

**Isolation and Antibiotic Susceptibility Pattern of
Shigella and *Campylobacter* from Acute Enteric
Infections in Yekatit 12 Hospital and Shiromeda
Health center, Addis Ababa**

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June 2011

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University In Partial Fulfillment of the Requirements for the Degree
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Abbreviations

AAU	Addis Ababa University
AIDS	Acquired Immune Deficiency Syndrome
CDC	Center for Disease Control and Prevention
CDT	cytolethal-distending toxin
CLSI	Clinical and Laboratory Standards Institute
CLT	Cholera-like toxins
EHEC	enterohemorrhagic <i>E. coli</i>
EIEC	enteroinvasive <i>E. coli</i>
EPEC	enteropathogenic <i>E. coli</i>
GBS	Guillain Barré Syndrome
HL	heat labile
HS	heat stable
IL-8	interleukin-8
Ipa	invasion plasmid antigens.
LPS	lipopolysaccharide
ShET	<i>Shigella</i> enterotoxin
STEC	shiga toxin producing <i>E. coli</i>
VNC	viable non-culturable
WHO	World Health Organisation

Abstract

Background -Acute infective diarrhoea and gastroenteritis are major causes of ill health and premature death in developing world due, in large part, to the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status. Among the leading causes of infectious diarrhoea, *Campylobacter* and *Shigella* contribute a lot. Antimicrobial resistance has developed among many of the major diarrheal bacterial pathogens and complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim–sulfamethoxazole.

Objective - To isolate and determine antibiotic susceptibility pattern of *Shigella*, and *Campylobacter* from acute enteric infections in Addis Ababa

Method - A cross sectional study was conducted from December 2010 to March 2011 at Shiromeda health center (n=254) and Yekatit 12 Hospital (n=140). All diarrheal stool specimens were cultured for isolation of *Shigella* and *Campylobacter* species. Antimicrobial susceptibility testing was performed for culture isolates according to the method of Clinical and Laboratory Standards Institute (CLSI) by disk diffusion method.

Result – A total of 163 enteropathogens were isolated from 394 patients that had acute diarrhea. The isolates were 37 (9.4%) *Shigella* species, 19 (4.8%) *Campylobacter* species, 23 (5.8%) *Salmonella* species and 84 (21.3%) parasites. 192 (48.7%) of the patients were females and 202 (51.3%) were males making the female to male ratio 1:1.05. The antimicrobial susceptibility pattern for 37 strains of *Shigella* isolates showed 67.6% resistance to ampicillin followed by, trimethoprim-sulfamethoxazole (64.7%), and chloramphenicol (40.5%). More than 90% of the strains were sensitive to nalidixic acid ciprofloxacin, norfloxacin and polymyxin B. Multiple resistances (resistant to two or more drugs) were observed in 23 (62.1%) of the isolates. All *Campylobacter* spp. were susceptible to chloramphenicol and showed low resistance rates (<60%) against, trimethoprim-sulfamethoxazole, ampicillin, nalidixic acid, ciprofloxacin and erythromycin.

Conclusion- the results of the present study showed the high prevalence for *Shigella* spp. while *Campylobacter* spp. showed a moderate one. Continuous surveillance of the prevalence and

antibiotic susceptibility pattern of diarrheal bacteria in hospitals and in the community is needed which should be the basis for empiric therapy.

Chapter I: Introduction

1.1 General Introduction

Acute infective diarrhoea and gastroenteritis are major causes of ill health and premature deaths in developing world due, in large part, to the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status. According to the latest available figures, an estimated 2.5 billion people lack improved sanitation facilities, and nearly one billion people do not have access to safe drinking water. These unsanitary environments allow diarrhoea-causing pathogens to spread more easily (UNICEF 2009).

Ranging from mild annoyances during vacations to devastating dehydrating illnesses that can kill within hours, acute gastrointestinal illnesses rank second only to acute upper respiratory illnesses as the most common diseases worldwide. In children <5 years old, attack rates range from 2–3 illnesses per child per year in developed countries to as high as 10–18 illnesses per child per year in developing countries (Kasper *et al.*, 2005).

Diarrhoea is one of the most common childhood illnesses, in both developing and developed countries. Estimation by World Health Organization (WHO) indicates that the world population suffered from 4.6 billion incidences of diarrhea causing 2.2 million deaths in the year 2004. While the disease is rarely a cause of death in developed countries, it is estimated that approximately 1.6 million children die each year from diarrhoea in the developing world. Africa and South Asia are home to more than 80 percent of child deaths due to diarrhoea. In addition, by contributing to malnutrition and thereby reducing resistance to other infectious agents, gastrointestinal illnesses may be indirect factors in a far greater burden of disease (UNICEF 2009, WHO 2004).

All over the world, severe acute bacterial gastroenteritis is caused mainly by *Shigella*, whereas *Salmonella*, *E. coli* (chiefly enteropathogenic *E. coli*, or EPEC, but also enterohemorrhagic *E. coli* or EHEC, enteroinvasive *E. coli* or EIEC and other types), *Campylobacter* and *Vibrio* spp.

have been shown to play a role in the epidemiology of diarrhea, especially in certain areas of the globe (Diniz-Santos *et al.*, 2005).

Antimicrobial resistance has developed among many of the major diarrheal bacterial pathogens and complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim–sulfamethoxazole. In developing countries resistance commonly emerges in outpatient populations and involves drugs that are taken orally; this pattern is presumably related both to the over-the-counter availability of such drugs without a prescription, with resultant overuse and abuse, and to sanitary conditions that favor the fecal-oral spread of microorganisms (Asrat *et al.*, 2008; Murray, 1986).

Diarrheal diseases are major causes of infant and child mortality and morbidity in Ethiopia. About 39,000,000 episodes of diarrhoea per year were estimated to occur in Ethiopia; out of which 230,000 deaths occur in children below five years of age. Studies done in different part of Ethiopia have shown that *Campylobacter* species and *Shigella* species were major etiologic agent of diarrheal illness (Muhe *et al.*, 1995; Mitikie *et al.*, 2000; Beyene and Haile-Amlak, 2004; Asrat *et al.*, 2008; Tiruneh 2009; Ewnetu and Mihret 2010).

1.2 Literature review

1.2.1 Infectious Diarrhea

Gastrointestinal infections encompass a wide variety of symptom complexes and recognized infectious agents. With the exception of *Helicobacter pylori* gastritis, the term *gastroenteritis* is applied to syndromes of diarrhea or vomiting that tend to involve non inflammatory infection in the upper small bowel or inflammatory infection in the colon. Infections of the gastrointestinal tract, especially infectious diarrhea, are among the most common debilitating infectious diseases, afflicting people of all ages around the world (Mandell *et al.*, 2005). There are three main forms of acute childhood diarrhoea, according to World Health Organization (2009). The first one is acute watery diarrhoea includes cholera and is associated with significant fluid loss and rapid dehydration in an infected individual. It usually lasts for several hours or days. The second type is, bloody diarrhoea, often referred to as dysentery, and is marked by visible blood in the stools. It is associated with intestinal damage and nutrient losses in an infected individual. The third is persistent diarrhoea is an episode of diarrhoea, with or without blood, that lasts at least 14 days. Undernourished children and those with other illnesses, such as AIDS, are more likely to develop persistent diarrhoea. Diarrhoea, in turn, tends to worsen their condition (UNICEF, 2009).

In 2009, The Food borne Diseases Active Surveillance Network (FoodNet) of CDC's Emerging Infections Program collects data on the incidence of diarrhoea attributable to nine Enteropathogens by population-based surveillance in 10 U.S. states for all laboratory-confirmed infections with select enteric pathogens transmitted commonly through food, a total of 17,468 laboratory-confirmed cases of infection were identified. The number of reported infections and incidence per 100,000 population, by pathogen, were as follows: *Salmonella* (7,039; 15.19), *Campylobacter* (6,033; 13.02), *Shigella* (1,849; 3.99), *Cryptosporidium* (1,325; 2.86), STEC O157 (459; 0.99), STEC non-O157 (264; 0.57), *Vibrio* (160; 0.35), *Listeria* (158; 0.34), *Yersinia* (150; 0.32), and *Cyclospora* (31; 0.07) (CDC, 2010).

Mortality from diarrhoea has declined over the past two decades from an estimated 5 million deaths among children under five to 1.5 million deaths in 2004, which parallels downward trends in overall under five mortality during this period. Despite these declines, diarrhoea remains the second most common cause of death among children under five globally, following closely

behind pneumonia, the leading killer of young children. Together, pneumonia and diarrhoea account for an estimated 40 per cent of all child deaths around the world each year. Nearly one in five child deaths is due to diarrhoea, a loss of about 1.5 million lives each year. The toll is greater than that caused by AIDS, malaria and measles combined (UNICEF, 2009).

1.2.2 The Genus Shigella

Microbiology of *Shigella* species

Bacillary dysentery was first differentiated from amoebic dysentery in 1887 and an etiologic agent, *Bacillus dysenteriae*, was isolated and described by Shiga in 1898. *Shigella flexneri* was originally described by Flexner in 1900. *Shigella sonnei* was first isolated in 1904, but it was not until 1915 that its pathogenic potential was recognized by Sonne (Gillespie and Hawkey, 2006). The subsequent painstaking process of epidemiological, physiological, and serological characterization of related dysentery bacilli culminated with the recommendations of the 1950. Congress of the International Association of Microbiologists *Shigella* Commission that *Shigella* be adopted as the generic name and that species subgroups be designated A (*Shigella dysenteriae*), B (*S. flexneri*), C (*S. boydii*), and D (*S. sonnei*) (Hale, 1991)

Shigella species are straight rods, 1–3× 0.7–1.0 µm, that conform to the general definition of the family *Enterobacteriaceae* and contain the enterobacterial common antigen. Gram negative, nonmotile, nonpigmented, facultatively anaerobic having both a respiratory and a fermentative type of metabolism. Catalase positive (with exceptions in *Shigella dysenteriae*), oxidase negative, chemoorganotrophic, ferment sugars without gas production (a few exceptions produce gas), salicin, adonitol, and *myo*-inositol are not fermented. Strains of *Shigella sonnei* ferment lactose and sucrose upon extended incubation; however, other species do not utilize these substances in conventional medium. Do not utilize citrate, malonate, or sodium acetate (with exceptions in *Shigella flexneri* for sodium acetate) as a sole carbon source. Do not grow in KCN (potassium cyanide) or produce H₂S. Do not decarboxylate lysine. Reduce nitrates to nitrites. Based on 16S rDNA sequencing, *Shigellae* belong in the *Gammaproteobacteria* (Nancy *et al.*, 2005).

