ADDIS ABABA UNIVERSITY

SCOOL OF GRADUATE STUDIES

THE PREVALENCE AND ANTIMICROBIAL RESPONSES OF *YERSINIA* ISOLATES IN COMPARISON TO ENTEROPATHOGENS CAUSING DIARRHOEA

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ABSTRACT

Among the stool samples of 205 patients tested for bacteriological cultures, only 6 (2.9%) were positive for *Yersinia*, 22 (10.7%) for *Salmonella,* 12 (5.8%) for *Shigella* and *11* (42.3%) for diarrhoeagenic *E. coli*. Out of the *Yersinia* spp. totally isolated in this study, only 3 (1.5%) were *Yersinia enterocolitica* and 3 (1.5%) were *Yersinia pestis*. Among *Yersinia enterocolitica* strains two were isolated from children while one was from adult diarrhoeal patients. Similarly, 2 of these strains were from male patients, while 1 was from female patients. With regard to *Yersinia pestis* all strains were isolated from males, of which 2 were from children and one from adult diarrhoeal patient.

The standardized agar disk-diffusion technique was used with disks of 13 antmicrobial agents (ampicillin, carbenicillin, cephalothins, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, sulphadiazene, tetracycline, and trimethoprim-sulphamethoxazole. All (51) strains of enteric pathogenic bacterial isolates were sensitive to nalidixic acid, norfloxacin, and polymyxin B and 46 strains were resistant to cephalothin. Sensitivity to gentamycin, nalidixic acid, norfloxacin and polymyxin B is greater than 90% of the tested strains, while resistance to cephalothin, ampicillin and tetracycline was greater than 50%.

All strains of *Yersinia enterocolitica* and *Yersinia pestis* were sensitive to carbenicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, sulphadiazene and trimethoprim-sulphamethoxazole. All strains of *Yersinia enterocolitica* were resistant to ampicillin. Among *Yersinia pestis* strains, 66.7% were resistant to ampicillin and cephalothin respectively while 33.3% of the strains were resistant to tetracycline.

Out of 22 *Salmonella* strains, all isolates were sensitive to norfloxacin, polymyxin B and nalidixic acid while 21 (95.5%) of strains were sensitive to gentamycin and kanamycin. Resistance was found 86.4% against cephalothin, 36.4% ampicillin and 27.0% sulphadiazene and both streptomycin and trimethoprim-sulphamethoxazole in 22.7% of

these strains. Eleven different combinations of resistant patterns were observed for all the *Salmonella* strains.

All of the 12 strains of *Shigella* were sensitve to nadixic acid, norfloxacin, polymyxin B, gentamycin and kanamycin. Ampicillin, carbenicillin, cephalothin, streptomycin, sulphadiazene, tetracycline and trimethoprim-sulphamethoxazole were sensitive against ≤ 50% of *Shigella* isolates. Among the isolates, 91.7% were resistant against tetracycline, 50% against cephlothin, 75% against each of ampicillin and strepomycin. Totally, 10 different patterns of resistance were noted for *Shigella* spp.

All strains of diarrhoeagenic *E. coli* were sensitive to gentamycin, kanamycin, nalidixic acid, norfloxacin and polymyxin B. While 90.9% of them were sensitive to chloramphenicol. Of all these isolates, 81.8% strains were resistant against cephalothin and 63.6% against sulphadiazene.

Based on the above summerized results the prevalnece and antimicrobial response of *Yersinia* spp. is discussed in comparison with those of *Salmonella, Shigella* and *E. coli* isolates. According to this study, gentamycin, kanamycin, nalidixic acid, norfloxacin and polymyxin B were effective to treat diarrhoeal patients against enteropathogens.

I. INTRODUCTION

Diarrhoea is one of the most common diseases throughout the world. Recently diarrhoea has been defined as an increased loss of stool in the frequency and fluidity greater than the usual habit of each individual (Plotkin *et al*, 1979) or it is an intestinal disorder characterized by abnormal frequency and fluidity of fecal evacuation Mims *et al.*, 1992). Diarrhoea is a worldwide problem that causes death among children in developing countries with high morbidity and mortality. It has a devastating effect particularly on small children, causing death to at least 4 to 5 million of those aged less than five years in Asia, Africa and Latin America. This is due to dehydration, which results from a rapid loss of fluid and electrolytes in diarrrhoeal stools (WHO, 1984). In general, diarrhoeal diseases are the major causes of morbidity and mortality in all age groups, but pediatric patients experience more frequent and more severe illnesses than adults (Giannella, 1993). Moreover, nowadays diarrhoeal illnesses are becoming one of the most common clincal observable gastrointestinal manifestations of AIDS problems, occurring at stages of HIV-1 infection, usually due to opportunistic infection (Colbunders *et al.*, 1990).

According to Guerrant *et al., 1991),* based on the basis of clinical symptoms and pathogenesis; diarrhoeal diseases are classified as acute watery diarrhoea, acute dysenteric diarrhoea, and chronic and persistence diarrhoea. Acute watery diarrhoea involves the small intestine and is characterized by the frequent passage of loss of watery stools without blood, while acute dysenteric diarrhoea is mainly localized to the large intestine and the

stool consists of blood and mucous. Persistent diarrhoea begins in acute form, such as watery diarrhoea or dysentry but can extend for a long period of time.

In developing countries, acute gastroenteritis which is complicated by malnutrition, constitutes a major cause of illness at all ages and kills millions of infants annually (Evans, 1979). The incidence and the consequences of diarrhoeal diseases is the major health problems in tropical and developing countries (Cutting, 1979). This, therefore, necessitate the study on the etiology of diarrhoea in different parts of the world (WHO, 1980). Among those etiological agents of diarrhoea, the most important infective agents are of bacterial, viral and parasitic origins. With regard to bacterial agents, many members of *Enterobacteriaceae* are significant etiological agents of infectious diarrhoea that occur in both developed and developing countries, at all seasons, and among people of all ages, (WHO, 1990). Particularly *Vibrio cholerae* and related *Vibrios, Salmonella, Shigella*, diarrhoeagenic *E.coli*, *Yersinia* and *Campylobacter* belong to the group of diarrhoeal causative agents.

In Ethiopia, despite the frequent occurrence of diarrhoea among the Ethiopian population caused by *Salmonella* and *Shigella* (Mogessie Ashenafi, 1983; Daniel Asrat *et al.*, 1999), and *E. coli* (Daniel Asrat *et al.*, 2001), the pattern of etiology of diarrhoeal agents caused by *Yersinia enterocolitica* has not been determined in all age groups (personal communication with Dr. Aberra Geyid). This is may be due to the series limitation of bacteriological diagnostic facilities in most laboratories and research centers. So still there is a need to study the prevalence of diarrhoea of these agents in all age groups in order to

determine their magnitude of infection in various parts of the country. The pattern of diarrhoea diseases, and the drug resistance patterns of the etiological agents are significant for health personnel in order to take the necessary measures. This study, therefore, attempts to investigate the etiological pattern by determining the prevalence of *Yersinia* isolates in comparison to the prevalence of *Salmonella*, *Shigella*, and diarrhoeagenic *E. coli* in acute diarrhoea of all age groups in some Addis Ababa hospitals and health centers. This investigation is restricted to these organisms mainly because of financial, technical and time limitations.

II. LITERATURE REVIEW

1. *YERSINIA*

1.1 General characteristics

The genus *Yersinia* belongs to the family *Entherobacteriaceae* and consists of 11 species and 5 biovars including *Y. pestis*, the cause of plague (Jay, 1996). The term yersinosis refers to infection with *Yersinia* species other than *Y. enterocolitica* (Joklik, 1984). The species of primary interest in diarrhoeal disease is *Y. enterocolitica*. This Gram-negative and rod shaped bacterium is somewhat unique in that it is motile below 30^0C but not at 37^0C. It produces colonies of 1.mm or less on nutrient agar. *Y. enterocolitica* is oxidase negative and ferments glucose with little or no gas production. Naturally *Y. enterocolitica* lacks phenylalanine deaminase but it is urease positive (Jay, 1996). The species negative to H_2S production, lysine decarboxylase, arginine dihydrolase, esculin and citrate (Simmons) reaction. However, it is positive to catalase, methyl red and ornithine decarboxylase tests.

Generally *Y. enterocolitica* ferments glucose, galactose, mannitol, sucrose, sorbitol and cellobiose but it does not ferment rhamnose, adonitol, dulcitol, raffinose and lactose. It is unique as a pathogen in being psychrotrophic (living and grow in cold temperature) (Joklik, 1984).

1.2 Growth requirements and cultural techniques

Growth requirements and metabolic pathways are similar to those of the other *Enterobacteriaceae.* The difference between the species is based on biochemical tests. The results of different tests are markedly affected by temperature (Joklik, 1984). There is no growth below -2^0C and above 45^0C and the optimal temperature is in between 22^0C and 29^0C. No growth is observed between pH 4.6 and 9.6 but the optimal PH is in the range of 7.0-8.0. *Y. enterocolitia* is destroyed in 1-3 min. at 60^0C (Hanna, *et al.,* 1977). It is rather resistant to freezing (Leistrer *et al*, 1975).

Cultural methods for the isolation of *Y. enterocolitica* from clinical and environmental samples compared with *Salemonella, Shigella* and diarrhoeagenic *E. coli* require special selective media such as Yersinia selective agar base or cefsulodin-Irgasan novobiocin (CIN) agar base and antibiotic supplement cefulodin novobiocin (CN). Yersinia selective agar base is a selective and differential medium that supports good growth of *Y. enterocolitica* and other *Yersinia* species. It has been found to be far superior to MacConkey, Salmonella-Shigella (SS) or other agar media (Power and McCuen, 1988). Biochemical tests should be carried out for further identification of isolates.

1.3 Epidemiology of enteric yersinosis.

Yersinia enterocolitica was first isolated from humans in Czechosloavakia in 1963 (Rakovsly 1973). *Yersinia enterocolitica* and the related spp. are widely distributed in the terrestrial and aquatic habitats, which are sources of the organisms to warm-blooded animals. It is found more often among human than the other species (Jay, 1996). Animals from which *Y.enterocolitica* has been isolated include wild and domestic mammals, birds and invertebrates. It is widely believed that swine constitutes the most common source of *Y.enterocolitica* in humans (Toma and Deidrick, 1975; Pedersen, 1976).

The majority of cases have come from Northern Europe, especially France, Germany and the Scandinavian countries. The sources of infection in humans are poorly known (Swaminathan *et al.*, 1982). It may will be direct contamination of food or water by infected animals (Joklik *et al.*, 1984). Several investigators agree that water might be a reservoir for *Y. enterocolitica*, and source of infection by consumption of contaminated water (Toma and Deidrick, 1975). The majority of human infections occur at cold climatic condition (Joklik *et al.*, 1984). In some areas these *Yersinia* species are as common causative gaents of serious gastrointestinal disease as is shigellosis (Mims *et al.*,1992).

The incidence of *Y. enterocolitica* infection with both human sexes is the same for all males and females. *Y.enterocolitica* is primarily a disease of infants, whereas *Y. pseudotuberculosis* more commonly affect persons 10 to 20 years of age. The frequency of isolation of *Y. enterocolitica* from persons suffering from intestinal diseases in the southern

part of Germany was 1.5% and was second only to the incidence of *Salmonella* (Swamiathan *et al.*, 1982).

As it had been observed in Canada, all Yersinia *enterocolitica* serotypes were more prevalent than O: 3 in adults (Caprioli *et al.*, 1978) as well as a family outbreak of yersiniosis have been reported (Martin, 1982). According to Weissfeld and Sonnewirth (1988), the incidence of *Y. entrocolitica* in adult was almost equivalent to that of the incidence of salmonellosis in USA. Several attempts were made to isolate this pathogen from diarrhoeal patients in Bangladesh. This lead to the conclusion that the pathogen is not important in tropical countries (Somadi *et al.*, 1982). However, *Y. enterocolitica* was found in 2.5% of the urban and 5.8% of the rural population in Nigeria (Jay, 1996). This pathogen was isolated more frequently from stools of urban children living in areas without pip-borne water than urban children residing at places connected by pip-borne water supplies (Oluanya and Olusany, 1987). In the case of Ethiopia, the pattern of etiology of diarrhoea agents caused by *Y. enterocolitica* has not been determined in all age groups (Mogesse Ashenafie, 1983).

1.4 Pathogenesis and pathophysiology of *Yersinia enterocolitica*

In addition to gastroenteritis, this organism is associated with different human syndromes such as invasion of the portal system, leading to liver involvement, and generalized septicemia with colonization in other parts of the body. The incidence of gastroenteritis syndrome is highest in the very young and old people. The symptoms are fever, diarrhoea, sever abdominal pain, vomiting, pharyngitis and headache. Symptoms of the gastroenteritis

syndrome develop several days following ingestion of contaminated food and are characterized by abdominal pain and diarrhoea. Children appear to be more susceptible than adults, and the organisms may be present in stools for up to 40 days following illness (Asakawa *et al.*, 1973).

The primary lesion results from the invasion of the wall of the small intestine, usually in the area of the ileum. Ulcers on intestinal mucosa at the site of lymphoid tissue may develop and lead to extensive loss of blood and fluid, strongly resembling the intestinal findings in typhoid fever (Joklik *et al.*, 1984).

Yersinia enterocolitica produces a heat-stable (ST) enterotoxin that survives 100^0C for 20 min. It is not affected by proteases and lipases but biological activity is lost upon treatment with 2-mercaptethanol (Okamoto *et al.*, 1981; and Okamoto *et al.*, 1982). When subjected to isoelectric focusing, two active fractions (ST-1) and (ST-2) have been found (Okamoto *et al.*, 1981). ST-1 is methanol soluble and stimulates guanylate cyclase and the cAMP (cyclic adenosine monophosphate) response in intestines, while the mechanism of ST-2 is not yet clear (Okamoto *et al.*, 1981; and *Robins*-Browine et al., 1979). It is produced only at or below 30^0C (Pai *et al.*, 1978) and its production is favored in the pH range 7-8.

