**An-Najah National University**
**Faculty of Graduate Studies**

# Diarrheagenic *Escherichia coli* Prevalence and Related Factors Among Children, Jenin Governorate

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# Dedication

***To***

***My lovely family (parents, sisters and brothers) for their support and encouraging me all the way since the beginning of my study.***

***To***

***My husband for helping me to overcome all the difficulties.***

***To***

***My daughter Asma, with my great love.***

***To***

***My uncle Mostafa with my respect.***

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**الإقرار**

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

# Diarrheagenic *Escherichia coli* Prevalence and Related Factors Among Children, Jenin Governorate

# معدل انتشار بكتيريا الاشريكية القولونية والعوامل المرافقة لها في فئة الأطفال في محافظة جنين

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء مـــا تمـــت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

## Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student's name:** **اسم الطالبة:**

**Signature:** **التوقيع:**

**Date:** **التاريخ:**

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**Diarrheagenic *Escherichia coli* Prevalence and Related Factors Among Children, Jenin Governorate**

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## Abstract

Diarrheagenic *Escherichia coli* (DEC) is one of primary causative organisms of diarrhea. It's recognized to be the most common cause of endemic and epidemic diarrhea worldwide. This study aim to determine the prevalence of DEC infections and the risk factors included socio-demographic, environmental factors, behavioral habits and complaints related to *E. coli* among children less than 12 years old in Jenin governorate.

**Method**: Stool samples from 145 children with diarrhea or dysentery (symptomatic) and 170 samples from asymptomatic children (without diarrhea) of both sexes were collected randomly from Governmental health service centers from Jenin governorate. The samples were transported to laboratory within an hour after microscopic examination and inculated on MacConkey agar (MA) and Sorbitol MacConkey agar (SMA). *E coli* were identified by colony characteristic and standard biochemical tests. DEC groping were done by Multiplex PCR for the suspected *E. coli* colonies. Family of each child filled a questionnaire regards socio-demographic, environmental factors and behavioral habits of their child.

**Results:** Result showed a total prevalence of 14.3% (45/315) among study population. No significant difference in the prevalence of DEC between symptomatic 15.2 (22/145) and asymptomatic (control) 13.5 (23/170) groups. The most prevalent pathotypes was ETEC (10.2%) followed by EAEC (2.5%) and EHEC (1.5%). The majority of DEC infection isolates (20.1%) were detected in children less than two years old. This result related to beginning of environmental exposure and increased introduction of solid foods to children whose immune system is still developing within the first year of age.

Significant association was found between *DEC* prevalence and the contact with animals and drinking un-boiled or unpasteurized milk. The water source was although associated with the infection, the use of municipal water or boiling water showed a significant reduction prevalence of infection.

In addition, our results provide evidence that washing child hands or vegetables before eating, helping mother for child and use of toilet tissues at the bathroom played an important role in decrease rate of infection.

**Conclusion and Recommendation**: The prevalence rate of DEC infection in Jenin area was 14.3%. However this ratio can also be reduced by different way depended on the factors studied in this study. The majority of DEC infection isolates (20.1%) was detected in children less than two years old and mostly found in both diarrheal and control patients without any significant difference.

Our society needs a further work to determine the prevalence of DEC among children in Palestine. In addition we can do courses education to educate mothers and children how to use proper hand-hygiene technique, drink pasteurized beverages, eat cooked meat avoid direct contact with animals and wash hands after handling raw meat.

# Chapter One

# Introduction and Literature Review

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# Introduction and Literature Review

## 1.1Background

Acute diarrhea is one of the main public health problems worldwide. It's one of the major causes of morbidity and mortality of all ages. It's rank to be the third and the sixth cause of morbidity and the mortality respectively, especially among infant and children leading to 2-2.5 million deaths and billion episodes annually [1, 2].

Diarrhea morbidity was reported to be associated with different factors included environmental, socio-demographic, hygienic and age (small age). This association has been reported by several previous studies that tackled the source of water and food as the most important environmental factors that were found to be associated with the diarrhea [3, 4]. Among the socio-demographic factor, place of residence, mother education and occupation, number of children <5 years and average monthly income were found to be the most common factors that lead to diarrhea, while type of bathroom and presence of toilet paper are the main hygienic factors [ 3, 4, 5]. Furthermore, children factors included age and sex were also reported to cause diarrheal infection [5, 6] .

Diarrhea is caused by different etiologic agents in both developed and developing countries [4]. These organisms included bacteria, viruses, and parasites (E*ntamoeba-hystolitica, Cryptosporidium* and *Giardia-lamblia)*. Most of diarrheal episodes that had been reported in developing countries were found to be associated with bacterial infections, while

rotavirus was reported as the most causes of diarrhea in developed countries [6].

The most predominant bacterial infections that cause diarrhea are *Salmonella spp, Shigellaspp, Campylobacter jejuni, Yersinia enterocolitica,* and Diarrheagenic *Escherichia coli* (DEC). DEC is recognized to be the most importance cause of diarrhea among children in the world and reported as one of the most common and important cause of endemic and epidemic diarrhea worldwide [7] .

### 1.2 Diarrheagenic *Escherichia coli* (DEC) infection

*Escherichia coli (E. coli*) belongs to the family *Enterobacteriaceae*, which contains mostly motile gram-negative bacilli and consider to be a major component of the normal intestinal flora of human and animals within hour of birth and is ubiquitous in the human environment, thereafter, *E. coli* and the host take mutual benefit from each other [8].However some of strains including *DEC* which are harmful and have been associated with a wide range of diseases including gastrointestinal infections which may developed to several types of diarrheal illnesses, urinary tract infection, sepsis and meningitis. These status can be result in cases of incapacitated or immunosuppressed host, or in case of fragile gastrointestinal barriers [7].

DEC have specialized fimbriae that allow them to bind to certain intestinal epithelial cells and to produce toxins that lead to severe food born disease [9]. Different mechanism of infection observed by different DEC strains on the basic of bacterial variant factors [7].

## 1.3 Modes of transmission of DEC infection

There are many ways associated with DEC infection, some of these are associated with surrounding persons and others with surrounding environment included food and water. Some modes of transmission can occur from person to person but is more frequent due to animal defecation or contamination with untreated human sewage, irrigation land with fecally-contaminated water, in cooked or uncooked foods prepared by infected food handlers, in water contamination with human sewage or animal wastes, contaminated swimming pool and unpasteurized milk or juice [10, 11].

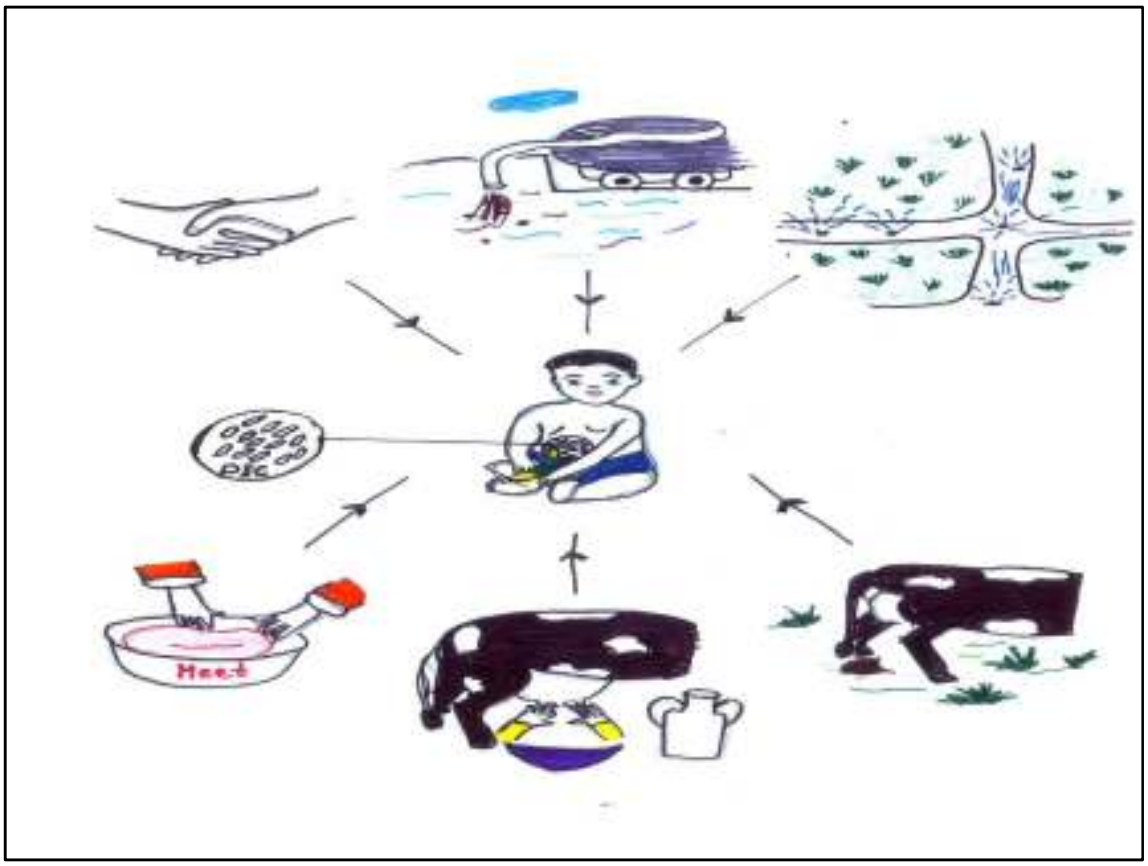


**Fig (1): Modes of transmission of DEC.**

## 1.4 Pathogenesis of DEC infection

The strategy of DEC infection include colonization of mucosal site, evasion of host defenses , multiplication and host damage.