The genus is divided into four serogroups with 47 serotypes: A (*S dysenteriae*, 12 serotypes); B (*S flexneri*, 15 serotypes), C (*S boydii* 18 serotypes); and D (*S sonnei*, two antigenic types, phase1 and phase2). This serotyping scheme uses the polysaccharide O antigen found in the outer part of the cell wall (Zafar *et al.*, 2005)

The Epidemiology of *Shigella*

The disease caused by *Shigella* spp., dysentery, was recognized by Hippocrates as a condition characterized by the frequent passage of stools containing blood and mucus. He also recognized some aspects of the epidemiology, noting that after a dry winter and a wet spring the number of cases became more frequent in the summer (Gillespie *et al.*, 2006).

Shigellosis is endemic throughout the world. Worldwide there are approximately 164.7 million cases, of which 163.2 million in developing countries and 1.5 million in industrialized countries. Each year 1.1 million people are estimated to die from *Shigella* infection and 580 000 cases of shigellosis are reported among travelers from industrialized countries (Kotloff *et al.*, 1999a). *Shigella* species are important pathogens that are responsible for 5 to 10% of diarrheal diseases and dysentery occurring all over the world (Vrints *et al.*, 2009) A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involve children less than 5 years of age. Since the late 1960s, pandemic waves of *Shigella* dysentery have hit sub-Saharan Africa, Central America and South and South-East Asia, often striking areas of political upheaval and natural disaster. *Shigella* incidence varied from 0.6 episodes/1000 person-years in Thailand to 107/1000 person-years in Egypt (Ram *et al.*, 2008). During the 1994 genocide in Rwanda, approximately 20,000 Rwandan refugees who had fled into the North Kivu region of Zaire died in the first month alone from dysentery caused by a strain of *Shigella* that was resistant to all commonly used antibiotics. (Kotloff *et al.*, 1999a)

In endemic areas of the developing world, shigellosis is predominantly a pediatric disease, with the urban poor being hardest hit. Disease is more severe in children who are malnourished. In developed countries it occurs more commonly where there is overcrowding or poor sanitation (e.g. in institutionalized individuals, children in day-care centers, prisoners, military recruits and residents of Native American reservations). Although these groups have higher rates of disease

than the population at large, food borne infection frequently occurs among individuals who are not in high-risk groups (Sake *et al.*, 2001).

The geographical distribution and the pathogenicity of the four species of *Shigella* are different predominant serogroup of *Shigella* circulating in a community appears to be related to the level of socioeconomic development. Attempts have been made to summarize published studies which quantify the proportion of isolates from hospitalized patients, presumed to be the most severe cases, throughout the world, recognizing that considerable variation exists among studies. *S. flexneri* is the main serogroup found in developing countries (median 60% of isolates), with *S. sonnei* being the next most common (median 15%). *S. dysenteriae* (which is seen most often in South Asia and sub-Saharan Africa) and *S. boydii* occur with equal frequency (median 6%) (Kotloff *et al.*, 1999b). One study in northwestern Ethiopia shows serotype distribution of *S. flexneri* 72.2%, *S. dysenteriae* 10%, *S. boydii* 8.9% and *S. sonnei* 8.9% among 90 isolates (Tiruneh 2009). In contrast, data from Spain, Israel and the United States consistently demonstrate that *S. sonnei* is the most common serogroup found in industrialized countries (median 77%), followed by *S. flexneri* (median 16%), *S. boydii* (median 2%) and finally *S. dysenteriae* (median 1%) (Kotloff *et al.*, 1999b). Study conducted in Belgium During the period 1990 to 2007, of the 7,307 strains of *Shigella* spp. isolated, 4,951 were serotyped as *S. sonnei* (67.8%), 1,856 as *S. flexneri* (25.4%), 244 as *S. boydii* (3.3%), and 163 as *S. dysenteriae* (2.2%). A total of 72 isolates were nonagglutinable (1%) but were biochemically confirmed as *Shigella* spp (Vrints *et al.*, 2009)

The wide variations in these distributions together with the rather limited number of countries for which serotype distributions have been studied underscore the need for more studies on this topic (Vrints *et al.*, 2009).

S. dysenteriae 1 is an unusually virulent enteric pathogen that causes endemic or epidemic dysentery with high death rates. It is the most common cause of large-scale, regional outbreaks of dysentery. In recent years, *S. dysenteriae* 1 has caused epidemic dysentery in Central America, south Asia and central and southern Africa. An epidemic in Central America from 1969 to 1973 was responsible for more than 500,000 cases and 20,000 deaths. The epidemic in central and southern Africa began in 1979, initially affecting eastern Zaire, Rwanda and Burundi. In the early 1990s, epidemic dysentery moved southward, affecting first Zambia, then Malawi,

Mozambique, Zimbabwe and southern Africa. A large rise in the number of cases associated with refugee camps was seen in central Africa in 1994 (Cheryl *et al.*, 1999).

Shigella is highly adapted to man, with humans and primates in captivity being the only known natural hosts. *Shigella* species are potentially the most communicable of bacterial pathogens. Shiga's bacillus is transmitted very efficiently through the fecal-oral route. The infectious dose for symptomatic infection of *Shigella* can be as low as 10 organisms. Large numbers of *Shigella* are present in stools of clinical cases and healthy carriers, facilitating transmission in areas where there are crowding, poor sanitation and poor hygiene. Shigellosis is transmitted by fecal-oral contamination directly through person-to-person contact direct, or indirectly through fecal contamination of food (food borne) and water sources (waterborne), and houseflies (fly-borne) (Sake *et al.*, 2001).

Person-to-person contact seems to be the most important mode of shigellosis transmission. It is believed that this may also be the case during epidemic circumstances. In Central Africa, direct transmission within families, hospitals, and institutions apparently played a more important role than other modes of transmission. However, a combination of direct and indirect contamination is likely to have occurred in the settings where inadequate hygiene and sanitary patterns prevailed. The relative importance of the housefly in comparison with other routes of transmission is not known, but is likely to be minimal (Cobra and Sack, 1996). Secondary cases during outbreaks of shigellosis are common. One study demonstrated that bacillary dysentery develops in 61% of the children younger than 1 year once an index case occurs in a household. The attack rate was approximately 40% for those aged 1 to 4 years and 20% for all ages once an index case was identified. Secondary attack rates are increased in houses having privies and are reduced in families once sanitary toilet facilities are installed (Mandell *et al.*, 2005)

There appears to be a seasonal incidence to the occurrence of bacillary dysentery in the tropics and subtropics. Epidemics are more common during the rainy season and shortly thereafter. During hot dry summers, epidemics are rare. In the rainy season in the tropics, people crowd together indoors and are more susceptible to chills. The rains inhibit people from defecating at a safe distance from their village and the damp soil allows the bacilli to flourish. Under these conditions, dysentery generally spreads rapidly. The house fly is a common carrier of the bacilli. The seasonal incidence of shigellosis corresponds directly to the maximum prevalence of

swarming flies. Contaminated water is another important mode of spread. The *Shigella* can survive in water for 3 weeks but for a much shorter time when exposed to sunlight. Several outbreaks of Flexner and Sonne dysentery have been traced to contaminated milk or food, especially in England and Europe. Susceptibility to infection is likewise important; persons new to the tropics, especially young children, are more likely to acquire dysentery than local inhabitants. Patients whose resistance has been lowered by other diseases, such as malaria, tuberculosis or AIDS, are also particularly susceptible (Kotloff *et al.*, 1999a).

Pathogenesis of *Shigella*

Shigellosis is highly communicable of the bacterial diarrheas. Experiments in volunteers have demonstrated that shigellosis is unique among bacterial enteropathogens in that fewer than 200 viable cells can readily produce the disease in healthy adults. The reasons for this low-dose response are not completely clear. One possible explanation is that virulent *Shigella* can withstand the low pH of gastric juice. *Shigella* infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare (Mandell *et al.*, 2005; Jawetz *et al.*, 2003).

Shigella causes disease by invading and replicating in cells lining the colonic mucosa. Structural gene proteins mediate the adherence of the organisms to the cells, as well as their invasion, intracellular replication, and cell-to-cell spread. *Shigella* species appear unable to attach to differentiated mucosal cells; rather, they first attach to and invade the M cells located in Peyer's patches. The type III secretion system mediates secretion of four proteins (**IpaA, IpaB, IpaC, IpaD**) into epithelial cells and macrophages. These proteins induce membrane ruffling on the target cell, leading to engulfment of the bacteria. *Shigella* lyse the phagocytic vacuole and replicate in the host cell cytoplasm. With the rearrangement of actin filaments in the host cells, the bacteria are propelled through the cytoplasm to adjacent cells, where cell-to-cell passage occurs. In this way, *Shigella* organisms are protected from immune-mediated clearance. *Shigellae* survive phagocytosis by inducing programmed cell death (**apoptosis**). This process also leads to the release of IL-1 β , resulting in the attraction of polymorphonuclear leukocytes into the infected tissues. This in turn destabilizes the integrity of the intestinal wall and allows the bacteria to reach the deeper epithelial cells (Murray *et al.*, 2005)

Following host epithelial cell invasion and penetration of the colonic mucosa, *Shigella* infection is characterized by degeneration of the epithelium and inflammation of the lamina propria. This results in desquamation and ulceration of the mucosa, and subsequent leakage of blood, inflammatory elements and mucus into the intestinal lumen. Patients suffering from *Shigella* infection will therefore pass frequent, scanty, dysenteric stool mixed with blood and mucus, since, under these conditions, the absorption of water by the colon is inhibited. This is in opposition to the diarrheal symptoms seen in patients suffering from extensive *Shigella* colitis, and the pathologic basis for this is unknown. It is possible that prostaglandin interactions induced by the inflammatory response to bacterial invasion contribute to diarrhea in patients with *Shigella* colitis (Todar, 2009)

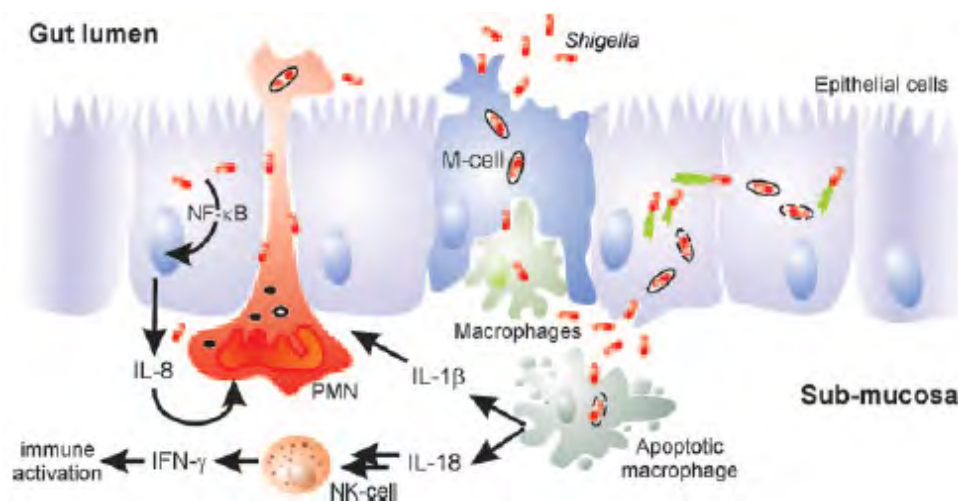


FIG. 1.1 Cellular pathogenesis of *Shigella* spp.

