



## Identification of Antibiotic-Resistant Genes in *Salmonella Typhi* Isolated From Typhoid Patient in Samawa City

Ali Anok Njum\*<sup>1</sup>, Rimia N. Hassan<sup>1</sup>, Jabbar Afat Alwan<sup>2</sup>

<sup>1</sup>Technical Institute/ Samawa Nursing Technique Department, Samawa, Iraq

<sup>2</sup>Veterinary medicine College, AL-Qadysia University, AL-Qadysia, Iraq

### Abstract

A total of 100 blood samples taken from patients with suspected typhoid fever aged between (1-60) years, were involved in this study. Blood samples were cultured directly on brain heart infusion broth. After that sub cultured of isolates on MacConkey agar and XLD agar and S.S agar to find the *Salmonella typhi* then identified by the biochemical and antibiotic sensitivity test. Resistant genes were identified by using *aacc2* gene and *cat* gene. Results showed that there was 7 *Salmonella typhi* isolates from blood culture, as well as, *aacc2* gene success in amplification of 450bp fragment for amino glycoside resistant, while not improve amplification for chloramphenicol resistant gene (*cat* gene) from *Salmonella typhi* isolates. also our isolates showed sensitivity 100% for ceftriaxone, cefepime, cefazolin, and chloramphenicol, while all isolates were resistant 100% for tetracycline and erythromycin.

**Keywords:** Resistant Genes, *Salmonella Typhi*, Antibiotic-Resistant.

## تحديد جينات مقاومة المضادات الحيوية في جرثومة السالمونيلا تايفي المعزولة من المرضى المصابين بحمى التايفوئيد في مدينة السماوة

علي عنوك نجم\*<sup>1</sup>، ريميا نعمت الله حسن<sup>1</sup>، جبار عفات علوان<sup>2</sup>

<sup>1</sup>المعهد التقني سماوة، قسم التمريض، السماوة، العراق

<sup>2</sup>كلية الطب البيطري، جامعة القادسية، القادسية، العراق

### الخلاصة

اجريت الدراسة الحالية وذلك بجمع 100 عينة دم من المرضى المصابين بحمى التايفوئيد Typhoid fever والتي تتراوح اعمارهم بين ( 1-60 ) سنة، زرعت عينات الدم مباشرة على مرق نقيع القلب والدماغ ومن ثم زرعت العزلات على وسط اكار الماكونكي MacConkey agar و اكار الاكس XLD agar الذي واكار السالمونيلا والشيكلا *Salmonella and Shigella* agar للتحري عن جرثومة السالمونيلا تايفي *Salmonella typhi* ومن ثم تم التعرف على الجرثومة بواسطة الفحوصات الكيموحيوية واختبار الحساسية للمضادات الحيوية. حددت جينات المقاومة باستخدام جين *aacc2* و جين *cat*. أظهرت النتائج بالحصول على 7 عزلات لجرثومة السالمونيلا تايفي من طريقة زرع الدم Blood culture وأظهرت النتائج أيضا بأن جين *aacc2* نجح في تضخيم الحزمة 450bp والخاصة بالمقاومة لعائلة الامينوكلايكوسايد Aminoglycosides، بينما لا نلاحظ تضخيم لجين *Cat* والخاص بالمقاومة للمضاد الحيوي الكلورمفينيكول. كذلك اظهرت العزلات الحالية حساسية 100% لكل من المضادات الحيوية السيفاتركزون ceftriaxone

\*Email: najumaok@gmail.com

والسيفيبيم cefepime والسيفازولين cefazolin والكلورمفنيكول. بينما كل العزلات كانت مقاومة 100% للمضادات الحيوية التتراسايكلين والايثرومايسين.

## Introduction

*Salmonella* is greatest essential genus of Enterobacteriaceae. *Salmonella* genus are Gram-ve , facultative anaerobes and live in the intestinal tract of animals and human also broad range of hosts such as human, swine, poultry, environment, and foods. Members of the genus *Salmonella* might be pathogenic to domestic or wild human and animals [1]. It's significant pathogen to the food manufacturing and has been commonly recognized as the etiological cause of food borne out breaks [2]. These bacteria cause systemic infection, enteritis and enteric fever [3].