In the United States, 43 strains of *Yersinia enteocolitica* from children with gastroenteritis and 18 laboratory strains were examined for ST production, and all clinical and 7 laboratory strains produced ST using the infant mouse assay, and all were negative in the

Y-1 adrenal cell assay (Pai *et al.*, 1980). Although pathogenic strains of *Y.enterocolitica* produce ST, it appears that this agent is not critical to virulence (Schiemann, 1981).

1.5 Treatment and control measures

Susceptibility of *Y. enterocolitica* and other species to ampicillin, tetracycline and other commonly used antibiotics is variable. So sensitivity testing is essential for each isolate (Cover and Aber 1989). Antibiotics are usually recommended in the case of severe disease or in patients with underlying illness. Trimethoprim-sulphamethoxazole, aminoglycosides, chloramphenicol, and the third generation cephalosporins are all appropriate choices for children (Giannella, 1993). Other forms of supportive care, such as fluid and electrolyte balance, are essential in the case of the severely ill patients. Gentamycin or chloramphenicol is recommended for treatment of *Yersinia* septicemia (Joklik, 1984).

Control measures against *Y. pestis* include the control of flea, rat, rodent and treatment of pneumonic plaque as well as restriction of movement in highly infected areas. In addition, garbage disposal and application of good personal hygiene are significant control measures. With regared to Y. enterocolitica, water sanitation and communal hygiene.

2. *SALMONELLA, SHIGELLA* AND DIARRHOEAGENIC *E. COLI*

2.1 *SALMONELLA*

2.1.1 General characteristics

Salmonella is in the family *Enterobacteriaceae*. Salmonellae are facultatively anaerobic, Gram-negative bacilli that are typically oxdase negative, non-lactose fermenting, H_2S positive and produce gas. They are indistinguishable from *E. coli* under microscopic or on ordinary nutrient media. In contrast to *Shigella, Salmonella* infect many animal spp. besides humans and are capable of invading external tissues and causing enteric fever, the most sever of which is typhoid fever. According to Ewing *et al.* (1986) there are only three species of *Salmonella: S. enteritidis, S. cholrae-suis, and S. typhi* (Ewing and Edwards, 1972). However, some significant changes have occurred in the taxonomy of *Salmonella*. All of them have been placed into two species: *S. enterica* and *S. bongori*, with the 2,324 serovars being divided into 5 subclasses or groups, most of which are classified under *S. enterica*, the type species (LeMinor and Popoff, 1987). These classifications are based on DNA-DNA hybridization and multilocus enzyme electrophoretic characterization of the genus.

2.1.2 Biochemical and cultural characteristics

Salmonella are able to grow on a large number of culture media and produce visible colonies within 24 h at about 37^0C. They can be isolated using differential and selective media of various types, such as McConkey and SS Agar (Ewing, and Edwards 1972). Salmonellae are generally unable to ferment lactose, sucrose or salicin. However, glucose and other monosaccharides are fermented with the production of gas. *S. typhi*, however, does not produce gas from glucose fermentation and H_2S production may be very slight. But most strains are motile and produce H_2S from thiosulfate. Although they normally utilize amino acids as N-sources, in the case of *S. typhimurium*; nitrate, nitrite, and NH_3 can also serve as sole source of nitrogen (Page and Solberg, 1980).

The optimal pH for growth is around neutrality i.e. 6.6 to 8.2 (Chung and Goepzert, 1970). The lowest temperature at which growth has been reported is 5.3^0C for *S. hedelberg* and 6.2^0C for *S. typhimurium* (Matches and Liston, 1968). Temperature around 45^0C has been reported by several authors to be the upper limit for growth. With regard to moisture, growth inhibition has been reported for a_w (water activity) values below 0.94 in media with neutral pH; with higher a_w as value being required as the pH is decreased toward growth minima. Unlike the *Staphlococci*, *Salmonella* are unable to tolerate high salt concentrations, brine above 9. 0% is reported to be bactericidal (Jay, 1996). All salmonellae are readily destroyed at milk pasteurization temperature.

2.1.3 Epidemiology of *Salmonella* infections

Salmonella is found in nature and occurs in the intestinal tract of domestic and wild animals. Although their primary habitat is the intestinal tract, they may be found in other part of the body from time to time. As intestinal forms, the organisms are excreted in feces from which they may be transmitted by insects and other living creatures to a large number of places. As intestinal forms, they may also be found in water, especially polluted water (Jay, 1996).

An organism carrier is defined as a person or an animal that repeatedly sheds *Salmonella* spp., usually through feces, without showing any sings or symptoms of the disease (Jay, 1996). Infants frequently become long-term carriers and may harbor the bacteria for longer periods than older persons and thereby transmit infection to other members of the family (Szanton, 1957). Transmission may be direct by means of the fecal-oral route, it may be air-borne or it may be indirect by means of foods (Chapman, 1980). Contaminated milk, poultry, meat, pork, egg products and beef products are associated with outbreaks of salmonellosis (Jay, 1996).

For epidemiological purpose, the salmonellae can be classified into three categories. (1) Those that infect humans only; which include *S. typhi*, *S. paratyphi A,* and *S. paratyphi C.* They are the agents of typhoid and the paratyphoid fever, which are the most severe of all diseases caused by *Salmonella*. Of all, typhoid fever has a longest incubation time, fever and has highest mortality rate (Jay, 1996). (2) The host-adapted serovars, some of which

are human pathogens and may be contracted from foods. (3) Unadapted serovars (no host preference). They are pathogenic of humans and other animals, and they include most of food borne serovars (Jay, 1996).

Salmonella spp. cause a variety of human diseases called salmonellosis. The range of disease is from mild self-limiting gastroenteritis to a more severe form, such as bacteremia or typhoid fever, which can be severe and life threatening. Salmonellae, the most common bacterial cause of gastroenteritis in the United States, are estimated to cause 1 million to 2 million infections per year (Hargrett-Bean *et al.*, (1988). The group at greatest risk for infection is infants.

In the case of developing countries, according to Wamola (1980), the isolation rate of *Salmonella* was 24-35% in Kenya. In most developing countries *S. typhi* was the most dominant strain followed by *S. typhimurium*. In 1979, from Vietnams refugees in Hong Kong the prevalence rate of *Salmonella* was 13.5% (Anton *et al.*, 1981). In Iraq, in between 1973 and 1976, the prevalence of *Salmonella* was 10.85% (Allos, 1978). In Ethiopia, forty-five strains of *Salmonella* was isolated from 1000 adult out-patients in 1983 (Mogessie Ashenafi, 1983) and the prevalence of *Salmonella* in all age groups were 3.8% (Daniel Asrate *et al.*, 1999 and Zeleke W/Tenssai (1990 Zeleke W/Tenssai (1990).

2.1.4 Pathogenesis and pathophysiology of *Salmonella*

Salmonella species cause a variety of human diseases called salmonellosis. Infection with the organism is known to cause a variety of clinical syndromes including (1) acute gastroenteritis; (2) local, non-intestinal infections; (3) bacteremia; (4) an asymptomatic carrier state; (5) enteric fever, including typhoid fever (Giannella, 1993). The severity of the diarrhoea disease depends on the virulence of the strain and the condition of the human host. Salmonellae cause disease through invasion of epithelial cells in the ileum and colon, leading to the typical clinical picture of watery stools; which often contain blood and mucous. Generally, the disease is characterized by diarrhoea, fever, and abdominal pain that is self-limiting and lasts for 2 to 5 days. *Salmonella* infection in man is almost acquired by ingestion of the organism. The incubation period varies between 8 and 72 hours and duration of illness is between 5 and 7 days (Plotkin *et al.*, 1979).

Pathogenicity is determined by surface antigens, invasiveness and toxins. Surface O and Vi antigens in *S. typhi* may be contributed to attach to host receptor cells. The Vi antigen may serve to protect the O antigen from antiserum and prevent phagocytosis (Joklik, *et al.*, 1984). Like *Shigella*, virulent *Salmonella* penetrate the epithelial lining of the small bowel. However, unlike *Shigella, Salmonella* do not reside in the epithelial lining but pass subepithelial tissue (Joklik *et al.*, 1984).

In the pathogenesis of *Salmonella,* mostly two types of toxins, such as an enterotoxin and a cytotoxin, are involved. The enterotoxin was first demonstrated in 1975 by Koupal and

Deibial (1975). As reported, more enterotoxin is produced during the stationary phase of growth, at a pH of 6-7 or higher, at 37^0C and with increased aeration (Koo and Peterson, 1981). This toxin was found to be heat-labile at 100^0C. Enterotoxin of salmonellae acts in a manner similar to those of *E. coli* by elevating intestinal cyclic adenosine monophosphate (cAMC). Unlike the *E. coli* enterotoxins, it is produced in much lower quantities and is more difficult to separate from producing cells. Furthermore, the *Salmonella* enterotoxin is quite similar to cholera toxin (CT) in biological and antigenic characteristics. The cellular damage that occurs to the intestinal mucosa during salmonellosis is caused by a cytotoxin. Once damaged, the intestinal mucosa is more easily invaded by the infecting organisms, which can then bring about additional tissue damage. Although the pathogenesis of shigellosis and salmonellosis appears to be closely related, more tissue destruction occurs in the former than in the latter. This is may be due to an additional toxin or to other factors. (Jay, 1996).

2.1.5 Treatment and control measures

Treatment is recommended for children who are immnocompromised, those with hematological disease, and those with artificial implants. Treatment is also recommended for patients at any age that appears toxic. In this case, ampicillin, chloramphenicol and trimethoprim-sulphamethoxazole (TMP-SMZ) as well as oral amoxicillin are recommended (Giannella, 1993). However, chloramphenicol resistance was common among isolates of *S. typhi* during a recent epidemic of typhoid fever in Mexico (Giannella, 1993). Control measures include water treatment, eat properly cooked and / or refrigerated food.

2.2 SHIGELLA

2.2.1 General characteristics

The genus *Shigella* is composed of gram-negative non-motile that belongs to the family *Enterobacteriaceae* as do the *Salmonella* and *Escherichia.* Only four species are recognized: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei.* *S. dysentriae* is a primary pathogen that causes classical bacillary dysentry; as few as 10 cfu are known to initiate infection in susceptible individuals. Unlike the salmonellae and escherchiae, shegillae have no known non-human animal reservoirs. The shigellae are phylogenetically closer to the escherichiae than to the salmonellae. The three species are etiologic agents of food borne gastroenteritis and are placed in separate serologic groups based on O antigens. These are *S. flexneri* in group B, *S. boydii* in group C, and *S. sonnei* in group D. (Jay, 1996).

2.2.2 Biochemical and Cultural Characteristics

Shigellae are non-motile, oxidase-negative and produce acid only from sugars but not gas (except certain type of *S. flexneri*). They do not grow on citrate as sole carbon source and also do not grow on KCN and do not produce H_2S (Jay, 1996). These factors help to distinguish shigellae from most salmolellae. In contrast to *E. cloi*, *Shigella* does not produce lysine decarboxylase but can utilize acetate as a carbon source. In addition to these, shigellae ferment lactose upon extended incubation (Joklik, et al.1984).

In the case of growth requirements of *Shigella*, the temperature is as low as 10^0C and as high as 48^0C but *S. sonnei* can grow at lower temperature than the other three species. The

optimum temperature for growth is 37°C. Growth at pH 5.0 has been recorded but best growth is recorded in the range of 6 to 8 pH. It is unclear whether shigellae can grow at acid values below those for *Salmonella* or E*scherchia*. But their heat resistance is appeared to be parallel with that of *E. coli* strains (Jay, 1996). They can grow in ordinary nutrient (beef extract) media.

2.2.3 Epidemiology of *Shigella* Infection

The most important reservoir of infection is the human carrier, either convalescent or with an important infection. The spread of the disease is due to the more or less direct transfer of the

specific *Shigella* strain from infected intestinal discharges to the alimentary tracts of another individual. Polluted water can be the medium of transmission (Hughes *et al.,* 1975; Weissman *et al.,* 1976; Katzenelson *et al.,* 1976) and the contamination of food by chronic carriers and convalescents (Donaldo and Gangaresa, 1969) appear to be the most important factors in the spread of bacillary adysentry.

In developed countries such as in North America, 73% were *S. sonnei*, 26% were *S. flexeri*, 0.7% were *S. boydii*, and 0.6% were *S. dysenteriae*. In contrast to developed countries, in developing countries where hygiene is poor, the isolation pattern is reversed: *S.dysenteriae*, and *S. boydi* are the most frequent isolates, followed by *S. flexneri* and then *S. sonnei* (Joklik *et al.,* 1984).

There are various reports on *Shigella* with variable prevalence rates from humans. For instance, 51-54 % in Kenyia between 1975 and 1979 (Wamola, 1980). In *Shegella* strains from 13,943 diarrhoeal patients during the period 1969-1973 (Ai *et al.,* 1975). In Bangladesh, out of 3550 patients in 1979, 11.6% of the isolates were *Shigella* (Stoll *et al.,* 1982). In Ethiopia, 165 *Shigella* strains were isolated in between 1974 and 1978 (Afeworki Gebreyohannes and Yetneberish Limeneh, 1980), and 90 strains of *Shigella* were isolated from 1000 adult diarrhoeal out-patients (Mogessie Ashenafi, 1983). About 50 *Shigella* strains were isolated from 700 diarrhoeal samples collected from adult diarrhoeal out-patients in Addis Ababa between January 1995 and July 1995 (Mache *et al.,* 1997). About 11.7% of *Shigella* species were isolated at Tikur Ambassa and Ethio-Swedish Children's Hospital, Addis Ababa, Ethiopia (Daniel Asrat *et al.,* 1999).