The Shiga bacillus (*S. dysenteriae* 1) was shown in the early part of the 20th century to produce a shiga toxin that is cytotoxic for a variety of cell types and is responsible for the development of vascular lesions in the colon, the kidney, and the central nervous system. Since then, it has been suspected that the toxin played an important role in the pathogenesis of clinical illness. Later, an exotoxin in the Shiga bacillus was shown to have enterotoxin activity in the ligated ileal loop model and also to have cytotoxic properties when intestinal mucosa was examined. Notably, *Shigella* enterotoxin 1 (ShET1) and ShET2, which are produced by several *Shigella* strains, were found to induce fluid secretion into the intestine, thus accounting for the watery phase of diarrhea (Schroeder and Hilbi 2008; Keusch *et al.*, 1972).

Antibiotic Therapy and resistance of *Shigellosis*

All cases of bloody diarrhoea should be treated promptly with an antimicrobial that is known to be effective against *Shigella*. This lessens the risk of serious complications and death, shortens the duration of symptoms, and hastens the elimination of *Shigella* from the stool. Other supportive measures used to treat acute diarrhoea, such as rehydration, feeding and zinc supplementation, should also be provided (WHO, 2005)

Most controlled clinical trials of antimicrobial chemotherapy have demonstrated that effective antibiotics shorten the duration of symptoms and eradicate *Shigellae* from the stool more quickly (compared to placebo or to ineffective antibiotics) (Bennish and Salam, 1991).

A variety of antibiotics are effective for treatment of shigellosis, although options are becoming limited due to globally emerging drug resistance. Originally, both sulfonamides and tetracycline were effective, but *Shigella* strains rapidly developed resistance to these agents. Ampicillin and TMP-SMZ were then used and continue to be effective in many industrialized countries. Unfortunately, in many parts of the world strains of all species of *Shigella* have become resistant to these low-cost agents, and neither can now be confidently used as empiric therapy for shigellosis. One of the few remaining, relatively inexpensive and effective drugs for shigellosis is the quinolone, nalidixic acid. In clinical trials, nalidixic acid treated groups achieved rates of clinical cure (absence of fever and of unformed stools by day 5 of treatment) and bacteriological cure (absence of *Shigella* from the stool by day 3 of therapy) comparable to the rates in individuals with ampicillin-sensitive infections treated with ampicillin. Unfortunately, resistance to nalidixic acid is also common in regions where it was introduced to treat epidemic shigellosis due to *Shiga* bacillus, resistance developed within six months. Resistance to fluoroquinolones has been rarely reported, and nearly all *Shigella* isolates are susceptible to these agents. Therefore, if treatment must be commenced before laboratory-based tests are available; it is still recommended that fluoroquinolone antibiotics, such as ciprofloxacin, should be used to treat adults but that nalidixic acid should be used to treat children. Fluoroquinolones are not currently approved for children (Bennish and Salam, 1991; Gillespie and Hawkey, 2006; Sack *et al.*, 2001).

Laboratory Diagnosis

The organisms are usually present in large numbers in the intestinal mucus or the faeces in the early stages. Freshly passed stools should be examined, although rectal swabs showing marked faecal staining may be used (Gillespie and Hawkey, 2006). When there is likely to be a delay in the specimen reaching the laboratory a suitable transport medium must be used to ensure viability of the organisms (Cheesbrough, 2006).

Microscopic examination of the mucus in the early stages of acute bacillary dysentery shows a marked predominance of polymorphonuclear cells and red cells. Microscopic examination of stools for leukocytes has been used to differentiate between bacillary and amebic dysentery; in Bangladesh 85 % of patients with shigellosis but only 28 % of patients with amebic dysentery had more than 50 white blood cells per high-power field (Echeverria *et al.*, 1991).

Isolation and identification of *Shigella* can be greatly enhanced when optimal laboratory media and techniques are employed. For optimal isolation of *Shigella*, two different selective media should be used: a general purpose plating medium of low selectivity, such as MacConkey agar (MAC), and a more selective agar medium, such as xylose lysine desoxycholate (XLD) agar. Desoxycholate citrate agar (DCA) and Hektoen enteric (HE) agar are suitable alternatives to XLD agar as media of moderate to high selectivity. *Salmonella-Shigella* (SS) agar is not suitable alternatives. As it frequently inhibit *Shigella dysenteriae* type 1, *S. sonnei* (Cheryl *et al.*, 1999; Vandepitte *et al.*, 2003).

Shigella appears as small colourless or slightly pink colonies on DCA and as pink or red colonies with, in some cases, a pink or yellow periphery on XLD. A few strains grow poorly on inhibitory media, and it is advisable to use MacConkey agar and to examine any non-lactose-fermenting colonies after overnight incubation (Cheesbrough, 2006; Gillespie and Hawkey, 2006).

Identification of *Shigella* spp. involves both biochemical and serologic testing. *Shigella* strains are oxidase-negative, non-motile, lysine-decarboxylase negative, and urea is not hydrolysed. On KIA they produce an alkaline slant and acid butt, no H₂S, and no gas, except for *S. flexneri* serotype 6 (Newcastle and Manchester varieties) and *S. boydii* serotype 14, which are aerogenic. Catalase is produced except for *S. dysenteriae* serotype 1, which is catalase negative. If these

criteria are fulfilled, report: “*Shigella* isolated (provisional identification)” (Vandepitte *et al.*, 2003).

1.2.3 Genus *Campylobacter*

Microbiology of *Campylobacter*

The family *Campylobacteraceae* included in the epsilon subdivision of Proteobacteria which is comprised of genera *Campylobacter*, *Arcobacter* and, more recently, genus *Sulfurospirillum*, as well as the generically misclassified *Bacteroides ureolyticus*. The complex taxonomy of Gram-negative spiral-shaped bacteria is continuously evolving with new information acquired from phylogenetic studies (Nachamkin and Blaser, 2000; Gillespie and Hawkey, 2006).

Awareness of the public health implications of *Campylobacter* infections has evolved over more than a century. In 1886, Escherich observed organisms resembling *Campylobacters* in stool samples of children with diarrhea. In 1913, McFaydean and Stockman identified *Campylobacters* (called related *Vibrio*) in fetal tissues of aborted sheep. In 1957, King described the isolation of related *Vibrio* from blood samples of children with diarrhea, and in 1972, clinical microbiologists in Belgium first isolated *Campylobacters* from stool samples of patients with diarrhea. The development of selective growth media in the 1970s permitted more laboratories to test stool specimens for *Campylobacter*. A convincing association of these organisms with human diarrhoea was demonstrated a decade later. Their significance to human health, however, was not established until a medium was designed for routine isolation of the organisms (Altekruse *et al.*, 1999; Gillespie and Hawkey, 2006).

Campylobacteraceae can be conveniently separated into three groups on the basis of phenotypic characteristics. The first and most important group to human health consists of five thermophilic enteropathogenic species. In this group are *C. jejuni* with two subspecies, *C. coli*, *C. lari*, *C. upsaliensis* and *C. helveticus*. A second group includes four species well known to veterinary microbiologists because of their frequent occurrence as pathogens or commensals in farm livestock, although some species have been shown to be pathogenic for humans. Included are *C. fetus* with two subspecies, *C. hyointestinalis* with two subspecies, *C. sputorum* with three biovars

and *C. mucosalis*. A third group, found in human periodontal disease, consists of *C. concisus*, *C. curvas*, *C. rectus*, *C. showae* and *C. gracilis* (Gillespie and Hawkey, 2006)

Campylobacter cells are mostly slender, spirally curved Gram-negative rods, 0.2 to 0.8 µm wide and 0.5 to 5 µm long. Cells of some species are predominantly curved or straight rods. Cells of most species are motile with a characteristic corkscrew like motion by means of a single polar unsheathed flagellum at one end or both ends. A characteristic common to all *Campylobacteraceae* is their ability to grow under microaerobic conditions, although some also grow under aerobic or anaerobic conditions. Growth is optimal between 30 and 37 °C. None of the species ferment or oxidize carbohydrates and all species utilize organic amino acids or tricarboxylic acid intermediates as sources of carbon. Spores are not produced but spherical coccoid forms have been observed in some species in older cultures or under unfavourable growth conditions. These coccoid forms have been coined viable non-culturable (VNC), and this form has been suggested to be a dormant state required for survival under conditions not supporting growth of *Campylobacter*, e.g. during transmission or storage. However, the existence and infectivity of a VNC form of *Campylobacter* is controversial. Thus, the role of the VNC state in *Campylobacter* transmission and colonization remains to be elucidated (Gillespie and Hawkey 2006; Nachamkin and Blaser 2000; Van Vliet and Ketley, 2001)

As with other bacteria whose ecologic niche is the gastrointestinal tract of mammals, the serotypic diversity of *C. jejuni* is enormous. Approximately 122 different serotypes based on heat-stable (HS) lipopolysaccharides antigens and 70 different serotypes based on heat-labile (HL) flagellar antigens have been identified (Asrat *et al.*, 1997)

Epidemiology of *Campylobacter*

Campylobacter is the leading cause of zoonotic enteric infections in developed and developing countries, and the incidence is increasing even in countries with adequate public health surveillance. The prevalence in developed countries range from 1-13% among children with acute diarrhea and 1.5% among children without diarrhea while in developing countries range from 5 - 35%. Case community based studies in developing countries have provided an incidence estimate of 40,000 to 60,000 per 100,000 for children less than five years of age (Nachamkin and Blaser, 2000; Diniz-Santos *et al.*, 2005; Murray, 1986)

This genus comprises an increasing number of species, there are numerous *Campylobacter* species but not all of these are important human pathogens. In developed Westernized communities *Campylobacter* infection is the commonest form of bacterial gastroenteritis. The major pathogens are known as the thermotolerant or thermophilic *Campylobacter* species. These include *Campylobacter jejuni*, *C. Coli*, *C. lari*, *C. upsaliensis*, but *C. jejuni* and *C. coli* are responsible for the majority of cases of human enterocolitis worldwide accounting more than 99% of *Campylobacter* strains isolated and identified in cases of human disease. The incidence of infection by *C. upsaliensis*, *C. lari*, *C. foetus*, and *C. hyointestinalis* is unknown, but these organisms seem to be much less common (Sack *et al.*, 2001; WHO, 2001). *Campylobacter jejuni* and *C. Coli* remain the two main species isolated from most studies in the developing countries; the isolation rate of *C. jejuni* exceeds that of *C. Coli*. One study in Ethiopia reports that of the *Campylobacters* that were differentiated at species level, *C. jejuni* accounted for 82.4% and *C. coli* for 17.6% of the isolates (Asrat *et al.*, 1997).

