

# Molecular Detection and Diagnosis of Pathogenic Escherichia Coli and Giardia Lamblia of Patients in Babylon Province

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## Abstract

In this study detected and diagnosis the parasites (Giardia lamblia) and Escherichia coli Bacteria causes of diarrhoea according molecular, morphological examination and biochemical test. intestinal parasite spread in different regions of the world, especially in the tropical and subtropical regions, more than any region in the world because the population density increase beside temperate and cold areas that few take into account public health rules as well as the ability of a number of types of insects like flies on the transfer of cyst and egg of different parasites. A total of (96) stool specimens were taken from patients with diarrhoea and vomiting, during the period from February to June (2022) admitted to the Al-Hillah Hospital, Al- Imam Al-Sadiq Hospital and laboratories out. Among them 2 (2.08%) of specimens were found to be with G.lamblia and 25 (26.04%) with E. coli pathogens. In the present study, In the current study, the polymerase chain reaction (PCR) technique was also used, through which 96 samples were examined for microscopic examination of the parasites that cause diarrhea (G.lamblia ), and the infection rates was (2.08%) ,As for the bacteria, 96 samples were examined for the bacteria that cause diarrhea ( E.coli), and the infection rates was (26.04%). Depending on the PCR technique, the highest rates of infection was shown in males (39.2%), while the infection rates in females decreased (35.5%) for parasites. As for bacteria, the study recorded an increase in the infection rates in females (31.7%), while the rates of infection decreased Infection in males (21.81%), and this study recorded the highest infection rates in the age group (6-10 years), reaching (50%), while the decrease in the infection rates was in the age group (21 years and over) and was (10%) for parasites. As for bacteria, the study also recorded an increase in the rates of infection in the age group (6-10), reaching (35.29%), while the study decreased in the age group (21 years and over), reaching also (10%).

**Keywords:** patients, Molecular detect, Escherichia coli, and Giardia lamblia.

## 1. Introduction

The world health organization defined diarrhoeas the voiding of more than two unformed watery stool in any 24-hour period, or any voiding of watery stool if accompanied by fever, abdominal pain and / or vomiting diarrheic accounts for more death in childhood than any other disease in the developing world. It has been point out that in the developing country, a child of less than seven years of age still has 50 % chance of dying from diarrhoeal diseases (Florez et al., 2020). diarrhoea remains one of the leading causes of childhood morbidity and mortality in developing countries. Children in the developing world have an average of 5-6 episodes a year. WHO has accordingly underlined the need for epidemiological surveys of infantile diarrhoea in all geographical areas (Coelho et al.,2018) Diarrhea occurs when the ability of a normal or a damaged colon to re-absorb all excess fluid in the intestines fluid is impairs, hence results in the loss of fluids and electrolytes. The end result is dehydration which is the main cause of death. There is a high mortality percentage in urban slums and in rural farming communities (Petit, et al., 2017). E.coli serotypes are types according to the somatic, flagella and capsular antigen groups O, H and k respectively, e.g. E.coli,

0111, k58, H12. E.coli produces diarrheic a using four main mechanisms: (i) through production of toxin by entero pathogenic E.coli (EPEC) ; (ii) through invasion by enteroinvasive E.coli (EIEC) ; (iii) through adhering and effacing of the membrane of the intestinal microvilli by entero pathogenic E.coli (EPEC) ; and (iv) through the production of verotoxin by verotoxin E.coli (VTEC) (Bessonov et al.,2021). The E.coli serotypes known as entero pathogenic Escherichia coli (EPEC) are those pathogenic E.coli strains which do not produce heat – labile (LT) and/or heat stable (ST) toxins and are not invasive. Study have reported as many as 170 serogroups as the causal agents of infantile diarrheic in many parts of the world (Sadeq et al., 2018). Giardia lamblia is a parasite that causes Giardiasis, a common human-animal disease Bogitsh, B. J et al (2018). Spreading in tropical and subtropical regions Waldram, A et al (2017). Giardiasis is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries. G.lamblia is considered to be one of the leading causative agents of diarrheic in both children (Addy PAK et al (2004), Noor Azian MY et al (2007), and adults (Nyarango RM et al (2008), Ayeh-Kumi PF et al (2009). the parasite has two phases, the trophozoite and the cyst phase, the infection occurs

when the bags are devoured with contaminated food and drink Lucia, S. M., et al (2018).

