 1929 by Alexandar Fleming, (Selwyn, 1980).

Penicillins belong to the β–lactam group of antibiotics. The basic nucleus of penicillin is 6–aminopenicillinic acid, (Range and Dale, 1987). Penicillins may be destroyed by the enzymes amidases and β–lactamases, (penicillinases).

(i) **TYPES OF PENICILLINS AND THEIR ANTIMICROBIAL ACTIVITY:**

It is useful to classify the penicillins according to their spectrum of antimicrobial activity, (Gilman *et al.*, 1980).

1- Penicillin G and its closely related penicillin V and phenethicillin are narrow–spectrum penicillins. They are highly active against Gram–positive cocci, but readily hydrolyzed by penicillinase, (Pratt, 1977).

2- The penicillinase resistant penicillins, (e.g. methicillin, oxicillin, cloxacillin and flucloxacillin) have less potent antimicrobial activity against microorganisms that are sensitive to penicillin G. methicillin is the drug of choice for infections caused by penicillinase producing *Staph. Aureus*, (Gilman *et al.*, 1980).
3- Ampicillin, amoxicillin and hetacillin are broad–spectrum penicillins and usually less active than penicillin G against most Gram–positive bacteria, but their activity is greater than that of penicillin G against Gram–negative bacilli, (Pratt, 1977).
4- The antimicrobial activity of carbencillin and its indanylester, ticarcillin and azlocillin is extended to include *Pseudomonas*, *Enterobacter*, and *Proteus* species. These are extended spectrum penicillins, (Gilman *et al.*, 1980).
5- A new group of penicillin included mezlocillin and piperacillin. These drugs have useful antimicrobial activity against *Klebsiella* species and certain other Gram–negative microorganisms, (Gilman *et al.*, 1980).

**(ii) MECHANISM OF ACTION:**

All β–lactam antibiotics interfere with the synthesis of the bacterial cell wall peptidoglycan that normally protects the bacterium from its environment. After attachment to binding sites on the bacterium, they inhibit transpeptidation enzyme that cross–links the peptide chains attached to the backbone of the peptidoglycan. The final bacterial event is the inactivation of an inhibitor of the autolytic enzymes in the cell wall; this leads to lyses of the bacterium, (Pratt, 1977; Gilman *et al.*, 1980; Range and Dale, 1987).

According to Rang and Dale, (1987), and Pratt, (1977), resistance to penicillin may be due to different causes:

1- The production of β–lactamases, (There are about 50 different types), which open the β–lactam ring and terminate the activity of penicillin.
2- A reduction in the permeability of the outer membrane and thus decreased ability of the drug to penetrate to the target site is influenced. This occurs with Gram–negative organisms.
3- The occurrence of modified penicillin–binding sites obscures the targets.

**1.2.4.1.1.1. BENZYL PENICILLIN, (PENICILLIN G):**

Penicillin G has a narrow spectrum of action. It is still the drug of choice for the treatment of non-penicillinase–producing strains of most cocci, Gram–positive bacilli, and *Spirochetes*, (Pratt, 1977).

The two major limitations to the use of penicillin G in the treatment of the common infections are the production of penicillinase by the infecting organism and the presence of penicillin allergy, (Pratt, 1977).

Penicillin G is highly effective *in vitro* against many, but not all species of Gram–positive and Gram–negative *Cocci* and *Streptococci* are very susceptible to the drug.

At present most *Staphylococci* isolated from individuals outside of hospitals, are resistant to penicillin G. In hospitalized patients, the incidence of such strains may be as high as 90 to 95%. Most anaerobic microorganisms, including *Clostridium* species, are highly sensitive. *Bacteroides fragilis* is an exception. Although many species of Gram–negative bacilli are resistant to penicillin G, some are affected by moderate - to – high concentrations, (Gilman *et al.*, 1980).

**1.2.4.1.1.2. AMPICILLIN:**

Ampicillin is amino substituted, broad–spectrum penicillin. It is acid stable and it was introduced many years ago, (Pratt, 1977). Ampicillin is bactericidal for both Gram–positive and Gram–negative bacteria. The viridians group of *Streptococci* is usually inhibited by very low concentration of ampicillin.

Although, most strains of *E. coli*, *Proteus mirabilis*, *Salmonella* spp. and *Shigella* spp were highly susceptible when ampicillin was first used in the early 1960, an increasing percentage of these species is now resistant. From 30 to 50% of *E. coli*, a significant number of *Proteus mirabilis*, and practically all species of *Enterobacter* are presently insensitive. Most strains of *Pseudomonas*, *Klebsiella*, and *Serratia* are also resistant.

**1.2.4.1.1.3. CARBENCILLIN:**

This drug is penicillinase susceptible derivative of 6–aminopenicillinic acid. The major advantage of this agent is that it often cures serious infections caused by *Pseudomonas* spp., *Proteus* strains resistant to ampicillin and certain other Gram–negative microorganisms, (Gilman *et al.*, 1980).

Penicillin G–resistant *Staphylococci*, *Klebsiella*, and *Serratia* are usually resistant to carbencillin; it is not absorbed by the gastrointestinal tract and, therefore, must be given parenterally, (Pratt, 1977; Gilman *et al.*, 1980).

**1.2.3.1.2. THE CEPHALOSPORINS:**

Culture of the fungus *Cephalosporium acremonium* obtained from the sea near a sewer outlet in Sardinia were found to yield extract which inhibited the growth of *Staphylococcus aureus.* These cultures fluids were found to contain three distinct antibiotics:

(i) Cephalosporin P, active only against Gram–positive microorganisms.

(ii) Cephalosporin N, a new type of penicillin with a side chain derived from D–α–aminodipic acid, effective against both Gram–positive and Gram–negative bacteria.

(iii) Cephalosporin C, less potent than cephalosporin N, but possessing the same range of antimicrobial effectiveness, (Gilman *et al.*, 1980).

The nucleus of cephalosporin C has been isolated and a very large number of semi synthetic broad–spectrum cephalosporins have been produced, (Gilman *et al.*, 1980; Rang and Dale, 1987).

**1.2.4.1.2.1. MECHANISM OF ACTION:**

The cephalosporins are bactericidal and appear to inhibit bacterial cell wall synthesis in a manner similar to that of penicillin. As with the penicillins, resistance occurs if organism generates enzymes that cleave the β–lactam ring or if it has an outer membrane that prevents penetration of the drug. The cephalosporins are not readily attacked by the plasmid – encoded β–lactamases, but there are reports of resistance due to mutations involving the binding–site proteins, (Gilman *et al.*, 1980; Rang and Dale, 1987).

The third generation of cephalosporins e.g. cefotaxime is less active against Gram–positive than the second generation, but more active against Gram–negative bacteria, (Rang and Dale, 1987).

**1.2.3.2. INHIBITORS OF PROTEIN SYNTHESIS:**

**1.2.3.2.1. THE AMINO GLYCOSIDES:**

The amino glycosides are a group of antibiotics resembling each other. They are derived from the bacteria of the genus *Streptomyces* or *Micromonospora***.** They are bactericidal antibiotics. The main agents are streptomycin, gentamycin, amikacin, kanamycin, tobramycin, netilmicin, newmycin and framycetin.

All these drugs contain amino sugars in glycoside linkage; they are poly cations, (Rang and Dale, 1987).

The amino glycosides are used almost exclusively to treat infections caused by Gram–negative bacteria. They affect protein synthesis in the bacterial cell similarly, but there are probably slight differences in their mechanisms of action, (Pratt, 1977).

Serious toxicity, (especially oto-toxicity) is a major limitation to the usefulness of the amino glycosides. Nephro-toxicity is an additional important problem, (Gilman *et al.*, 1980).

**(i) ANTIBACTERIAL ACTIVITY:**

The amino glycosides are effective against many aerobic Gram–negative and some Gram–positive organisms. Gentamycin is the most commonly used; tobramycin is a preferred member of this group for *Pseudomonas aeroginosa* infection. In general, gentamycin, tobramycin and amikacin are more active than kanamycin, (Gilman *et al.*, 1980).

**(ii) MECHANISM OF ACTION AND MICROBIAL RESISTANCE:**

The amino glycosides are rapidly bactericidal; they act directly on the bacterial ribosome. To reach the ribosome, the drug must be transported across the cell membrane.

All binds to the sites on the 30 S subunit of the bacterial ribosome, causing an alteration incodon: anticodon recognition. The thing that results in the

misreading of the messenger RNA and thence in the production of defective bacterial proteins, (Pratt, 1977; Gilman *et al.*, 1980; Rang and Dale, 1987).

Bacteria may become resistant to the antimicrobial activity of the amino glycosides because of failure of permeation of the antibiotic, low affinity of the drug for the bacterial ribosome or inactivation of the drug by microbial enzymes. Most acquired resistance to amino glycosides results from their enzymatic inactivation. There are multiple enzymes involved in. Those are localized in the bacterial membrane at or near the site of drug transport, (Gilman *et al.*, 1980).

**1.2.4.2.1.1. GENTAMYCIN:**

Gentamycin is an important agent for the treatment of many serious Gram–negative bacillary infections. It has a broad–spectrum. It is very effective against *E. coli*, *Proteus mirabilis*, *Klebsiella*, *Enterobacter*, *Serratia* and *Pseudomonas aeroginosa*. Gentamycin is active against most strains of *S. aureus*, (Pratt, 1977).

However, emergence of resistant microorganisms in some hospitals has become a serious problem and may limit the future use of this agent. In some hospitals, the nosocomial flora has undergone considerable alterations in susceptibility to antibiotics during the last 25 years, with a gradual increase in resistance to gentamycin, (Gilman *et al.*, 1980).

**1.2.4.2.1.2. TOBRAMYCIN:**

This is a new amino glycoside. It is significantly more active *in vitro* against *Pseudomonas* than gentamycin, (Levison *et al.*, 1992). Most Gram–negative bacilli except *Ps. aeroginosa* that are resistant to gentamycin because of plasmid mediated inactivating enzymes that, also, inactivate tobramycin.

**1.2.4.2.1.3. KANAMYCIN:**

Kanamycin has a broad antibacterial activity among Gram–negative bacilli. It is not effective against *Pseudomonas*. Kanamycin is less active on a molar basis than either gentamycin or tobramycin, and it is more toxic. Gentamycin is now utilized in many cases for which Kanamycin was formerly the drug of choice, (Pratt, 1977).

**1.2.4.2.1.4. AMIKACIN:**

Amikacin is the newest of the amino glycosides and it is a new synthetic derivative of kanamycin. The spectrum of antimicrobial activity of amikacin is the broadest of the group, and because of its unique enzymes resistance, it has a special role in hospitals where gentamycin, kanamycin and tobramycin resistant microorganisms are prevalent, (Pratt, 1977; Gilman *et al.*, 1980).

**1.2.3.3. BACTERIOSTATICS OF PROTIEN SYNTHESIS:**

This group includes: Chloramphenicol, Erythromycin, Lincomycin, Clindamycin, Spectinomycin and the tetracyclines.

The antibiotic chloramphenicol, erythromycin, lincomycin and clindamycin all inhibit bacterial protein synthesis, and they all bind to the 50 S subunit of the bacterial ribosome, (Pratt, 1977).

**1.2.4.3.1. CHLORAMPHENICOL:**

This antibiotic was originally isolated from a soil *Actinomycetes*, *Strepotmyces venezuela*, and is now produced by chemical synthesis, (Pratt, 1977).

**1.2.4.3.1.1. MECHANISM OF ACTION:**

The drug readily penetrates into bacterial cell, probably by a process of facilitated diffusion. Chloramphenicol acts primarily by binding reversibly to the 50S ribosomal subunit. It is clear that the interaction of chloramphenicol with the ribosome is responsible for the inhibition of protein synthesis by the drug, (Pratt, 1977; Gilman *et al.*, 1980).

**1.2.4.3.1.2. ANTIMICROBIAL ACTIVITY:**

This drug is active against wide range of Gram–positive and Gram–negative bacteria. It is primarily bacteriostatic, although it may be bactericidal to certain species, such as *Haemophylus influenzae*, *N. meningitides*, *Salmonella typhi*, *Brucella* species and *Bordetella pertusis*. The Enterobacteriaceae members have a variable sensitivity to chloramphenicol, (Gilman *et al.*, 1980).

**1.2.4.3.1.3. RESISTANCE TO CHLORAMPHENICOL:**

Resistance is due to the production of chloramphenicol acetyl–transferase and is plasmid–mediated, (R. plasmid containing determinants for multiple drug resistance for chloramphenicol) and may be transferred from one bacterial species to another by promiscuous plasmids, (Rang and Dale, 1987).

**1.2.4.3.2. ERYTHROMYCIN:**

Erythromycin is the only one of the macrolides antibiotics that is still used clinically. It is the pro-type, (Pratt, 1977).

**1.2.4.3.2.1. MECHANISM OF ACTION:**

The mechanism of action of erythromycin is the inhibition of bacterial protein synthesis by an effect of translocation. The drug may be bactericidal or bacteriostatic; the effect is depending on its concentration and on the type of the microorganism. The free base is bound to the 50S subunit of the bacterial ribosome, (Rang and Dale, 1987).

**1.2.4.3.2.2. ANTIMICROBIAL ACTIVITY AND RESISTANCE:**

Erythromycin has a good therapeutic index; the antimicrobial spectrum is very similar to that of penicillin, but not against most Gram–negative organisms. It is an effective alternative choice for penicillin allergic patients

infected with *Streptococcus pyogens*, *Streptococcus pneumoniae*, or *Treponema pallidum*, (Pratt, 1977; Rang and Dale, 1987).

Resistance may occur and is due to a plasmid-controlled alteration of the receptor for erthromycin on the bacterial ribosome, (Rang and Dale, 1987).

**1.2.4.3.3. CLINDAMYCIN:**

Clindamycin is a semi synthetic modification of lincomycin, an antibiotic isolated from *Streptomyces lincolnensis.* Its mechanism of action involves inhibition of protein synthesis and is similar to that of erythromycin and chloramphenicol. In general clindamycin is active against many strains of *Staph. aureus* but may not inhibit methicillin–resistant strains, (Gilman *et al.*, 1980). In some hospitals resistance to clindamycin has been found in 20% of isolates, (Nunnery and Rites, 1964).

Clindamycin is more active than erythromycin against many anaerobic bacteria, especially *Bacteroides fragilis*, (Gilman *et al.*, 1980).

**1.2.4.3.4. THE TETRACYCLINES:**

The tetracycline antibiotics were isolated from various species of *Streptomyces***.** The structures of tetracyclines are all very closely related, (Pratt, 1977).

Tetracyclines are broad–spectrum antibiotics that have a polycyclic structure. The first tetracyclines used, chlortetracycline, oxytetracyclin, and demeclocycline were derived from cultures of *Streptomyces*. More recently developed compounds; tetracycline, methocycline, dexycycline, minocycline, clomocycline and lymecycline are synthetic or semi synthetic, (Rang and Dale, 1987).

**1.2.4.3.4.1. MECHANISM OF ACTION:**

Tetracyclines are bacteriostatic. They inhibit protein synthesis by blocking the binding of aminoacyl-tRNA to the mRNA–ribosome complex. Tetracycline

binds to both ribosome and mRNA. This binding is largely reversible and the bulk of the bound tetracycline is associated with the 30S ribosomal subunit, (Pratt, 1977).

**1.2.4.3.4.2. ANTIMICROBIAL ACTIVITY:**

The tetracyclines possess a wide range of antimicrobial activity against Gram–positive and Gram–negative bacteria. In general, Gram–positive microorganisms are affected by lower concentration of tetracyclines than are Gram–negative species, (Gilman *et al.*, 1980).

**1.2.4.3.4.3. RESISTANCE TO TETRACYCLINES:**

Resistance to the tetracyclines produced *in vitro* appears slowly in a graded manner. Resistance is also mediated by a plasmid and is an inducible trait, that is, the bacteria become resistant only after exposure to the drug. Plasmid that impact resistance contains genetic information for a number of proteins that appear to affect transport of the drug into the cell, (Gilman *et al.*, 1980).

**1.2.4.3.5. FUSIDIC ACID:**

Fusidic acid is a narrow spectrum steroid antibiotic active mainly against Gram–positive bacteria. It acts by inhibiting protein synthesis. It is used mainly for infections caused by penicillin resistant *Staphylococci*.

**1.2.3.4. INHIBITORS OF BACTERIAL GROWTH:**

This group includes: the sulphonamides, the sulphones, Para–aminosalicylic acid, trimethoprim and trimethoprim–sulphamethoxazole combination, (Pratt, 1977).

**1.2.4.4.1. SULPHONAMIDES:**

The sulphonamide drugs were the first effective chemotherapeutic agents to be employed systematically for prevention and cure of bacterial infections in man, (Gilman *et al.*, 1980).

#### 1.2.4.4.1.1. MECHANISM OF ACTION:

Sulphonamide is a structural analogue of *p*–aminobenzoic acid, (PABA) that is essential for the synthesis of folic acid in bacteria. These drugs arrest cell growth by inhibiting the synthesis of folic acid by the bacterium. There is a lag period, before cell growth is arrested; the bacterium is exhausting its stores of folic acid, (Pratt, 1977; Rang and Dale, 1987).

#### 1.2.4.4.1.2. ANTIMICROBIAL ACTIVITY:

Sulphonamides have a wide range of antimicrobial activity against both Gram–positive and Gram–negative microorganisms. Among the microorganisms highly susceptible *in vitro* to *sulphonamide*s are *Streptococcus pyogens*, *Streptococcus pneumoniae*, some strains of *Bacillus anthracis*, and *Corynebacterium diphtheriae*, *Haemophilus influenzae* and *Chlamydia trachomatis*.

#### 1.2.4.4.1.3. RESISTANCE:

Bacteria initially sensitive to *sulphonamide*s can acquire resistance to the drug both *in vitro* and *in vivo*. Resistance to *sulphonamide*s is probably the consequence of an altered enzymatic constitution of the bacterial cell. The alteration may be characterized by, (1) an alteration in the enzyme that utilizes PABA, (2) an increased capacity to destroy or inactivate the drug., (3) An alterative metabolic pathway for synthesis of an essential metabolite, or, (4) an increased production of an essential metabolite or drug antagonist. Acquired bacterial resistance to *sulphonamide*s plays a significant role in limiting the activity of these drugs, particularly infections caused by *Staphylococci* and *Streptococci*, (Gilman *et al.*, 1980).

### 1.2.4.4.2. TRIMETHOPRIM:

In structure, trimethoprim has some resemblance to the pteridine moiety of foliate. Trimethoprim is active against most common bacterial pathogens, and

is bacteriostatic rather than bactericidal. It is frequently given as a mixture with sulphamethoxazole as co–trimoxazole, (Rang and Dale, 1987). The use of the combination appears to slow the development of resistance. Trimethoprim resistance has increased over the past decades whilst resistance to co–trimoxazole has remained unchanged.

### 1.2.5. ANTIBIOTIC RESISTANCE:

When antibiotics are used to treat an infection, a favourable therapeutic outcome is influenced by numerous factors. However, in simple terms, success is dependent on achieving a level of antibacterial activity at the site of infection that is sufficient to inhibit the bacteria. When host defenses are maximally effective, the alteration required may be minimal, (Gilman *et al.*, 1980). Resistance of microorganism to an antibiotic may be intrinsic, (natural) or acquired.

#### 1.2.5.1. NATURAL RESISTANCE:

Bacterial resistance to antimicrobial agents has long been recognized, (Brayan, 1984). The most obvious determinant of bacterial response to an antibiotic is the presence or absence of the target for the drug action. If an organism lacks the receptor for the drug, it will not respond and is therefore, inherently resistant to the antibiotic action, (Pratt, 1977). For an antibiotic to be effective, it must first gain access to the target sites of action on or in the bacterial cell. Microorganism may resist this passage by several mechanisms. Some produce enzymes at or within the cell surface that inactivate the drug. Still others lack the transport systems that are required for entrance of the drug into the bacterial cell, (Dickie *et al.*, 1978).

Bacteria often contain the drug receptor but they do not respond because the concentration of antibiotic at the target site is inadequate. Although the

organism is not insensitive, for all practical purposes, it behaves as if it was with respect to the chemotherapeutic goal, (Pratt, 1977).

Interaction of the antibiotic with the target may be directly lethal or may disrupt a cellular function in lethality, (Tomasz, 1981). The drug must pass through the outer layer of bacterial cell to reach the target site within the cytoplasm.

**1.2.5.2. ACQUIRED ANTIBIOTIC RESISTANCE:**

When the antimicrobial activity is first tested, a pattern of sensitivity and resistance is usually defined. The development of resistance to antibiotics involves a stable genetic change, heritable from generation to generation. Any of the mechanisms that result in alteration of bacterial genetic composition can operate. While mutation is frequently the cause, resistance to antimicrobial agents may be acquired through transfer of genetic material from one bacterium to another, (Gilman *et al.*, 1980).

**1.2.5.2.1. TRANSFORMATION:**

This process involves the passage of naked DNA from donor to recipient through the medium. It is limited to Gram–positive bacteria, (David, 1980).

**1.2.5.2.2. TRANSDUCTION:**

This process involves transfer of DNA, which is carried, from donor to recipient by a phage, (David, 1980). If this genetic material includes a gene for drug resistance, the newly infected bacterial cell may become resistant to the agent and capable of passing the trait to its progeny, (Gilman *et al.*, 1980).