Campylobacter spp. are part of the normal intestinal flora of wild and domesticated animals and birds. Of particular importance to humans is their colonization of animals used in food production including poultry, cattle, sheep and swine (Blaser, 1997). Poultry comprise the most significant reservoir worldwide; *Campylobacter* spp. can be isolated from 30–100% of the birds in many domestic and wild avian species. In one study in Bahr Dar, Ethiopia show the prevalence of thermophilic *Campylobacters species* is 72.7% in chickens (Ewnetu and Mihret, 2010). Household pets such as dogs, cats and birds are additional animal reservoirs. Although excretion of *Campylobacter* is not associated with symptoms in poultry, diarrheal illnesses have been documented in mammalian pets and livestock, and this contributes to the contamination of surface water. Post-slaughter processing does not reduce the extent of colonization of poultry and may, in fact, lead to cross-contamination of previously uncontaminated carcasses. Ingestion of contaminated water, interaction with colonized pets (especially puppies and kittens), and consumption of unpasteurized milk or undercooked poultry or meat are all associated with human disease. Human-to-human transmission, while uncommon, has been documented via faecal exposure, particularly from young, incontinent children (Blaser, 1997; WHO, 2001).

In developed country most cases of human Campylobacteriosis are sporadic, *Campylobacter* enteritis outbreak are most frequently transmitted by food such as contaminated milk and water. Eating undercooked chicken appears to be the most common course of *Campylobacter* enteritis

(Nachamkin and Blaser 2000). In studies in many parts of the United States, Europe, and Australia, 50%–70% of all *Campylobacter* infections have been attributed to consumption of chicken (Allos 2001). Consumption of raw milk was implicated as the source of infection in 30 of the 80 outbreaks of human Campylobacteriosis reported to CDC between 1973 and 1992. Outbreaks caused by drinking raw milk often involve farm visits (e.g., school field trips) during the temperate seasons (Altekruse *et al.*, 1999).

The sources of human infection with *Campylobacter's* in developing countries include environmental contamination by wild and domestic animals, humans excreting the organisms, or foods. Wild birds and domestic animals are known as reservoirs for *Campylobacter's* and shedding of the bacteria causes contamination of the environment (WHO, 2001). One study in south west Ethiopia indicate domestic animal could serve as reservoir of thermotolerant *Campylobacter* that probably risk the public at large. *Campylobacter species* was isolated in this study from 68.1% of chicken, 38% of sheep, 12.6% of cattle and 50% of pigs in (Kassa *et al.*, 2007). Human with Campylobacteriosis are potential sources of infection. In developing countries, the mean duration of convalescent-phase excretion of *Campylobacter* organisms after an acute infection is 8 days (WHO, 2001). *Campylobacter's* present in foods for consumption in developing countries as a result of poor sanitations are also an important potential source of infection in humans. In Addis Ababa, Ethiopia *Campylobacter's* were isolated from different retail raw meats were - beef: 14/227=6.2%; sheep: 12/114=10.5%; goat: 7/92=7.6%; pork: 4/47=8.5%; chicken: 13/60 (21.7%) (Dadi and Asrat, 2008).

Pathogenesis of *Campylobacter*

Not all *Campylobacter* infections produce illness. Although all factors responsible for this phenomenon are not known, three of the most important appear to be the dose of organisms reaching the small intestine, the virulence of the infecting strain, and the specific immunity of the host to the pathogen ingested. Among exposed persons who become ill, the incubation period varies from 1 to 7 days, a characteristic that is probably inversely related to the dose ingested. Most infections occur 2 to 4 days after exposure (Mandell *et al.*, 2005). One study show *Campylobacter* species ingested by adult volunteers, in doses ranging from 8×10^2 to 2×10^9 organisms, caused diarrheal illnesses (Black *et al.*, 1988)

Clinical disease is characterized by acute diarrhea accompanied by intense abdominal pain. Campylobacteriosis is inflammatory enteritis that is initially found in the small bowel and later affects the colon and the rectum. The diarrhea can be either watery or, in almost one-third of the cases, bloody, indicating that the extents of intestinal inflammation vary among individuals. . It has been shown that this is in part related to differences in properties of the infecting strain. Limited evidence suggests that different *Campylobacter* strains can harbor different virulence traits (Sack *et al.*, 2001; Janssen *et al.*, 2008).

Campylobacter establish infection, first in the small intestine and later in the colon, and to cause diarrheal illnesses with many of the features of naturally acquired infection (Black *et al.*, 1988). Colonization of human intestine with *C. jejuni* results in the expression of several putative virulence factors, yet the mechanisms of pathogenesis are unclear (Van Vliet and Ketley 2001). Colonization may be influenced by the expression of lipopolysaccharides (LPS) on the bacterial cell surface. A mutation in the *galE* gene involved in the synthesis of LPS reduced the ability of *C. jejuni* to adhere and invade INT 407 cells, suggesting that LPS is a virulence factor for *C. jejuni* infection, although the *C. jejuni* was still able to colonize chickens (Gillespie and Hawkey, 2006)

Flagella are an important determinant in virulence since colonization of intestinal tracts does not occur with non-motile mutants of *C. jejuni*. Nonflagellated mutants and flagellated but nonmotile (paralyzed) mutants are also unable to invade eukaryotic cells in vitro, further suggesting either a role for motility in invasion or a coordinate regulation of motility and other virulence determinant (Guerry, 1997)

After colonization of the intestine, clinical disease may occur. Based on clinical syndromes found in patients, two mechanisms by which *Campylobacter* can induce disease were postulated: (i) adherence of *Campylobacter* to the intestine and the production of toxins, which alter the fluid resorption capacity of the intestine, resulting in secretory diarrhea, and (ii) bacterial invasion and replication within the intestinal mucosa accompanied by an inflammatory response resulting in blood-containing, inflammatory diarrhea (Janssen *et al.*, 2008)

All strains of *C. jejuni* produce cytolethal-distending toxin (CDT), a nuclease that results in cell-cycle arrest and host DNA damage. Separately *C. jejuni* invasion of the mucosa or CDT alone

triggers basolateral IL-8 release from the epithelium. In vitro data suggest that *C. jejuni* can survive within macrophages/monocytes for several days, which might allow for some localized bacterial dissemination. The proinflammatory cytokine IL-8 triggers an influx of PMNs from the lamina propria which prevents further spread of *C. jejuni*. The resulting focal necrosis of the epithelium could result from the local inflammatory response and host cell death caused by CDT (Kopecko *et al.*, 2001)

The role of enterotoxin production in *Campylobacter* pathogenesis is highly debated. Cholera-like toxins (CLT) have been reported, and they may play a role in the enterotoxigenic, noninflammatory diarrhoea typical in the developing world (Sack *et al.*, 2001)

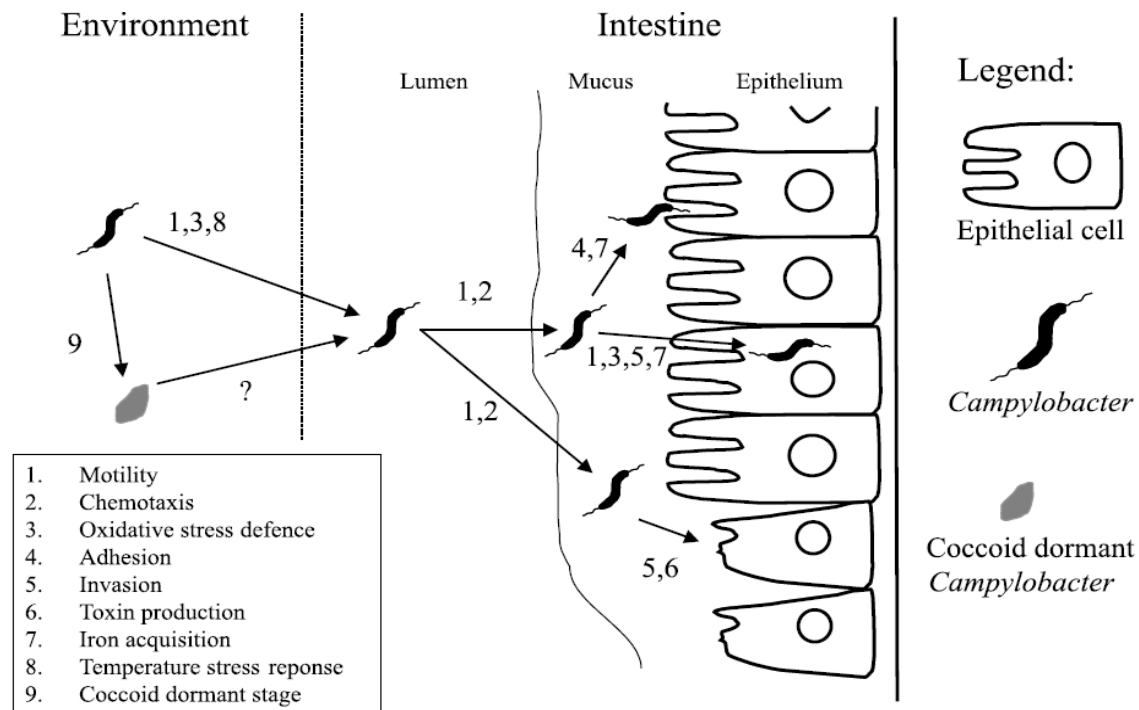


Fig. 1.2 Overview of the different phases of *Campylobacter* colonization of the intestine. Putative virulence factors discussed are indicated, together with the phase(s) in which these are thought to be expressed.

Campylobacter outer membranes contain lipopolysaccharides (LPSs) with typical endotoxic activity. The structure of the LPS O antigen is highly variable. Many *C. jejuni* O antigens possess sialic acid-containing structures. Their close resemblance to those seen in human gangliosides

such as GM₁, GD_{1a}, GD₃, and GT_{1a}, and their presence in strains isolated from patients who developed the Guillain-Barré syndrome (GBS) suggest a role in the pathogenesis of this disorder (Mandell *et al.*, 2005) resistance

Antibiotic Therapy and Resistance of *Campylobacter*

In immunocompetent individuals, *Campylobacter* enterocolitis is generally self-limited, with mild to moderate symptoms, and antibiotic therapy is not required for most patients. Supportive care with oral rehydration is the preferred treatment (Mandell *et al.*, 2005). Antibiotic therapy may be prudent for patients who have high fever, bloody diarrhea, or more than eight stools in 24 hours; immunosuppressed patients, patients with bloodstream infections, and those whose symptoms worsen or persist for more than 1 week from the time of diagnosis. When indicated, antimicrobial therapy soon after the onset of symptoms can reduce the median duration of illness from approximately 10 days to 5 days. When treatment is delayed (e.g., until *C. jejuni* infection is confirmed by a medical laboratory), therapy may not be successful (Altekruse *et al.*, 1999). Macrolides and fluoroquinolones are normally considered for treatment of *Campylobacter* enteritis (Alfredson and Korolik, 2007; Blaser, 1997; Taylor *et al.*, 1987; Ternhag *et al.*, 2007)

Erythromycin has traditionally been the first-line therapy, although newer macrolides such as azithromycin are quickly gaining popularity (Sack *et al.*, 2001). Erythromycin comes to be considered the optimal drug for treatment of *Campylobacter* infections. Despite decades of use, the rate of resistance of *Campylobacter* to erythromycin remains quite low. Resistance against erythromycin was not detected until the second year of the study period; it persisted, although it remained below 2% (Talsma *et al.*, 1999). Other advantages of erythromycin include its low cost, safety, ease of administration, and narrow spectrum of activity. Unlike the fluoroquinolones and tetracyclines, erythromycin may be administered safely to children and pregnant women and is less likely than many agents to exert an inhibitory effect on other fecal flora. Erythromycin stearate is acid-resistant, stable, and incompletely absorbed. Therefore, in addition to its systemic effects, it may be capable of exerting a contact effect throughout the bowel (Allos, 2001).