When *E. coli* strains bind to specific fimbrial antigens enhance colonization process which mean presence, growth and multiplication of the organism in one or more body site without observable clinical symptoms or immune reaction. Mostly DEC colonize to the bowel mucosa (a site that is not normally colonized) [7]. This colonization result with different and specific pathogenetic strategies for each bacterial category to cause diarrhea including enterotoxin production such as (ETEC and EAEC), invasion (EIEC) and intimate adherence with membrane signaling (EPEC and EHEC).

## 1.5 Symptoms of DEC infection

Symptoms depend largely on the virulence of the infecting *E. coli* strain. They range from vomiting to profuse watery diarrhea, to severe cramps and bloody diarrhea which in some cases develop to Hemolytic uremic syndrome**.** Fever is not usually prominent and recovery occurs within 10 days in most cases [7, 12].

## 1.6 Diagnoses of DEC infection

There are many methods used for the purpose of diagnosis of DEC, but the most important methods used are summarized in the following ways:

### 1.6.1 Isolation and identification of DEC infection

Most of *E. coli* groups can be recovered from clinical specimens by using general or selective media as MacConkey or eosin methylene-blue (EMB) agar at 37$^{o}$C under aerobic condition. These media are used for differentiation *E. coli* from other enteric organisms on the basis of morphology and fermentation of lactose and this method should be used carefully, because only about 90% of *E. coli* strains are lactose positive but some DEC strains like many of enteroinvasice *E.coli* (EIEC) strains are lactose negative, the indole test is positive in 99% of *E.coli* strains to help in *E.coli* detection [7].

There is no biochemical test used for specific differentiation between DEC strains, except a few biochemical tests used for differentiation of EHEC strains such as O157: H7. Its performed using MacConkey agar containing sorbitol instead of lactose. This differential medium facilitates the primary screening of *E. coli* O157:H7 which does not ferment sorbitol in 48 hours, a characteristic that differentiates it from most *E.coli* groups [12].

### 1.6.2 Serotyping

Identification of DEC requires differentiated it from nonpathogenic members of the normal flora. The traditional method was serotypic markers, *E.coli* is made on the basis of their O (somatic), H (flagellar), and K (capsular) antigens. At least 137 serologic types are known, of which 11 have been correlated with infantile diarrhea [9] however, this method was

insufficient for characterization of diarrheagenic strains because its expensive and limited sensitivity and specificity [7].

### 1.6.3 Polymerase chain reaction (PCR)

Recently the most popular and reliable techniques is molecular method including nucleic acid-based probe technologies as well as PCR methods[7] this method differentiating diarrheagenic strains from normal flora of the stool and distinguishing on category from another on the basic of presence different chromosomal and plasmid-encoded virulence genes that are absent in nonpathogenic *E. coli* [10].

Each cycle of PCR consist of three steps: a denaturation step, in which the target DNA is incubated at high temperature to made accessible to annealing by specific oligo-nucleotide primers. The second step is an annealing step, in which the reaction mixture is cooled to allow the primers to anneal to target sequences and finally an extension step which usually done at an intermediate temperature, in which the primers are extended on the DNA template by a DNA polymerase [13].

Advantages of PCR include great sensitivity in sit detection of target templates, therefore, this method used to distinguish and differentiate the different types of DEC from each other and from normal flora [7].

## 1.7 Categories of DEC infection

DEC can be divided into at least five different categories with distinct pathogenic patterns; enterotoxigenic *E. coli* (ETEC) a major cause

of travelers' diarrhea and infant diarrhea in less-developed countries, enteroinvasive *E. coli* (EIEC) a cause of dysentery , enteropathogenic *E. coli* (EPEC) an important cause of infant diarrhea , and enterohemorrhagic *E. coli* (EHEC) a cause of hemorrhagic colitis and hemolytic uremic syndrome, and enteroaggregative*E. coli*(EAEC) which consider as the main cause of diarrhea in children in developing countries and can lead to both acute and persistent diarrhea among children, adults and HIV infected person in developing and developed countries and adherence E. coli (ADEC) [14, 15].

### 1.7.1 Enterotoxigenic *Escherichia coli* (ETEC)

ETEC is an important category of DEC which was first recognized in piglets as causes of diarrheal disease [11]. Different studies showed that ETEC is the most common type particularly in the developing world [7] and in areas of poor sanitation and are uncommon in developed countries [12].

ETEC recognized as one of the most important cause of infants diarrhea and in tropical and subtropical climates it's was associated with diarrhea of infants and adults. In developing world ETEC remain the main causative of infant diarrhea, usually varies from 10 to 30% [7] and consider to be the most frequently isolated enteropathogen, accounting for approximately 210 million diarrhea episodes and approximately 380 000 deaths annually (WHO).This high incidence of infection result from fecal

contamination of water and foods sources which are the most vehicles for ETEC infection [7].

ETEC infection occurs most frequently in infants, the percentage of cases of sporadic endemic diarrhea which are due to ETEC ranged from 10 to 30% and very low cases of ETEC infection recorded among school-age children and adults [7].

ETEC also recognized as the causative agent of traveler's diarrhea responsible for 50-65% of all traveler's diarrhea [16]. While there is another study showed that ETEC responsible for 20 to 40% of cases of travelers' diarrhea [7]. In the United States, ETEC is the most common cause of a diarrheal disease which often acquired by travelers from industrialized countries to developing countries [12]. Annually, there are an estimated 10 million cases of ETEC travelers' diarrhea worldwide (WHO).

The infection is self-limiting disease which may last from 1-5 days often being with watery diarrhea usually without blood, mucus and accompanied by variable symptoms similar to that seen in V. cholera infection [18]. The infection required approximately $10^8$ organisms, therefore person to person transmission is uncommon [10].

ETEC differentiate from other categories of DEC by specific virulence factors such as colonization factors (CFs) and enterotoxins.

Colonization factors (CFs) allow the organism to adhere to and colonize the intestinal epithelium. More than 22 colonization factors (CFs)

have been recognize damong human ETEC and many more are about to be characterized [19]. Usually ETEC express fimbriae that permit the bacteria to bind to specific receptors on the microvilli and then give them the ability to release one or both of the toxins in to small intestine: a heat – labile enterotoxin (LT) that is very similar in size, sequence (80% identity), antigenicity and function to the Vibrio cholera toxin. The second toxin is a heat-stable toxin (ST) that have low molecular size and resistant to boiling for 30 min Some member of ST associated with diarrheal disease in both humans and animals (STb) and the another one associated primarily with diarrhea in piglets (STa).

### 1.7.2 Enteroinvasive *Escherichia coli* (EIEC)

EIEC strains are different from other types of DEC in its ability to cause dysentery with direct penetration, invasion, and destruction of intestinal mucosa. This infection produce an enterotoxin that leads to cause shigellosis- like symptoms in patients of all ages [12].

Genetically EIEC are non motile, lactose negative and lysine decarboxylase negative while most *E.coli* strains de-carboxylate lysine [17].

It's may be easily misidentified and less widely reported because of their similarity to *Shigellae*. Studies suggested that EIEC cause about 5% of all diarrhoeas in areas of poor hygiene [18]. Although EIEC and *Shigellae* have been found to be similar in morphology and clinical

presentation, the infective dose of EIEC necessary to produce disease is much higher that of *shigellae* [12] so that the potential for person-to-person transmission is low [7].

Dysentery syndrome are shown in minority of patients observed in 0-7% of person infected while in most patients with EIEC, watery diarrhea are the most commonly symptoms [7].

A small number of bacteria need to be swallowed from 10-100 organisms as they are relatively resistant to gastric acid and bile, and pass readily in to the large intestine where they multiply in the gut lumen. The current model of EIEC pathogenesis includes five steps: epithelial cell penetration, lysis of the endocytic vacuole. Intracellular multiplication, directional movement through the cytoplasm and extension into adjacent epithelial cells[7, 18]. The infection is characterized by fever, severe abdominal cramps, malaise and watery diarrhea accompanied by toxemia [12].

### 1.7.3 Enterpathogenic *Escherichia coli* (EPEC)

EPEC strains associated with infants chronic diarrhea and persistent diarrhea. Cases with severe diarrhea in children younger than 1 year, should suspected that the infection associated with EPEC, while cases among adults are rarely seen [18].

The illness is characterized by low-grade fever, malaise, vomiting and acute or persistent watery, non-bloody or mucoid diarrhea [12].

Transmission occurs through contaminated hands and weaning foods with infective dose equal $10^{10}$. Reservoirs of infection are both children with and without infection and adult carriers [7].

Infection with EPEC in developed countries has limited importance in contrast to the 1940s-1950s while in many developing countries it's continuo to be a major cause of infant's diarrhea [12].

Belong to EPEC strains; there were two different sets of serotypes which differ in genetic characteristics, serotypes, and virulence properties: Typical and atypical EPEC. Typical EPEC a mainly cause of infantile diarrhea in developing countries and associated strongly with diarrhea in children less than 1 year of age. This type have only humans as reservoir while atypical EPEC seems to be a more important cause of diarrhea with both reservoirs animals and humans and characterized with its closely related to Shiga toxin–producing *E. coli* (O157:H7) in genetic characteristics, serotypes, production of toxins, reservoir, and other epidemiologic aspects [7, 10].

### 1.7.4 Entero-aggregative *E. Coli* (EAggEC)

Entero-aggregative *E.coli*, first referred to as enteroadherent-enteroaggregatrive *E.coli* [12] , which responsible for different cases of acute and persistent diarrhea among children, adults and HIV-infected persons in the world [20].The infection with EAggEC characterized with cytotoxic effects when adhering to the mucosal surface of the intestine and

induced damage. These strains have the ability to adhere to Hep2 cells, packed in aggregative pattern on the cells and in between the cells[7]. To initiate disease approximately $10^{6}$-$10^{8}$ of bacteria are required with appearing of different symptoms such as watery and mucoid diarrhea, vomiting, dehydration and occasionally abdominal pain and bloody stools [12]. The mechanisms by which EAggEC cause diarrhoeal illness are poorly understood [18].