Transduction is known to occur for both Gram–positive and Gram–negative bacteria and it is particularly important in the transfer of antibiotic resistance among strains of *Staphylococcus aureus*, where some phages can carry plasmids that code for penicillinase, (Gilman *et al.*, 1980).

**1.2.5.2.3. CONJUGATION:**

In this process DNA passes from the donor, (or male) cell to the recipient, (or female) cell via a bridge linking the two, (sex pilus), (David, 1980). This is now recognized as an extremely important mechanism for spread of antibiotic resistance. Although transfer seems to be confined to Gram–negative bacteria, the process allows the passage of any number of genes, thus multiple drug resistance may occur at a single step, (David, 1980).

**1.2.5.2.4. CONJUGATION AND R–FACTORS:**

Resistance by conjugation, "infectious" resistance or multiple resistance, was first recognized in Japan in 1959 after an outbreak of bacillary dysentery caused by *Shigella flexneri* that was resistant to four different classes of antibiotics, (Wantanabe, 1966). They demonstrated that the phenomenon was due to resistance genes passing from *E. coli* in the gut to the infecting *Shigella*, (David, 1980).

It is known that the conjugation process is responsible for the transfer of extra chromosomal (or episomal) genetic elements called resistance–factors, (R–Factors), (David, 1980). For example, in the case of resistance to amino glycosides or chloramphenicol, the R–Factor codes for the synthesis of drug inactivating enzymes. The second sequence codes for a sex factor for the transfer apparatus and is termed Resistance Transfer Factor, (RTF), or transfer factor plasmid. This DNA specifies the synthesis of sexual apparatus or pili that are essential for the conjugal transfer of the genetic material, (Wilson and Miles, 1977).

Each of these two components can exist alone, but they must be joined in order to transfer antibiotic resistance successfully.

**1.2.5.3. MUTATION:**

Antibiotic susceptible bacteria are likely to contain some mutant strains that are relatively resistant to the drug, (Gilman *et al.*, 1980).

The infective organism produces resistant mutants during treatment. Resistant mutants are particularly liable to arise during treatment with streptomycin; *sulphonamide*s, isoniazid, nalidixic acid and rifamycins are also serious offenders, (Thomas, 1988).

The emergence of resistant mutants is encouraged by inadequate dosage, prolonged treatment and the presence of closed focus of infection, (Thomas, 1988).

**1.2.5.4. EPIDEMIOLOGY OF ANTIBIOTIC RESISTANCE:**

In a study carried out in Japan in 1997, Shigatoxin–producing *E. coli* (STEc) O26: H11 were isolated from thirty-two children suffering from watery or bloody diarrhoea. The isolates have possessed multiple antimicrobial resistance, which were encoded by a transmissible plasmid.
(Hiruta *et al.*, 2001).

A study carried out in Yemen among children who are suffering from diarrhoea to identify the bacterial etiology and their antimicrobial resistance to the commonly used antibiotics, (Banajeh *et al.*, 2001) revealed that more than two-thirds of the *Salmonella* isolates were resistant to nalidixic acid, chloramphenicol, co–trimoxazole, gentamycin and amoxycillin, while 42% were resistant to cefotaxime. Most of the *Shigella* isolates were susceptible to nalidixic acid and cefotaxime, and resistant to other antibiotics. All the tested Enteropathogenic *E. coli* isolates were resistant to amoxycillin, 83% were resistant to co–trimoxazole, 62% to chloramphenicol, and 54% to gentamycin, while only 6% were resistant to nalidixic acid and 6% to cefotaxime, (Banajeh *et al.*, 2001).

Microorganisms associated with infants' diarrhoea were studied in Uruguay. Enteropathogenic *E. coli* and *Shigella flexneri* showed usually

frequent antimicrobial resistance, especially towards β–lactam antibiotics, (Torres *et al.*, 2001).

## 2. AIM OF THE WORK

In diarrhoeal diseases, especially in infants and small children, the use of antibiotics must be recommended after isolation of the causative organism and its sensitivity testing. Also the over–use of these antibiotics increases the levels of drug resistance.

The aims of the present work are:

1- To investigate the strains of the genus *Escherichia* which are associated with diarrhoea in infants in Sudan and to, comparatively, tackle bacterial causatives of infantile diarrhoea other than *Escherichia* as foil.

2- To detect the antibiotic sensitivity for isolates causing diarrhoea in infants.

# 3. EXPERIMENTAL WORK

## 3.1. MATERIALS:

### 3.1.1. CULTURE MEDIA:

| | |
|---|---|
| **Bacteriological Peptone** | **Oxoid Ltd., England** |
| Carry-Blair Transport Medium | Oxoid Ltd., England |
| Eosin Methylene Blue agar | Oxoid Ltd., England |
| Glucose Phosphate Peptone Water | Oxoid Ltd., England |
| Kligler Iron Agar | Oxoid Ltd., England |
| MacConkey Agar | Oxoid Ltd., England |
| Muller & Hinton Sensitivity Medium | Oxoid Ltd., England |
| Nutrient Agar | Oxoid Ltd., England |
| Nutrient Broth | Oxoid Ltd., England |
| Peptone Water | Oxoid Ltd., England |
| Simmon's Citrate Medium | Plasmatec Labs. U.K. |
| Urea Agar Base | Plasmatec Labs. U.K. |

### 3.1.2. ANTIBIOTICS:

| | **Exp. Date** |
|---|---|
| ***Ampicillin*** | ***Oxoid Ltd., England 06.2004*** |
| Chloramphenicol | Oxoid Ltd., England 06.2004 |

| | |
|---|---|
| Gentamycin | Oxoid Ltd., England 06.2004 |
| Sulphamethoxazole | Oxoid Ltd., England 06.2004 |
| Trimethoprim | Oxoid Ltd., England 06.2004 |
| Co-trimoxazole | Oxoid Ltd., England 06.2004 |
| Nalidixic Acid | Oxoid Ltd., England 06.2004 |
| Erythromycin | Oxoid Ltd., England 06.2004 |
| Tetracycline | Oxoid Ltd., England 06.2004 |
| Amoxicillin | Oxoid Ltd., England 06.2004 |
| Ciprofloxacin | Oxoid Ltd., England 06.2004 |

**3.1.3. SUGARS:**

| | |
|---|---|
| Adonitol | Hopkin & Williams Ltd. |
| Arabinose | Hopkin & Williams Ltd. |
| Cellobiose | Hopkin & Williams Ltd. |
| Dulcitol | Hopkin & Williams Ltd. |
| Glucose | Hopkin & Williams Ltd. |
| Glycerol | Hopkin & Williams Ltd. |
| Inositol | Hopkin & Williams Ltd. |
| Lactose | Hopkin & Williams Ltd. |
| Maltose | Hopkin & Williams Ltd. |
| Mannitol | Hopkin & Williams Ltd. |
| Raffinose | Hopkin & Williams Ltd. |
| Rhamnose | Hopkin & Williams Ltd. |
| Salicin | Hopkin & Williams Ltd. |
| Sorbitol | Hopkin & Williams Ltd. |
| Starch | Hopkin & Williams Ltd. |
| Sucrose | Hopkin & Williams Ltd. |
| Trehalose | Hopkin & Williams Ltd. |
| D(+)Xylose | Hopkin & Williams Ltd. |

### 3.1.4. CHEMICALS AND REAGENTS:

| | |
|---|---|
| Amyl alcohol | The British Drug Houses Ltd. |
| Bromothymol Blue | George T. Gurr, Ltd. |
| Bromocresol purple | George T. Gurr, Ltd. |
| Crystal Violet | The British Drug Houses Ltd. |
| Di-Potassium Hydrogen Phosphate Anhydrous | The British Drug Houses Ltd. |
| Ethyl alcohol | The British Drug Houses Ltd. |
| Hydrochloric Acid | The British Drug Houses Ltd. |
| Hydrogen Peroxide | The British Drug Houses Ltd. |
| Immersion Oil | The British Drug Houses Ltd. |
| Iodine | Hopkin & Williams Ltd. |
| Methyl Red Indicator | The British Drug Houses Ltd. |
| Para-dimethyl amino-Benzaldehyde | The British Drug Houses Ltd. |
| Paraffin Oil | The British Drug Houses Ltd. |
| Potassium Iodide | The British Drug Houses Ltd. |
| Safranin Red | The British Drug Houses Ltd. |
| Savlon (Hibicet Hc) | Imperial Chemical Industries Ltd. |
| Sodium Chloride | Oxoid Ltd. England. |
| Sodium Hydroxide | The British Drug Houses Ltd. |
| Tetramethyl-*p*-Phenylene Diamine dihydrochloride | The British Drug Houses Ltd. |
| Urea | Abbott Ltd. U.K. |

### 3.1.5. INSTRUMENTS AND EQUIPMENT:

| | |
|---|---|
| Autoclave | Baird & Tatlock, England. |

| | |
|---|---|
| Balance | Adam Equipment Co. England |
| Colony Counter | Gollenkamp. England. |
| Glass Ware etc… | Griffin & George Ltd. England and Kimax Ltd. U.S.A. |
| Hot Air Oven | Memmert, Germany. |
| Incubator | Baird & Tatlock, England. |
| Microscope | Olympus, type CH20, Japan. |
| Microscope Slides 1mm thick | Horwell Limited, London, U.K. |
| Swabs | Medical Disposable Industrial Complex (MDIC), Saudi Arabia |
| Water Bath | Grant Ltd., England. |
| Wire Loop Holder | Baird & Tatlock, England |

**3.1.6. TEST CONTROL ORGANISMS:**

Standard strains of the test organisms were obtained from the National Collection of Type Cultures (NCTC), England. The following organisms were used:

| | |
|---|---|
| *Escherichia coli* | NCTC 10418 |
| *Staphylococcus aureus* | NCTC 7447 |
| *Pseudomonas aeruginosa* | NCTC 6750 |

**3.1.7. Pathogenic *E.coli* Immune Sera:**

| | |
|---|---|
| Polyvalent 1: | Denka Seiken Co., Ltd. Japan. |
| Polyvalent 2: | Denka Seiken Co., Ltd. Japan. |
| Polyvalent 3: | Denka Seiken Co., Ltd. Japan. |
| Polyvalent 4: | Denka Seiken Co., Ltd. Japan. |
| Polyvalent 5: | Denka Seiken Co., Ltd. Japan. |
| Polyvalent 6: | Denka Seiken Co., Ltd. Japan. |
| Polyvalent 7: | Denka Seiken Co., Ltd. Japan. |

| | |
|---|---|
| Polyvalent 8: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O1: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O26: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O86a: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O111: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O119: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O127a: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O128: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O44: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O55: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O125: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O126: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O146: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O166: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O18: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O114: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O142: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O151: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O157: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O158: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O6: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O27: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O78: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O148: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O159: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O168: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O20: | Denka Seiken Co., Ltd. Japan. |

| | |
|---|---|
| Monovalent O25: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O63: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O153: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O167: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O8: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O15: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O115: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O169: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O28ac: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O112ac: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O124: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O136: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O144: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O29: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O143: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O152: | Denka Seiken Co., Ltd. Japan. |
| Monovalent O164: | Denka Seiken Co., Ltd. Japan. |

### 3.2. STUDY POPULATION (INFANTS):

Two hundred stool samples were collected from infants, aged 1 – 24 months, who suffered from diarrhoea and were admitted to different emergency units in Khartoum State hospitals.

### 3.3. STUDY AREA:

The survey included different hospitals in Khartoum state – Sudan. These were: Muzammil Aba-Yazied hospital (Mayo area), Ibrahim Malik hospital (Sahafa area), Omdurman Pediatric Teaching Hospital (Omdurman Governorate) and Ahmed Gasim Pediatric Hospital (Khartoum North).

As a supplement to data collection a questionnaire was distributed to be filled up by children's parents or guardians. The questionnaire involved, amongst common particulars, inquiries on place of residence, water supply sources, breast or bottle-feeding, previous hospitalization or clinic visits and previous treatment, where it occurred. The age of the infants was classified into two categories; 1 – 12 months and 13 – 24 months, postulating a possible variance in susceptibility to diarrhea.

### 3.4. METHODS:

#### 3.4.1. SPECIMENS:

For the purpose of present work a Carry-Blair transport medium was used. The medium was prepared, sterilized and distributed into 10ml amounts to 20ml sterile universal bottles with screw caps. The specimens were collected in the early stage of the disease, when the pathogens usually were present in the stool in high numbers. Ideally, a stool is preferred rather than a rectal swab. Practically, however, there are situations in which a rectal swab may be used, such as, when it is desirable to collect the faecal specimens immediately or

when rapid transport of the stool to the laboratory is difficult (WHO, 1987.; 1999).

### 3.4.2. SAMPLING:

For the collection of stool, clean containers of sufficient size and having a tight–fitting leak-proof lid were used. The container was autoclaved or rinsed with boiling water before use. When the collected stool was returned to the laboratory, it was processed immediately or as soon as possible, but not longer than two hours after collection (WHO, 1987).

The specimens, which were collected and returned back to the hospital laboratory, were investigated immediately for general routine tests such as: Consistency; Colour; Presence of Protozoa, Helminthes, Eggs, Blood, Mucus, Pus cells; Chemical reaction.

Before the general investigation a swab-full was taken from the stool container and immersed in a bottle of Carry-Blair transport medium.

The inoculated Carry-Blair medium was returned back to the Bacteriology Research Laboratory in the Department of Pharmaceutics – University of Khartoum. There the bacteriological investigations were carried out.

### 3.4.3. CULTIVATION & ISOLATION OF ORGANISMS:

When they were received at the bacteriology laboratory, the swabs, already inserted in Carry-Blair medium, were then inoculated on suitable Petri dishes of differential and selective culture media. The following media were used:

**(a) MacConkey's agar:**

As a differential-selective medium, MacConkey's agar was used for the cultivation of Enterobacteria and for the differentiation between lactose-fermenting and non-lactose fermenting enterobacteria.

**(b) Nutrient agar:**

It was used as an ordinary culture medium for culturing bacteria that have no special nutritional requirements.

**(c) Peptone water:**

As a tryptophane-containing medium it was used for detecting indole production (an important character of *E. coli*).

**(d) Kligler's Iron Agar (KIA):**

This differential medium was used to differentiate between members of enterobacteriaceae. It was significantly used for the detection of lactose and glucose fermentation, evolution of gas during fermentation and production of hydrogen sulphide.

### 3.4.3.1. INOCULATION OF THE PLATES

A swab from Carry-Blair bottle was inoculated on the sterile plates of MacConkey's agar and then streaked by means of a wire loop. The plates were incubated at 37ºC for 24 hours. All samples were cultured and incubated under aerobic conditions. On MacConkey's agar plates, the lactose and non-lactose fermenting colonies were isolated. Subcultures were made on a new sterile MacConkey's agar and Nutrient agar plates.

### 3.4.4. PURIFICATION OF THE ISOLATED COLONIES:

The purification was done by streaking the organisms on Nutrient agar. Single colonies of purified cultures were further subcultured on a sloping agar incubated aerobically at 37ºC for 24 hours and then preserved in the refrigerator at 4ºC until they were used.

### 3.4.5. MICROSCOPICAL EXAMINATION OF THE ISOLATES:

All these isolates were subjected to microscopical examination to study their morphology and staining properties using the Gram's staining technique, which is the most important of staining methods in bacteriology, and must be employed for the diagnostic identification of various organisms (Cruickshank *et al*., 1975).

The bacterial films were prepared, fixed and stained with crystal violet stain for 30 – 60 seconds; then the film was washed with water and followed by iodine application, which acts as a mordant, for one minute. Then the iodine was washed off with water. The film was decolorized rapidly with ethyl alcohol for few seconds and washed immediately with water. The smear was covered with safranin, as a counter-stain, for two minutes, and then washed with clean water. The film was, then, dried and examined microscopically with the oil immersion objective lens to look for bacteria. If Gram's method is properly carried out, Gram–negative organisms are stained red because they lose their color, after being stained with crystal violet, when treated with decolorizer (ethanol) and stained red with counter–stain (safranin), whilst the Gram–positive organisms are stained dark violet and will not lose their violet color with decolorizer (ethanol).

### 3.4.6. CULTURAL CHARACTERISTICS OF THE ISOLATES:

*Escherichia coli* isolates on MacConkey's agar were lactose fermenting, having 2 – 4 mm colonies, which were pink in colour.

### 3.4.7. BIOCHEMICAL TESTS ADOPTED FOR IDENTIFICATION OF BACTERIAL ISOLATES:

#### 3.4.7.1. INDOLE PRODUCTION TEST:

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. This test

is important for the identification of enterobacteria (Cruickshank *et al*., 1975). Most strains of *E.coli* breakdown the amino acid tryptophane with the formation of indole.

The 0.5ml of kovac's reagent is added to a 48 hours culture of the test organism grown on a suitable tryptophane rich medium, mainly peptone water. A red colour in the alcohol layer indicates a positive reaction.

**3.4.7.2. OXIDASE TEST:**

This test depends on the presence of oxidase enzyme, in certain bacteria, that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye (tetramethyl-*p*-phenylene-diamine dihydrochloride) (Cruickshank *et al*., 1975). A piece of filter paper is soaked with a few drops of oxidase reagent (tetramethyl-*p*-phenylene-diamine dihydrochloride). A colony of the test organism is then smeared on the filter paper. If the organism is oxidase–producer, the phenylenediamine in the reagent will be oxidized into a deep purple colour.

**3.4.7.3. UREASE TEST:**

This test was run to test urease enzyme activity, and it was important in recognizing enterobacteria. The test is carried out by heavily inoculating slopes of Christensen's medium, which contains urea, with test organism and incubated at 37ºC for up to five days and examined daily. When the strain is urease producer, the enzyme would breakdown the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium became alkaline as shown by a change in colour of the indicator (Phenol Red) to red-pink (Cheesbrough, 1985).

**3.4.7.4. CITRATE UTILIZATION TEST:**

This test was based on the ability of an organism to use citrate as its only source of carbon and ammonia, also as its only source of nitrogen. The test was

carried out by inoculation of Simmon's citrate medium (by zigzag form) with a broth culture of the test organism. The medium was incubated at 37ºC for up to four days and checked daily for growth in the medium shown by appearance of visible colonies and change in colour of the indicator (Bromothymol Blue) from light green to blue had occurred, due to the alkaline reaction, following citrate utilization. Positive cultures were further subcultured on new bottles of Simmon's citrate medium to confirm positivity (Cheesbrough, 1985).

**3.4.7.5. OXIDATION FERMENTATION (O-F) TEST:**

This test was run to differentiate those organisms that oxidize carbohydrates (aerobic utilization), from those organisms that fermented carbohydrates (anaerobic utilization). The test organism was inoculated into two tubes of a peptone agar medium containing glucose and the indicator Bromothymol Blue. The inoculated medium in one tube was sealed with a layer of sterile liquid paraffin to exclude oxygen. The tubes were incubated at 37ºC for up to 14 days. Fermentative organisms utilized the carbohydrate in both the open and sealed tubes as shown by a change in colour of the medium from green to yellow. Oxidative organisms were, however, able to use the carbohydrate in the open tube only (Cheesbrough, 1985).

**3.4.7.6. VOGES – PROSKAUER (V-P) TEST:**

This test was used to assist in the recognition of enterobacteria. Many bacteria fermented carbohydrates with the production of acetyl methyl carbinol (acetoin) or its reduction product 2,3,butylene glycol. The test organism was cultured in Glucose Phosphate Peptone Water for 48 hours at 37ºC. Sodium Hydroxide and a small amount of creatine were then added. Under alkaline conditions and exposure to the air, a pink colour within 2 – 5 minutes, which

becomes crimson in 30 minutes, indicated a positive reaction for acetoin production (Cruickshank *et al*., 1975).

**3.4.7.7. METHYL RED (M. R.) TEST:**

The methyl red test was implicated to detect the production of sufficient amount of acid during the fermentation of glucose. The test was performed by inoculating a colony of the test organism in 5ml of sterile Glucose Phosphate Peptone Water. After overnight incubation at 37ºC, a drop of Methyl Red solution was added. A positive methyl red test was shown by the appearance of a bright red colour, indicating acidity.

**3.4.7.8. KLIGLER'S IRON AGAR (KIA)**

The test organism was subcultured onto KIA by stabbing the agar and then streaking the slant with a zigzag configuration. Screw–cap KIA tubes were incubated overnight at 37ºC. All members of the family *Enterobacteriaceae* fermented glucose, producing acid (yellow butt) or acid and gas on KIA. A yellow butt, yellow slant indicated that the organism has fermented glucose and lactose, whereas a yellow butt, red slant (alkaline) indicated that the organism has fermented glucose but not lactose. A red butt, red slant indicated that the organism couldn't ferment neither glucose nor lactose. Cracks or vacuoles in lower part or inside the medium indicated the production of gas during fermentation. Hydrogen sulphide production is indicated by the blackening that occurred along the stab line or throughout the medium (WHO, 1987).

**3.4.7.9. SUGAR FERMENTATION TEST:**

10ml of indicator solution (Bromocresol purple 0.2%) were added to 900 ml of peptone water and sterilized at 115ºC for 20 min. 5 – 10g of the

appropriate sugar dissolved in 90 ml of sterile distilled water and then steamed for 30 min. The steamed sugar solution has been added to the sterile peptone water with indicator. After mixing the fluid was distributed into sterile test tubes with inverted inner (Durham) tubes and then steamed for 30 min. (Cowan & Steel, 1999).

Peptone water sugar medium was inoculated with the test organism and examined, daily for 7 days, for acid or acid and gas production. The negative tests were examined at regular intervals for up to 30 days.

