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Isolation and Antibiogram of *Salmonella enterica* from Children Under Five Years with Diarrhea in Thi-Qar Province

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Abstract:

The present study aimed to determine the prevalence of *Salmonella* in children under five years and to determine the antimicrobial susceptibility profiles among the isolates. Our study was carried out during the period from November 2017 to May 2018. Four hundred fecal samples were collected from children aged (1day-5 years) of both sexes (216 Male, 184 Female) that had suffering from diarrhea in Mohammed Al -Mosawi Hospital and public health laboratory in Thi-Qar province/southern Iraq. Twenty isolates (5%) were identified as *Salmonella* using API-20E system and molecular detection using *invA* gene. Antimicrobial susceptibility testing was done using 9 Antibiotics from different classes showed that all isolates were sensitive to Chloramphenicol and Tetracycline. Resistance ratio to Cefotaxime, Ampicillin, Amikacin, Ceftriaxone, Gentamycin, Nalidixic acid, and Ciprofloxacin at (55%), (45%), (35%), (30%), (30%), (30%) and (15%) respectively.

Keywords: *Salmonella enterica,* antimicrobial susceptibility, *invA.*

1-Introduction:

Diarrhea considers globally one of leading causes of death among children under the age of five, with diarrhea accounting for about 40 per cent of under-five mortality in sub-Saharan Africa and about half a million about 25 per cent in South Asia. diarrhea has killed almost 2 million children in 2013 and accounts for nearly a third of all under-five deaths worldwide (Vakili *et al.*, 2015). Acute diarrheal diseases, a major public-health problem in developing countries , are often associated with significant morbidity and mortality, especially among children (Lee *et al*., 2005 ; Paula *et al*., 2010).

Salmonella is a bacterial genus within the Family Enterobacteriaceae that consists of a large group of genetically similar organisms with the ability to infect a large number of animal hosts (Costa *et al.*, 2012). *Salmonella enterica* is one of the most commonly detected, in terms of both numbers of human infections and severe disease; the widespread of *S. enterica* in humans and animals worldwide have always been a major public health concern (Yoke-Kqueen *et al*., 2008). The children are the highest risk for *Salmonella* infections have higher rates of *Salmonella* infection than any other age group. The young children, older adults, and people with weakened immune systems are the most likely to have severe infections (CDC, 2014). Antimicrobial agents, of natural or synthetic origin, commonly work by inhibiting or disrupting vital processes within the bacterial cell, targeting structures or pathways sufficiently different or absent in mammalian cells. Antibiotics can be grouped according to several different criteria: spectrum of activity, inhibitory effect and molecular target (Pietsch, 2015). Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Genes can be transferred between bacteria in a horizontal fashion by transduction, conjugation, or transformation. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. The primary cause of antibiotic resistance is genetic mutation in bacteria (WHO, 2005). The continuous evolution of *Salmonella* at the genetic and genomic levels contributes to the increased virulence and resistance to multiple antibiotics, leading to a phenotype of multidrug resistance. This resistance is a significant public health concern (Fàbrega, 2013).

This study aimed to determine the prevalence of *Shigella* and *Salmonella* isolates in children under five year and to determine the antimicrobial susceptibility profiles among the isolates.

2- Methods:

Four hundred fecal samples were collected from children with aged ranged (1 day-5 years) suffering from diarrhea, from both sexes in Mohammed Al-Mosawi Hospital and public health lab. in Thi-Qar province\southern Iraq. Stool samples were directly cultured on MaCconkey agar incubated at 37°C for 18-24 hours and after incubation period, non-lactose ferment colonies were cultured on XLD and HE agar at 37°C overnight, After incubation period *Salmonella* suspect colonies cultured on nutrient agar plates for further experiments.

The biochemical tests were conducted: Motility test, Catalase test, Oxidase test, Urease test, Kligler iron (KI), Lactose fermentation, Indole test, Citrate utilization test, Methyl red test (MR), and Voges-Proskaur test (VP).

Genomic DNA was extracted from *Salmonella* isolates by using Geneaid Genomic DNA Purification Kit (UK) and done according to company instructions in many steps. All isolates of *Salmonella* were detected by polymerase chain reaction (PCR assay) using *invA* gene (Table1). The reaction was performed in final amount (20µl), (3µl DNA, 2µl of each primer and 13µl of ddH_2O). PCR was performed in Thermocycler (Eppendrof, Germany) and consisted of the following steps: 95°C for 5 minutes (initial denaturation), followed by 30 cycles of 94°C for 40 seconds, annealing temperature at 66.5°C for 60

seconds and 72°C for 90 seconds. The final extension step was performed at 72°C for 10 minutes, followed by cooling to 4°C. The fragments obtained were analyzed by horizontal electrophoresis on 1% Agarose gel loaded with ethidium bromide at 100V in TBE buffer and visualized on a transilluminator.

