HE ISLAMIC UNIVERSITY - GAZA

Biological Sciences Master Program

Bacterial and Parasitic Etiologic Agents among Acute Gastroenteritis Patients in Gaza strip, Palestine.

Submitted in partial fulfillment for the degree of Master of Science in Biological Sciences- Medical Technology

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Abstract

Enteric illnesses are common and costly problem that cause significant morbidity and mortality worldwide in children. In Palestine, diarrhea is one of the major causes of many outpatient visits and hospitalizations. These diseases account for approximately 5-10 million deaths each year in Asia, Africa and Latin America.

The identification and diagnosis of enteric pathogens in stool samples in Palestinian health laboratories is done only for *Salmonella* spp. and *Shigella* spp, through culture, biochemical and serological assays, while parasites are diagnosed by direct microscopic slide method. The other pathogens, however, are not routinely diagnosed.

In the present study conventional culture techniques will be used for analyzing 150 stool samples collected from patients suffering acute diarrhea for the presence of the common bacterial enteropathogens; *Salmonella spp., Shigella spp., Campylobacter spp., Yersinia enterocolitica* and to determine their susceptibility to antibiotics.

Moreover, the occurrence of parasites in these samples will be investigated by direct microscopic examination.

Keywords; Diarrhea, Gastroenteritis, Gaza Strip.

Chapter 1
Introduction

1.1 Introduction

Acute gastroenteritis or infectious diarrhea is one of the leading causes of illnesses and death in infants and children throughout the world, especially in developing countries. This is so in Asia, Africa and Latin America, where an estimated 2.5 million deaths occur each year in children less than 5 years of age **(1,2).** Diarrhea is also one of the leading causes of deaths among the population in Gaza strip **(3).**

Worldwide, the most common pathogens that cause acute gastroenteritis are: *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *E. coli O157:H7*, *Listeria monocytogenes, Vibrio cholera, Yersinia enterocolitica*, Rotavirus, *Cryptosporidium spp, Entamoeba histolytica,* and *Giardia lamblia.* These pathogens can cause potentially serious diseases which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks **(4).**

Only *Salmonella* spp. and *Shigella* spp, are routinely investigated through routine culture in Gaza strip. Other pathogens, however, are not routinely diagnosed **(5).**

This often causes misdiagnosis and other pathogens are overlooked by physician and thus epidemiological data are inaccurate.

Data from the health laboratories all over Gaza strip show that the detection rate of *Salmonella* spp. is very low (about 0.4% in the year 2004) and even lower for *Shigella* spp. (0.4,1.2 and 0.12%, in the 2002, 2003 and 2004 respectively).

Moreover, data concerning cases of Campylobacter, and Yersinia and their relation to infection in Palestinian children are extremely scarce **(5-7).**The objectives of this study is to perform a detailed microbiological investigation of some potential pathogens associated with diarrhea, to characterize the isolates, and the

epidemiological factors related to the diarrheal disease in patients from Gaza, Palestine.This method of investigation is a matched Case-control study with prospective data record.

1.2 Objectives:

General objective:

To determine the etiologic agents (bacteria and parasites) of diarrhea among diarrheal patients in Gaza strip, Palestine.

Specific Objective:

1. To detect and identify enteric bacterial pathogens in fecal samples from diarreal patients.
2. To assess the antibiotic susceptibility of isolated bacteria.
3. To determine the prevalence of common parasites causing diarrhea.
4. To investigate possible risk factors for contracting diarrhea.

1.3 Significance:

Poor sanitation and restriction to water access may favour the spread of communicable diseases, especially infectious diarrhea, which is one the leading cause of morbidity in the Gaza strip and is one of the more frequent reasons for primary health care center (PHCC) attendance in addition to respiratory tract infections. Infectious diarrhea affects mainly the children who are at risk of complications, especially when they suffer from malnutrition, which is common in Palestinian children. In 2007, in a local study they found a rate of 17.3% of bacteriological diarrhea in 150 children ≤ 5 years old, using a PCR technique (*Shigella*: 6%; *Campylobacter*: 4.7%; *E coli 0157:H7*: 4.7%, *Salmonella*: 2%) **(8).**

Moreover, uncontrolled use of antibiotics due to the lack of antibiotic prescribing policy in diarrhea but also for any kind of suspected infection has lead to the emergence of bacterial resistance which has become a major concern in Gaza **(9-11)**.