The indicator bromocresol purple gave a purple color in the alkaline condition, and changed to a yellow color when the pH was acidic. When the test organism attacked the carbohydrate in the sugar medium and produced sufficient amount of acids the pH became acidic and the color of the medium would change to a yellow color. Where there was a production of gas, it was collected by means of inverted Durham tube.

**3.4.8. SEROTYPING:**

**3.4.8.1. SLIDE AGGLUTINATION TEST USING LIVE ORGANISM:**

Polyvalent and Monovalent agglutination tests were carried out on a clean glass slide. By means of a straight wire, a portion of the growth was removed from nutrient agar plates and emulsified in a drop of physiological saline. It was mixed thoroughly by tilting back and forth for about 30 seconds, and then examined carefully to ensure that the suspension was smooth and did not show clumping due to autoagglutination. Where clumping occurred, the culture was rough and could not be serotyped. When the suspension was smooth (turbid and free–flowing), one drop of antiserum was added, mixed well using the loop and observed for agglutination over a period of 60 seconds against a dark background. When the reaction was positive, clumping appeared within 30 seconds to 1 minute (WHO, 1987).

When a positive reaction was observed with one of the polyvalent sera, a slide agglutination test was carried out in the same way as described above using monovalent sera comprising the polyvalent serum which was agglutinated.

**3.4.8.2. SLIDE AGGLUTINATION TEST USING HEATED CELLS:**

When one monovalent serum showed agglutination, the live cell suspension densely suspended in physiological saline heated at 100°C for one hour to check that the serum produced agglutination of the heated cells as well as of the live cells.

**3.4.8.3. INTERPRETATION OF RESULTS:**

When both the live and heated cells of a bacterium, whose biochemical characteristics were found to be identical to those of *E.coli*, agglutinated in the reaction with one of the monovalent sera, the organism was regarded as pathogenic *E.coli* belonging to the sero-group representing the monovalent serum. However, where only live cells agglutinated (and heated cells did not), then the case was excluded from the interpretation.

**3.4.9. ANTIBIOTIC SENSITIVITY TEST FOR CLINICAL ISOLATES USING THE DISC – DIFFUSION TECHNIQUE:**

Laboratory antimicrobial sensitivity testing was performed using a diffusion technique or a dilution technique. Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity, because this proved to be the easiest method and the technique was recommended by the WHO committee and the association of clinical pathologists (David, 1980).

Amongst the numerous diffusion methods the paper-disc was selectively chosen, for being simple and less complicated, despite the fact that, unlike the cup-plate method, the drug had to diffuse first from the disc and then through the agar medium, the thing that might alter the size of the resultant inhibition

zone, (Kavangh,1972). Yet the inhibition zones could be interpreted in accordance with the WHO standards, which gave accurate results.

**3.4.9.1. PREPARATION OF PLATES:**

Twenty ml of sterile Muller& Hinton Sensitivity Agar medium was poured into sterile plates. After medium has solidified, the plates were dried for 30 minutes in an incubator at 37ºC to remove excess moisture from the surface.

**3.4.9.2. PREPARATION OF INOCULUM AND INOCULATION:**

This preparation was done by emulsifying 2 – 3 colonies of the test organism in a small amount (about 3 – 5ml) of sterile normal saline or nutrient broth. In order to prevent further growth the diluted and standardized inoculum was not allowed to stand longer than 15 – 20 minutes before the plates are inoculated. A sterile cotton swab was dipped into a suitable diluted culture or suspension and rotated; the swab is turned against the side of the bottle to remove excess fluid, and was streaked across the medium (David, 1980).

**3.4.9.3. APPLICATION OF SENSITIVITY TEST:**

The bacteriostatic action of the individual antibiotics against the clinical isolates was determined using disc-diffusion technique.

This method involves paper disc, onto which a known amount of drug was applied and dried. Not more than six discs were tested on a plate and certainly not more than eight (Cruickshank *et al*., 1975).

Multiple discs containing a variety of different drugs were, as commonly, used during this study. Single discs from Oxoid Ltd. were removed from their respective containers and carefully placed on the surface of the inoculated medium with the help of disc-dispenser. Finally, the disc pressed lightly with the sterile forceps to make complete contact with the surface of medium.

The antimicrobial diffused from the disc into the medium. Following overnight incubation at 37ºC, the culture was examined for areas of no growth around the disc (inhibition zone).

### 3.4.9.4. THE INTERPRETATION OF THE INHIBITION ZONE DIAMETER DATA:

The interpretation of the inhibition zone diameters was based on the following data (WHO, 1997):

**Ampicillin:**

≥ 17mm: Susceptible-S; 14-16mm: Intermediate-I;

≤ 13mm: Resistant-R.

**Gentamycin:**

≥ 15mm: Susceptible-S; 13-14mm: Intermediate-I;

≤ 12mm: Resistant-R.

**Nalidixic acid:**

≥ 19mm: Susceptible-S; 14-18mm: Intermediate-I;

≤ 13mm: Resistant-R.

**Tetracycline:**

≥ 19mm: Susceptible-S; 14-18mm: Intermediate-I;

≤ 14mm: Resistant-R.

**Chloramphenicol:**

≥ 18mm: Susceptible-S; 13-17mm: Intermediate-I;

≤ 12mm: Resistant-R.

**Sulphamethoxazole:**

≥ 17mm: Susceptible-S; 13-16mm: Intermediate-I;

≤ 12mm: Resistant-R.

**Trimethoprim:**

≥ 17mm: Susceptible-S; 13-16mm: Intermediate-I;

≤ 12mm: Resistant-R.

**Amoxicillin:**

≥ 18mm: Susceptible-S; 14-17mm: Intermediate-I;

≤ 13mm: Resistant-R.

**Ciprofloxacin:**

≥ 21mm: Susceptible-S; 16-20mm: Intermediate-I;

≤ 15mm: Resistant-R.

# 4. RESULTS

## 4.1 Introduction:

In the present work, 234 bacterial isolates were isolated from 200 stool specimens, obtained from different Khartoum and Omdurman hospitals. All the specimens were collected from infants of 1 to 24 months of age, who suffered diarrhoea. The clinical isolates gave an appreciable bacterial growth of organisms on culture.

The tests used for the identification of individual bacteria were inapplicable to single cells and required to be done on a large population of a

culture. Therefore, the ultimate unit to be identified was the pure culture. The pure cultures that were considered to consist, exclusively, of the progeny of a single cell and not to include any demonstrable mutant cells were described as colonies. A population of bacteria presumed to descend from a single ancestral bacterium, as found in a natural habitat and in primary cultures, from that habitat and subcultures, from the primary cultures, was called strain according to Duguid *et al*, (1978).The method of Cowan and Steel, (1999), was adopted for the identification steps, which were started by microscopical examination of properly prepared films that had been stained by Gram's staining technique.

The samples were inoculated on appropriate differential and selective culture media and the purified isolated colonies were subjected to an identification scheme based on the cultural characteristics, the microscopical examination and the biochemical reactions, besides the ability to produce acids from selectively prescribed carbohydrates.

The isolates, which were identified as *Escherichia coli* had, then, been subjected to serotyping.

## 4.2 Main Findings:

All the microorganisms, involved as causatives of infantile diarrhoea were isolated from the stool samples and their percentage representation was tabulated, (table 4-1). An explicit survey over the representation of each isolate obtained from the samples was demonstrated by fig 4-1

.

**Table 4-1: Intestinal Organisms Isolated From Infants With Diarrhoea**

| Organisms | No of Cultures | Percentage[%] |
|---|---|---|
| *E. coli* | 141 | 60.3 |
| *Proteus* | 23 | 9.8 |
| *Shigella* | 14 | 6.0 |
| *Salmonella* | 13 | 5.6 |
| *Klebsiella* | 13 | 5.6 |
| *Pseudomonas* | 13 | 5.6 |
| *Citrobacter* | 12 | 5.1 |
| *Morganella* | 4 | 1.7 |
| *Enterobacter* | 1 | 0.4 |
| **Total** | **234** | **100** |

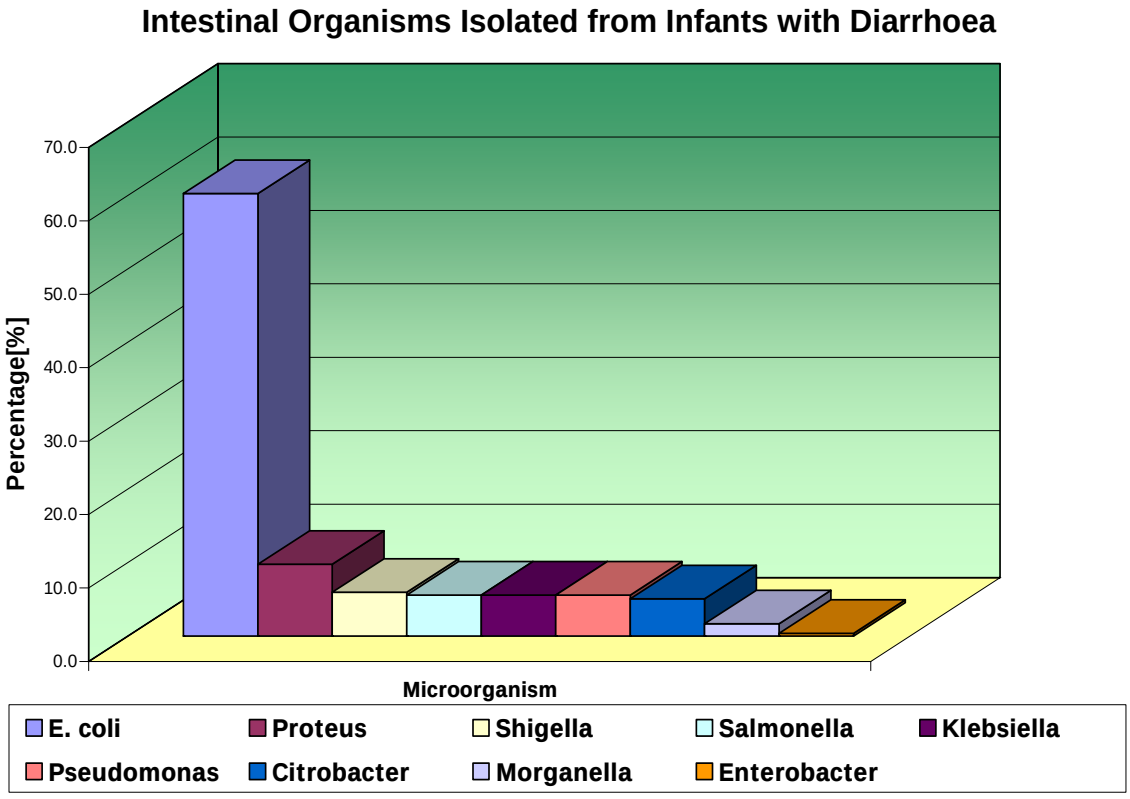


**Fig 4-1**

### 4.2.1 *Escherichia coli* Isolates:

#### 4.2.1.1 Cultural characters:

On MacConkey agar medium large rose colonies were observed, indicating lactose fermentation.

On Eosine Methylene blue agar, metallic green sheen colonies were detected.

#### 4.2.1.2 Microscopical Examination

Gram-negative non- spore forming rods were seen.

#### 4.2.1.3 Biochemical Reactions:

All the isolates were found to be lactose fermenters, indole-positive, methyl red-positive, Voges-Proskauer-negative, oxidase-negative, urease-negative, citrate-negative and $H_2S$ gas production-negative (table 4-2). Acids and gas were produced from the majority of sugars tested, (table 4-4).

On Kligler's iron agar, yellow slope and yellow butt were observed, indicating the fermentation of both glucose and lactose. Also cracks due to the evolution of a produced gas, but not $H_2S$, were clearly observed.

**Table 4-2:** **Biochemical Identification of Organism**

**141 Isolates of *E. coli***

| Result / Test | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| **Gram Reaction*** | 0 | 0.0% | 141 | 100.0% |
| **Oxidase** | 0 | 0.0% | 141 | 100.0% |
| **Motility** | 141 | 100.0% | 0 | 0.0% |
| **Indole** | 141 | 100.0% | 0 | 0.0% |
| **M.R** | 141 | 100.0% | 0 | 0.0% |
| **V-P** | 0 | 0.0% | 141 | 100.0% |
| **Citrate** | 0 | 0.0% | 141 | 100.0% |
| **Urease** | 0 | 0.0% | 141 | 100.0% |
| **KIA Slope R** | 0 | 0.0% | 141 | 100.0% |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | **Y** | 141 | 100.0% | 0% | 0.0% |
| | **Butt** | **R** | 0 | 0.0% | 141 | 100.0% |
| | | **Y** | 141 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 0 | 0.0% | 141 | 100.0% |
| | **Gas** | | 141 | 100.0% | 0 | 0.0% |
| **O-F** | | **O** | 0 | 0.0% | 141 | 100.0% |
| | | **F** | 141 | 100.0% | 0 | 0.0% |

| | | | |
|---|---|---|---|
| R | Red | KIA | Kligler's Iron Agar |
| Y | Yellow | O | Oxidative |
| | | F | Fermentative |

**4.2.1.4 Serotyping:**

The isolates were tested with polyvalent and monovalent specific antisera. Agglutination occurred on the spot, (table 4-3a). On the basis of the results of these serotyping tests, it was found that out of 234 isolates from hospitals, 141 were *Escherichia coli*, (table 4-3b), of which:

34 (24%) were classified as Enteropathogenic *E. coli,*

8 (5.7%) were classified as Enteroinvasive *E. coli,*

12 (8.5%) were classified as Enterohaemorrhagic *E. coli,*

16 (11.3%) were classified as Enterotoxigenic *E. coli,*

7 (5%) were classified as Enteroaggregative *E. coli* and

64 (45.5%) were classified as Untypable *E. coli*.

**Table 4-3a Distribution of Pathogenic E. coli O-Serotypes Isolated from Infants with Diarrhoea**

| *E. coli Serotype* | Number | Percentage [%] |
|---|---|---|
| O1 | 2 | 2.6 |
| O18 | 3 | 3.9 |
| O26 | 5 | 6.5 |
| O29 | 7 | 9.1 |
| O55 | 8 | 10.4 |
| O78 | 3 | 3.9 |
| O111 | 10 | 13.0 |
| O114 | 11 | 14.3 |
| O127 | 3 | 3.9 |
| O128 | 7 | 9.1 |
| O142 | 6 | 7.8 |
| O146 | 2 | 2.6 |
| O153 | 5 | 6.5 |
| O157 | 5 | 6.5 |
| **Total** | **77** | **100.0** |

**Table 4-3b: Distribution of Pathogenic *E. Coli* According to Their Virulent Groups**

| Virulent group | Number of Isolates | % out of Typed *E. coli* | % out of all *E. coli* | % out of all Findings |
|---|---|---|---|---|
| Enteropathogenic *E. coli* | 34 | 44.2 | 24.0 | 14.5 |
| Enterohaemorrhagic *E. coli* | 12 | 15.6 | 8.5 | 5.1 |
| Enterotoxigenic *E. coli* | 16 | 20.8 | 11.3 | 6.8 |

| | | | | |
|---|---|---|---|---|
| Enteroinvasive *E. coli* | 8 | 10.4 | 5.7 | 3.4 |
| Enteroaggregative *E. coli* | 7 | 9.0 | 5.0 | 3.0 |
| Total | 77 | 100.0 | 54.5 | 32.8 |

Like many other bacteria, as Taylor, (1961; 1966), had explained, *Escherichia* was subdivided into numerous serotypes; some of which cause infection in man and are especially associated with gastroenteritis of infants. The graphic representation of the different serotypes in fig 4-2 gives a clearer picture of their distribution in the isolates scrutinized.

## Distribution of Pathogenic E. coli According to Their Virulent Groups

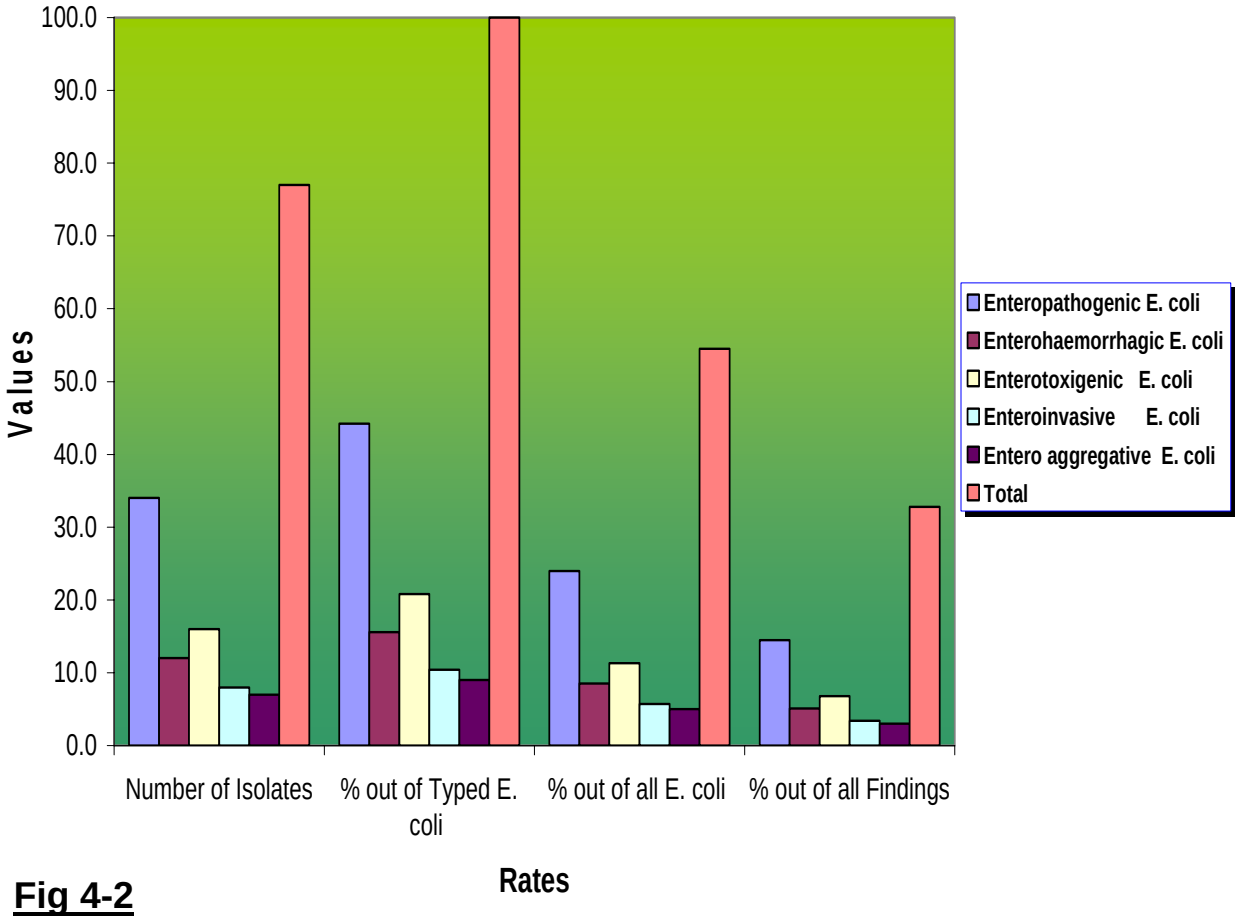


**Fig 4-2**

**Table 4-4**: **Production of Acid and Gas from Sugar**

**141 Isolates of *E. coli***

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 141 | 100% | 0 | 0% |
| Adonitol | 0 | 0% | 141 | 100% |
| Arabinose | 141 | 100% | 0 | 0% |
| Cellobiose | 0 | 0% | 141 | 100% |
| Dulcitol | 141 | 100% | 0 | 0% |
| Glycerol | 141 | 100% | 0 | 0% |
| Inositol | 0 | 0% | 141 | 100% |
| Lactose | 141 | 100% | 0 | 0% |
| Maltose | 141 | 100% | 0 | 0% |
| Mannitol | 141 | 100% | 0 | 0% |
| Raffinose | 141 | 100% | 0 | 0% |
| Rhamnose | 141 | 100% | 0 | 0% |
| Salicin | 141 | 100% | 0 | 0% |
| Sorbitol | 141 | 100% | 0 | 0% |
| Sucrose | 141 | 100% | 0 | 0% |
| Trehalose | 141 | 100% | 0 | 0% |
| Xylose | 141 | 100% | 0 | 0% |
| Starch | 0 | 0% | 141 | 100% |

### 4.2.2 *Proteus* Isolates:

#### 4.2.2.1 Cultural Characters:

On MacConkey agar medium, pale-coloured colonies of non-lactose–fermenting organisms were detected. On nutrient agar, distinctive small colonies with swarming appearance showed up.

Gram-negative non-spore forming and non-capsulated rods were seen.

#### 4.2.2.2 Microscopical Examination:

*Proteus* species showed Gram-negative, non-capsulated, motile rods,.

#### 4.2.2.3 Biochemical reactions:

All of the isolates were non-lactose fermenters, and urease-positive. On Kligler's iron agar, red slope and yellow butt were observed, indicating the fermentation of glucose, but not lactose, accompanied by the evolution of $H_2S$ gas, (Table 4-5). They exhibited variable indole-positive activities. *Proteus* isolates produced acids only from 4 sugars of the 18 test sugars (table 4-6).

