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## Bacteriological study of diarrheal cases in children under 5 years in the Tikrit city

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### ABSTRACT

Diarrheal disease is common among children worldwide, especially developing countries, and common causes of mortality in children under the age of five. One hundred and fifty stool samples were collected from children with diarrhea below five years from the beginning of August 2016 until the end of December 2016. The prevalence of diarrhea was different among males and females: 47.11% of males were collected, while 52.88% of bacterial females. The study revealed that 104 isolates were identified from patients with diarrhea. These bacteria were isolated and diagnosed based on phenotype and biochemical tests. The most isolated bacteria were *Serratia odorifera* (17.30%), followed by *E.coli* (14.42%), *Salmonella* (9.61%), *K.pneumoniae* (5.76%) and *S.saprophyticus* (5.76%), the results of the sensitivity test for 13 antibiotics were shown using the disc method and there was a different the levels of resistance to different antimicrobial agents.

## **Introduction:**

The diarrhea in infants is a common cause of death between in children under of age under five years<sup>(1)</sup>.

The causes of diarrhea are different types, may be viral or bacteriological or parasitic. The rotaviruses cause about 40% of cases of acute diarrhea in the first 5 years, while adenoviruses cause 30% . They are found about 20% of the children infected by bacterial of *Campylobacter jejuni* , *Yersinia*, *Salmonella*, *Shigella*, pathogenic *E. coli* and *Clostridium difficile*<sup>(2)</sup>.

The incidence of the diarrhea because of pathogens in the cavity of the intestine of children because the contamination of the food and drinks and hands by pathogens or the changes of some normal flora in the intestines to the pathogens or by changes in the bowel environment by taking drug of these a microorganisms<sup>(3)</sup>. The study aims to: Isolate and diagnose of bacterial causes of diarrhea in children and test the sensitivity of these isolates towards some types of antibiotics.

## **Material and Methods**

### **Preparation of culture media:**

Culture media were prepared according to manufacturer's instructions installed on the packagings. The circles were sterilized at a temperature of 121 ° C and pressure of 15 lb / kg for 15 min. After incubation, incubate at 37 ° C for 24 hours before use to make sure it was not contaminated Until use<sup>(4)</sup>.

**Collection of Samples:** One hundred fifty samples were collected from children with diarrhea under five years in the emergency of Salah Aldin General Hospital for the period from the beginning of August 2016 to the end of December 2016. These samples were collected in sterile plastic bottles. Child who had taken antibiotics before entering Hospital emergency was excluded from study.

**Culture of Sample :** Samples were planted on the blood agar , Maconkey and Manitol salt agar, incubated at 37 ° C for 24 hours. The developing colonies were then

purified to obtain individual colonies for diagnosis through their phenotypic and chemical properties<sup>(5)</sup>.

### **Diagnosis of bacteria using API 20**

**E:** The tape contains 20 small test tubes for chemical tests. Then, a homogeneous suspension consisting of a saline solution and a bacteria to be diagnosed was then transferred to 0.12 ml of bacterial suspension for each test tube and 0.28 ml of suspension for GEL, VP, CIT and 0.1 ml oil for the ODC, LDC, ADH, URE, H<sub>2</sub>S Provide anaerobic conditions. The reagents were added to the incubator at 37 ° C for 24 hours. The reagents were added according to the requirements of each test and the results were read, turn the results were turned into numbers and then compare a with the codes in the index supplied by the company (Bio-Merieux / France) to give the name of the species and type of bacteria.

**Antibiotic Susceptibility Test of Bacterial Isolates:** The antibiotic

sensitivity test was conducted on the Muller Hinton agar using the antibiotics prescribed in Table 1. A single colony was transferred into tubes containing 5 mL of the Nutrient broth and incubated at 37°C for 24 hours, and then compared turbid growth with turbid constant solution standard turbid (McFarland solution). In the case of unequal turbidity, the salt was added solution to these tubes so that the turbidity is equal to the McFarland solution. Using Swab Cotton, the bacteria to be tested were spread on the surface of Muller Hinton agar. The discs were placed on the surface of the plate using sterile forceps. Then the inhibition zone was measured<sup>(6)</sup>.

### **Results :**

The results showed that 116 samples gave bacterial growth. Children were classified into four age groups. The highest percentage of diarrhea 81.73% was found in the 12-month age group, followed by 11.53% among the 13-24 month age group.