2.2.4 Pathogenesis and pathophysiology of *Shigella*

Bacillary dysentery follows attachment to and penetration of the epithelial cells of the mucousal surface of the terminal ileum and the colon by *Shigella.* Local inflammation occurs by cell death and shallow ulcers (Jokilik *et al.,* 1984). Much is know about the virulence properties of *S. dysenteriae* than the other three species. The pathogenic properties of *S. flexineri* are similar to enteroinvasive strains of *E. coli* and this species appears to be more invasive than *S. sonnei* and *S. boidi.* However, *S. flexineri* lacks the Shiga toxin but it has four virulence-associated loci on large plasmids. Three of them are necessary for the adherence and invasiveness as well as for intracellular survival and multiplication (Finlay and Fedkow, 1988).

The specimen of disease varies from asymptomatic infection to severe bacillary dysentery with high fever, chills, convulsions, abdominal cramps, tenesmus and frequent bloody stools. First presents as watery diarrhoeaa and fever and subsequently changed in frequent small volume stools with blood and mucous. The organisms multiply initially in the small bowel to a concentration of 108 organisms per milliliter. Penetration of epithelial cells causes inflammation and superficial ulceration (Jokilik *et al.*, 1984). The incubation period in acute onset of diarrhoea is 1-3 days and duration of illness is between 2 and 20 day (Plotkin *et al.*, 1979).

Virulent *Shigella* possesses three factors that contribute for pathogenesis: smooth lipopolysacchride (LPS), invasiveness and toxin production. Smooth LPS structure has been demonstrated with *S. flexneri* and *S. sonnei* (Joklik *et al.*, 1984). In the case of invasiveness, virulent *Shigella* penetrates the epithelial cells of the colon in an uneven manner. The organisms rarely penetrate to the lamina propria, and exist intracellularly in cytoplasmic vacuoles. Once inside the cell, *Shigella* induces a number of degenerative changes in ultrastracture, indicating cell injury. Cellular damage may be related to the production of toxin.

The toxin that is produced by *S. dysenteriae* is an enterotoxin (Shiga toxin) which is thought to be responsible for the convulsions seen in some children and responsible for the higher mortality. This toxin is cytotoxic for various tissue cultures and has enterotoxic activity, producing fluid accumulation in ligated rabbit ileal loops. The enterotoxic activity does not appear to be due to an increase in adenyl cyclase activity as with *E. coli* heat-labile

or cholera enterotoxin. The toxin may play two roles in the pathogenesis of *Shigella* infection. First, it may act as an enterotoxin in the jejunum, producing the watery diarrhoea associated with early disease. Second, its ability to inhibit protein synthesis and cytotoxicity may account for the cellular death and production of chronic ulcers (Joklik *et al.,* 1984).

2.2.5 Treatment and Control Measures

Shigellae are generally resistant to most commonly used antibiotics and are also known to develop multiple drug-resistance. Multiple resistance is believed to be received from *E. coli* already present in the patient's intestinal tract (Mitsuhashi, 1969; Watanabe, 1963; and Watson, 1967).

In Ethiopia, multiple drug resistance and R-factors were reported by Melesse Gedebou and Alebachew Tasew (1979 and 1982) and a comparative drug resistance within *Shigella* strains by Afeworki Gebreyohannes and Yetnebersh Limeneh (1980) (Mache et al., 1997).

The use of antibiotics in the treatment of *Shigella* is controversial. The arguments against antibiotic use are based on that the disease is usually self-limited, antibiotic use may increase the number of resistant strains, and the risk of developing the hemolytic uremic syndrome is increased after antibiotic therapy (Butler *et al.,* 1987). However, in sever cases of dehydration, fluids should be provided through intravenous as well as glucose-electrolyte solutions are used orally. Tetracycline, ciprofloxacin and norfloxacin are all effective

against *Shigella* but are not recommended for use in young children. Although ampicillin is a suggested agent, amoxillin is not effective. Trimethoprim-Sulphamethoxazole is the drug of choice when the organisms sensitivity is unknown or the patient is allergic to penicillin-type drugs.

In the case of control measures, carriers should be treated and not allow to handle foods. Proper sewage disposal and water chlorinating are important measures required for controlling all gram-negative enteropathogen including *Yersinia (*Joklik *et al.,* 1984).

2.3 DIARRHOEAGENIC *E. COLI*

Escherichia coli bacteria of most strains are non-pigmented, motile, produce lysine decarboxylase and utilized acetate as the sole source of carbon. Serotyping of the O antigen, K antigen, and H antigen of *E. coli* is a useful epidemiological tool. At this time, at least 150 O antigen types, 90 K antigen types, and 5 H antigen types have been described. The K antigens are further divided on the basis of physical behaviour into three main types: L, A and B.

E. coli produce at least two different types of fimbria: mannose-sensitive, or common pilli and mannose resistant. Both types have been shown to be important for colonization of the host tissues. The K, capsular antigen is frequently found in *E. coli* isolates obtained from patients with bacteremia and neonatal meningitis. Certain serotypes have a higher probability of acquiring and retaining the enterotoxin plasmids. For instance, nearly all O78:H11 and O78:H12 serotypes of *E. coli* are enterotoxigenic, where as *E. coli* O78

possessing other H antigens are less likely to be toxin producers. Now *E. coli* is established as one of the most important bacteriological agents of enteritis, and several extraintestinal diseases such as urogenital infection, wound infection, bovine mastitis, septicemia, and meningitis.

Enteropathogenic *E. coli* is an important agent of diarrhoea in children of developing countries, where personal hygiene and public sanitation is deficient. In addition, enterotoxigenic *E. coli* is major causes of traveler's diarrhoea that occurs when people travel to endemic region.

2.3.1 Epidmology of *Escherichia coli*

In 1885, Theodore Escherich was the first bacteriologist that describes *E. coli* as bacterium and provided the name *E. coli*. *E. coli* has long been known as a harmless bacteria, living inside the intestine and probably palying a useful role in providing a regular supply of vitamins and growth factors for the body. Under special conditions, it was known to cause urinary infections and could cause bacteraemia or even septicemia and meningitis only in infants and old subject under unusual conditions where there was a breakdown of the defense mechanisms.

The role of *E. coli* as a diarrhoeagenic organism became obvious in the late 1940s with the identification of enteropathogenic *E. coli* (EPEC). Later enterotoxigenic *E. coli* (ETEC) became and identified as one of a major cause of diarrhoea all over the wold. Soon after,

the interoinvasive *E. coli* (EIEC) was added to the list of diarrhoeagenic *E. coli*. It is now known that EIEC also share antigenic components and other characteristics with *Shigella* (O'Brien *et al.*, 1982). It is known that plasmids obtained from EIEC and *Shigella* share considerable homology (Sansonetti *et al.*, 1983). In the Sudan, EIEC strains were found 3.5% diarrhoea patients and 2.4% non-diarrhoea patients. The over all isolation rate was 2.7%. Similar pattern of EIEC prevalence among adults (2.8%) and children (2.5%) was seen (Omer and Ibrahim, 1985). Lastly in 1982, haemorrhagic *E. coli* (EHEC) strains have been recognized as a significant pathogen in the causes of haemorrhagic colitis and the hemolytic-uremic syndrome (HUS). EHEC outbreaks were reported in USA in 1982 for the first time and since then sporadic cases have been recognized in several European countries and there have been a number of outbreaks in the US (Nataro *et al.*, 1983).

Recently, the enteroaggregative *E. coli* (EAggEC) has been detected that characterized by aggregates of bacteria with a formation of a "stacked brick" or "honey comb" appearance adhering to both the surface of the Hep-2 cells and the glass coverslide (Nataro *et al.*, 1983a cited in Aberra Geyid, 1995). Recent publications indicate that EPECs and ETECs could also be present in asymptomatic individuals and the same is true with EIECs (Omer *et al.*, 1985); however, they are present only in small numbers. Even though the low numbers of EPECs, ETECs and EIECs known to be present in low numbers in the asymptomatic population, they produce the disease when they find a favorable condition to develop as the predominant strain of *E. coli* population in the gut.

Serotyping can be used to identify different strains. But this is a time-consuming process, and only a few laboratories in the world are equipped to carry out serotyping of all *E. coli* strains. Therefore, it is not very suitable for the large-scale routine testing of all *E. coli* strains that may be present in any one individual specimen. Some workers are now interested in involving a sensitive, specific and practical new diagnostic tool (Levine and Edelman, 1984). Candidate methods include genetic probes, identification of page lysotypes, plasmid profile and perhaps simple tests for identification of specific antigens by development of co-agglutination techniques for each individual antigen. Based on the basis of disease syndromes and characteristics, and also on their effect on certain cell cultures and serologic groupings, there are five virulence groups of *E. coli.*

1. **Enteropathogenic *E. coli* (EPEC):** Historically, this group of EPEC was the first of this species to be identified as causative agents of diarrhoea and until recently were defined by belonging to certain O: H serotypes that do not produce enterotoxins. EPEC strains also do not have invasive ability. More recently, the adherence ability of EPEC in tissue-culture assay, in a manner described as localized adherence, has been recognized as an important characteristic of this group (Scaletsky *et al.,* 1985). EPEC strains were the first to be described as causative agents in an epidemic of infantile enteritis with diarrhoea. EPECs exhibit localized adherence to tissue culture cells and autoagglutinate in tissue culture medium. The adherence to HEP-2 cells in a localized pattern is known to be associated with a high-molecular weight plasmid. EPEC infection is also characterized by attachment and effacement of the eucaryotic cell surface with a characteristic "pedestal" formation. The

chromosomal gene, *eae A* controls this attachment-effacement, and *att-eff* lesions are produced by EPEC strains in both the small and large intestines.

2. Enterotoxigenic *E. coli* (ETEC): Enterotoxigenic *E. coli* has the ability to produce entrotoxins and specific adhesive fimbriae. The bacteria colonizes the proximal part of the small intestine and induces watery diarrhoea without any damage to the intestinal mucosa in the same way as cholerae (Levine, 1987). The clinical features of ETEC acute diarrhoea are abdominal pain, low-grade fever, and result in water diarrhoea with usual duration of 4-5 days. The severity of this infection rate vary from mild traveler's diarrhoea to sever, or dehydrating neonatal diarrhoea in infants (Drasar and Barrow, 1985 cited in Aberra Geyid, 1995).

Enterotoxigenic *E. coli* that isolated from patients with diarrhoea has been shown to produce two types of enterotoxins: a heat-labile enterotoxin (LT) and a heat stable enterotoxin (ST) (Donta *et al.,* 1974). The ability to produce these enterotoxins is associated with two transferable plamids, one coding for both enterotoxins and the second coding for the ST only. So that, a given strain of ETEC can produce either LT or ST only or both. The LT enterotoxin is similar in many respects to the enterotoxin of *Vibrio cholerae.* Both the LT and cholera enterotoxin stimulates the adenyl cyclase in epithelial cells of the small intestinal mucosa. Moreover, LT, like Chloera toxin (CT), consists of one polypeptide A, and five smaller and identical polypeptide B subunits. The B subunits bind to the intestinal epithelial cells and subunit A move across the cell membrane and catalyses the adenylate cyclase to cause an increase in the concentration of cyclic adenosine-5-monophosphate (cAMP). Increased cAMP inhibits the coupled influx of Na^+/Cl^- leading to

a decrease in the active absorption of sodium and, therefore, water. Simultaneously, increased cAMP stimulates an increase in active secretion of chloride out of crypt cells into the bowel lumen. The next result is diminished absorption of water and electrolytes, an increase in the quantity of water within the intestinal lumen that leading to a profuse watery diarrhoea.

Unlike the LT and the cholera enterotoxin, the ST does not stimulate adenyl cyclase activity and is not reactivity in the rabbit skin test. The ST appears to activate guanylate cyclase to produce cyclic guanosine monophosphate, impairing net chloride and sodium absorption. Furthermore, the STs of *E. coli* are further divided into two: STI (STA) and STII (STB). STI is methanol soluble polypeptide that biologically active in sucking mice or piglets and STII is a methanol insoluble polypeptide active in ligated intestines of neonatal and weaned pigs.

STI has a biological activity to stimulate guanylate cyclase, which is found on the apical membrane of enterocytes. When activated, this enzyme produces cyclic GMP, which activate a protein kinase that phosphorylated the brush border proteins. As the result, there will be a net decrease in chloride ion absorption rather than chloride ion secretion take place like cholera toxin. The mechanism of STII is not known, but it appears to be act neither via cAMP nor cGMP. The ST also appears to decrease the motility of the small bowel. The LT toxin is destroyed at 60^0C in about 30 min, whereas ST toxin can resist heating at 120^0C for over 30 min.

ETEC gastroenteritis is caused by the ingestion of 10^6-10^{10} viable cells per gram that must colonize the small intestines and produce enterotoxin(s). In conclusion ETEC strains have been shown to be associated with the causes of diarrhoea in infants and adults in several countries including Great Britain (Rowe *et al.*, 1977) and the United States (Ryder, 1976). They are also known to cause traveler's diarrhoea in adults.

3. Enteroinvasive *E. coli* (EIEC): In 1971, Dupont *et al.* described certain *E. coli* strains that caused an invasive, dysenteric form of diarrhoea (Levine, 1987). These do not produce interotoxins as do ETECs, but they enter and multiply in colon epithelial cells and then spread to adjacent cells in a manner similar to the shigellae. These strains closely resembled *Shigella* in many ways. Like *Shigella*, EIECs are non-motile; unable to ferment lactose; resist gastric acidity, bile salts and pancreatic enzymes in order to survive the passage through the upper intestine; and have smooth LPS cell wall structure. Furthermore, like shigellae, EIECs possess 140-Mda enteroinvasive plasmids (PINV) that are quite similar to those found in *Shigella flexneri* and are essential for their invasiveness.

EIECs are a major cause of diarrhoea and dysentery in children in developing countries and have been observed in food-brone outbreaks in the industrialized countries (Fasano *et al.*, 1990). EIECs are common in very young and very old individuals. The epidemiological and ecological studies of EIECs are poor and there is no evidence of an animal or environmental reservoir. Infections are usually food-borne but there is also evidence of person to person or animal to person cross-infection (Levine *et al.*, 1993).

