

**Antimicrobial resistance, Virulence profiles of *Salmonella enterica* serovar Typhimurium isolated from diarrheal children in Thi-Qar province during 2015**

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**Abstract**

A total of 14 *Salmonella enterica* serovar (ser.)Typhimurium isolates were isolated from diarrheal children in Thi-Qar province during 2015. these isolates were subjected to testing and analyzed for antibiotic resistance and virulence genes by using PCR all isolates were sensitive to the Amikacin and Gentamycin, while three isolates resistance to chloramphenicol, and nalidixic acid and one isolates was resistance to Ciprofloxacin in addition to four isolates were resistance to Ampicillin. All isolates were positive for *invA* and *sipB* genes, eight isolates were positive to *sopB* gene as and only three isolates were positive to *spvB* gene. These results suggest that *S. Typhimurium* from clinical is virulent and able to cause salmonellosis in human and it may contribute to pathogenesis. This article is a part of master thesis.

Keywords: - Antimicrobial resistance, virulence genes, *Salmonella typhimurium*, diarrheal Children

**المقاومه للمضادات الحياتيه وملاح الضراوه لبكتريا السالمونيلا تايفيموريوم المعزوله من العينات السريره للاطفال المصابين بالاسهال في محافظه ذي قار سنه 2015**

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**الخلاصه:**

تضمنت الدراره عزل 14 عزله من السالمونيلا التايفيموريوم المعزوله من العينات السريره للاطفال المصابين بالاسهال في محافظه ذي قار سنه 2015 وتعرضت هذه العزلات لاختبار وتحليل المقاومه وضراوه الجينات باستعمال ال PCR العادي وكانت جميع العزلات حساسه للجنتاميسين والاميكاسين بينما ثلاثه عزلات كانت مقاومه للكلورامفينيكول و الناليدياكسك اسيد وعزله واحده فقط مقاومه للسبروفلوكساسين بالاضافه الى اربع عزلات

مقاومه للامبسلين .وكانت جميع العزلات موجبه للجين *invA*/*invB* والجين *SipB* فقط ثلاث عزلات موجبه للجين *SopB* بينما ثمانى عزلات كانت موجبه للجين *SpvB* .وتشير هذه النتائج ان السالمونيلا تايقميويوم هي الاكثر ضراوه و امراضيه للبشر .

## 1-Introduction:

*Salmonella enterica* consists of more than 2668 different serotype. It can cause the disease in both humans and animals (Akbarmehr, 2011). *Salmonella* 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. Salmonellosis is common disease throughout the world. This disease in humans usually takes the form of a self-limiting food poisoning but occasionally manifested as a serious systemic infection or enteric fever (Cardinale *et al.*, 2005).

The contaminated food and water are the major mode of transmission for non typhoidal *Salmonella* because salmonellosis is a zoonosis and has an enormous animal reservoir. The most common of these animal reservoirs are chickens, turkeys, pigs and cows (Akbarmehr, 2011). Non- typhoidal *Salmonella* causes an estimated 1 million illnesses with approximately 20,000 hospitalizations and approximately 378 deaths each year (Scallan *et al.*, 2011). It is kills 3 million children each year in both developed and developing countries (Cardinale *et al.*, 2005). *Salmonella typhimurium* is the most common *Salmonella* serotype isolated from humans suffering from infectious gastroenteritis and correspondingly has long been recognized as a public health problem. Gastroenteritis is called nontyphoidal salmonellosis or enterocolitis; it is caused by at least 150 *Salmonella* serotypes with *S. Typhimurium* and *S. Enteritidis* being the most common serotypes in the United States (Finlay and Falkow, 1990).

This infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste. The emergence of multidrug-resistant *S. Typhimurium* DT104 has been associated with infection related to beef contamination and resulted in hospitalization rates twice than that of other foodborne salmonellosis (Gray and Fedorka-Cray, 2002; Yousef and Carlstrom, 2003).