Table (1): The sequence of *invA* gene, annealing temperature and size product

Gene	Sequence		Annealing Temp.	Size product	Reference
invA	F	CTGGCGGTGGGTTTTGTTGTCTTCTCTATT	66.5°C	1070 bp	(Galan and Curtiss,1989)
	R	AGTTTCTCCCCCTCTTCATGCGTTAC			

All isolates of *Salmonella enterica* were tested for resistance to eight antimicrobials on Mueller-Hinton agar by a disc diffusion method as described by Bauer *et al.* (1966). The following antimicrobials were used: Ampicillin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Gentamicin (10 µg) and Nalidixic acid (30 µg). The Sensitivity and resistance were determined by the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2017).

3-Results:

Among 400 stool samples were collected from Children only 20 patients were positive (5%) to *Salmonella* using conventional biochemical test, API-20E system and molecular detection using *invA* gene.

A. Macroscopic Features & Biochemical Identification

Different morphology characteristics were observed of *Salmonella enterica* which grew on different media then Incubated Overnight (24 hour) at 37°C. Also biochemical tests was applied to all isolates of *Salmonella enterica* (Table 2).

Table (2): The results of morphology characteristics and biochemical tests of *Salmonella enterica*

No.	Test or Media	Result
1	XLD agar	small, smooth, rounded, red in color with black center
2	Hekton enteric agar	Clear colonies with black centers
3	MaCconkey agar	Smooth, colorless colonies
4	Indole test	-
5	Urea test	-
6	Motility test	+
7	Kligler test	K\A , with H2S, With gas or Without
8	Citrate utilization test	V*
9	Methyl Red test	+
10	Vogus Proskauer test	-
11	Lactose fermentation	None lactose ferment
12	Oxidase test	-
13	Catalase test	+

V*: Variable

B. Api-20E System Identification

The result of Api-20E test has exposed the codes number (6744752) and (4404540) as confirmed diagnostic test for *S. enterica* as demonstrated in Table (3).

Table (3) Result of API-20 E tests of *S. enterica*

Biochemical Test	Result	
	Salmonella spp.	***Salmonella typhi***
ONPG (β-galactosidase)	-	-
ADH (Arginine dihydrolase)	+	-
LDC (Lysine decarboxylase)	+	+
ODC (ornithine decarboxylase)	+	-
CIT (Citrate utilization)	+	-
H2S (H_2S production)	+	+
URE (Urease test)	-	-
TDA (Tryptophan deaminase)	-	-
IND (Indole test)	+	-
VP (Acrtoin production)	-	-
GEL (Gelatinase)	-	-
GLU (Glucose)	+	+
MAN (Mannitol)	+	+
INO (Inositol)	+	-
SOR (Sorbitol)	+	+
RHA (Rhaminose)	+	-
SAC (Sucrose)	-	-
MEL (Melibiose)	+	+
AMY (Amygdaline)	-	-
ARA (Arabinose)	+	-
Code Number	**6744752**	**4404540**

C. Molecular Diagnosis

Simplex PCR assay was used to detect *invA* gene then migrate on Agarose gel and signified by a single band in the equivalent region of the DNA ladder (2000bp).The data demonstrated that the presence this gene in all isolates of *S. enterica* with size product (1070 bp) (Figure 1).

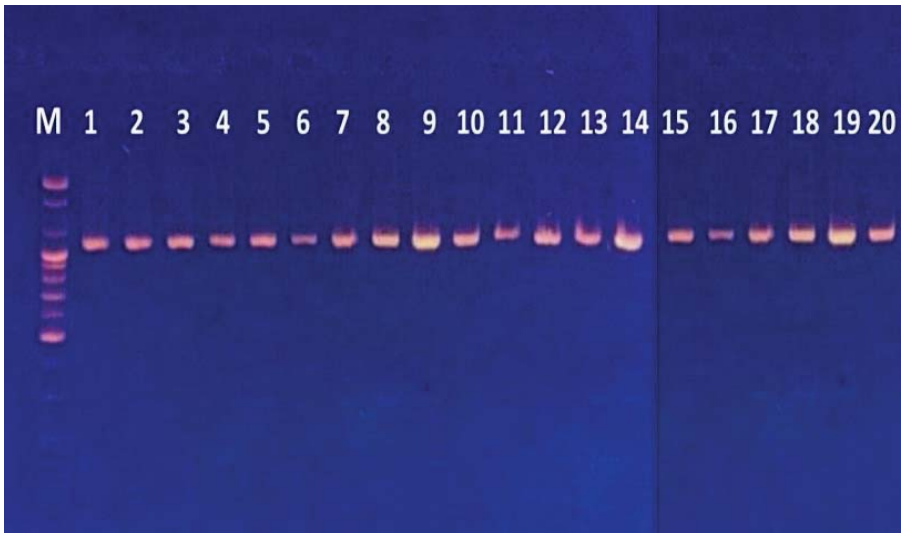


Figure (1) Gel electrophoresis *invA* PCR Product (M: DNA ladder ,1-20 positive result)

