

## Molecular detection of *Salmonella typhi* isolated from diarrheal patients in Al-Najaf governorate

Ahmed AlObaidi<sup>1</sup>; Najat Mohammed Flyyih<sup>2</sup>; Dr. Mohammed Abdulrazzaq Assi<sup>3\*</sup> and Basima Basim M.A.<sup>2</sup>

<sup>1</sup>Department of Medical Lab Techniques, College of Health and Medical Techniques, Al\_FuratAl\_Awsat Technical University, 31003 Al-Kufa, Iraq.

<sup>2</sup>Middle Technical University, Collage of Health & Medical Technology, Baghdad, Iraq.

<sup>3</sup>Department of Community Health, College of Health and Medical Techniques, Al\_FuratAl\_Awsat Technical University, 31003 Al-Kufa, Iraq.

\*Corresponding author: Mohammed Abdulrazzaq Assi, E-mail: [razaq\\_assi@yahoo.com](mailto:razaq_assi@yahoo.com), [kuh.muh@atu.edu.iq](mailto:kuh.muh@atu.edu.iq)

### Summary

All of (80) stool samples isolated from affected persons with diarrheal problems from (11/ 2018 to 12/ 2018). All samples tokened from patients admitted to AL-Sadder Medical hospital and AL-Hakem General Hospital through the studying time. The samples were grown on the S-S agar media and incubated for 18 - 24 hr. at 37°C with the aerobic condition. About 80 patient samples, results include 38 (47.5%) were males and 42 (52.5%) were females. Many cultural, morphological, and biochemical tests were made in order to recognize bacterial isolates. Results revealed that *Salmonella typhi* constitute twelve (12) isolates from all specimens. A collection of 12 (15%) *Salmonella typhi* isolates demonstrated by the cultural, biochemical and morphological features, the investigation was specified by molecular techniques to investigate the *fliC* gene presence. The results revealed that only 6 (50%) samples were carrying the *fliC* gene which is considered as *Salmonella typhi*. The assays of antibiotic susceptibility test showed that samples of *Salmonella typhi* exhibited complete sensitivity to Gentamicin and Ciprofloxacin, moderate sensitivity against Trimethoprim, whereas the findings showed complete resistant to amoxicillin and piperacillin. According to these results it can have considered that Gentamicin and Ciprofloxacin as better choice antibiotics for treatment against *Salmonella typhi*.

**Keyword:** *Salmonella typhi*, diarrhoea, *fliC*

**How to cite this article:** Assi MA, Al Obaidi A, et al (2019): Molecular detection of *Salmonella typhi* isolated from diarrheal patients in Al-Najaf governorate, *Ann Trop & Public Health*; 22(9): S263. DOI: <http://doi.org/10.36295/ASRO.2019.22097>

### Introduction

Typhoid fever is one of the most important infectious and serious diseases of wide distribution<sup>(1)</sup>. It is a very important systemic disease mainly caused due to *S. typhi* and is carried via fecal-oral mode by the ingestion of polluted eggs, poultry, milk, and meat. The disease is most frequently in the elderly and children at a young age with the high occurrence at the end of summer<sup>(2)</sup>. The disease is transported by the consumption of food or water mixed with feces. This infection is carried by direct hands mixed with urine or feces of patients or by consumption of polluted food, water, and dairy products indirectly all of which are considered as rich sources of infection<sup>(3,11)</sup>.

Because *S. typhi* is a specific-human pathogen, those carriers serve as a critical source for more spread of the disease through bacterial dissemination in feces, which is an intermittent and sporadic event<sup>(4)</sup>. *Salmonella* as a pathogenic expresses a wide variety of virulence factors that promote the organism's pathogenicity. These factors may include polymorphic carbohydrates in the membrane, many fimbria adhesions, flagella with phase and antigenic variation property and best structural mechanisms for survival and invasion of host macrophages and other cells. A pathogenicity island is one of the genetic elements that occur as a separate and distinct unit in the bacterial chromosome<sup>(5)</sup>.

Flagella are thin, filamentous, rigid projections of bacteria, and are considered bacterial locomotion organelles. A flagellum has three main parts: the outermost and longest part is a filamentous shape which consists of around 20,000 protein subunits of a single protein called flagellum (Flic) have a molecular weight of 50 to 60 KDa<sup>(6)</sup>. Flagella provide the *S. typhi* with rigidity, motility and may play a role in cell entry, are controlled inside cells, and it has been supposed that they are used for escape from an intracellular location<sup>(7)</sup>.

