

# Epidemiology and Hematological study of certain enteric bacteria Isolated from Children with Acute Diarrhea in Sulaymania and Erbil Governarates/Iraq

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## Abstract

Diarrhea in children occurs for multiple reasons, especially after exposure to intestinal pathogens present in their environment due to contamination of food and water. There are social and economic reasons that aid in the transmission of pathogens to children. The study aims through research to detect it using sociodemographic determinants and expose complications of the disease using a complete blood count. The cross-sectional study was conducted on children brought to Sulaymania and Erbil children hospital. A total of 200 children (52.5% male and 47.5% female) were included. Data analysis showed that there was a significant correlation between general stool examination and bacterial infection ( $P < 0.05$ ). The infection rate among cases was 50% *Klebsiella spp.*, 3% *E. coli*, and 46.5% for both. Demographic data showed that both gender and location were significantly associated with the type of bacterial infection ( $P > 0.05$ ), and the type of food intake was significantly associated with increased diarrhea. As for the hematological parameters, the study showed that the total white blood cell count (WBC) and platelet count could be increased in the group of patients. There was a consistent decrease in the mean red blood cell (RBC) count in healthy subjects compared to controls using mean prevalence. We concluded through this study that the socio-economic conditions show the hidden causes of the infection rate of *E. coli* and *Klebsiella*. Accordingly, deal with it and reduce its spread

**Keywords:** Diarrhea, sociodemographic determinants, hematological parameters.

## INTRODUCTION

Diarrhea is defined as having loose or watery stools at least three times a day or more than usual for an individual. More than 1 billion cases and at least 4 million deaths annually are attributed to diarrhea worldwide (Randy P, 2018)

Diarrhoeal disease remains a major public health problem and is considered a major cause of morbidity and mortality after pneumonia, especially in infants and children (Margaret M. et al, 2017).

Several types of bacteria can enter in the body through contaminated food or water and cause diarrhea. Common bacteria that cause diarrhea include *Campylobacter*, *Escherichia coli*, (*E. coli*), *Salmonella*, *klepsiella spp.* and *Shigella* (Kim Hodges, and Ravinder Gill, 2010).

The pathogenic *E. coli* strains can be further classified into intestinal diarrheagenic *E. coli* which causes loose bowels and extraintestinal *E. coli* (ExPEC) which causes a wide range of illnesses in humans such as neonatal meningitis, chronic urinary tract infections, septicemia, and hemolytic uremic syndrome (Croxen and Finlay, 2010).

Many people with an *E. coli* and *klepsiella spp.* infection make a full recovery, but it can lead to serious life-threatening complications. People with weakened immune systems, pregnant people, young children, and older adults are at increased risk of developing these complications (Suzanna Flack 2017).

Our capacity to control and manage *E. coli* disease in humans and to limit the scale of outbreaks is dependent upon quick diagnosis and identification of the source of infection, as well as the development of therapeutic and preventative strategies (James C. & Adrienne W. 1998).

*Klebsiella spp.* shares some chemical and immunological properties with *Escherichia coli*. *Klebsiella* infection is a major problem in pediatric wards, as it can cause various infections in hospitals such as serious diarrhea in children and infants (Adriana et al, 2009).

The contribution of the various pathogens to diarrhea may vary basically between regions depending on local meteorological, geographic, and socio-economic conditions (Reither et al., 2007).

Underlying reasons for the spread of diarrhoeal diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as in inadequate education of health care providers and recipients (Curtis et al., 2000).

Morbidity rates in younger children are a particular problem because early childhood is a critical period in terms of development. Physical growth during this period is faster than at any other time. Disruption of these processes by diarrheal diseases in general and *E. coli*, in particular, can lead not only to short-term mortality but also to poor growth and a tendency to develop other diseases in adulthood, due to the child being given large amounts of drugs and antibiotics. The widespread and inappropriate use of antibiotics has also led to the emergence of the phenomenon of bacterial resistance to various antibiotics (Margaret M. et al, 2017).