Bactericidal fluoroquinolones have become the drugs of choice for treating travelers' diarrhoea and are therefore used as first-line therapy of *Campylobacter* infections contracted abroad. Tetracycline, doxycycline and TMP-SMZ have been used as second line agents, although

increasing resistance limits their use. Gentamicin, kanamycin, imipenem and ampicillin-clavulanic acid are reserved for systemic, refractory illness (Allos, 2001)

Laboratory Diagnosis

Clinical diagnosis of enteric Campylobacteriosis may be established by demonstration of the organisms by direct examination of feces, or by isolation of the organisms.

Bacteria of the genus *Campylobacter* have a characteristic morphology and a darting type of motility that permits their identification by direct examination of broth suspensions of faeces. However, *C. jejuni* cannot be distinguished from *C. coli* by this procedure and the test is considerably less sensitive than isolation by culture (Gillespie and Hawkey, 2006).

A confirmed diagnosis requires culture of the organism from faeces or blood. Faecal samples should arrive in the laboratory within a few hours after collection or, if a delay is likely, should be inoculated into a transport medium (e.g. Cary-Blair medium) (Sack *et al.*, 2001).

The first method for the isolation of *Campylobacters* from human faeces was differential filtration of a saline extract through a 0.65, µm filter, which allows *Campylobacters* and other small bacteria to pass through. The filtered fluid, which contains the filter-passing *Campylobacteria*, is inoculated on a solid isolation medium. Thereafter the introduction of selective agars proved successful for isolation from human faeces and so established *Campylobacters* as an important cause of enteritis in man. Several different blood-based and non-blood-based media (Blaser, Butzler, Skirrow) containing different antimicrobial supplements and growth factors have been developed for the isolation of *Campylobacters* from faecal specimens (Bolton and Robertson, 1982; Cheesbrough, 2006).

Plates for the isolation of *Campylobacter* spp. should be incubated at 42–43°C. The growth of the normal faecal flora is inhibited at this temperature while the thermotolerant *Campylobacter* species are unaffected (Gillespie and Hawkey, 2006). Since *Campylobacteria* are microaerophilic it is essential that they be cultured in an atmosphere with reduced oxygen concentration. The recommended gaseous mixture is 5% oxygen, 10% carbon dioxide and 85% nitrogen for most *Campylobacter* species. The most convenient method of achieving such an

atmosphere is with commercially available gas-generating envelopes that are activated in the anaerobic jars. Plates should be incubated for 48 – 72 hours and examined daily for growth (Vandepitte *et al.*, 2003).

Colonial morphology varies and colonies may appear gray, flat, irregular and spreading or round, convex and glistening. They are often found to spread along the lines of inoculation. *Campylobacter coli* colonies tend to be creamy-grey, moist and more discrete than those of *C. jejuni* subsp. *jejuni*. Colonies of *C. lari* are generally grey and discrete but more variable, resembling either *C. jejuni* subsp. *jejuni* or *C. coli* colonies (Gillespie and Hawkey, 2006; Nachamkin 2003).

Suspect colonies should be screened with three presumptive tests: oxidase test, wet mount preparation under dark-field or phase-contrast microscope, and Gram stain. If a dark-field or phase-contrast microscope is not available, colonies may be rapidly screened for typical cell morphology by staining with Gram's crystal-violet solution. For the Gram stain 0.3% carbol fuchsin is recommended as counterstain (Vandepitte *et al.*, 2003).

Biochemical inertness of *Campylobacter* prevents effective discrimination of the species by phenotypic methods, such as tolerance to different temperatures and to agents that inhibit growth. However, tests for oxidase, catalase, hippuricase, urease, indoxyl acetate hydrolysis, nitrate and nitrite reductases, hydrogen sulfide production, susceptibility to nalidixic acid and cephalothin and growth at 15, 25 and 42 °C are the most reliable for species differentiation (Gillespie and Hawkey, 2006)

Any colonies isolated from selective media under microaerobic conditions that are oxidase positive, Gram-negative curved, S-shaped or spiral rods 0.2–0.9 mm wide and 0.5–5mm long may be reported as *Campylobacter* species (Asrat *et al.*, 1997). The ability of many *C. jejuni* to hydrolyse hippurate allows its differentiation from *C. coli*, which lacks this ability. Speciation becomes more difficult for *C. jejuni* strains that are unable to hydrolyse hippurate (Gillespie and Hawkey, 2006).

1.3 Relevance of the study

Diarrheal diseases represent a major problem in many areas of the world and are associated with severe morbidity and mortality. The greatest mortality occurs in infants and children. Even though economic development and progress in health care delivery are expected to catalyse substantial improvements in infectious-disease-related morbidity and mortality during the next 30 years, it is predicted that diarrhoea will remain a leading health problem. *Campylobacter* spp. and *Shigella* spp. remain among the bacteria most frequently isolated from stool samples obtained from diarrhea patients, especially from developing countries (Kotloff *et al.*, 1999a).

Shigellosis is highly infectious disease of worldwide significance. Its prevalence is highest in tropical and subtropical region of world, where living standard are very low and access to safe and adequate drinking water and to proper excreta disposal system are often very limited or even absent. In Ethiopia, various studies have invariably concluded that shigellosis is major causes of mortality and morbidity. Infant and young children are the most affected. *Shigella* isolate from different part of Ethiopia as in other developing countries shows higher rate of resistant to commonly used antimicrobial agent (tetracycline, co-trimoxazole, ampicillin, and chloramphenicol (Asrat, 2008; Tiruneh, 2009; Mandomando *et al.*, 2009). Because *Shigella* species have acquired multiple antimicrobial resistances, the challenge for clinical management is identifying which drugs retain their activity and clinical effectiveness, (Sack *et al.*, 2001) patterns of antibiotic resistance, which vary considerably from place to place and which are in a continuous state of evolution, must be updated (Kotloff *et al.*, 1999b).

C. jejuni and related species are increasingly recognized as important causative agents of enterocolitis worldwide (Nachamkin and Blaser, 2000). *Campylobacter* is one of the most frequently isolated bacteria from stools of infants with diarrhoea in developing countries resulting from contamination of food or water. The rate of *Campylobacter* infections has been increasing, with the number of cases often exceeding those of *Salmonella* and *Shigella* (WHO, 2001). However, screening for *Campylobacter* in acute enteric infections is often not a routine matter due to its relatively recent link to human disease and the complexity of procedures for its isolation and identification. As a result, there is little information available specifically on *Campylobacter* in acute enteric infections or antimicrobial resistance from the developing world including Ethiopia (Mitike *et al.*, 2000; Wasfy *et al.*, 2000).

Therefore this study attempted to isolate *Campylobacter* and *Shigella* from diarrheal stool specimen and determine their antibiotic susceptibility pattern.

1.4 Objectives of study

General objective

To isolate and to determine their antibiotic susceptibility pattern of *Shigella*, and *Campylobacter* spp. from acute enteric infections in Addis Ababa

Specific objective

To determine prevalence of Shigellosis and Campylobacteriosis in patient with acute enteric infections

To determine the antimicrobial resistance pattern of *Shigella*, and *Campylobacter* isolates.

To assess the risk factors associated with *Shigella*, and *Campylobacter* infection.

Chapter II: Materials and Methods

2.1 Study design

A cross sectional study was conducted from December 2010 to March 2011 to isolate *Shigella* and *Campylobacter* species from human diarrheic stool specimens and to determine the antimicrobial susceptibility of the isolates.

2.2 Study area

The study was conducted in Addis Ababa and including both hospital and health center setting. Yekatit 12 hospital and Shiromeda health center.

Addis Ababa is the capital city of Ethiopia. It is the largest city in Ethiopia, based on the preliminary 2007 census results Addis Ababa has a total population of 2,738,248, consisting of 1,304,518 men and 1,433,730 women and contains 22.9% of all urban dwellers in Ethiopia. With an estimated area of 530.14 square kilometers (204.69 sq mi), this chartered city has an estimated density of 5,165.1 inhabitants per square kilometre (13,378 /sq mi).

Shiromeda health center located in Gulele subcity serving 111,005 catchment population. It provides both primary and curative service. Yekatit 12 Hospital is a referral hospital under the Addis Ababa City Government Health Bureau (AACG-HB) and has a total of 265 beds. It functions through a referral system but accepts emergency cases without referral. The hospital has 9 major departments and 6 units; in pediatrics and maternity alone, Yekatit 12 treats well over 15,000 patients annually. During the study period pediatrics unit was not functional due to renovation.

2.3 Study population

All patients presented with acute diarrhea visiting the Yekatit 12 hospital and Shiromeda health center in Addis Ababa.

Diarrhoea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual (WHO, 2009).

Dysentery, defined as diarrhea with visible blood (Cheryl *et al.*, 1999).

2.4 Sample size

Sample size calculated using the prevalence of *Campylobacter* from diarrheal stool in Addis Ababa 10.8 (Asrat *et al.*, 1997) Using the following formula:

$$\begin{aligned}\text{Total study subject } N &= Z^2 p \times (1-p) / d^2 \\ &= (1.96)^2 \times 0.108 \times (1-0.108) / (0.03)^2 = 411\end{aligned}$$

Where N = patient who should be sampled

Z = the standard normal deviation corresponding, 95% of Confidence level = 1.96

P = prevalence (10.8%)

d = the degree of accuracy desired (3% = 0.03)

A total of 394 patients with diarrheal illness from Yekatit 12 hospital (n=140) and Shiromeda health centre (254) were investigated for *Shigella* and *Campylobacter* infection.

Histories were taken from informed and consented patients or parents/guardians before sample collection by principal investigator and qualified health worker. All the relevant data (Demographic, Clinical and risk assessment data) were recorded and transferred to the questionnaire prepared for this study (see appendix I)

Patients with acute diarrhea who took antibiotic were excluded from the study.

2.5 Collection, handling, and transport of specimen

Freshly passed stool swabs specimens were collected, placed immediately in Cary Blair transport medium (Oxoid Ltd, England) and transported to the AAU Department of Microbiology laboratory in ice cold box within 4 hours of collection.