**Methodology**

**Samples isolation**

All (80) clinical samples were isolated from individuals who suffered from diarrheal events through time from 11/ 2018 to 12/ 2018). Concerning these samples were isolated from patients who were admitted to AL-Sadder Medical hospital and AL-Hakem General Hospital through the studying. All samples were isolated and cultured on S-S agar media and incubation under the aerobic conditions for 18 - 24 hr.at 37°C.

**Molecular Studies of Salmonella typhi**

**Purification of Chromosomal bacterial DNA**

Whole DNA was isolated from pure cultures of agar plates via boiling technique according to <sup>(8)</sup>. Pure isolates of the bacterial colonies were harvested by using sterilized swab from agar media surfaces, suspended with 40 µl Tris -EDTA buffer. The solution boiled for 15 minutes at 100 °C then transformed on ice rapidly for 5 min. The DNA-suspended solution was kept at -20 °C to be later utilized for PCR.

**Primers Selection**

Selected primers were manufactured by Korean Bioneer Company, table (1).

**Table (1): Size of products & sequences of all primers.**

Kind of Primer	(5'-3') Primer		Size of product(bp)	References
<i>fliC</i>	F.	ACTCAGGCTTCCCGTAACGC	763	(9)
	R.	GGCTAGTATTGTCTTATCGG		

**Table (2): PCR cycling by thermo cycler instrument**

Gene Name	Temp. (°C) / Time				Final Extension of DNA	Number of cycles
	Denaturation Initial	Cycling programs				
		Denaturation	Annealing of primer	Extension of primer		
<i>fliC</i>	95/5 min.	94/35 s	55/30 s	72/40 s	72/5min	35

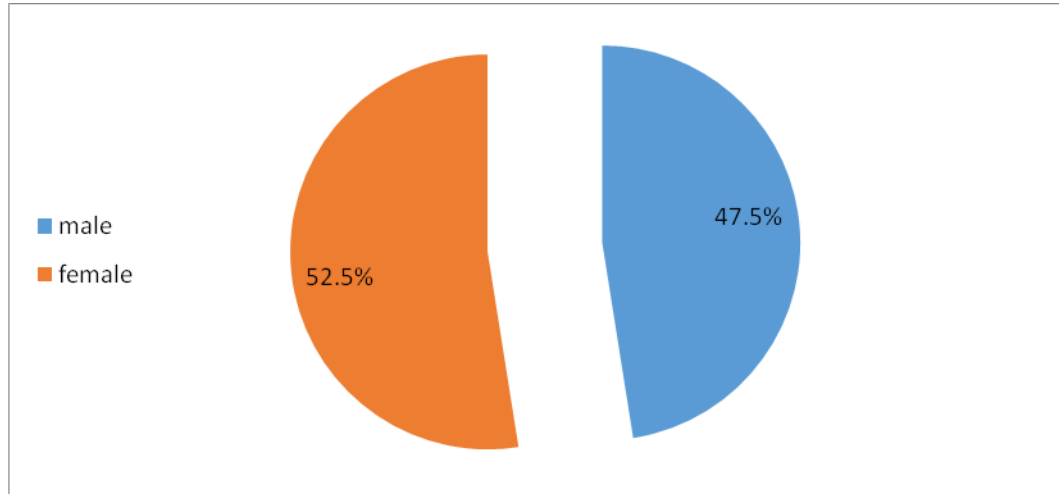
**Sensitivity tests for antibiotics**

Demonstration of Salmonella typhi susceptibility to several types of antibiotics by disks diffusion test was performed depending on <sup>(10, 12, 13)</sup>. The culture of pure colonies from identified bacteria previously was demonstrated by adding an isolated colony in culture to 5 ml sterilized saline with a density identical to (0.5) McFarland turbidity which mainly identical with bacterial broth density of 1.5x10<sup>8</sup> cells/ml. A sterilized swab was used to make inoculation that plated on Muller Hinton Agar (MHA). The disks of antibiotics were situated on the medium surface at intervals with forceps sterilized by flaming and incubated at 37°C for 18 hr. The inhibition zone of antibiotics was tested by using a specific ruler. The diameter of the zone was correlated with standard results of the recommended clinical laboratory standards institute (CLSI).

## Results & Discussion

### Bacterial Isolation

From overall 80 patients, 38(47.5%) were males and 42 (52.5%) were females. Many cultural, morphological, biochemical tests were done in order to identify bacterial cultures. This result disagrees with other results of studies in AL-Musaib district <sup>(11)</sup> and Diyala<sup>(12)</sup>, where found males were more infected than females with diarrheal conditions due to these bacteria.