The aim of this study is to research and find *E. coli* in the stools of children with diarrhea and whether it is caused by *E. coli* or others pathogens.

Recognize the complications of infection and diarrhea through complete blood count. Meanwhile the demographic information (questionnaire), the study aims to estimate the prevalence and sociodemographic determinants of diarrhea.

## MATERIALS AND METHODS

### Samples and subjects

From September 2021 to December 2021, samples were consecutively collected from patients whose guardians had agreed to participate in the study. Samples were collected from (200) patients from sulaymania children hospital 100 patients and erbil children hospital 100 patients, the study included (105 Male) and (95 Females). All patients were children aged between (1 month to 12 years) suffering from diarrhea, this study included a set of tests.

### Fecal sample collection

Stool samples were collected randomly from children with diarrhea, Diarrhea is objectively defined as passing a stool weight or volume greater than 200 g or 200 mL per 24 hours (Davies GJ, et al, 1986), and the samples were transferred directly to the laboratory for the following analyzes:

### General Stool Examination (GSE):

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract. 5 ml of stool was collected from children suffering from diarrhea, equivalent to 2 g of dry stool, in a clean, dry container and sent to the laboratory for examinations by the naked eye, including color, structure, shape, presence of blood, and mucus, and then microscope, including looking for bacteria, parasites, eggs, pus cells, and blood cells These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer (Al-Abri SS, et al, 2005).

### Blood sample collection

Drawing blood samples then transferred to anticoagulant tested tube from the same children from whom stool was taken, and transmitting the sample to a hematology laboratory for (CBC test) examination, complete blood counts were performed with a

device (Auto Hematology Analyzer, HEMA-D6031).

### Demographic information

A questionnaire was completed during the interview to collect the demographic information by asking demographic questions in surveys, and can gather demographic information at scale and, in turn, help you design a strategy to reach the goal.

### Bacterial isolation

Stool samples from patients were collected with signs and symptoms of diarrhea. A loop of stool (20 ul) was inoculated on three differential Medias (MacConkey agar, Blood agar, and Mannitol salt agar) and incubated at 37°C for 16 hours. For isolation of *E. coli*, a typical colony was chosen on the MacConkey agar and streaked again on eosin methylene blue (EMB) for further confirmation through the development of metallic sheen green.

### Biochemical tests

IMViC reactions are a set of four chemical reactions or a series of tests used to study the physiological properties of bacteria of the family Enterobacteriaceae. It is designed to distinguish Gram-negative Enterobacteriaceae of the family Enterobacteriaceae which it contains a large number of genera that are chemically and genetically related to each other, and each letter in "IMViC" stands for one of these tests (I - indol, M - methyl red, V-Voges-Proskauer, C- jackets, and the letter "i" is only for the rhyming purpose), was used for further bacterial identification and confirmation. (Sylvia, 2009)

### Data processing and analysis (Statistical Analyses)

Immediately after the completion of data collection, both the questionnaire and medical analysis were carefully reviewed for completeness and consistency. Then the data was entered into the Excel program and transferred to the statistical analysis program for analysis using SPSS which is the abbreviation of Statistical Package for the Social Sciences, (IBM"SPSS" Statistics Base 22.0) by ANOVA test, T test used and the P value 0.05 was considered significant value. Descriptive statistical analysis, was used to calculate the frequency, percentages, and average results of this study. The results were presented using tables and graphs.

## RESULTS

A total of 200 stool samples were included in the study stool appearance with 55% of semi-liquid, 32% watery stool, regarding stool color 54% yellow, 52% brown,. Pus cell 75.5% seen, RBCs 82% presented, while the remainder 18% there were no RBCs in the stool samples, there was no significant association between bacterial infection and stool examination ( $P > 0.05$ ), while data analysis shows there was statistically significant links between types of bacterial infections and stool appearance ( $P < 0.05$ ), as shown in table (1).

