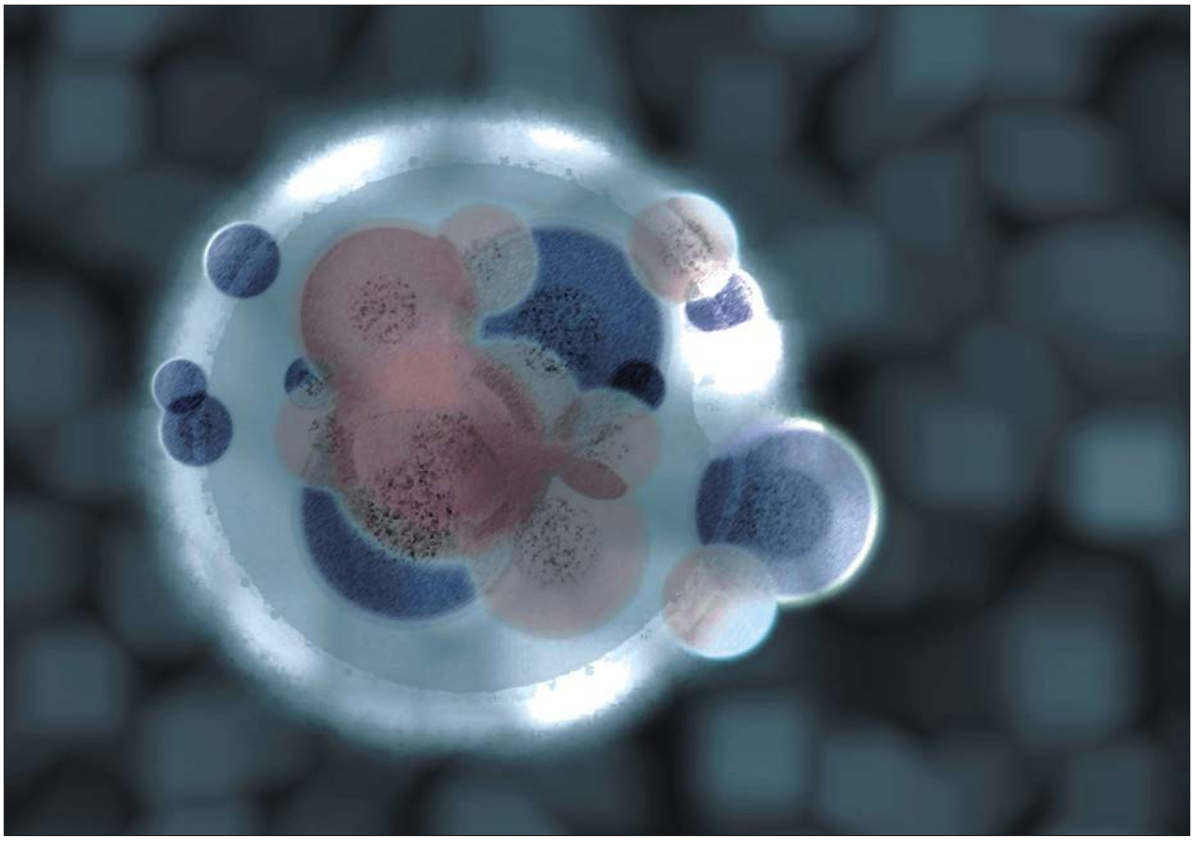

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2017.171.184

Research Article
Prevalence of Some Antimicrobials Resistance Associated-genes in *Salmonella typhi* Isolated from Patients Infected with Typhoid Fever

Ahmed Abduljabbar Jaloob Aljanaby and Ali Reyadh Medhat

Department of Microbiology, Faculty of Science, University of Kufa, Al-Najaf City, Iraq

Abstract

Background and Objective: In developing countries such as Iraq, every year there are thousands peoples infected with typhoid fever caused by *Salmonella typhi*. These infections became difficult to treat by different types of antimicrobials. Therefore, the main aim of this study was to investigate the ability of *S. typhi* strains isolated from blood of inpatients and outpatients infected with typhoid fever to produce some antimicrobial resistance associated-genes. **Materials and Methods:** Disc diffusion method was used to investigate the ability of *S. typhi* to resistance of 12 antibiotics and PCR technique were used to investigate the prevalence of 13 antimicrobials resistance genes. Fisher's exact test was used for the comparison between samples of the study and then analyzed with graph pad prism version 5. **Results:** The results proved that 61.53 and 51.28% of *S. typhi* strains were resistant to ampicillin and chloramphenicol, respectively and all strains (100%) were susceptible to ceftriaxone 30 µg. Among 13 antimicrobial resistance genes, the most prevalent was *floR* (74.35%), while there was no prevalence of *blaCMY-2, Cat3* and *strA-strB* genes. **Conclusion:** The *floR*, *Cat1* and *pse-1* genes were the most prevalent in *S. typhi* strains isolated from blood of inpatients with typhoid fever more than those isolated from blood of outpatients.

Key words: *Salmonella typhi*, inpatients, outpatients, typhoid fever, antimicrobials resistance associated-genes

Received: January 16, 2017 Accepted: March 10, 2017 Published: April 15, 2017

Citation: Ahmed Abduljabbar Jaloob Aljanaby and Ali Reyadh Medhat, 2017. Prevalence of some antimicrobials resistance associated-genes in *Salmonella typhi* isolated from patients infected with typhoid Fever). J. Biol. Sci., 17: 171-184.

Corresponding Author: Ahmed Abduljabbar Jaloob Aljanaby, Department of Microbiology, Faculty of Science, University of Kufa, Al-Najaf City, Iraq
Tel: +96407816118353

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Typhoid fever or enteric fever remains the one of the most recurrent health problems worldwide and there are no advanced methods to prevent this infection except by using of antimicrobial. Typhoid fever is a most important systemic disease worldwide. Typhoid is the only remaining enteric disease for which the rates of mortality have not been declining and it is common in areas with inadequate hygiene and sanitation[1].