Figure(1): Distribution of patients according to gender

### *Salmonella typhi* Isolation with Identification

#### Morphological with Biochemical Test Identification

Microscopically *S. typhi* considered as gram-negative bacilli, motile with peritrichous, without any spore and surrounded by a tiny capsule of polysaccharide. All *S. typhi* isolates have the ability to fermenting arabinose. The isolates were having facultative anaerobic and aerobic conditions and cultured on bacterial media in temperature mainly 37°C, also need enrichment medium enriched with amino acids & vitamins. *Salmonella typhi* was isolated by special selective media from clinical specimens such as S-S agar. Results obtained showed that the *S. typhi* appearing on S-S agar is a highly selective special medium designed to stop the growth of many *E. coli* bacteria and permit only the growth of *Shigella* and *Salmonella* species from clinical specimens.

The current study results showed that colonies of many strains *S. typhi* were large with 2-3 mm after 24 hr. at 37°C. The colonies of *Salmonella typhi* on MacConkey agar appeared pale yellow at 37°C after 18-24 hour and 1-3 mm in diameter with easily recognized from the lactose fermenter pink colonies of commensal coliform bacilli. IMVC tests were used for differentiation genus *S. typhi* from *Citrobacter* and *Shigella*. The reports of the study revealed that *S. typhi* isolates have negative results to indole, Voges-Proskauer, and Simmons citrate while positive to methyl red test and H<sub>2</sub>S with no gas production.

In Slants of TSI agar, both the slant and butt changed into red and yellow color respectively, indicating the glucose was fermented only without acid in the butt. The *S. typhi* isolates revealed H<sub>2</sub>S production without gas in TSI. All the results (morphology and cultural) were similar to (13), table (3).

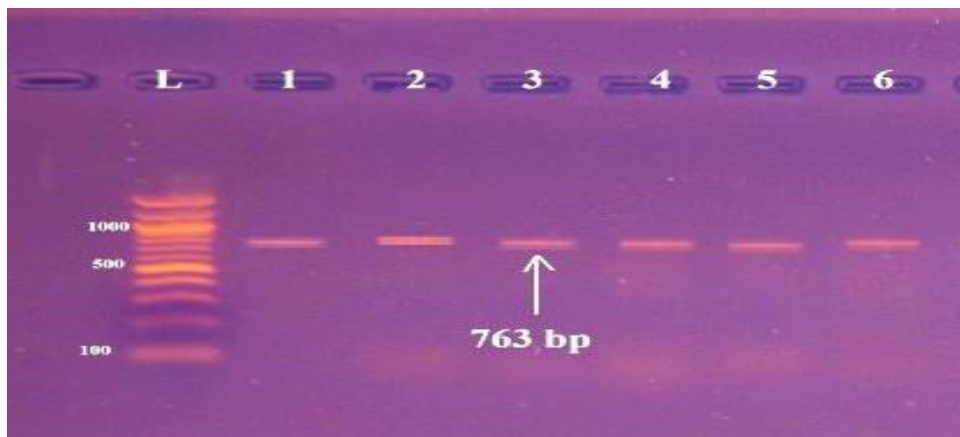
Table (3): Biochemical tests of *S. typhi*

Test	Result
------	--------

Cell shape	Bacilli
Production of Indole	-ve
Methyl red production	ve+
Voges –Pproskauer (V.P.)	ve_
Simmons Citrate production	ve_
Triple sugar iron (TSI)	(for H <sub>2</sub> S)+

**Detection of *Salmonella typhi* genes by molecular techniques**

Sum of 12 (15%) *Salmonella typhi* isolates demonstrated via the, cultural and biochemical features and other morphological, the investigation was specified with molecular biology assays in order to demonstrate *fliC* gene. The study results revealed that 6 (50%) isolates only carried *fliC* which are considered as *Salmonella typhi*. This result is identical with (14) who found from overall 80 typhoid fever patients, flagellum gene (*fliC-d*) was investigated by PCR technique in 56 (70%) cases which related with the reports of identical study conducted in Bangladesh where PCR was positive in 88.7% of typhoid fever cases. The reported positivity rate of PCR is 65%- 71.9% in different studies (14), Figure (2).



**Figure (2):** Ethidium bromide-stained agarose gel electrophoresis of PCR amplification products of *S. typhi* isolates that amplified with *fliC* gene primers with product 763 bp for 1 hr. at 80 volt/cm.