**Table -1** - Distribution of the study groups according to types of bacterial infection with General stool examination test

Study groups	<i>E coli</i>	%	<i>Klebsella</i>	%	Both	%	Total	%	P value
<b>Colour</b>									
Yellow	3	50	57	56.4	48	51.6	108	54	0.873
Greenish Yellow	0	0.0	2	2	4	4.3	6	3	
Brown	2	33.3	30	29.7	32	34.4	64	32	
Yellowish Brown	1	16.7	9	8.9	5	5.4	15	7.5	
White	0	0.0	3	3	4	4.3	7	3.5	
<b>Appearance</b>									
Liquid	1	16.7	27	26.7	43	46.2	71	35.5	0.011
Semi-Liquid	5	83.3	74	73.3	50	53.8	129	54.5	
<b>Pus cell</b>									
seen	3	50	82	81.2	66	71	151	75.5	0.97
Not Seen	3	50	19	18.8	27	29	49	24.5	
<b>RBCs</b>									
seen	4	66.7	83	82.2	77	82.8	164	82	0.653
Not Seen	2	33.3	18	17.8	16	17.2	36	18	

### Diagnose of *Escherichia coli* and *Klebsiella spp.*

In the first step, we used EMB ( Eosin Methylene Blue Agar) medium which is a selective and differential agar medium used to isolate Gram-negative in stool samples. The bacteria which ferment the lactose in the medium form colored colonies, while those that do not ferment lactose appear as colorless colonies. Then we used biochemical tests to diagnose the bacteria present.

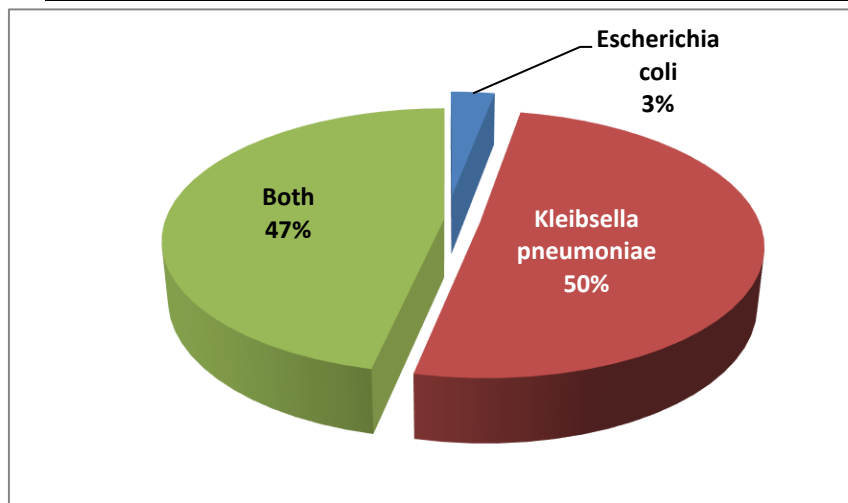
The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. Indole test Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase, Production of indole is detected using Ehrlich's reagent, Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top. - Formation of a red or pink coloured ring at the top is taken as positive(*Escherichia coli*: Positive; *Klebsiella spp.*: Negative).

Methyl Red test This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less. Development of red color is taken as positive. MR negative organism produce yellow color.( *Escherichia coli*: Positive; *Klebsiella spp.*: Negative).

Citrate utilization test This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. If the organism has the ability to utilize citrate, the medium changes its color from green to blue it is citrate Positiv.( *Escherichia coli*: Negative; *Klebsiella spp.*: Positive).

**Figure -1-** Distribution of the study groups according to stool culture results.

Study groups	Frequency	%
<i>Escherichia coli</i>	6	3.0
<i>Klebsiella spp.</i>	101	50.5
Both	93	46.5
Total	200	100



## Demographic information

Demographic information provides data regarding research participants and includes different concepts necessary to understand such as sex, age, blood group ....etc (Table 2).