### 1.7.5 Enterohemorrhagic *E.coli* (EHEC)

EHEC recognized as a classic diarrheal illness characterized by a watery diarrhea that progresses to a bloody diarrhea and cramp abdominal pain with low grade fever. The infection considers being fatal especially in young children and elderly and results from ingestion of contaminated uncooked or undercooked meat as hamburger and raw milk [7, 21].

The most prevalent serotype of EHEC which was first recognized in 1982 was O157:H7 that has been associated with hemorrhagic diarrhea, colitis and hemolytic-uremic syndrome (HUS) [10]. The infection results from producing a potent cytotoxins as Verotoxin I and II that are identical to the Shiga toxin (expressed by Shigella dysenteriae 1) which are a major virulence factor for O157:H7 that may lead to death and many other symptoms range from mild diarrhea to severe manifestations such as HUS which characterized with low platelet count, hemolytic anemia and kidney failure and approximately 10% of cases mostly patients younger than 10 years developed to HUS [7, 11].

The infection dose is low around 10 to 100 organism which may facilitate spread from person to person at any age but usually affects the very young and the elderly and where hygiene conditions are poor [10].

In USA, *E. coli* O157:H7 causes at least 20000 cases (only about 10% are probably reported) of food infection and 250 death each year)[21] and more than 800 cases with EHEC are reported each year [18].

Ruminant animals as cattle and sheep and their gastrointestinal tract consider being the main reservoir of *E.coli* O 157. Also it has been isolates from other species, including pigs, rodents and wild birds. All infected animals excreting *E.coli* O 157 are normally asymptomatic and transmission of infection occur via direct contact with the animals or their feces

Unlike other DEC groups, *E.coli O157:H7* does not ferment sorbitol and appears colorless when the bacteria is culturing on MacConkey agar containing sorbitol that facilitates the primary screening *E.coli O157:H7* [12] .

## 1.8 *Literature review*

### 1.8.1 DEC in developing countries

Prevalence of *E. coli* in developing countries was studied in many different countries among different target groups, all of these studies concern *E. coli* as one of the most common bacterial pathogens lead to

diarrhea especially among children groups included infants, preschool children and school children.

In Palestine, most studies focused in prevalence of parasites and there was no study concern in prevalence of DEC in stool samples, only one study was done in West Bank determine the prevalence of UTI among children urine samples. Across sectional study was done among 1338 children of primary schools in Nablus found that the prevalence of UTI calculated to be 4%. The most predominant pathogen was *E. coli* with 51.8% followed by *Staphylococcus aureus* 29.6% [22].

In Arab world, a growing number of studies have supported the association of DEC groups with infection .In Jadah (Saudi Arabia), a total of 576 fecal samples were collected from children aged 0-5 years suffering from acute diarrhea and attending hospitals and outpatient clinics. They found that the prevalence of *E. coli* was (13%) of which 3.8% were EPEC and 1.9% were EHEC [23]. While in Egypt the prevalence of ETEC isolates from children less than 5 years who were seeking hospital care was 320/1,540 (20.7%) [24]. In comparison with Jordanian children under 5 years of age. 265 stool samples were taken from children admitted to the Rahma hospital, detected ETEC (5.7%) as one of the main isolated enteropathogens [25]. Another study was done in Baghdad found that EPEC was the most frequently isolated organism (13%) of the children with diarrhea. This study appeared that the male to female ratio was 1.6:1.0 [26].

In Tunisia a case control study was done among 115 children with diarrhea and 54 controls. The most frequently isolated enteric pathogens from diarrheagenic children were ETEC (32.3%), EAEC (11.3), EIEC (11.3%), EHEC (10.4%) and Salmonella spp (9.5%), while in controls, ETEC (37%), EAEC (15%), EHEC and EPEC (11.1%) (27).Another case control study was done in Kuwait among 537 children less than 5 years old hospitalized with acute diarrhea and 113 matched controls. This study showed that the prevalence of DEC varied from 0.75% for EHEC to 8.4% for EPEC in diarrhoeal children with no significant differences compared to that in controls [28].

Infants were the main target group for the study of the *E.coli* prevalence in many different countries. In rural Mexican villages the incidence of diarrhea during the 1$^{st}$ year of life was 98% and 93% during the 2$^{nd}$ year. 75% of cases with diarrhea were associated with EPEC and ETEC strains, along with rotaviruses and possibly s*higella* [29]. While EAEC (25%) was the most prevalent pathogen associated with acute diarrhea in Brazil followed by ETEC (10%), EPEC (1.7%), EIEC (1.4%) [30].

A big review included different descriptive studies were done in low and middle countries in five hospitals in China, India, Mexico, Myanmar and Pakistan among children aged 0-35 months. A total of 3640 children with acute diarrhea and 3279 age-and sex matched controls showed that the most strongly pathogens associated with diarrhea were ETEC (16% of

cases and 5% of control), rotavirus ( 16% of cases and 2% of control) and *shigella* spp (11% of cases and 1% of control) [31] .

Among preschool children different cases of diarrheal infection occur. The increasing numbers of studies among pre-school children in which DEC groups are implicated suggest that DEC is important emerging agents of diarrhea. In developing countries, a study was done in low and middle income countries included all descriptive studies of pathogens in the stool of children with diarrhea. This study showed that EPEC types were commonly found in children with persistent diarrhea (up to 63%) [32].In another study which was done in Porto Velho (Rondonia, Western Amazon region, Brazil) among 470 children less than 72 months of age with diarrhea found that the prevalence of DEC was 18.2%. Another result in the study was found that EPEC had a significant difference between diarrhea and control groups [33]. Among Nicaraguan children ward aged 0-60 months, an outbreak of DEC was (53.8%) in the diarrhea group and (53.1%) in the non-diarrhea group. Detecting of ETEC, EPEC, EIEC and EAEC among children with diarrhea were (20.5, 16.0, 0.8 and 27.8%, respectively) and among children without diarrhea were (8.3, 20.7, 1.4 and 33.1%, respectively), this result showed that ETEC is an important agent associated with diarrhea in children from Nicaragua [34]. While In Salvador, A total of 1233 pre-school children with acute diarrhea were be isolated. The most frequently identified DEC were a-EPEC (10%), followed by ETEC (7.5%) and EAEC (4.2%)[35].

A clear association of *DEC* with diarrhea was shown in Tazaniain study which detected DEC as the predominant enteropathgens with prevalence rate equal 35.7% [36]. In India there were two studies concern in the role of *E. coli* in causing acute diarrhea. In rural northern India a cohort study was done among children aged 0-71 months showed that the incidence of diarrhea was 6.3 per 100 child-years, ETEC was the common a etiologic agent of diarrhea, accounting for 9.3% of cases. There were no significant sex-related differences and seasonal distribution in the Incidence of the disease [37]. Other study was done among 780 preschool children with diarrhea of central India showed that the commonest DEC was EAEC which isolated from 64 children, followed by EPEC (27), ETEC (10) and EHEC (2) [38].

Among school children different studies were be done among this target group and showed that DEC groups were one of the most common lead of diarrhea. In Dhaka, Bangladesh a study was done among children were up 5 years found that enteric pathogen was isolated from 74.8% of diarrheal children and 43.9% of control children included rotavirus, *Campylobacter jejuni*, ETEC, *Shigella* spp [39]. In the same country with different population a study was done among children aged more than 5 years showed that *DEC* isolated from 45.2% of children with diarrhea (EPEC had the highest prevalence, followed by ETEC) compared with 26.6% of children without diarrhea [40].

In Thailand a study showed that the prevalence of DEC among 2629 children less than 12 years were 16.9 % [41]. While in Korea , a case

control study was done and showed that rotavirus was the most frequently identified pathogen (47%of cases), followed by ETEC (22%), entero-adherent E. coli (15%) and EPEC(6%) [42]. And among Mongolian children the incidence of EAEC was 15.1% [43].

In Switzerland a study showed that EAEC was the most frequently detected bacteria associated with diarrhea with prevalence rate equal 10.2% among children with diarrhea and 2.2% among children without diarrhea [44]. In addition the prevalence of DEC among 1207 children in Salvador, Bahia and Brazil were 18.7% with diarrhea and control group, this result reflecting that the prevalence of infection in children with diarrhea was not significantly different from control group [45]. And in Bosnia and Herzegovina a case control study was done among 380 children less than 12 years showed that the prevalence of E. coli was 22% and the most prevalent path types was EPEC (54%), followed by ETEC (22.3%), EHEC (21.1%) and EIEC (2.3%) [46].

### 1.8.2 DEC in developed countries

In developed countries, most studies interesting with prevalence and epidemiology of *E. coli* O157.H7 as a main enteritis infection, but some of studies consider DEC as an important, unrecognized cause of childhood diarrhea not only in developing countries but also in developed areas such as Italy, a total of 160 infants with a diagnosis of acute gastroenteritis. They found that the presence of DEC was 6.3% but the ratio decreasing because

of the small sample size becomes 5% considering *E.coli* as a unique agent responsible for diarrhea [47].

Another study was done. In U.S, 1327 children with acute gastroenteritis were identified in the Emergency Department (ED) and inpatient units at Hospital Medical Center showed that diarrheal children infected with EAEC more than healthy children (1.4%)[48], where in Australia a different results were be found that Rotavirus (63%) and EPEC (59%) were the most common pathogens identified among cases less than 2 years [49].

### 1.8.3 The relationship between prevalence of *DEC* with types of water

A significant association between incidence of diarrhea and water showed in different studies. A across-sectional cohort study was done in Santiago among 340 children aged birth to 47 months was collected in a low socioeconomic level community with access to chlorinated water, the incidence of diarrhea was low (2.1 episodes/infant/year). Nevertheless, EPEC was found in a large proportion of diarrheal episodes, particularly during the summer. This result showed that *E. coli* is occurring despite the availability of potable water) [50]. In Hanoi, Vietnam (where the use of untreated wastewater in agriculture and aquaculture is a common practice) two different studies were done with different population. Among 111 pairs children with diarrhea and healthy controls less than five years of age, the pathogens most often associated with diarrhea were rotavirus (17% of cases) and *Entamoeba histolytic* (15%) followed by *Shigella* (5%). DEC

was found in 23% of both patients and controls [51]. Another study was showed that the prevalence of DEC types among 249 Vietnamese children less than 5 years old in the diarrhea and control groups were 25.7 and 10.5%, respectively [52].