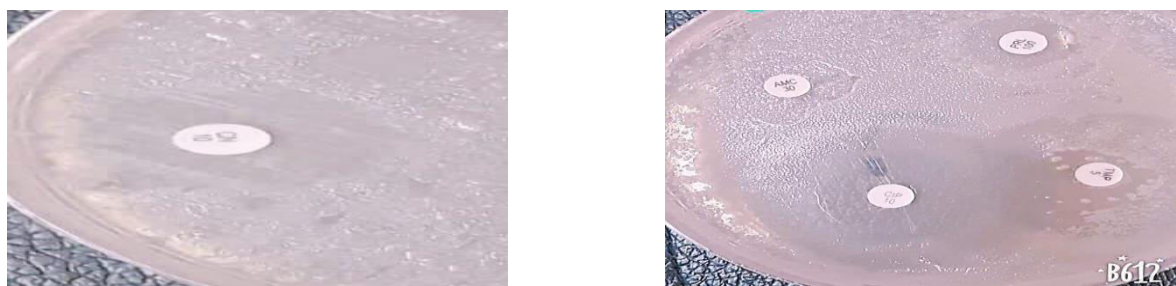
**Antibiotic Susceptibility Test**

The antibiotic sensitivity results showed that the samples of *Salmonella typhi* have risen sensitivity to Gentamicin and Ciprofloxacin, moderate sensitivity for Trimthoprime, whereas the results showed complete resistant to amoxicillin and piperacillin. According to these results it can be consider that Gentamicin and Ciprofloxacin as the better treatment against *Salmonella typhi*, table (4)

No.of specimens	Amoxicilli	Gentamicin	Trimethoprim	piperacillin	ciprofloxacin
1	R	S	R	R	S
2	R	S	R	R	S
3	R	S	S	R	S
4	R	S	R	R	S
5	R	S	S	R	S
6	R	S	S	R	S
<b>Sensitivity</b>	<b>0%</b>	<b>100%</b>	<b>50%</b>	<b>0%</b>	<b>100%</b>
<b>Resistant</b>	<b>100%</b>	<b>0%</b>	<b>50%</b>	<b>100%</b>	<b>0%</b>

**Table (4): Antibiogram for *Salmonella typhi* isolates**

In many cases resistant to ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole is positioned on plasmids <sup>(15)</sup>. Through the 1990's quinolones mainly ciprofloxacin became the treatment of choice for the cure of MDR typhoid infections but intermittent cases with epidemic resistance spread to these drugs also were recorded through a few years after their starts. *S. typhi* strains with little sensitivity to fluoroquinolones considered as the most important equal in the Asian countries <sup>(16)</sup>. About 90% *S. typhi* MDR isolates consist of integrons Class I indicating their high frequency of presence in *S. typhi*. Class I-integrons have been shown carrying 1-4 resistance genes of antibiotics. The isolates carrying integrin are mainly multi antibiotic-resistant, while the isolates without integron are more susceptible to antibiotics like (Amikacin, Ampicillin, Gentamycin, Trimethoprim Piperacillin, Chloramphenicol, and trimethoprim /sulphamethoxazole). A high correlation of class I integrons and many resistance to antibiotics has been attributed as a part of the presence of resistant genes for gentamicin and kanamycin <sup>(17)</sup>. The results revealed a complete sensitivity of 6 (100%) to Ciprofloxacin. This study does not coincide with the results of <sup>(18)</sup> who found resistant rates were 20% to Ciprofloxacin. With the emergence of MDR in *S. typhi*, Ciprofloxacin was considered the drug of choice; however, there have been many reports of treatment failure with Ciprofloxacin in patients with enteric fever. These strains were Ciprofloxacin- susceptible by former CLSI breakpoints <sup>(19,20)</sup>



**Figure (3): Kirby-Bauer disk diffusion method of *S. typhi* isolates**

## Conclusions

In this study, the *FliC-d* gene was a major-specific gene for the detection of *S. typhi* in diarrheal patients by the diagnostic laboratories. However, *S. typhi* isolated from diarrheal patients show susceptibility to such as Gentamicin and Ciprofloxacin, moderate sensitivity for Trimethoprim, while complete resistant to amoxicillin and piperacillin. PCR method was more sensitive for *S. typhi* diagnosis among diarrheal patients.