4. Enterohaemoragic *E. coli*, (EHEC): EHEC strains are agents of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). These strains of *E. coli*, which are now termed enterohaemorrhagic, produce one or more cytotoxins active against vero cells (verotoxins) and Hela cells in much the same manner as Shiga toxin of *Shigella dysenteriae* type I. This class of *E. coli* toxins are now referred to as Shiga-like cytotoxins (SLTs). There are two antigenically distinct groups of SLT: SLT-I and SLT-II. The SLT-I type appears to be antigenically homogeneous and more closely related to Shiga toxin. The SLT-II group appears to be more diverse antigenically (Gannon *et al.,* 1990).

Clinical syndrome is bloody diarrhoea, which is different from the classical dysentery caused by *Shigella* and EIEC strains. EHEC is similar to EPEC in possession of the chromosomal gene *eae A* in the production of *att-eff* lesions. In contrast EIEC, EHEC strains affect only the large intestine (in piglet models). EHEC also produce large quantities of Shiga-like toxins and 60-Mda plasmid that encodes fimbriae that mediate attachment to culture cells, and they do not invade HEP-2 or INT 407 cell lines.

5. Enteroaggregative *E. coli* (EaggEC): According to Nataro et al (1985) the enteroaggregative *E. coli* strains exhibit a "stacked-brick" or "honey comb" of adherence to HEP-2 cells, and carry a 60 fimbriae that are responsible for the aggregative expression, and for a specific outer membrane protein (OMP). Antibodies raised against the OMP of a strain prevented adherence to the HEP-2 cells (Dallas *et al.,* 1979). Some EAggEC strains produce a heat stable enterotoxin (ST), which has been designed EASTI (Picken *et al.,*

1983). They are common causes of infantile blood diarrhoea (Baudry *et al.*, 1990 cited in Aberra Geyid, 1995). Initially EAggEC were isolated from adult travelers with diarrhoea among isolates of the serotype O44: H18 (Scotland *et al.*, 1991 cited in Aberra Geyid).

2.3.2 Treatment and control measures

The best treatment of diarrhoea caused by *E. coli* appears to be management of fluid and electrolyte balance. Although infantile diarrhoea has been controlled by a number of antibiotics, resistant forms can be treated by trimethoprim-sulphamethoxazole (Joklik *et al.*, 1984).

3. SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

Antibiotic is refered as a substance produced by a microorganism or to a similar substance produced wholly or partially by chemical synthesis that, in low concentration, inhibits the growth of other microorganisms (Joklik, 1984). There are four main target sites for antimicrobial action: such as inhibition of cell wall synthesis, protein synthesis, nucleic acid synthesis and cell membrane function (Mims *et al.*; 1992).

Inhibition of cell wall synthesis: Beta-lactams, such as cephalothin, ampicillin and carbenicillin, are antimicrobial agents whose inhibition action is attributed to an interference with cell wall synthesis (Mims *et al.*, 1992).

Inhibition of protein synthesis: protein synthesis is the end result of two major processes (transcription and translation). An antibiotic that inhibits either of these processes will inhibit protein synthesis in the bacteria. During transcription, the genetic information in the DNA nucleotides by the enzyme RNA is transferred to a complementary sequence of RNA nucleotides by the enzyme RNA polymerase. For instance, actinomycin inhibits both DNA and synthesis and DNA-dependent RNA synthesis. It blocks RNA synthesis by preventing the progression of RNA polymerase along the DNA tempelate. Another example is rifampin, which inhibits protein synthesis by selective inactivating the DNA-dependant RNA polymerase (Joklik, 1984). Inhibition of translation by antibiotics is carried out either by binding to the 30s or 50s ribosomal subunits. Streptomycin is the best example of this extent. It binds irreversibly to the 30s ribosomal subunit, drastically interupting the ribosome cycle at the intition of protein synthesis. In addtition to treptomycin, the aminoglycoside antibiotics such as Kanamycin and gentamycin have the same mechanism of action (Brewer, 1977). In conclusion, all of aminoglycosides act on protein synthesis at the level of 30s ribosomal subunit Franklin, (1989).

Tetracyclines are a familly of aminocyclitol antibiotics that inhibit protein synthesis at the 50s ribosomal subunit. Chloramphenicol is another antibiotic that inhibits growth of bacteria by interfering with protein synthesis. It binds exclusively to the 50s ribosomal subunit (Joklik. 1984).

Nnucleic acid synthesis: Sulphonamides are antimicrobial agents act directly on DNA synthesis because of their effect on folic acid metabolism. Sulphonamides exhibit inhibitory activity against the synthesis of folic acid. For instance, trimethoprim-sulphamethoxazole is one of the inhibitory of dihydrofolate reductase. Quinolones are drugs act on the α-subunit of DNA gyrase. Nalidixic acid and norfloxacin are belonging to these drugs (Greenwood *et al.*, 1992). Polymyxin B act like cationic detergents to disrupt the cell membrane of the pathogens.

3.1 Drug resistance

Drug resistance arises by a random chromosomal mutation that results in an altered susceptability to the drug, the drug serving only as as a selective agent favouring the survival of resistant over sensitive organisms once the genetic alternation has taken place. For instance, sreptomycin resistance via the alternation in a ribosomal protein and a single amino acid change in the enzyme dihydropteroat synthetase resulting in a lowered affinity for sulphonamides (Mims *et al.*, 1992). Bacteria are also able to acquire resistant gens on the transmissible plasmids. Such plasmids often code for resistance determinants to several unrelated families of antimicrobial agents. Thus, a cell may acquire resistance as many as six different drugs at once. Resistance genes may also occur on transposons and can disseminate rapidly from chromosome to plasmids or from one plasmid to anther (Garrod *et al.*, 1992).

Resistance mechanisms can be broadly classified into three main types. (1) Alternation in target site. The target enzyme may be altered so that it has a lowered afinity for the antimicrobial but still functions adequately for normal metabolism to proceed. (2) Alternation in access to the target (altered up take). This mechanism involves decreasing the amount of drug that reaches the target, either by increasing the impermeability of the cell wall, or by pumping the drug out of the cell. (3) Production of enzymes which modify or destroy the antimicrobial agent (drug inactivation). There are many examples of such enzymes, the most importants are beta-lactamases, aminoglycoside-modifying enzymes and chloramphenicol acetyl transferases (Mims *et al.*, 1992).

3.2 Antimicrobial druge resistant enteric pathogenic bacteria

A comomonly prevalence of bacterial resistance to commonly prescribed antimicrocrobials, especially in developing countries poses a major concern in the management of infection (Shanahan *et al.*, 1993). This has resulted mainly from the extensive use, and often misuse, of antimicrobial in both human and verterinary medicine (Mitsuhashi *et al.* 1993). Several workers have reported to a positive correlation between usage and the increase in resistance of enteric bacteria (King *et al.*, 1992). The widespread use of antimicrobial agents has accelerated the spread of genetic coding for resistance by giving a selective advantage to resistant enteric bacteria (Mazodier *et al.*, 1991).

Excessive antimicrobial resistance in the community particularly in developing countries may also be enhanced due to environmental conditions such as crowding, poor sanitation,

or contamination of food, that tends to disseminate resistant strains (Lpaton *et al* 1991). Other practices such as the excessive and inappropriate use of antibiotics in the treatment of childhood gastroenteritis in many developing countries may result in the development of resistance in enteric pathogens (Levy, 1990).

The indiscriminate use of antimicrobial agents for animal treatment is one of the causes of the development of resistant strains of bacteria. Nearly half of all of the antibiotics produced in the US is used in animals and this is related to the risk of human infection with multidrug resistance of *Salmonella* of animal origin (Bennett, 1980). Resistance to one or more drugs had significantly increased within 8 years (21% to 31%). The increase in frequency of resistance was significant for streptomycin, sulphonamides, ampicillin and kanamycin (Ryder *et al.*, 1980). In Algeria, 91% of most *Salmonella* serotypes showed resistance to 3-7 antibiotics.

According to Messale Gedebou and Alebachew Tassew (1979), 89 *Salmonella* and 69 *Shigella* isolates from 5 hospitals in Addis Ababa were studied for their antibiotic susceptibility. About 25% of *Salmonella* strains were susceptible to all 11 different drugs and 52% resistant to only one drug. According to Mogessie Ashenafi (1983) 45 isolates of *Salmonella* were sensitive to only gentamycin, polymyxin B and trimethoprim sulphamethoxazole out of 11 different antimicrobial drugs. Sensitivity to the remaining 8 drugs was fairly high and varied between 73.3% and 82.2%. About 31% of *Salmonella* strains were resistant to one or more drugs. Of the 45 *Salmonella* strains, 26.7% were multiple resistant. In all, 7 different pattens of resistance were noted.

In many countries a high incidence of antibiotic resistance has been observed in *Shigella*. In India, streptomycin resistance was found in 29.3% of *Shigella* strains, (Rao *et al.*, 1979). Shigellae are generally resistant to most commonly used antibiotics and are also known to develop multiple drug-resistance. Multiple resistance is believed to be received from *E.coli* already present in the patient's intestainal tract (Mitsuhashi, 1969). Multiple drug resistance was first noted in Japan in the early 1950s and by the end of in 1967. About 80% of *Shigella* strains isolated in Japan were resistant to two or more drugs (Falkow, 1975).

In Ethiopia, multiple drug resistance and R-factors were reported by Messele Gedebu and Alebachew Tassew (1979) and a comparative drug resistance within *Shigella* strains by Afeworki Gebreyohannes and Yetnebersh Limenh (1980). About 19 patterns of drug resistance were exhibited from the 360 *Shigella* isolates (Afeworki Gebreyohannes and Yetnebersh Limenh , 1980). This problem of multiple drug resistance is a worldwide and is the result of indiscriminate use of antibiotics by man and animals.

According to Mogessie Ashenafi (1983), all (90) strains of *Shigella* were sensitive to kanamycine, gentamycine, polymyxin B and trimethoprim-sulphamethoxazole out of 11 different antibiotics. Most (95.6%) were sensitive to cephalothin. Sensitivity to ampicillin, chloramphenicol, streptomycin, sulphadiazine and tetracycline varied between 31.1% and 57.7%. Ampicillin, carbencillin, strepthomycin, sulphadiazine and tetracycline were mostly ineffective (< 50%) while only 57.7% of the strains were sensitive to chloramphenicol. Aacccording to Zeleke W/Tenssai (1990), of the 11 antimicrobial drugs;

kanamycin, gentamycin, trimethoprim-sulphamethoxazole, chloramphenicol and polymyxin B were effective against all 6 (100%) the *Shigella* isolates. Of the 6 *Shigella* isolates, 5 (83.3%) were resistance to ampicillin and carbenicillin. Similarly, 4 (66.7%), and 3 (50%) of *Shigella* tested were resistant to cephalothin, teteracycline and sulphadiazine respectively.

Most of the *Shigella* isolates in Mache et al. Study (1997) contained between two and six plasmids per strain, however, some strains contained only a single small plasmid. Afeworki and Drasar (1990) also isolated multiple plasmids from *Shigella* strains in Ethiopia and noted that some plasmids were contained in all of the isolates while some other in a few of the strains. This variabilitiy could be due to loss of plasmids or to chromosomal integration, which are common encountred phenomena among plasmid containg strains (Mache *et al.*, 1997).

About 24 (92.3%) of *E. coli* isolates in the Zeleke W/ Tenssai (1990) were sensitive to kanamycin. According to Zeleke W/ Tenssai study (1990), only 17 (65.4%), 11 (42.3%) and 18 (69.2%) of the isolates were sensitive against cephalothin, teteracycline, and trimethoprim-sulphamethoxazole respectively.

III. OBJECTIVE

A. General objectives

To determine and describe the prevalence of diarrhoea caused by *Yersinia* isolates in comparison with the commonly encountered diarrhoeagenic *Salmonella*, *Shigella* and *E. coli* and resistance and susceptable strains among all age group Ethiopians in Addis Ababa.

B. Specific objectives

1) To isolate and identify the specific diarrhoeal causing organisms in the stool specimens by using specific isolation and biochemical tests.
2) To document and report the prevalence of *Yersinia* and other common diarrhoeagenic enteric bacterial spp. in the stool specimens that are collected from diarrhoeal out-patientts of all age groups.
3) To determine antimicrobial susceptibility and resistance of *Yersinia*, *Salmonella*, *Shigella* and *E. coli* isolated strain fromt diarrhoeal pateints.

IV. MATERIALS AND METHODS

A) **The sample size.** The sample size for the study was determined by using sampling method in **EPI** (Epidemiology info.) statistical computer software to be 205 by considering the population size of 7549 (second hand data in 1999-2000) visiting the study sites of hospital and health center from where the stool specimens were collected. Expected rate of frequency prevalence of diarrhoeac out-patientts of all age groups were 9% (data source from Region 14 Health Bureau) and 95% level of confidence. The responsible physicians or nurses and laboratory technicians filled questionnaires related to the history and clinical status of each subject.

B) **Study area.** The study sites were Yekatit 12 hospital and Arsho Laboratory as well as Shero-Meda, Arada, Addis Ketema, Kebele 18 public and Tekle-Himanote health centers, in Addis Ababa. The study sites were selected by personal judgement in consideration with population size, location, cost, time and other statistical elements.

C) **Patients.** All age group diarrhoeal out-patients in some Addis Ababa health centers, hospital and laboratory (as mentioned in the study area). In the case of diarrhoeagenic *E. coli*, only children (< yrs) were tested due to lack of anti-O157 antisera. It is well known that serogroup anti-O157 antisera strain of *E. coli* is pathogenic to both children and adults. The rest strains of *E. coli* are normal flora for children (> 5 yrs) and adults.

D) **Duration of the study.** Stool specimens were collected in sterile containers from diarrhoeal out-patientts between Novober 2000 and April 2001. Diarrhoea in this study was defined as three or more loose watery stools passed in a day (WHO, 1989)

E) **Collection, Handling and Transport of specimens.** Specimens from hospitals and health centers were collected daily by using Cary and Blair transport medium (BBL).

