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IDENTIFICATION, ANTIMICROBIAL RESISTANCE OF *SALMONELLA ENTERICA* ISOLATED FROM DIARRHEAL CHILDREN IN THI-QAR PROVINCE DURING 2015

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ABSTRUCT

This study was carried out to isolate *Salmonella* species from children, 300 fecal samples were collected from children aged (1day-13 years) of both sexes that had suffering from diarrhea in Mohammed Al -Mosawi Hospital in Thi-Qar province. Twenty-four isolates were diagnosed as genus *Salmonella* and ensured by National center of *Salmonella* in Baghdad, four *Salmonella* serotypes were determined as: *S .typhimurium* (14) (58.3%), *S. typhi* (4) (16.6. %), *S. enteritidis* (3) (12.5%), and *S. muenchen* (3) (12.5. %). The results revealed that the rate of *Salmonella* isolates in fecal samples of children was (8%). According to the age the rate of *Salmonella* isolation in different age of children; age (1 day-2 years) showed the highest rate. Antimicrobial susceptibility testing to 24 *Salmonella* isolated showed that all isolates were sensitive to the Amikacin and Gentamycin, while 7 isolates resistance to chloramphenicol, 12 isolates were resistance to nalidixic acid and 6 isolates were resistance to Ciprofloxacin in addition to 8 isolates were resistance to Ampicillin.

KEYWARDS: *Salmonella enterica*, diarrheal Children.

1-INTRODUCTION

Salmonella enterica is one of the most commonly detected, in terms of both numbers of human infections and severe disease (numbers of hospitalizations and deaths associated with infections), **(**Scallan *et al*., 2011**).** The species *Salmonella enterica* comprises a group of gram negative bacteria that are important pathogens for humans and animals (Eisenstei, 1999; Akbarmehr, 2011). This organism consists of more than 2668 different serotype. It is one of the major causative pathogens of food borne disease outbreaks (Onyango *et al*., 2009) and also a public health concern all over the world (Cardinale *et al*., 2005). The infectious dose is usually greater than 10^2 to 10^3 organisms and may vary with age and health status of the host. In some cases, it can be as few as 15 to 20 cells (FDA /CFSAN Bad Bug Book). The children are at the highest risk for *Salmonella* infections have higher rates of *Salmonella* infection than any other age group (CDC, 2014). The young children, older adults, and people with weakened immune systems are the most likely to have severe infections (CDC, 2012). One of the most severe *Salmonella* infections is typhoid fever, which causes diarrhea and systemic disease. *S.* Typhi is highly adaptive in humans and is responsible for persistent as well as life-threatening systemic infections (Haghjoo & Galan, 2004). In contrast, nontyphoidal *Salmonella* serovars can cause illness to a broad host range, including humans, ruminants, birds and reptiles and has a rapid onset and short incubation period. According to Food Net, which was established in 1996 as a collaboration of the CDC, USDA, FDA, and selected state health departments, 3.6 million (39%) foodborne illnesses were caused each year by bacteria, in which nontyphoidal *Salmonella* caused 1 412 498 cases of illness while *S.* typhi caused 824 cases in the United States (Chen et al., 2006). It is kills 3 million children each year in both developed and developing countries (Cardinale *et al*., 2005). Typhoid fever is one of the major health problems in the developing countries, because many interrelated factors, including increased urbanization, inadequate supplies of clean water, antibiotic resistance, the variable efficacious of vaccine preparations, and the increased regional movement of large number of migrant workers (Thong *et al*., 1994). In Iraq, electricity generating plants, water purification and sewage treatment plants were destroyed during the 1991 war. These, coupled with overcrowded conditions and lack of sanitation facilities, have led to increase cases of typhoid fever .WHO (2003[a]) have reported 1812 cases in 1989 while the number increased to 21356 in 2001. The number of cases after the last war in 2003 is expected to be much higher with the collapse of the health care system. The clinical management of patients infected with *Salmonella enterica* serotypes is difficult

due to the emergence of multidrug-resistant (MDR) strains (Mermin *et al*., 1999; Kariuki *et al*., 2004). In this study *Salmonella enterica* is isolated from diarrheal children in Thi-Qar province and the serovars of *Salmonella enterica* isolated is determinant in addition to antimicrobial susceptibility testing to *Salmonella* isolates.

