

ISOLATION AND SEROTYPING OF SALMONELLA SPECIES IN DIARRHEAL CHILDREN

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SUMMARY

This study was carried out to isolate *Salmonella* species from children, 170 fecal samples were collected from children aged (5-12) years of both sexes that had suffering from diarrhea in Teaching laboratories of Al- Hussein Hospital in Thi-Qar governorate. Nineteen isolates were diagnosed as genus *Salmonella* and ensured by National center of Salmonella in Baghdad, five *Salmonella* serotypes were determined as (*Salmonella typhimurium* (8) (42.1%), *S.braenderup* (6) (31.58%), *S.hadar* (3) (15.78%), *S.eneritidis* (1) (5.27%), and *S. munchen* (1) (5.27%). The results revealed that the rate of *Salmonella* isolates in fecal samples of children was (11.17%). According to gender of children the rate of *Salmonella* isolates was (68.42%) in males and (31.58%) in females. In respect to the rate of *Salmonella* isolation in different age of children; age (5-8) years showed the highest rate respectively.

This article is a part of master thesis

INTRODUCTION

Salmonella is recognized as an important pathogen to public health and notified as a cause of bacterial diarrhea worldwide (1-Polo *et al.*, 1999; 2-Medeiros *et al.*, 2001). Contaminated food is the usual source of human infections, and poultry products are considered the major infectious route for humans (3-Obi and Bessong, 2002; 4-Noda *et al.*, 2010). *Salmonella enterica* is a common cause of gastroenteritis and bacteremia in people of all ages, severe invasive disease in infants, elderly persons, and immunocompromised persons; and a wide variety of animals, particularly food animals, have been identified as reservoirs for non-typhoidal *Salmonella* (5-Hald *et al.*, 2007; 6-Wales *et al.*, 2010). Non-typhoid *Salmonellae* causes an estimated 1.4 million illnesses in the United States each year, resulting in an estimated 15,000 hospitalizations and 400 deaths (7-Galanis *et al.*, 2006). Two thousand five hundred forty one *Salmonella* serovars have been identified worldwide and cause more outbreaks of food borne illnesses than any other bacteria, most human infections are caused by a limited number of serovars (8-Popoff *et al.*, 2004).

MATERIALS & METHODS

Isolation and identification of *Salmonella* from Children

samples collection: One hundred seventy fecal samples were collected from children (5-12years) suffering from diarrhea, from both sexes in Teaching laboratories of Al-Hussein Hospital in Thi-Qar governorate. Fecal samples (1gm) were put immediately in a sterile tube contained selenite F broth, and transmitted immediately to the laboratory with cooling box (9-Quinn *et al.*, 2004;10- Cherneck and Berger, 2008).

Culturing of samples: Selenite F broth contained the fecal sample was incubated into at 37C° for 24 hours, and then subculture streaked on Xylose – lysine deoxycholate agar, Hekton enteric agar, *Salmonella- Shigella* (SS) agar, MacConkey agar and Brilliant green agar plates and then incubated at 37C° for 24 hrs (9-Quinn *et al.*, 2004).The growing colonies were examined by naked eye concerning the color, shape and size and bacterial cells examined by gram stains

Gram stain: According to (11) bacterial cells examined by gram stains

Biochemical tests: Important biochemical

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tests { Triple Sugar Iron (TSI) ,Catalase test,Oxidase test, Lactose fermentation, Urease test ,Indole test, Citrate utilization test} were conducted according to (12).

Api-20E system (Analytical profile index for Enterobacteriaceae test):

It was done according to (13), this test (Api-20E system) is used clinically for the rapid identification of the bacterial isolates

Slide agglutination test: According to (14), all isolates were examined with polyvalent O and H antisera by using slide agglutination test as follows:

1-One drop from physiological normal saline was placed on each of the glass slides at each side, and then a loopful from bacterial culture was mixed with each drop.

2-One drop from each O, H polyvalent antisera was added to one of the previous drop and then mixed by plastic rod and rocked. The other drop was used as control.

3-The clear agglutination occurred within 1-2 minute indicated a positive result.

Serotyping diagnosis: Suspected *Salmonellae* genus was sent to the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad) on Kligler iron medium for final serotyping diagnosis.

RESULTS

Isolation and identification of Salmonellae spp: Results showed the different morphology characteristics of *Salmonellae spp* which grow on different media (table 1).

Microscopic examination: Results of microscopic examination has showed that these isolates were gram negative rod, non spore forming after (18–24) hours post incubation at 37 °C

Biochemical identification: The results of the biochemical tests showed that these isolates gave negative results for oxidase, urease, and indole tests, while gave positive results for catalase and citrate utilization test as shown in table (2)

Api-20E system identification:

The result of Api-20E test has revealed the numerical profile (6704752) as confirmed diagnostic test for *Salmonella* isolate in table (3) and figure (1).