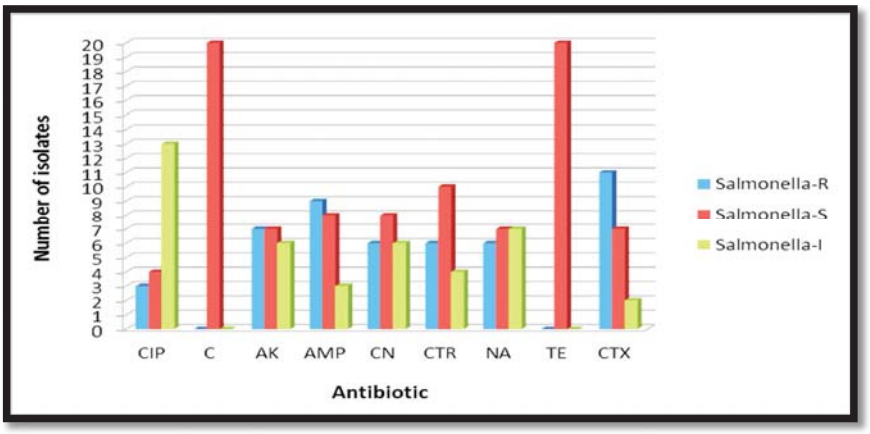
D. Antimicrobial Susceptibility Testing

The results indicated that all *Salmonella* isolates (100%) are sensitive to Chloramphenicol and Tetracycline, while 11/20 isolates (55%) were resistant to Cefotaxime, 9/20 isolates (45%) were resistant to Ampicillin ,7/20 isolates (35%) were resistant Amikacin, 6/20 isolates (30%) were resistant to Ceftriaxone, Gentamycin and Nalidixic acid , however only 3/20 isolates (15%) were resistant to Ciprofloxacin (Table 4 and Fig. 2).

Table (4) The Result of Susceptibility Test for *Salmonella*

Antibiotic	*Salmonella* isolates (n=20)					
	No. R	% of R	No. I	% of I	No. S	% of S
Ampicillin	9	45%	3	15%	8	40%
Amikacin	7	35%	6	30%	7	35%
Chloramphenicol	-	-	-	-	20	100%
Cefotaxime	11	55%	2	10%	7	35%
Ceftriaxone	6	30%	4	20%	10	50%
Ciprofloxacin	3	15%	13	65%	4	20%
Gentamicin	6	30%	6	30%	8	40%
Nalidixic acid	6	30%	7	35%	7	35%
Tetracycline	-	-	-	-	20	100%

CIP: Ciprofloxacin;



C: Chloramphenicol; AK: Amikacin; AMP: Ampicillin
CN: Gentamicin; CTR: Ceftriaxone; NA: Nalidixic acid; TE: Tetracycline; CTX: Cefotaxime

Figure (2) Antibiotics Resistance Profile of *Salmonella*

The data demonstrated that nine (45%) of isolates considered as multi-drug resistant because the isolates were totally non-susceptible to equal or more than one antibiotic in equal or more than three antimicrobial categories, two isolates were resistant to three antibiotics, five isolates were resistant to four antibiotics, and one isolate was resistant to five and six antibiotics (Table 5).

Table (5) Multi Drug Resistance to Antibiotic of *S. enterica*

No. of antimicrobial resistance values	*Salmonella enterica* (n=20)		
	Antimicrobial resistance patterns	No. of isolates (%)	Total (%)
Three	CTX/ CN/ AMP	1 (5%)	2 (10%)
	CTX/ AMP/ AK	1 (5%)	
Four	CTX/ NA/ CN/ AMP	1 (5%)	5 (25%)
	CTX/ CTR/ CN/ AK	1 (5%)	
	CTX/ CTR/ AMP / AK	2(10%)	
	NA/ CTR/ AMP/ CIP	1 (5%)	
Five	NA/ CTR/ CN/ AK/ CIP	1 (5%)	1 (5%)
Six	CTX/ NA/ CTR/ CN/ AMP/AK	1 (5%)	1 (5%)

4-Discussion:

Salmonellosis still remains a public health problematic especially in developing countries where poor sanitation, poverty, poor personal hygiene and water supply promote the spread of enteric diseases. Malnutrition and the lack of suitable medical intervention partakes to the high mortality rate, especially for young children. *Salmonella* spp. mostly inhabit the intestinal tract of vertebrate and invertebrate, consequently they are excreted in feces and resulting in contamination of food, water and environment (Hale *et al.*, 2012).