2-MATERIALS AND METHODS

Children samples collection

Three hundred fecal samples were collected from children (1 day-13years) suffering from diarrhea, from both sexes in Mohammed Al-Mosawi Hospital in Thi-Qar province. Fecal samples (25 gm.) were put immediately in sterile tube contained buffer peptone water.

Isolation and identification of *Salmonella*

Weigh out 25 g faeces with a sterile wood spatula, put it into an Erlenmeyer flask etc. and add 225 ml buffered peptone water to obtain 1 part sample + 9 part buffer. Mix. Incubate at 37^{o}C overnight (16-20 hours) then Transfer 1 ml of the pre-enrichment with a pipette to 10 ml Tetrathionate broth (Müller-Kaufmann). Incubate at 41.5^{o}C ± 0.5^{o}C overnight (18-24 hours) then Spread a 10 µl loop full from the inoculated and incubated Tetrathionate broth on XLD and on BGA agar plates and incubate at 37^{o}C overnight (18-24 hours) and read the XLD plates and BGA plates .Salmonella suspect colonies on XLD and BGA agar onto non-selective media, (nutrient agar) plates for biochemical confirmation of *Salmonella*.

Biochemical tests

The important biochemical tests were conducted according to (Winn *et al.*, 2007). Tests {Triple Sugar Iron (TSI) and Kligler iron (KI), Catalase test, Oxidase test, Lactose fermentation, Urease test, Indole test, Citrate utilization test}.

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

It was done according to (Leboffe and Piercr, 2005); this test (Api-20E system) is used clinically for the rapid identification of the bacterial isolates

Serotyping diagnosis

The positive results for *Salmonellae* isolates in biochemical tests has been sent to the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad) on Kligler iron medium for final serotyping diagnosis.

Antimicrobial susceptibility testing by disk diffusion

All isolates *Salmonella* spp. in this study were tested for resistance to eight antimicrobials on Mueller-Hinton agar (Difco Laboratories, Detroit, MI) by a disk agar diffusion method (Khan *et al*., 2006). The following antimicrobials were used: ampicillin (10 mg), Ciprofloxacin (5 mg), Chloramphenicol(30), gentamicin (10 mg), Amikacin (30 mg) and nalidixic acid (30 mg). The Sensitivity and resistance were determined by the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2006).

Maintenance of bacterial isolates

The isolates after definitive diagnosis were cultured on slant of brain heart infusion agar, incubated 37° C for 24 hours; they may be re-cultured monthly to maintain their viability and activity. The definitive isolates were cultured on brain heart infusion broth and glycerol 20 % and incubated at 37° C for 24 hours, then after turbidity occurred, stored in a deep freezing (Feltham *et al.*, 1978)

3-RESULTS

The results showed the different morphology characteristics of *Salmonellae* spp. which grow on different media (table 1).

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

Culture Media	Morphology of colonies
Brilliant green agar	small, rounded, translucent rosy
Xylose-Lysine Deoxycholate agar	small, smooth, rounded, red in color with black center

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).

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

Biochemical test	Result
KI and TSI	Red/Yellow with H2S production With or without gas production
Catalase test	+
Oxidase test	_
Lactose fermentation	Non-lactose fermenter
Urease test	_
Indole	_
citrate utilization test	(+) for *Salmonella* (majority) and (-)for *Salmonella* Typhi

Api-20E system identification

The result of Api-20E test has revealed the numerical profile (6704752) as confirmed diagnostic test for *Salmonella* isolate as table (3) and figure (1)B and the numerical profile(4004500) as confirmed diagnostic test for *Salmonella typhi* as table (4) and figure (1)A.

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

(+): The test positive. (-): The test negative. (4004500) and (6704752): The numerical profile.

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

Biochemical test	**Results**	**Biochemical** test	**Results**
(ONPG) β-galactosidase	-	(ADH) arginine dehydrolase	+
(LDC) lysine decarboxylase	+	(ODC) ornithine decarboxylase	+
(CIT) citrate utilization	+	(H2S) H2S production	+
(URE) urease	-	(TDA) tryptophan deaminase	-
(GEL)gelatinase	–	indole(IND), acrtoin production(VP), & cytochrome oxidase(OX)	-
fermentation/oxidation of these sugars (SAC)sucrose, (AMY)amygdaline	-	fermentation/oxidation glucose(GLU), mannitol(MAN) inositol(INO), sorbitol(SOR), rhaminose(RHA), melibiose(MEL) &arabinose(ARA)	+

Table (4): The results of Api-20 E tests of *Salmonellae* typhi.