**Table -2** - Distribution of the study groups according to the Demographic information

Study groups		Frequency	%
Gender			
	Male	105	52.5
	Female	95	47.5
Age groups			
	Infant	58	29
	Toddler and preschool	85	42.5
	School Age and Above	57	28.5
Blood group			
	A+	55	27.5
	B+	29	14.5
	AB+	21	10.5
	O+	88	44
	A-	1	0.5
	B-	0	0
	AB-	3	1.5
	O-	3	1.5
Feeding type			
	Breast Milk	13	6.5
	Bottle Milk	48	24
	Mix	27	13.5
	Neither	112	56
Complementary Foods			

	Yes	99	49.5
	No	101	50.5
Water Supplement			
	Tap Water	131	65.5
	Boiled Water	34	17
	Bottle Water	28	14
	Well Water	7	3.5
Place of residence			
	Kindergarten	10	5
	School	57	28.5
	Home	133	66.5
Residency			
	Urban	178	89
	Rural	22	11
Types of the House			
	Rent	69	34.5
	Ownership	131	65.5
Number of Children			
	1.00	30	15
	2.00	60	30
	3.00	76	38
	4.00	19	9.5
	5.00	8	4
	6.00	4	2
	7.00	3	1.5
Mother Education			

	Illiterate	17	8.5
	Read and Write	19	9.5
	Primary School	48	24
	Secondary School	57	28.5
	Institute	26	13
	College and Above	33	16.5
<b>Mother Occupation</b>			
	Employed	43	21.5
	Unemployed	28	14
	self-employed	2	1
	House Wife	126	63
	Retired	1	0.5
<b>Monthly Family Income</b>			
	Sufficient	88	44
	Barely Sufficient	83	41.5
	Insufficient	29	14.5
<b>How long diarrhea lasts</b>			
	Within 2 days	121	60.5
	Within 1 week	62	31
	More than 1 week	17	8.5

A total of 200 children (52.5% male and 47.5% female) were included. The highest percentages were for children's ages (42.5% for toddlers and preschoolers) and blood types (44% o+). As for the type of feeding, the largest number of children who completed the age of milk intake was (56% of those who did not drink milk) because they eat the usual food. The water given to the child was (65.5% tap water) and for the place of residence it was (66.5% in the house), and the majority of the children lived in the city (89% in the urban) and the type of housing. . The women residing in it (65.5% owners), the educational level of the mother (28.5% at the secondary level), and the highest percentage of sons were (38% and they have three children). The percentage of employed was few (77% House Wife), the monthly Family Income was (44% Sufficient) and for the largest child's diarrhea period was (60.5% within two days).

The (table2) shows the sociodemographic attributes of the participants. It is illustrated the univariate analysis of socioeconomic properties variables and investigations of sample study. A total of 200 children (52.5% male and 47.5% female) were included. The highest percentages were for children's ages (from birth to 12 year old) (42.5% for toddlers and preschoolers) predominate blood types was O+(44%). The largest number of children who completed the age of milk intake was (56% of those who did

not drink milk). Mostly drink tap water (65.5%) unfortunately most of the participants not went to school (66.5%), and the majority of the children lived in the city urban (89%) and the type of habitation of The family is (65.5% owners), the educational level of the mother (28.5% at the secondary level), and the highest percentage of sons were (38% and they have three children). The percentage of employed was few (77% House Wife), the monthly Family Income was (44% Sufficient) and for the largest child's diarrhea period was (60.5% within two days).