Salmonella enterica serovar typhi (*S. typhi*) is the main causative agent of enteric fever which is members of the family of Enterobacteriaceae and it is the most important Gram-negative motile bacterium cause typhoid fever in Iraq and other developing countries[2]. The rate of morbidity of typhoid fever over than 21 million cases and some of this cases lead to death per year worldwide[3]. The recurrent use of the same antimicrobials against infections with typhoid fever led to an increase in multidrug resistance *S. typhi* and other bacterial species[4]. In recent years, *S. typhi* became more resistance to different antimicrobials such as chloramphenicol, ampicillin, sulfonamides and tetracycline and became resist to another different types of antimicrobials and therefore, called multidrug-resistant (MDR)[5]. Multidrug resistant *S. typhi* is defined as *S. typhi* isolates which are resistant to three different classes of antimicrobials[6]. The use of first-line antimicrobial for the treatment of typhoid fever such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole had been recommended to be replaced with other antimicrobials such as ceftriaxone and cefotaxime[7].

Different studies showed that there were many prevalence of antimicrobials resistance-associated genes like, β-lactamase, ampicillin, chloramphenicol, aminoglycosides in both clinical and non-clinical *Salmonella enterica* isolates[8,9]. This study advance new knowledge by investigation the prevalence of new antimicrobial resistance associated genes in *S. typhi* strains.

In Iraq, there is no study focusing on isolation and investigation about antimicrobial resistance-genes in *S. typhi* strains isolated from clinical and non-clinical sources. Therefore, the main aim of this study was to investigate the prevalence of thirteen antimicrobial resistance associated-genes in *S. typhi* strains isolated from blood of inpatients and outpatients infected with typhoid fever to provide a clear image about the relationship between virulence of hospital *S. typhi* isolates and nonhospital *S. typhi* isolates.

MATERIALS AND METHODS

***Salmonella typhi* isolates, collection, culture and identification:** During the period from June, 2016 to February, 2017 a total 187 blood samples were collected from inpatients (97 samples) and outpatients (90 samples) clinically suspected infected with typhoid fever in Al-Najaf Hospital in AL-Najaf Covernorate, Iraq. By sterile syringes (Hamilton, China), Five milliliter of blood samples were collected in sterile inoculated bottles (Oxoid,UK) containing sterile of 45 mL brain heart infusion broth (Oxoid,UK) and incubated aerobically at 37°C for 7 days with continuous shaking every day. Streaked by sterile loop (Himedia, India) on blood agar (Oxoid,UK), MacConkey agar (Oxoid,UK) and *Salmonella-Shigella* agar (SS agar) (Oxoid,UK) plates[10]. All grown colonies on the surface of agar plates were identified as *S. typhi* according to standard bacteriological tests according to MacFaddin[11] such as, pale colonies on MacConkey agar surface, black colonies on SS agar surface, Gram-negative, motile, Alkaline/Acid, without gas and weak production of H2S according to Triple Sugar Iron test, cultivation on Chrome agar medium (Orientation Company-France) and all isolates were identified by using Vitek2® system (BioMerieux®-France).

Antimicrobial susceptibility testing: This test was done by the method of Kirby-Bauer according to the Clinical Laboratory Standards Institute (CLSI.)[12]. Antimicrobial susceptibility and resistance was determined by strain growth zone diameter according to CLSI guidelines[12]. All the following 12 antimicrobial discs were provided from (Bioanalyse-Turkey) were used in this study: Ampicillin (AM) 10 µg, amoxicillin (AX) 25 µg, cefotaxime (CTX) 30 µg, ceftriaxone (CRO) 30 µg, gentamicin (CN) 10 µg, amikacin (AK) 30 µg, tobramycin (TM) 10 µg, streptomycin (S) 10 µg, tetracycline (TE) 30 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (C) 30 µg, sulfonamide(SSS) 300 µg. *Escherichia coli* ATCC 25922 strain was used as controls.

DNA extraction: DNA was exacted according to the method of Aljanaby and Alfaham[13].

Molecular detection of antimicrobials resistance-associated genes: Polymerase Chain Reaction Protocol (PCR) was used to detect 13 antimicrobials resistance-associated genes. All PCR products were loaded on a 1% (w/v) agarose gel with 0.5 mg mL^{-1} safe stain and were analyzed by gel

Table 1: Sequencing of primers used in PCR for 13 antimicrobials resistance-associated genes of *S. typhi*

Antibiotics	Target genes	Primer sequences (5'–3')	Size (bp)	References
Ampicillin	*Pse-1*	F:CGCTTCCCGTTAACAAGTAC	419	Bacci *et al.*[14]
		R:CTGGTTCATTTCAGATAGCG		
β-lactamase	*blaTEM*	F:CAGCGGTAAGATCCTTGAGA	643	Ensor *et al.*[15]
		R: ACTCCCCGTCGTGTAGATAA		
β-lactamase	*blaSHV*	F:GGCCGCGTAGGCATGATAGA	714	Ensor *et al.*[15]
		R:CCCGGCGATTTGCTGATTTC		
β-lactamase	*blaCMY-2*	F:TGGCC GAACTGACAGGCAAA	354	Alcaine *et al.*[16]
		R:TTTCTCCTGAACGTGGCTGGC		
Chloramphenicol	*Cat1*	F:CACCGTTGATATATCCCAATGGCATCGT	582	De Vito *et al.*[17]
		R:CTGCCGACATGGAAGCCATCACAAAC		
Chloramphenicol	*Cat2*	F:CCTGCCACTCATCGCAGTAC	547	Ma *et al.*[18]
		R:AACGGCATGAACCTGAA		
Chloramphenicol	*Cat3*	F:ATCGGCATCGGTTACCATGT	310	Ma *et al.*[18]
		R:ATCCCCTTCTTGCTGATATT		
Chloramphenicol	*floR*	F:ATGACCACCACACGCCCCG	198	Ma *et al.*[18]
		R:AGACGACTGGCGACTTCTTCG		
Chloramphenicol	*cmlA*	F:ATCCCCTTCTTGCTGATATT	662	Ma *et al.*[18]
		R:GGCCTCGCTCTTACGTCATC		
Gentamicin	*ant (3")-la*	F:GTGGATGGCGGCCTGAAGCC	526	Bacci *et al.*[14]
		R:ATTGCCCAGTCGGCAGCG		
Tetracycline	*tet A*	F:GCTACATCCTGCTTGCCTTC	210	Bacci *et al.*[14]
		R:CATAGATCGCCGTGAAGAGG		
Tetracycline	*tet B*	F:TTGGTTAGGGGCAAGTTTTG	659	Bacci *et al.*[14]
		R:GTAATGGGCCAATAACACCG		
Streptomycin	*strA-strB*	F:ATGGTGGACCCTAAAACTCT	891	Tamang *et al.*[19]
		R:GT-CTAGGATCGAGACAAAG		

electrophoresis. All primers used in this study are listed in Table 1 and all PCR thermo cycling conditions are listed in Table 2.