There are differentiations of clinical signs of Salmonellosis in human, some of them are related to the microorganism itself, such as the infectious dose, serotype, and the virulence of bacteria; other depend on host, age, and immune system (Zaidi *et al.*, 2006). The

clinical spectrum with infection by nontyphoidal *Salmonella* that ranges from asymptomatic carriage to a severe and potentially fatal illness (Miller and Pegues, 2000). In generally, diarrhea a common symptom of human salmonellosis, possibly abdominal cramps, vomiting and fever are clinical signs usually begin between 12 and 72 hours after infection, however the incubation period ranges 5 hours to 7 days (Zaidi *et al.*, 2006). In some cases, the organism may pass into the blood stream and spread throughout the body causing organ damage and possibly death (Colville and Berryhill, 2007). *Salmonella* spp. possesses a number of structural and physiological virulence factors, enabling them to cause acute and chronic disease in humans. The virulence of *Salmonella* spp. varies with the length and structure of the O side chains of lipopolysaccharide molecules at the surface of the bacterial cell. (Jay *et al.*, 2003). Other important virulence factors include the presence and type of fimbriae, which is related to the ability of *Salmonella* spp. to attach to host epithelium cells, as well as the expression of genes responsible for invasion into cells (Jones, 2005). Some of these virulence genes are encoded on *Salmonella* pathogenicity islands (SPI). SPI-1 is required for bacterial invasion into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* spp. are dependent on the function of SPI-2 (Valle and Guiney ,2005). In addition, *Salmonella* spp. produces a heat labile enterotoxin, resulting in the loss of intestinal fluids (causing diarrhea). This enterotoxin is closely related functionally, immunologically and genetically to the toxin of *Vibrio cholerae* and the heat labile toxin of pathogenic *Escherichia coli* (Jay *et al.*, 2003).

Most *Salmonella* serovars also produce heat labile cytotoxin which may cause damage of the intestinal mucosal surface and results in general enteric symptoms and inflammation (Jay *et al.*, 2003; Hanes, 2003). Other important *Salmonella* spp. virulence factors are found on virulence plasmids. All of the virulence plasmids share a highly conserved region designated *spvRABCD* (*Salmonella* plasmid virulence). The *spv* region promotes rapid growth and survival of *Salmonella* spp. within the host cells and it is important for systemic infection (Libby *et al.*, 1997). However, the presence of virulence plasmids has been associated

with non-typhoidal *Salmonella* spp. surviving in phagocytes and spreading from the small intestine to the spleen and liver (Jay *et al.*, 2003; Hanes, 2003).

Strain identification is necessary for effective investigation of source outbreaks in addition to the molecular tools are important for monitoring and prevention the diseases. among molecular –based techniques used recently polymerase chain reaction (PCR). The clinical management of patients infected with *Salmonella enterica* serotypes is difficult due to the emergence of multidrug-resistant (MDR) strains (Rowe *et al.*, 1997 and Mermin *et al.*, 1999).

In this study *S. Typhimurium* isolates from diarrheal children in Thi-Qar province during 2015 were examined for antimicrobial resistance and PCR for virulence genes.

## 2. Materials and methods

### 2.1. Bacterial strains

Fourteen isolates of ser. Typhimurium were selected for this study. These strains were isolated from children (1 day-13years) suffering from diarrhea, from both sexes in Mohammed Al-Mosawi Hospital in Thi-Qar province. All isolates were stored in brain heart broth containing 20% glycerol at -70 °C.

### 2.2. Antimicrobial susceptibility testing by disk diffusion

All isolates *Salmonella typhimurium* 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).

### 2.4. PCR detection of virulence genes

All isolates of ser. Typhimurium were screened for 4 virulence genes (*invA*, *SopB*, *sipB*, *SpvB*) by a simplex PCR method (Skyberg *et al.*, 2006). The Primers used for our study are listed in Table 1. Total genomic DNA from the isolates was extracted from overnight cultures by using the Presto Mini g DNA Bacteria Kit from Geneaid,USA). The composition of

the PCR mixture was and 1 µl of template DNA 1µl PCR buffer, 200 mM of each dNTP, 0.25 mM of forward and reverse primers, 2.5 units of Taq DNA polymerase (Bioneer). The PCR cycling conditions were 5 min at 95 °C; 30 cycles of 40 s at : 94 °C, 60 s at 66.5 °C, and 90 s at 72 °C, with an additional extension for 10 min at 72 °C. The PCR products were visualized by electrophoresis on 1.2% agarose gels in 1X TBE buffer at 50 V for 85 min.