**Table 1: Types of antibiotics used in the study and their concentration ( $\mu$  / disc)**

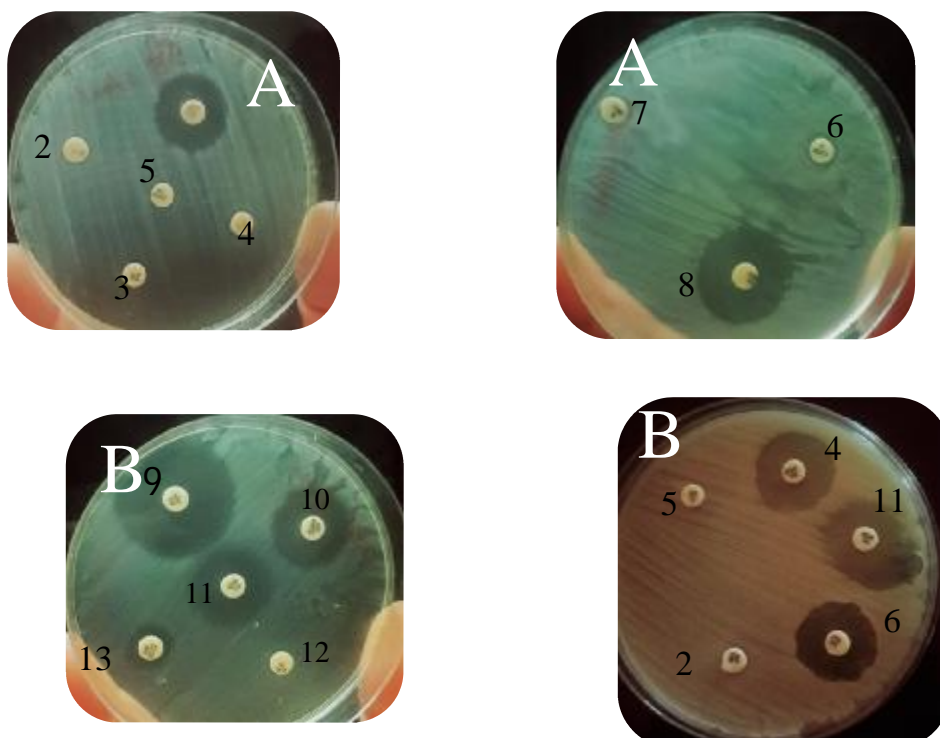
No.	Antibiotics	Symbol	Concentration disc/ $\mu$ g
1	Nitrofurantion	NI	300
2	Cefixime	CFM	5
3	Trimethoprim-Sulfamethoxaole	TS	23.75 /1.25
4	Penicillin-G	P2	10
5	Amoxicillin	AMX	30
6	Nalidixic Acid	NA	30
7	Cepodoxime	CPD	10
8	Cefotaxime/clavulanic acid	CEC	10/30
9	Cloxacillin	CX	5
10	Gentamicin	GM	10
11	Imipenem	IMI	10
12	Ampicillin	SAM	10
13	Cefuroxime	CXM	30

**Table 2: Distribution of samples by sex and age group of children with Diarrhea**

Age in months	Sex		Total	Total percentage
	Male	Female		
<b>Less than12</b>	41	44	85	%81.73
<b>24-13</b>	5	7	12	%11.53
<b>36-25</b>	2	2	4	%3.84
<b>48-37</b>	1	2	3	%2.88
<b>Total</b>	49	55	104	

**Table 3: types of isolated bacteria from patients with diarrhea**

<b>Isolated Bacteria</b>	<b>No.</b>	<b>%</b>
<i>Serratia odorifera</i>	18	17.30
<i>E.coli</i>	15	14.42
<i>Salmonella</i>	10	9.61
<i>K.pneumoniae</i>	6	5.76
<i>Shigella sonni</i>	4	3.84
<i>Klebsiella oxtoca</i>	5	4.80
<i>S.saprophyticus</i>	6	5.76
<i>Roultella orinithiolytica</i>	5	4.80
<i>Echerichia fergusonii</i>	2	1.92
<i>Serratia fonticola</i>	4	3.84
<i>Roultella orinithiolytica</i>	5	4.80
<i>Serratia liquefaciens</i>	3	2.88
<i>Hafnia alvei</i>	3	2.88
<i>Raoultella terrigena</i>	1	0.96
<i>Kluyvera cryocrescens</i>	1	0.96
<i>Kluyvera ascarbata</i>	1	0.96
<i>S.aureus</i>	5	4.80
<i>Stenotrophomonas maltophilia</i>	5	4.80
<i>Serratia marcescens</i>	2	1.92
<i>Enterobacter sakazakii</i>	2	1.92
<i>Pasteurella pneumotropica</i>	1	0.96
<i>Providencia alcalifaciens</i>	1	0.96
<i>Vibrio parahaemolyticus</i>	1	0.96
<i>Citrobacter freundii</i>	2	1.92
<i>Burkholderia cepacia</i>	1	0.96
<b>Total</b>	<b>104</b>	<b>100</b>