1. Culture and identification

A) ***Yesinia***: *Yersinia spp.* were isolated by using Yersinia Selective Base Agar or Cefsulodin-Irgasan novobiocin (CIN). The CIN was prepared from the dehydrated product according to Power and McCuen (1988). As soon as the specimens were received in the laboratory for primary isolation, cold enrichment procedures were carried out. The specimen containing swabs were inoculated into phosphate buffered saline and kept at 4^0C for up to 21 days (Weissfeld and Sonnenwirth, 1982; and Kelly, Brenher and Farmer, 1985 cited in Power and McCuen 1988). Then it has been subcultured onto Yersinia Selective Base Agar plates by streaking with sterilized loop for isolation. The plates were incubated at 25°C for 24-48 hrs.

B) ***Shigella, Salmonella*** and Diarrhoeagenic ***E. coli.*** The collected specimens were plated on McConkey agar and Salmonella-Shigella (SS) agar plates by inoculating with the swab and streaking with sterile loop. At the same time, in order to enrich the collected specimens,

the swab specimens were inserted into the enrichment Selenite-F broth as soon as the specimen arrive at the laboratory.

All the inoculated plates of McConkey and SS agar were incubated at 35-37^{0}C for 24 h. In the case of enrichment broth, the tubes were incubated with loosened caps at 35-37^{0}C and subcultured again after 18 to 24 h of incubation. The culture growth on the McConkey and SS plates were examined for lactose and non-lactose fermenting colonies. By using a sterile straight wire, a single colony of both types were picked and preserved in TSY (Trypticase Soy broth with 20% glycerol) broth tubes for further biochemical test by the ***Api 20E assay systems.***

2. Biochemical identification

Biochemical tests were carried out by using ***Api 20E system*** that consists of microtubes containing dehydrated substrates, which are used to detect enzymatic reactions and fermentation of carbohydrates. These microtubes were inoculated with a bacterial suspension. During the incubation period, metabolic activities occur and producing colour changes that are either spontaneous or reveled by the addition of reagents. The reactions were read according to the interpretation Table and the identification was obtained by referring to the identification Table.

a) **Preparation of the strip:** 5 ml of water were distributed into the honeycombed wells of the tray in order to create a humid chamber. The reference code was recorded on the elongated tab of the tray. Then, the strip was placed in the tray. Before preparation of a

bacterial suspension, oxidase test was carried out according to the manual of Api ***20E system*** (Avril, 1994).

b) **Preparation of the inoculum:** the ampoule of suspension medium (ref. 20110) was opened. With the aid of a pipette, a single well-isolated colony was taken from the MacConkey agar plates and carefully emulsified to achieve a homogenous bacterial suspension. The suspension was standardized by using McFarland 0.5 (Avril, 1994).

c) **Inoculation of the strip:** with the same pipette, both the tube and capule of tests such as sodium citrate (CIT), pyruvate (VP), and Kohn's gelatin (GEL) were filled with the bacterial suspension in accordance to the manual of the system where by tubes were carefully filled (and not the capules) of the other tests. Anaerobiosis was created in the tests of Arginine dihydrolase (ADH), Lysine decarboxylase (LDC), Ornithine decarboxylase (ODC), Urease (URE) and H_2S by overlaying with mineral oil. Lastly, the incubation box was closed and incubated at 35-37^{0}C for 18-24 h.

d) **Reading of the strip:** after 18-24 hours at 35-37^{0}C, the strip was read by referring to the interpretation Table. Reactions (colour changes) were recorded on the report sheet and the rest activities have been carried out in accordance to the A***pi E 20 system*** (Avril, 1994).

e) **Identification:** by using the identification Table the recorded results on the report sheets were compared with those given in the identification Table and the name of the identified bacterium was written on the report sheet.

3.Antimicrobial sensitivity testing for *Yersinia*, *Salmonella*, *Shigella* and Diarrhoeagenic *E. coli*

Susceptibility testing of all strains were carried out by using the standard agar disk diffusion technique (Bauer et al, 1966). Each isolate was sub-cultured on a plate of MacConkey agar and incubated at 35° C -37° C for 18 to 24 h. From a pure culture of each isolate 4 to 5 similar colonies were randomly selected and transferred to a test tube of Trypticase Soy Broth by touching the top of each colony with a sterilized wire loop (Power and McCuen, 1988). Trypticase Soy broth was incubated at 35°C for 2 to 8 h. to obtain moderate turbidity. The suspension (a portion of it) was diluted with a sterile saline to obtain turbidity visually equal to that of the standard of McFarland 2 (Avril, 1994).

A sterile cotton swab was dipped into the inoculum. The excess fluid was removed by pressing the swab against the side of the tube. The entire surface of a Muller-Hinton agar plate was inoculated by swabbing with the broth culture in three different directions. The inoculated plate was left at room temperature to dry for 3 to 5 minutes. With the help of an automatic dispenser (BBL), the set of 13 sensitivity disks with the following concentrations were plated on the surface of each Muller-Hinton plate, and the disks were gently pressed by sterile forceps to ensure firm contact. The concentration of 13 antibiotic discs were ampicillin (Amp), 10μg; carbenicillin (Car) 100μg; cephalothins (Cep) 30μg; chloramphenicol (Chl) 30μg; gentamycin (Gen) 10μg; kanamycin (Kan) 30μg; nalidixic acid (Na) 30μg; norfloxacin (Nor) 10μg; polymyxin B (Pol) 300μg; streptomycin (Str)

10μg; sulphadiazene (Sul) 300μg; tetracycline (Tet) 30μg; and trimethoprim-sulphamethoxazole (Sxt) 25μg. The plates were then incubated at 35 °C to 37°C

for 18 to 24 h. The standard reference *E. coli* (ATCC25922), sensitive to all of the antimicrobial drugs were tested at the same time control in the experiment. Finally, diameters of inhibition zones were measured in millimeters using a caliper. The interpretation of the measurement as sensitive, intermediate and resistance were made according to a standard zone size interpretive chart (Matsen and Barry, 1974). The very few `intermediate` readings were considered as sensitive for a purpose of assessment of the data.

4. Reference strains

Reference strains for *Yersinia* (**NBL 482**), *Salmonella* (**NBL IS-11**), *Shigella* (**NBL SC 530**) and diarrhoeagenic *E. coli* (**LMG 6440**) were used for control purposes throughout the study.

5. Statistical methods

Statistical analyses were done using the **EPI** (Epidemiology info.) statistical computer software.

V. RESULT

The prevalnce of enteric bacteria: Two hundred five stool specimens from all age-group patients were collected from five health centers, one laboratory and one hospital in Addis Ababa: 56 specimens from Arada health center, 25 specimens from Tekele-Hiymanote Health Center, 24 specimens from Addis Ketema Health Center, 10 specimens from Kebele 18 public Health Center, 20 specimens from Yekatite 12 Hospital and 54 specimens from Arsho Laboratory (Table-1) The frequency of isolation of *Salmonella*, *Shigella* and *Yersinia* as well as diarrhoeagenic *E. coli* (from infants) are shown on Table-2. Of 205 stool specimens cultured, 6 (2.9%) were positive for *Yersinia*, 22 (10.7%) for *Salmonella*, 12 (5.8%) for *Shigella* and 11 out of 26 samples for diarrhoeagenic *E. coli* in under five years old children (n = 26) (Table-1 and Table-2).

Out of a total of 205 diarrhoeal samples, only 3 (1.5%) of the strains of *Yersinia enterocolitica* and 3 (1.5%) of the strains of *Yersinia pestis* were isolated. 66.7% of *Yersinia entrocolitica* isolates were from children and 1 from adults. In addition to this, 2 were isolated from male diarrhoeal patients, while 1 was from the female diarrhoeal patients. With regard to *Yersinia pestis*, all strains were isolated from males, of which some were from children patients and a few from adults respectively.

Table 1: Frequency of isolation of *Salmonella*, *Shigella*, *Yersinia* and diarrhoeagenic *E. coli* (from < 5 yrs) from diarrhoeal patients.

Health center, laboratory and Hospital	No of specimens	*Yersinia*	(%)	*Salmonella*	(%)	*Shigella*	(%)	Diarrhoeagenic *E.coli(from* <5 years)	(%)
Arsho Laboratory	54	1	(1.9)	2	(3.7)	4	(7.4)	1	(1.9)
Arada Health center	56	2	(3.6)	5	(8.9)	1	(1.8)	5	(8.9)
Yekatit Hospital	20	-	-	1	(5)	-	-	-	-
Shero-Meda	16	2	(2.5)	1	(6.3)	1	(6.3)	2	(2.5)
Teklehimanot Health center	25	-	-	7	(28)	3	(12)	1	(4)
Addis Ketema Health center	24	1	(4.2)	4	(16.6)	2	(8.3)	1	(4.2)
Kebele 18 Public Health center	10	-	-	2	(20)	-	-	1	(10)
Total	205	6	(2.9)	22	(10.7)	12	(5.9)	11	(42.3)

Table 2: Distribution of enteric pathogenic bacteria.

Enteric pathogenic bacteria	Positive sample	(%)	Negative sample	(%)
Yersinia (n = 205)	6	(2.9)	199	(97.1)
Salmonella (n = 205)	22	(10.7)	183	(89.3)
Shigella (n = 205)	12	(5.9)	193	(94.1)
E. coli (<5 yrs) (n = 26)	11	(42.3)	15	(57.7)

Age specific distribution of enteric pathogenic bacterial isolates of *Yersinia, Salmonella* and *Shigella* are presented in Figure-1. In the age group of 5-14 years, infection rate of *Yersinia* spp. is 33.2%. With regard to *Shigella*, 25% of infection was obtained in the age group of 5 to 24. High frequency of *Salmonella* isolates (22.7%) was observed in the age group under five years. Relatively, high rate of *Shigella* isolates (16.7%) were in the age group of 55-64 years.

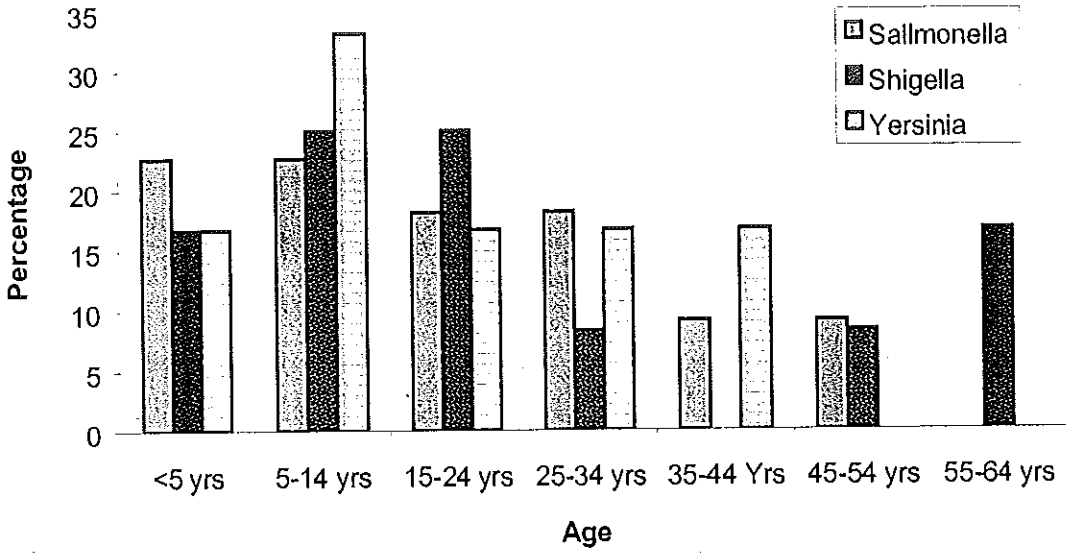

Figure-1: Age specific distribution of enteric pathogenic bacterial isolates,

The risk and selected factors of enteric bacteria: A comparison of enteric bacteria pathogen distribution by sex among the differences of adult and children patients with pathogenic bacteria is shown in Table-3. The distribution of *Yersinia*, *Salmonella* and *Shigella* were not different in adult males and females ($P \geq 0.26$). The same is true in the distribution of *Yersinia*, *Salmonella* and *Shigella* in male and female children ($P \geq 0.15$) with regard to children under five years, there is no statistically significant differences in the distribution of diarrhoeagenic *E. coli* in males and females ($P \geq 0.49$). Although not statistically significant, females had a higher percentage of culture positivity than males (54.5% versus 33.3%) (for the *E. coli* isolates).

Table-4 compares selected risk factors with the isolation rate of *Yersinia*, *Salmonella*, *Shigella* and diarrhoeagenic *E. coli*. The history of recent antibiotic use or hospitalization over the past one week was not significant in those who were positive for *Yersinia*, *Shigella*

and diarrhoeagenic *E. coli* than those with culture negative ($P \geq 0.40$). However, this was statistically significant in those with culture positive for *Salmonella* than those with negative ($P \leq 0.05$).

The diarrhoeal status compared with the detection of enteric pathogenic bacteria is given in Table-5. The duration of diarrhoea was not significantly associated with culture positivity for *Yersinia* and *Shigella* ($P \geq 0.27$). However, the duration of diarrhoea was significantly associated for *Salmonella* and *E. coli* ($P \leq 0.05$). The diarrhoea nature was not significantly associated with culture positivity for *Yersinia* and diarrhoeagenic *E. coli* ($P \geq 0.61$), while it was statistically associated with culture positivity for *Salmonella* and *Shigella* ($P \leq 0.01$). Reported symptoms such as high fever, abdominal pain, abdominal tenderness and others were not different in those which were culture positivity and negativity for *Yersinia, Salmonella, Shigella* and diarrhoeagenic *E. coli* ($P \geq 0.21$). Similarly, frequency of passing watery stools per day was not statistically associated with culture positivity for enteric pathogenic bacteria under study ($P \geq 0.48$). Moreover, the presence or absence of vomiting was not different in those who were culture positive and negative for these investigted enteric bacteria ($P \geq 0.18$).