### 1.9 Prevention and control

The most effective means of preventing infection is to avoid exposure to the infecting agent as contaminated food and water by provision of safe supplies of water together with education in hygienic practice in the handling and production the food. Another prevention steps include: hand washing, using pasteurization drinks and milk, thorough cooking food especially meat which mean that it should appear gray or brown and juices should be clear. Some of countries approved the meat irradiation as an acceptable means of dealing with food infection bacteria [7,11]

Travelers to countries with poor hygiene should select eating places with care, consume only hot food and drinks or bottled water and avoid eating salads and unheated milk [12].

For infants breast-fed play an important role in minimizing enteritis infection because in the preparation of infant formulas and the sterilization of bottles and nipples required hygienic care which is uncommon in breast-fed baby [8].

## 1.10 Treatment of DEC infection

In the absence of acquired resistance DEC can be treated by different antibacterial agents such as amplicillin, cephalosporeins, tetracyclines, aminoglycosids. However some strains have resistance to one or more drugs and patient should have laboratory test to take the best antimicrobial therapy [7, 18].

For gastroenteritis, *E. coli* treatment includes replacing the fluid lost from diarrhea and vomiting (dehydrated) should receive oral administration of solutions with electrolytes.

No vaccines have been approved, but some vaccine candidates have advanced to clinical trials, and other experimental treatments are being developed[12].

## 1.11 The objectives

To investigate the prevalence of *DEC* pathotypes among children less than 12 years old in Jenin Districts. In addition, the risk factors such as socio-demographic, environmental factors, behavioral habits were also investigated.

# Chapter Two

# Methodology

# Chapter Two
# Methodology

## 2.1 Study population

During the study period, 315 stool samples from children less than 12 years old were investigated for DEC. Of them, 145 were with diarrhea and 170 without (control group). Inclusion criteria of enrolled in the study for patients with diarrhea were the occurrence of three or more loose, liquid or watery stools or at least one bloody loose stool in 24 h period (WHO, 2000). Control subjects were healthy children without history of diarrhea for at least one month. A written consent from the children parents or guardians was obtained. The general characteristic and the clinical history were collected by interview using structured questionnaires and from the clinical files respectively.

### Study area

Among children less than 12 years old attending the primary health service centers in Jenin city and neighboring towns. The study was conducted during the period of November, 2009 to December 2012**.**

### Study design

This case control study was designed to investigate the prevalence of Diarrheagenic *Escherichia coli* and their risk factors including socio-demographic, environ mental factors, behavioral habits.

**Sample size**

Number of children who visited Jenin Health Centers and need stool examination / number of all children who visited Jenin Health Centers and need stool examination * 100 =315/ 760 *100=41.44%

**2.2 Inclusion criteria**

Children less than 12 years old from Jenin Government.

Children that had been staying in Jenin District no less than three month.

**2.3 Exclusion criteria**

Children with surgery such as appendectomy.

Children on antibiotic therapy such as prodrospectom and anti-gram negative bacteria.

Children with catheters.

**2.4 Data collection tools**

**2.4.1 Questionnaire**

The general characteristic and the clinical history of the patients and the control group including clinical diseases, socio-demographic, environmental features and behavioral habits were collected using questionnaire prepared for the this study showed in appendix 1.

The researcher filled the questionnaire by interviewing the parents or guardian of the children after they signed a written consent.

### 2.4.2 Materials and Reagents

- MacConkey agar (MA) .
- Sorbitol MacConkey agar (SMA).
- Brain Heart infusion broth.
- Absolute Ethanol.
- TaqDNApolymerase.
- Tris-Hcl (pH 8.3)
- Master Mix PCR.
- Primers: stored separately at -20 C (table 1).
- DNA marker.
- Agarose gel contain: agarose agar, tax x5 BP423 buffer and distilled water.
- Ethidium Bromide and DNA Ladder

**Table (1): Primers used in the detection of DEC.**

| Designation | Sequence (5′ to 3′) | Target gene | Amplicon size (bp) |
|---|---|---|---|
| SK1 | CCCGAATTCGGCACAAGCATAAGC | Eae | 881 |
| SK2 | CCCGGATCCGTCTCGCCAGTATTCG | | |
| VTcom-u | GAGCGAAATAATTTATATGTG | Stx | 518 |
| VTcom-d | TGATGATGGCAATTCAGTAT | | |
| AL65 | TTAATAGCACCCGGTACAAGCAGG | Est | 147 |
| AL125 | CCTGACTCTTCAAAAGAGAAAATTAC | | |
| $LT_L$ | TCTCTATGTGCATACGGAGC | Elt | 322 |
| $LT_R$ | CCATACTGATTGCCGCAAT | | |
| ipaIII | GTTCCTTGACCGCCTTTCCGATACCGTC | ipaH | 619 |
| ipaIV | GCCGGTCAGCCACCCTCTGAGAGTAC | | |
| aggRks1 | GTATACACAAAAGAAGGAAGC | aggR | 254 |
| aggRkas2 | ACAGAATCGTCAGCATCAGC | | |
| Eaggfp | AGACTCTGGCGAAAGACTGTATC | CVD432 | 194 |
| Eaggbp | ATGGCTGTCTGTAATAGATGAGAAC | | |
| aspU-3 | GCCTTTGCGGGTGGTAGCGG | aspU | 282 |
| aspU-2 | AACCCATTCGGTTAGAGCAC | | |

## 2.5 Methods

### 2.5.1 Samples collection

The stool samples were collected using sterile wide-mouthed containers with tight-fitting leak proof lids. All stool samples were investigated microscopically for parasitic infection in situ (collected lab). Then, all the samples were transfer as soon as possible to Al-Basha Scientific Centre for studies and research in Jenin District.

Upon arrival the samples were inoculated into MacConkey (MA) and Sorbitol MacConkey agar (SMA) and incubated overnight at 37$^{o}$C. *E.coli* was identified based on colony morphology and biochemical tests.

### 2.5.2 DNA templates for PCR

#### 2.5.2.1 DNA extraction

The DNA of the E. coli was extracted as follows;

1. Three suspected colonies were selected and added to sterile tube with 100 □l PBS
2. Tube was boiled for 15 minutes.
3. Tube was centrifuged for 10 minutes at 14.000 rpm.
4. The supernatant that contain the DNA was collected into new sterile tube and store at -20$^{o}$C till tested.

#### 2.5.2.2 PCR assay

The extracted DNA was amplified by adding **5** µl of the extracted DNA to 45 µl of the PCR master mix according to the manufacturer instructions (Reddymix PCR Master Mix from westburg-UK) using primers describe previously (53). The primer name's and ratios used as follows; a 0.125 µM concentration (each) of primers SK1, SK2, ipaIII, and ipaIV; a 0.25 µM concentration (each) of primers VTcom-u, VTcom-d, $LT_L$, $LT_R$, aggRks1, and aggRkas2; a 0.5 µM concentration (each) of primers AL65 and AL125. The PCR protocol that used was; 95°C for 5 min, then, 95 for 1 min, 52°C for 1 min, and 72°C for 1 min, for 30 cycles, and final extentsion at 72°C for 10 min (53 )

Ten microliter of the PCR products was analyzed using 2.5% agarose gel (AmpliSize; Bio-Rad Laboratories), stained with ethidium bromide, and visualized by UV transillumination. The buffer in the electrophoresis chamber and in the agarose gel was 0.5× Tris-borate-EDTA.

## 2.6 Statistical analysis

Data was tabulated and analyzed using the Statistical Package for Social Sciences SPSS software. Chi-square analysis ($x^2$) was used to find any statistical value by comparing of positive DEC cases according to individual characteristics. Evaluations were carried out at 95% confidence level and $P < 0.05$ was considered statistically significant.

## 2.7 Study limitation

Delayed access of missing materials, difficulty of collection samples and lack of finance resource in addition of health workers strikes through the time of the study.

## 2.8 Ethical issues

Office permission obtained from Palestinian Ministry of Health to conduct the present study and collect samples. In addition, a written consent was obtained from children parents after explaining the main goal of the research.

# Chapter Three

# The Results

# Chapter Three
# The Results

## 3.1 Distribution of participant children

Of the 328 children that were selected for the study, only 315 (96.03%) enrolled the study criteria and agreed to participated in the study and gave stool samples and made interview to filling the study questionnaire. Three stool samples were rejected because the children were on antibiotics therapy.

Of the 315 children enrolled in the study, 167 (53%) were boys and 148 (47%) were girls (Fig 2), with mean 4.5 years (1 month -12 years old).

The number and the percent of children according to sex, age group and their place of residence are shown in table 2 and 3 and 4 respectively.