Chapter 2
Literature review

Gastroenteritis is strictly inflammation of the stomach and intestine. The term is used to describe a variety of infections that cause symptoms of vomiting and/or diarrhea.

Many of these infections are transmitted by food or water, but some can also spread directly or indirectly via person-to-person transmission **(12).**

Two hundred fifty children presenting with diarrhea at 2 teaching hospitals in Mosul, Iraq over a 9-month period were studied for the presence of *Yersinia* spp. in stools by cold-enrichment culture at 4 °C for 21 days. *Yersinia* spp. were isolated from the stools of only 4 patients; 3 isolates were identified as *Y. enterocolitica* and 1 was *Y. pseudotuberculosis*. The blood isolated *Yersinia* culture was also positive for *Y. enterocolitica* in 1 case. The antibiogram test for the isolates was determined. Cross-reaction between *Y. pseudotuberculosis* and *Salmonella typhi* or *S. paratyphi* B, and between *Y. enterocolitica* and *Brucella* was detected serologically **(13).**

The frequency of isolation was 1.8% (65/3570) for *Salmonella* spp. and 0.8% (28/3570) for *Shigella* spp. *Shigella flexneri* (16/28) was the most frequently isolated *Shigella* species. Most of the *Shigella* isolates were resistant to trimethoprim-sulfamethoxazole (89%), ampicillin (79%) and chloramphenicol (46%) and most of the *Salmonella* isolates showed resistance to ampicillin (62%), trimethoprim-sulfamethoxazole (35%), chloramphenicol (35%) and cephalexin (26%) **(14).**

In an Egyptian study on 714 patients with diarrhea, they found that 14% were *ETEC*-associated diarrhea, 1.0% *Campylobacter*-associated diarrhea and *Shigella*-associated diarrhea represented 2%. Children with Shigella- or *Campylobacter*-associated diarrhea were reported as watery diarrhea and rarely dysentery. ETEC did not have any clinically distinct characteristics **(15).**

A group of researcher in Denmark studied foodborne bacterial infection and hospitalization among 52,121 patients. They found that (14.4%) were hospitalized

with a diagnosis of gastroenteritis. A total of (17.7%) of the hospitalized patients with infections due to *S. enterica* and (10.8%) of the infections were due to *Campylobacter* species **(16).**

The epidemiological and microbiological aspects of acute bacterial diarrhea in 260 positive stool cultures of children between 0 and 15 years of age during two years were studied. *Shigella* was the commonest pathogen, being found in 141 (54.3%) of the cultures, while *Salmonella* was found in 100 (38.4%) of the cultures and enteropathogenic *E. coli* in 19 (7.3%). *Shigella* specimens presented a very high resistance rate to trimethoprim-sulfamethoxazole (90.1%) and to ampicillin (22.0%), while *Salmonella* presented very low resistance rates to all drugs tested **(17).**

In a study in Jordan investigated the polymicrobial infections in 220 children with diarrhea in a rural population. Potential pathogenic agents isolated from 143 (65%) children were identified by molecular and standard microbiological methods. Co-infections with two or more agents were detected in 50 (35%) cases. *Escherichia coli*, *Shigella* spp, *Giardia* and *Entamoeba histolytica* were found to be predominant **(18).**

Another study in Jordan investigated the enteropathogens associated with cases of gastroenteritis in a rural population in 180 children. Pathogens and potential enteropathogens were identified in 140 (77.8%) of the patients, with more than one pathogen being recovered from 67 (37.2%) of the patients. Potentially pathogenic parasites were observed in 90 (50%) patients; those that were associated significantly with diarrhea were *Giardia lamblia, Blastocystis hominis, Cryptosporidium spp., Entamoeba histolytica* and *Cyclospora cayetanensis*. Pathogenic bacteria were isolated from 72 (40%) patients, and, of these, 62.5% were resistant to at least one antibiotic, and 30.6% of these were multidrug resistant. Diarrheagenic *Escherichia coli* strains were found in 14.3% of the specimens. The most common enteropathogenic bacteria found were *Shigella spp., Campylobacter jejuni* and *Yersinia enterocolitica* **(19).**