Statistical analysis: Statistical analysis was performed with graph pad prism version 5 software. Fisher's exact test[20] was used for the comparison between samples of the study. p-values less than the 0.05 level of significance were considered statistically significant.

RESULTS

Total isolates and *S. typhi* strains: The result proved that, out of total 187 blood samples there were 39 *S. typhi* strains isolated from inpatients (22 strains, 11.76%) and outpatients (17 strains, 9.09%) (Fig. 1, 2).

Antimicrobial susceptibility testing: The results of the present study proved that the antimicrobial resistance rates of the 93 strains of ampicillin were high. The moderate resistance rate was observed for chloramphenicol and amoxicillin, while the lowest resistance rate was observed for cefotaxime and ceftriaxone (Fig. 3, Table 3). On the other hand, the results demonstrated that there were 17 strains

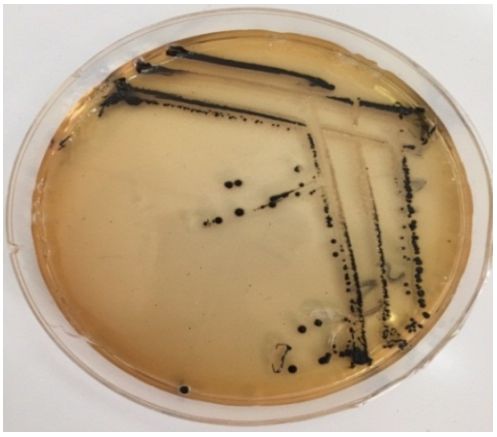

Fig. 1: Black colonies of *S. typhi* on SS agar surface after 24 h at 37°C of incubation

(43.58%) were MDR (Table 4). Phenotypic resistance profile of 39 *S. typhi* strains are given in Table 5.

Molecular detection of antimicrobials resistance-associated genes: The results of the current study proved that out of the 39 *S. typhi* strains there were 18 strains (46.15%) positive for *pse-1* gene, 6 strains (15.38%) were positive for *blaTEM* gene, 5 strains (12.82%) were positive for *blaSHV* gene, 24 strains (61.53%) were positive for *Cat1* gene, 8 strains

Table 2: Thermo cycling conditions of PCR for 13 antimicrobials resistance-associated genes of *S. typhi*

		Cycling condition					
Genes	Initial denaturation	Denaturation	Annealing	Extension	Number of cycles	Final extension	References
Pse-1	94°C/12 min	94°C/1 min	57°C/30 sec	72°C/5 min	34	72°C/7 min	Bacci *et al.*[14]
TEM	95°C/5 min	94°C/30 sec	52°C/45 sec	72°C/45 sec	30	72°C/7 min	Ensor *et al.*[15]
SHV	95°C/5 min	94°C/30 sec	55°C/60 sec	72°C/45 sec	30	72°C/7 min	Ensor *et al.*[15]
blaCMY-2	95°C/9.5 min	95°C/45 sec	60°C/45 sec	72°C/60 sec	40	72°C/7 min	Alcaine *et al.*[16]
Cat1	95°C/5 min	94°C/30 sec	62°C/45 sec	72°C/5 min	30	72°C/7 min	De Vito *et al.*[17]
Cat2	95°C/5 min	94°C/30 sec	55°C/30 sec	72°C/30 sec	35	72°C/10 min	Ma *et al.*[18]
Cat3	95°C/5 min	94°C/30 sec	55°C/30 sec	72°C/30 sec	35	72°C/10 min	Ma *et al.*[18]
floR	95°C/5 min	94°C/30 sec	55°C/30 sec	72°C/30 sec	35	72°C/10 min	Ma *et al.*[18]
cmlA	95°C/5 min	94°C/30 sec	55°C/30 sec	72°C/30 sec	35	72°C/10 min	Ma *et al.*[18]
ant (3")-la	94°C/3 min	94°C/60 sec	58°C/60 sec	72°C/60 sec	35	72°C/5 min	Bacci *et al.*[14]
tetA	94°C/5 min	94°C/25 sec	55°C/30 sec	72°C/50 sec	34	72°C/5 min	Bacci *et al.*[14]
tetB	94°C/5 min	94°C/25 sec	55°C/30 sec	72°C/50 sec	34	72°C/5 min	Bacci *et al.*[14]
strA-strB	94°C/2 min	94°C/60 sec	55°C/60 sec	72°C/60 sec	30	72°C/5 min	Tamang *et al.*[19]

Table 3: Antibiotics sensitivity test of 39 *S. typhi* strains isolated from blood of patients with typhoid fever