## 2. Material and Methods

### Sample collection

A total 100 stool samples were collected from some patient with suffer from diarrheic infection based on symptoms, these samples are collecting from October 2021 till February 2022, diagnosed by the physion and stool microscope examination, the age groups ganged from less than one year to 65 years, the sample were collected from AL-Hilla city, Babylon maternity and children hospital, privates laboratories. And transferred to the advance parasitology and bacteriology laboratories in the Science Collage for Women Babylon University. then examined samples during a period not exceeding half an hour from time obtained by optical microscope using a direct smear methods for G.lambliia . As for bacteria, it was examined by culture dishes. After that we doing extracting DNA process and subjected to polymerase chain reaction technique.

### Stool Sample Processing

Stool samples were examined grossly for color, consistency, presence or absence of blood, mucus and worms. Routine stool microscopic examination for trophozoites and cysts of parasite. Stool samples was culture on several media for maximal yield. Sample was inoculated directly on MacConkey's agar, and blood agar. The plates were incubated for 18-24 hour at 37°C. Identity was established by a battery of bio chemical tests like: in dole test, Gram stain

### Genomic DNA extraction

Genomic DNA was extracted from fecal samples using stool DNA extraction Kit, Pioneer (Korea) after determining the target parasite using light microscope. The extraction was achieved according to the protocol that supplied from the company. DNA concentration was measure using Nano drop spectrophotometer. All the DNA sample were then store at -20 °C until need.

Primers	Sequence	Size
G.lambliia	F GTTGAAACGCCCGTAGTTGG	574
	R CTCGCTCGTTGTCGCAATG	
E. coli	F AGAGTTTGATCCTGGCTCA	544
	R GGTTACCTGTTACGACTT	

### PCR reaction mixture

The polymerase chain reaction (PCR) was used to amplify fragment of DNA using Maxima PCR PreMix Kit (I-Ta q) (Intronbio/Korea). The template DNA, forward and reverse primer, and RNase - free water were add to PCR tube, containing i-Taq DNA polymerase, dNTP mixture, and reaction buffer mixed together as in table (3-10).

Contents of reaction mixture	Volume ( µl )
Master Mix	12.5
Template DNA	8.5
Forward primer	2
Reverse primer	2
Total	25

PCR Steps	Temp	Time	Repeat
Initial Denaturation	95 °C	5 min	1
Denaturation	95 °C	30 sec	35 cycle
Annealing	57 °C	30 sec	
Extension	72 °C	55 sec	
Final extension	72 °C	5 min	1

### PCR product analysis

The PCR product of positive samples was analyzed by agarose gel electrophoresis following these steps

1.5 % agarose gel was prepared in using 1x TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

Then 3 microliter of Ethidium bromide stain were add into agarose gel solution.

Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidification for 15 minutes at room temperature. Then the comb were remove gently from the tray and 10 µl of PCR product is add in to each comb well and five ml of (100bp Ladder) in one well.

4-The gel tray was fixed in electrophoresis chamber and filled by 1 x TBE buffer. Then electric current was performed at 100 volt and 80 AM for one hour.

5-PCR product were visualized by using UV transilluminator and then photography of bands that reveal by digital camera.