**Table -3** Relation of Demographic and socioeconomic characteristics *E coli* and *Klebsiella spp.* Infection

Study groups	Characteristics	<i>E coli</i>	%	<i>Kleib sella spp.</i>	%	Both	%	Total	%	P value
Age groups										
	Infant	1	16.7	32	31.7	25	26.9	58	29.0	0.154
	Toddler and preschool	4	66.7	47	46.5	34	36.6	85	42.5	
	School Age and Above	1	16.7	22	21.8	34	36.6	57	28.5	
Gender										
	Male	0	0.0	57	56.4	48	52.5	105	52.5	0.008
	Female	6	100	44	43.6	45	48.4	95	47.5	
Blood group										
	A+	1	16.7	32	31.7	22	23.7	55	27.5	0.866
	B+	1	16.7	15	14.9	13	14	29	14.5	
	AB+	0	0.0	11	10.9	10	10.8	21	10.5	
	O+	4	66.7	39	38.6	45	48.4	88	44	
	A-	0	0.0	0	0.0	1	1.1	1	0.5	
	B-	0	0.0	0	0.0	0	0.0	0	0.0	
	AB-	0	0.0	2	2.0	1	1.1	3	1.5	
	O-	0	0.0	2	2.0	1	1.1	3	1.5	

Feeding type										
	Breast Milk	0	0.0	11	10.9	2	2.2	13	6.5	0.093
	Bottle Milk	1	16.7	28	27.7	19	20.4	48	24	
	Mix	1	16.7	14	13.9	12	12.9	27	13.5	
	Neither	4	66.7	48	47.5	60	64.5	112	56	
Complementary Foods										
	Yes	2	33.3	50	49.5	47	50.5	99	49.5	0.716
	No	4	66.7	51	50.5	46	49.5	101	50.5	
Water Supplement										
	Tap Water	4	66.7	65	64.4	62	66.7	131	65.5	0.998
	Boiled Water	1	16.7	17	16.8	16	17.2	34	17.0	
	Bottle Water	1	16.7	15	14.9	12	12.9	28	14.0	
	Well Water	0	0.0	4	4.0	3	3.2	7	3.5	
Place of residence										
	Kindergarten	1	16.7	8	7.9	1	1.1	10	5.0	0.025
	School	1	16.7	22	21.8	34	36.6	57	28.5	
	Home	4	66.7	71	70.3	58	62.4	133	66.5	
Mother Education										
	Illiterate	0	0.0	9	8.9	8	8.6	17	8.5	0.120
	Read and Write	1	16.7	8	7.9	10	10.8	19	9.5	

	Primary School	0	0.0	29	28.7	19	20.4	48	24	
	Secondary School	3	50	22	21.8	32	34.4	57	28.5	
	Institute	0	0.0	18	17.8	8	8.6	57	13	
	College and Above	2	33.3	15	14.9	16	17.2	33	16.5	
<b>Mother Occupation</b>										
	Employed	3	50	21	20.8	19	20.4	43	21.5	0.512
	Unemployed	1	16.7	15	14.9	12	12.9	28	14.0	
	self-employed	0	0.0	2	2.0	0	0.0	2	10	
	House Wife	2	33.3	62	61.4	62	66.7	126	63.0	
<b>Monthly Family Income</b>										
	Sufficient	3	50	47	46.5	38	40.9	88	44	0.662
	Barely Sufficient	2	33.3	34	42.6	38	40.9	83	41.5	
	Insufficient	1	16.7	11	10.9	17	18.3	29	14.5	
<b>How long diarrhea lasts</b>										
	Within 2 days	4	66.7	60	59.4	57	61.3	121	60.5	0.839
	Within 1 week	2	33.3	31	30.7	29	31.2	62	31.0	
	More than 1 week	0	0.0	10	9.9	7	7.5	17	8.5	
<b>Appearance of Stool</b>										
	Watery	1	16.7	23	22.8	40	43.0	64	32	0.50
	Semi-liquid	4	66.7	61	60.4	45	48.4	110	55	

Mucoid	1	16.7	15	14.9	8	8.6	24	12
Bloody Diarrhea	0	0.0	2	2.0	0	0.0	2	1.0

The p-value (gender and place of residence) were  $P < 0.05$  the result is significant statistically, the Feeding type is Eloquent to significant, and other was  $P > 0.05$  the results are not significant statistically.

### Complete blood count test (CBC)

We are aware that CBC parameters have poor ability to distinguish between *Escherichia coli* and *Klebsiella* because the results are very close. The blood tests showed that the high probability value ( $P > 0.05$ ) means that the results are not statistically significant except for the MID% that was ( $P < 0.05$ ), which is statistically significant.