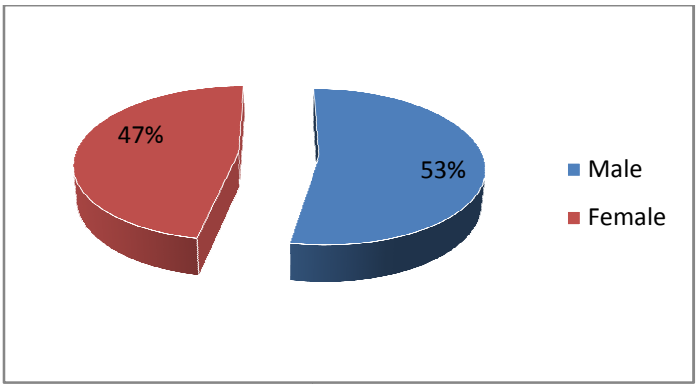


**Fig (2): Gender distribution of the study population**

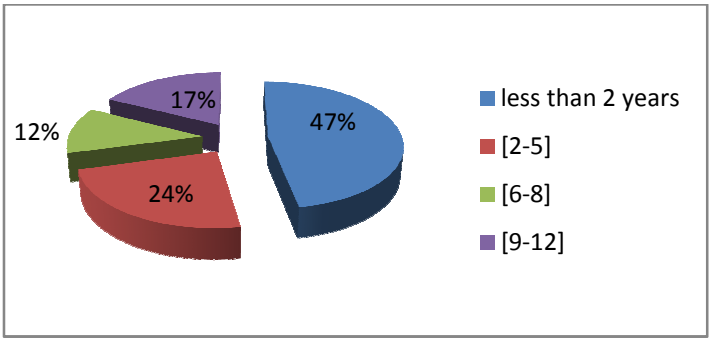


**Fig (3): Distribution of participant children age in the study**

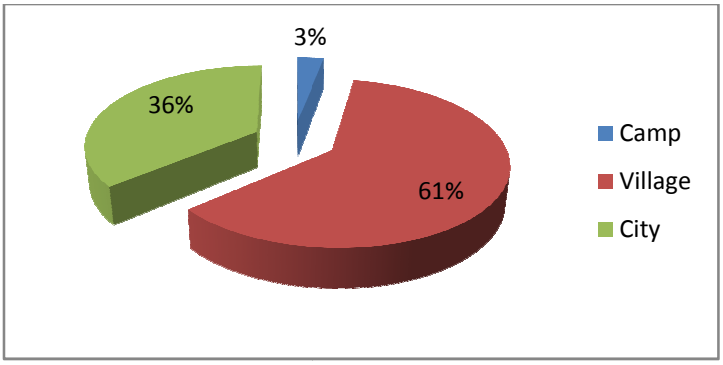


**Fig (4): Distribution of participant children residence in the study**

### 3.2 Microscopy results

Wet-mount microscopic examination for stool samples for parasitic infection was recorded in Jenin health centers by laboratory technicians. Records showed that 75/315 (23.8%) and 1/315 (0.3) were infected with *Entameba histolytica* and *Giardia lambelia* respectively. These results should be reevaluated since there is a misdiagnosis of *Entameba histolytica* cyst with *Entameba dispar* cyst and with pus cells [54].

**Table (2): Microscopic results**

| | | Frequency | Percent |
|---|---|---|---|
| **Valid** | **Nell** | 239 | 75.9 |
| | ***Entameba histolytica*** | 75 | 23.8 |
| | **Giardia Lamblia** | 1 | 0.3 |
| | **Total** | 315 | 100.0 |

### 3.3 Culture and PCR

Of the 315 inoculated stool samples on Mackonkey and MS agar, 169 (53.6%) yield positive test for indole (suspect *E. coli*). Forty five of the 169 (26.6%) samples showed positive result by multiplex PCR for DEC specific plasmid genes.

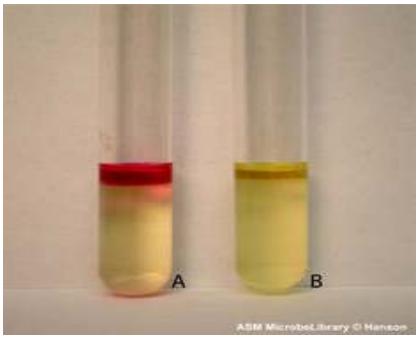


**Fig. (5): Result of indol test**

## 3.4 Prevalence of DEC

The prevalence of DEC infection among children in Jenin area was found to be 14.3% (45/315) (Table 5). The prevalence of DEC infection among children with diarrhea was15.2% comparing to 13.5% among control (table 6). The main prevalent pathotypes of DEC were ETEC and EAEC with prevalence rate 10.2%, 2.53% respectively (table 7).

Specific genes were shown in double bands for eae and stx for EHEC and single band for either elt or est for ETEC and single band for aggR for EAEC as shown in gel electrophoresis (fig 4)

**Table (3): Percentage of DEC infection among participant children**

| The Result | | | |
|---|---|---|---|
| Not Infection With DEC | | Infected With DEC | |
| Count | Percent | Count | Percent |
| 270 | 85.7 | 45 | 14.3 |

**Table (4): Percentage of DEC infection cases and control**

| | Not Infection With DEC | | Infected With DEC | |
|---|---|---|---|---|
| | Count | Percent | Count | Percent |
| Diarrhea | 123 | 84.8 | 22 | 15.2 |
| Without Diarrhea | 147 | 86.5 | 23 | 13.5 |

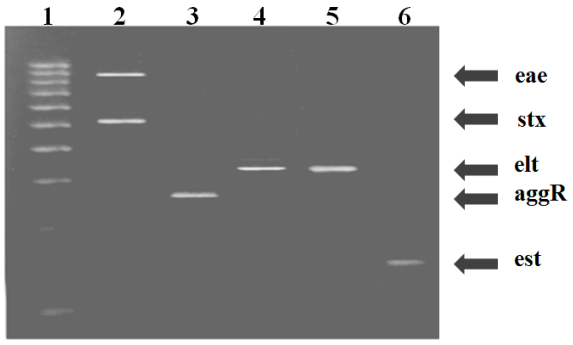


**Fig. (6): Sample of PCR products in agarose gel electrophoresis. 1-100 bp ladders; 2- S52 (EHEC); 3- S27 (EAEC); 4- S4 (ETEC) 5-S20 (ETEC) 6- S87 (ETEC).**

**Table (5): Results of Multiplex PCR.**

| Gene (s) | No. of positives | Percentage of population | Category |
|---|---|---|---|
| eae + stx | 5 | 1.6 | EHEC |
| Elt | 23 | 7.3 | ETEC |
| aggR | 8 | 2.5 | EAEC |
| Est | 9 | 2.9 | ETEC |

### 3.4.1 Prevalence of DEC and demographic characteristics

The result of this study showed that DEC was insignificant with diarrhea and sex factors with *p*= (0.678) (0.963) respectively. While place of residency was a significant factor for DEC infection. Children who lives in village showed prevalence of 18.8% compared to Jenin city (7%) and Jenin camp (11%) (*p*=0.016), Also DEC infection was strongly associated

with age group under 1 years (20.1%) ($p$= 0.037) compared to other age groups as shown in table (6).

**Table (6): The association between DEC and demographic characteristics**

| | | The result | | | | |
|---|---|---|---|---|---|---|
| | | Not infection with DEC | | Infected with DEC | | |
| | | Count | percent | Count | Percent | P-value |
| Type of stool | Diarrhea | 123 | 84.8 | 22 | 15.2 | .678* |
| | without diarrhea | 147 | 86.5 | 23 | 13.5 | |
| Residence | Camp | 8 | 88.9 | 1 | 11.1 | .016* |
| | Village | 155 | 81.2 | 36 | 18.8 | |
| | City | 106 | 93.0 | 8 | 7.0 | |
| Age of child | less than 2 years | 119 | 79.9 | 30 | 20.1 | .037* |
| | 3-5 | 69 | 93.2 | 5 | 6.8 | |
| | 6-8 | 33 | 89.2 | 4 | 10.8 | |
| | 9-12 | 49 | 89.1 | 6 | 10.9 | |
| Sex | Male | 143 | 85.6 | 24 | 14.4 | .963 |
| | Female | 127 | 85.8 | 21 | 14.2 | |
| Season | Summer | 36 | 90.0 | 4 | 10.0 | .652 |
| | Autumn | 106 | 86.9 | 16 | 13.1 | |
| | Winter | 74 | 82.2 | 16 | 17.8 | |
| | Spring | 54 | 85.7 | 9 | 14.3 | |

### 3.4.2 Prevalence of DEC and social characteristics

The statistical results showed that DEC infection was strongly associated with family size with $p$ value= 0.007. Children who live in family consists of 3 members their prevalence was 4.7%, also the prevalence of DEC among children who live in family consists of 4-5 members was10.7%,the prevalence was 21.7% among children who live in family consists of 6-8 members and 6,5% among children who live in family consists of more than 8 members.

Family financial situation is an important social factor which affects the prevalence of infection and showed clear association with DEC

infection ( $p$= 0.001), the results were as follow: the prevalence was 6.1% among children lives in excellent family situation, 14.1% among children lives in middle family situation, and 34.5% among children lives in less middle family situation.

In addition table 7 showed that there is a significant association between DEC infection and number of students in the class with $p$ values (0.037). The prevalence of DEC in the class with number of students less than 30 were 3.6% and 15.4% among children in class containing more than 30 students.

