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### Detection of *Salmonella*, *Shigella* and *Candida* spp. in stool from diarrheal children and evaluation the heating effect on *Salmonella* phage in Kirkuk city

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#### ABSTRACT

**Background:** *Salmonella* spp. and *Shigella* are two pathogenic members within the Enterobacteriaceae family, and they are causing food poisoning and diarrhea that transmits via oral route by contaminated food and water especially in children from ages 1 day to 14 years.

**Objective:** focus on the study of *Salmonella* spp., *Shigella* and *Candida* spp. isolated from diarrheal children in Kirkuk city and diagnose via in vitro bacterial diagnosis with traditional fermentation chemical tests, API 20E, RapIDTM ONE technology and Vitek 2 compact system, as well as study the thermal stability of isolated *Salmonella* phage,

**Method:** Collecting (200) cases of diarrhea from children in Kirkuk hospitals, through an epidemiological statistical study and conventional methods for diagnosis enteric bacteria with API 20E and RapIDTM ONE, and non-typhi *Salmonella* isolates (*S.typhimurium*) were identified with Vitek 2 compact System. Bacterial sensitivity tests to antibiotics were verified by Kerby - Bauer disk diffusion method and *Candida* spp. with CHROM agar *Candida* and API 20C AUX ; The *Salmonella* phage was isolated by spot assay, then exposed to different temperatures before the observation of degradation range of the exposed phage to the salmonella non-typhi, on Tryptic Soy Agar medium (TSA).

**Results:** *Salmonella* spp., *Shigella* and *E. coli* isolated in rate of 6%, 0.5% and 51.5% respectively while intestinal *candida* detected in rate of 89.5% in total of 200 children were diagnosed, in which 113 samples of males (56.5%) and 87 samples of females (43.5%) ( $p > 0.05$ ), the degradation range was observed to the exposed phage to degrees (35, 40) C. for (15, 30) minutes, and stopped at the exposed *Salmonella* phages to temperatures (45) at 30 minutes.

**Conclusion:** The prevalence of *salmonella* and *shigella* was relatively low and the highest incidence of *Salmonella* was within the age groups ranging between (11-14) and (1-6) year compared to other age groups. Isolated strains showed multidrug resistance (MDR), As well the genus *Candida albicans* was the most common type compared to other intestinal *Candida* species in children with diarrhea. and it has been found that *salmonella* phage can be isolated from sewage water and chicken's droppings by simple methods.

#### Introduction

Diarrheal disease is one of the threats to human health that could cause significant economic losses in terms of medicines and other expenditures [22; 14; 9] Non-typhoid *salmonella* (NTS) infection occurs via mouth and mucosal systemic invasion, causing gastroenteritis in humans, The incubation: 8-48 hrs.; duration for diarrhea 3-7 days and 72 hrs. for fever

limited to GI tract straight accompanied by diarrhea associated with fever ( $> 39$ ) ° C: diarrhea, nausea, and vomiting, abdominal cramps after (12-72) hours of exposure, also accompanied by loose, bloody stool; Pseudo appendicitis (rare). Stool culture will remain positive for 4-5 weeks •  $< 1\%$  will become carriers and [2]. Once it crosses the hostile

environment of the stomach, it is absorbed by the intestinal epithelial cells and released into the underlying connective tissue where it begins to multiply indicated that NTS infection progresses to bacteremia and complications that extend beyond the intestine, such as (UTI) urinary tract infection, meningitis, endocarditis, and pneumonia [7].

Shigellosis is characterized by inflammation and ulceration in the large intestine, and the loss of their fluids results from infection with the *Shigella* species, causing gastroenteritis in humans, accompanied by diarrhea. *Shigella* has an effective acid-fighting system (the glutamate decarboxylase system) that gives *shigella* the ability to withstand a very acidic stomach environment and cause inflammation and ulceration in the large intestine [23].