	Susceptible (100%)		Intermediate (100%)		Resistance (100%)		Total of resistance and percentage
Antibiotics	Inpatients 22 isolates	Outpatients 17 isolates	Inpatients 22 isolates	Outpatients 17 isolates	Inpatients 22 isolates	Outpatients 17 strains	N = 39 isolates
AM	6 (27.27%)	9 (52.94%)	0 (0.0%)	0 (0.0%)	16 (72.72%)	8 (47.05%)	24 (61.53%)
AX	9 (40.90%)	12 (70.58%)	0 (0.0%)	0 (0.0%)	13 (59.09%)	5 (29.41%)	18 (46.15%)
CTX	21 (95.45%)	17 (100%)	0 (0.0%)	0 (0.0%)	1 (4.54%)	0 (0.0%)	1 (2.56%)
CRO	22 (100%)	17 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.00%)
S	14 (63.63%)	14 (82.35%)	2 (9.09%)	0 (0.0%)	6 (27.27%)	3 (17.64%)	9 (23.07%)
CN	15 (68.18%)	17 (100%)	3 (13.63%)	0 (0.0%)	4 (18.18%)	0 (0.0%)	4 (10.25%)
AK	14 (63.63%)	17 (100%)	3 (13.63%)	0 (0.0%)	5 (22.72%)	0 (0.0%)	5 (12.82%)
TM	14 (63.63%)	16 (94.11%)	0 (0.0%)	0 (0.0%)	8 (36.36%)	1 (5.88%)	9 (23.07%)
TE	8 (36.36%)	12 (70.58%)	2 (9.09%)	0 (0.0%)	12 (54.54%)	5 (29.41%)	17 (43.58%)
SSS	10 (45.45%)	12 (70.58%)	0 (0.0%)	0 (0.0%)	12 (54.54%)	5 (29.41%)	17 (43.58%)
CIP	19 (86.36%)	17 (100%)	1 (4.54%)	0 (0.0%)	2 (9.09%)	0 (0.0%)	2 (5.12%)
C	8 (36.36%)	11 (64.70%)	0 (0.0%)	0 (0.0%)	14 (63.63%)	6 (35.29%)	20 (51.28%)

AM: Ampicillin 10 µg , AX: Amoxicillin 25 µg, CTX: Cefotaxime 30 µg, CRO: Ceftriaxone 30 µg , S: Streptomycin 10 µg, CN: Gentamicin 15 µg, AK: Amikacin 30 µg, TM: Tobramycin 10 µg, TE: Tetracycline 30 UI, SSS: Sulfonamide 300 µg, CIP: Ciprofloxacin 5 µg, C: Chloramphenicol 30 µg

Table 4: Numbers and percentage of multidrug resistance *S. typhi* strains isolated from blood of patients infected with typhoid

	Inpatients*		Outpatients		Total	
Strains	No.	%	No.	%	No.	%
S. typhi	22	56.41	17	43.59	39	100.00
MDR *S. typhi*	14	63.63	3	17.64	17	43.58
XDR *S. typhi*	0	0.00	0	0.00	0	0.00
PDR *S. typhi*	0	0.00	0	0.00	0	0.00

MDR: Multidrug resistance, XDR: Extensive drug resistance, PDR: Pandrug resistance,*p<0.05

Table 5: Phenotypic resistance profile of 39 *S. typhi* strains isolated from blood of patients with typhoid fever

No.	Source of strain	Phenotypic resistance profile	No.*	%*	MDR
1	Inpatients	AM, AX, CTX, S, CN, TM, TE, SSS, C	9	75.00	+
2	Inpatients	AM, AX, S, CN, SSS, C	9	75.00	+
3	Inpatients	AM, AX, S, CN, AK, TM, TE, SSS, C	9	75.00	+
4	Inpatients	AM, AX, S, AK, TM, C	9	75.00	+
5	Inpatients	AM, AX, S, TE, SSS, C	9	75.00	+
6	Inpatients	AM, AX, S, TE, SSS	5	41.66	+
7	Inpatients	AM, AX, SSS	3	25.00	-
8	Inpatients	AM, AX, TE, CIP, C	5	41.66	+
9	Inpatients	AM, AX, TE, CIP, C	5	41.66	+
10	Inpatients	AM, AX, TE, C	4	33.33	+
11	Inpatients	AM, AX, TE, C	4	33.33	+
12	Inpatients	AM, AX, CN, TE, C	5	41.66	+
13	Inpatients	AM, AX, C	3	25.00	-
14	Inpatients	AM, TE, C	3	25.00	+
15	Inpatients	AK, SSS, C	3	25.00	+
16	Inpatients	AK, SSS	2	16.66	-
17	Inpatients	AK, TM	2	16.66	-
18	Inpatients	TM, SSS	2	16.66	-
19	Inpatients	TM, SSS	2	16.66	-
20	Inpatients	TM, SSS	2	16.66	-
21	Inpatients	AM, S, TE	2	16.66	-
22	Inpatients	AM, S, TM, TE, C	5	41.66	+
23	outpatients	SSS	1	8.33	-
24	outpatients	SSS	1	8.33	-
25	outpatients	AM, AX, S, TE, C	5	41.66	+
26	outpatients	AM, AX, TE, C	4	33.33	+
27	outpatients	AM, AX, TE, C	4	33.33	+
28	outpatients	AM, AX, C	3	25.00	-
29	outpatients	AM, AX. C	3	25.00	-
30	outpatients	AM	1	8.33	-
31	outpatients	AM	1	8.33	-
32	outpatients	AM	1	8.33	-
33	outpatients	TM	1	8.33	-
34	outpatients	TE, SSS	2	16.66	-
35	outpatients	TE, SSS	2	16.66	-
36	outpatients	SSS	1	8.33	-
37	outpatients	C	1	8.33	-
38	outpatients	SSS	1	8.33	-
39	outpatients	SSS	1	8.33	

*Total numbers (n) and percentage (%) of *S. typhi* strains that were resistant to 12 antibiotics, MDR: Multidrug resistance, +: Positive, -: Negative, No. (100%)

(20.51%) were positive for *Cat2* gene and 4 strains (10.25%) were positive for *cmlA* gene, 29 strains (74.35%) were positive for *floR* gene (Fig. 4-9). Also, the results proved that there were one strain (2.56%) positive for *ant (3")-la* gene, 19 strains (48.71%) were positive for *tetA*, 11 strains (28.20%) were positive for *tetB* (Fig. 10, 11). On the other hand, the results indicated that, there was no prevalence of *bla CMY-2, Cat3* and *strA-strB* genes in any strain. The prevalence and distribution of antimicrobials resistance genes are given in Table 6, 7 and Fig. 12, 13.