The illness may well latest from 3 to 4 weeks and death rate range between (12% and 30%). Although the worldwide burden of typhoid fever has less , emergence of multi drug resistant *Salmonella typhi* (MDRST) is still a threat to public health. Currently, "107 strains of this organism have been isolated; many containing varying metabolic characteristics, levels of virulence, and multi-drug resistance genes that complicate treatment in areas that resistance is prevalent" [4]. *S.typhi* gained resistance to antibiotics like ceftriaxone, co-trimoxazole and ampicillin, also developing resistance to efficient medicines like ciprofloxacin. The emergence of multidrug-resistance(MDR) to the generally used antibiotics has further complicated the management and treatment of enteric fever and this is known as one of the highest challenges in the management of this disease [5, 6]. The purpose of the research was to study antibiotic resistant genes of *Salmonella typhi* in typhoid patient by molecular and bacteriological assay.

## Materials and Methods

**Patients:** The study included 100 patient who attended Al-Hussein-Teaching Hospital – Al-Sammawa city aged (1-60) during the period extended from December 2018 to Jun 2019. Clinical signs of typhoid patient were recorded by the physician and firstly diagnosed with Widal test

**Bacterial isolation:** specific isolation of *Salmonella typhi* from blood specimens, 5 ml of blood were cultured on Brain-heart infusion broth(BHI broth) then incubated at 37C°/24 hr. , then purified via sub cultured on blood agar and macConkey agar . The identification tests for the isolate, including cultural (macConkey agar and XLD agar), morphological and biochemical characteristics (catalase, oxidase, urease, Triple sugar iron, indole, VP-MR, and citrate utilization) was done for each isolate.

## Antibiotic susceptibility test

The isolates will be tested for susceptibility to 13 diverse antibiotic agents include

**Table 1**-Antimicrobial disks

No	Antibiotics	Symbol	mcg \ disk	Manufacturing company
1.	Amikacin	AK	10	Bioanalyse(Turkey)
2.	Ampicillin	AM	10	Bioanalyse(Turkey)
3.	Cefazoline	CZ	4 MIC	Biomerieux( France)
4.	Cefepime	FEP	1 MIC	Biomerieux ( France)
5.	Ceftriaxone	CRO	30	Bioanalyse(Turkey)
6	Chloramphenicol	C	10	Bioanalyse(Turkey)
7	Ciprofloxacin	CIP	5	Bioanalyse(Turkey)
8	Erythromycin	E	15	Bioanalyse(Turkey)
9	Gentamycin	G	10	Bioanalyse(Turkey)
10	Nitrofurantion	NF	300	Bioanalyse(Turkey)
11	Penicillin G	P	10 unit	Himedia (India)
12	Sulfamethazole/Trimethoprim	SXT	23.75/1.25	Bioanalyse(Turkey)
13	Tetracyclin	TE	30	Himedia (India)

The disc diffusion of these antibiotic agents was determined by a standard method which recommended in "the National Committee for Clinical of Laboratory Standards" (NCCLS) [7]. Molecular Identification for resistant genes by PCR-based assay:

The system used was Flic primers system. As advised by [8, 9]. And provided by Alpha DNA Company, as described.

Primer	Sequence 5-3		Amplicon
<i>Aacc2</i>	F	5'GGCAATAACGGAGGCAATTCTGA3'	450bp
	R	5'CTCGATGGCGACCGAGCTTCA3'	
<i>CatP 4</i>	F	"5'CCT GCC ACT CAT CGC AGT 3'"	623bp
	R	"5'CCA CCG TTG ATA TAT CCC 3'"	

The major basic process use for DNA purification is executed according to handbook information of Norgen,Canada

The PCR amplification process.

-The reaction solution: Enzymatic amplification of DNA was approved out in a final volume of (25µl) according to the recommendations of manufacture

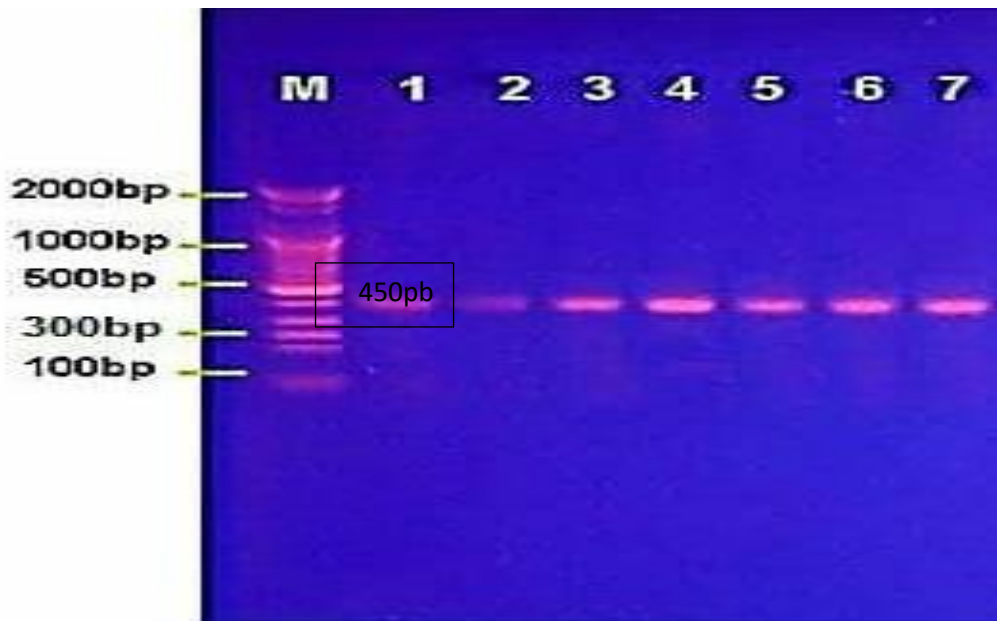
- Cycling condition: the reaction was executed in a DNA thermal cycler with no mineralize oil .following numerous tests, the subsequent program was relied, PCR composed of a preheating in 95C for 5min following the start denaturation step, the mixture was undergone to 40 amplification cycles.