In order to find a relationship between existing data, we analyzed CBC parameters using the model of mean difference (more correctly, "difference in means") is a standard statistic that measures the absolute difference between the mean value of two groups in a clinical trial (Juthatip C. et al., 2018), (Table 4.).

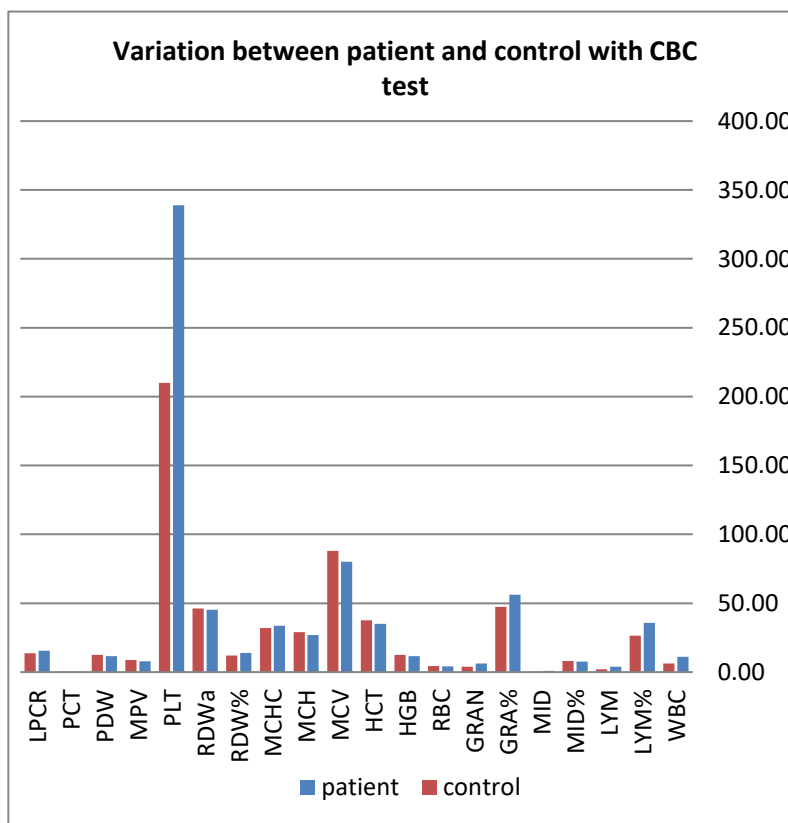
$$\text{Mean Difference} = \frac{\sum x_1}{n} - \frac{\sum x_2}{n}$$

$x_1$  = Mean of group one     $x_2$  = Mean of group two     $n$  = Sample size

**Table-4-** Comparison of the mean data between patients and control of hematological parameter

	patient	control	variation	unit
WBC	11.19	6.23	4.96	$10^9/L$
LYM%	35.78	26.4	9.38	%
LYM	3.98	2.02	1.96	$10^9/L$
MID%	7.73	8.2	-0.47	%
MID	0.88	0.5	0.38	$10^9/L$
GRA%	56.10	47.4	8.70	%
GRAN	6.14	3.91	2.23	$10^9/L$
RBC	4.05	4.47	-0.42	$10^{12}/L$
HGB	11.52	12.5	-0.98	g/dL
HCT	34.97	37.6	-2.63	%
MCV	80.21	87.9	-7.69	fL
MCH	26.91	28.9	-1.99	pg

MCHC	33.63	32.1	1.53	g/dL
RDW%	13.80	12.1	1.70	%
RDWa	45.22	46.1	-0.88	fL
PLT	338.96	210	128.9	10 <sup>9</sup> /L
MPV	7.88	8.8	-0.92	fL
PDW	11.59	12.6	-1.01	%
PCT	0.27	0.128	0.14	%
LPCR	15.59	13.7	1.89	%



As we seen, the means of WBC, LYM, GRN, PLT, PCT, and LPCR in the patient group were higher than those in the control group. The mean RBC, HCT, MCV, and MCH in the patient group were lower than the control group. Many conditions affect the number and characteristics of red and white blood cells and platelets. A CBC can determine if these changes are different from what they should be and whether or not they require attention.