## References

1. House, D.; Bishop, A.; Parry, C. M.; Dougan, G. and Wain, J. (2001). Typhoid fever: pathogenesis and disease. *Curr Opin Infect Dis*. 14:573-8.
2. Jerrold, R. and Turner. (2010). The gastrointestinal tract: Salmonellosis, in Kumar, V, Abbas, AK, Fausto, and Aster, JC, editors, *Robins and Cotran Pathologic Basis of Disease*, 8th edition India, Elsevier, p. 801.
3. Singh, B. (2001). Typhoid fever: epidemiology, *Journal of Indian Academy of Clinical Medicine*, vol. 2, pp. 17-20.
4. Bhan, MK.; Bahl, R. and Bhatnagar, S. (2005). Typhoid and paratyphoid fever, *Lancet* 366:749–762.
5. Hensel, M. (2004). Evolution of pathogenicity islands of *Salmonella enterica*. *Int. J. Med. Microbiol.* 294:95-102.
6. Chang, G-N.; Chen, C-S. and Chieh Ho, K.(2007). Molecular cloning and expression of a phase I flagellin gene from *Salmonella entericaserovarCholerasuis*. *Taiwania*. 53(3): 201-209.
7. Ibarra, JA .and Steele-Mortimer, O. (2009). *Salmonella* –the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cell Microbiol* 11: 1579 – 1586.
8. Yi,H.;Zhang, L.;Tuo,Y.; Han, X. and Du, M.(2010).A novel method for rapid detection of class II bacteriocin –producing lactic acid bacteria. *Food control*.21.426-430.
9. Levy, H.; Diallo, S.; Tennant, SM.; Livio, S.; Sow, SO.; *et al.* (2008). PCR method to identify *Salmonella entericaserovarstyphi*, *Paratyphi A*, and *Paratyphi B* among *Salmonella* isolates from the blood of patients with clinical enteric fever. *J Clin Microbiol* 2008, 46(5):1861-1866.
- 10- Perilla, M.J.; Ajello, G.; Boop, C.; Elliot, J.; Facklan, R.; *etal.* (2003). Manual for laboratory identification and antimicrobial susceptibility testing of bacterial pathogen of public health importance in developing world .Center for Diseases Control and Prevention ,Atlanta, Georgia, USA.
- 11- AL-Khafaji, J.K.; Yasarri, H.F. and Altaei, M.H. (2006). Prevalence of typhoid fever among pediatric patients at almusaiib district. *Medical Journal of Babylon*.3, 1-2.
- 12- Saleh, M. A. D. (2013). Comparison of widal agglutination test with ELISA *typhi* test for serological diagnosis of typhoid fever in some Iraq patients in Diyala governorate. 1(5):59-64.
- 13- Nalbantsoy, A. (2012). Prevention and identification of *Salmonella* 4: 1614.1622.
- 14- Khan, B.N.; Harish, G.A.; Menezes, N.S.; Acharya. and S.C., Parija. (2012). Early diagnosis of typhoid fever by nested P.C.R. for flagellin gene of *Salmonella enterica serotype typhi*. *Indian J Med Res* 136: 850-854.
- 15- Parkhill, J.; Dougan, G.; James, K. D., Thomson, N. R.; Pickard, *et al.* (2001). Complete Genome Sequence of A Multiple Drug Resistant *Salmonella Enterica Serovar Typhi* CT18. *Nature*.413:848-852.
- 16- Threlfall E. J. and Ward L. R. (2001). Decreased Susceptibility To Ciprofloxacin In *Salmonella Enterica Serotype Typhi*. United Kingdom. *Emerg Infect Dis*; 7: 448-450.
- 17- Miko, A.; K. Pries, A. Schroeter and Helmuth, R. (2005). Molecular Mechanisms of Resistance In Multidrug-Resistant Serovars of *Salmonella Enterica* Isolated From Foods In Germany. *Journal of Antimicrobial Chemotherapy* 56, 1025-1033.
- 18- Doughari, J. H.1.; Elmahmood, A. M1. and Nggada, H. P.2(2007). Retrospective study on the antibiotic resistant pattern of *Salmonella typhi* from some clinical samples. *African Journal of Microbiology Research* pp. 033-036, August, 2007.
- 19- Renuka K, Kapil A, Kabra SK, Wig N, Das BK, Prasad VV, Chaudhry R, Seth P.(2004). Reduced Susceptibility to Ciprofloxacin and Gyra Gene Mutation in North Indian Strains of *Salmonella Enterica Serotype typhi* and *Serotype paratyphi A*. *Microb Drug Resist* 10: 146-153. *Research*, 167:199-210.
- 20 Al Shareefi, E. and Cotter, S.C., 2018. The nutritional ecology of maturation in a carnivorous insect. *Behavioral Ecology*, 30(1), pp.256-266.