### Results and Discussion

A total of one hundred "blood samples" collected from a patient with suspected typhoid fever 7 isolates (7%) recovered from the blood of the patient. typhoid fever continues to be the major cause of mortality and morbidity . in Iraq, rare studies on typhoid fever were approved out .these studies give emphasis to on the epidemiological of *Salmonella typhi* , has studied the out breaks of typhoid fever and reported that the distribution of cases over a large zone within a small time favored the possibility of water-borne disease [10]. A similar findings are obtained by Tarrish et al., [11], who reported that blood yield more positive culture during first week of fever ,delayed hospital admission may have contributed to the low salmonella isolation rate from blood. This low rate may be due to pre hospital antibiotic administration, Blood culture has promised diagnosis in the first week and is very specific, but its sensitivity poor due to various factors. The most important factor is the very few numbers of bacteria that need to cause severe infection, which can be as low as 10/ml Hence, positive culture yield is very low and elude definitive diagnosis. Other limiting factors ,beside bacteriostatic effect of antibiotics (already administrate before the culture sample is taken ), may be the nature of culturemedium employed. The time of blood collection, the hosts immune response system and intracellular characteristics of *S.typhi* [12].

all *S.typhi* isolates are sensitive (100%) for ceftriaxone ,cefepime, cefazolin and chloramphenicol, while other sal.typhi isolates were showed resistance rates (27.57%) for ampicillin, penicillin G, ciprofloxacin also s.typhi isolates were resistance (41%) for amikacin , (70.22%)for gentamicin ,nitrofurane and trimethoprime-sulfa and (83.61%) for tobramycin respectively . All *S. typhi* isolates are resistance (100%)for tetracycline and erythromycin.

The present study used 2 primers to detect the resistance genes for gentamicin in isolates, Figure-1. The primer pairs that used to detect the resistance genes for gentamicin *Acc2* 450bp succeeded in the amplification of a 450bp fragment from *S.typhi* isolates before and after antibiotic sensitivity test meanwhile , the DNA extracted from all *S. typhi* isolates harboring the gentamicin resistance gene and these results different from results that obtained from antibiotic sensitivity test ,where isolates of *S.typhi* were sensitive for gentamicin while the primer pair for *cat* gene that used to detect chloramphenicol resistant gene in *S.typhi* not succeeded in amplification of a 450 bp fragment from *S.typhi* isolates culture that were studied ,that means , the DNA extracted from all *S.typhi* isolates non harboring the chloramphenicol resistance gene and this results were significant associated with that gain from antibiotic sensitive test .



**Figure 1-** agarose gel electrophoresis for PCR products of *S. typhi*, where *aacc2* gene (450bp), PCR products appear as positive results as follow.

Lan M: DNA marker (1000-100bp). Lan 1: salmonella isolate 1, positive. Lan 2: salmonella isolate 2, positive. Lan 3: salmonella isolate 3, positive. Lan 4: salmonella isolate 4, positive. Lan 5: salmonella isolate 5, positive. Lan 6: salmonella isolate 6, positive. Lan 7: salmonella isolate 7, positive.