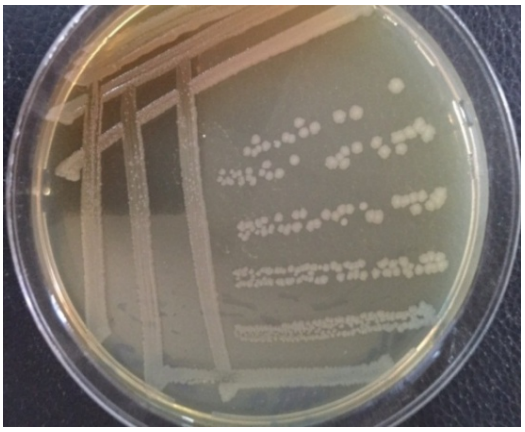

Fig. 2: Brown-pale colonies of *S. typhi* on chrome agar surface after 24 h at 37°C of incubation

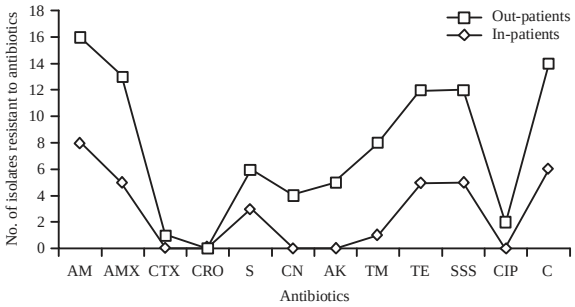

Fig. 3: Numbers of *S. typhi* strains that were resistant to 12 antibiotics

AM: Ampicillin 10 µg, AMX: Amoxicillin 25 µg, CTX: Cefotaxime 30 µg, CRO: Ceftriaxone 30 µg, S: Streptomycin 10 µ,CN: Gentamicin 15 µg, AK: Amikacin 30 µg, TM: Tobramycin 10 µg, TE: Tetracycline 30 UI, SSS: Sulfonamide 300 µg, CIP: Ciprofloxacin 5 µg, C: Chloramphenicol 30 µg

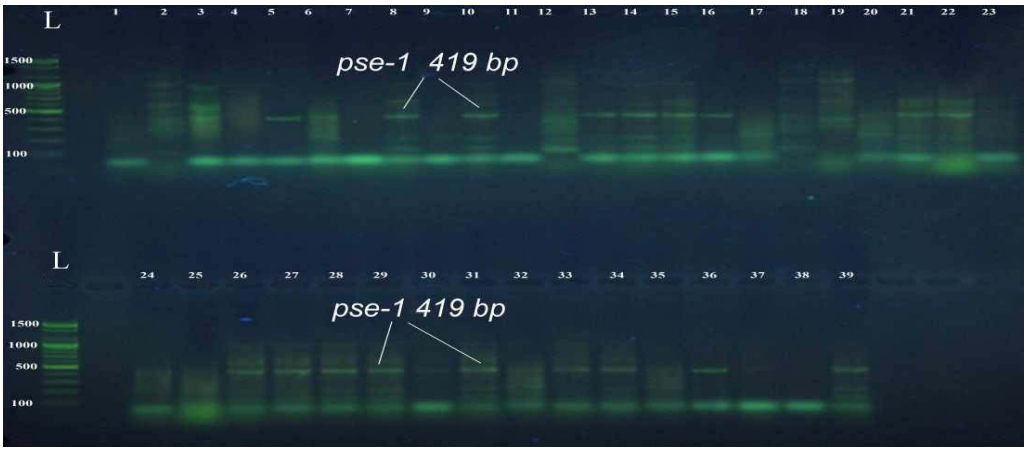

Fig. 4: PCR amplified products from extracted DNA of *S. typhi* strains

Amplified with diagnostic gene *pse-1* show positive results at 419 bp. L: DNA molecular size marker. Lane 1-39: Samples numbers

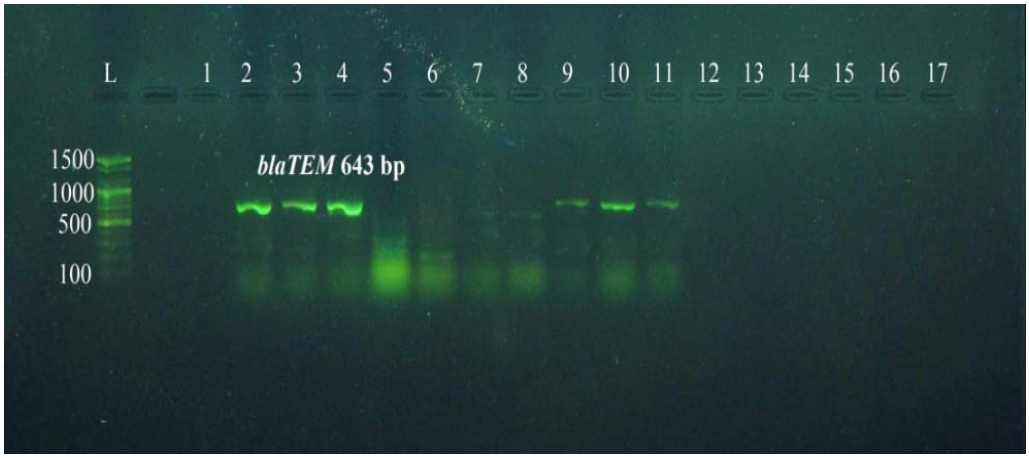

Fig. 5: PCR amplified products from extracted DNA of *S. typhi*

Amplified with diagnostic gene *blaTEM* show positive results at 643 bp. L: DNA molecular size marker. Lane 1-17: Samples numbers

Fig. 6: PCR amplified products from extracted DNA of *S. typhi* strains
Amplified with diagnostic gene *blaSHV* show positive results at 714 bp. L: DNA molecular size marker. Lane 1-17: Samples numbers