## DISCUSSION

Diarrhea will remain the main life-threatening problem for children. The direct stool examination shows that 55% of Semi-liquid, 32% Watery, Pus cell 75.5% seen, *E. histolytica* 8.5% seen, there is no appearance of (Ova, *Giardia lamblia*, and *T*

*hominia*). However, this percentage *E. histolytica* in this study is lower than that obtained by (Rabatti,2008) (60.3%) in 70 patients reported from Erbil.

Demographic information is used in this study to estimate the prevalence of diarrhea and its determinants and causes of *E. coli* and *Klebsiella sp.*, (gender and place of residence) were  $P < 0.05$  the result was significant, feeding type is Eloquent to significant, and other was  $P > 0.05$  or non-significant. This does not mean that other factors have no effect on the infection, the largest sufferers are females, and the reason may be due to a technical error when collecting samples by mixing drops of urine with stool, and this does not happen in males because of the physiological difference. Children infected with *E. coli* range in age from one to five years, which is consistent with large numbers of research that confirm that children under five years of age are exposed to diarrhea that threatens their lives (Mbugua et al, 2014).

The relationship of infection with blood groups was the largest percentage of patients with blood type O + and this is normal because the number of children participating in the study was the percentage of blood types of type O + They are similar to the results obtained (Amit Agrawal,et al. 2014).

Regarding nutrition, children who do not drink milk are more susceptible to infection than those who drink milk, and children who drink breast milk do not get any of the *E. coli*. As for the sources of drinking water, a large percentage of children use tap water for drinking, and the cause of infection may be drinking water. Children infected with *E. coli* are those who have diarrhea for a short period, i.e. acute type, which is a sign of *E. coli*, and children who suffer from diarrhea for more than a week, which means chronic, have a low incidence of *E. coli* and may suffer from other problems (Penders J, et al. 2006).

The complete blood count means of WBC, and PLT in the patient group were higher than in the control group. While the mean of RBC was lower. When the white blood cell count increases, it indicates infection and inflammation with pathogens such as bacteria As an immune response, when infected with bacteria, it attacks the endothelial tissues of the digestive system and this leads to its damage, and this stimulates and activates the platelets and assembles quickly because of the multiple receptors on their surface, which increases their numbers and also has a role in stimulating the nearby white blood cells, when bacteria attack the endothelial tissues of the digestive system leads to the loss of red blood and a decrease in its number as stated in (Liberki et al, 2020).

According to the stool culture and biochemical tests result the most frequent bacteria was (*E.coli* 3% and *Klebsiella spp.* 50%, and both were 46.5% done from September to December as it is wet and cool seasons , which is different with the result reported in kirkuk city by (Thekra et al, 2016) which was *E.coli* 29.40% and *Klebsiella* 26.5% from May to September .

This result can be linked to several factors such as environmental conditions, health practices, patient conditions, personal hygiene, the number of patients involved in the study, and laboratory procedures.

The difference may be due to the technique of collecting samples or the examination process and its accuracy, and the temperature may have an important role in the high rate of diarrhea and *E. coli* infection. Research done in warm seasons will have a higher rate than research done in cold seasons with the pathotypes being more frequent in the dry seasons than in the wet seasons. A global meta-analysis of 28 individual studies reported an 8% increase in the incidence of diarrheagenic *E. coli* per 1C° increase in the mean monthly temperature ( Philipsborn et al, 2016).

## CONCLUSION

Diarrhea is a public health risk for children. There are many causes of diarrhea; According to this study, acute diarrhea is caused by bacteria (*E. coli* and *Klebsiella spp.*) among children. *Klebsiella spp.* was dominant among children with diarrhea, and socio-economic conditions played a role in the spread of diarrheal disease.

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