### 4.2.3 Pseudomonas Isolates:

#### 4.2.3.1 Cultural Characters:

On MacConkey's agar medium, pale-coloured colonies were detected. A green pigmentation was observed. On nutrient agar, most of the isolates produced blue-green pigments and brown pigments, which diffused in the surrounding medium.

#### 4.2.3.2 Microscopical Examination:

Gram-negative rods were seen. They were non-spore-forming and non-capsulated.

#### 4.2.3.3 Biochemical Reactions:

All the strains showed non-lactose fermentation and were oxidase-positive, (Table 4-7).

*Pseudomonas* species were capable of producing acid from carbohydrates in Ammonium Salt medium,(table 4-8). A large number of *Pseudomonas* species produced water-soluble pigments and sometimes fluorescent pigments as had been described by Skerman, (1967), and Cowan, (1999).

**Table 4-5: Biochemical Identification of Organism**

**23 Isolates of *Proteus***

| Test \ Result | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 23 | 100.0% |
| **Oxidase** | | | 0 | 0.0% | 23 | 100.0% |
| **Motility** | | | 23 | 100.0% | 0 | 0.0% |
| **Indole** | | | 15 | 65.2% | 8 | 34.8% |
| **M.R** | | | 20 | 86.9% | 3 | 13.1% |
| **V-P** | | | 2 | 8.7% | 21 | 91.3% |
| **Citrate** | | | 16 | 69.6% | 7 | 30.4% |
| **Urease** | | | 23 | 100.0% | 0 | 0.0% |
| **KIA** | **Slope** | **R** | 23 | 100.0% | 0 | 0.0% |
| | | **Y** | 0 | 0.0% | 23 | 100.0% |
| | **Butt** | **R** | 0 | 0.0% | 23 | 100.0% |
| | | **Y** | 23 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 23 | 100.0% | 0 | 0.0% |
| | **Gas** | | 17 | 73.9% | 6 | 26.1% |
| **O-F** | | **O** | 0 | 0.0% | 23 | 100.0% |
| | | **F** | 23 | 100.0% | 0 | 0.0% |

R Red

| | | | |
|---|---|---|---|
| Y | Yellow | O | Oxidative |
| KIA | Kligler's Iron Agar | F | Fermentative |

*All isolates exhibited the rod shape.

**Table 4-6: Production of Acid and Gas from Sugar 23 Isolates of *Proteus***

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 23 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 23 | 100.0% |
| Arabinose | 0 | 0.0% | 23 | 100.0% |
| Cellobiose | 0 | 0.0% | 23 | 100.0% |
| Dulcitol | 0 | 0.0% | 23 | 100.0% |
| Glycerol | 23 | 100.0% | 0 | 0.0% |
| Inositol | 0 | 0.0% | 23 | 100.0% |
| Lactose | 0 | 0.0% | 23 | 100.0% |
| Maltose | 0 | 0.0% | 23 | 100.0% |
| Mannitol | 0 | 0.0% | 23 | 100.0% |
| Raffinose | 0 | 0.0% | 23 | 100.0% |
| Rhamnose | 0 | 0.0% | 23 | 100.0% |
| Salicin | 0 | 0.0% | 23 | 100.0% |
| Sorbitol | 0 | 0.0% | 23 | 100.0% |
| Sucrose | 0 | 0.0% | 23 | 100.0% |
| Trehalose | 23 | 100.0% | 0 | 0.0% |
| Xylose | 23 | 100.0% | 0 | 0.0% |
| Starch | 0 | 0.0% | 23 | 100.0% |

On Kligler's iron agar, a red slope and a red butt, indicating neither the fermentation of glucose nor of lactose, were shown.

### 4.2.4 *Shigella* Isolates:

#### 4.2.4.1 Cultural Characters:

On the MacConkey's agar medium, non-lactose fermenting colonies were detected.

#### 4.2.4.2 Microscopical Examination:

Non-spore forming and non-capsulated Gram-positive rods were seen.

#### 4.2.4.3 Biochemical Reactions:

On Kligler's iron agar medium, red slope and yellow butt were observed, indicating the fermentation of glucose, but not lactose. There was no sign of $H_2S$ gas production. As, previously, mentioned by Cruickshank *et al*., (1975), and Cowan, (1999), the organism showed indole-positive, urease negative, non-motile reactions, (table 4-9).All of the strains produced acid from the majority of sugars tested,(table 4-10).

**Table 4-7: Biochemical Identification of Organism**

**13 Isolates of *Pseudomonas***

| Test \ Result | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 13 | 100.0% |
| **Oxidase** | | | 13 | 100.0% | 0 | 0.0% |
| **Motility** | | | 13 | 100.0% | 0 | 0.0% |
| **Indole** | | | 0 | 0.0% | 13 | 100.0% |
| **M.R** | | | 0 | 0.0% | 13 | 100.0% |
| **V-P** | | | 0 | 0.0% | 13 | 100.0% |
| **Citrate** | | | 13 | 100.0% | 0 | 0.0% |
| **Urease** | | | 13 | 100.0% | 0 | 0.0% |
| **KIA** | **Slope** | **R** | 13 | 100.0% | 0 | 0.0% |
| | | **Y** | 0 | 0.0% | 13 | 100.0% |
| | **Butt** | **R** | 13 | 100.0% | 0 | 0.0% |
| | | **Y** | 0 | 0.0% | 13 | 100.0% |
| | **$H_2S$** | | 0 | 0.0% | 13 | 100.0% |
| | **Gas** | | 0 | 0.0% | 13 | 100.0% |
| **O-F** | | **O** | 13 | 100.0% | 0 | 0.0% |
| | | **F** | 0 | 0.0% | 13 | 100.0% |

R Red

| | |
|---|---|
| Y | Yellow |
| KIA | Kligler's Iron Agar |
| O | Oxidative |
| F | Fermentative |

*All isolates exhibited the rod shape.

**Table 4-8**: **Production of Acid and Gas from Sugar**

**13 Isolates of *Pseudomonas* in Ammonia Salt Medium**

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of –ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 13 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 13 | 100.0% |
| Arabinose | 13 | 100.0% | 0 | 0.0% |
| Cellobiose | 0 | 0.0% | 13 | 100.0% |
| Fructose | 13 | 100.0% | 0 | 0.0% |
| Dulcitol | 0 | 0.0% | 13 | 100.0% |
| Glycerol | 13 | 100.0% | 0 | 0.0% |
| Inositol | 0 | 0.0% | 13 | 100.0% |
| Lactose | 0 | 0.0% | 13 | 100.0% |
| Maltose | 0 | 0.0% | 13 | 100.0% |
| Mannitol | 13 | 100.0% | 0 | 0.0% |
| Raffinose | 0 | 0.0% | 13 | 100.0% |
| Rhamnose | 0 | 0.0% | 13 | 100.0% |
| Salicin | 0 | 0.0% | 13 | 100.0% |
| Sorbitol | 0 | 0.0% | 13 | 100.0% |
| Sucrose | 0 | 0.0% | 13 | 100.0% |

| | | | | |
|---|---|---|---|---|
| Trehalose | 12 | 92.3% | 1 | 7.7% |
| Xylose | 13 | 100.0% | 0 | 0.0% |

**Table 4-9: Biochemical Identification of Organism**

**14 Isolates of *Shigella***

| Result / Test | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 14 | 100.0% |
| **Oxidase** | | | 0 | 0.0% | 14 | 100.0% |
| **Motility** | | | 0 | 0.0% | 14 | 100.0% |
| **Indole** | | | 14 | 100.0% | 0 | 0.0% |
| **M.R** | | | 14 | 100.0% | 0 | 0.0% |
| **V-P** | | | 0 | 0.0% | 14 | 100.0% |
| **Citrate** | | | 0 | 0.0% | 14 | 100.0% |
| **Urease** | | | 0 | 0.0% | 14 | 100.0% |
| **KIA** | **Slope** | **R** | 14 | 100.0% | 0 | 0.0% |
| | | **Y** | 0 | 0.0% | 14 | 100.0% |
| | **Butt** | **R** | 0 | 0.0% | 14 | 100.0% |
| | | **Y** | 14 | 100.0% | 0 | 0.0% |

| | | | | | | |
|---|---|---|---|---|---|---|
| | $H_2S$ | | 0 | 0.0% | 14 | 100.0% |
| | **Gas** | | 0 | 0.0% | 14 | 100.0% |
| **O-F** | | **O** | 0 | 0.0% | 14 | 100.0% |
| | | **F** | 14 | 100.0% | 0 | 0.0% |

R Red O Oxidative
Y Yellow F Fermentative
KIA Kligler Iron Agar

*All isolates exhibited the rod shape.

**Table 4-10: Production of Acid and Gas from Sugar**

**14 Isolates of *Shigella***

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 14 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 14 | 100.0% |
| Arabinose | 14 | 100.0% | 0 | 0.0% |
| Cellobiose | 0 | 0.0% | 14 | 100.0% |
| Dulcitol | 14 | 100.0% | 0 | 0.0% |
| Glycerol | 14 | 100.0% | 0 | 0.0% |
| Inositol | 0 | 0.0% | 14 | 100.0% |
| Lactose | 0 | 0.0% | 14 | 100.0% |
| Maltose | 14 | 100.0% | 0 | 0.0% |
| Mannitol | 14 | 100.0% | 0 | 0.0% |
| Raffinose | 14 | 100.0% | 0 | 0.0% |
| Rhamnose | 14 | 100.0% | 0 | 0.0% |
| Salicin | 14 | 100.0% | 0 | 0.0% |

| | | | | |
|---|---|---|---|---|
| Sorbitol | 14 | 100.0% | 0 | 0.0% |
| Sucrose | 14 | 100.0% | 0 | 0.0% |
| Trehalose | 14 | 100.0% | 0 | 0.0% |
| Xylose | 14 | 100.0% | 0 | 0.0% |
| Starch | 0 | 0.0% | 14 | 100.0% |

### 4.2.5 *Salmonella* Isolates:

#### 4.2.5.1 Cultural Characters:

On the MacConkey's agar medium, pale-coloured colonies were observed indicating non-lactose fermenting nature.

#### 4.2.5.2 Microscopical Examination:

Non-spore forming, Gram-negative rods were seen.

#### 4.2.5.3 Biochemical Reactions:

On Kligler's iron agar medium, the red slope and yellow butt were observed, indicating the fermentation of glucose, but not lactose, (Table 4-11) The $H_2S$ gas evolution was observed. Different results were detected when tested for production of acid from carbohydrates (table 412).

### 4.2.6 *Klebsiella* Isolates:

#### 4.2.6.1 Cultural Characters:

On the MacConkey's agar medium, large mucoid Ping colonies were observed, indicating lactose fermentation.

#### 4.2.6.2 Microscopical Examination:

Gram-negative rods were observed. They were non-spore-forming, and non-capsulated.

#### 4.2.6.3 Biochemical Reactions:

On Kligler's iron agar medium, yellow slope and yellow butt were observed, indicating the fermentation of both glucose and lactose. Cracks due to a gaseous evolution were detected but no $H_2S$ gas was produced.

The isolate showed a VogesPreskauer-positive and a Methyl red-negative reaction, (table 4-13). Acid produced from all sugars tested, (4-14).

### 4.2.7 *Citrobacter freundii* Isolates:

#### 4.2.7.1 Cultural Characteristics:

On MacConkey's agar medium, lactose fermenting colonies of a faint pink colour appeared.

#### 4.2.7.2 Microscopical Examination:

Gram-negative rods were observed. They were nonspore-forming and non-capsulated.

#### 4.2.7.3 Biochemical Reactions:

On Kligler's iron agar medium a yellow slope and a yellow butt indicated that the fermentation of both glucose and lactose occurred. The evolution of $H_2S$ gas was evident and the reactions proved positive for indole as well as urease and citrate test, as described in table, (4-15). Acid produced from the most sugars selectively tested, (table 4-16).

**Table 4-11: Biochemical Identification of Organism**

**13 Isolates of *Salmonella***

| Result / Test | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| **Gram Reaction*** | 0 | 0.0% | 13 | 100.0% |
| **Oxidase** | 0 | 0.0% | 13 | 100.0% |
| **Motility** | 13 | 100.0% | 0 | 0.0% |
| **Indole** | 0 | 0.0% | 13 | 100.0% |
| **M.R** | 13 | 100.0% | 0 | 0.0% |

| | | | | | | |
|---|---|---|---|---|---|---|
| **V-P** | | | 0 | 0.0% | 13 | 100.0% |
| **Citrate** | | | 13 | 100.0% | 0 | 0.0% |
| **Urease** | | | 0 | 0.0% | 13 | 100.0% |
| **KIA** | **Slope** | **R** | 13 | 100.0% | 0 | 0.0% |
| | | **Y** | 0 | 0.0% | 13 | 100.0% |
| | **Butt** | **R** | 0 | 0.0% | 13 | 100.0% |
| | | **Y** | 13 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 13 | 100.0% | 0 | 0.0% |
| | **Gas** | | 10 | 76.9% | 3 | 23.1% |
| **O-F** | | **O** | 0 | 0.0% | 13 | 100.0% |
| | | **F** | 13 | 100.0% | 0 | 0.0% |

R Red F Fermentative O Oxidative
Y Yellow
Kligler Iron
KIA Agar
*All isolates exhibited the rod shape.

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 13 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 13 | 100.0% |
| Arabinose | 8 | 61.5% | 5 | 38.5% |
| Cellobiose | 0 | 0.0% | 13 | 100.0% |
| Dulcitol | 12 | 92.3% | 1 | 7.7% |
| Glycerol | 13 | 100.0% | 0 | 0.0% |
| Inositol | 0 | 0.0% | 13 | 100.0% |
| Lactose | 0 | 0.0% | 13 | 100.0% |
| Maltose | 13 | 100.0% | 0 | 0.0% |
| Mannitol | 13 | 100.0% | 0 | 0.0% |
| Raffinose | 0 | 0.0% | 13 | 100.0% |
| Rhamnose | 8 | 61.5% | 5 | 38.5% |
| Salicin | 0 | 0.0% | 13 | 100.0% |
| Sorbitol | 13 | 100.0% | 0 | 0.0% |
| Sucrose | 0 | 0.0% | 13 | 100.0% |
| Trehalose | 13 | 100.0% | 0 | 0.0% |
| Xylose | 5 | 38.5% | 8 | 61.5% |

**Table 4-13: Biochemical Identification of Organism**

**13 Isolates of *Klebsiella***

| Test \ Result | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 13 | 100.0% |
| **Oxidase** | | | 0 | 0.0% | 13 | 100.0% |
| **Motility** | | | 0 | 0.0% | 13 | 100.0% |
| **Indole** | | | 0 | 0.0% | 13 | 100.0% |
| **M.R** | | | 0 | 0.0% | 13 | 100.0% |
| **V-P** | | | 13 | 100.0% | 0 | 0.0% |
| **Citrate** | | | 13 | 100.0% | 0 | 0.0% |
| **Urease** | | | 13 | 100.0% | 0 | 0.0% |
| **KIA** | **Slope** | **R** | 0 | 0.0% | 13 | 100.0% |
| | | **Y** | 13 | 100.0% | 0 | 0.0% |
| | **Butt** | **R** | 0 | 0.0% | 13 | 100.0% |
| | | **Y** | 13 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 0 | 0.0% | 13 | 100.0% |
| | **Gas** | | 13 | 100.0% | 0 | 0.0% |
| **O-F** | | **O** | 0 | 0.0% | 13 | 100.0% |
| | | **F** | 13 | 100.0% | 0 | 0.0% |

Y Yellow KIA Kligler's Iron Agar F Fermentative

R Red O Oxidative

*All isolates exhibited the rod shape.

**Table 4-14: Production of Acid and Gas from Sugar**

**13 Isolates of *Klebsiella***

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 13 | 100.0% | 0 | 0.0% |
| Adonitol | 13 | 100.0% | 0 | 0.0% |
| Arabinose | 13 | 100.0% | 0 | 0.0% |
| Cellobiose | 13 | 100.0% | 0 | 0.0% |
| Dulcitol | 13 | 100.0% | 0 | 0.0% |
| Glycerol | 13 | 100.0% | 0 | 0.0% |
| Inositol | 13 | 100.0% | 0 | 0.0% |
| Lactose | 13 | 100.0% | 0 | 0.0% |
| Maltose | 13 | 100.0% | 0 | 0.0% |
| Mannitol | 13 | 100.0% | 0 | 0.0% |
| Raffinose | 13 | 100.0% | 0 | 0.0% |
| Rhamnose | 13 | 100.0% | 0 | 0.0% |
| Salicin | 13 | 100.0% | 0 | 0.0% |
| Sorbitol | 13 | 100.0% | 0 | 0.0% |
| Sucrose | 13 | 100.0% | 0 | 0.0% |
| Trehalose | 13 | 100.0% | 0 | 0.0% |
| Xylose | 13 | 100.0% | 0 | 0.0% |
| Starch | 13 | 100.0% | 0 | 0.0% |

### 4.2.8 *Morganella morganii* Isolates:

#### 4.2.8.1 Cultural Characteristics:

On MacConkey's agar medium non-lactose fermenting colonies were observed with a faint colour.

#### 4.2.8.2 Microscopical Examination:

Gram-negative, nonspore-forming and non-capsulated rods showed up.

#### 4.2.8.3 Biochemical Reactions:

On Kligler's iron agar medium the appearance of a red slope and a yellow butt indicated the fermentation of glucose but not lactose, (table 4-17)
The evolution of gas was evident, but not an $H_2S$ production. The isolates gave urease and indole positive reactions and formed no acids with most sugars except for glucose and glycerol, (table 4-18).

**Table 4-15: Biochemical Identification of Organism**

**12 Isolates of *Citrobacter***

| Test \ Result | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 12 | 100.0% |
| **Oxidase** | | | 0 | 0.0% | 12 | 100.0% |
| **Motility** | | | 12 | 100.0% | 0 | 0.0% |
| **Indole** | | | 4 | 33.3% | 8 | 66.7% |
| **M.R** | | | 12 | 100.0% | 0 | 0.0% |
| **V-P** | | | 0 | 0.0% | 12 | 100.0% |
| **Citrate** | | | 12 | 100.0% | 0 | 0.0% |
| **Urease** | | | 12 | 100.0% | 0 | 0.0% |
| **KIA** | **Slope** | **R** | 0 | 0.0% | 12 | 100.0% |
| | | **Y** | 12 | 100.0% | 0 | 0.0% |
| | **Butt** | **R** | 0 | 0.0% | 12 | 100.0% |
| | | **Y** | 12 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 12 | 100.0% | 0 | 0.0% |
| | **Gas** | | 8 | 66.7% | 4 | 33.3% |
| **O-F** | | **O** | 0 | 0.0% | 12 | 100.0% |
| | | **F** | 12 | 100.0% | 0 | 0.0% |

R Red
Y Yellow

KIA Kligler Iron Agar
O Oxidative
F Fermentative

*All isolates exhibited the rod shape

**Table 4-16: Production of Acid and Gas from Sugar**

**12 Isolates of *Citrobacter***

| Test \ Result | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 12 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 12 | 100.0% |
| Arabinose | 12 | 100.0% | 0 | 0.0% |
| Cellobiose | 12 | 100.0% | 0 | 0.0% |
| Dulcitol | 0 | 0.0% | 12 | 100.0% |
| Glycerol | 12 | 100.0% | 0 | 0.0% |
| Inositol | 0 | 0.0% | 12 | 100.0% |
| Lactose | 12 | 100.0% | 0 | 0.0% |
| Maltose | 12 | 100.0% | 0 | 0.0% |
| Mannitol | 12 | 100.0% | 0 | 0.0% |
| Raffinose | 9 | 75.0% | 3 | 25.0% |
| Rhamnose | 12 | 100.0% | 0 | 0.0% |
| Salicin | 0 | 0.0% | 12 | 100.0% |
| Sorbitol | 12 | 100.0% | 0 | 0.0% |
| Sucrose | 8 | 66.7% | 4 | 33.3% |
| Trehalose | 12 | 100.0% | 0 | 0.0% |
| Xylose | 12 | 100.0% | 0 | 0.0% |
| Starch | 0 | 0.0% | 12 | 100.0% |

### 4.2.9 *Enterobacter* Isolates:

#### 4.2.9.1 Cultural Characteristics:

On MacConkey's agar medium lactose fermenting colonies, which were irregular around the edges that went from 1 up to 1.5 mm diameter, and mucoid nature, appeared.

#### 4.2.9.2 Microscopical Examination:

Gram-negative short rods appeared.

#### 4.2.9.3 Biochemical Reactions:

On Kligler's iron agar medium a yellow slope and a yellow butt showed up indicating the fermentation of both glucose and lactose, accompanied by the evolution of a gas but no production of $H_2S$ was evident. The isolates reaction proved positive for citrate but neither to urease nor to indole, (table 4-19) and produced acid from the majority of sugars under test, (4-20).

**Table 4-17: Biochemical Identification of Organism**

**4 Isolates of *Morganella***

| Test \ Result | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 4 | 100.0% |
| **Oxidase** | | | 0 | 0.0% | 4 | 100.0% |
| **Motility** | | | 4 | 100.0% | 0 | 0.0% |
| **Indole** | | | 4 | 100.0% | 0 | 0.0% |
| **M.R** | | | 4 | 100.0% | 0 | 0.0% |
| **V-P** | | | 0 | 0.0% | 4 | 100.0% |
| **Citrate** | | | 0 | 0.0% | 4 | 100.0% |
| **Urease** | | | 4 | 100.0% | 0 | 0.0% |
| **KIA** | **Slope** | **R** | 4 | 100.0% | 0 | 0.0% |
| | | **Y** | 0 | 0.0% | 4 | 100.0% |
| | **Butt** | **R** | 0 | 0.0% | 4 | 100.0% |
| | | **Y** | 4 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 0 | 0.0% | 4 | 100.0% |
| | **Gas** | | 3 | 75.0% | 1 | 25.0% |
| **O-F** | | **O** | 0 | 0.0% | 4 | 100.0% |
| | | **F** | 4 | 100.0% | 0 | 0.0% |

R Red
Y Yellow
KIA Kligler Iron

Agar
O Oxidative
F Fermentative

*All isolates exhibited the rod shape.