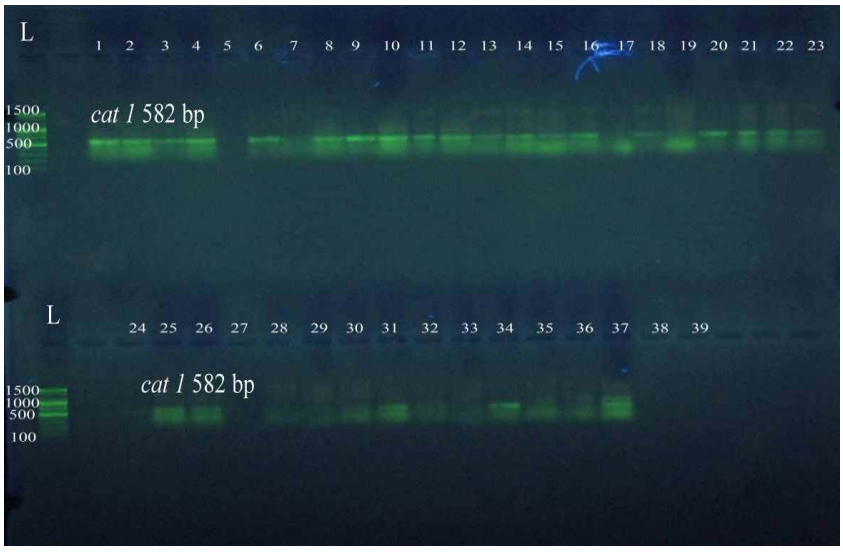

Fig. 7: PCR amplified products from extracted DNA of *S. typhi* strains
Amplified with diagnostic gene *Cat1* show positive results at 582 bp. L: DNA molecular size marker. Lane 1-39: Samples numbers

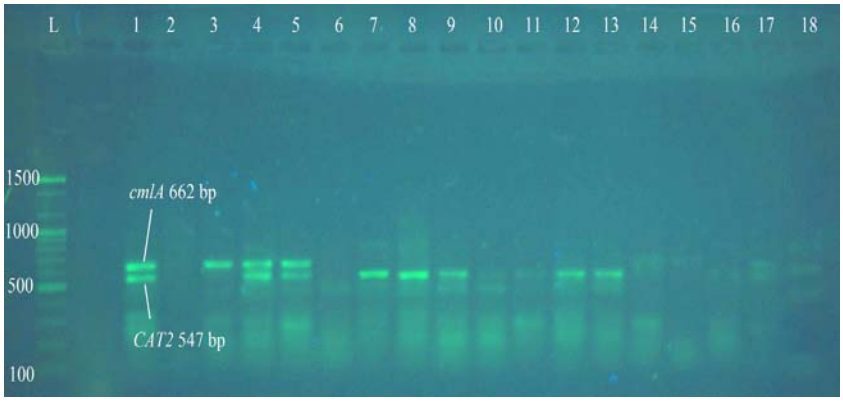

Fig. 8: Multiplex PCR amplified products from extracted DNA of *S. typhi* strains
Amplified with diagnostic genes *Cat2* and *cmlA* show positive results at 547 bp and 662 bp respectively. L: DNA molecular size marker. Lane 1-18: Samples numbers

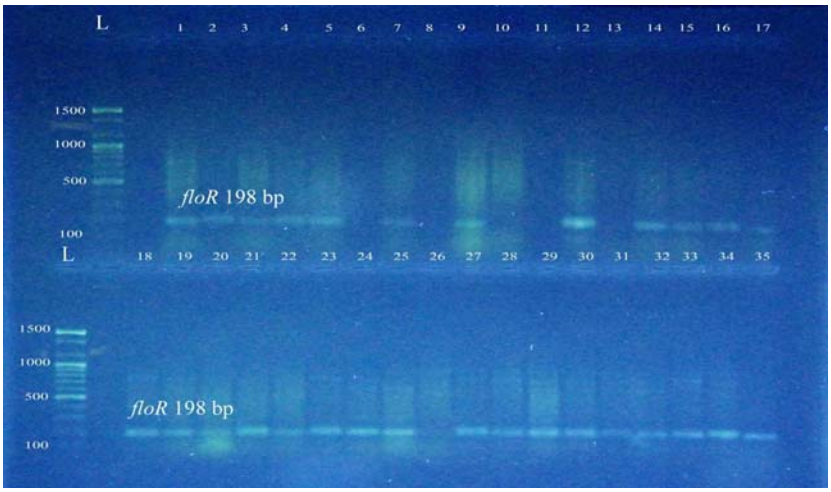

Fig. 9: PCR amplified products from extracted DNA of *S. typhi* strains
Amplified with diagnostic gene *floR* show positive results at 198 bp. L: DNA molecular size marker. Lane 1-35: Samples numbers

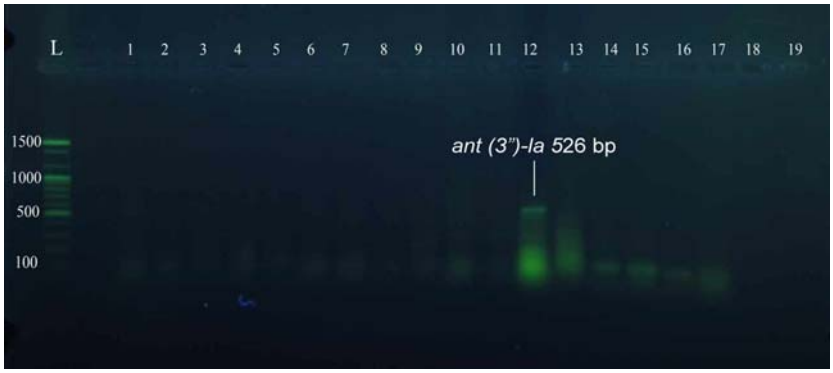

Fig. 10: PCR amplified products from extracted DNA of *S. typhi* strains
Amplified with diagnostic gene *ant (3")-la* show positive results at 526 bp. L: DNA molecular size marker. Lane 1-19: Samples numbers