Table (1): primers used in PCR for detection of virulence genes in *S. Typhimurium*

Gene Name	DNA Sequences (5'-3')	Product Size Bp	Function of gene
<i>sipB</i>	F GACGCCGCCGGGAAAACTCTC	875	Entry into non - phagocytic cells
	R CACTCCCGTCGCCGCTTCACAA		
<i>spvB</i>	F CTATCAGCCCCGACGGAGAGCAGTTTTTA	717	Growth within host
	R GAGGAGGCGGTGGCGGTGGCATCATA		
<i>invA</i>	F TGGCGGTGGGTTTTGTGTCTTCTCTATT	1070	Host recognition /invasion
	R GTTCTCCCCCTCTTCATGCGTTACCC		
<i>sopB</i>	F GGACCGGCCAGCAACAAAACAAGAAGAAG	220	Host recognition /invasion
	R AGTGATGCCCGTTATGCGTGAGTGATT		

## 3. Results

Antimicrobial susceptibility testing to 14 *S. Typhimurium* isolated showed that all isolates were sensitive to the Amikacin and Gentamycin, while three isolates resistance to chloramphenicol, and nalidixic acid and one isolates was resistance to ciprofloxacin in addition to four isolates were resistance to ampicillin, as in table( 2 ).

Table (2): Antimicrobial susceptibility testing

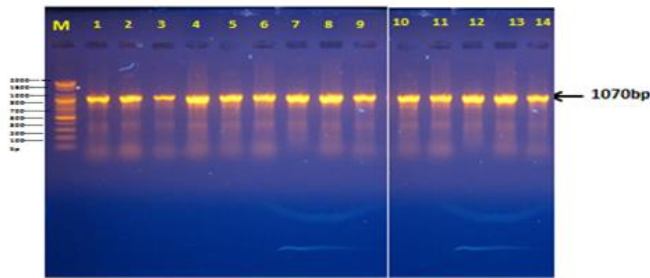
Antimicrobial Agents	<i>Salmonella enterica</i>					
	No. R	% of R	No. I	%of I	No. S	%of S
Amikacin (AMC)	-	-	-	-	14	100%
Gentamycin (CN)	-	-	-	-	14	100%
Ciprofloxacin(CIP)	1	7.1%	11	78.5%	2	14.2%
Nalidixic acid (NA)	3	21.4%	8	57.1%	3	21.4%
Ampicillin(AMP)	4	28.6%	10	71.4%	-	-
Chloramphenicol(C)	3	21.4%	1	7.1%	10	71.4%

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

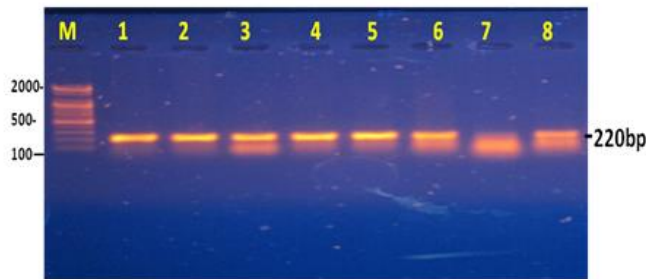
All *S. Typhimurium* isolates are screened for four virulence genes (*SpvB*, *SopB*, *invA*, *sipB*) by using PCR .All isolates were positive for *invA* and *sipB* genes as in (fig.1) and (fig.3), eight isolates were positive to *sopB* gene as in ( fig.2) and only three isolates were positive to *spvB* gene as in (fig.4) (table 3)