Biochemical test	**Results**	**Biochemical test**	**Result**
(ONPG) β-galactosidase	-	(ADH) arginine dehydrolase	-
(LDC) lysine decarboxylase	+	(ODC) ornithine decarboxylase	-
(CIT) citrate utilization	-	(H2S) H2S production	-
(URE) urease	-	(TDA) tryptophan deaminase	-
(GEL)gelatinase	–	indole(IND), acrtoin production(VP), & cytochrome oxidase(OX)	-
fermentation/oxidation of these sugars (SAC)sucrose, (AMY)amygdaline	-	fermentation/oxidation glucose(GLU),mannitol(MAN) sorbitol(SOR),	+
		fermentation/oxidation inositol(INO), rhaminose(RHA),melibiose(MEL) &arabinose(ARA)	-

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. typhi, S. enteritidis and S. muenchen.*

Percentage of infection with *Salmonella spp*. in diarrheic children

The results of isolation of *Salmonella* spp. from 300 fecal samples of diarrheic children, (24) *Salmonella* spp. isolates with (4) different serotypes were recognized according to Central Public Health Laboratories. These serotypes isolated were *S. typhimurium* (58.3 %) from (14) patients, *S. typhi* (16.6 %) from (4) patients followed by *S. enteritidis* from (3) patients (12.5 %),.and *S. muenchen* with(3)isolate also (12.5 %) as in table (4),figure (2). The important clinical signs have been appeared in children were the vomiting, abdominal pain and fever. In addition to watery and sometimes bloody diarrhea with mucus. According to age effect of patient, the study revealed that most of the diarrheal cases was in age (1 - 2) years at percentage (58.3 %) of all *Salmonella* isolates.

Table (4): Different serotypes of Salmonellae isolated from diarrheic children (National center of *Salmonella* in Baghdad)

Species	Number	Percentage%
Salmonella typhimurium	**14**	**58..3**
Salmonella typhi	**4**	**16.6**
Salmonella enteritidis	**3**	**12.5**
Salmonella muenchen	**3**	**12.5**
Total	**24**	

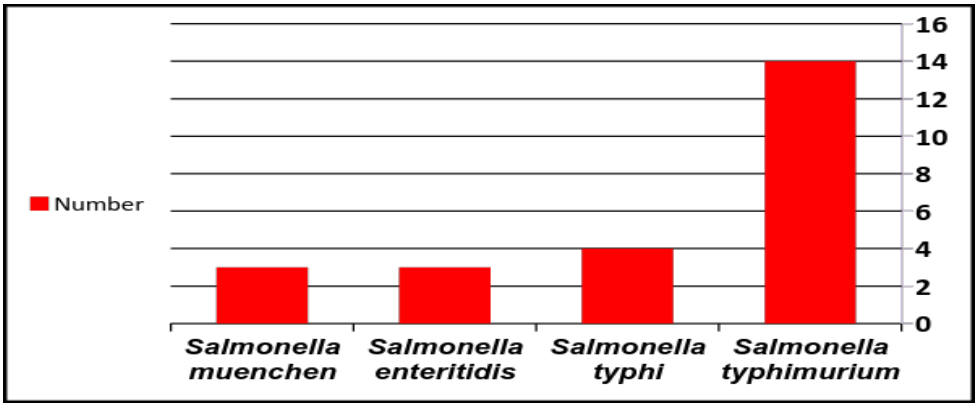

Figure (2): diagram show Different serotypes of *Salmonellae* isolated from diarrheic children

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing to 24 *Salmonella* isolated showed that all isolates were sensitive to the Amikacin and Gentamycin, while seven isolates resistance to chloramphenicol, twelve isolates were resistance to nalidixic acid and six isolates were resistance to Ciprofloxacin in addition to eight isolates were resistance to Ampicillin, as in table(5).