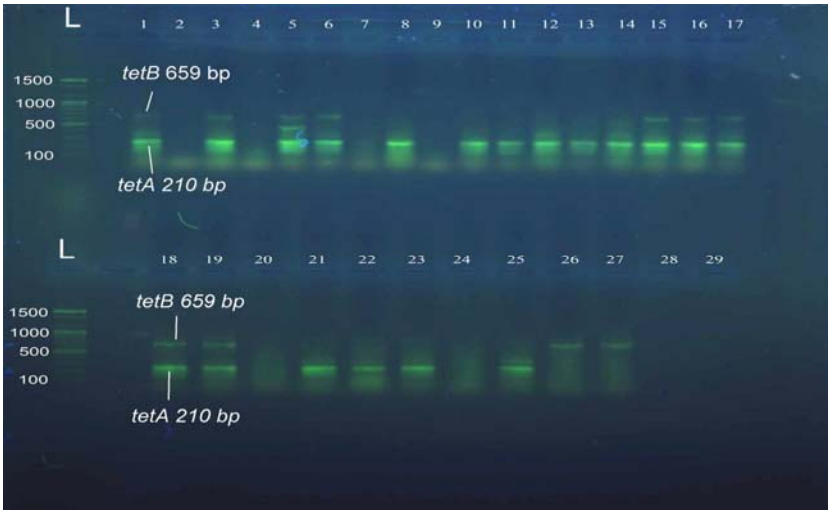

Fig. 11: Multiplex PCR amplified products from extracted DNA of *S. typhi* strains
Amplified with diagnostic genes *tetA* and *tetB* show positive results at 210 bp and 659 bp respectively. L: DNA molecular size marker. Lane 1-29: Samples numbers

Table 6: Numbers and percentages of 13 anti-microbials resistance-associated genes among 39 *S. typhi* strains isolated from patients infected with typhoid fever

		Source of strain		
Genes	Antibiotics	Inpatients*22 strains (%)	Outpatients 17 strains (%)	Total 39 strains (%)
pse-1	Ampicillin	9 (40.90%)	9 (52.94%)	18 (46.15%)
blaTEM	lactamase	6 (27.27%)	0 (0.0%)	6 (15.38%)
blaSHV	β-lactamase	5 (22.72%)	0 (0.0%)	5 (12.82%)
blaCMY-2	β-lactamase	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cat1	Chloramphenicol	18 (81.81%)	6 (35.29%)	24 (61.53%)
Cat2	Chloramphenicol	8 (36.36%)	0 (0.0%)	8 (20.51%)
Cat3	Chloramphenicol	0 (0.0%)	0 (0.0%)	0 (0.0%)
floR	Chloramphenicol	17 (77.27%)	12 (70.58%)	29 (74.35%)
cmlA	Chloramphenicol	4 (18.18%)	0 (0.0%)	4 (10.25%)
ant (3")-la	Gentamicin	1 (4.54%)	0 (0.0%)	1 (2.56%)
tet A	Tetracycline	17 (77.27%)	2 (11.76%)	19 (48.71%)
tet B	Tetracycline	9 (40.90%)	2 (11.76%)	11 (28.20%)
strA-strB	Streptomycin	0 (0.0%)	0 (0.0%)	0 (0.0%)

*p<0.05

Table 7: Genotypic resistance profile of 39 *S. typhi* strains isolated from blood of patients with typhoid fever

Source of strain	Genotypic resistance profiles	No.	%
Inpatients	*blaSHV, Cat1, Cat2, floR, cmlA, tet A, tet B*	7	53.84
Inpatients	*blaTEM, blaSHV, Cat1, floR*	4	30.76
Inpatients	*blaTEM, blaSHV, Cat1, floR, cmlA, tet A, tet B*	7	53.84
Inpatients	*blaTEM, Cat1, Cat2, floR, cmlA*	5	38.46
Inpatients	*pse-1, Cat2, floR, cmlA, tet A, tet B*	6	46.15
Inpatients	*Cat1, tet A, tet B*	3	23.07
Inpatients	*Cat2, floR*	2	15.38
Inpatients	*pse-1, Cat1, Cat2, tet A*	4	30.76
Inpatients	*blaTEM, blaSHV, Cat1, Cat2, floR*	5	38.46
Inpatients	*pse-1, blaTEM, blaSHV, Cat1, tet A*	5	38.46
Inpatients	*blaTEM, Cat1, floR, tet A*	4	30.76
Inpatients	*Cat1, Cat2, ant (3")-la, tet A, tet A*	5	38.46
Inpatients	*pse-1, Cat1, Cat2, floR, tet A*	5	38.46
Inpatients	*pse-1, Cat1, floR, tet A*	4	30.76
Inpatients	*pse-1, Cat1, floR, tet A, tet B*	5	38.46
Inpatients	*pse-1, Cat1, floR, tet A, tet B*	5	38.46
Inpatients	*floR, tet A, tet B*	3	23.07
Inpatients	*Cat1, floR, tet A, tet B*	4	30.76
Inpatients	*floR, tet A, tet B*	3	23.07
Inpatients	*Cat1, floR*	2	15.38
Inpatients	*pse-1, Cat1, floR, tet A*	4	30.76
Inpatients	*pse-1, Cat1, floR, tet A*	4	30.76
Outpatients	*Cat1, floR, tet A*	3	23.07
Outpatients	*floR*	1	7.69
Outpatients	*Cat1, floR, tet A*	3	23.07
Outpatients	*pse-1, Cat1, tet B*	3	23.07
Outpatients	*pse-1, floR, tet B*	3	23.07
Outpatients	*pse-1, floR*	2	15.38
Outpatients	*pse-1, floR*	2	15.38
Outpatients	*floR*	1	7.69
Outpatients	*pse-1, floR*	2	15.38
Outpatients	*floR*	1	7.69
Outpatients	*pse-1, floR*	2	15.38
Outpatients	*pse-1, Cat1, floR*	3	23.07
Outpatients	*floR*	1	7.69
Outpatients	*pse-1*	1	7.69
Outpatients	*Cat1*	1	7.69
Outpatients		0	0.0
Outpatients	*pse-1*	1	7.69

Total numbers (n) and percentage (%) of *S. typhi* strains that were carriers of antimicrobial resistance genes (total: 13 genes)