Culture and identification of *Shigella* species

Stool samples was streaked on Salmonella – Shigella agar and MacConkey agar (DIFCO, Detroit, USA) and incubated at 37 °C for 18-24 hrs, the resulting colonies which exhibited

characteristics of *Shigella* spp. were identified by conventional biochemical methods. All biochemical test tubes had been incubated at 37 °C for 18-24 hrs.

Culture and identification of *Campylobacter* species

Stool sample were inoculated on *Campylobacter* agar and incubated at 42 °C in a microaerophilic atmosphere containing 5-10% O₂ and 10% CO₂ produced from gas generating sachets (Campy-GenTM; Oxoid Ltd) for 48hr.

Thermotolerant *Campylobacter* species were identified by their characteristic appearance on culture media, Gram staining reaction, and oxidase and catalase reaction.

Gram stained morphology showed a Gram negative organism with an 'S'-shaped appearance. Positive results with oxidase and catalase tests identified thermotolerant *Campylobacter* genera (Nachamkin and Blaser, 2000).

Microscopic examination of stool examination

Parasites were identified through direct microscopy using saline wet mount at both sites by laboratory technicians.

Antimicrobial sensitivity test for *Shigella* species

The disk diffusion method was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI). A standard inoculum adjusted to 0.5 McFarland was swabbed onto Muller-Hinton agar. Susceptibility testing of all isolates were done using the single disc diffusion technique against ampicillin (Amp), 10µg; chloramphenicol (Chl) 30µg; nalidixic acid (Na) 30µg; norfloxacin (Nor) 10µg; polymyxin B (Pol) 30µg; kanmycin (Kan) 30µg, and ciprofloxacin (Cip) 5µg (CLSI, 2007).

Antimicrobial sensitivity test for *Campylobacter* species

Briefly, well-isolated colonies of the same morphological type were selected from an agar plate culture and turbidity of the inoculum was matched with the turbidity standard McFarland 0.5.

Swab was dipped into suspension and used to inoculate Muller Hinton Agar supplemented with 5% sheep blood. After incubation at 42°C for 24 h under microaerobic conditions, a sterile cotton swab was dipped into the suspension and streaked on the entire surface of Mueller–Hinton agar (DIFCO, Detroit, USA) with 5% sheep blood. The inoculum was allowed to dry for 5min. Antibiotic discs were placed on the plate and after 16-18 h of microaerobic incubation at 42°C, the diameter of the inhibition zone were measured with calipers. Interpretation of the result, break point recommended by the CLSI for bacteria isolate grown aerobically was performed (Nachamkin and Blaser, 2000).

The following antibiotic-impregnated discs were used: ampicillin (10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), nalidixic acid (30 µg), Chloramphenicol 30 (µg), Trimethoprim-Sulfamethoxazole (SXT) 25(µg) (Oxoid Ltd.).

Multidrug resistant in this study defined as resistant to two or more drugs.

Quality control

Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) strains were used for quality control throughout the study.

2.6 Statistical Methods

Data were organized and summarized in simple descriptive statistics methods.

Moreover, all components of the data entered and analyzed using SPSS 16.0 computer software. Chi-square test (χ^2) results were used and a *p*-value of less than 0.05 was considered statistically significant.

2.7 Ethical consideration

Study approval was obtained from department of Microbiology, Immunology and Parasitology, Addis Ababa University (AAU). Official permission was requested from both study sites where the study was conducted. The study was explained to the patients/guardians, and informed consent for participation in the study was obtained prior to collecting the specimens.

Chapter III: Results

3.1 Socio-demographic data

A total of 394 patients visiting the outpatient departments with acute diarrhoea were investigated for *Shigella* and *Campylobacter* and other enteropathogens from Shiromeda health center (n=254) and Yekatit 12 Hospital (n=140), between December 2010 and March 2011. The median age of the patients was 11 yrs (range 3 month to 80 years). 144 (36.5 %) of the patients were younger than 5 years. Out of the 394 patient that had diarrhea, 192 (48.7%) were females and 202 (51.3%) were males making the female to male ratio 1:1.05 The age and sex distribution of the 394 patients are shown in Table 3.1.

Table 3.1: Age and sex distribution of 394 patients investigated for *Campylobacter* and *Shigella* and other enteropathogens at Shiromeda health center and Yekatit 12 Hospital Addis Ababa Ethiopia, 2010/2011

Age in year	Shiromeda Health Center		Yekatit 12 Hospital	
	Female	Male	Female	Male
	No. (%)	No. (%)	No. (%)	No. (%)
0 - 4	73 (54.1%)	71 (59.7%)	-	-
5 - 14	35 (25.9%)	15 (12.6%)	-	-
15 - 24	12 (8.9%)	16 (13.4%)	29 (50.9%)	37 (44.6%)
25 - 34	9 (6.7%)	7 (5.9%)	11 (19.3%)	15 (18.1%)
35 - 44	3 (2.2%)	4 (3.4%)	8 (14.0%)	10 (12.0%)
45 - 54	2 (1.5%)	4 (3.4%)	7 (12.3%)	8 (9.6%)
55+	1 (0.7%)	2 (1.7%)	2 (3.5%)	13 (15.7%)

3.2 Clinical Features

Among all patients abdominal pain was reported by 229 (58.1%) of patients, fever 135 (34.3%), tenesmus 132 (33.5%), and vomiting 121 (30.7%). Among 37 patients who were positive for *Shigella*, abdominal pain was the commonest clinical findings 26 (70.3%), followed by tenesmus 17 (45.9%), fever 13 (35.1%), and vomiting 11 (29.7 %) and of the 19 *Campylobacter* positive patients 14 (73.7) had abdominal pain, vomiting 6(31.6%), tenesmus 8(42.1%), and fever 8 (42.1%). There were no statistically significant difference detected between the percentage of patients with abdominal pain, Vomiting, Tenesmus and fever in those who were culture positive and negative ($p>0.05$).

Shigella species were significantly more likely to be isolated from patients with bloody diarrhea or mixed (mucus and blood) ($p<0.05$). On the other hand isolation of *Campylobacter* species was not significantly associated with bloody or mixed (mucus and blood) ($p>0.05$) diarrhea. (Table 3.2)

The duration of illness prior to the patients' initial visit ranged from one day to two weeks. Seventy four point nine percent (74.9%) of the patients presented with an acute illness of 0- 5 days duration, 24.4 % had been ill for 6 to 12 days and only 0.8% for two weeks or longer. There was no significant association between the duration of diarrhoea and culture positivity though the majority were from 1 to 5 days duration ($p > 0.05$).

Table 3.2 Clinical findings and their association with positivity of *Shigella* and *Campylobacter* species among 394 children with diarrhoea at Shiromeda health center and Yekatit 12 Hospital Addis Ababa Ethiopia, 2010/2011

		<i>Shigella</i> spp.				<i>Campylobacter</i> spp.			
		Negative %	Positive%	OR	P-value	Negative %	Positive%	OR	P-value
Types of diarrhea	Bloody	18(5.0%)	6(16.2%)	–	0.001	23(6.1%)	1(5.3%)	–	0.060
	Loose	210(58.8%)	11(29.7%)			216(57.6%)	5(26.3%)		
	Mixed	13(3.6)	4(10.8%)			16(4.3%)	1(5.3%)		
	Mucoid	85(23.8%)	14(37.8%)			89(23.7%)	10(52.6%)		
	Watery	31(8.7%)	2(5.4%)			31(8.3%)	2(10.5%)		
Abdominal pain	NO	154(43.1)	11(29.7%)	1.85 CI (0.88-3.8)	0.161	160(42.7%)	5(26.3%)	1.02 CI (0.4–2.6)	0.233
	YES	203(56.9%)	26(70.3%)			215(57.3%)	14(73.7%)		
Vomiting	NO	247(69.2%)	26(70.3%)	0.95 CI (0.4– 1.9)	0.892	260(69.3%)	13(68.4%)	1.04 CI(0.38–2.8	0.933
	YES	110(30.8%)	11(29.7%)			115(30.7%)	6(31.6%)		
Tenesmus	NO	242(67.8)	20(54.1%)	1.78 CI(0.9–3.5)	0.092	251(66.9%)	11(57.9%)	1.47 CI(0.57–3.7)	0.415
	YES	115(32.2%)	17(45.9%)			124(33.1%)	8(42.1%)		
Fever	NO	235(65.8%)	24(64.9%)	1.04 CI(0.5–2.1)	0.907	248(66.1%)	11(57.9%)	1.42 CI(0.55–3.6)	0.460
	YES	122(34.2%)	13(35.1%)			127(33.9%)	8(42.1%)		
Duration of diarrhea	0 – 5	264 (73.9%)	31 (83.8%)	–	0.109	280 (74.7%)	15 (78.9%)	–	0.051
	6 – 12	91 (25.5%)	5 (13.5%)			93 (24.8%)	3 (15.8%)		
	12+	2 (0.6%)	1 (2.7%)			2 (0.5%)	1 (5.3%)		

3.3 Patterns of Etiological agents

Bacterial pathogens were isolated from 79 (20%) of the 394 patient presented with acute diarrhea. The isolation rates were 9.4 % for *Shigella* species, 5.8 % for *Salmonella* species, and 4.8 % for *Campylobacter* species. Twenty one percent (84/394) of the patients were infected by one or more parasites. Co-infections were found in 6 (1.5%) of the patients, of these 2 were bacteria/ parasite and 4 were parasite/parasite co-infection. The number and the percentage of detection of enteropathogens in stool samples obtained from diarrheic patients by culture and microscopic examination are shown in Table3.3.

Table 3.3 Bacterial pathogens and parasites detected from 394 patients with diarrhoea at Shiromeda health center and Yekatit 12 Hospital Addis Ababa Ethiopia, 2010/2011

Enteropathogens	Shiromeda Health center No. (%) n= 254	Yekatit 12 Hospital No. (%) n= 140	Total No. (%) n= 394
<i>Shigella</i> Spp.	24 (9.4%)	13 (9.3%)	37 (9.4%)
<i>Campylobacter</i> Spp.	17 (6.7%)	2 (1.4%)	19 (4.8%)
<i>Salmonella</i> Spp.	14 (5.5%)	9 (6.4%)	23 (5.8%)
Total	55 (21.6%)	24 (17.1%)	79 (20.0%)
Parasites			
<i>Giardia lamblia</i>	29 (11.4%)	11 (7.8%)	40 (10.2%)
<i>Entamoeba histolytica</i>	20 (7.9%)	16 (11.4%)	36 (9.1%)
<i>Hymenolopsis nana</i>	3 (1.2%)	1 (0.7%)	4 (1.0%)
<i>Ascaris lumbricoides</i>	3 (1.2%)	2 (1.4%)	5 (1.3%)
Total	55 (21.6%)	30 (21.4%)	85 (21.5%)

Among *Campylobacter* positive patients 68.4% were from children less than 5 year of age, whereas in *Shigella* positive patients 78.4% were from those whose age is above five years. The rate of isolation of *Campylobacter* in children less than 5 year of age was significantly higher than in older age groups ($p < 0.05$). On the other hand, the rate of isolation of *Shigella* in patient was higher in the 24 – 34 and 45 – 55 age groups. The distribution of both *Campylobacter* and *Shigella* was not different in males and females ($P > 0.05$).