In Brazil the prevalence of enteropathogens associated with diarrheal disease in 94 infants < 5 years old was investigated. *Cryptosporidium* (85.1%) topped the list of parasite isolates, followed by *Entamoeba histolytica* (56.4%) and *Giardia intestinalis*

(4.3%). Four samples contained enteropathogenic *Escherichia coli* (4.3%). *Samonella* and *Shiguella,* however*,* were not detected, and only one sample contained rotavirus (1.1%) **(20).**

In Sao Paulo (Brazil) the etiologic profile of acute diarrhea in 154 children aging less than 5 years was studied. Intestinal pathogens were detected in 112 (72.8%) cases. The association of two or more intestinal pathogens occurred in 47 (30.5%) cases. The pathogens identified were, rotavirus: 32 (20.8%), bacteria: 53 (34.4%), both: 25 (16.2%), and 2 (1.4%) with *Giardia intestinalis* (in one case associated with rotavirus and in another one associated with bacteria). Altogether, there were 105 bacterial isolates; 90 were *Escherichia coli* (EPEC 27, DAEC 24, ETEC 21 and EAEC 18), 12 were *Shigella* sp, 2 were *Salmonella* sp, and one was *Yersinia* sp. Children with mixed infections (viral and bacterial) had increased incidence of severe vomiting, dehydration and hospitalization **(21).**

The trend in isolation of *Vibrio cholerae, Shigella,* and *Salmonella* in neonates with diarrhea in Bangladesh was investigated. The study population included 240 neonates who were admitted with acute diarrhea and other medical complications to the inpatient department of ICDDR hospital, Dhaka, Bangladesh, in 2001. A single enteric pathogen was detected in 71 (29.5%), and multiple pathogens were detected in 12 (5%) of the neonates. Enteropathogens identified were as follows: *V. cholerae* O1 (17.5%), *Shigella* spp*.* (9.1%), *Salmonella* spp*.* (3.3%), *Aeromonas* spp*.* (3.7%), and *Hafnia alvei in* (0.8%) of the neonates **(22).**

A group of researcher in Gaborone, Botswana investigated the *Shigella* and *Salmonella* strains isolated from 221 children under 5 years, and their antibiotic susceptibility patterns. They isolated *Shigella* from (21%) and *Salmonella* (3%). *S. boydii* (13%) was the most prevalent *Shigella* species followed by *S. flexneri* (6%) and *S. sonnei* (2%). *Salmonella* species were *S. typhimurium* and *S. paratyphi*. Antibiograms of the predominant isolates showed that most *Shigella* species were resistant to ampicillin but susceptible to chloramphenicol. The *Salmonella* species were susceptible to chloramphenicol, collistin-sulphate, gentamicin, cotrimoxazole, and ampicillin **(23).**

In a study in Jeddah, Saudi Arabia, investigating the prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhea in Jeddah, Saudi Arabia, in 576 fecal samples collected from children <5 years old suffering from acute diarrhea and attending hospitals and outpatient clinics. One or more enteropathogens were identified in 45.6% of the stool specimens. Mixed infections were detected in 12.2% of the diarrheal cases. Rotavirus was detected in 34.6% of the specimens of the hospitalized patients and in 5.9% of the specimens of the outpatients. Other etiologic agents recognized included *Escherichia coli* (13%), of which 3.8% were enteropathogenic *E. coli (EPEC)* and 1.9% enterohaemorrhagic *E. coli*. Other detected pathogens were: *Klebsiella pneumoniae* (4%), *Giardia intestinalis* (3.1%), *Salmonella* sp. (3%), *Shigella flexneri* (2.6%), *Entamoeba histolytica* (2.2%), *Trichuris trichiura, Hymenolepis nana*, and *Ascaris lumbricoides* (0.7% each), and *Candida albican*s (0.5%) **(24).**