Our study is the same as shown by Shymapada *et al.*, [13] who found that chloramphenicol was the most active and usually used drug for typhoid fever is most broadly used anti-microbial agents. The action by "inhibiting bacterial enzymes DNA gyrase" which is responsible for coiling, division and super coiling of bacteria DNA during reproduction from present study showed all strain of local *S. typhi* were resistance for gentamycin as shown by results of *aacC2* gene and this results as like mentioned by Onyango *et al.*, [14] who said that Reference to amino glycosides resistance amplification was detected for *aacC2* gene, mechanism of antibiotic resistance in *S. typhi* is refereed by two aspects. This first acquisition of foreign genes by plasmids and mutation on chromosome, resistance can be attained by horizontal acquisition of resistance-genes, mobilized by conjugative plasmids, insertion sequences and transposons, by "recombination of foreign- plasmids DNA into the chromosome or by mutations in different chromosomal loci". Consistent with the reported of Randall *et al.*, [15] there was numerous instance in this study when an isolate was resistant to an anti-microbial drug, but the characteristics of the gene conferring resistance was not ascribed with the primers used in the study. This difference exists for the reason that there are frequently several genes related to the improvement of phenotypic resistance to a single-drug. It is impossible to cover all reported genes in one study. There were also some isolates in which drug resistance-related-genes were identified but corresponding phenotypic resistance was lacking.

## References

1. Akinyemi, KO, Oshundare, YO, Oyeyinka, OG, and Coker, AO.A. **2012**. retrospective study of community acquired salmonella infections in patients attending public hospitals in lagos, Nigeria. *J infect Dev Ctries*, **6**: 387-395.
2. Baker, S., Favorov, M. and Dougan, G. **2010**. Searching for the elusive typhoid diagnostic. *BMC Infectious Diseases*, **10**: 45.
3. Beig, F. K., Ahmed, F., Ekram, M., Shukla, I. **2010**. Typhoid and Diazo test vis-à-vis blood culture and Widal test in the early diagnosis of typhoid fever in children in a resource poor setting. *Braz. J. Infect. Dis.* **14**(6): 589-593.
4. Berkley, J. A., Lowe, B. S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., Ngetsa, C., Slack, M. P. E., Njenga Sally, Hart, C. A., Maitland, K., English, M., Kevin, M. and Scott, J. A. G. **2005**.

- Bacteremia in children admitted to a rural hospital in Kenya. *The New England Journal of Medicine*, **352**(1): 39-47.
5. Bhan, M. K., Bahl, R. and Bhatnagar, S. **2005**. Typhoid and Paratyphoid fever. *Lancet*, **366**: 749-762.
  6. Buckle, G.C., Fischer Walker, CL. and Black, RE. **2012**. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality, for 2010. *J. Glob. Health*. **2**(1).
  7. NCCLS. **2017**. M2-A7-disk diffusion. Performance standards for antimicrobial disk susceptibility test, in CLS document M2-A7. Wayne (PA): NCCLS; 2017.
  8. Beatriz, G., Soto, SM., Argüelles, JM. and Mendoza MC. **2001**. Multidrug resistance is mediated by large plasmids carrying a Class 1 Integron in the emergent *Salmonella enterica* serotype [4, 5, 12:i:- ]. *Antimicrobial Agents Chemotherapy*, **45**(4): 1305-1308.
  9. Frana, T.S., Carlson, S.A. and Griffith, R.W. **2001**. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. *Appl Environ Microbiol*, **67**: 445-448.
  10. Abu-Shanab, B., Adwan, G., D.Abu-Safiya, K. Adwan. And M.Abu Shanab **2005**. Antibacterial activity of *Rhus coriaria*. L extracts growing in Palestine. *J. Islam. Univ. Gaza*. **13**(2): 147-153.
  11. Tarrish, A.H., Al-Khayatt, J.Q. and Al-Dujaily, A.A., Prevalence of typhoid fever among patients attending to Saddam hospital in Tikrit. *Med. J. Tikrit Univ.*1997, **3**(2): 196-199.
  12. Beig, F.K., Ahmed, F., Ekram, M., Shukla, I. **2010**. Typhoid and Diazo test vis-à-vis blood culture and Widal test in the early diagnosis of typhoid fever in children in a resource poor setting. *Braz. J.Infect.Dis.* **14**(6): 589-593.
  13. Shyamapada, M., Manisha, D. and Nishith, K. P. **2012**. Antibiotic resistance of *Salmonella enteric serovar typhi* in Kolkata, India, and in vitro experiments on effect of combined chemotherapy. *The Scientific World Journal*, **1**: 1-4
  14. Onyango, D., Machioni, F., Kakai, R. and Waindi, EN. 2008. Multidrug resistance of *Salmonella enterica serovars Typhi and Typhimurium* isolated from clinical samples at two rural hospitals in Western Kenya. *J Infect Dev Ctries*, **2**: 106-111.
  15. Randall, L., Cooles, S., Osborn, M., Piddock, L. and Woodward, M. **2004**. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *J Antimicrob Chemother*, **53**: 208.