The types of intestinal *Candida* yeasts density increase in the feces of malnourished children, and become pathological when appropriate environmental conditions are provided and an opportunistic infection in healthy people. Candidiasis can occur as a result of a dysfunction in the epithelial layer of the human intestine [15]. It is often associated with antibiotic-related diarrhea in children and has been reported as the only "pathogen" in the feces of children with diarrhea [5; 12; 9].

*Salmonella* is facultative anaerobic, bacilli not forming spheres of approximate size (2-5)  $\mu$  length (0.8-1.5)  $\mu$  width, it moves with Peritrichous flagella except for two spp. such as *S. pullorum* and *S. gallinarum*. The optimum growth temperature for both ranges between (35-37) °C, but some species grow at (5-47) °C and kill at the normal cooking temperature of (70) °C [17]. These organisms grow on mainly nutrient media Salmonella Shigella agar (SSA), Xylose lysine deoxycholate (XLD), and (TSA) [11]. *Salmonella* has a respiratory and fermentary metabolism, and recognize by its ability to metabolize citrate as the only source of carbon and lysine as a source of nitrogen, gas production and hydrogen sulfide over Kligler Iron (KI) and triple iron sugar (TSI) Motile, indole positive urease and oxidase negative. *Salmonella* can ferment glucose but not lactose or sucrose, resistance of *salmonella* 55° c – 1 hour, 60° c [16; 21].

While *shigella* has the advantage of not producing H<sub>2</sub>S, urease, and gas for glucose, and negative for decarboxylase, lysine, sucrose, lactose (for 2 days), donitol, inositol, KCN, malunite, stearate, and salicin, while it is positive for red methylation test [8]. So, we focus on this study on:

1) The study of disease history, epidemiology, pathology, and classification of *salmonella*, *shigella*, and intestinal candidiasis and isolate.

2) diagnose of *salmonella* spp., *shigella* and intestinal *Candida* spp. from diarrheal children via in vitro bacterial diagnosis with traditional fermentation chemical tests such as IMVC, Kligler and, enzymatic tests, means of Api 20E and RapIDTM ONE technology, and identification of bacterial isolates (*S.*

*Typhimurium*) *salmonella* non-typhi using the Vitek 2 compact system.

3) Study of the resistance of *Salmonella* spp. for antibiotics and their sensitivity.

4) To study the thermal stability of *Salmonella* phage isolated from multiple water sources and chicken's droppings.

### Samples and methods

One stool sample (3-5 g) was divided into three parts. The first one was taken for direct cultivated on MacConkey agar plates and incubated at 35.5 ° C for 24 hours, and the second part of it was for direct cultivation on the medium of sabouraud dextrose agar (SDA) and incubated at a temperature of 35.5° C for (24-48) hours. A third section were collected in rich media, as tetra thionate broth with a drop of iodine (0.5 ml) was added, and the tubes were incubated at a temperature of 37° C for a period of 6-14 hours. For isolate *Salmonella*, *Shigella*, all stool samples were examined as soon as they were received microscopically to check for white and red blood cells in the stool, and isolation of *Salmonella*, *Shigella* done on differential media XLD, SSA, and *Candida* spp. on CHROM agar.

*Candida* and *Salmonella* and *Shigella* were diagnosed by bacterial enzymatic tests (Oxidase, Catalase) and the biochemistry tests on Clinical Laboratory Diagnostic Media (IMVC) and KIA Kligler Iron Agar. More accurate techniques were used in diagnosing intestinal bacteria, by API 20E, RapIDTM ONE, and API 20C AUX used to identified *Candida* spp. *S. typhimurium* isolates were identified with the Vitek 2 compact System, and the sensitivity of bacterial isolates to antibiotic was verified by Kerby-Bauer disc diffusion method.

Moreover, the current study included the thermal stability study of *Salmonella* bacteriophage, which was isolated from multiple water sources and chicken's droppings according to the method used by previous researchers. The phage tubes were exposed to different temperatures on a thermal water bath (35, 40, 45, 60, 70, 80) °C., then exposed to non-typhi *salmonella*, on Tryptic Soy Agar medium (TSA) by spot assay method.