**Table 4-18: Production of Acid and Gas from Sugar**

**4 Isolates of *Morganella***

| Result / Test | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 4 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 4 | 100.0% |
| Arabinose | 0 | 0.0% | 4 | 100.0% |
| Cellobiose | 0 | 0.0% | 4 | 100.0% |
| Dulcitol | 0 | 0.0% | 4 | 100.0% |
| Glycerol | 4 | 100.0% | 0 | 0.0% |
| Inositol | 0 | 0.0% | 4 | 100.0% |
| Lactose | 0 | 0.0% | 4 | 100.0% |
| Maltose | 0 | 0.0% | 4 | 100.0% |
| Mannitol | 0 | 0.0% | 4 | 100.0% |
| Raffinose | 0 | 0.0% | 4 | 100.0% |
| Rhamnose | 0 | 0.0% | 4 | 100.0% |
| Salicin | 0 | 0.0% | 4 | 100.0% |
| Sorbitol | 0 | 0.0% | 4 | 100.0% |
| Sucrose | 0 | 0.0% | 4 | 100.0% |
| Trehalose | 0 | 0.0% | 4 | 100.0% |
| Xylose | 0 | 0.0% | 4 | 100.0% |
| Starch | 0 | 0.0% | 4 | 100.0% |

| Test \ Result | | | Total No of +ve Samples | Percentage of +ve Samples | Total No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|---|---|
| **Gram Reaction*** | | | 0 | 0.0% | 1 | 100.0% |
| **Oxidase** | | | 0 | 0.0% | 1 | 100.0% |
| **Motility** | | | 1 | 100.0% | 0 | 0.0% |
| **Indole** | | | 0 | 0.0% | 1 | 100.0% |
| **M.R** | | | 0 | 0.0% | 1 | 100.0% |
| **V-P** | | | 1 | 100.0% | 0 | 0.0% |
| **Citrate** | | | 1 | 100.0% | 0 | 0.0% |
| **Urease** | | | 0 | 0.0% | 1 | 100.0% |
| **KIA** | **Slope** | **R** | 0 | 0.0% | 1 | 100.0% |
| | | **Y** | 1 | 100.0% | 0 | 0.0% |
| | **Butt** | **R** | 0 | 0.0% | 1 | 100.0% |
| | | **Y** | 1 | 100.0% | 0 | 0.0% |
| | $H_2S$ | | 0 | 0.0% | 1 | 100.0% |
| | **Gas** | | 1 | 100.0% | 0 | 0.0% |
| **O-F** | | **O** | 0 | 0.0% | 1 | 100.0% |
| | | **F** | 1 | 100.0% | 0 | 0.0% |

***All isolates exhibited the rod shape.**

R Red
Y Yellow
KIA Kligler Iron Agar
O Oxidative
F Fermentative

**Table 4-20**: **Production of Acid and Gas from Sugar**

**1 Isolate of *Enterobacter***

| Test \ Result | No of +ve Samples | Percentage of +ve Samples | No of -ve Samples | Percentage of -ve Samples |
|---|---|---|---|---|
| Glucose | 1 | 100.0% | 0 | 0.0% |
| Adonitol | 0 | 0.0% | 1 | 100.0% |
| Arabinose | 1 | 100.0% | 0 | 0.0% |
| Cellobiose | 1 | 100.0% | 0 | 0.0% |
| Dulcitol | 0 | 0.0% | 1 | 100.0% |
| Glycerol | 1 | 100.0% | 0 | 0.0% |
| Inositol | 1 | 100.0% | 0 | 0.0% |
| Lactose | 1 | 100.0% | 0 | 0.0% |
| Maltose | 1 | 100.0% | 0 | 0.0% |
| Mannitol | 1 | 100.0% | 0 | 0.0% |
| Raffinose | 1 | 100.0% | 0 | 0.0% |
| Rhamnose | 1 | 100.0% | 0 | 0.0% |
| Salicin | 1 | 100.0% | 0 | 0.0% |
| Sorbitol | 0 | 0.0% | 1 | 100.0% |
| Sucrose | 1 | 100.0% | 0 | 0.0% |
| Trehalose | 1 | 100.0% | 0 | 0.0% |
| Xylose | 1 | 100.0% | 0 | 0.0% |
| Starch | 0 | 0.0% | 1 | 100.0% |

## 4.3 Distribution of Microorganisms Involved in Diarrhoea among the Different Age Groups and according to Gender:

### 4.3.1 Age Groups:

It was observed that isolates of samples from infants with diarrhoea are not distributed evenly amongst children of different age categories. As is shown, in fig 4-12 below, the infection with diarrhoea is more frequent amongst the category of infants of age between 1 to 12 months. Infection with diarrhoea at this stage registered 71.8% of all the isolates under scrutiny, whilst infections amongst the infants' age category from 13 to 24 months constituted the remaining 28.2%.

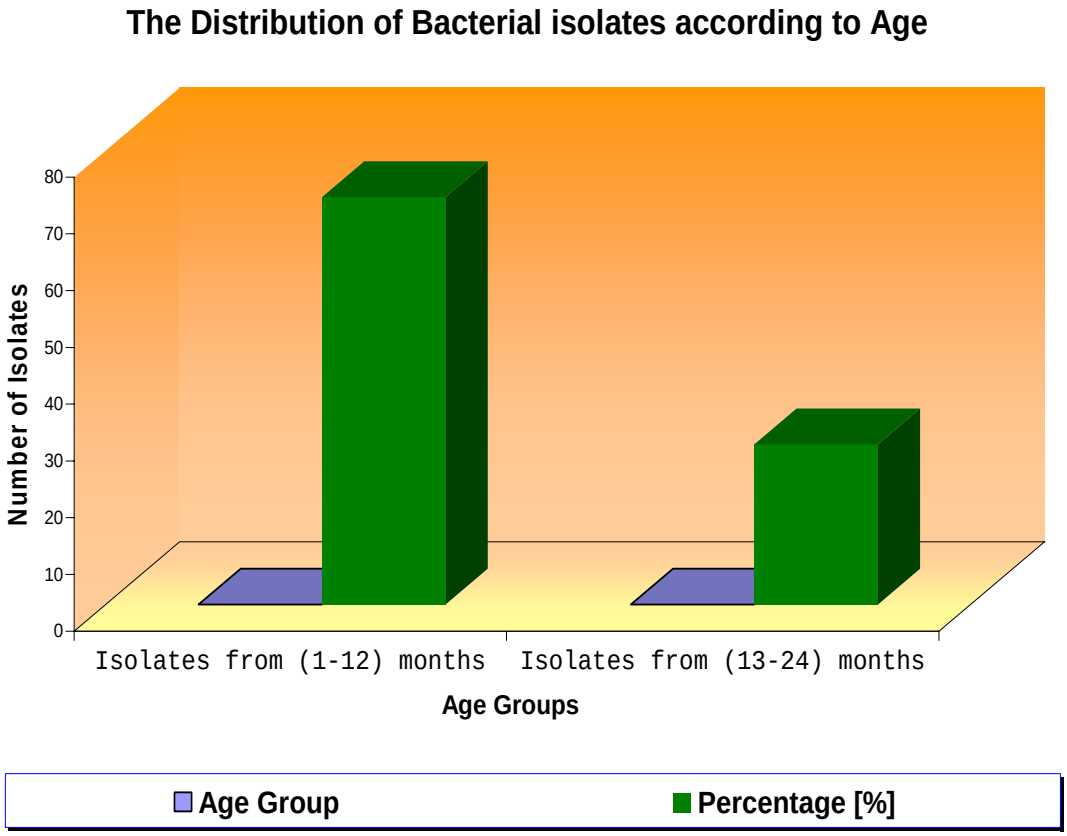


**Fig 4-12**

### 4.3.2 Distribution of Bacterial Isolates According to Gender:

A significant difference was not registered in the infection rate of diarrhoea amongst infants from the two sexes. The results revealed a rate of

51.1% to 49.9% occurrence of diarrhoea for male and female infants, respectively. Also, no significant variations in the types of organism isolates in both sexes as in fig 4-13 below.

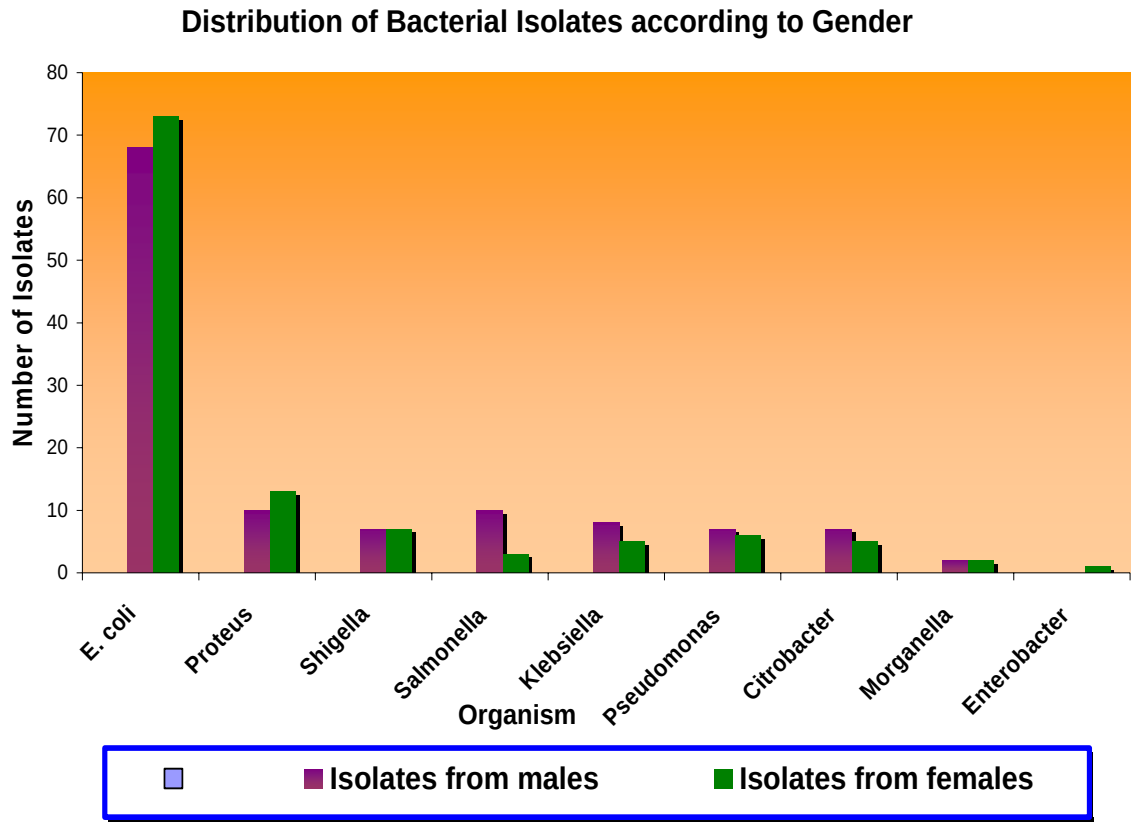


**Fig 4-13**

## 4.4 Sensitivity of the Clinical Isolates to Antibiotics

The total number of clinical isolates was 234 isolates. The selected bacterial strains were tested by the disk diffusion method against different

commonly used antibiotics and their sensitivity patterns were presented in tables and histograms. The tests were done on Moeller-Hinton agar.

**4.4.1 The Clinical isolates of *E. coli*:**

The total number of *E. coli* was 141 out of the 234 total isolates. They were tested against Tetracycline, Nalidixic acid, Sulphamethoxazole, Gentamycin, Trimethoprim, Chloramphenicol, Amoxicillin/Clavulonic acid, Ampicillin and Ciprofloxacin. In table 4-21 the reaction to the different concentrations of antibiotics (in µg/disc) are depicted in three major categories; sensitive, intermediate and resistant according to the degree of inhibition of the antibiotic to the bacterial growth.

**Table 4-21: Sensitivity Reaction of E. coli to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 28 | 19.9 | 12 | 8.5 | 101 | 71.6 |
| Nalidixic Acid 30µg | 120 | 85.1 | 6 | 4.3 | 15 | 10.6 |
| Sulphamethoxazole 300µg | 31 | 22.0 | 4 | 2.8 | 106 | 75.2 |
| Gentamycin 10µg | 77 | 54.6 | 18 | 12.8 | 46 | 32.6 |
| Trimethoprim 5µg | 30 | 21.3 | 4 | 2.8 | 107 | 75.9 |
| Chloramphenicol 30µg | 61 | 43.3 | 16 | 11.3 | 64 | 45.4 |
| Amoxicillin/Clavulonic Acid 20/10µg | 50 | 35.5 | 26 | 18.4 | 65 | 46.1 |
| Ampicillin 10µg | 10 | 7.1 | 0 | 0.0 | 131 | 92.9 |
| Ciprofloxacin 5µg | 135 | 95.7 | 3 | 2.1 | 3 | 2.1 |

The most conspicuous feature is the great sensitivity of *E. coli* to Ciprofloxacin and Nalidixic acid (95.7%, and 85.1, respectively), the fact that places both Ciprofloxacin and Nalidixic acid as the drugs of choice for these isolates. Gentamycin, Chloramphenicol and Amoxicillin come half the way in

the inhibition of *E. coli*'s growth. *E. coli* is from the major part resistant to Ampicillin, (92.9% resistant). On the other hand a weak response is relatively shown toward other antibiotics. The graphic representation of the patterns of reaction gives a clearer survey in fig 4-3.

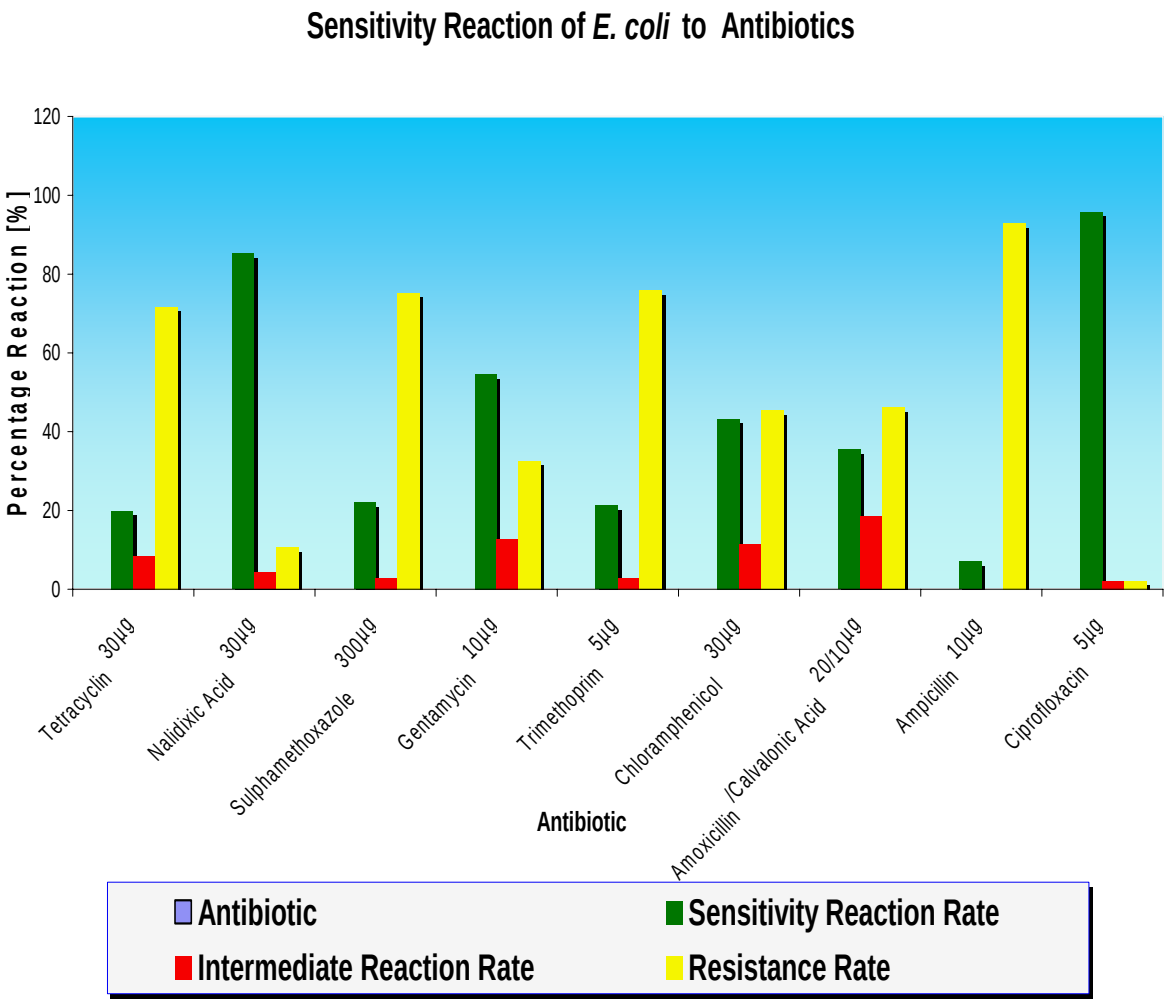


**Fig 4-3**

### 4.4.2 The Clinical isolates of *Proteus mirabilis*:

*Proteus mirabilis* isolates from intestines of infants with diarrhea counted to 23, i.e. 9.8% of total isolates, and the results of their sensitivity testing were summarized below, in table 4-22.

**Table 4-22:Sensitivity Reaction of Proteus to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 3 | 13.0 | 1 | 4.3 | 19 | 82.6 |
| Nalidixic Acid 30µg | 13 | 56.5 | 3 | 13.0 | 7 | 30.4 |
| Sulphamethoxazole 300µg | 5 | 21.7 | 1 | 4.3 | 17 | 73.9 |
| Gentamycin 10µg | 17 | 73.9 | 1 | 4.3 | 5 | 21.7 |
| Trimethoprim 5µg | 3 | 13.0 | 2 | 8.7 | 18 | 78.3 |
| Chloramphenicol 30µg | 4 | 17.4 | 2 | 8.7 | 17 | 73.9 |
| Amoxicillin/Clavulonic Acid 20/10µg | 6 | 26.1 | 4 | 17.4 | 13 | 56.5 |
| Ampicillin 10µg | 2 | 8.7 | 0 | 0.0 | 21 | 91.3 |
| Ciprofloxacin 5µg | 20 | 87.0 | 3 | 13.0 | 0 | 0.0 |

Of these isolates it is clear from the table, that 20 sensitivity response to Ciprofloxacin. That represents a ratio of 87.0% of all the isolates of *Proteus*, whilst only 3 showed intermediate sensitivity reaction. Next follows the sensitivity to Gentamycin and Nalidixic acid, (73.9% and 56.5%, respectively). The rest of antibiotic sensitivity tests yielded, relatively, low percentage reactions, especially the isolates of *Proteus mirabilis* did not, mostly, seem to be inhibited by Ampicillin, Tetracycline, and Trimethoprim nor by Chloramphenicol. A clear comparison of the variant rates of sensitivity and resistance reaction rates would, best, be depicted by fig 4-4 below.

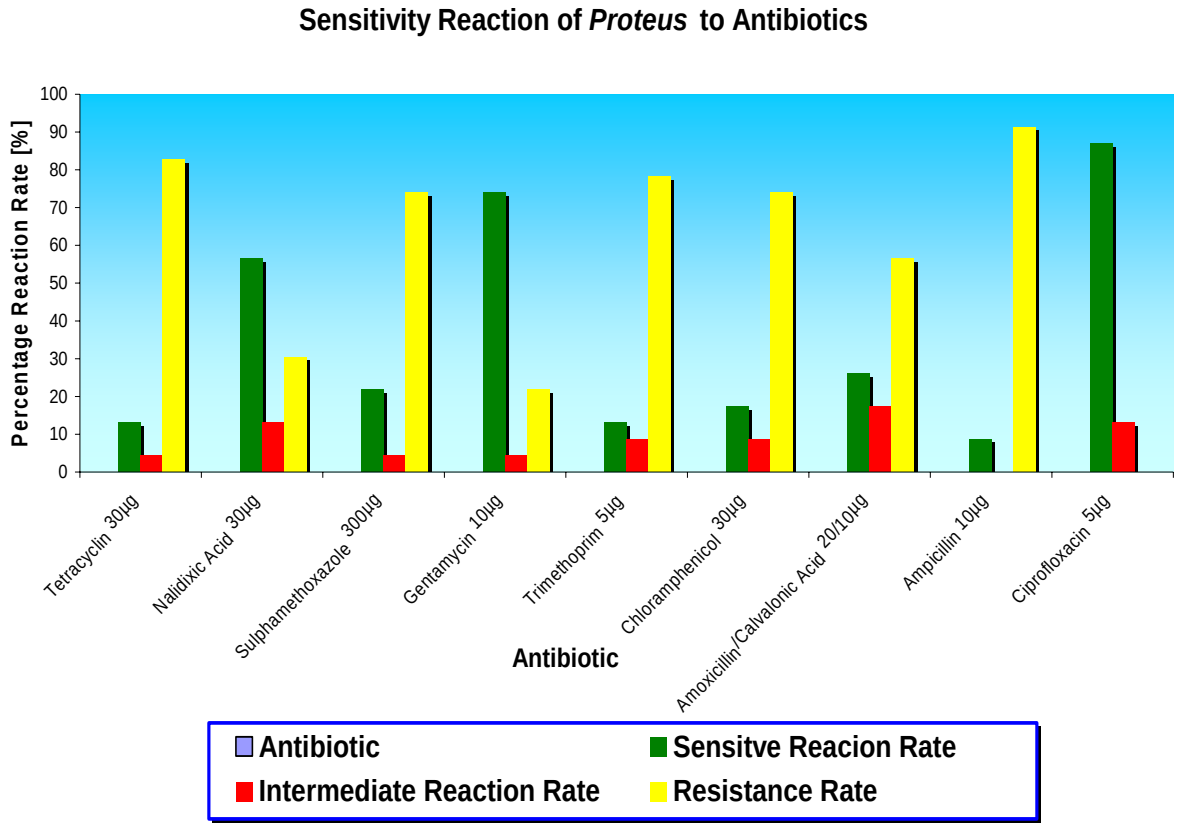


**Fig 4-4**

The drug of choice for these isolates remains to be Ciprofloxacin followed by Gentamycin as ersatz.