**Figure 1: Antibiotic Sensitivity testing of**

**A: *E.coli* , B: *S.saprophyticus***

- 1: Cefotaxime , 2: Amoxycillin , 3 :Cefpodoxime ,4 : Penicillin-G  
 5:Nalidixic Acid, 6 :Nitrofurantion ,7 : Trimethoprim, 8 :Cefixime  
 9:Imipenem ,10:Ampicillin ,11: Gentamicin ,12: Cloxacillin  
 13: Cefuroxime**



**A**



**B**

**Figure 2: Result of API tests 20 E for**

**A: *Serratia odorifera***

**B: *E.coli***

Table 4: Resistance and sensitivity of isolated bacteria from patients with diarrhea to antibiotics used in the study (Nitrofurantion, Cefixime, Trimethoprim-Sulfamethoxaole, Penicillin-G)

Penicillin-G				Trimethoprim-Sulfamethoxaole				Cefixime				Nitrofurantion				Antibiotics	
%	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	No.	Isolated Bacteria
0	0	100	18	11.1	2	88.9	16	22.2	4	77.8	14	44.4	8	55.6	10	18	<i>Serratia odorifera</i>
0	0	100	15	13.3	2	86.7	13	20	3	80	12	93.3	1	6.7	1	15	<i>E.coli</i>
0	0	100	10	10	1	90	9	10	1	90	9	60	6	40	4	10	<i>Salmonella</i>
0	0	33.3	2	16.7	1	83.3	5	16.7	1	83.3	5	50	3	50	3	6	<i>K.pneumoniae</i>
0	0	100	5	40	2	60	3	40	2	60	3	80	4	20	1	5	<i>Roultella orinitholytica</i>
0	0	100	2	0	0	100	2	0	0	100	2	50	1	50	1	2	<i>Echerichia fergusonii</i>
0	0	100	4	0	0	100	4	0	0	100	4	100	4	0	0	4	<i>Serratia fonticola</i>
0	0	100	4	25	1	75	3	0	0	100	4	75	3	25	1	4	<i>Shigella sonnei</i>
0	0	100	3	0	0	100	3	0	0	100	3	66.7	2	33.3	1	3	<i>Serratia liquefaciens</i>
0	0	100	3	0	0	100	3	0	0	100	3	66.7	2	33.3	1	3	<i>Hafnia alvei</i>
0	0	100	1	0	0	100	1	0	0	100	1	0	0	100	1	1	<i>Raoultella terrigena</i>
0	0	100	5	20	1	80	4	40	2	60	3	60	3	40	2	5	<i>Klebsiella oxtoca</i>
0	0	100	1	0	0	100	1	0	0	100	1	100	1	0	0	1	<i>Kluyvera cryocrescens</i>
0	0	100	1	0	0	100	1	0	0	100	1	0	0	100	1	1	<i>Kluyvera ascarbata</i>
0	0	100	5	0	0	100	5	20	1	80	4	80	4	20	1	5	<i>S.aureus</i>
0	0	100	6	16.7	1	83.3	5	0	0	100	6	83.3	5	16.7	1	6	<i>S.saprophyticus</i>
0	0	100	5	40	2	60	3	0	0	100	5	80	4	20	1	5	<i>Stenotrophomonas maltophilia</i>
0	0	100	2	0	0	100	2	0	0	100	2	0	0	100	2	2	<i>Serratia marcescens</i>
0	0	100	2	50	1	50	1	50	1	50	1	50	1	50	1	2	<i>Enterobacter sakazakii</i>
0	0	100	1	0	0	100	1	0	0	100	1	100	1	0	0	1	<i>Pasteurella pneumotropica</i>
0	0	100	1	0	0	100	1	0	0	100	1	100	1	0	0	1	<i>Providencia alcalifaciens</i>
0	0	100	1	0	0	100	1	0	0	100	1	100	1	0	0	1	<i>Vibrio parahaemolyticus</i>