**Table (7): The association between DEC and the social characteristics**

| | | Not infection with DEC | | Infected with DEC | | |
|---|---|---|---|---|---|---|
| | | Count | Percent | Count | Percent | P-value |
| Family size | 3 | 41 | 95.3 | 2 | 4.7 | .007* |
| | 4-5 | 92 | 89.3 | 11 | 10.7 | |
| | 6-8 | 108 | 78.3 | 30 | 21.7 | |
| | more than 8 | 29 | 93.5 | 2 | 6.5 | |
| Number of children < 5 years | 1 | 125 | 87.4 | 18 | 12.6 | .47* |
| | 2-4 | 106 | 83.5 | 21 | 16.5 | |
| | more than 4 | 9 | 100.0 | 0 | .0 | |
| | 0 | 30 | 83.3 | 6 | 16.7 | |
| The number of adult children at home | 1 | 40 | 85.1 | 7 | 14.9 | .660* |
| | 2-4 | 54 | 81.8 | 12 | 18.2 | |
| | more than 4 | 8 | 80.0 | 2 | 20.0 | |
| | 0 | 168 | 87.5 | 24 | 12.5 | |
| Level of education mother | secondary or less | 186 | 86.5 | 29 | 13.5 | .176* |
| | Diploma | 17 | 100.0 | 0 | .0 | |
| | University | 66 | 80.5 | 16 | 19.5 | |
| | high education | 1 | 100.0 | 0 | .0 | |
| Job of mother | Housewife | 242 | 85.8 | 40 | 14.2 | .770* |
| | Employee | 26 | 83.9 | 5 | 16.1 | |
| Level of education father | secondary or less | 200 | 86.2 | 32 | 13.8 | .675* |
| | Diploma | 23 | 79.3 | 6 | 20.7 | |
| | University | 44 | 86.3 | 7 | 13.7 | |
| | high education | 3 | 100.0 | 0 | .0 | |
| Job of father | official employee | 78 | 84.8 | 14 | 15.2 | .555* |
| | Business | 51 | 83.6 | 10 | 16.4 | |
| | Worker | 102 | 85.0 | 18 | 15.0 | |
| | without job | 39 | 92.9 | 3 | 7.1 | |
| Family financial situation | Excellent | 62 | 93.9 | 4 | 6.1 | .001* |
| | Middle | 189 | 85.9 | 31 | 14.1 | |
| | less than middle | 19 | 65.5 | 10 | 34.5 | |
| # of children at class | Less than 30 | 96 | 96.4 | 4 | 3.6 | 0.037* |
| | More than 30 | 84 | 84.6 | 16 | 15.4 | |

### 3.4.3 Prevalence of DEC and environmental characteristics

**Table (8): The association between DEC and environmental characteristics.**

| | | The Result | | | | P-Value. |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| Number Of Rooms Home | Two Rooms Or One Room | 57 | 96.6 | 2 | 3.4 | .062* |
| | 3 Rooms | 107 | 84.3 | 20 | 15.7 | |
| | 4 Rooms | 85 | 81.7 | 19 | 18.3 | |
| | More Than 4 Rooms | 21 | 84.0 | 4 | 16.0 | |
| Ownership Home | Own | 234 | 84.8 | 42 | 15.2 | .328* |
| | Rent | 26 | 89.7 | 3 | 10.3 | |
| | Own+Family | 10 | 100.0 | 0 | .0 | |
| Type Of School | Governmental | 83 | 81.2 | 18 | 18.8 | .123* |
| | Private | 6 | 100.0 | 0 | .0 | |
| | Agency | 4 | 66.7 | 2 | 33.3 | |
| | Not Applicable (Children With Small Age) | 171 | 83.0 | 35 | 17.0 | |
| | Kindergarten | 6 | 100.0 | 0 | .0 | |
| Place Of Breeding Animals | Didn't breed animals | 95 | 91.3 | 9 | 8.7 | .194* |
| | Family | 75 | 85.2 | 13 | 14.8 | |
| | Neighbor | 80 | 81.6 | 18 | 18.4 | |
| | In The Neighborhood | 20 | 80.0 | 5 | 20.0 | |

An important environmental factor is animals breeding, this factor consists of two main ideas, place of breeding animals and types of this animals. Place of breeding animals was insignificant factor with *p* value = (0.194), while types of this breeding animals such as sheep and rabbit showed significant association with infection with *p* values = (0.008) and (0.029) respectively. The prevalence of DEC infection among children who breeding sheep and rabbits at their home or in their neighborhoods were

23.3% and 0.00% while breading of cows, chickens, dogs, cats donkeys, horses and dove did not show any significant difference with *p* values = (0.223), (0.297), (0.144), (0.966), (0.483), (0.305) and (0.644) respectively.

**Table (9): The association between DEC and breeding animals**

| | | The result | | | | P-value |
|---|---|---|---|---|---|---|
| | | not infection with DEC | | Infected with DEC | | |
| | | Count | Percent | Count | Percent | |
| Place of breeding animals | Family | 75 | 85.2 | 13 | 14.8 | .742* |
| | Neighbor | 80 | 81.6 | 18 | 18.4 | |
| | In the neighborhood | 20 | 80.0 | 5 | 20.0 | |
| Sheep | No | 86 | 90.5 | 9 | 9.5 | .008* |
| | Yes | 89 | 76.7 | 27 | 23.3 | |
| Cows | No | 129 | 81.1 | 30 | 18.9 | .223* |
| | Yes | 46 | 88.5 | 6 | 11.5 | |
| Rabbits | No | 154 | 81.1 | 36 | 18.9 | .029* |
| | Yes | 21 | 100.0 | 0 | .0 | |
| Chickens | No | 104 | 85.2 | 18 | 14.8 | .297* |
| | Yes | 71 | 79.8 | 18 | 20.2 | |
| Dogs | No | 143 | 81.3 | 33 | 18.8 | .144* |
| | Yes | 32 | 91.4 | 3 | 8.6 | |
| Cats | No | 127 | 83.0 | 26 | 17.0 | .966* |
| | Yes | 48 | 82.8 | 10 | 17.2 | |
| Donkeys | No | 159 | 82.4 | 34 | 17.6 | .483* |
| | Yes | 16 | 88.9 | 2 | 11.1 | |
| Horses | No | 170 | 82.5 | 36 | 17.5 | .305* |
| | Yes | 5 | 100.0 | 0 | .0 | |
| Dove | No | 151 | 83.4 | 30 | 16.6 | .644* |
| | Yes | 24 | 80.0 | 6 | 20.0 | |

### 3.4.4 Prevalence of DEC with food and drinks characteristics

According to source of water, children who depended on municipal water were less associated with DEC infection ( 8%) followed with 15.1% among children who depended on collection of wells, 26.2% among children who depended on both municipal water and wells collection and

16.7% among children who depended on spring water and there were statistically significant differences, with *p* value (0.008).

**Table(10): The association between DEC and nature of water.**

| | | The Result | | | | P-value |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| Source Of Water | Municipal Water | 127 | 92.0 | 11 | 8.0 | .008* |
| | Collection Wells | 73 | 84.9 | 13 | 15.1 | |
| | Municipal Water + Collection Wells | 45 | 73.8 | 16 | 26.2 | |
| | Spring Water | 25 | 83.3 | 5 | 16.7 | |
| Having Filter Of Water | Yes | 27 | 81.8 | 6 | 18.2 | .499* |
| | No | 243 | 86.2 | 39 | 13.8 | |

The prevalence of DEC among children who always use pasteurized milk is 5.9%, 8.2% among children who sometimes use pasteurized milk and 18.4% among children who don't use pasteurized milk, the result is statistically significant with *p* value ( 0.028). In addition, there is significant association between DEC infection and boiled cows and sheep milk before use with *p* value (0.046); The prevalence of DEC among children who drink boiled cows and sheep milk is 8.6% and 17.6% among children who did not boil cows and sheep milk .

**Table (11): The association between DEC and foods and drinks characteristics.**

| | | The Result | | | | P-Value |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| Using Of Pasteurized Milk | Always | 32 | 94.1 | 2 | 5.9 | .028* |
| | Sometimes | 78 | 91.8 | 7 | 8.2 | |
| | Children who didn't use pasteurized milk | 160 | 81.6 | 36 | 18.4 | |
| Reliance Of Fast Food | Yes | 82 | 91.1 | 8 | 8.9 | .083* |
| | No | 188 | 83.6 | 37 | 16.4 | |
| Average Monthly Consumption Of Fast Food | Once Or Less | 148 | 82.2 | 32 | 17.8 | .123* |
| | 2-5 | 83 | 90.2 | 9 | 9.8 | |
| | More Than 5 | 39 | 90.7 | 4 | 9.3 | |
| Boiled Cows And Sheep Milk Before Use | Yes | 85 | 91.4 | 8 | 8.6 | .046* |
| | No | 145 | 82. 4 | 31 | 17.6 | |

### 3.4.5 Prevalence of DEC with behavioral characteristics

Both washing children's hands before eating and washing vegetables before eating showed significant association with DEC infection ($p$=0.031) and ($p$=0.000) respectively. Prevalence of DEC among children who always wash their hands before eating is 8.6% and 20.6% among children who sometimes wash their hands before eating while the prevalence of DEC among children who always wash vegetables before eating is 8.7% and 33.3% among children who sometimes wash vegetables before eating.

**Table(12): The association between DEC and hand washing**

| | | The Result | | | | P-value |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| Washing Child Hands Before Eating | Always | 128 | 91.4 | 12 | 8.6 | .031* |
| | Sometimes | 50 | 79.4 | 13 | 20.6 | |
| | Not Applicable | 92 | 82.1 | 20 | 17.9 | |
| Washing Vegetables Before Eating | Always | 158 | 91.3 | 15 | 8.7 | .000* |
| | Sometimes | 20 | 66.7 | 10 | 33.3 | |

Table 13 shows clearly association between infection and number of bathroom ( $p$= 0.006). Children who have one bathroom at their home appeared the highest infection percent (20.6%) followed with children who have 2 bathrooms at their home (8.4%) and the lowest percent was showed among children who have 3 bathroom (6.9%) but, type of bathroom and place of sink didn't show any significant association with $p$ values ($p$=0.868) and ($p$=0.497).

Mother care associated with DEC infection ($p$=0.028). The prevalence of DEC infection among children who are helped by their mother is 16.7% and 6.6% among children who depends on their self. This may reflect high immunity rate of children Also the analysis showed that the presence of toilet tissues is significant factor with DEC infection ($p$=0.033).The prevalence of DEC among children who always have toile tissues in their bathroom are 9.7%, 19% among children who sometimes have toilet tissues and 20.8% among children who don't have toilet tissues at their bathroom. While washing hands after using bathroom statistically

not associated with DEC infection ($p$=.097). But the results showed that washing hands reduce DEC infection. Children who always wash their hands after using bathroom showed the lowest prevalence infection rate 10.2% while the prevalence of DEC infection among children who sometimes wash their hand with soap or with water only was 15.4%.