### 4.4.3 The Clinical isolates of *Shigella* species:

From the total isolates, isolated from infantile diarrhoea infection, *Shigella* isolates constituted 6.0%. That means 14 isolates of *Shigella* were subjected to sensitivity tests. The results, (table 4-23), revealed a sharp contrast of 100% sensitivity to Ciprofloxacin to full resistance of 100% to Ampicillin.

**Table 4-23:Sensitivity Reaction of *Shigella* to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 2 | 14.3 | 1 | 7.1 | 11 | 78.6 |
| Nalidixic Acid 30µg | 13 | 92.9 | 1 | 7.1 | 0 | 0.0 |
| Sulphamethoxazole 300µg | 1 | 7.1 | 3 | 21.4 | 10 | 71.4 |
| Gentamycin 10µg | 5 | 35.7 | 0 | 0.0 | 9 | 64.3 |
| Trimethoprim 5µg | 1 | 7.1 | 1 | 7.1 | 12 | 85.7 |
| Chloramphenicol 30µg | 5 | 35.7 | 2 | 14.3 | 7 | 50.0 |
| Amoxicillin/Clavulonic Acid 20/10µg | 3 | 21.4 | 2 | 14.3 | 9 | 64.3 |
| Ampicillin 10µg | 0 | 0.0 | 0 | 0.0 | 14 | 100.0 |
| Ciprofloxacin 5µg | 14 | 100.0 | 0 | 0.0 | 0 | 0.0 |

Besides Ampicillin, Trimethoprim and Tetracycline showed also high resistance rates, (85.7% and 78.6%, respectively). On the other hand Ciprofloxacin followed by Nalidixic acid remain to be the drugs of choice. For the different reactions of *Shigella* to other antibiotics fig 4-5 gives a clearer picture.

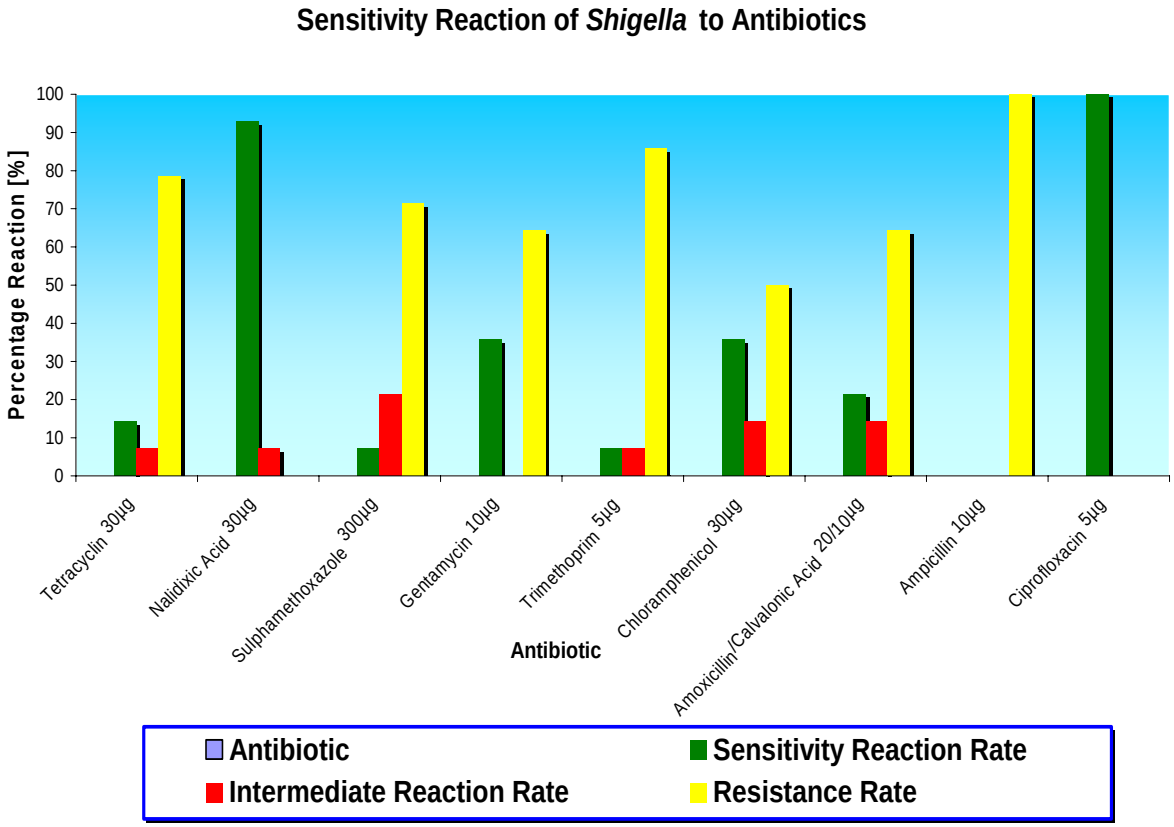


**Fig 4-5**

### 4.4.4 The Clinical isolates of *Salmonella* species:

*Salmonella* species isolated from infants with diarrhoea were 13 isolates in all. They were subjected to sensitivity tests to different antibiotics, using the standard disks. The results were shown in table 4-24.

It is noticeable that all the isolates were fully resistant to Ampicillin concentrations of 10µg/disc, whilst all of them showed full sensitivity reaction to a concentration of 5µg/disc of Ciprofloxacin.

**Table 4-24: Sensitivity Reaction of *Salmonella* to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 1 | 7.7 | 2 | 15.4 | 10 | 76.9 |
| Nalidixic Acid 30µg | 10 | 76.9 | 2 | 15.4 | 1 | 7.7 |
| Sulphamethoxazole 300µg | 3 | 23.1 | 1 | 7.7 | 9 | 69.2 |
| Gentamycin 10µg | 10 | 76.9 | 1 | 7.7 | 2 | 15.4 |
| Trimethoprim 5µg | 3 | 23.1 | 1 | 7.7 | 9 | 69.2 |
| Chloramphenicol 30µg | 6 | 46.2 | 3 | 23.1 | 4 | 30.8 |
| Amoxicillin/Clavulonic Acid 20/10µg | 3 | 23.1 | 1 | 7.7 | 9 | 69.2 |
| Ampicillin 10µg | 0 | 0.0 | 0 | 0.0 | 13 | 100.0 |
| Ciprofloxacin 5µg | 13 | 100.0 | 0 | 0.0 | 0 | 0.0 |

Ciprofloxacin registered the highest rate of inhibition of *Salmonella* species growth (100% sensitivity ratio), followed by Nalidixic acid and Gentamycin, which equally registered a ratio of 76.9% recorded as sensitivity of the organism to both antibiotics. The most commonly chosen antibiotic against *Salmonella* infections, Chloramphenicol, was found only half the way in the sensitivity test, (46.2% sensitive, 23.1% intermediate and 30.8% resistant). The rest of antibiotics demonstrated variable but lower capabilities of inhibiting *Salmonella* growth, as is best described by fig 4-6.

From the table it is obvious that the drug of choice for these isolates is Ciprofloxacin, in the first place, followed then by Nalidixic acid and Gentamycin.

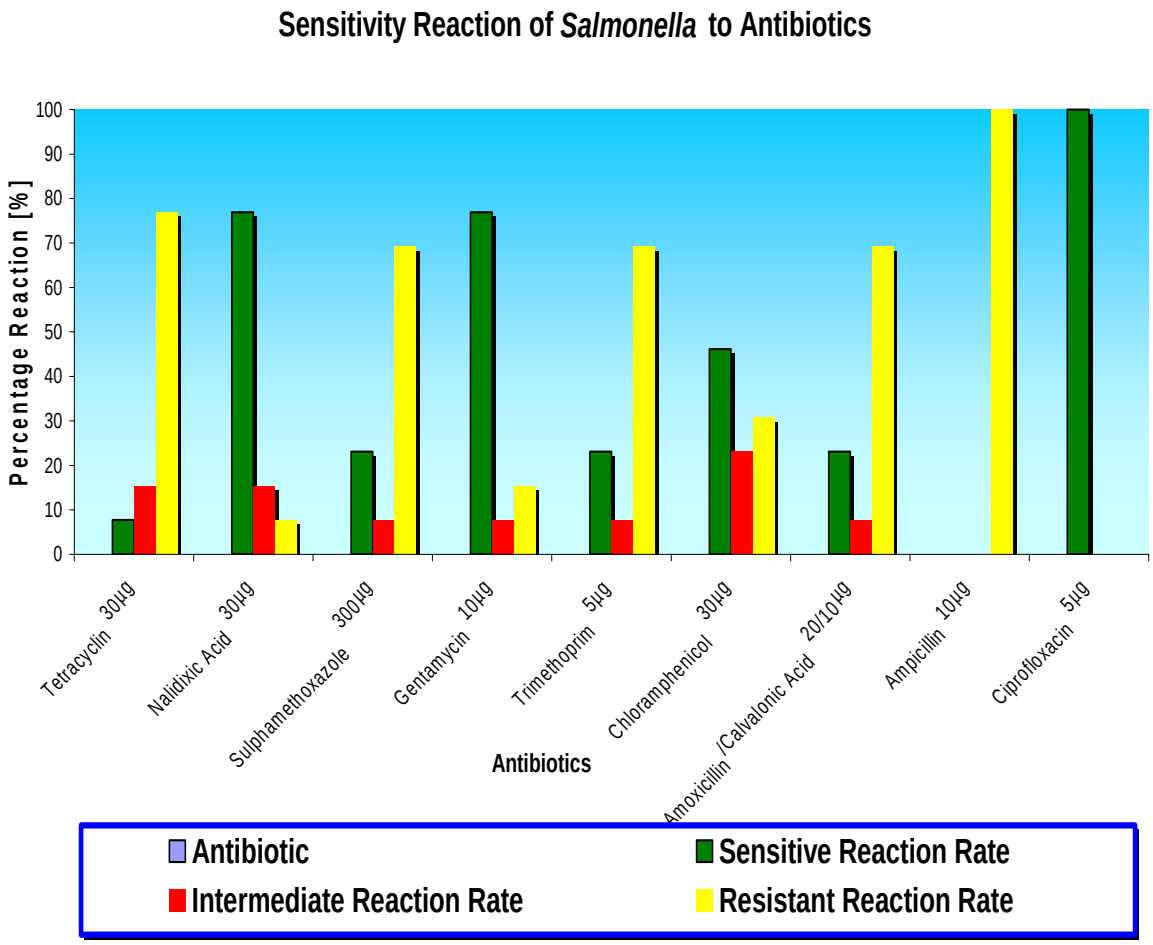


**Fig 4-6**

### 4.4.5 The Clinical isolates of *Klebsiella pneumoniae* species:

The number of total isolates of *Klebsiella pneumoniae* from infants suffering diarrhea counted to 13 isolates, (i.e. 5.6% of all isolates). They were subjected to sensitivity tests to commonly used antibiotics. The results were shown in table 4-25 below.

**Table 4-25: Sensitivity Reaction of *Klebsiella* to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 3 | 23.1 | 1 | 7.7 | 9 | 69.2 |
| Nalidixic Acid 30µg | 11 | 84.6 | 2 | 15.4 | 0 | 0.0 |
| Sulphamethoxazole 300µg | 4 | 30.8 | 0 | 0.0 | 9 | 69.2 |
| Gentamycin 10µg | 7 | 53.8 | 1 | 7.7 | 5 | 38.5 |
| Trimethoprim 5µg | 4 | 30.8 | 0 | 0.0 | 9 | 69.2 |
| Chloramphenicol 30µg | 7 | 53.8 | 2 | 15.4 | 4 | 30.8 |
| Amoxicillin/Clavulonic Acid 20/10µg | 2 | 15.4 | 3 | 23.1 | 8 | 61.5 |
| Ampicillin 10µg | 1 | 7.7 | 0 | 0.0 | 12 | 92.3 |
| Ciprofloxacin 5µg | 13 | 100.0 | 0 | 0.0 | 0 | 0.0 |

*Klebsiella pneumoniae* demonstrated a very high resistance response to Ampicillin and to a considerable extent toward Sulphamethoxazole, Tetracycline and Trimethoprim. On the other hand, an utterly high sensitivity to Ciprofloxacin places the antibiotic as the ultimate drug of choice for these isolates. The descending placement of other antibiotics can be deducted from the graphic representation in fig 4-7.

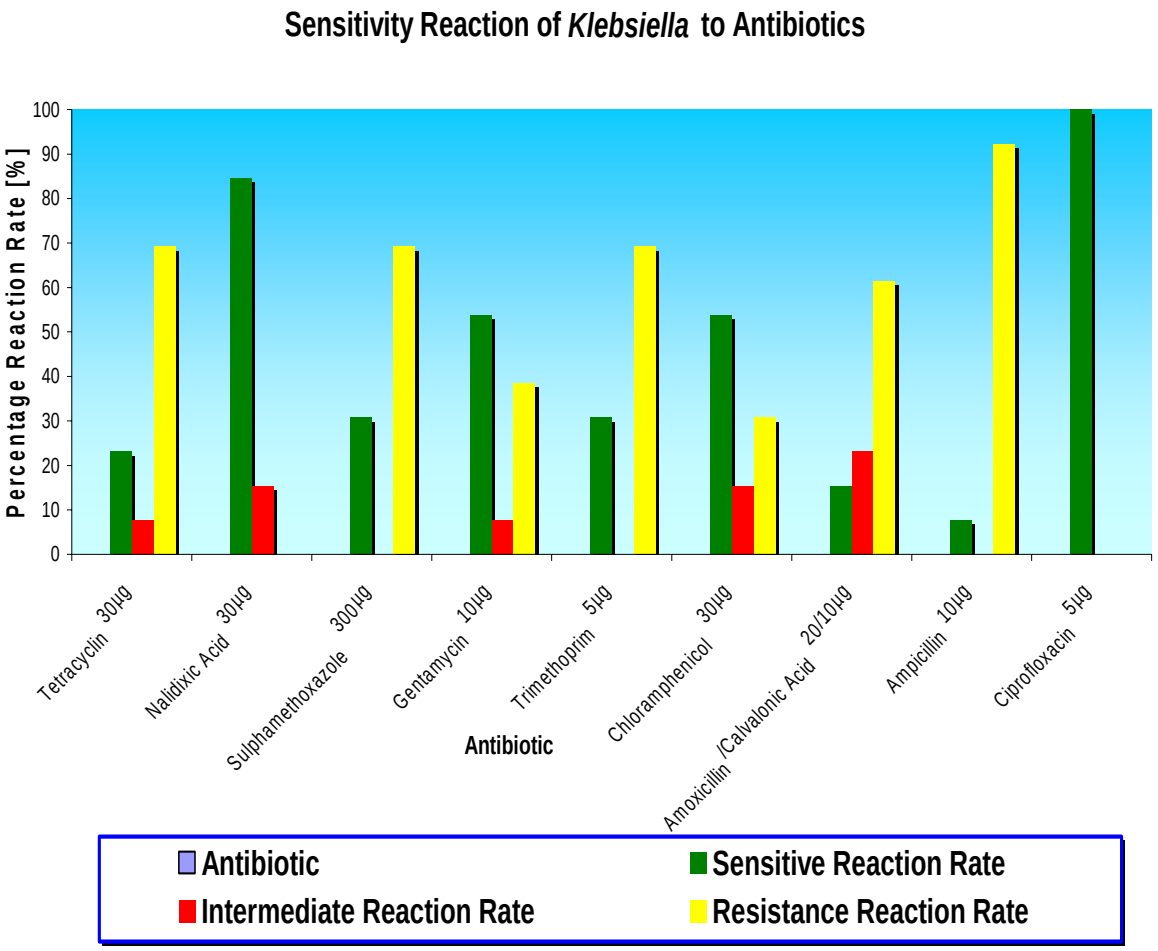


**Fig 4-7**

### 4.4.6 The Clinical isolates of *Pseudomonas aeruginosa* species:

The isolates of these species isolated from the samples taken from infants suffering diarrhea counted to 13 isolates constituting 5.6% of all the isolates. The 13 isolate underwent sensitivity tests to antibiotics.

Their attitude to the different antibiotics, recorded in table 4-26, showed unspeakably full resistance, (100%), to Sulphamethoxazole, Trimethoprim and Ampicillin in the following concentrations of 300µg, 5µg, and 10µg respectively.

**Table 4-26: Sensitivity Reaction of *Pseudomonas* to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 1 | 7.7 | 2 | 15.4 | 10 | 76.9 |
| Nalidixic Acid 30µg | 8 | 61.5 | 0 | 0.0 | 5 | 38.5 |
| Sulphamethoxazole 300µg | 0 | 0.0 | 0 | 0.0 | 13 | 100.0 |
| Gentamycin 10µg | 5 | 38.5 | 0 | 0.0 | 8 | 61.5 |
| Trimethoprim 5µg | 0 | 0.0 | 0 | 0.0 | 13 | 100.0 |
| Chloramphenicol 30µg | 1 | 7.7 | 1 | 7.7 | 11 | 84.6 |
| Amoxicillin/Clavulonic Acid 20/10µg | 0 | 0.0 | 3 | 23.1 | 10 | 76.9 |
| Ampicillin 10µg | 0 | 0.0 | 0 | 0.0 | 13 | 100.0 |
| Ciprofloxacin 5µg | 12 | 92.3 | 1 | 7.7 | 0 | 0.0 |

*Pseudomonas aeruginosa*, being regarded as naturally resistant to most of the commonly used antibacterial agents, proved through this study, that most of their strains exhibited a high resistance to the agents previously regarded as active, namely Gentamycin. Yet the sole agent to which the majority of strains were sensitive was Ciprofloxacin, which remains to be the drug of choice.

The graphic representation, in fig 4-8, reveals clearly the response of strains to antibiotics.

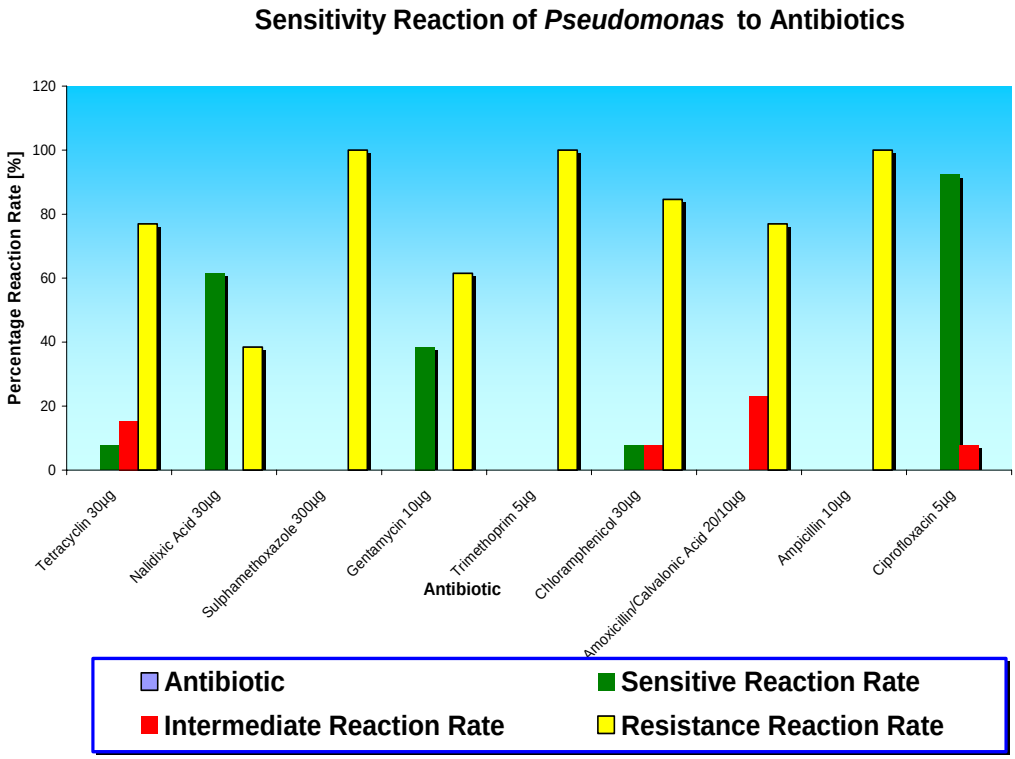


**Fig 4-8**

### 4.4.7 The Clinical isolates of *Citrobacter freundii* species:

*Citrobacter freundii* isolates constituted 5.1% of all the isolates detected in the samples taken from infants with diarrhea, (i.e. 12 isolates). On subjecting these isolates to the sensitivity test, (table 4-27), the strains revealed, amongst other attitudes, an utter resistance to Ampicillin at a concentration of 10µg/disc, (100% resistant). Tetracycline, Sulphamethoxazole and Trimethoprim showed, also, incapable of inhibiting the growth of *Citrobacter freundii* strains.