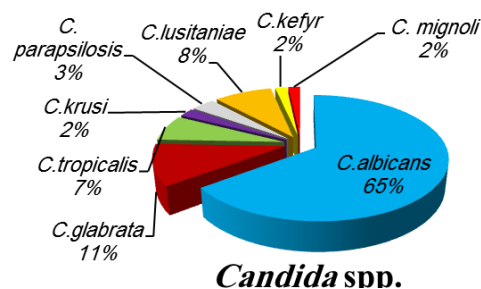
### Results

The study showed (12:200) isolates from stool samples positive with *Salmonella* bacteria at a rate of 6%, one isolation of 0,5% for *Shigella* spp. and by 51.5% for *E. coli* and other pathogenic bacteria (*Enterobacter*, *Citrobacter*, *Klebsiella*, *Pseudomonas*), as shown in table (1).

The results of the study also showed that, (179) were positive isolates of intestinal *Candida* spp. with a percentage of 89.5%, among them was the presence of more than one type of intestinal *Candida* spp. The results showed that *C. albicans* was the most common with (115) samples at a rate of (64.24%), which was diagnosed on *Candida* Chrome agar medium, while it showed varying proportions of non-white *Candida* isolates. The *C. glabrata*, *C.*

*lusitaniae*, *C. tropicalis* and *C. parapsilosis* were 8.4%, 6.7%, 5.6%, and 3.5% respectively as shown in Figure (1).

All isolates of *Salmonella* spp. were resistant to beta-lactam and trimethoprim, high sensitivity to imipenem and cefixime (100%) and were moderate to Ceftriaxone (58,3%), Amoxicillin (41,6%) and Azithromycin (33,3%) and low to Furoxone (16,6%), as shown in table (3). In the thermal stability study of *Salmonella* phage, the degradation range was observed to the exposed phage to degrees (35, 40) °C. for (15, 30) minutes, and stopped at the exposed *Salmonella* phages to temperature (45) °C at 30 minutes. as shown in Table (2).



**Fig. 1: Types of candida and its percentage in cases of diarrhea in children.**

**Table 1: The statistical epidemiological study of *Salmonella*, *Shigella* and *Candida***

Variants	Diarrhea %	Positive salmonella	Negative Salmonella	Candida infection	P-value	Collabora- tive Inf.	P-value	Chi square
Gender								
Male	56.5	8	103	104	0.0666	7	0.029	2.7468
Female	43.5	4	83	75		4		
Age group								
year>	50	0	100	91	0.0473	0	0.105	3.1448
2-6) years(	30	5	55	51		4		
7-10) years(	14	2	26	26		2		
11-14) years(	6	5	7	11		5		
Residency								
Urban	73.5	2	145	134	0.1555	2	0.178	4.6314
Rural	26.5	10	43	45		8		
Water Sources								
Liquefaction Water	71	2	140	134	0.1437	2	0.140	8.1872
Well Water	19.5	6	33	28		5		
River Water	9.5	4	15	17		4		
Nutrition								
Artificial Feeding	65	2	11	12	0.0098	2	0.009	8.2575
Breast Feeding	19.5	0	39	28		0		
Artificial & Breast Feeding	29	0	58	54		0		
Home food	30	5	55	56		5		
Non-home food	15	5	25	29		5		

**Table 2: shows the temperature tolerance of salmonella phages.**

Phage sample	Time period	incubation temperature					
		35C	40C	50C	60C	70C	80C
1	15	+	+	+	-	-	-
2	20	+	+	+	-	-	-
3	30	+	+	-	-	-	-
4	45	+	+	-	-	-	-

(+) A positive result is the presence of lytic activity of the phage.

(-) the negative result of the absence of lytic activity of the phage.

Table 3: Minimal Inhibitory Concentration Breakpoints ( $\mu\text{g/mL}$ ) for *Salmonella* Species.