Table-6 shows the educational and economical status of the patient and his/her family. The educational status of the patients was not associated with culture positivity and negative for *Yersinia* and *Salmonella* ($P \geq 0.07$). However, the educational status of the patients was statistically related with culture positivity and negativity for *Shigella* ($P \leq 0.02$). In the case

of children's mother educational status, it was not associated with culture positivity and negativity for *Yersinia, Shigella* and diarrhoeagenic *E. coli* ($P \geq 0.48$) but statistically associated with that of *Salmonella* ($P \leq 0.009$).

Table 3: The prevalence distribution of the enteric bacterial pathogens by sex of patients.

Sex	*Yersinia*		*Salmonella*		*Shigella*		*E. coli*	
	Positive sample (%)	P-value	Positive sample (%)	P-value	Positive sample (%)	P-value	Positive sample (%)	P-value
a) Adult								
Male (n=68)	2 (2.9)		8 (11.8)		5 (7.4)		-	
Female (n=62)	1 (1.6)	0.53*	5 (8.1)	0.68	2 (3.2)	0.26*	-	
b) Children								
Male (n=41)	3 (7.3)		3 (7.3)		2 (4.9)		-	
Female (n=34)	-	0.15 *	6 (17.6)	0.15*	3 (8.8)	0.41 *	-	
Childern (<15 yrs)								
d) Diarrhoeagenic *E. coli* under 5 years								
Male (n=15)	-		-		-		5 (33.3)	
Female (n=11)	-		-		-		6 (54.5)	0.49

* = Fisher exact: 1-tailed

P-value: ** = statistically significant

Table 4: Selected risk factors and enteric bacterial pathogens.

Selected risk factors	*Yersinia*		*Salmonella*		*Shigella*		*E. coli*	
	Positive samples (%)	P-value	Positive samples (%)	P-value	Positive samples (%)	P-value	Positive samples (%)	P-value
a) Expected route of contamination								
Water (n=8)	- (-)		1 (12.5)		2 (25)		(0)	
Food (n=71)	2 (2.8)		7 (9.9)		7 (9.9)		3 (4.2)	
Unknown (n=126)	4 (3.2)	0.87*	14 (11.1)	0.95*	4 (3.2)	0.01**	8 (6.3)	0.64*
b) Closely related persons With similar disease								
Yes (n=45)	1 (2.2)		4 (8.9)		4 (8.9)		4 (8.9)	
No (n=160)	5 (3.1)	0.60*	18 (11.3)	0.44+	10 (5.6)	0.31*	7 (4.4)	0.18*
c) Recent antibiotic use or hospitalization over past a week								
Yes (n=36)	2 (5.6)		1 (2.8)		4 (8.7)		4 (8.7)	
No (n=169)	4 (2.4)	0.28+	21 (12.4)	0.05+	9 (5.7)	0.36*	7 (4.4)	0.19*
d) Other medication								
Yes (n=46)	1 (2.2)		5 (10.9)		4 (8.7)		4 (8.7)	
No (n=159)	5 (3.1)	0.59+	17 (10.7)	0.86 +	9 (5.7)	0.36*	7 (4.4)	0.19*

+ = Fisher exact: 1-tailed P-value: ** = statistically significant

Table 5: Diarrhoeal status of enteric pathogenic bacteria.

Selected risk factors	Yersinia			Salmonella			Shigella			E. coli		
	(+ve)	(%)	P-value	(+ve)	(%)	P-value	(+ve)	(%)	P-value	(+ve)	(%)	P-value
a) Duration												
<3 days (n=64)	0	(0)		4	(6.2)		6	9.4		3	4.7	
3-7 days (n=74)	4	(5.7)		15	(20.3)		5	6.8		4	5.4	
8-14 days(n=26)	1	(4)		0	(0)		2	7.7		4	15.5	
>14 days (n=41)	1	(2.5)	0.30*	3	(12.2)	0.007**	0	0.0	0.27*	0	0	0.05**
b) Nature												
Watery (n=84)	2	(2.4)		3	(3.6)		2	2.4		3	5.4	
Bloody (n=24)	0	(0.0)		1	(4.2)		5	20.8		1	1.2	
Mucoid (n=62)	2	(3.2)		7	(11.3)		3	4.8		5	32.1	
Mixed (n=35)	2	(5.7)	0.61*	11	(31.5)	0.00009**	3	8.6	0.01**	2	5.7	0.68*
c) Symptoms												
High fever (n=89)	2	(2.2)		13	(14.6)		6	6.7		3	3.4	
Abdominal pain (n=116)	4	(3.4)		17	(4.6)		7	6.0		7	6.0	
Abdominal Enderness (n=28)	0	(0.0)		1	(3.6)		2	7.1		1	3.6	
Others (n=24)	1	(4.2)	0.73*	1	(4.2)	0.21*	1	4.2	0.96*	0	0	0.54*
Pass of stool	2	(3.1)		7	(10.8)		2	3.1		4	6.2	
Three times (n=65)												
Four times (n=140)	4	(2.9)	0.61*	15	10.7	0.81*	11	7.9	0.15*	7	5.0	0.48*
e) Vomiting												
Present (n=72)	2	(2.8)		11	15.28		7	9.7		5	6.9	
Absent (n=133)	4	(3.0)	0.64*	11	8.18	0.18*	6	4.5	0.24	6	4.5	0.67*

* = Fisher exact: 1-tailed
P-value: ** = statistically significant
:+ve = Positive

Table 6: Educational and economical status of the patient and his/ her family in association with the isolated enteric bacterial pathogens.

Educational and economical status	Yersinia			Salmonella			Shigella			E. coli		
	Positive			Positive			Positive			Positive		
	No	(%)	P-value	No	(%)	P-value	No	(%)	P-value	No	(%)	P-value
a) Educational status												
Lliterate (n=37)	0	(0)		2	(5.4)		3	(8.1)		-	-	-
Read and write (n=25)	0	(0)		3	(12.0)		0	(0.0)		-	-	-
Elementary (n=38)	1	(2.6)		6	(15.8)		3	(7.9)		-	-	-
High school (n=66)	2	(3.0)		3	(4.5)		2	(3.0)		-	-	-
University (n=6)	0	(0.0)	0.73*	2	(33.3)	0.07*	2	(33.3)	0.02**	-	-	-
b) If the patient is a child, mothr's ed. status												
Illiterate (n=9)	1	(11.1)		2	(22.2)		1	(11.1)		4	(44.4)	
Read and write (n=11)	0	(0.0)		0	(0.0)		0	(0.0)		4	(36.4)	
Elementary (n=11)	2	(18.2)		2	(8.2)		2	(18.2)		2	(18.2)	
High school (n=2)	0	(0.0)	0.48*	2	(100)	.009**	0	(0.0)	0.48*	1	(50.0)	0.58*
c) Family econ. status												
< 100 Birr	2	(3.2)		11	(17.7)		6	(9.7)		4	6.5	
101-400 Birr	4	(3.8)		9	(8.7)		5	(4.8)		7	6.7	
401-600 Birr	0	(0.0)		2	(6.9)		2	(6.9)		0	0	
6001- 1000 Birr	0	(0.0)		1	(0.0)	0.33*	0	(0.0)		0	0	
>1000 Birr	0	(0.0)	0.82*	0	(0.0)		0	(0.0)	0.68*	0	0	0.60*

* = Fisher exact: 1-tailed
P-value: ** = statistically significant
:+ve = Positive

Antibiotic sensitivity test and resistant patterns of enteric bacteria: The sensitivity pattern of all 51 strains isolated from diarrhoeal patients against 13 chosen antibacterial agents are presented in Figure-2. All strains were sensitive to norfloxacin and all but one strains were resistant to cephalothin. Resistance to cephalothin, ampicillin and tetracycline was greater than 50%. Sensetivity to gentamycin, kanamycin, nalidixic acid, norfloxacin and polymyxin B is greater than 90%. 45 (88.2%) enteric bacterial isolates resistant to at least one antibiotic.

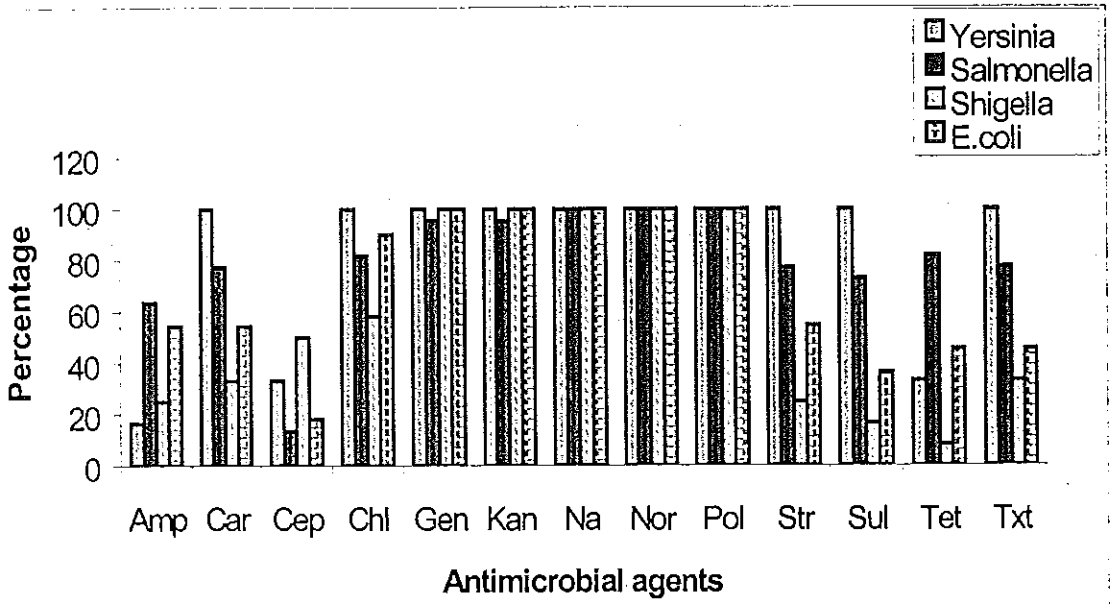

Figure-2. Antimicrobial susceptibility of 51 enteric pathoginic bacterial isolates from diarrhoea patients against 13 animicrobial agents.

Am: ampicillin
Car: carbinicillin
Cep: cephalothin
Chl: chloramphenicol
Gen: gentamycin
Na: nalidixic acid
Nor: norfloxacin
Pol: polymyxin
Str: streptomycin
Kan: kanamycin
Sul: sulphadizene
Tet: teteracycline
Sex: trimethoprim-sulphamethoxazole

***Yersinia* isolates sensitivity test agnist 13 antimicrobial agents:** Sensitivity of all 6 (2.9%) isolates of *Yersinia (Yersinia entrocolitica and Yersinia pestis)* against 13 chosen antimicrobial agents is presented in Figure-3. All strains were sensitive to carbenicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, sulphadizene and trimethoprim-sulphamethoxazole. Sensitivity to the remaining antimicrobial agents was varied between 33.3% and 50%. About 66.7% of the strains were sensitive to tetracycline. 83.3% of *Yersinia* isolates were resistant to at least one antibiotic. Next to ampicillin (83.3%), resistant strains were found against cephalothin (66.7%) and tetracycline (33.3%) respectively.

The sensitivity test of *Yersinia entrocolitica and Yersinia pestis* is shown in Table-7. All strains were sensitive to carbenicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, sulphadiazene and trimethoprim-sulphamethoxazole. All strains were resistant to ampicillin. In this study, all strains of *Yersinia pestis* were sensitive to carbenicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, sulphodiazene and trimethoprim-sulphamethoxazole. 66.7% of *Yersinia pestis* strains were resistant to ampicillin and cephalothin. About 50% of the strains were also resistant to tetracycline.

The resistance of *Yersinia* isolated strains to one or more drugs is shown in Table-8. Resistance to one or more drugs was detected in 83.3% of *Yersinia* isolates. Multiple drug resistance was detected in 66.7% of the isolates. Of all, 3 different patterns of resistance were noted.

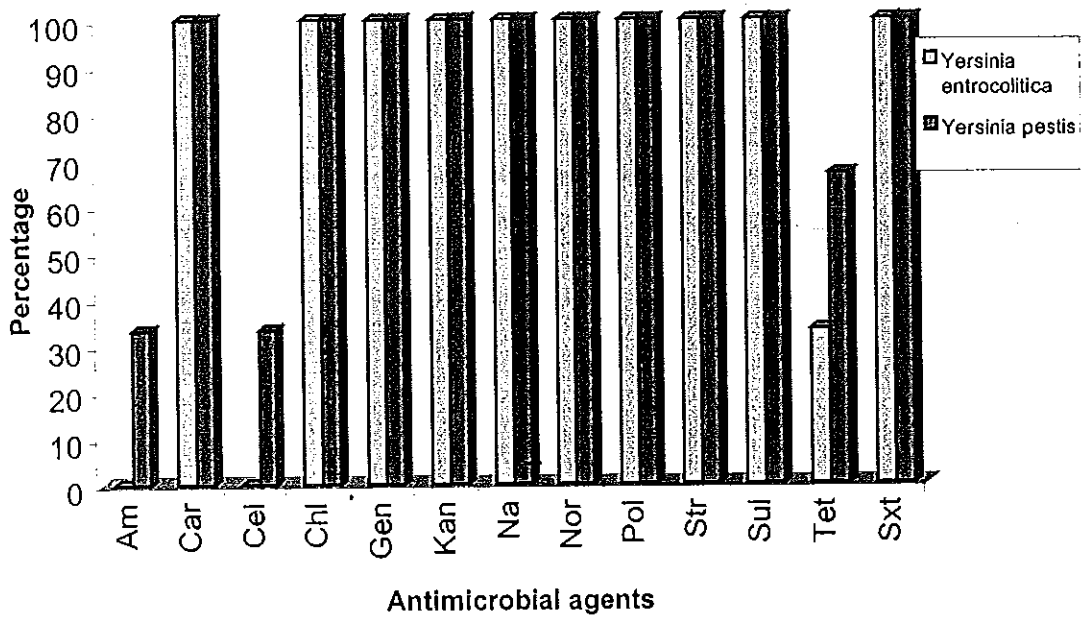

Figure-3. The sensitivity of *Yersinia entrocolitica* and *Yersinia pesti* against 13 antimicrobial agents.