Fig 4-9 gives a clear picture of the different patterns of sensitivity reactions of strains of *Citrobacter freundii* toward the different antibiotics.

**Table 4-27**: **Sensitivity Reaction of Citrobacter to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 2 | 16.7 | 0 | 0.0 | 10 | 83.3 |
| Nalidixic Acid 30µg | 10 | 83.3 | 1 | 8.3 | 1 | 8.3 |
| Sulphamethoxazole 300µg | 2 | 16.7 | 1 | 8.3 | 9 | 75.0 |
| Gentamycin 10µg | 10 | 83.3 | 1 | 8.3 | 1 | 8.3 |
| Trimethoprim 5µg | 2 | 16.7 | 1 | 8.3 | 9 | 75.0 |
| Chloramphenicol 30µg | 3 | 25.0 | 2 | 16.7 | 7 | 58.3 |
| Amoxicillin/Clavulonic Acid 20/10µg | 4 | 33.3 | 1 | 8.3 | 7 | 58.3 |
| Ampicillin 10µg | 0 | 0.0 | 0 | 0.0 | 12 | 100.0 |
| Ciprofloxacin 5µg | 11 | 91.7 | 0 | 0.0 | 1 | 8.3 |

It is noticeable from the table that only one of the isolates imparted resistance to Ciprofloxacin, whilst the whole rest yielded sensitivity, which makes it the drug of choice followed by Gentamycin.

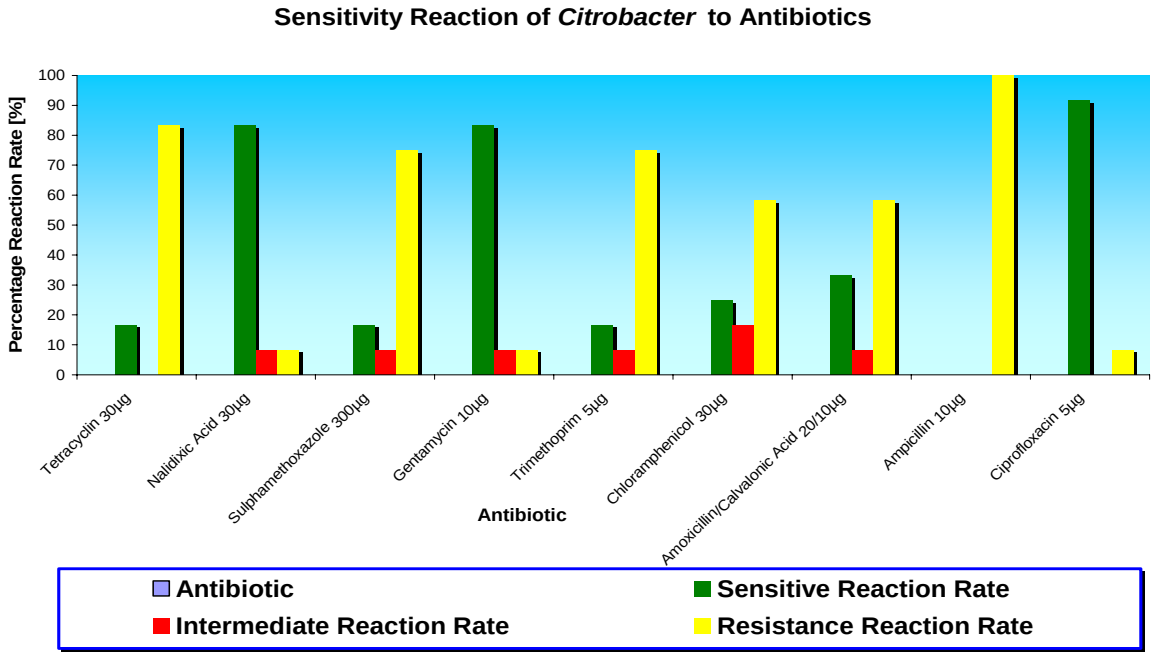


**Fig 4-9**

### 4.4.8: The Clinical isolates of *Morganella morganii* species:

From the samples taken under study from infants suffering from diarrhea four isolates, (1.7% of all isolates), of *Morganella morganii* were separated and subjected to sensitivity tests to the different antibiotics in common use. The results registered in table 4-28 show the different sensitivity patterns to the individual antibiotic.

**Table 4-28**: **Sensitivity Reaction of *Morganella* to Antibiotics**

| Reaction / Antibiotic | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 1 | 25.0 | 0 | 0.0 | 3 | 75.0 |
| Nalidixic Acid 30µg | 2 | 50.0 | 1 | 25.0 | 1 | 25.0 |
| Sulphamethoxazole 300µg | 1 | 25.0 | 0 | 0.0 | 3 | 75.0 |
| Gentamycin 10µg | 2 | 50.0 | 1 | 25.0 | 1 | 25.0 |
| Trimethoprim 5µg | 1 | 25.0 | 0 | 0.0 | 3 | 75.0 |
| Chloramphenicol 30µg | 2 | 50.0 | 0 | 0.0 | 2 | 50.0 |
| Amoxicillin/Clavulonic Acid 20/10µg | 2 | 50.0 | 0 | 0.0 | 2 | 50.0 |
| Ampicillin 10µg | 1 | 25.0 | 0 | 0.0 | 3 | 75.0 |
| Ciprofloxacin 5µg | 3 | 75.0 | 0 | 0.0 | 1 | 25.0 |

Half the strains showed sensitivity to both Nalidixic acid and Gentamycin, one quarter showed resistance and other quarter showed intermediate reactions. Chloramphenicol and Amoxicillin/Clavulonic acid inhibited the growth of half the isolates, whilst they failed to inhibit the other half. As shown in the graph, in fig 4-10, Ciprofloxacin showed the highest level of inhibition of *Morganella morganii* strains, and remains as the suggested drug of choice.

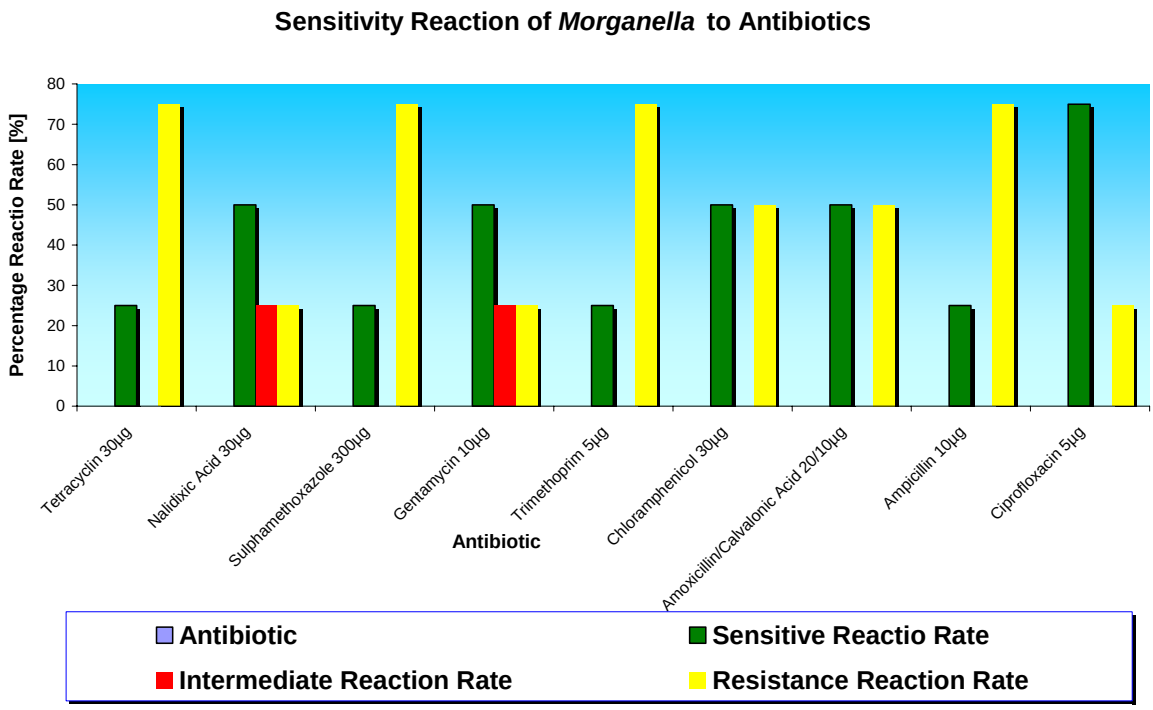


**Fig 4-10**

### 4.4.9 The Clinical isolates of *Enterobacter aerugens* species:

Only one single isolate of *Enterobacter aerugens* was procured out of all isolates extracted from the samples taken from infants with diarrhoea. This fact made it impossible to assure reliably reproducible results. In spite of that the results were presented the way they came. The isolate was tested against different antibiotics and the results were shown in table 4-29 below.

The single isolate showed sensitivity to all antibiotics, except for Trimethoprim, Sulphamethoxazole and Amoxicillin/Clavulonic acid, where it showed resistance reaction as clearly shown on fig 4-11.

**Table 4-29**: **Sensitivity Reaction of Enterobacter[@] to Antibiotics**

| Antibiotic \ Reaction | Sensitive | | Intermediate | | Resistant | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Tetracycline 30µg | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Nalidixic Acid 30µg | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Sulphamethoxazole 300µg | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| Gentamycin 10µg | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Trimethoprim 5µg | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| Chloramphenicol 30µg | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Amoxicillin/Clavulonic Acid 20/10µg | 0 | 0.0 | 0 | 0.0 | 1 | 100.0 |
| Ampicillin 10µg | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Ciprofloxacin 5µg | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |

**@ Only one isolate was detected**

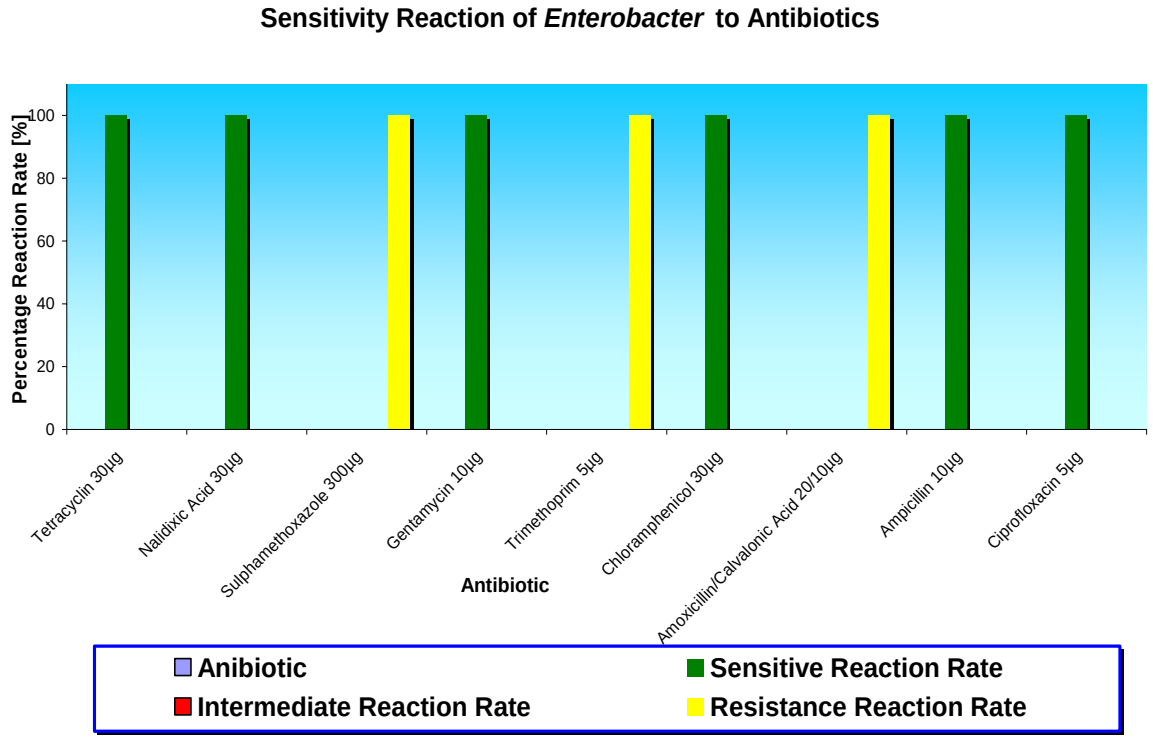


**Fig 4-11**

## 5. DISCUSSION

The hospitals attended for the stool samples of infants suffering from diarrhoea are situated in the Khartoum, Khartoum North and Omdurman Governorates. Yet, they house, from the major part, a population from the outskirts and suburbs of these towns, inside their wards in severe cases of diarrhoea. Parents, carrying their infants afflicted with diarrhoea, from surrounding villages and districts are the main visitors to these hospitals seeking urgent medical services.

The vast majority of infants, as the questionnaire revealed, were breast-fed, so it was unlikely to put the blame of infection on bottle feeding or bad use of utensils. Yet, there remains an undebatable assumption that the high rates of diarrhoea infections in these societies are attributed to the defective community hygiene and personal hygiene. Mothers are not educated enough to realize the

dangers of not bothering to wash their hands and breasts properly prior to feeding their babies. Improper sanitation, bad food storage and handling and poor hygiene of water supply could, also, contribute unfavourably. This situation if far from being uncommon, for it was reported by the WHO, in 1982, that acute diarrhoea used to be an extremely wide spread disorder, particularly serious in the young and old, affecting hundreds of millions of people every year. Among infants and children, it happened to hold responsible for more deaths than any other cause in the world. Isabel and Affonso, from a worldwide study in 2001, reported that there were 744 to 1000 million episodes of diarrhoea and 4 to 6 million deaths each year in the previous decade from diarrhoeal disease in children younger than five years in Africa, Asia, and Latin America, and concluded that acute diarrhoea is still a leading cause of infantile death in less-developed countries and the most important symptom associated with malnutrition and bad hygiene practice.

Diarrhoea is a symptom, not a disease in itself, of any condition that affects the integrity of the intestines. It could be concisely defined as the frequent passage of watery motions. The contents of the small intestine are always liquid. The main function of the large intestine is to absorb most of the water, leaving a soft but not watery residue of faeces to be passed. If the passage through the large intestine is unduly delayed, too much water is absorbed and the faeces become dry, hard and difficult to pass; that is constipation. But if the passage is too rapid or if there is an excess of water, the faeces are still liquid when they reach the rectum and defaecation is frequent and urgent. Most diarrhoea is due to inflammation of the intestine, with excessive production of watery mucus and over-activity of the intestinal muscles both contributing to the symptoms. The inflammation comes as a result of pathogenic bacterial action or a viral one amongst many other causals.

In the present study different strains of bacteria were isolated from 200 stool samples taken from infants that suffered from diarrhoea. The infants came from different areas of residence, but had similar environmental surroundings of poor hygiene. The total amounted to 234 isolates, of which alone *E. coli* constituted 141 isolates. The rest of 93 isolates were distributed unevenly among other 8 bacterial microorganisms recognized as pathogens. The distribution ran as such: *Proteus mirabilis* showed in 23 of the isolates, *Shigella* species in 14 isolates, each of *Salmonella*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* appeared in 13 isolates, *Citrobacter freundii* was detected in 12 isolates, *Morganella morganii* was determined in 4 of the isolates, whilst *Enterobacter cloacae* showed up in one single isolate. Now then the question looms; taking into consideration that these strains are well recognized pathogenic microorganisms and diarrhoea is one feature of their individual infection's symptoms: which of these organisms hold responsible for the infantile diarrhoea?

However, it is legitimate to stick to the rule that states that the organism, which has the capability of maximum growth in the culture medium, is the one that holds responsible for diarrhoea. In this view *E. coli* strains are, beyond doubt, the main causative of diarrhoea, for they have surpassed the other rivals in growing the largest colonization.

Once again, another question comes into view. What makes a person certain about this result? May not, also, the other pathogenic microorganisms have contributed to the occurrence of diarrhoea, as well? The questions may not be answered spontaneously but it can be postulated that the other microorganisms are, indeed, suspected for contribution to the infantile diarrhoea, especially the isolates of relatively significant counts. In this respect *E. coli* count for the major causative agent of infantile diarrhoea, followed by

*Proteus mirabilis*. *Shigella* species pose on the third place and *Salmonella* species, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* share together the fourth place. The next on the row are *Citrobacter freundii*. Perhaps, *Morganella morganii* are insignificant participants in infantile diarrhoea but *Enterobacter cloacae* can hardly be regarded as contributing to diarrhoea amongst infants, since only one isolate of the microorganism was found amongst the 234 detected. Ismail, 1994, reported 76.3% prevalence of *E. coli* in diarrhoea samples taken from children in Khartoum State.

The present study revealed a higher liability of younger infants, below one year of age, to diarrhoea infection more than the elder infants. This could be an indication of the fact that the infants acquire gradually immunity through continuous breast-feeding. That denotes that prolonged breast-feeding reinforces the sucklings with antibodies from their mothers. Moreover, it is most probable that infants could have gradually gained immunity through the past experiences of infections.

The results arrived at, in the current study, showed that the enteropathogenic *E. coli* had demonstrated the highest representation amongst the other typable pathogenic strains of *E. coli*, i.e. 44.2% of typable *E. coli* and 24% out of all *E. coli* isolates. These results coincide with reports by Trabulsi *et al*., (2002), in Brazil. Banajeh *et al*., (2001), reported after a study carried on Yemeni children suffering diarrhoea that the majority were infants in the age range of 1 – 12 months and constituted 58.0% of the positive cultures, besides alone (EPEC) proved responsible for 58.4% of the diarrhoea infections due to *E. coli*. Similar results were obtained by Torres, et *al*., (2001), in Uruguay, and Asrat., (2001), in Ethiopia. Marc *et al*., (1978) studied the (EPEC) in Canadian infants with diarrhoea and found out that 6% of the infections were constituted by (EPEC), namely in infants below 12 months of age. Also, Oliva *et al*.,

(1997) concluded from a study, in Sao Paulo, that (EPEC) were the most prevalent enteropathogens amongst infants with severe acute diarrhoea. In his long term studies, in Sudan and Khartoum North in particular, Erwa *et al.*, in 1971 and 1975 found that (EPEC) scored 72.8%, whereas Untypable *E. coli* constituted 12.7% among children suffering diarrhoea.

The results obtained in this study proved a considerable multiple drug resistance among *E. coli* isolates especially towards Ampicillin, Tetracycline, Amoxicillin/Clavulonic acid, Sulphamethoxazole and Trimethoprim. Other microorganisms involved in infantile diarrhoea, also, imparted almost a similar behaviour toward commonly used antibiotics. This would most probably denote that these microorganisms have gained some kind of tolerance to antibiotics of common use. This resistance the organisms impart on these antibiotics can only be interpreted as an eventual consequence of the drugs abuse, during the last decades. These bacteria do not possess a natural or intrinsic resistance, since the antibiotics in issue used to be very effective drugs against their infections in the past, so it is rather a question of acquired resistance that they have built through bad use. Perhaps, the antibiotics Chloramphenicol, Sulphamethoxazole and Trimethoprim were until recently regarded as drugs of choice against *Salmonella* infections. They proved incapable during the present study of inhibiting the growth of *Salmonella* species and were confronted with a resistance of 30.8%, 69.2% and 69.2% respectively. Banajeh *et al.*,(2001), reported that ⅔ of *Salmonella* isolates showed multiple drug resistance to Chloramphenicol, Co-trimoxazole, Gentamycin, and Amoxicillin, in a study amongst Yemeni Children suffering diarrhoea. They, also, concluded from the same study that all *E. coli* isolates were resistant to Amoxicillin and 83% were resistant to Co-trimoxazole, 62% to Chloramphenicol, 54% to Gentamycin, while only 6% were resistant to

Nalidixic acid and Cefotaxime, respectively. From the same research study it was proved that *Shigella* isolates were susceptible to Nalidixic acid and Cefotaxime, whilst they were resistant to the other antibiotics.

In a study carried out in Uruguay targeting infants' diarrhoea, Enteropathogenic *E. coli* and *Shigella flexneri* showed usually frequent antimicrobial resistance, especially towards β–lactam antibiotics, (Torres et al., 2001).

One conspicuous finding of the present work is that most of the *E. coli* isolates and most of the other diarrhoeagenic microorganisms detected responded almost fully to Ciprofloxacin and Nalidixic acid in contrast to other antibiotics. Similar results were obtained by Vila *et al.*, (1999) after a study on the prevalence and antimicrobial susceptibility of *E. coli* in children under 5 years of age in Tanzania. They found out that 38% of the cases of diarrhoea were due to multiresistant *Enterotoxigenic, Enteroaggregative* or *Enteropathogenic E. coli.* All of these strains exhibited high resistance to Ampicillin, Tetracycline, Co-trimoxazole and Chloramphenicol, but were highly susceptible to quinolones.