Antimicrobial Agent	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ceftraxone	2	16.6	3	25	7	58.5
Rifampicillin	12	100	-	-	-	-
Azithromycin	3	25	3	25	4	33.3
cefexim	2	16.6	3	25	7	58.5
Trimethopri	9	75	2	16.6	1	8.3
imipenem	-	-	-	-	12	100
Pencillin	12	100	-	-	-	-
carpencillin	12	100	-	-	-	-
Tetracycline	12	100	-	-	-	-
Amoxicillin	4	33.3	3	25	5	41.6
Ampicillin	12	100	-	-	-	-
Chloromphilicol	10	83.3	2	16.6	-	-
furazolidone	6	50	3	25	3	25
Spiramycin	7	58.5	2	16.6	3	25
Clindamycin	12	100	-	-	-	-
Cephalexin	12	100	-	-	-	-

## Discussion

The results of the current study showed that among (200) children with diarrhea, the infection with *Salmonella* spp. was more in the age group between (11-14) years for rate (41.6%) with a value of P ( $P > 0.05$ ), the reason may be due to excessive exposure to contaminated food sources, contaminated drinks and toilets [19], and also due to the unsanitary conditions of children and immunodeficiency, high mucosal permeability, malabsorption [3] and the percentage of male samples (56.5%) and the percentage of female samples (43.5%), ( $P > 0.05$ ). The reason may be due to the different social habits in place that allow males more freedom to be outside the home, and to own food from street vendors, and thus they become more vulnerable to infection. There is the biological hypothesis which assumes that there may be pathophysiological differences between girls and boys regarding acute diarrhea, which makes boys more vulnerable to infection [20].

Environmental theories suggest different hypotheses according to gender. For example, older children may be allowed more freedom to walk around the house or go to work with parents, which disproportionately exposes them to infectious pathogens [6; 13; 20]. The incidence was more in rural areas in of Kirkuk city and some cases of stunting, wasting and being underweight may be among the main risks of diarrhea in children younger than 5 years old as shown in Table (1).

The decrease in *Salmonella* and *Shigella* infections among the isolated samples compared to the results recorded by researchers [4] in Karbala, where the

percentage of *S. Typhimurium* isolates was (12.7%) and *Shigella* spp. With (8.6%) of the total (648) cases of diarrhea, and the rates was close to the results obtained by [1] at the College of Medicine / University of Baghdad, where there were 19 isolates of *Salmonella* (1.39%) and 31 isolates of *Shigella* with a percentage of (2.21%) of 1,399 cases of diarrhea. may be due to the early administration of antibiotics before sending the patient's stool for culture diagnosis, While the results were similar to [24], with (6.8%) positive for *Salmonella*, *Shigella* infection may in fact be twice what was estimated, and because it is sensitive to acidic conditions in the environment in which it is present, or has been inhibited by colonies of commensal microbes such as *Escherichia*, *Enterobacter* and *Proteus*, and the reduction in *salmonella* and *Shigella* infections among the isolated samples may be due to early antibiotic intake before sending the patient's stool for culture diagnosis.

As recommended or self-use at home medication and strong correlations have been observed between the presence of intestinal candidiasis, diarrhea, and age ( $<0.05$  P) and antibiotic use, it was in the age of less than a year and by 91%, which affects children mainly in the first six months after birth and after five years of age it is less, as mentioned by the study [18] due to weak immunity of infants, especially those with artificial feeding exposed to pollution and disease [10]. As recommended or self-use of home medicines, and the reason may be due to the increase in the level of pollution, to the area of residence, and to the cultural health awareness.

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## التحري عن السالمونيلا والشيغيلا والمبييضات. في البراز من الأطفال المصابين بالإسهال وتقييم تأثير الحرارة على عاثية السالمونيلا في مدينة كركوك

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### الملخص

**الخلفية:** ان بكتريا *Shigella* و *Salmonella* spp. تمثلان عضوين مرضيين للعائلة Enterobacteriaceae، ومن مسببات للتسمم الغذائي والإسهال عن طريق الفم من خلال الطعام والماء الملوثين خصوصاً عند الأطفال من اعمار (يوم-14 سنة).