The prevalence of enteropathogens associated with diarrheal disease in 130 infants living in the poor urban areas of Porto Velho, Brazil, was investigated. 80% of diarrheal cases were observed in the groups under 2 years of age. Rotavirus (19.2%) was the most frequent enteropathogen associated with diarrhea, followed by *Shigella flexneri* (6.15%) and *S. sonnei* (1.5%) and *Salmonella* sp. (6.9%). Enterotoxigenic *E. coli* infections (3.1%), enteropathogenic *E. coli* (2.3%), enteroinvasive *E. coli* (0.8%) and *Yersinia enterocolitica* (0.8%). Mixed infections were frequent, associating rotavirus, *EPEC* and *Salmonella* sp. with *Entamoeba histolytica* and *Giardia intestinalis* **(25).**

The incidence of enteric pathogens in 265 children with gastroenteritis by PCR & conventional methods in Jordan was investigated; they detected enteropathogens in 66.4% of the examined patients. A single enteric pathogen was detected in 50.9% of the children, and multiple pathogens were detected in 15.5%. The prevalence of enteropathogens identified was as follows: rotavirus (32.5%), enteropathogenic *Escherichia coli* (12.8%), enteroaggregative *E. coli* (10.2%), enterotoxigenic *E. coli* (5.7%), *Shigella* spp. (4.9%), *Entamoeba histolytica* (4.9%), *Salmonella* spp. (4.5%), *Campylobacter jejuni/coli* (1.5%), *Cryptosporidium* spp. (1.5%), enteroinvasive *E. coli* (1.5%), *Giardia intestinalis* (0.8%) and *Yersinia enterocolitica* (0.4%). No *Vibrio*

cholerae, Shiga toxin-producing *E. coli*, microsporidia, adenovirus or small round viruses were detected **(26).**

In a local study investigating the intestinal parasites and diarrhea in children, the intestinal parasites were found to be prevalent in Gaza, with an overall prevalence of 24.5%. *Giardia intestinalis* (62.2%) was the most common parasite, followed by *Ascaris lumbricoides* (20.0%), then *Entamoeba histolytica* (18.0%) **(27).**

In the USA the regional variation in the incidence of laboratory-confirmed bacterial food borne illnesses was studied. 12,125 cases were identified. The incidence per 100,000 population was highest for Campylobacter (15.7%), followed by Salmonella (14.4%), and *Shigella* (7.9%). Lower incidences were reported for *E. coli* O157 (2.1%), Yersinia (0.4%), Listeria (0.3%) and Vibrio (0.2%). The incidence of *Campylobacter* and *Salmonella* among infants proved particularly high, although substantial regional variations were observed **(28).**

In Bangladesh, the enteropathogens associated with childhood diarrhea, in 814 cases of diarrhea were investigated. A potential enteric pathogen was isolated from 74.8% of diarrheal children. The study identified these pathogens as being significantly associated with diarrhea; the study identified the enteropathogenic *E. coli*, *Aeromonas* spp., *V. cholerae* O139, enterotoxigenic *Bacteroides fragilis*, *Clostridium difficile*, and *Cryptosporidium parvum*, as being significantly associated with diarrhea. *Plesiomonas shigelloides*, *Salmonella* spp., diffusely adherent *E. coli*, enteroaggregative *E. coli*, *Entamoeba histolytica,* and *Giardia lamblia* were not significantly associated with diarrhea. The major burden of diseases due to most pathogens occurred in the first year of life, and infections with multiple pathogens were common **(29).**

In Japan the bacteriological and virological etiologies of sporadic acute gastroenteritis in 1,564 samples were studied, 722 (46.2%) were enteropathogen positive cases, and mixed infection was observed in about 15% of the positive cases. Among 13 different kinds of enteropathogens identified, the most prevalent one was pathogenic *E. coli* (20.7%), followed by *Campylobacter* spp. (10.0%), rotavirus (8.8%), *Salmonella* spp. (3.9%), adenovirus (1.9%), ECHO virus (0.9%), *Vibrio*

parahaemolyticus (0.8%), poliovirus (0.7%), *Aeromonas* spp. and *Coxsackie B virus* (both 0.6%). In addition, *Shigella sonnei* (3 cases), *S. paratyphi-A* (1 case) and enterohemorrhagic *E. coli O157: H7* (2 cases) were also detected. A higher detection ratio was recorded in February, August and November, reflecting respectively by month a higher frequency of Rotavirus and food-poisoning causing enteric bacteria **(30).**