The overall prevalence of *Salmonella* in this study was twenty isolates from 400 cases of diarrhea among children under five year and constituted (5%), this finding is supported by earlier studies done by AL-Taie, (2009) mentioned that *Salmonella* account at (5.66%). also Alrifai *et al.* (2008) isolated (6.2%) *Salmonella* from patients with diarrhea under five year in Tikrit city. Getamesay *et al.* (2014) and Abbas *et al.* (2017) asserts that appearance (2.5%) and (1.39%) children infected with *Salmonella* respectively, this percentage is slightly lower than present study. This study is irreconcilable with earlier study in Al-Hawijah city by Ali (2012), revealed (15.6%) from diarrheal state in children visiting Al-Hawijah public hospital. The variability of isolation of *Salmonella* may be attributable to the difference in study areas and period because the features of the disease vary from place to place and time to time depending on the local meteorology, geography and socioeconomic elements. Poor access to safe water, inadequate sanitary conditions, lower literacy rate, and unavailability of healthcare facilities in the remote area are the major factors for Salmonellosis infection.

The polymerase chain reaction is an alternative method to identify *Salmonella enterica,* in our study *invA* gene is carried by all *Salmonella* isolates (100%), this ratio accepted with previous studies by Shanmugasamy *et al.*(2011), Karmi (2013), and Elgohary *et al.* (2017) they found *invA* gene in all *Salmonella* isolates from different sources. *invA* gene is essential for the invasion of epithelial cells by *Salmonella* (Al-Kaaby *et al.,* 2014).

In this study the antimicrobial susceptibility testing for twenty *Salmonella* isolates showed that all isolates were sensitive to Chloramphenicol and Tetracycline, Lamboro *et al.* (2016) showed that (94%) of *salmonella* isolates sensitive to chloramphenicol. Tallal and Youssef (2010) reported that isolates of *Salmonella* (76.9%) sensitive to Tetracycline. Much of the resistance to chloramphenicol and tetracycline is associated with the acquisition and expression by efflux pumps that remove toxic of the drug from the bacterial cells (Butaye *et al.,* 2003). Microbial resistance develops through acquisition of resistance that involves drugs inactivation, decreasing drugs uptake, decrease receptors sites and metabolic pathway attacked by the drugs. Moreover widespread indiscriminate prescribing of antibiotics favors resistance to all common drugs (Omar, 2015).

In current study *Salmonella* isolates showed varying resistance ratios between antibiotic, it was highest percentage observed against Cefotaxime (55%), followed by Ampicillin (45%), Amikacin (35%), resistance to Ceftriaxone, Gentamicin and Nalidixic acid was detected in (30%), while Ciprofloxacin (15%). Former study in Tanzania indicated that resistance proportion to Ampicillin (46%), Ciprofloxacin (15%), this results agreement with our results, however disagree in resistance to Gentamicin (9.1%) (Omar, 2015). Lamboro *et al.* (2016) showed that (26.3%) resistance to Nalidixic acid, this ratio in line with our result. Whereas Tosisa (2015) reported lower ratio Resistance to Cefotaxime was (33.3%).

Antibiotic resistance has been around for as long as antibiotics have been used to treat infection. The origin of antibiotic resistance extends much further back in evolutionary terms and reflects the attack and counterattack of complex microbial flora in order to establish ecological niches and survive (Denyer *et al.,* 2011). Early treatment failures with antibiotics did not represent a significant clinical problem because other classes of agents, with different cellular targets were available. The major problem in the clinic today is the emergence of multiple-drug resistance, i.e. resistance to several types of antimicrobial agent (Amenu, 2014). Our result show that (45%) of isolates were MDR, so it in line with study done by Raza *et al.* (2003) they reported that 53.3% of their isolates were (MDR). These findings reinforce the need for continuous surveillance program and strengthened infection control system to reduce the rate of infection and to apply appropriate guidelines for the use of therapeutic antibiotics.

As concluded to this study: The occurrence of *Salmonella* was considered low when compared to other causes of gastroenteritis among children as enteropathogenic *Escherichia coli* or parasite causes. 45% of *Salmonella* isolates were multidrug resistance to antibiotic (MDR).

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