Content of reaction mixture	Volume (µl)
Master Mix	12
Template DNA	4
Forward primer (10 pmol / µl)	2
Reverse primer (10 pmol / µl)	2
Nuclease free water	up to 20 ul

PCR Step	Temp	Time	Repeat
Initial Denaturation	95 °C	5min	1
Denaturation	95 °C	30sec	35 cycle
Annealing	55 °C	45sec	
Extension	72 °C	45sec	
Final extension	72 °C	5min	1

### Agarose Gel Electrophoresis of E. coli

Agarose gel electrophoresis is the most effective way of separating DNA fragments. The concentration of agarose in a gel depends on the size of the DNA

fragment need to be separated, ranging between (0.5 % - 2 %). A (0.7 %) gel was used to obtain good separation of genomic DNA (5-10 kb) after extraction while (1.5 % - 2 %) was used to gain good resolution for small fragment of PCR product (0.2 - 1 kb). However, the specific weight of agarose was added to 100 ml of 1 × TPE buffer and then melted in microwave until the solution become clear. Once the agarose was cooled to (50 - 55°C), 5 µl of simply safe dye (10 mg / ml ) was add to 100 ml of melting agarose gel to get final concentration 0.5 µg / ml (Sambrook & Rusell, 2001).

The agarose was poured in the gel tray with sealed ends, comb placed properly, and then left to dry. The samples were loaded in a separate well of the gel, with marker in one well. Electrodes were connected correctly and the run was applied according to the gel rate and size of gel, (the time of agarose gel electrophoresis is 45 minute for genomic DNA and 1 hour and 30 minute for PCR DNA sequencing of 16sRNA of tested isolate).

### 3. Results and Discussion

The first direct examination of the faces samples (96) from diarrhea patients in different ages and from (males and females) was performed using light microscope. The results showed that (2) isolates were Giardia lamblia and 25 isolates were Escherichia coli. This results were confirmed later via PCR assay, the present study showed the highest rates and lowest rates according to the gender and age groups for the parasite and bacteria that causes diarrhea. According to the table (1) showed the incidence total of infection parasite and bacterial where was the infection total of G.lamblia (2.08%) and the infection total of E.coli (26.04%), this study disagree with what recorded by Beena Uppal et al.(2015) where he scored the rates of infection for parasites (4.78%) and the rates of infection for bacteria (8.90%). According to the tables (2) and (3) for bacterial E.coli, Table (2) according to the gender showed the highest rates of infection in the females (31.7 %) and the lowest rates of infection in the males (21.81%), this this study is disagree Mitra et al (2011), where the highest rate of females was recorded (58.82%), and the rate of male was recorded (41.18%). while table (3) according to the age groups ( from age - less than one year to age 21 and over ), reveals the highest rates of infection bacteria (35.29%) were in the age groups (6-10), and the lowest rate of infection bacteria (10%) were in the age groups (21 and more). While in the other age groups ( less than one year, 1-5, 11-15, 16-20 ) the following percentages were recorded ( 30.76%, 26.66%, 28.57%, 16.66% ) respectively, This study is disagree with Nazek Al-Gallas, et al (2007) in Tunisia, who scored the highest rates of infection bacteria E.coli in children (70.4%) and in the adult scored the highest rate of infection bacteria E.coli (16.4%). according to the tables (4) and (5) for parasite G.lamblia, Table (4) according to the gender showed the highest rates of infection in the females (2.2 %) and the lowest rates of infection in the males (1.9 %) ,This study disagree with (Al-mussawi, H.S. (2012) Al-

ibraimi, L.A.(2013) who recorded that males have more rate of infection with giardiasis than female. while table (5) according to the age groups showed the highest rates of infection were (12.5%) in the age groups (6-10), whereas the another age groups records the lowest rates of infection (0%) for (less than one year, 1-5, 11-15, 16-20, 21 and more), this study disagree with Suad et al (2019).

**Table (6): Incidence of infection parasites and bacterial**

Kind of parasite and bacteria	Examined No.	Infected No.	(%)	Total
G.lamblia	96	2	2	2.08
E. coli	96	25	25	26.04

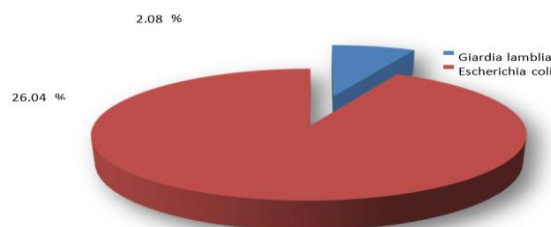


Figure (3): showed Total rates of Infection for parasite and bacteria that cause diarrhea in the present study.