Table 5: Resistance and sensitivity of isolated

0	0	100	2	0	0	100	2	0	0	100	2	50	1	5
0	0	100	1	0	0	100	1	0	0	100	1	100	1	0

bacteria from patients with diarrhea to antibiotics used in the study

Cloxacillin				Cefotaxime/clavulanic acid				Cefpodoxime				Nalidixic Acid				Amoxicillin				N o.	Antibiotics
%	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R		Isolated Bacteria
0	0	100	1	22.2	4	77.8	1	16.7	3	83.3	1	55.6	1	44.4	8	5.6	1	94.4	1	1	<i>Serratia odorifera</i>
0	0	100	1	6.7	1	93.3	1	13.3	2	86.7	1	53.3	8	46.7	7	0	0	100	1	1	<i>E.coli</i>
0	0	100	1	20	2	80	8	10	1	90	9	40	4	60	6	0	0	100	1	1	<i>Salmonella</i>
0	0	100	6	16.7	1	83.3	5	16.7	1	83.3	5	0	0	33.3	2	0	0	33.3	2	6	<i>K.pneumoniae</i>
0	0	100	5	20	1	80	4	40	2	60	3	80	4	20	1	0	0	100	5	5	<i>Roultella orinitholytica</i>
0	0	100	2	0	0	100	2	0	0	100	2	50	1	50	1	0	0	100	2	2	<i>Echerichia fergusonii</i>
0	0	100	4	0	0	100	4	0	0	100	4	50	2	50	2	0	0	100	4	4	<i>Serratia fonticola</i>
0	0	100	4	0	0	100	4	0	0	100	4	75	3	25	1	0	0	100	4	4	<i>Shigella sonnei</i>
0	0	100	3	33.3	1	66.7	2	0	0	100	3	0	0	100	3	0	0	100	3	3	<i>Serratia liquefaciens</i>
0	0	100	3	33.3	1	66.7	2	0	0	100	3	0	0	100	3	0	0	100	3	3	<i>Hafnia alvei</i>
0	0	100	1	0	0	100	1	0	0	100	1	100	1	0	0	0	0	100	1	1	<i>Raoultella terrigena</i>
0	0	100	5	20	1	80	4	0	0	100	5	80	4	20	1	0	0	100	5	5	<i>Klebsiella oxtoca</i>
0	0	100	1	0	0	100	1	0	0	100	1	0	0	100	1	0	0	100	1	1	<i>Kluyvera cryocrescens</i>
0	0	100	1	0	0	100	1	0	0	100	1	0	0	100	1	0	0	100	1	1	<i>Kluyvera ascarbata</i>
0	0	100	5	0	0	100	5	0	0	100	5	20	1	80	4	0	0	100	5	5	<i>S.aureus</i>



0	0	1	6	0	0	100	6	0	0	10	6	66	4	33	2	0	0	10	6	6	<i>S.saprophytic</i>
0	0	1	5	0	0	100	5	0	0	10	5	40	2	60	3	0	0	10	5	5	<i>Stenotrophomonas maltophilia</i>
0	0	1	2	0	0	100	2	0	0	10	2	0	0	10	2	0	0	10	2	2	<i>Serratia marcescens</i>
0	0	1	2	50	1	50	1	50	1	50	1	50	1	50	1	0	0	10	2	2	<i>Enterobacter sakazakii</i>
0	0	1	1	0	0	100	1	0	0	10	1	0	0	10	1	0	0	10	1	1	<i>Pasteurella pneumotropica</i>
0	0	1	1	100	1	0	0	10	1	0	0	10	1	0	0	0	0	10	1	1	<i>Providencia alcalifaciens</i>
0	0	1	1	0	0	100	1	0	0	10	1	0	0	10	1	0	0	10	1	1	<i>Vibrio parahaemolyticus</i>
0	0	1	2	0	0	100	2	0	0	10	2	50	1	50	1	0	0	10	2	2	<i>Citrobacter freundii</i>
0	0	1	1	0	0	100	1	0	0	10	1	10	1	0	0	0	0	10	1	1	<i>Burkholderia cepacia</i>
																					Total
																					104

( Nitrofurantion, Cefixime, Trimethoprim- Sulfamethoxaole, Penicillin-G)

Table 6: Resistance and sensitivity of isolated bacteria from patients with diarrhea to antibiotics used in the study