Table (5): Antimicrobial susceptibility testing

Antimicrobial Agents	*Salmonella enterica*					
	No.R	% of R	No. I	%of I	No. S	%of S
Amikacin (AMC)	-	-	-	-	**24**	**100%**
Gentamycin (CN)	-	-	-	-	**24**	**100%**
Ciprofloxacin(CIP)	**6**	**25%**	**15**	**62.5%**	**3**	**12.5%**
Nalidixic acid (NA)	**12**	**50%**	**9**	**37.5%**	**3**	**12.5%**
Ampicillin(AMP)	**8**	**33.3%**	**13**	**54.1%**	**3**	**12.5%**
Chloramphenicol(C)	**7**	**29.1%**	**1**	**4.1%**	**16**	**66.6%**

(R) Resistance, (S) sensitive and (I) intermediate

4-DISCUSSION

Salmonella enterica is known worldwide as a significant causing the diseases for both human and animal (Guibourdenche *et al*., 2010; Mezal *et al*., 2014). Our study showed that *Salmonella enterica* isolation and identification has been done according to cultural characteristics, conventional biochemical identification and using the Api-20E system which is specific for identification of *Enterobacteriaceae* from other bacteria and serological test, this was matching with the characterization of genus *Salmonella* as fixed in (Quinn *et al*.,2004, Jawetz *et al*.,2007). In the present study, data demonstrated that (Oie, 2008).) *Salmonella enterica* isolates were isolated from 300 fecal samples from children (1 day-13 years) suffering from diarrhea for both sexes and constituted about (8 %) from all diarrheal cases. There are four serotypes isolated there are: *S.* typhimurium ,was the highest rate (58.3%) of *Salmonella* isolated this result is accepted with the study by (Harab and Yousif ,2011) that appeared *S.* typhimurium was the highest rate also (42.1%), *S.* typhi (16.6%) but this serotype was not isolated by (Harab and Yousif ,2011) and each of *S.* enteritidis and *S.* muenchen show (12.5%) in study by (Harab and Yousif ,2011) *S.* enteritidis and *S.* muenchen were appeared(5.27%) %). In contrast, study of (Al-Janabi,2001) on Salmonellosis in children, revealed highest proportion (14.47%) of all (608) diarrhea cases in Al – Qadsia province, while (Al-Taayi,2002) found the lowest percentage at (2.8%) in Baghdad, and (Al-Shamaa,2001) in Mosul found that the percentage of *Salmonella* isolations strains account at (6%) from (450) human patients stools, and a study of (Rowe *et al*.,2010) recorded a percentage (3.9%) of Salmonellosis in diarrheal human. In the last years, the number of supermarkets and restaurants in Thi-Qar province has been growing considerably because the favorable socioeconomic conditions. These supermarkets selling meat in parallel with other different matters as

poultry products, fish and meats. These products are acquired directly from special farms or through importing of these products from other countries. In our study, this is consistent with the record of (WHO, 1997) 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 (Goldberg and Rubin, 1988; Bartlett, 1996). The results of age is incompatible with a previous study of Salmonellosis in Iraq by (Al-Hayali, 1993) who found that children more than five years old are more susceptible to Salmonellosis. But our study were appeared that the children (1day -2 year) are more susceptible to Salmonellosis ,this accepted with study of (Czerwinski,2008) who record that the Children aged 2 are the most affected age group (incidence 391.6 per 100 000 population). *Salmonella* was the first organism that was monitored for antimicrobial resistance by NARMS.(Prevention, C. f. D. C. a. 2001). In this study the antimicrobial susceptibility testing for 24 *Salmonella* isolated showed that all isolates were sensitive to the Amikacin and Gentamycin this accepted with (Faik,2007) .in other hand, seven isolates showed resistance to chloramphenicol, however all isolates Al-Hashimy(2012) were resistance to chloramphenicol. Twelve strains were resistance to nalidixic acid in our study although Faik(2007)reported one isolate was resistance to nalidixic acid and Al-Hashimy(2012) reported all isolates were sensitive to nalidixic acid, In the recent years, the portion of *Salmonella* which is resistant to the important drugs such as fluoroquinolones (e.g., ciprofloxacin) is increasing (Barza,.2002) in this study six isolates were resistance to ciprofloxacin .our study showed eight isolates were resistance to Ampicillin. These results are agree with Mezal (2014), Faik (2007) reported all isolates were sensitive to ciprofloxacin and ampicillin.

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