**Table (13): The association between DEC and behavioral characteristicsz**

| | | The Result | | | | P-Value |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| Number Of Bathroom | 1 | 123 | 79.4 | 32 | 20.6 | .006* |
| | 2 | 120 | 91.6 | 11 | 8.4 | |
| | 3 | 27 | 93.1 | 2 | 6.9 | |
| Type Of Baths | Traditional Bath | 85 | 85.9 | 14 | 14.1 | .868* |
| | Bath Syphilis | 86 | 84.3 | 16 | 15.7 | |
| | Traditional Bath + Bath Syphilis | 99 | 86.8 | 15 | 13.2 | |
| Place Of The Bathroom Sink | Inside The Bathroom | 63 | 81.8 | 14 | 18.2 | .497* |
| | Outside The Bathroom | 133 | 86.4 | 21 | 13.6 | |
| | Inside And Outside The Bathroom | 74 | 88.1 | 10 | 11.9 | |
| Helping Mother For Child | Yes | 199 | 83.3 | 40 | 16.7 | .028* |
| | No | 71 | 93.4 | 5 | 6.6 | |
| Presence Of Toilet Tissues | Always | 158 | 90.3 | 17 | 9.7 | .033* |
| | Not At All | 61 | 79.2 | 16 | 20.8 | |
| | Sometimes | 51 | 81.0 | 12 | 19.0 | |
| Washing Hands Child After Using Bathroom | Wash His Hands With Water Only Or Sometimes With Water And Soap | 33 | 84.6 | 6 | 15.4 | .097* |
| | Always Wash His Hands With Soap And Water | 141 | 89.8 | 16 | 10.2 | |
| | Not Applicable: (children with small age) | 96 | 80.7 | 23 | 19.3 | |

### 3.4.6 Prevalence of DEC with medical symptoms

As a result of SPSS analyses, there is no statistically association between DEC infection in our society and symptoms including: abdominal pain, diarrhea, dysentery, fever, loss of appetite and constipation with *p* values: (0.860), (0.678), (0.782), (0.664), (0.622) and (0.118) respectively.

**Table (14): The association between DEC and associated symptoms.**

| | | The Result | | | | P-Value |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| Abdominal Pain | No | 51 | 86.4 | 8 | 13.6 | .860* |
| | Yes | 219 | 85.5 | 37 | 14.5 | |
| Diarrhea | No | 147 | 86.5 | 23 | 13.5 | .678* |
| | Yes | 123 | 84.8 | 22 | 15.2 | |
| Dysentery | No | 262 | 85.6 | 44 | 14.4 | .782* |
| | Yes | 8 | 88.9 | 1 | 11.1 | |
| Fever | No | 177 | 86.3 | 28 | 13.7 | .664* |
| | Yes | 93 | 84.5 | 17 | 15.5 | |
| Loss Of Appetite | No | 207 | 85.2 | 36 | 14.8 | .622* |
| | Yes | 63 | 87.5 | 9 | 12.5 | |
| Constipation | No | 256 | 85.0 | 45 | 15.0 | .118* |
| | Yes | 14 | 100.0 | 0 | .0 | |

**Table (15): The association between DEC and suffering of a rounded people.**

| | | The Result | | | | P-Value |
|---|---|---|---|---|---|---|
| | | Not Infection With DEC | | Infected With DEC | | |
| | | Count | Percent | Count | Percent | |
| A Family Member Suffering From The Same Symptoms | Yes | 88 | 74.6 | 30 | 25.4 | .081* |
| | No | 182 | 92.4 | 15 | 7.6 | |
| One Of The Neighbors Suffering The Same Symptoms | Yes | 28 | 93.3 | 2 | 6.7 | .210* |
| | No | 242 | 84.9 | 43 | 15.1 | |
| The Suffering Of One Of His Colleagues With The Same Symptoms | Yes | 17 | 89.5 | 2 | 10.5 | .641* |
| | No | 88 | 85.4 | 15 | 14.6 | |

# Chapter four

# Discussion and Conclusion

# Chapter four
# Discussion and Conclusion

## 4.1Discussion

There were no previous studies have been carried out in Palestine regard DEC. This present study aimed to study the prevalence of DEC and its association with socio-demographic, environmental and behavioral factors within children in Jenin governorate in the north part of Palestine. Results showed prevalence of 14.3% of DEC infection among total population similar with that reported in Oman and in Iraq [6, 26].

Present study demonstrated that no significant correlation between DEC infection and diarrhea among children. This was in contrast with results reported in India, Chile and Peru [37, 50, 55] but in parallel with other in Oman, Kuwait and Jordan [6, 26, 56]. This may due to food habits and traditions which lead to exposure for different type of *E.coli* or other coliform bacteria within environment which enhance the individual immunity.

High prevalence of infection was found in villages and family with low salary. The absence of sanitary facilities and poor child hygiene beside the use of drinking-water from collection wells and springs which are simply to be contaminated could be the main reason for this result.

Families depend only on municipal water showed a decrease in the prevalence of DEC infection. That is because of the water treatment by chlorination or other methods which limit the growth of bacteria.

Similar to a Jordanian report [56] our study showed that DEC infection was significantly higher in children less than one year old compare to those above with no difference between male and female in agreement with other studies done in Bagdad and India. [26, 37]. This result could be related to beginning of environmental exposure and increased introduction of solid foods to children whose immune system is still developing within the first year of age [57].

Prevalence of infection showed a clear association with family size, when the members of the family increase the mother care becomes less because of the increase in her responsibilities. This finding agrees with study done in rural area of Zaire [58].

Direct contacts with animals affect the prevalence rate of DEC infection because animals play a role as receiver for different type of infection [11]. There were different studies ensure this relationship. A study was done in US found that DEC isolated from liver, spleen and blood of cattle, sheep, chicken and pig [59]. Another study was done in chicken in Korea showed that ETEC was present in the feces of flocks chickens[60].Also a Germany study showed that DEC can be transmitted between dogs and humans and appeared a clear association reported between EPEC and ETEC with enteric disease in young dogs [61]. This study didn't show any association between animals farm animals and DEC infection except chickens, doves and rabbits.

Presence of chickens and doves showed high association (20.2% ,20%) respectively due to easily transmission from one place to another and their waste can be found in any place. Also rabbits showed significant association with DEC infection. But this result mustn't be considered, because the number of children who breed rabbits is low, so there will be a bias in calculation Chi-square.

Although DEC was isolated from different traditional and fast food as chicken, shwarma in different places [7], there was no association with DEC infection in our population. This result suggested that the immunity rate of children in Jenin area is high or reflect presence of another type of pathogens associated with diarrhea such as salmonella which is the common cause of food borne illness, particularly in undercooked chicken [62].

Behavioral variables are associated with increase or decrease of the rate of DEC infection, In this study, children who washes their hands or vegetables before eating reduce the prevalence rate of DEC infection. Another study was done in Nairobi City showed clear association with DEC and salmonella as a result of poor hygienic and unsanitary practices due to absent of washing hands and vegetables before preparation, sneezing and coughing over food during preparation [63]. This explains high diarrheal morbidity especially in children less than five years in this area.

Our results showed that washing hands after defecation play an important role in minimize rate of DEC infection. This factor particularly

an important measure at the individual level to reduce spread of pathogens [64] also hand washing especially with soap most effective in reducing diarrhea illness[65].

Different clinical symptoms associated with DEC such as fever, abdominal pain, diarrhea, loss of appetite as a study done in Nicaragua [34]. But this results didn't showed statistical association with DEC. Another study was done in Salvador, Bahia and Brazil recorded bloody stools, fever, vomiting and cough as a main symptoms associated with DEC [35].

Transmission of infection from any member of the family or neighbors or colleagues of the child's didn't see any associated with prevalence of DEC infection. All of these factors ensure that DEC insignificant cause of disease among children in Jenin area.

## 4.2 Conclusion

- The prevalence rate of DEC infection in Jenin area was 14.3%. However this ratio can also be reduced by different way depended on the factors studied in this study.
- The majority of DEC infection isolates (20.1%) were detected in children less than two years old.
- DEC isolates were mostly found in both diarrheal and control patients without any significant difference.

- To minimize rate of DEC infection we can educate mothers and children to avoid direct contact to animals as a sheep, boiling sheep and cows milk before using or using pasteurized milk and avoid touch to animal feces. Also using municipal water or boiling water before using at home. In addition to the above washing child hands or vegetables before eating, helping mother for child and use of toilet tissues at the bathroom play an important role in decrease rate of infection.

## 4.3 Recommendation

Further work is still needed to determine the prevalence of DEC among children in Palestine. In addition we can educate mothers and children how to use proper hand-hygiene technique, drink pasteurized beverages, eat cooked meat avoid direct contact with animals and wash hands after handling raw meat.

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# Appendix

**جامعة النجاح الوطنية**

**كلية الدراسات العليا / برنامج الصحة العامة**

**أخي المواطن ,أختي المواطنة:**

**تحية طيبة وبعد:**

إن هذا الاستبيان الذي بين أيديكم يهدف الى أغراض علمية تتعلق بمعرفة معدل انتشار بكتيريا (**Diarrheagenic *Escherichia coli***) بين الأطفال واهم الأسباب التي قد تــؤدي إلى الإصابة بهذا النوع من البكتيريا بين الفئة المستهدفة في بلادنا والتي قد تنتج عن تلوث المياه والأطعمة والاتصال المباشر مع احد المصابين شاكرين لكم حسن تعاونكم.

**الطالبة: روان أمين**

**الاسم:**---------- رقم العينة:---

الحالة:1-□ اسهال 2- □سليم

**العمر:**-- □ اقل من 1. □2-5. □6-8 . □ 9-12. التاريخ:----

**الجنس:** 1: □ ذكر 2:□ انثى الهاتف:---

**مكان المعيشة:** ------ 1 □ مخيم 2: □ قرية 3: □مدينة

الفصل : 1:□صيف 2:□خريف 3:□شتاء. 4: □ ربيع

Household Demographic Information"-

**عدد أفراد الأسرة :-**

1. ☐ ثلاث

2. ☐ 5-4

3. ☐ 8-6.