(Gentamicin, Imipenem, Ampicillin ,Cefuroxime)

Cefuroxime				Ampicillin				Imipenem				Gentamicin				No.	Antibiotics
%	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R		Isolated Bacteria
11.1	2	88.9	16	38.9	7	66.7	11	88.9	16	11.1	2	72	13	27.8	5	18	<i>Serratia odorifera</i>
13.3	2	86.7	13	33.3	5	66.7	10	86.7	13	13.3	2	66	10	33.3	5	15	<i>E.coli</i>
20	2	80	8	40	4	60	6	100	10	0	0	10	10	0	0	10	<i>Salmonella</i>
33.3	2	66.7	4	16.7	1	83.3	5	83.3	5	16.7	1	66	4	33.3	2	6	<i>K.pneumoniae</i>
40.0	2	60	3	40	2	60	3	60	3	40	2	80	4	20	1	5	<i>Roultella orinithiolytica</i>
0	0	100	2	0	0	100	2	50	1	50	1	50	1	50	1	2	<i>Echerichia fergusonii</i>

0	0	100	4	75	3	25	1	100	4	0	0	75	3	25	1	4	<i>Serriatia fonticola</i>
0	0	100	4	50	2	50	2	75	3	25	1	50	2	50	2	4	<i>Shigella sonni</i>
0	0	100	3	33.3	1	66.7	2	100	3	0	0	100	3	0	0	3	<i>Serratia liquefaciens</i>
0	0	100	3	33.3	1	66.7	2	100	3	0	0	100	3	0	0	3	<i>Hafnia alvei</i>
0	0	100	1	0	0	100	1	100	1	0	0	100	1	0	0	1	<i>Raoultella terrigena</i>
0	0	100	5	60	3	40	2	80	4	20	1	80	4	20	1	5	<i>Klebsiella oxtoca</i>
0	0	100	1	0	0	100	1	100	1	0	0	100	1	0	0	1	<i>Kluyvera cryocrescens</i>
0	0	100	1	100	1	0	0	100	1	0	0	100	1	0	0	1	<i>Kluyvera ascarbata</i>
0	0	100	5	0	0	100	5	80	4	20	1	20	1	80	4	5	<i>S.aureus</i>
16.7	1	83.3	5	0	0	100	6	66.7	4	33.3	2	83.3	5	16.7	1	6	<i>S.saprophytic</i>
0	0	100	5	0	0	100	5	100	5	0	0	60	3	40	2	5	<i>Stenotrophomonas maltophilia</i>
0	0	100	2	0	0	100	2	100	2	0	0	100	2	0	0	2	<i>Serriatia marcessens</i>
50	1	50	1	0	0	100	2	50	1	50	1	50	1	50	1	2	<i>Enterobacter sakazakii</i>
0	0	100	1	0	0	100	1	100	1	0	0	100	1	0	0	1	<i>Pasteurella pneumotropica</i>
100	1	0	0	100	1	0	0	100	1	0	0	100	1	0	0	1	<i>Providencia alcalifaciens</i>
0	0	100	1	100	1	0	0	100	1	0	0	100	1	0	0	1	<i>Vibrio parahaemolyticus</i>
0	0	100	2	0	0	100	2	50	1	50	1	50	1	50	1	2	<i>Citrobacteria freundii</i>
0	0	100	1	0	0	100	1	100	1	0	0	100	1	0	0	1	<i>Burkholderia cepacia</i>
																104	total

### Discussion:

A low percentage 3.48% in the 25-36 month age group and 2.88% in the 37 to 48 month age group. In comparison to previous studies, Jameel<sup>(7)</sup> in Salah Aladdin found that

the percentage of persistent diarrhea was 45%. Alrifai<sup>(8)</sup> found that the highest incidence of diarrhea in children under 12 months was 67.9%.

The prevalence of diarrhea was different among males and

females. 49 (47.11%) were males, while 55 (52.88%) were females.. Table 2.

The study revealed that 104 isolates from patients with diarrhea reported, the most isolated bacteria were *Serratia odorifera* 18 (17.30%), followed by 15 (14.42%) isolates belonging to *E.coli*, and 10 (9.61%) isolates belonging to *Salmonella*, isolate of *k.pneumoniae* and *S.saprophyticus* 6(5.76%). As for the distribution of *E. coli*, this study does not agree with obtained by Tawfeeq<sup>(9)</sup> (34.48%), (Table 3).