Slide agglutination test (Polyvalent):

The genus *Salmonella* detected by using polyvalent O and H antisera showed that clear agglutination indicated a positive result in comparison to clear homogeneity of the control reaction in each one.

Serotyping of Salmonella (Monovalent):

The result of the serotyping of the bacteria in the Central Public Health Laboratories/ Ministry of Health has ensured that these bacteria are *S. typhimurium*, *S. braenderup*, *S. hadar*, *S. enteritidis*, and *S. munchen* .

Percentage of infection with Salmonella spp in diarrheic children:

The results of isolation of *Salmonella spp* from 170 fecal samples of diarrheic children, (19) *Salmonella spp* isolates, five different serotypes were recognized according to Central Public Health Laboratories. These serotypes isolated were *S. typhimurium* (42.1%) from (8) patients, *S. braenderup* (31.58%) from (6) patients followed by *S. hadar* from (3) patients (15.78%), *S. enteritidis* and *S. munchen*, with one isolate for each (5.27%) as in table (4). the important clinical signs appeared in children were vomiting, fever and abdominal pain. In addition watery, mucus and blood stools.

According to age effect, the study revealed that most of the diarrheal cases was in age (5-8) years at percentage (78.94%) of all *Salmonella* isolates, while the percentage (21.06%) appeared in age (9-12) years as in table (5). This study revealed the distribution of *Salmonellae* was according to gender effect that from total (19) *Salmonellae* serotypes, (13) serotypes isolated from male (68.42%), and (6) serotypes isolated from female (31.58%) as in table (6). The number of separate serovars during the period of investigation was different. The highest percentage of *Salmonellae* infected samples was detected in male in comparison with female respectively.

DISCUSSION

Our study showed that the bacterial identification was done according to cultural characteristics, microscopic examination, primary biochemical identification, using the Api-20E system which is specific for identification of *Enterobacteriaceae* from other bacteria and slide agglutination test, this was matching with the characterization of genus *Salmonella* as fixed in (9,11). In the present study, data demonstrated that (19) *Salmonella* isolates were isolated from 170 fecal samples and constituted about (11.17%), this result is consistent with (15) who recorded that proportion of isolations bacterium *Salmonellae* from diarrheal cases of children in Baghdad (10%). In contrast, study of (16) on Salmonellosis in children, revealed highest proportion (14.47%) of all (608) diarrhea cases in Al – Qadsia province, while (17) found the lowest percentage at (2.8%) in Baghdad, and (18) in Mosul found that the percentage of *Salmonella* isolations strains account at (6%) from (450) human patients stools, and a study of (19) recorded a percentage (3.9%) of *Salmonellosis* in diarrheal human. In the present study, five different *Salmonella* species were recorded in children, and this is incompatible with a study by (20) who detect five serotypes (*S. enteritidis* , *S. typhimurium*, *S. hadar*, *S. infantis* and *S. virchow*) . Salmonellosis has remained one of the three most common meat associated diseases in human (21), animal products are easily contaminated with microorganisms and

support their growth if not properly handled, processed and preserved (22, 23), and the disease caused by *Salmonellae* organisms is the most common and important zoonotic diseases (24). Over the last years, the number of supermarkets and restaurants in Nasiria city has been growing considerably due to the favorable socioeconomic condition. These supermarkets selling meat in parallel with other different items as poultry products, fish and meats. These products are acquired directly from private farms or through importation of these products from other countries. In our study, , so this is consistent with the record of (25) that Salmonellosis has remained one of the most common causes diarrheal diseases in human, and gastroenteritis is the typical disorder caused by non typhoidal *Salmonella* infection as recorded by (26 , 27). The results of age is compatible with a previous study of Salmonellosis in Iraq by (28) who found that children more than five years old are more susceptible to Salmonellosis. and with study of (20) who record that the Children aged 2 are the most affected age group (incidence 391.6 per 100 000 population). In the present study, we observed differences in the prevalence rate of diarrhea between male and female. Male cases were of higher percentage than females. A similar result has been observed by (15, 16, 17) in Iraq.

On the contrary (29) reported that high percentage of *Salmonella* (4%) in female and low in male (2.4%), also (30) found that female constituted about (4.81%) and in male (3.66%).