Table (3) distribution virulence genes in *S. Typhimurium*

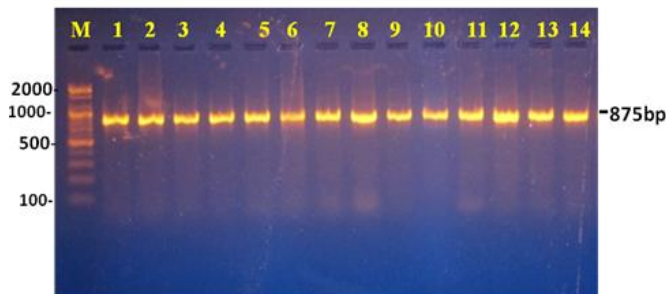
Virulence gene	Number of <i>S. Typhimurium</i>	Percentage of <i>S. Typhimurium</i> %
<i>invA</i>	14	100
<i>sopB</i>	8	57.1
<i>sipB</i>	14	100
<i>spvB</i>	3	21.4



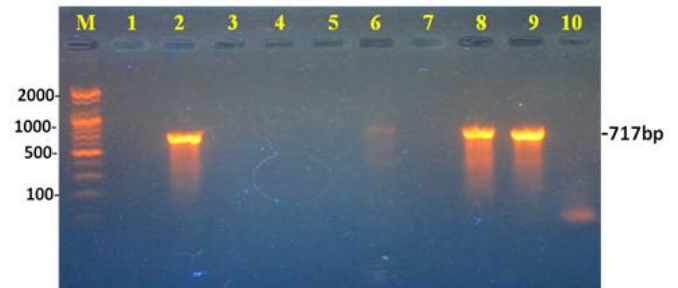
**Figure 1.** Agarose gel electrophoresis of *invA* gene .PCR products amplified from *S. Typhimurium* .M: marker (2 kb ladder); 1 to 14 lanes: *S. typhimurium* isolates.



**Figure 2.** Agarose gel electrophoresis of *sopB* gene. PCR products amplified from *S. Typhimurium* .M: marker (2 kb ladder); 1 to 8 lanes: *S. Typhimurium* isolates



**Figure 3.** Agarose gel electrophoresis of *sipB* gene .PCR products amplified from *S. Typhimurium* .M: marker (2 kb ladder); 1 to 14 lanes: *S. Typhimurium* isolates.



**Figure 4.** Agarose gel electrophoresis of *spvB* gene .PCR products amplified from *S. Typhimurium* .M: marker (2 kb ladder); 1 to 14 lanes: *S. typhimurium* isolates

#### 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). There are 3.6 million (39%) foodborne illnesses have been caused each year by the bacteria, in which nontyphoidal *Salmonella* caused 1 412 498 cases of illness in the United States According to the Food Net, which was established in 1996 as a collaboration of the CDC, USDA, FDA, and selected state health departments, (Chen *et al.*, 2006; Mezal *et al.*, 2014). Our study showed that *S. Typhimurium* isolates have many virulence genes such as *spvB*, *invA*, *sipB*, *sopB*, which might essential role in invasion and survival in the host and in growth within host in addition to entry into non-phagocytic cells .recently Akiyama *et al.*(2011) and Mezal *et al.*, (2013) indicated that same virulence genes in *S. enteritidis* and *S. saintpaul* isolated from clinical sources in united states of America ,which are able to cause infections to 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 such 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, so 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) In this

study the antimicrobial susceptibility testing for 14 *S. Typhimurium* isolates showed that all isolates were sensitive to the Amikacin and Gentamycin this accepted with (Mezal,2015) .in other hand , three isolates showed resistance to chloramphenicol and nalidixic acid, however all isolates were resistance to chloramphenicol and Al-Hashimy(2012) reported all isolates were sensitive to chloramphenicol and 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 one isolates were resistance to ciprofloxacin .our study showed four isolates were resistance to Ampicillin. This results are agree with Mezal (2015).

### Conclusion:

*Salmonella Typhimurium* can present virulence genes (*spvB*, *sopB*, *invA*, and *sipB*) related to invasion and survival in the host and in growth within host in addition to entry into non-phagocytic cells and that have high prevalence in antimicrobial resistance.

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