In Switzerland *a* total of 13,965 specimens from 7,124 patients (1.96 specimens per patient) were cultured, yielding 369 (2.6%) *Salmonella* spp., 408 (2.9%) *Campylobacter* spp., and 79 (0.6%) *Shigella* spp. The cumulative positivity rate of 6.1% decreased to 2.7% when patients received antimicrobial agents. The positivity rate for 5,912 specimens obtained from patients hospitalized for <3 days was 12.6%, whereas it dropped to 1.4% for patients hospitalized for >3 days. Of 3,837 stool samples originating from pediatric patients, 8.8% were positive, and 5.1% of 10,128 samples from adults were positive. Rotaviruses were detected in 190 of 1,601 (11.9%) samples **(31).**

A study in Jordan carried out to determine the bacterial and parasitic causes of acute diarrhea in Northern Jordan in 200 patients. One or more bacterial or parasitic enteropathogens were isolated from 79 patients (39.5%). Prevalence rates for these pathogens were as follows: *enterotoxigenic Escherichia coli*, 9%; enteropathogenic *E. coli*, 9%; *Salmonella* spp. 7%; *Campylobacter* spp, 5.5%; *Yersinia enterocolitica*, 4.5%; *Shigella* spp, 4%; *Aeromonas* spp, 3.5%; enterotoxigenic *Clostridium perfringens*, 2%; *Vibrio* spp, 2%; and *Plesiomonas shigelloides*, 0.5%. Both *Giardia intestinalis* and *Entamoeba histolytica* were detected in 2% of the stool samples examined **(32).**

Chapter 3

Proposed Methodology

3.1Study area

The study will be done in Primary Health Care Clinics at Ministry of Health (MOH) in Gaza Strip. The study will be conducted from Jan 2010 to Sep 2010.

Patients are recruited in 6 primary health care centres in Gaza strip located in Gaza area, northern area, middle area and Khan Yunis area (table 1) from January 2010 to March 2010.

Table 1: PHCC participants

Name	**location**
Jabalia Martyrs	Northern area
Al Zaitoun	Gaza area
Sabha	Gaza area
Der Al Balah Martyrs	Middle area
Al Nusairat Martyrs	Middle area
Bander Khan Yunis	Khan Yunis area

3.2 Samples

A total of 150 stool samples will be collected from patients with acute diarrhea (gastroenteritis). Fresh passed stool samples without any preservatives will be collected in two containers one for culture, and the other for wet mount of parasites.

Cases are patients attending the primary health care centre for diarrhea (defined as the passage of 3 or more loose or liquid stools per day). The age categories of patients are <5; 5-9; 10-14; 15-20; >20 years old).

3.3 Sample transport

All stool samples will be transported to the laboratory in the sterile container, and transported in an ice box immediately after collection. Sample will be completely labeled by the necessary data (date, time of collection, sample type etc....,).

3.4 Detection of pathogenic enteric bacteria in stool by routine culture:

Salmonella and Shigella: All stool samples will be cultured onto *Salmonella-Shigella* agar, XLD agar, Hektoen enteric agar media, *Salmonella* enrichment in (SF) Selenite F broth.

Campylobacter: All stool samples will be cultured onto charcoal cefoperazone deoxycholate agar CCDA media in microaerophilic condition at 42 ^{0}C for 48 hour.

***Y. enterocolitica*:** All stool samples will be cultured on Cefsulodin-irgasan novobiocin (CIN media) at 28 ^{0}C.

Parasites: are diagnosed by wet mount slide method.

3.5 Antimicrobial surveillance for the bacterial isolates

Antimicrobial susceptibility testing (AST) will be performed by growing the isolates in the presence of a given antibiotic. Disk diffusion method for routine susceptibility testing of bacterial isolates will be used. Antibiotic-impregnated paper disks are placed on the surface of an agar plate which has been seeded with the isolate being tested. If the organism is susceptible to the antibiotic tested its growth will be inhibited and a zone of inhibition will result around the antibiotic disk **(33).** The diameter of the zone of inhibition of growth is proportional to the MIC value. The zone size is measured (in mm), the value is compared to the interpretive criteria developed by the manufacturer. The isolate is assigned a sensitive, intermediate or resistant category for each antibiotic after comparison with the appropriate manufacturer table.