## 6. CONCLUSION AND RECOMMENDATIONS

Diarrhoea is one of the major causes of deaths amongst infants the world over, and is specifically widespread in less developed societies. The poor personal and community hygiene practice, lack of health education and health service, bad sanitation, unhealthy water supply sources and neglect of food hygiene altogether contribute to the spread of the infection.

Bacterial infantile diarrhoea is caused from the major part through the infection by *Enterobacteriaceae* member microorganisms. The most dominant of these microorganisms with the highest representation ratio in infantile

diarrhoea is *E. coli* especially *Enteropathogenic, Enterotoxigenic* and *Enterohaemorrhagic E. coli*. *Proteus mirabilis, Shigella species, Salmonella species, Klebsiella pneumoniae, Citrobacter freundii, Morganella morganii* are all represented in infantile diarrhoea in descending prevalence rates. The only diarrhoea causative that does not belong to the *Enterobacteriaceae* family that was detected in this study and had a significant prevalence rate was *Pseudomonas aeruginosa*.

The microorganisms isolated from stool samples of infants with diarrhoea exhibited high multidrug resistance to antibiotics in common use. This phenomenon is most probably attributed to the abuse of drugs. Ciprofloxacin, Nalidixic acid and to a great extent Gentamycin proved capability of inhibiting the bacterial growth, but these diarrhoeagenic microorganisms showed variable but significant resistance rates to the rest of antimicrobial agents.

Infants younger than 12 months of age are more vulnerable to diarrhoea infection than the elder ones, since they gradually procure immunity through continuous breast feeding. Also the fact that the elder are less exposed could probably be due to the acquired immunity from their previous experiences.

On basis of the findings obtained in this study and the status of infants in the previously-mentioned underdeveloped communities, it would be highly recommended that:

- The concerned national and international authorities to supply poor communities with proper water supply sources, to afford proper sanitation and proper waste disposal installations. Not only that but to provide these communities with sufficient health education. The latter could

be accomplished through allotting an experienced staff of health workers or paramedicals to hold lessons about community health, personal and community hygiene. Mothers should be in focus.

- High health authorities should firmly forbid the sale of antibiotic drugs without an authorized medical prescription. Neglecting this point would facilitate the abuse of these drugs and eventually the loss of their efficacy.
- Medical authorities should bother themselves to immediately test for the sensitivity of the diarrhoeal causative microorganism, before issuing the prescription with the hypothetical antimicrobial agent. It would be even recommendable if they avoid, *where possible*, prescribing antibiotics and resort to rehydration as a substitute solution.
- Prolonged breast feeding should be encouraged nationwide.
- A careful further pharmacological research on Ciprofloxacin as a potent antimicrobial agent should be established as to maintain it in the appropriate manner that it could be given to children.

## 7. REFERENCES


- **Agus, S.G.; Dolin, R.; Wyatt, R.G.; Tousimis, A.J. and Northrup, R.S. (1973).**

Acute infectious non – bacterial gastroenteritis, intestinal histopathology. Ann. Internal. Med. 79 : 18.

- **Alekseen, P.A.; Berman, M.I.; and Korneava, R.A. (1960).**

Clinical and pathological picture of *S.typhimurium* infection in children. J. Microbiol. Epidemiol. Immunol. 3:III.

- **Asrat, D. (2001).**

Screening for enteropathogenic *E.coli* (EPEC) in pediatric patients with diarrhoea and controls using pooled antisera.

Ethiop. Med. J. 2001 Jan; 39(1): 32 – 8.

- **Baffone, W.; Ciaschini, G.; Pianetti, A.; Brandi, G.; Casaroli, A.; Bruscolini, F.**

Detection of *Escherichia coli* O 157: H7 and other intestinal pathogens in patients with diarrhoeal disease. Eur. J. Epidemiol. 2001; 17 (1): 97 – 9.

- **Banajeh, S.M.; Ba-Oum, N.H.; Al-Sanabani, R.M. (2001).**

Bacterial etiology and anti – microbial resistance of childhood diarrhoea in Yemen. J. Trop. Pediar. 2001 Oct; 47 (5):301 – 3.

- **Banwell, J.G. and Sherr, H. (1973).**

Effect of bacterial enterotoxins on the gastrointestinal tract. J. gastroenterology 65: 467.

- **Barrow, G.I. and Feltham, R.K.A. (1999).**

Gram – negative bacteria. In: COWAN AND STEEL'S manual for the identification of medical bacteria. Third edition. Cambridge University Press, UK. PP. 128, PP. 135.

- **Bradley, R.S.T.; Hon, C.R. and Weisfield, D.A. (1980).**

Laboratory Diagnosis of Bacterial Diarrhoeas. American Society for Microbiology, Washington D.C.

- **Brenner, D.J.; Davis, B.R.; Steigerwalt, A.G.; Riddle, C.F.; McWhorter, A.C.; Allen, S.D.; Farmer, J.J.III.; Saitoh, Y. and Fanning, G.R. (1982a).**

Atypical biogroups of *Escherichia coli* found in clinical specimens and description of *Escherichia hermanii* species.

Nov. J. Clin. Microbiol. 15,703.

- **Brenner,D.J.; McWhorter, A.C.; Knuston, J.K.L. and Steigerwalt, A.G. (1982b).**

*Escherichia vulneris* : a new species of Entrobacteriaceae associated with human wounds. J. Clin. Microbiol. 15,1133.

- **Brooks,G.F.; Butel, J.S.; Morse,S.A. (1998).**

Enterobacteriaceae, In: Jawetz, Melnick, and Addelberg's Medical Microbiology, 21edition "Middle East Edition". Librairie, P.O.Box 945 Beirut,Lebanon. Appelon & Lange, Norwalk, Connecticut/ Sam Mateo, California – USA. PP. 218 – 230.

- **Burgess, N.R.H.; McDermott, S.N. and Whiting, J. (1973).**

Laboratory transmission of Enterobactericeae by the oriental cockroach, *Blatta orientalis*. J.Hyg. Camb. 71, 9.

- **Chakrabarti, M.K.; Sinha, A.K. (2001).**

Studies on the binding of *E.coli* heat stable – enterotoxin to intestinal epithelial cell and brush – border membranes of different animals

**Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H. (1975).**

Medical Microbiology, Churchill Livingston Pub., Edinburgh, 12th Ed.

- **Curran, P.F. and Schultz, S.G. (1968).**

Transport across membranes: General principles in: Hand book of Physiology, Alimentary Canal, Vol. 111, code, C.F.(ed), Am. Physiol. Soc., Washington D.C.

- **David, I.E. (1980).**

In: Antimicrobial Drug Action. Edwards, David Macmillan press Ltd., London and Basingtoke, pp. 15 & 57 – 60.

- **Davidson, G.P.; Goller, I.; Bishop, R.F.; Townley, R.W.; Holmes, I.H. and Buck, B.J. (1975).**

Immuno-fluorescence in duodenal mucosa of children with acute enteritis due to a new virus. J. Clin. Pathol., 28: 163.

- **Davis Green Wood (2002).**
- Richard, C.B. S.; John, F.P.

Escherichia, In Medical Microbiology, Sixteen Edition, Churchill Livingston, UK. PP. 265 – 274.

- **Donaldson, R.M., Jr. (1964).**

Normal bacterial population of the intestine and their relation to intestinal function. III. New. Engl. J. Med. 270: 1050.

- **Dubose, R.J. (1958).**

In: Bacterial and Mycotic infections of man. J.B. Lippiun cott company 3rd Ed., pp. 218 – 22.

- **Dutta, S.; Nair, G.B.; Saha, M.R.; Niyogi, S.K.; Bahattacharya, S.K.; Dutta, P. (2001).**

Studies on the role of enteroaggregative *E.coli* as a cause of diarrhoea with special reference to its virulence properties.

- **El – Sheikh, S.M. and El – Assouli, S.M. (2001)**

In: Prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Jeddah, Saudi Arabia. J. Health Popul. Nutr. 2001 Mar; 19(1): 25 – 30.

- **Erwa, H.H.; Yousif, M. and Gumaa, S.A. (1971).**

Bacterial diarrhoeal diseases in Sudanese children.

J. Trop. Med. Hyg. 74: 177.

- **Erwa, H.H. (1975).**

Bacterial enteritis in Sudanese children.

Thesis submitted for the M.D. Degree to the University of Khartoum, Sudan.

- **Ewing, E.H. and Fife, M.A. (1972).**

*Enterobacter agglomerans* (Beijerinck) Comb. nov. (the herbicola-Lathyribacteria). Int. J. Sys. Bact. 22, 4.

- **Farmer, J.J.III; Fanning, G.R.;Davis, B.R.; O'Hara, C.M.; Riddle, C.; Hickman-Brenner, F.W.; Asbury, M.A.; Lowery, V.A. III and Brenner, D.J. (1985).**

*Escherichia fergusonii* and *Enterobacter taylorae*, two new species of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol.21, 77.

- **Farmer, J.J. III. (1995).**

Eterobacteriaceae: introduction and identification. In: Manual of Clinical Microbiology, 6th edition. Murray PR *et al* (editors).

American Society for Microbiology, (1995).

- **Flewett, T.H.; Bryden, A.S.; Davis, H.; Woode, G.N.; Bridger, J.C. and Derrick, J.M. (1974).**

Relation between viruses from acute gastroenteritis of children and newborn calves. Lancet 2: 61.

- **Garrod, L.P.; Lambert, H.P. and O'Grady, F. (1981).**

In: Antibiotics and chemotherapy, Churchill Livingston Pub. Edinburgh, 5th ed.

- **Gianella, R.A.; Broitman, S.A. and Zamcheck, N. (1971).**

*Salmonella enteritis*. I. Role of reduced gastric secretion in pathogenesis.

Am. J. Dig. Diseases 26: 1000.

- **Gilman, A.G.; Goodman, L.S. and Gilman, A. (1980).**

In: The Pharmacological Basis of Therapeutics.

Macmillan Pub. Co., London, 6th Ed., pp. 1154 – 1253.

- **Gorbach, S.L. (1971).**

Intestinal Microflora.

Gastroenterology 60 : 1110.

- **Gorbach, S.L.; Kean, B.H.; Evans, D.G.; Evans, D.J. and Bessudo, D. (1975).**

Traveler's diarrhoea and toxogenic *E. coli*.

New Engl. J. Med. 292: 933.

- **Gordon, J.E.; Behar, M. and Scrimshaw, N.S. (1964).**

Acute diarrhoeal disease in less developed countries I. An epidemiological basis for control. Bull. World Health Organization 31: 1.

- **Hiruta, N.; Murase, T.; Okamura, N. (2001).**

An outbreak of diarrhoea due to multiple antimicrobial resistant Shiga toxin – producing *Escherichia coli* O26 : H11 in a nursery.

Epidemiol. Infect. 2001 October; 127(2): 221 – 7.

- **Holmes, I.H.; Rodger, S.M.; Schnall, R.D.; Ruck, R.J. and Gust, I.D. (1976).**

Is lactase the receptor and uncoating enzyme for infantile enteritis (rota) viruses ? Lancet 1: 26.

- **Hugo, W.B.; Russel, A.D.(2002).**

In: Antibiotics in: Pharmaceutical Microbiology, 6th edition. Printed by MPG Books Ltd., Bodmin, Cornwall, Great Britain.

- **Isabel, C.; Affonso, S. (2000).**

*Escherichia coli* infections and malnutrition, Lancet prespective – 536 – December 2000.

- **Ismail, A.A. (1994).**

Microbiological Identification and Antibiotic Resistance Patterns of Clinical Isolates From Children with Gastroenteritis.

Thesis submitted for the Degree of M.Sc. to the University of Khartoum.

- **Leclerc, H. (1962).**

Étude biochemique d'Enterobacteriaceae pigmentées.

Annls. Ins. Pasteur, Paris 102, 726.

- **Lifshitz, F.; Coello-Ramirez, P. Gutierrez-Topete, G. and Cornado,-Cornet, M.C. (1971).**

Carbohydrate intolerance in infants with diarrhoea.

J. Pediatr. 79: 760.

- **Lippincoh, V. B.; Welsley, A.V.; Benjamin; Kadur and Parsons (1991).**

Enteric and related gram – negative organisms, in Essentials of Medical Microbiology, fourth edition (1991).

- **Loureiro, I.; Frankel,G.; Adu-Bobie,J.; Dougan, G.; Trabulsi, L.R.; Carneiro-Sampaio, M.M. (1998).**

Human colostrums contains IgA antibodies reactive to enteropathogenic *E. coli* virulence – associated proteins: intimin. BfpA, EspA, and EspB,

J. Pediatr. Gastroenterol. Nutr. 1998 Aug; 27(2): 166 – 71.

- **Marc Gurwith, Dorothy Hinde, Roger Gross, and Bernard Rowe. (1978).**

In: A prospective study of enteropathogenic *E. coli* in endemic diarrhoeal disease , J. Infect. 1978 March; 137 (3): 292 – 297.

- **McClung, H.J.; Butler, D.G.; Kerzner, B.; Gall, D.G.; Hamilton, J.R. (1976).**

Transmissible gastroenteritis. Mucosal transport in acute viral enteritis.

Gastroenterology 70: 1091.

- **Mlatezou, H.C.; Zafiropoulou, A.; Mavrikou, M.; Bozavoutoglou, E.; Foustoukou, M.; Kafetzis, D.A. (2001).**

Acute diarrhoea in children treated in an outpatient – setting in Athena, Greece.

J. Infect. 2001 Aug; 43(2): 122 – 7.

- **Nataro, J.P.; Kaper, J.B. (1998).**

Diarrhoegenic *Escherichia coli*

Clin. Microbiol. Rev 1998; 11: 142 – 201.

- **Nunnery, A.W. and Riley, H.D. (1964).**

Clinical and laboratory studies of Lincomycin in children.

Antimicrob. Agents Chemo.; 4: 142 – 146.

- **Oliva, C.A.; Scaletsky, I.; deMorais, M.B.; Fagundes Neto, U. (1997).**

Severe acute diarrhoea associated to classic enteropathogenic *E. coli* (EPEC): clinical features and fecal losses in hospitalized infants.

Rev. Assoc. Med. Bras. 1997 Oct-Dec; 43(4): 283 – 9.

- **Olsen, S.J.; Miller, G.; Kennedy, M.; Higgins, C.; Walford, J.; McKee, G.; Fox, K.; Bibb, W.; Meal, P. (2002).**

A waterborne outbreak of *Escherichia coli* O157:H7 infections and hemolytic uremic syndrome: implications for rural water systems.

Emerg. Infect. Dis. 2002 Apr; 8(4): 370 – 5.

- **Omer, El-E., (1990).**

*Escherichia coli*, In: Review of Medical Bacteriology, First Edition Almarwah Printing, Khartoum – Sudan, 1990 pp. 236 – 239.

- **Oo, K.N.; Aung, W.W.; Thida, M.; Toe, M.M.; Lwin, H.H.; Khin, E.E. (2000).**

Relationship of breast – feeding and hand – washing with dehydration, in infants with diarrhoea due to *Escherichia coli*.

J. Health. Popul. Nutr. 2000 Sept; 18 (2):93 – 6.

- **Panhotra, B.R. (1983).**

Comparative study of four tests for the detection of *E. coli* – heat labile enterotoxin. Indian J. Med. Res. 1983, 77(6): 295 – 298.

- **Panigrahi, D; Roy, P. and Chakrabarti, A. (1991).**

Enterotoxigenic *Klebsiella pneumoniae* in acute childhood diarrhoea.

Indian J Med Res. 1991 Sept;93:293-6.

- **Pleczar, Reid and Chan (1982).**

Host – Microbe interactions. In: Microbiology, McGraw – Hill, Inc. New York. Tata McGraw – Hill Publishing Company. India pp. 499.

- **Pratt, W.B. (1977).**

Chemotherapy of infection, Oxford University Press, New York 2nd Ed. pp. 10 – 15.

- **Range, H.P. and Dale, M.M. (1987).**

Pharmacology. Longman group, U.K. Ltd., Churchill Livingston, 1st ed.

- **Reynolds, J.E.F. (1989).**

In: Martindale, The extra pharmacopoeia. The pharmacological press, London, 29th ed.

- **Rout, W.R.; Formal, B.S.; Giannella, R.A.; Dammin, G.J. (1975).**

Pathophysiology of *Shigella* diarrhoea in the rhesus monkey: intestinal transport morphological and bacteriological studies.

Gastroenterology 68 : 270.

- **Selwyn, S., (1980).**

The β–lactam antibiotics: Penicillins and Cephalosporins in perspectives, Hodder and Stoughton Pub., London.

- **Sharaf,A.A. (1986).**

Diarrhoeal Diseases in children below five years of age in Juba and district. Thesis submitted for the M.D. Degree to the University of Khartoum.

- **Sharpe, M.E., (1962).**

Group D *Streptococci* in faeces of healthy infants and of infants with neonatal diarrhoea.

J. Hygiene 50 : 209.

- **Takeuchi, A.; Formal, S.B.; Sprinz, H. (1966).**

Experimental acute colitis in the Rhesus monkey following per oral infection with *Shigella flexneri*, an electron microscope study .

Am. J. Pathol. 52: 503.

- **Tamura, K.; Sakazaki, R.; Kosako, Y. and Yashizaki, E. (1986).**

*Leclercia adecarboxylata* – gen. nov., comb.nov., formerly known as *Escherichia adecarboxylata*.

Curr. Microbiol. 13, 179.

- **Thomas, C.G.A., (1988).**

In: Medical Microbiology.

Bailliere Tindall, London 6th ed. pp. 205.

- **Tomasz, A.,(1981).**

In: β–lactam antibiotics. M. Salton and G.A. Shockman (eds),

Academic Press, New York. pp. 125.

- **Torres, M.E.; Pirez, M.C.; Schelotto, F.; Varela, G.; Parodi, V.; Allende, F.; Falconi, E.; Dell'Acqua, L.; Gaione, P.; Mendez, M.V.; Ferrari, A.M.; Montano, A.; Zanneta, E.; Acuna, A.M.; Chiparelli, H.; Ingold, E. (2001).**

Etiology of children's diarrhoea in Montivideo, Uruguay: associated pathogens and usual isolates.

J. Clin. Microbiol. 2001 June; 39(6): 2134 – 9.

- **Trabulsi, Luiz R.; Rogéria Keler.; Tânia A. Tradelli Gomes, (2002).**

Typical and Atypical Enteropathogenic *Escherichia coli*

J. Emerging Infectious Diseases, may (2002) Vol. 8, No. 5.

- **Vila J.; Vargas M.; Casals C.; Urassa H.; Mshinda H.; Schellemberg D.; Gascon J. (1999)**

Antimicrobial resistance of diarrhoeagenic *Escherichia coli* isolated from children under the age of 5 years from Ifakara, Tanzania.

Antimicrob Agents Chemother 1999 Dec;43 (12): 3022-4

- **Waddell, W.R. and Kunz, L.J. (1956).**

Association of *Salmonella* enteritis with operation on the stomach.

New Eng. J. Med. 255: 555.

- **Waksman, S.A. and Lechevalier, H.A. (1962).**

In: The Actinomycetes. Vol. 3. Bailliére, London.

- **Watanabe, T. (1966).**

Infectious drug resistance in enteric bacteria. New Eng. J. Med., 275: 888 – 894.

- **Weil, A.J.; Ramchand, H.D.; Arisa, M.E. (1966).**

Nosocomial infection with *Klebsiella* type 2s.

New Eng. J. Med. 275: 17.

- **WHO Bulletin, (1982).**

Diarrhoeal Diseases

Vol. 89, No. 9.

- **WHO, (1987).**

Manual for laboratory investigation of acute enteric infections. World Health Organization document No. CDD/83 – 3.

- **WHO, (1995).**

Supplementation with vitamin A and other micronutrients.

Division of Diarrhoeal and acute Respiratory Disease Control.

Interim report 1994

WHO Geneva 1995, pp. 90.

- **WHO, (1996).**

Golden Rules for safe food preparation.

Food Safety Unit, World Health Organization, 1211 Geneva 27, Switzerland.

- **Williams, P.; Baldy, T.; Withers, H.; Dodson, S.; Griffin, C.; Snape, S.; Enfield, C. (2001).**

*Echerichia colii,* in: *E. coli* Research Group, Institute of Infections and Immunity, Queen's Medical Centre, Nottingham, U.K.

- **Wilson, G.S. and Miles, A. (1964).**

Topley and Wilson's Principles of Bacteriology and Immunity.

Vol. I. and II. Edward and Arnold Pub., London.

- **Wilson, G.S. and Miles, A. (1977).**

Bacterial variation. In: Topley and Wilson's principles of Bacteriology, Virology and Immunity.6$^{th}$ ed. vol.1. The Williams & Wilkins Co., Baltimore, pp. 379 – 82.

- **Young, V.M.; Sochard, M.R.; Gillem, H.C. (1960).**

Proc. Soc. Exptl. Biol. Med. 105: 635.

- **Youssef, M.; Shurman, A.; Bougnoux, M.; Rawashdeh, M.; Bretagne, S.; Strockbine, N. (2000).**

Bacterial, Viral and Parasitic enteric pathogens associated with acute diarrhoea in hospitalized children from northern Jordan.

FEMS Immunol. Med. Microbiol. 2000 June; 28(3): 257 – 63.