Table 3.4 Distribution of *Shigella* and *Campylobacter* spp. among different age group investigated at Shiromeda health center and Yekatit 12 Hospital Addis Ababa Ethiopia, 2010/2011.

Age group (Year)	Number (n=394)	<i>Shigella</i> spp. (n=37)	<i>Campylobacter</i> spp. (n=19)
0 - 4	144	8 (5.6%)	13 (9.0%)
5 - 14	50	5 (10.0%)	3 (6.0%)
15 - 24	94	11 (11.7%)	1 (1.1%)
25 - 34	42	6 (14.3%)	1 (2.4%)
35 - 44	25	2 (8.0%)	1 (4.0%)
45 - 54	21	3 (14.3%)	0
55+	18	2 (11.1%)	0
Total	394	37 (9.4%)	19 (4.8%)

3.4 Risk Factors

There is no significant association between consumption of raw meat or milk and culture positivity. Of the 19 patients who found to be positive for *Campylobacter*, 10 (52.6%) were had contacts with dogs or cats with OR=1.8. In hand washing practices, 290 (73.6%) of patients had no habit of hand washing before meal regularly (Table 3.5).

Table 3.5 Selected factor and culture positivity of *Campylobacter* and *Shigella* at Shiromeda health center and Yekatit 12 Hospital Addis Ababa Ethiopia, 2010/2011

		<i>Campylobacter</i> Spp.				<i>Shigella</i> spp.			
		Negative	Positive	p-value	OR	Negative	Positive	p-value	OR
Sex	Female	170(47.6%)	22(59.5%)	0.727	0.849 CI (0.3 – 2)	182(48.5%)	10(52.6%)	0.170	0.700 CI (0.3- 1.3)
	Male	187(52.4%)	15(40.5%)			193(51.5%)	9(47.4%)		
contact with pets	NO	242 (64.5%)	9 (47.4%)	0.346	1.800 CI (0.7-4.5)	229 (64.1%)	25(67.6%)	0.208	0.696 CI (0.3 – 1.4)
	YES	133 (35.5%)	10(52.6%)			128(35.9%)	12(32.4%)		
Consume raw milk	NO	320 (85.3%)	15(78.9%)	0.447	1.552 CI (0.4 – 4.8)	302(84.6%)	33(89.2%)	0.456	0.666 CI (0.2 – 1.9)
	YES	55(14.7%)	4(21.1%)			55(15.4%)	4(10.8%)		
Consume raw meat	NO	285(76%)	18(94.7%)	0.089	0.176 CI (0.02 – 1.3)	277(77.6%)	26(70.3%)	0.315	1.465 CI (0.7 – 3)
	YES	90(24%)	1(5.3%)			80(22.4%)	11(29.7%)		
hand washing habit before meal	Never	31(8.3%)	3(15.8%)	0.355	–	29(8.1%)	5(13.5%)	0.295	–
	Regularly	101(26.9%)	3(15.8%)			92(25.8%)	12(32.4%)		
	Sometimes	243(64.8%)	13(68.4%)			236(66.1%)	20(54.1%)		

* Statistically significant

3.5 Antimicrobial Sensitivity

The antimicrobial susceptibility testing was done on all *Shigella* and *Campylobacter* spp. isolates using disk diffusion method and the results are presented in Table 3.5.

Thirty seven *Shigella* isolates were tested against Ampicillin, Chloramphenicol, trimethoprim-sulphamethoxazole, nalidixic acid, ciprofloxacin, norfloxacin, polymyxin B and kanamycin. The overall rates of resistance were high for ampicillin 25(67.6%), chloramphenicol 15(40%), trimethoprim-sulphamethoxazole 24(64.9%). Low level of resistance observed against nalidixic acid 1 (2.7%), norfloxacin 1 (2.7%), kanamycin 1 (2.7%), ciprofloxacin 1 (2.7%), and polymyxin B 2 (5.4%). Susceptibility to all drugs tested in *Shigella* isolates was observed for 9 (24.3%) of the isolates. Multiple resistance (resistant to two or more drugs) was observed in 23 (62.1%) of the isolates. The frequency of resistance for ampicillin, trimethoprim-sulphamethoxazole, and chloramphenicol is the highest patterns of resistance.

Nineteen isolated *Campylobacter* species were tested against six antibiotics. All isolated *Campylobacter* species were sensitive to chloramphenicol. Ampicillin and trimethoprim-sulfamethoxazole were found to have resistance against *Campylobacter* species of 42.1% and 52.6%, respectively. The majority of the strains were sensitive to nalidixic acid, ciprofloxacin and erythromycin. Out of the 19 *Campylobacter* 8 (42.1%) isolates, were multiple resistant (resistant to two or more drugs). (Table 3.6)

Table 3.6 Antimicrobial susceptibility patterns of *Shigella* and *Campylobacter* isolates

		Antimicrobial agent (%)								
		Amp	Chl	Na	Nor	Cip	Sxt	Pol B	K	
<i>Shigella</i> spp. (n=37)	S	24.3	59.5	97.3	97.3	97.3	35.1	86.5	97.3	ND
	I	8.1	0	0	0	0	0	8.1	2.7	ND
	R	67.6	40.5	2.7	2.7	2.7	64.9	25.4	0	ND
<i>Campylobacter</i> spp. (n=19)	S	57.9	100	84.2	ND	89.5	47.4	ND	ND	94.7
	I	0	0	0	ND	0	0	ND	ND	0
	R	42.1	0	15.8	ND	10.5	52.6	ND	ND	5.3

ND: not done

Chapter IV: Discussion

Gastroenteritis-causing pathogens are the second leading cause of morbidity and mortality worldwide; it is mainly children under the age of 5 years who are at risk. Globally, *Salmonella* and *Shigella* remain major contributors to acute enteric infections with nontyphoidal *Salmonella* isolated in increasing numbers in diverse geographic regions. *Campylobacter* has emerged as a significant cause of gastroenteritis. When clinical laboratories include a screen for *Campylobacter* in routine enteric culture procedures, the recovery for this organism often exceeds that of *Salmonella* and *Shigella* (Guerrant *et al.*, 2001; Wasfy *et al.*, 2000).

These organisms are readily transmitted via food, water, environmental contacts, pets and from person to person, with morbidity rates in developing countries 3-to-6-fold higher than in developed countries (Asrat 2008)

The predominant bacterial pathogen isolated in this study was *Shigella* (9.4%), followed by *Salmonella* (5.8%) and *Campylobacter* (4.8%) indicating that they remain significant threats to health in this region.

The prevalence of *Salmonella* in this study was 5.8% (Table 3.3). Which is in agreement with studies conducted in Ethiopia at different times, 5.8% (Andualem and Geyid, 2003), 4.9 % (Beyene and Haile-Amlak, 2004), and higher than (3.8%) (Asrat *et al.*, 1999) and (1%) (Aseffa *et al.*, 1997). which could be due to difference in study time, place and age group of the study subject.

The rate of isolation of *Shigella* spp. (9.4%) in this study was comparable with studies conducted in Ethiopia at different times, 11.7 % (Asrat *et al.*, 1999), 8.7% (Andualem *et al.*, 2006), and higher than (5.8%) and (7.5%) reported by (Andualem and Geyid, 2003) and (Tiruneh, 2009) respectively. This could be due to difference in study period and geographical location.

Common presenting features of shigellosis include diarrhoea that is bloody or watery, with or without mucus, fever, abdominal pain, and tenesmus. From these preliminary data, the most useful signs and symptoms for the diagnosis of shigellosis were the complaints of stool with blood and mixed (mucus and blood) and have also been reported in other studies (Taylor *et al.*, 1991; Stoll *et al.*, 1982; Brooks *et al.*, 2006).

The overall prevalence of *Campylobacter* in this study was 4.8%. This rate is comparable to the rate in developing countries ranges from 5-20 % (WHO, 2001). Isolation of *Campylobacter* in this study is low compared to other findings conducted in Ethiopia at different times 10.8% (Asrat *et al.*, 1997), 10.5 % (Mitikie *et al.*, 2000), 11.6 % (Beyene and Haile-Amlak, 2004) and 8 % (Ewnetu and Mihret, 2010). This could be explained by the fact that studies were conducted in different laboratory technique, age groups, study period, and other factors which were not studied/ considered in this study.

In this study fever, vomiting, abdominal pain and tenesmus were not associated with isolation of *Campylobacter* species. *Campylobacter* infection was clinically indistinguishable from diarrheal illnesses caused by other pathogens that are common in the same population. However, symptoms such as fever, bloody stool and, abdominal pain were reported as early symptoms due to *Campylobacter* enteritis (WHO, 2001).

In this study the infection rate was significantly higher in children below five years (9.9%) compared to (2.4%) in those above five years ($p < 0.05$). which is a similar finding to other studies in Ethiopia (Asrat *et al.*, 1999) and other developing countries such as Uganda (Mshana *et al.*, 2009), and Kenya (Brook *et al.*, 2006). This nature of Campylobacteriosis appears to be related to acquired immunity in developing nations due to the hyperendemic nature of the disease (Sack *et al.*, 2001). On the other hand in patients in industrialized countries, there is a bimodal age distribution, with one peak in children under the age of 1 year and the second peak in young adults between the ages of 15 and 34 usually experience inflammatory diarrhoea with severe abdominal cramping and fever (Blaser *et al.*, 1979). The substantial age-related difference in the infection-to-illness ratios in developed and developing countries appears primarily to be due to differences in age- or exposure-related immunity of the populations rather than to differences in the isolates. (Allos and Blaser, 2005)

In contrast to the studies conducted previously (Mitikie *et al.*, 2000; Beyene and Haile-Amlak, 2004), contact with pets like dog and cat was not statistically associated with culture positivity to *Campylobacter*. Though Contact with animals, particularly pet dogs and cats, has been repeatedly identified as a risk factor for *Campylobacter* infections (WHO, 2001). This result may reflect differences in geographical location, sample size and methodology.

The emergence and dissemination of antimicrobial resistance among *Shigella* spp. are increasing global health problems that complicate the therapeutic management of severe shigellosis case.

Antimicrobial therapy for shigellosis reduces the duration and severity of the disease and can also prevent potentially lethal complications. However, over the past few decades *Shigella* spp. have become resistant to most of the widely used antimicrobials (Tiruneh, 2009; Vrints *et al.*, 2009).