3.6 Isolation Procedures of enteropathognic bacteria

Salmonella

Stool samples will be cultured onto XLD agar, *Salmonella Shigella* (SS), Hektoen enteric agar . Approximately 1 g of each sample will be inoculated into 10 ml of Selenite F broth. The tubes and plates will be incubated at 37 ºC for 18 to 24 hr. Selective Selenite F broth will be subcultured onto XLD agar, *Salmonella Shigella*

(SS), and Hektoen enteric agar then the plates will be incubated at 37 °C for 18 to 24 hr. The suspected colonies will be identified by colony morphological and biochemical characteristics. *Salmonella* species appears on SS, XLD and Hektoen enteric agar as colorless colonies with black center owing to H_2S production. Standard biochemical tests like API20E system and specific anti sera will be used for confirmation.

Shigella

Stool samples will be cultured onto XLD agar, *Salmonella Shigella* (SS), Hektoen enteric agar. Approximately 1 g of each sample will be inoculated into 10 ml of Selenite F broth. The tubes and plates will be incubated at 37 ºC for 18 to 24 hr. Selective Selenite F broth will be subcultured onto XLD agar, *Salmonella Shigella* (SS), and Hektoen enteric agar then the plates were incubated at 37 °C for 18 to 24 hr. The suspected colonies will be identified by colony morphological and biochemical characteristics. The suspected colonies will be identified on SS agar *as* colorless while on XLD and Hektoen enteric agar the colonies appeared as transparent red, Also the biochemical test will be carried out by API 20E system and specific Antisera.

Campylobacter

The stool samples will be cultured onto charcoal cefoperazone deoxycholate agar CCDA (*Campylobacter* selective media). The alkaline peptone water will be used as enrichment media in which samples will be incubated at 42 ºC for 24 hr then inoculated onto CCDA. The plates will be incubated at 42 ºC for 48 hr in microaerophilic conditions of growth with Gas pack system (5% O_2, 10% CO_2 and 85%N_2)**.** *Campylobacter jejuni* colony morphology may appear as small, mucoid, grayish, flat colonies with irregular edges and no hemolytic patterns after 24-48 hr. They may also appear as round, convex, entire, glistening colonies 1-2mm in diameter. Certain strains of *C. jejuni* may appear lightly pink or tan in color. The suspected colony of Campylobacter will be identified by biochemical tests such as oxidase, catalase tests and API campy system.

Yersinia enterocolitica

For isolation of Yersinia, stool samples will be cultured onto selective media CIN agar which will be incubated at 28 ºC for 48 hr. The cold enrichment method will be used in which samples will be incubated at 4 ºC for 3 weeks with phosphate buffer saline then inoculated onto selective media CIN and MacConkey agar which are incubated at 28ºC and 37 ºC for 48 hr. The suspected colony growth; red center, transparent borderlines will be identified by biochemical testes (API20E system).

3.7 Direct method for identification of parasites:

Saline and iodine wet mount for direct detection of parasites, presence of RBCs, and the presence of WBCs.

3.8 Identification of Enteropathogenic bacteria

Biochemical tests

Oxidase test reagent.

Kovacs reagent: 1% Tetramethyl-p-phenylenediamine dehydrochloride.

Gram's stain reagents

Crystal violet, Grams Iodine, Ethyl alcohol 95% v/v, Safranin and Distilled water .

API 20 E test: (BioMerieux France)

The analytical profile index (API) 20E strips (BioMerieux) will be used as biochemical system for identification of gram-negative rod bacteria. The API 20E strip consists of 20 micro tubes containing dehydrated substrates. These strips will be inoculated with bacterial suspension, which reconstitutes the media. The strip were incubated for 18 to 24 hours at 37ºC during incubation, metabolism produces changes that are either spontaneous or revealed by the addition of reagents. The standard will be scored according to reading table and the identification will be obtained by referring to the API20E catalogue.

API campy test:

The API Campy strip consists of 20 microtubes containing dehydrated substrates. It is made up of two parts. The first part of the strip (enzymatic and conventional tests)

is inoculated with a dense suspension which rehydrates the substrates. During incubation (in aerobic conditions), metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The second part of the strip (assimilation or inhibition tests) is inoculated with a minimal medium and incubated in microaerophilic conditions. The bacteria grow if they are capable of utilizing the corresponding substrate or if they are resistant to the antibiotic tested.