**الهدف:** عزل وتشخيص *Shigella* و *Salmonella* spp. والمبييضات المعوية من الأطفال المصابين بالإسهال في مدينة كركوك ودراسة الثبات الحراري لـ *Salmonella* phage المعزولة بطريقة Spot assay.

**الطريقة:** جمعت (200) حالة إسهال لأطفال في مستشفيات مدينة كركوك من خلال استخدام دراسة إحصائية وبائية والطرق الزرعية وفحوصات المختبرية الانزيمية والتخميرية التقليدية في تشخيص البكتريا المعوية (IMVC) واختبار Kligler Iron Agar (KIA) . واستعمال تقنيات أكثر دقة مثل Api 20E و RapidTM ONE و *Candida* spp. باستخدام CHROM Agar *Candida* وتقنية API 20C AUX وُحدت عزلات بكتريا (*S.Typhimurium*) السالمونيلا غير التيفية بجهاز Vitek 2 compact System وتم التحقق من حساسية العزلات البكتيرية للمضادات الحيوية بطريقة نشر القرص Kerby – Bauer وعزلت *Salmonella* phage من مصادر مياه متعددة وخروج الدجاج حسب طريقة Spot assay، وعُرضت أنابيب الحاوية للعائيات لدرجات حرارية على حمام مائي (35، 40، 45، 60، 70، 80)°م، ثم تم تعريضها على بكتريا السالمونيلا غير التيفية المنشورة على وسط اكار صويا تريبتيز (TSA) وحضنت لـ 24 ساعة بدرجة 37م.

**النتائج:** تم عزل وتشخيص (12) عزلة الايجابية ببكتريا *Salmonella* من عينات الاسهال بنسبة 6%، وعزلة واحدة لبكتريا *Shigella* spp بنسبة 0,5%، وبنسبة 51,5% كانت للإشريشية القولونية المرضية *E.coli*؛ و (179) عزلة إيجابية لـ *Candida* spp بنسبة 89,5%، من مرضى الإسهال حيث شخضت ما مجموع 200 طفلاً منها 113 عينات من الذكور (56,5%) و 87 عينات من الإناث (43,5%) ( $P > 0.05$ )، حيث أظهرت نتائج الدراسة الحالية إن إصابة الاطفال ببكتريا *Salmonella* كانت أكثر لدى الفئة العمرية ما بين (11-14) سنة بالنسبة (41,6%) ( $P \geq 0.051$ ). معظم العزلات كانت مقاومة لمضادات البنيتا لاكتام والتراي ميثوبرايم والحساسية العالية لـ cefixim و imipenem (100%) وكانت متوسطة للمضاد الحيوي Ceftraxone 58,3% و Amoxicillin 41,6% و Azithromycin 33,3% ونسبة منخفضة للمضاد الحيوي Furoxone 16,6%. ولوحظت نطاق تحلل للعائيات المعرضة لدرجات (35، 40) م لمدة (15، 30) دقيقة وتوقفت النشاط التحليلي للعائيات المعرضة لدرجات الحرارة المختلفة عند (45) م بعد 30 دقيقة.

**الاستنتاج:** كان انتشار السالمونيلا والشيغيلا منخفضاً نسبياً وكان أعلى معدل للإصابة بالسالمونيلا ضمن الفئات العمرية التي تراوحت بين (11-14) و (1-6) سنة، وأظهرت السلالات المعزولة مقاومة للأدوية المتعددة. وان *C. albicans* هو النوع الأكثر شيوعاً مقارنة بأنواع المبييضات الأخرى عند الأطفال المصابين بالإسهال، وتبينت انه يمكن عزل عاثية السالمونيلا من مياه الصرف الصحي وخروج الدجاج بطرق بسيطة وتبينت ايضا ان مقاومة العائيات المعزولة للحرارة تصل الى (45) م.