Table (1): The results of culture characteristics of *Salmonellae spp.*

Culture Media	Morphology of colonies
Brilliant green agar	small, rounded, translucent rosy
Hekton enteric agar	small, smooth, rounded, green with black center
MacConkey agar	small, smooth , and pale
<i>Salmonella-Shigella</i> agar	small, smooth, rounded, pale with black center
Xylose-Lysine Deoxycholate agar	small, smooth, rounded, red in color with black center

Table (2): The results of some biochemical tests of *Salmonellae spp.*

Biochemical test	Result
Oxidase test	-
Catalase test	+
Urease test	-
Indol	-
KI and TSI	Red/Yellow with H ₂ S production
simmon citrate test	+

Table (3): The results of Api-20 E tests of *Salmonellae spp.*

Biochemical test	results	Biochemical test	results
(LDC) lysine decarboxylase	+	(ONPG) β-galactosidase	-
(ODC) ornithine decarboxylase	+	(ADH) arginine dehydrolase	-
(CIT) citrate utilization	+	(URE) urease,(GEL) gelatinase	-
(H ₂ S) H ₂ S production	+	(TDA) tryptophane deaminase	-
fermentation/oxidation glucose (GLU), mannitol (MAN) , inositol (INO), sorbitol (SOR), rhaminose (RHA), melibiose (MEL) & arabinose (ARA)	+	fermentation/oxidation of these sugars (SAC) sucrose, (AMY) amygdaline	-
(ADH) arginine dehydrolase	+	indol (IND), acrtoin production (VP), & cytochrome-oxidase (OX)	-

Figure (1): Calculate the numerical profile in Api-20E system

(+) : The test positive. (-) : The test negative. (6704752): The numerical profile.

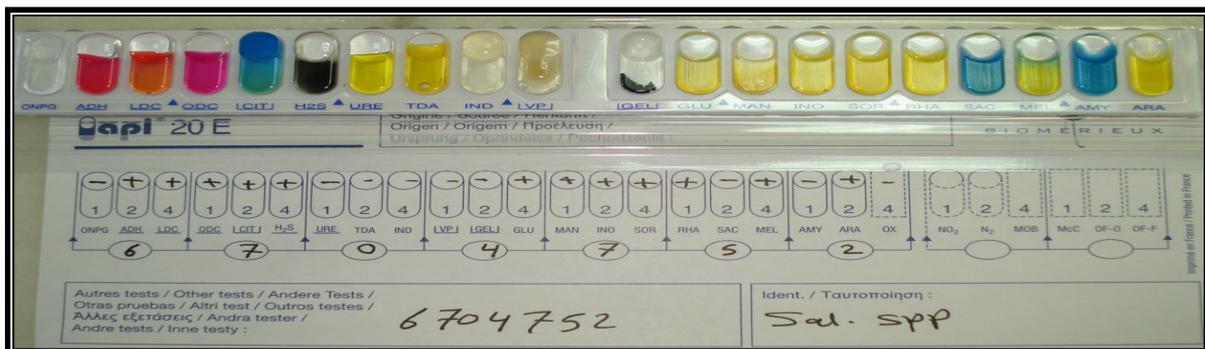


Table (4): Different serotypes of *Salmonellae* isolated from diarrheic children.

Species	Number	Percentage%
<i>Salmonella typhimurium</i>	8	42.1
<i>Salmonella braenderup</i>	6	31.58
<i>Salmonella hadar</i>	3	15.78
<i>Salmonella enteritidis</i>	1	5.27
<i>Salmonella munche</i>	1	5.27
Total	19	

Table (5): Distribution of *Salmonellae* serotypes according to age.

Age-year	No.of diarrheal case	No.of salmonella isolates	%
5-8	105	15	78.94
9-12	65	4	21.06
Total	170	19	11.17

Table (6): Distribution of *Salmonellae* serotypes according to gender.

Sex	No.of diarrheal case	NO. of salmonella isolates	%
Male	93	13	68.42
Female	77	6	31.58
Total	170	19	

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عزل وتصنيف بكتريا السالمونيلا *Salmonella* من الاطفال المصابين بالأسهال

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الخلاصة

أجريت هذه الدراسة لعزل انواع بكتريا السالمونيلا *Salmonella* من 170 نموذج براز من الأطفال تراوحت أعمارهم بين (5- 12 سنة) ومن كلا الجنسين يعانون من الأسهال، في مختبرات مستشفى الحسين التعليمي في محافظة ذي قار.

تم عزل (19) عزلة سالمونيلا من براز الأطفال، وتم التأكد من تشخيص العزلات في المركز الوطني للسالمونيلا في بغداد وكانت عائدة الى خمسة أنماط مصلية وهي: (8) *S. typhimurium*، (6) *S. braenderup*، (3) *S. hadar*، (1) *S. enteritidis* و(1) *S. munche*.

أظهرت النتائج ان نسبة عزل انواع السالمونيلا *Salmonellae spp* (11.17%)، واعتمادا على جنس الأطفال كانت نسبة عزل جراثيم السالمونيلا في الذكور (68.42%) فيما كانت في الاناث (31.85%)، اما نسبة عزل جرثومة السالمونيلا في الفئات العمرية المختلفة في الأطفال فكانت أعلى نسبة عزل لهذه الجرثومة في الفئة العمرية (5- 8) سنة على التوالي

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