The reactions are read according to the Reading table and the identification is obtained by consulting the profile list in the package insert or the Identification table if necessary, or using the identification software.

Specific Antisera

Confirmation of the results will be done for salmonella, shigella by specific antisera.

3.9 Questionnaire

The questionnaire will be used in this study include questions about age, sex, domestic animals in the house, sources of drinking water, symptoms, including the presence of diarrhea….etc (see annex). Data will be collected by interviewing patients or patient's guardians.

3.10 Permissions and ethical consideration

Ethical approval will be taken from the concerned authorities for sample collection, including Helsinki committee, Doctors of the World (MdM for Médecins du Monde) which will help me to collect data and achieve the thesis. Informed consent will be signed by the participating patient's guardians.

3.11 Data analysis

Data will be collected, summarized, tabulated and analyzed using Statistical Package for Social Sciences (SPSS) software. The results will be presented through histograms, tables, Chi-square test and pie charts.

3.12 Timeline

Research activities schedule:

Activity	Jan 2010	Jan- AUG 2010	SEP 2010	OCT 2010	NOV 2010	Dec 2010
Literature survey and obtaining permission	****					
Field work and lab analysis		**********				
Data processing and analysis					******	
Evaluation of results					******	
Writing the thesis						******

3.13 Estimated budget:

Item	Estimated cost	Quantity	Total
Media	50$	8	400$
Antisera	100$	2	200$
Biochemical tests	2$	250	500$
Supplements	30$	30	600$
Antibiotics	10$	30	300$
Thesis preparation			400$
TOTAL			**2400$**

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Questionnaire

PHCC	Name	Code	

	CASE	CONTROL
Diarrhea	❑ yes	❑ no diarrhea within the 3 last months or birth
File number		
Address		
Telephone No.		
Date of Birth Age category	_____/_____/_____ ❑ <5 ❑ 5-9 ❑ 10-14 ❑ 15-20 ❑ >20	_____/_____/_____ ❑ <5 ❑ 5-9 ❑ 10-14 ❑ 15-20 ❑ >20
Gender	❑ male ❑ female	❑ male ❑ female
Refugee status	❑ no ❑ yes	❑ no ❑ yes
No. of household member	Total: /_____/ No. <5 years old: /_____/	Total: /_____/ N. <5 years old: /_____/
No. of rooms in the house	/_____/	/_____/
No. of household workers	❑ none ❑ ≥1 If ≥1: **1** ❑ formal employment ❑ private employee ❑ public employee ❑ both ❑ Informal sector **2** ❑ formal employment ❑ private employee ❑ public employee ❑ both ❑ Informal sector **3** ❑ formal employment ❑ private employee ❑ public employee ❑ both ❑ Informal sector	❑ none ❑ ≥1 If ≥1: **1** ❑ formal employment ❑ private employee ❑ public employee ❑ both ❑ Informal sector **2** ❑ formal employment ❑ private employee ❑ public employee ❑ both ❑ Informal sector **3** ❑ formal employment ❑ private employee ❑ public employee ❑ both ❑ Informal sector