Antimicrobial resistance has complicated the treatment of shigellosis since the 1940s, when sulfa resistance among *Shigella* organisms was first recognized in Japan (Tauxe *et al.*, 1990). By 1967, due to high levels of resistance to sulfonamides, ampicillin became the drug of choice for treatment of shigellosis. However, by the early 1970s the prevalence of ampicillin resistance had markedly increased. When trimethoprim-sulfamethoxazole (TMP-SMZ) was first introduced, almost all enteric pathogens were susceptible, since 1980, however *Shigella* spp. have demonstrated a frequent and alarming resistance to TMP-SXT (WHO 2001, Murray, 1986).

In this study the *Shigella* isolates were more susceptible to kanamicin (97.3%), nalidixic acid (97.3%), ciprofloxacin (97.3%) and norfloxacin (97.3%) than to drugs commonly used to treat shigellosis such as ampicillin, TMP-SXT and chloramphenicol (Table 3.6).

As opposed to studies done in Addis Ababa, Ethiopia in the early 1980, that indicate majority of *Shigella* spp. were susceptible to TMP-SXT (100%) and ampicillin (79%) (Gedebu and Tassew, 1980). The frequency of sensitivity of *Shigella* strains in this study against TMP-SXT and ampicillin were 35.3%, 32.5% respectively; but comparable to recent studies that have been reported from different part of Ethiopia (Tiruneh, 2009; Asrat, 2008; Andualem *et al.*, 2006). The high prevalence of resistance to these drugs could be explained by the longtime use of this antibiotic to treat shigellosis, thereby ensuring selection pressure and maintenance of this resistance (Vrints *et al.*, 2009). Despite the high proportion of antimicrobial resistance observed among *Shigella* isolates, these organisms remain highly susceptible to quinolones (Na) and fluoroquinolones (ciprofloxacin and norfloxacin), which are now the drugs of choice in many areas. This study has revealed *Shigella* isolates were uniformly sensitive to quinolones and fluoroquinolones. These findings are in agreement with the previous data obtained from Ethiopia (Asrat 2008; Tiruneh 2009; Mache 2001)

Multiple antimicrobial resistances among strains of *Shigella*, including resistance to ampicillin and TMP-SMZ, have been reported from a number of different geographic locations (Bannish *et al.*, 1992). Earlier studies from Ethiopia have reported drug resistant to as many as six drugs

(Tiruneh, 2009; Mache, 2001). In this study multidrug resistance to two or more antibiotics was more commonly observed 23 (62.1%) than resistance to a single drug 5(13.5%).

Many socioeconomic and behavioral factors contribute to increasing antimicrobial resistance. Shigellosis is more prevalent in individuals of low socioeconomic status and education level in developing countries, where there are frequently inadequate treatment and poor compliance. In many developing countries antimicrobial agents are readily available without a prescription which promotes selection for resistance (WHO, 2001).

In the present investigation, susceptibilities of all isolated *Campylobacter* species to the antimicrobials tested were >80% except trimethoprim sulfamethoxazole and ampicillin (Table 3.5). Similar findings have been observed in a previous study conducted in Ethiopia where 80-100% of isolates from food, food animal and human were sensitive to these antimicrobial agents (Dadi and Asrat, 2008; Kassa *et al.*, 2007; Beyene and Hileamlak, 2006). This could be because of the fact that these antibiotics are prescribed less frequently in treating diarrheal cases either due to lesser availability or cost. On the other hand 42.1% of the isolates were resistant to ampicillin while the 52.3% were resistant to trimethoprim-sulfamethoxazole. As is indicated in another similar study, this could be either because they are commonly prescribed or are sold on the open market and private pharmacies without prescription (Beyene and Hileamlak, 2006)

Limitation of the study

This study was conducted among patients presenting with acute diarrhea visiting the Yekatit 12 hospital and Shiromeda health center. Thus, the result may not represent the whole population in the community.

It was not possible to identify up to species level due to budget constraints and laboratory facilities.

The study does not include Yekatit 12 Hospital Pediatric unit due to renovation of pediatric unit building in which high diarrheal infections are suspected.

Fastidious nature of *Campylobacter* spp. may have somewhat underestimated the true burden of disease among persons captured in this study.

Chapter V: Conclusion and Recommendations

In conclusion, the results of the present study showed that high prevalence for *Shigella* spp. while *Campylobacter* spp. shows a moderate one. Patients with diarrhea who were infected with *Campylobacter* spp. were younger than those infected with *Shigella*. Nalidixic acid, norfloxacin, kanamycin, ciprofloxacin, and polymyxin B showed low resistance against *Shigella* spp. The majority of *Campylobacter* spp. were sensitive to nalidixic acid, ciprofloxacin and erythromycin. Resistance to one or more drugs in *Shigella* isolates was observed for majority of the isolates. The frequency of resistance for ampicillin, trimethoprim-sulphamethoxazole, and chloramphenicol is the highest patterns of resistance.

It could be concluded from this and previous studies in Ethiopia that the recent isolates of *Shigella* isolate examined tend to be more resistant to commonly used antimicrobial agent than earlier one.

Based on these findings the following recommendations are made: -

There is a need of continuous surveillance of the prevalence and antibiotic susceptibility pattern of diarrheal bacterial isolates in hospitals and in the community which should be the basis for empiric therapy.

Campylobacter species is an important enteropathogen prevalent in children younger than 5 years of age, and therefore every pediatric faecal sample examined for enteric pathogens, such as *Salmonella* and *Shigella*, should also be examined for *Campylobacter* at least at the hospital level.

Bacteriological laboratory should be strengthened to be able to identify *Shigella* to the level of serotype.

Concentrating on antimicrobial therapy does not seem to be a solution to the control of diarrhea as there are strong resistances among prevalent pathogens to the antimicrobials most frequently prescribed for diarrhea in the community, and many persons receive inappropriate medications. Improving hygiene, providing safe potable water and intensive health education, are public health priorities that, together with more judicious use of antimicrobials, could preserve antimicrobial efficacy and substantially reduce diarrheal illness.

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Appendix I

Questionnaire

Questionnaire for Isolation and antibiotic susceptibility of, *Shigella*, and *Campylobacter* from acute enteric infection.

I. PATIENT IDENTIFICATION

Name _____ Sex _____ Age _____ Card no. _____

Date _____

Site/place of collection _____

Woreda _____ Kebele _____

II. CLINICAL DATA

1. Onset of diarrhoea _____

2. Frequency of defecation in a day _____

3. Antibiotics taken before (during) the last 5 days _____

4. Types of diarrhoea.

€ Loose

€ Watery

€ Mucoid

€ bloody

€ mucoid + bloody

€ Other

5. Symptoms

€ Vomiting-----

€ Nausea -----

€ Headache -----

€ Fever-----.

€ Abdominal pains -----.

€ Other symptoms -----

6. Oral rehydration taken during last few days (ORS) -----

7. Are there domestic animals in your household? _ yes €/ no €

8. If yes, circle them and mention the numbers.

€ Cattle

€ Sheep/ Goats

€ Dog

€ Cat

€ Chickens

€ Others (please specify)_____

9. Have you use to consume raw milk? Yes €/No€, Raw meat? Yes €/No€

10. Do you have latrine? Yes€/ No€

Appendix II

Culture and identification

Suspected growth of *Campylobacter* spp. from the stool on *Campylobacter* agar

Positive/present € Negative/absent €

Identification steps for suspected colonies

€ Gram stain result _____

€ Catalase test positive€/negative€

€ Oxidase test positive€/negative€

Antibiotics susceptibility result for *Campylobacter* species

Antimicrobial	Amp	Chl	Na	Sxt	Cip	Ery
S						
I						
R						

Non lactose fermenter colony from S-S/MacConkey agar

Lactose fermenter€non lactose fermenter€

Biochemical	Urea	Man	Ox	Cit	Mot	Ind	LDC	KIA medium			
								Slope	Butt	H ₂ S	Gas
<i>Shigella</i> spp.	–	D	–	–	–	d	–	R	Y	–	– ¹
<i>E. coli</i>	–	+	–	–	+ ²	+ ³	+	Y ⁴	Y	–	+ ³
<i>Salmonella</i> Typhi	–	+	–	–	+	–	+	R	Y	+ weak	–
<i>Salmonella</i> Paratyphi	–	+	–	–	+	–	–	R	Y	–	+
Most other salmonellae	–	+	–	+	+	–	+	R	Y	+ ³	d

Key: LDC = Lysine decarboxylase, **Man** = Mannitol (mannite), **Ox** = Oxidase test, **Cit** = Citrate test, **Mot** = Motility, **Ind** = Indole test, **Urea** = Urease, **H₂S** = Hydrogen sulphide (blackening), **R** = Red-pink (alkaline reaction), **Y** = Yellow (acid reaction), **d** = different strains gave different results.

Notes

1. A minority of strain give a positive result.
2. A few strain are non motile
3. A minority of strain give a negative result
4. A few strain give reaction similar to *Shigella* spp.

Indole Test: - The indole test is used to identify bacteria capable of producing indole using the enzyme tryptophanase. The enzyme tryptophanase can convert the amino acid, tryptophan, to indole, ammonia, and pyruvic acid. The by-product, indole, is the metabolite identified by this test. When Kovac's reagent, which contains hydrochloric acid and dimethylaminobenzaldehyde and amyl alcohol, a red layer form when indole is present. No color in this layer is a negative result.

Kligler Iron slant agar: - both the butt and the slant were streaked, to determine fermentation of glucose, lactose and to see the production of hydrogen sulfide. *Shigella* and *salmonella* species characteristically produce an alkaline (red) slant and an acid (yellow) butt, little or no gas, and with no or with Hydrogen sulfide, respectively.

Urea slant agar: - The test organism was cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

Motility test: - To determine whether the organism is motile or not it was stabbed with a straight inoculating needle, making a single stab about 1–2 cm down into the medium. Motility is indicated by the presence of diffuse growth (appearing as clouding of the medium) away from the line of inoculation.

Simmons citrate agar: - first the slope was streaked with a saline suspension of the test organism and then stab the butt was streaked to check utilization of citrate as a sole carbon source (Cheesbrough, 2006).

Antibiotics susceptibility result for *Shigella* species

Antimicrobial	(Amp)	(Chl)	(Na)	(Nor)	(Pol B)	(Kan)	(Cip)	(Sxt)
S								
I								
R								

Appendix III

Patient consent form (To be translated in to the patient's language)

Serial no.....

Card no.....

The objective of this study is to isolate the *Campylobacter* and *Shigella* species and pattern of antimicrobial resistance from acute enteric infections. Because the type of organisms and pattern of antimicrobial resistance in enteric infections are different, the results of this study are believed to be important to treat patients appropriately. Therefore, I am requesting you to participate in the study, which would require your response to an interview, and to provide samples (stool) for laboratory examination. Results was reported to the requesting physician for appropriate treatment and management.

Adults

I_____ here by give my consent for giving of the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Children

I_____ parent/guardian here by give my consent for giving of the requested information and specimens from my child as the doctors find best for her/him.

Signature: _____ Date_____

DECLARATION

I the undersigned assure that this is my original work. It has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

Principal Investigator

- Name: Getnet Worku Mengistu (BSc.)
- Signature:_____
- Date of submission_____

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