**Table (7): PCR incidence of studied bacterial infection according to gender of the patient.**

The sex	Examined No.	Infected No.	Bacteria that cause diarrhea		Total percentage of infection (%)
			Escherichia coli		
			Infec. No.	(%)	
Males	55	12	12	21.81	21.81
Females	41	13	13	31.7	31.7
Total	96	25	25	26.04	26.04

**Table (8): PCR incidence of studied bacterial infection according to age groups of patients.**

The age	Examined	Infected No.	Bacteria that cause diarrhea		Total percentage of infection (%)
			Escherichia coli		
			Infec. No.	(%)	
Less than one year	13	4	4	30.7	30.76
1-5	30	8	8	26.66	26.66
6-10	17	6	6	35.2	35.29
11-15	14	4	4	28.5	28.57
16-20	12	2	2	16.6	16.66
21 and more	10	1	1	10	10
Total	96	25	25	26.04	26.04

**Table (9): PCR incidence of studied parasites infection according to gender of the patient.**

The sex	Examined No.	Infected No.	Parasite that causes diarrhea		Total percentages of infection (%)
			G.lamblia		
			Infec. No.	(%)	
Males	51	1	1	1.9	1.9
Females	45	1	1	2.2	2.2
Total	96	2	2	2	2

**Table (10): PCR incidence of studied parasites infection according to age groups of patients.**

The age	Examined No.	Infected No.	Parasite that causes diarrhea		Total percentage of infection (%)
			<i>G.lambli</i> a		
			Infec. No.	(%)	
Less than one year	15	0	0	0	0
1-5	32	0	0	0	0
6-10	16	2	2	12.5	12.5
11-15	12	0	0	0	0
16-20	11	0	0	0	0
21andmore	10	0	0	0	0
Total	96	2	2	2	2.08

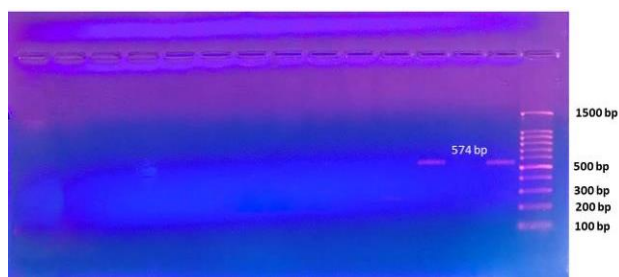


Figure (1): Agarose gel electrophoresis image that show the PCR product analysis of 18s RNA gene from genomic DNA of human stool samples. Where M: Marker (1500 – 100bp), lana (1-2) positive samples for *G.lambli* at 574 bp

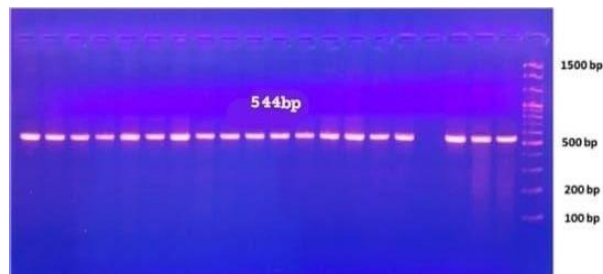


Figure (2): Agarose gel electrophoresis image that show the PCR product analysis of 16s RNA gene from genomic DNA of human stool samples. Where M: Marker (1500 – 100bp), lana (1-19) positive samples for *E. coli* at 544bp.

## 4. Conclusions

The pervasion of parasite and bacteria that cause diarrhea in Babylon area is so high when the microscope examination procedure for parasites and by culture for bacteria and the polymer chain reaction technique PCR is detected compared to previous studies. The PCR technique is more sensitive than direct smear procedure to determine the two parasite and culture method to determine *E.coli*. The single infection the highest rates when using PCR technique comparison with double infection. The *G.lambli*a parasite were the lowest pervasion from another *E.coli* bacterial and its infection focus on the little age group.

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