Am: ampicillin	Na: nalidixic acid	Sul: sulphadizene
Car: carbinicillin	Nor: norfloxacin	Tet: teteracycline
Cep: cephalothin	Pol: polymyxin	Chl: chloramphenicol
Str: streptomycin	Gen: gentamycin	Kan: kanamycin

Sex: trimethoprim- sulphamethoxazole

Table-7: Antimicrobial sensitivty test results of *Yersinia enterocolotica* and *Yersinia pestis* strains against 13 commonly used drugs.

Antimicrobial drugs	Organisms tested					
	Yersinia enterocolotica			*Yersinia pestis*		
	Sensitive No (%)	Intermediate No (%)	Resistance No (%)	Sensitive No (%)	Intermediate No (%)	Resistance No (%)
Ampicillin (Amp)	- -	- -	3 (100)	1 (33.3)	- -	2 (66.7)
Carbinicillin (Car)	2 (66.7)	1 (33.3)	- -	3 (100)	- -	- -
Cephalothin (Cep)	- -	1 (33.3)	2 (66.7)	1 (33.3)		2 (66.7)
Chloramphenicol (Chl)	3 (100)	- -	- -	3 (100)	- -	- -
Gentamycin (Gen)	3 (100)	- -	- -	3 (100)	- -	- -
Kanamycin (Kan)	3 (100)	- -	- -	3 (100)	- -	- -
Nalidixicacid (Na)	3 (100)	- -	- -	3 (100)	- -	- -
Norfloxacin (Nor)	3 (100)	- -	- -	3 (100)	- -	- -
Polymyxin B (Pol)	3 (100)	- -	- -	3 (100)	- -	- -
Streptomycin (Ttr)	3 (100)	- -	- -	3 (100)	- -	- -
Sulphadiazene (Sul)	3 (100)	- -	- -	3 (100)	- -	- -
Tetracycline (Tet)	1 (33.3)	- -	2 (66.7)	2 (66.7)	- -	1 (33.3)
Trimethoprim-Sulphamethoxazole (Sxt)	3 (100)	- -	- -	3 (100)	- -	- -

Table 8: Drug resistance pattern of *Yersinia entrocolitica* and *Yersinia pestis* isolates.

Resistance pattern of *Yresinia enrocolitica*	No	%	Resistance pattern of *Yersinia pestis*	No	%
A	1	(33.3)	A, Cf	1	(33.3)
A, T, Cf	2	(66.7)	A, T, Cf	1	(33.3)
Sensitive to all	0	(0)	Sensitive to all	1	(33.3)
Resistance to all	0	(0)	Resistance to all	0	(0)

A: Ampicillin

Cf: Cephalothin

T: Tetracycline

Antimicrobial sensitivity test of *Salmonella* isolates against 13 antimicrobial agents: The sensitivity of all 22 isolates of *Salmonella* against 13 chosen antimicrobial agents are presented in Table-9. All strains were sensitive to norfloxacin, polymyxin B and nalidixic acid. 19 strains were resistant to cephalothin. Sensitivity to the remaining antimicrobial agents was varied between 63.6% and 95.5%. 21 (95.5%) of strains were sensitive to gentamycin and kanamycin respectively. Next to cephalothin (86.4%), resistance was found against ampicillin 8 (36.4%), sulphadiazene 6 (27.0%) and both streptomycin and trimethoprim-sulphamethoxazole 5 (22.7%).

Resistance of *Salmonella* isolates to one or more drugs is shown in Table-10. Resistance to one or more drugs was detected in 21 (95.5%) of *Salmonella* isolates. Multiple drug resistance (resistance to two or more drugs) was detected in 11 (50%) of *Salmonella* strains. In all, 11 different patterns of resistance were noted.

Table-9: Antimicrobial sensitivty test results of *Yersinia* and *Salmonella* strains against 13 commonly used drugs.

Antimicrobial drugs	Organisms tested					
	Yersinia			*Salmonella*		
	Sensitive No (%)	Intermediate No (%)	Resistance No (%)	Sensitive No (%)	Intermediate No (%)	Resistance No (%)
Ampicillin (Amp)	1 (16.7)	- -	5 (83.3)	14 (50)	3 (13.6)	8 (36.4)
Carbinicillin (Car)	5 (83.3)	1 (16.7)	- -	17 (68.2)	2 (9.1)	3 (13.6)
Cephalothin (Cep)	- -	2 (33.3)	4 (66.7)	- -	3 (13.6)	19 (86.4)
Chloramphenicol (Chl)	6 (100)	- -	- -	18 (81.8)	- -	4 (18.2)
Gentamycin (Gen)	6 (100)	- -	- -	21 (95.5)	- -	1 (4.5)
Kanamycin (Kan)	6 (100)	- -	- -	20 (90.9)	1 (4.5)	1 (4.5)
Nalidixic acid (Na)	6 (100)	- -	- -	20 (90.9)	2 (9.1)	- -
Norfloxacin (Nor)	6 (100)	- -	- -	22 (100)	- -	- -
Polymyxin B (Pol)	6 (100)	- -	- -	21 (95.5)	1 (4.5)	- -
Streptomycin (Ttr)	6 (100)	- -	- -	14 (63.6)	3 (13.6)	4 (18.2)
Sulphadiazene (Sul)	6 (100)	- -	- -	16 (73.0)	- -	6 (27)
Tetracycline (Tet)	2 (33.3)	1 (16.7)	3 (50)	13 (59.1)	5 (22.7)	4 (18.2)
Trimethoprim-Sulphamethoxazole (Sxt)	6 (100)	- -	- -	17 (77.3)	- -	4 (18.2)

Table 10: Drug resistance pattern of *Salmonella* isolates.

Resistance pattern	No. of isolates (%)
Cf	10 (45.5)
C, Cf	1 (4.5)
A, Cf	2 (9.0)
S, Sul, Sxt	1 (4.5)
S, A, Sxt, Cb, Cf	1 (4.5)
S, Sul, A, T, Cb, Cf	1 (4.5)
S, Sul, A, C, Cb, Cf	1 (4.5)
S, Sul, A, Sxt, Cb, Cf	1 (4.5)
S, A, Sxt, C, Cb, Cf	1 (4.5)
G, A, Sxt, T, C, Cb, Cf	1 (4.5)
S, K, Sul, Gm, A, Sxt, T, C, Cb, Cf	1 (4.5)
Sensitive to all	2 (9.0)
Resistance to all	0 (0)

A: Ampicillin

C: Chloramphenicol

Cb: Carbenicillin

Cf: Cephalothin

Gen: Gentamycin

S: Streptomycin

Sul: Sulphadiazene

Sxt: Trimethoprim-Sulphmethoxazole

Antimicrobial sensitivy test of *Shigella* isolates against 13 antimicrobial agents: The sensitivity of all 12 *Shigella* isolates against 13 chosen antimicrobial agents is shown in Table-11. All the strains were sensitive to nalidixic acid, norfloxacin, polymyxin B, gentamycin and kanamycin. Sensitivity of the remaining drugs was varied between 8.3% and 58.3%. Ampicillin, carbenicillin, cephalothin, streptomycin, sulphadiazene, tetracycline and trimethoprim-sulphamethoxazole were mostly ineffective ($\leq$ 50% sensitivity). The most frequent is tetracycline (91.7%) following by resistant to sulphadiazene (83.4%), ampicillin and streptomycin (75%). Resistance to one or more drugs in S*higella* spp. was observed for 9 (75%) of the isolates as shown in Table-12. Of the 12 *Shigella* isolated strains 58.3% were multiple resistant. In all, 9 different patterns of resistance were noted.

Diarrhoeagenic *E. coli* isolates sensitivity test against 13 antimicrobial agents: The sensitivity test results of all 11 diarrhoeagenic *E. coli* strains that isolated from children under five years against 13 antmicrobial agents is presented in Table-11. All strains were sensitive to gentamycin, kanamycin, nalidixic acid, norfloxacin and polymyxin B. Sensitivity to the rest of antimicrobial agents was varied between 27.3% and 90.9%. 90.9% of the *E. coli* isolates were sensitive to chloramphenicol.

Resistance of diarrhoeagenic *E. coli* isolates to one or more drugs is shown in Table-13. Resistance to one or more drugs was detected in 10 (90.9%) of diarrhoeagenic *E. coli* isolates and 1 (9.1%) sensitive to all drugs. Multiple drug resistance was detected in 63.6% of the strains. Of all isolates, 81.8% strains were resistant against chephalothin and 63.6%

sulphadiazene. Morover, 54.5% of the isolates were resistant against tetracycline and trimethoprim-sulphamethoxazole respectively. In all, 9 different patterns of resistance were noted.

Table-11: Antimicrobial sensitivty test results of *Shigella* and *E. coli* strains against 13 commonly used antbiotics.

Antimicrobial drugs	Organisms tested					
	Shigella			*E. coli*		
	Sensitive No (%)	Intermediate No (%)	Resistance No (%)	Sensitive No (%)	Intermediate No (%)	Resistance No (%)
Ampicillin (Amp)	3 (25)	- -	9 (75)	6 (54.5)	- -	5 (45.5)
Carbinicillin (Car)	4 (33.3)	- -	8 (66.7)	6 (54.5)	- -	5 (45.5)
Cephalothin (Cep)	1 (8.3)	5 (41.7)	6 (50)	- -	2 (18.2)	9 (81.8)
Chloramphenicol (Chl)	7 (58.3)	- -	5 (41.7)	10 (90)	- -	1 (9.1)
Gentamycin (Gen)	11 (91.7)	1 (8.3)	- -	11 (100)	- -	- -
Kanamycin (Kan)	9 (75)	3 (25)	- -	10 (90)	1 (9.1)	- -
Nalidixic acid (Na)	12 (100)	- -	- -	11 (100)	- -	- -
Norfloxacin (Nor)	12 (100)	- -	- -	11 (100)	- -	- -
Polymyxin B (Pol)	12 (100)	- -	- -	11 (100)	- -	- -
Streptomycin (Ttr)	3 (25)	- -	9 (75)	3 (27.3)	3 (27.3)	5 (45.5)
Sulphadiazene(Sul)	2 (16.6)	- -	10 (83.4)	4 (36.4)	- -	7 (63.6)
Tetracycline (Tet)	1 (8.3)	- -	11 (91.7)	4 (36.4)	1 (9.1)	6 (54.5)
Trimethoprim-Sulphamethoxazole (Sxt)	4 (33.3)	- -	8 (66.7)	5 (45.5)	- -	6 (54.5)

Table 12: Drug resistance pattern of *Shigella* isolates.

Resistance pattern	No. of isolates (%)
Cb	1 (8.3)
A, T, Cf	1 (8.3)
S, Sul, Sxt, T	1 (8.3)
S, Sul, A, T, Cb, Cf	1 (8.3)
Sul, A, Sxt,T, C, Cb	1 (8.3)
S, Sul, A, Sxt, T, C	1 (8.3)
S, Sul, T, C, Cb, Cf	1 (8.3)
S, Sul, A, Sxt, T, Cf	1 (8.3)
S, Sul, A, Sxt, T, Cb, Cf	1 (16.7)
Sensitive to all	0 (0)
Resistance to all	0 (0)

A: Ampicillin
C: Chloramphenicol
Cb: Carbenicillin
Cf: Cephalothin
Sul: Sulphadiazene
S: Streptomycin
Sxt: Trimethoprim-Sulphamethonazole
T: Tetracycline

Table-13: Drug resistance pattern of Diarrhoeagenic *E. coli* isolates.

Resistance pattern	No. of isolates (%)
T	1 (9.1)
Cf	2 (18.2)
S, Sul, Sxt, Cf	1 (9.1)
S, Sul, T, Cf	1 (9.1)
Sul, A, Sxt, Cb, Cf	1 (9.1)
A, Sul, Sxt, T, Cb, Cf	2 (18.2)
S, Sul, A, Sxt, T, Cb, Cf,	1 (9.1)
S, Sul, A, Sxt, T, C, Cb, Cf	1 (9.1)
Sensitive to all	1 (9.1)
Resistance to all	0 (0)

A: Ampicillin

C: Chloramphenicol

Cb: Carbenicillin

Cf: Cephalothin

Sul: Sulphadiazone

Sxt: Trimethoprim-Sulphamethonazole

S: Streptomycin

T: Tetracycline

VI. DISCUSSION

In this study, the prevalence rate of *Yersinia* species found to be 2.9%. In the age group of 5-14 years, the isolation rate of *Yersinia* species was higher than that of *Shigella* and *Salmonella*. However, the frequency of *Yersinia* isolates was found to be less in children less than five years is much lower than *Salmonella* isolates. Moreover, the prevalence rate of *Yersinia* in children under five years was much lower than diarrhoeagenic *E. coli* 11. The age group from 15-34, show relatively high prevalence of enteric pathogenic bacteria in comparison to 35 to 54 age groups. In children, high rate of isolation of enteric pathogenic bacteria was observed. This may be due to immatured immune system and poor hygiene conditions in comparison to adults. In this study, *Yersinia entrocolitica* did not seem to be the main aetiological enteric pathogenic agent when compared with the well-studied diarrhoeacogenic bacteria agents like *Salmonella*, *Shigella*, and *E. coli* strains, although this finding was better than Mogessi Ashenafi (1983) and Danel Asrate *et al* (1999), a possible explanation for the lower isolation rate of *Yersinia entrocolitica* from patients in this study as compared to reports from some other different countries may be related to the relatively hotter climatic conditions (Toma and Deidrick, 1975). It has already been reported most sporadic infections caused by *Yersinia entrocolitica* occur in countries with colder climatic condtions (Swaminathan *et al.*, 1982).