Bacterial isolates showed different patterns of antibiotic resistance , as shown in Table 4,5,6 .Bacteria were multi-resistant and is difficult to choose the appropriate treatment for the patients<sup>(10)</sup> .Of the 15 strains of *E. coli*, the percentage of resistance was 100% for amoxycillin, penicillin-G and cloxacillin which was agree with Abed Jabuk<sup>(11)</sup> in Babylon, 100% for amoxycillin and penicillin-G Akter<sup>(12)</sup> in Bangladesh 96.93% were resistant to Cloxacillin.

The isolates of *E.coli* were 86.7% resistant to cefuroxime, cefpodoxime and trimethoprim- sulfamethoxaole. This is agree with Jameel<sup>(7)</sup>, who found that the resistance to trimethoprim was 89.5%. Schumacher<sup>(13)</sup> in Denmark, which found the percentage of resistance was 4.4% towards cefuroxime. *E.coli* was 80% resistant to cefixime and this is not agree with Smith and Eng<sup>(14)</sup> in New York who found the resistance ratio 1% and Akter<sup>(12)</sup> 86.5%. The percentage of resistance to nalidixic Acid 46.7% was agree with Tawfeeq<sup>(9)</sup>, 45% and did not agree with Ahmad and Ali<sup>(16)</sup> 14.3%. The percentage of resistance to gentamicin 33.3% was agree with Tawfeeq<sup>(9)</sup> was 30% and Akter<sup>(12)</sup> was 45.4%.

The percentage of resistance to Imipenem was 13.3% The resistance percentage to nitrofurantion was 5.5%

and the result was agree with Tawfeeq<sup>(9)</sup>12.5% and did not agree with AL-Tayar<sup>(18)</sup>. Libya,(40%). Of the 10 isolates of *Salmonella*, the

percentage of resistance 100% for amoxicillin, penicillin-G and cloxacillin was agree with Tawfeeq<sup>(9)</sup>, which found that the percentage of penicillin-G 100% and the percentage of resistance to amoxicillin 45.8%. In another study, *Salmonella* sensitive to amoxicillin and this agrees with Lucky<sup>(17)</sup>. The percentage of resistance to cefixime, cefpodoxime and trimethoprim-sulfamethoxazole is 90%. This is agrees with Moehario<sup>(18)</sup>98.6%. The percentage of resistance to cefotaxime / clavulanic acid and cefuroxime 80% was not agree with Muthu<sup>(19)</sup>3.75%. The percentage of resistance to ampicillin and nalidixic acid was 60%. Tawfeeq<sup>(9)</sup>found that the percentage of resistance to nalidixic acid was 66.7% and ampicillin was 62.5%. The percentage of nitrofurantion resistance was 40%, which is not agree with result of Al-Sorchee<sup>(20)</sup> who in Erbil, which was sensitive 100%. This study was agreed with Tawfeeq<sup>(9)</sup>, which found the sensitivity of imipenem 100% and was not agree with Patel<sup>(21)</sup> in India,

which found the percentage of resistance gentamicin 20%. Among the four isolates of *Shigella sonni* found the percentage of resistance 100% for each of cefixime and penicillin-G and amoxicillin and cefodoxime and cefotaxime / clavulanic acid and cloxacillin and cefuroxime This result is agree with Tawfeeq<sup>(9)</sup> in the percentage of resistance to the penicillin-G 66.7% and do not agree with Trivedi<sup>(22)</sup> in the United States of America who found most of the isolates were sensitive to cefotaxime. The percentage of resistance to the gentamicin and ampicillin was 50% and these are not agree with Pourakbari<sup>(23)</sup> in Iran who have found the most isolates were sensitive to gentamicin. The percentage of resistance was 25% for both imipenem and nitrofurantion and nalidixic acid this is agreed with Lestari<sup>(24)</sup> in Indonesia, who revealed that isolates were sensitive to nalidixic acid, but agree with the Tawfeeq<sup>(9)</sup>, which found the resistance to nitrofurantion was 33.3%.

## Conclusion

The most common isolated bacteria were *Serratia odorifera* (17.30%), followed by *E.coli* (14.42%), followed by *Salmonella* (9.61%), followed by *K.pneumoniae* (5.76%).

The results of the sensitivity tests showed that the optimal antibiotics for diarrhea in children were Imipenem and Nitrofurantion while Amoxicillin, Penicillin-G and Cloxacillin were not effective against all bacterial isolates

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