4. ☐ أكثر من 8

**عدد الأبناء الاطفال** (أقل من 5):

1: ☐ 1

2: ☐من 2-4

3: ☐أكثر من 4

4: ☐لا يوجد

**عدد الأبناء البالغين:**

1: ☐ 1

2: ☐من 2-4

3: ☐أكثر من 4

4: ☐لا يوجد

**عدد غرف المنزل:** (باستثناء المطبخ والحمامات): 1: ☐ غرفتين أو أقل. 2☐ ثلاث غرف 3: ☐أربع غرف 4☐ اكثر من اربع غرف.

**المنزل الذي تسكنه العائلة:**

: ☐ملك .

2: ☐أجار.

3: ☐ مع العائلة.

4: ☐ملك + مع العائلة

**المدرسة التي يدرس فيها الطفل** 1:□حكومي. 2:□خاص. 3: □وكالة. 4:□لا ينطبق 5: □ روضة

**عدد طلاب الفصل:** 1: □اقل من 30 طالب. 2: □أكثر من 30 طالب. 3: □لا ينطبق

**تربى هذه الحيوانات لدى:0:** □لايوجد 1:□ العائلة 2:□ الجيران 3: □في الحي.

**نوع الحيوانات:** 1: □أغنام 2□:أبقار 3:□ أرانب 4:□ دواجن 5:□ كلاب 6: □قطط 7□حمير 8: □خيول 9:□ حمام.

**Association between parents work and education with infection**

**مستوى تعليم ألام:**

**1:**□ ثانوي أو أقل 2:□ دبلوم.

**3:**□جامعي 4 :□ دراسات عليا.

**وظيفة ألام:**

1:□ ربة منزل 2 :□ موظفة

**مستوى تعليم الأب:**

**1:**□ ثانوي أو أقل 2 :□ دبلوم.

**3:**□ جامعي. 4 :□ دراسات عليا.

**وظيفة الأب:**

**1:**□موظف رسمي. 2: □عمل حر

3: □عامل. 4: □بلا وظيفة.

**وضع الأسرة المالي:**

1: □ممتاز 2:□ متوسط 3: □أقل من متوسط

Association between infection and source of water

**مصدر مياه الشرب:**

1:☐ مياه بلدية. 2: ☐آبار جمع.

3:☐ مياه بلدية وآبار جمع. 4: ☐مياه نبع

**هل يوجد فلتر للمياه داخل المنزل:**

1: ☐ نعم

2:☐لا

Association between infection and types of food

**هل تستخدم العائلة الحليب المبستر**

1:☐دائما

2:☐احيانا

3:☐لا

**في حالة استخدام الحليب المأخوذ من البقر مباشرة هل يتم غليه قبل تناوله**

1:☐نعم

2:☐ لا

3 :☐ العائلة لا تستخدم الحليب

**هل تعتمد العائلة على الوجبات السريعة:**

1:□ نعم

2:□لا

**معدل استهلاك العائلة للوجبات السريعة شهريا:**

1:□مرة واحدة او أقل

2:□خمس مرات أو أقل

3:□أكثر من خمس مرات

Association between infection and Hygienic condition

**هل يغسل الطفل يديه قبل تناول الطعام**

1: ☐دائما

2: ☐احيانا

3: ☐لا

4: ☐ لا ينطبق الطفل صغير

**هل يغسل الطفل الخضار قبل تناولها**

1☐دائما

2: ☐احيانا

3: ☐لا

4: ☐ لا ينطبق الطفل صغير

**عدد دورات المياه (الحمامات) في المنزل**

1: ☐واحد.

2: ☐اثنان.

3: ☐ثلاث.

4: ☐أربعة

**نوع الحمام الموجود في المنزل**

1: ☐عربي.

2: ☐ إفرنجي.

3: ☐ عربي وإفرنجي.

**مغسلة الحمام موجودة**

1: ☐داخل الحمام

2: ☐خارج الحمام

3: ☐داخل وخارج الحمام

**هل تساعد ألام (او احد أفراد الأسرة) طفلها في قضاء حاجته**

1: ☐نعم.

2: ☐لا.

**هل يستخدم الطفل يده في بعض الأحيان لتنظيف البراز بعد الانتهاء من قضاء حاجته**

1: ☐ نعم.

2: ☐ لا.

3: ☐ لا ينطبق

**هل يعتبر ورق حمام التواليت عنصر مهم (لا يمكن الاستغناء عنه) في الحمام**

1: ☐ نعم.

2: ☐ لا.

3: ☐ أحيانا.

**بعد انتهاء الطفل من قضاء حاجته فانه**

1: ☐ يغسل يديه بالماء.

2: ☐ يغسل يديه بالماء والصابون.

3: ☐ أحيانا يغسل يديه بالماء والصابون.

4: ☐ لا ينطبق

**هل عانى الطفل من أي من هذه الأعراض**

1: ☐ ألم في البطن (مغص)

2: ☐ اسهال

3: ☐ اسهال مصحوب بدم

4: ☐ ارتفاع في درجة الحرارة

5: ☐ فقدان الشهية.

**هل عانى احد من العائلة بنفس الأعراض التي عانى منها الطفل وفي نفس الفترة الزمنية**

1: ☐ نعم

2: ☐ لا

**هل عانى احد من الجيران بنفس الأعراض التي عانى منها الطفل وفي نفس الفترة الزمنية:**

1: ☐ نعم

2: ☐ لا

**هل عانى احد من زملائه في المدرسة بنفس الأعراض التي عانى منها الطفل وفي نفس الفترة الزمنية:**

1: ☐ نعم

2: ☐ لا

3: ☐ لا ينطبق الطفل صغير

جامعة النجاح الوطنية
كلية الدراسات العليا

# معدل انتشار بكتيريا الإشريكية القولونية والعوامل المرافقة لها في فئة الأطفال في محافظة جنين

إعداد
روان أمين طاهر أبو الحوف

إشراف
د. وليد باشا

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الصحة العامة بكلية الدراسات العليا في جامعة النجاح الوطنية بنابلس فلسطين.

2013م

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## الملخص

الإشريكية القولونية المسببة للأسهال(DEC) هي واحدة من أهم المسببات الرئيسة للإسهال وتعتبر بأنها السبب الأكثر شيوعا في جميع أنحاء العالم لحدوث الإسهال المتوطن والوبائي .**الهدف** :نظرا لعدم وجود بيانات حول الاصابة ب DEC في فلسطين فقد أجريت هذه الدراسة لتحديد مدى انتشار الإصابات DEC وعلاقتها مع العوامل، الاجتماعية والديمغرافية البيئية، والعادات السلوكية والاعراض المرضية المتعلقة بالإشريكية القولونية بين الأطفال أقل من 12 سنة في محافظة جنين.

**الطرق:** تم جمع عينات البراز من 145 طفل مصاب بالإسهال أو الزحار و 170 عينة من أطفال غير مصابين من كلا الجنسين عشوائيا من مراكز الخدمات الصحية الحكومية في محافظة جنين .تم نقل العينات إلى المختبر في غضون ساعة بعد الفحص المجهري وتم زراعتها على أجار ماكونكي (MA) وأجار سوربيتول ماكونكي (SMA) . وقد تم تحديد الإشريكية القولونية من اعتمادا على شكل المستعمرة والاختبارات الكيميائية الحيوية القياسية .تم تحديد انواع ال DEC عن طريق تفاعل البلمره المتسلسلة المركب من المستعمرات التي حددت كإشريكية قولونية .عائلة كل طفل طلب منها تعبئة استبيان يتعلق بالعوامل الاجتماعية السكانية، البيئية والعادات السلوكية لأطفالهم.

**النتائج:** أظهرت النتيجة الإجمالية لمعدل الانتشار 14.3٪ (315/45) بين مجتمع الدراسة .لم يكن هناك اختلاف كبير في مدى انتشار DEC بين المصابين بالإسهال أو الزحار 15.2 (145/22) وغير المصابين 13.5 (170/23). النوع الممرض من DEC الأكثر انتشارا كان

الذراري المنتجة للذيفان المعويETEC(10.2%) يليه الذراري الإشريكية القولونية المتكدسة في الأمعاءEAEC (2.5%) و الذراري المنزفة للأمعاءEHEC (1.5%). معظم عزلات DEC كانت في الأطفال أقل من سنتين من العمر.

الدراسة بينت ان هنالك ارتباط مهم بين انتشار DEC و الاتصال مع الحيوانات وشرب الحليب الغير مغلي او الغير مبستر .وأظهرت ان الإصابة مرتبط مع مصدر المياه بحيث أن استخدام مياه البلدية أو الماء المغلي حد بدرجة كبيرة من انتشار العدوى.

الإشريكية القولونية المسببة للأسهال (DEC) هي واحدة من أهم المسببات الرئيسة للإسهال وتعتبر بأنها السبب الأكثر شيوعا في جميع أنحاء العالم لحدوث الإسهال المتوطن والوبائي .

بالإضافة إلى ما سبق، نتائجنا تقدم دليلا على أن غسل اليدين أو الخضار قبل الأكل، ومساعد الأم للطفل واستخدام المناشف الورقية في المرحاض لعبت دورا هاما في انخفاض معدل الإصابة.

**الاستنتاجات والتوصيات:** لا تزال هناك حاجة إلى مزيد من العمل لتحديد مدى انتشارDEC بين الأطفال في فلسطين .يجب ان يكون هنالك الكثير من العمل على تثقيف الأمهات والأطفال حول حول افضل الممارسات الصحية للاعتناء بأيديهم وضرورة تناولهم للمشروبات المبسترة وتناولهم للحوم المطهية جيدا بالإضافة الى تجنبهم للاتصال المباشر مع الحيوانات وغسلهم أيديهم بعد ملامستهم للحوم النيئة.