Domestic animals in the house		
Poultry	❑ no ❑ yes	❑ no ❑ yes
Rabbit	❑ no ❑ yes	❑ no ❑ yes
Sheep	❑ no ❑ yes	❑ no ❑ yes
Pigeon	❑ no ❑ yes	❑ no ❑ yes
Donkey	❑ no ❑ yes	❑ no ❑ yes
Kitchen equipment		
Fridge	❑ no ❑ yes	❑ no ❑ yes
Washing machine	❑ no ❑ yes	❑ no ❑ yes
Cooker	❑ no ❑ yes	❑ no ❑ yes
Latrine or WC in the house	❑ no ❑ yes	❑ no ❑ yes
Current water access		
Public water access at home	❑ no ❑ yes	❑ no ❑ yes
Water from private provider	❑ no ❑ yes	❑ no ❑ yes
Roof Jerrican	❑ no ❑ yes	❑ no ❑ yes
Filtered water at home	❑ no ❑ yes	❑ no ❑ yes
Drinking water	❑ no ❑ yes	❑ no ❑ yes
Tap water	❑ no ❑ yes	❑ no ❑ yes
Filtered water	❑ no ❑ yes	❑ no ❑ yes
Boiled water	❑ no ❑ yes	❑ no ❑ yes
Bottle water	❑ no ❑ yes	❑ no ❑ yes
Well	❑ no ❑ yes	❑ no ❑ yes
Other	❑ no ❑ yes → /_____/	❑ no ❑ yes →/_____/
Did your water access change since January 2009? **Comments:**	❑ no ❑ yes If yes: ❑ improved ❑ worsened **If worsened, previous situation:** ❑ Public water access at home ❑ Water from private provider ❑ Roof Jerrican ❑ Filtered water at home	❑ no ❑ yes If yes: ❑ improved ❑ worsened **If worsened, previous situation:** ❑ Public water access at home ❑ Water from private provider ❑ Roof Jerrican ❑ Filtered water at home
To save water for your basic needs, do you	- buy water from private provider, ❑ never ❑ sometime ❑ always - reuse the same water for several tasks ❑ never ❑ sometime ❑ always - flush toilets less frequently	- buy water from private provider, ❑ never ❑ sometime ❑ always - reuse the same water for several tasks ❑ never ❑ sometime ❑ always - flush toilets less frequently

	❑ never ❑ sometime ❑ always - wash less regularly ❑ never ❑ sometime ❑ always - wash clothes and floors less regularly ❑ never ❑ sometime ❑ always	❑ never ❑ sometime ❑ always - wash less regularly ❑ never ❑ sometime ❑ always - wash clothes and floors less regularly ❑ never ❑ sometime ❑ always
Sewage disposal Barrel Socking pits sewers	❑ no ❑ yes ❑ no ❑ yes ❑ no ❑ yes ❑ no ❑ yes	❑ no ❑ yes ❑ no ❑ yes ❑ no ❑ yes ❑ no ❑ yes
Did your sewage disposal change since January 2009? **Comments:**	❑ no ❑ yes If yes: ❑ improved ❑ worsened **If worsened, previous situation:** ❑ Barrel ❑ Socking pits ❑ Sewers ❑ Other: /_____/	❑ no ❑ yes If yes: ❑ improved ❑ worsened **If worsened, previous situation:** ❑ Barrel ❑ Socking pits ❑ Sewers ❑ Other: /_____/
Sea swimming	❑ no ❑ yes If yes how many times the last month: /_____/	❑ no ❑ yes If yes how many times the last month: /_____/

Clinical data (for case only)	
Duration of diarrhea (days)	❑ < 24h ❑ other: /_____/ days
No. of stools the last 24h	/_____/ per day
No. of household members reported diarrhea within 10 days before patient's illness	/_____/
Received antibiotic within 4 weeks before the beginning of the diarrhea	❑ no ❑ yes → name of AB: /_____/
Chronic disease	❑ no ❑ yes →/_____/
Fever during diarrhea	❑ no ❑ yes
Temperature at attendance time (please check the temperature)	/_____/ °C
Chills during diarrhea	❑ no ❑ yes
Vomiting during diarrhea	❑ no ❑ yes
Bloody diarrhea	❑ no ❑ yes
Glairous diarrhea	❑ no ❑ yes
Lost of weight	❑ no ❑ yes

Management (for case only)	
Stool culture	❑ no ❑ yes
Antibiotic therapy for diarrhea	❑ no ❑ yes If yes: Name of antibiotic: /_____/ Duration of AB therapy: /_____/ days
Rehydration with ORS or equivalent	❑ no ❑ yes
Needs to be referred to the hospital	❑ no ❑ yes If yes → hospital: /_____/
Follow-up (1 week after time admission)	
Recovered	❑ no ❑ yes
Persistence of diarrhea	❑ no ❑ yes
Referred to hospital	❑ no ❑ yes
New attendance at the same PHCC	❑ no ❑ yes
Death due to diarrhea	❑ no ❑ yes
Lost to follow-up	❑ no ❑ yes
Stool culture and parasites results	
Positive culture	❑ no ❑ Not performed ❑ yes → Attach all the results to the data sheet If yes ❑ *Salmonella* ❑ *Shigella* ❑ *Yersinia enterocolytica* ❑ *Campylobacter* ❑ Giardiasis ❑ Amoebiasis ❑ Other: /_____/