The frequency of isolation of *Yersinia entrocolitica,* in this study, 3 (1.5%) was the same as its frequency of isolation from persons suffering from gastrointestinal problems in the southern region of Germany (1.5%) (Swamiathan *et al.*, 1982). However, *Yersinia*

entrocolitica isolated from diarrhoeal patients of this study 3 (1.5%) was lower than that of *Yersinia entrocolitica* isolated from urban (2.5%) and rural (5.8%) patients in Nigeria (Olusany, 1987).

In the studies of Mogessi Ashnafi (1983) and Daneil Asrate *et al.* (1999), *Yersinia entrocolitica* was not isolated from any of the tested samples. The possible conclusion of these results may be due to being unable to use Yersinia selective agar base medium and cold enrichment procedure. Moreover, in this study, cold enrichment medium (phosphate buffered saline) and Yersinia selective agar base medium was used, which was completely different from the method and media used in the above mentioned previous studies.

The frequency of isolation of *Salmonella* in this study (10.7%) was much higher than the frequnce of these strains in all age groups (3.8%) reported by Daniel Asrate et al. (1999). Similarly, the prevalence rate of *Salmonella* isolates in adults (10%), of this finding, was higher than the previous study results of the prevalence of *Salmonella* isolates (4.5%) from adult out-patient (Mogessie Ashenafi, 1983). On the other hand, according to Wanuola (1980), the isolation rate of *Salmonella* was 24-35% in Kenya, which was much higher than that of this study (10,7%). Furthermore, the isolation frequency of *Salmonella* (10.7%) in this study was relatively smaller than that (13.5%) obtained from Vietnamese refugees in Hong Kong (Anton, 1981). However, the prevalence of *Salmonella* in this investigation (10.7%) was almost the same as the prevalence of *Salmonella* (10.85%) in Iraq in between 1973 and 1976, (Allos, 1978).

The 5.8% frequency of isolation of *Shigella* in all age group of this study was lower than 11.7% isolation rate of the study by Daneil Asrate *et al.* (1999) in Addis Ababa at Tikur Anbassa and Ethio-Swedish Children's Hospital. The prevalence rate of *Shigella* isolates (5.8%) in this work was also lower than 7.1% of *Shigella* strains isolated by Mache *et al.* (1995) in Addis Ababa between January and July 1995. Of 130 adult diarrhoeal patients Shigella strains were isolated in 5.4% of the cases which was lower than the isolated (9%) strains reported by Mogesse Ashenafi (1983). This rate of isolation was also lower than the 7% isolation rate reported by Ai *et al.* (1975) from South Vietnam and the 11.6% isolation rate of Stool *et al.* (1982) from Bangladesh but was higher than 1.2% isolation rate from Malasia (Kan and Chan, 1980). In this study, only children under five years have been considered for the isolation of *E. coli* since most strains are diarrhoeagenic mainly in children under five years old. Of the 26 diarrhoeal patient children, 11 (42.3%) were positive for sole isolation of *E. coli* strains.

In conculsion, the prevalence rates of the commonly enteric pathogenic bacteria flactuate from time to time. The possible reason is that, their prevalence rate largely dependes on environmental and seasonal factors as well as degree of personal and communal hygienes (Jay, 1996).

In this study, the sensitivity results showed different resistance patterns of 51 strains (22 from *Salmonella*, 12 from *Shigella,* 11 from *E. coli* and 6 from *Yersinia*) against 13 chosen antimicrobial agents. All the 6 strains of *Yersinia* were sensitive to carbenicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B,

streptomycin, sulphadiazene and trimethoprim-sulphamethoxazole. Next to ampicillin (83.3%), resistance was found in the frequency of 66.7% and 50% against cephalothin and tetracycline respectively. Multiple drug resistance was detected in 66.7% of the isolates with 3 different combinations of drug resistance patterns.

In the case of *Yersinia* isolates, all strains were sensitive to carbenicillin, chloramphenicol, gentamycin, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, sulphadiazene and trimethoprim-sulphamethoxazole. Most strains were resistant to ampicillin. This result showed that ampicillin may be ineffective to treat for diarrhoeal diseases that are caused by *Yersinia* isolates. In order to confirm this, however, sensitivity test shoud be carried out by using large number of *Yersinia* strains that will be isolated from large sample size.

The sensitivity of all 22 isolates of *Salmonella* strains against 13 chosen antimicrobial agents showed that all of them were sensitive to nalidixic acid, norfloxacin and polymyxin B. According to Mogessie Ashenafi (1983), all isolated strains were sensitive to gentamycin Polymyxin B and trimethoprim-sulphamethoxazole. However, as opposed to his report, in this investigation only 95.4% of the isolated *Salmonella* strains were sensitive to gentamycin and 77.3% to trimethoprim-sulphamethoxazole. The occurrence of resistance strains in this study may be the result of the wide spread use of these drugs at this moment. This may cause selective pressure of resistance problems on the enteric bacteria circulating in the community (Bennett, 1980). The sensitivity results of the rest of antimicrobial agents was varied between 63.6% and 95.5%, the highest sensitivty was recorded by gentamycin

and kanamycin. This implies that there is still better treatment efficacy of these antimicrobial agents against the studied enteric pathogenic bacteria.

The frequency of resistant *Salmonella* strains to one or more antimicrobial agents was found to be 95.5% in this study. This was much higher than that 31.1% reported by Mogessie Ashenafi (1983) and the 79.4% reported by Messele Gedebou and Alebachew Tassew (1981). In this study, 50% of the totally isolated *Salmonella* strains showed multiple drug resistance (a strain resitant to two or more drugs), which was much higher than the 26.7% reported by Mogessie Ashenafi (1983) in Addis Ababa. Out of the 13 tested antibiotics, single drug resistance was found in 45.5% of the isolates only against cephalothin.

In this study *Shigella* strains were all sensitive to nalidixic acid, norfloxacin, polymixn B, gentamycin and kanamycin. As opposed to the 100% sensitivty of cephalothin and trimethoprim-sulphamethoxazole that has been reported by Mogessie Ashenafi (1983), the frequency of sensitivity of *Shigella* strains against cephalothin and trimethoprim-sulphamethoxazole was 50% and 33.3%, respectively. The frequency of sensitiviy to all the drugs, observed in this study (33.3%) was higher than 16.7% and 30% that were reported by Mogessie Ashenafi (1983); and Afeworki Gebreyohannes and Yetnebersh Limeneh (1980), respectively. In this investigation, the sensitivity of *Shigella* isolates to ampicillin, carbenicillin, cephalothin, streptomycin, sulphadiazene, tetracycline and trimethoprim-sulphamethoxazole were $\leq$50%. All 90 (100%) of *Shigella* were sensitive to trimethoprim-sulphadiazene (Mogessi Ashenafi, 1983) but out of 12 isolates only 33.3% of the isolated

strains of *Shigella* were sensitive to trimethoprim-sulphamethoxazole. According to Mogessi Ashenafi (1983), about 95.6% of the isolated strains were sensitive to cephalothin, while in this study, only 50% of the isolates were sensitive to cephalothine. According to Zeleke W/ Tenssai (1990), about 50%, 83.3%, 40%, 100% and 100% of the isolated *Shigella* strains were sensitive against tetracycline, strepomycin, sulphadiazene, trimethoprim-sulphamethoxazole and chloramphenicol respectively, while 8.3%, 25%, 16.6%, 33.3% and 58.3% of *Shigella* isolates of this study were sensitive against teteracycline, streptomycin, sulphadiazene, trimethoprim-sulphamethoxazole and chloramphenicol respectively. The high rate of occurrance of resistance to these antimicrobial drugs may by due to the extensive use of these drugs for the treatment of diarrhoeal diseases.

As shown in this study, 75% of *Shigella* isolates were resistant to one or more drugs. This frequency was much lower than that of 82.5% reported by Mogessie Ashenafi (1983) in Addis Ababa, 85% of Messele Gedebou and Alebachew Tassew (1982) in Addis Ababa, 78% from the study of Afeworki Gebreyohannes and Yetnebersh Limeneh (1980) from various parts of the country, 80% of strains from Korea (Shunand and Seol, 1978) and 90.4% from India (Kaliyugaperunal et al., 1987). Of the drugs tested, single drug resistance was found against carbenicillin in 8.3% of the strains, which was different from the report of single drug resistants against chloramphenicol, sulphadiazene, tetracycline, sreptomycin or cephlothin reported by (Mogessie Ashenafi, (1983). The most frequent resistant patterns of this study was against tetracycline (91.7%) followed by sulphadiazene (83.4%), ampicillin (75%) and streptomycin (75%), respectively. The frequency of ampicillin

resistance (75%), in this study, was much higher than that (52.3%) reported by Mogessie Ashenafi (1983) and (22%) by Afeworki Gebreyohannes and Yetnebersh Limeneh (1980). From this observation, one can conclude that there is increased emergence of resistant strains against ampicillin. Such a trend was also reported by various investigators (Byers et al., 1976; Ngu, 1977 and Stoll *et al.*, 1982).

Emergence of resistant *Shigella* strains against the commonly used antimicrobial agents is being reported in Ethiopia. This indicates the need for a newer and more effective drugs of choice for the treatment of shigellosis (Messele Gedebou and Alebachew Tassew, 1982). Gentamycin, kanamycin, nalidixic acid, norfloxacin and polymyxin B may be the choice of drugs for the treatment of shigellosis, particularly in regions where there is high prevalence of multiple drug resistant strains.

All strains of *E. coli* isolated from diarrhoeal disease patients of children under five years of age were sensitive to gentamycin, kanamycin, nalidixic acid, norfloxacin and polymyxin B. Among the 11 tested isolates of *E. coli,* 90.9% were found sensitive to chloramphenicol. Similarly, the results showed that 90.9% of diarrhoeagenic *E. coli* were resistance to one or more drugs with 63.6% of the strains showing multiple drug resistance. In all strains, 10 different drug combinations of resistance patterns were noted for *E. coli.* They showed high rate of resistance to ampicillin, carbenicillin and cephalothin (45.5%, 45.5% and 81.8% respectively). The resistant pattern of *E. coli* to ampicillin was found to be lower than the resistant rate of isolates (> 50%) of the study reported by Zeleke W/Tenssai (1990). *E. coli*

strains were sensitive against cephalothin, sulphadizene and trimethoprim-sulphamethoxazole in this ivestigation.

VII. CONCLUSION

In general, most of the bacterial isolates from clinical stool samples were resistant against the commonly available antimicrobial agents. The number of bacterial isolates tested in this study, was relatively low, however, the results of the antimicrobial susceptibility test observed, are comparable to the previous works related.

According to this investigation, norfloxacin, nalidixic acid and polymyxin B have broad-spectrum activity against several tested enteric pathogenic bacteria, which are cause suspected to diarrhoeal illnesses. On the other hand; cephalothin, tetracycline, ampicillin and streptomycin may not be used as the drugs of choice for the treatment of enteric bacteria unless culture and sensitivity tests are done prior to treatment.

Sever consequences may result from bacteria becoming resistant to antimicrobial drugs. Besides limiting only therapteutic options and the high cost of alternative effective agents, resistant organisms may lead longer hospitalization and an increased risk of death. The development of new antimicrobial agents may offer short-term solutions to this problem but in the long term, more effective measures such as health education and further research on the prevation of infections by vaccination and immunization should be emphasized.

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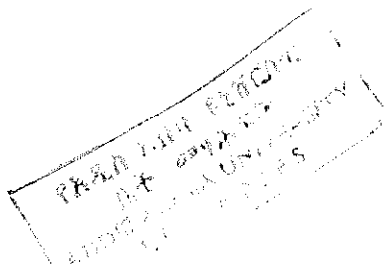

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Appendix-1

Clinical Data Collection Form.

Put X mark when needed.

1. Address and other information of the patient.

1.1 Date_____
1.2 Study number_____
1.3 Hospital or Health Center number _____
1.4 Hospital or Health Center_____
1.5 Patients name_____
1.6 Residence (Woreda _____Keftegna_____House number _____)
1.7 Patients age_____Sex_____

2. The epidemiological setting in which the diarrhoeal illness origin.

2.1 Recent types of food eaten or waters a) water____
b) food_____
c) I don't Know_____

2.2 Closely relate persons with similar disease problem. a) Yes _____
b) No _____

2.3 Recent antibiotic use or hospitalization over past a week.
a) Yes_____
b) No_____

2.4 If antibiotic is taken, state the type of antibiotics used _____

2.5 Other medication a) Yes _____b) No _____

2.6 If yes, specify the type of medication _____

3. Diarrhoea Status

3.1 The duration of the diarrhoeal illness in days a) less than 3 days____

b) 3-7 days____
c) 8-14 days____
d) Greater than 14 days___

3.2 Diarrhoea nature a) watery_____ b) bloody_____ c) mucoid ____ d) mixed such as

formed _____, semi-formed_____, soft_____

3.2 Symptoms of diarrhoea a) High fever _____ b) Abdominal pain ____
c) Abdominal tenderness_____d) Others _____

3.3 Pass of watery stools per day. a) 3_____ b) 4 and over_____

3.4 Vomiting a) present_____ b) absent_____-

4. Background information of the patient and his / her family

4.1 Educational status of patients (for adults)

a) Illiterate _____
b) Read and write only ____
c) Elementary _____
d) High school _____
e) University level _____

4.2 If the patient is a child,

4.2.1 Mother's educational status

a) Illiterate
b) Read and write only
c) Elementary
d) High school
e) University level

4.3 The family economical status income per month if possible

a) Below 100.00 Birr
b) 100.00-400.00 Birr
c) 401.00-600.00 Birr
d) 601.00-1000.00 Birr
e) Above 1000.00 Birr