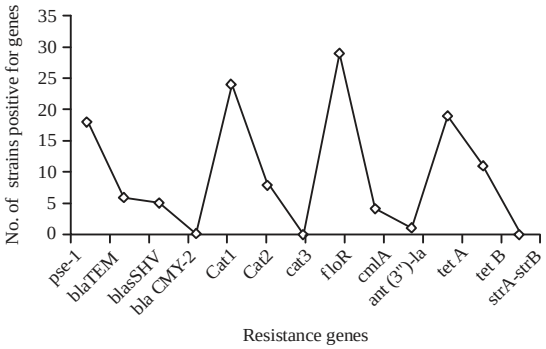

Fig. 12: Total numbers of antimicrobials resistance genes among 39 *S. typhi* strains isolated from blood of total patients infected with typhoid fever

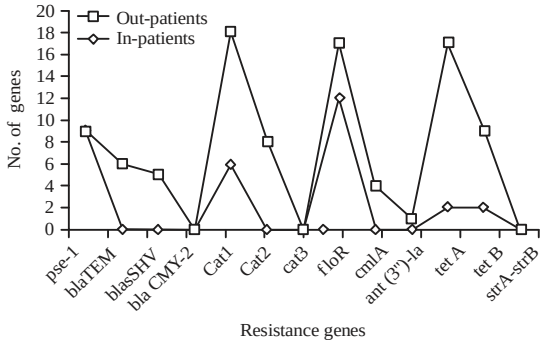

Fig. 13: Prevalence of 13 antimicrobials resistance genes among 39 *S. typhi* strains isolated from blood of inpatients and outpatients infected with typhoid fever

DISCUSSION

Salmonella typhi is one of the most drug-resistant Gram-negative bacteria cause typhoid fever and it is the one of the most major public health concern in developing Asian countries and a common cause of bloodstream infection in the Middle East[20,21].

The antimicrobials susceptibility test of 39 *S. typhi* strains proved that 61.53% of the strains were resistant to ampicillin, 51.28% were resistant to chloramphenicol, 46.15% were resistant to amoxicillin and 43.58% were resistant to tetracycline and sulfonamide. Most *S. typhi* strains were susceptible to tobramycin, streptomycin, amikacin, gentamicin, ciprofloxacin and cefotaxime and all *S. typhi* strains were susceptible to ceftriaxone (Fig. 3). *Salmonella typhi* strains isolated from blood of inpatients were more resistant to antimicrobials from those isolated from blood of outpatients (Table 4) and there were 17 strains (43.58%) were MDR most of them (14 strains, $p<0.05$) isolated from blood of inpatients (Table 5).

Multi-drug resistant *S. typhi* is endemic in Iraq and in some Asian countries such as Pakistan and India, also, MDR *S. typhi* has been reported in some parts of the world such as Finland, United Kingdom and United States[22-24]. Recently, the incidence of Multi-drug resistant *S. typhi* increased in the different countries worldwide[25,26].

In developing countries, typhoid fever is a common infection in both urban and rural areas and there were over 120 million infections and more than 500000 annual deaths, worldwide[27].

In Iraq and other developing countries, different antimicrobials such as ampicillin, chloramphenicol, amoxicillin and aminoglycosides were used as the first line antimicrobials typhoid fever caused by multidrug-resistant *S. typhi* and other type of *salmonella* therefore most Gram-negative bacteria including *S. typhi* developed new resistant against these antimicrobials[27,28]. In Nigeria, Fashae *et al.*[29] proved that there were resistance rate ranged from 59-36% against ampicillin, trimethoprim, sulfamethoxazole and chloramphenicol. In previous years, multi-drug resistant *S. typhi* strains have been reported in different studies[30-33]. Recently, 986 *S. typhi* isolates were collected in Asia and Middle East, from Iraq, Jordan, Qatar, Egypt, Uzbekistan and Pakistan and were tested for antibiotic susceptibility to 5 antimicrobials using the Kirby-Bauer method and the results proved that 83% of strains were MDR in Iraq, 52% were MDR in Pakistan and 14% in Qatar, these *S. typhi* strains were resistance to chloramphenicol, trimethoprim-sulfamethoxazole and ampicillin[27]. In Kuwait, Dimitrov *et al.*[34] found in his study that there were 50 clinical *S. typhi* strains (37%) were MDR . In Saudi Arabia, out of total 33 *S. enterica* strains there were 26 strain (78.78%) were MDR[35]. Also, in the current study, all *S. typhi* strains were susceptible to Ceftriaxone 30 µg, this result was observed in other Gulf countries such as, Kuwait and United Arab Emirates[36]. The results of the present study are in agreement with results by El-Tayeb *et al.*[35], who proved that there were 2 *S. enterica* strains (6.1%) were resistance to third-generation cephalosporin such as cefotaxime. While the results are disagreement with Burke *et al.*[37], who reported that 11% of the *S. enterica* strains were resistance to third-generation cephalosporin. Ceyssens *et al.*[38] reported that there were high prevalence in quinolone resistance-associated genes in *S. enterica* strains.

Infected by multi-drug resistant *S. typhi* strains have rapidly increased over the past 25 years in Asia and the Middle East[39,40]. In this study, most of *S. typhi* strains isolated from blood of inpatients infected with typhoid fever were MDR while others strains isolated from blood of outpatients were non-MDR, this might be due to vertical transfer of

antimicrobials resistance gene from other hospitalized Gram-negative bacteria such as *blaSHV*, *blaTEM* and *blaCTX-M*. Hassing *et al.*[41] suggested that the presence of a mutation in some antimicrobials resistance-association genes are responsible for the emergence of this reduced susceptibility. Conjugative transfer of bacterial plasmids cause acquired antimicrobial resistance phenotypes between different bacteria[42-44]. Class I integrons may be localized in plasmids, which are mobile DNA elements that are important in the proliferation of MDR Gram-negative enteric bacteria and others[45,46].

Molecular analysis of the 39 *S. typhi* strains shows there were high prevalence in *floR* gene (74.35%) followed by *Cat1* gene (61.53%) and there were moderate prevalence in *tetA* gene (48.71%) and *pse-1* gene (46.15%), while *blaTEM*, *blaSHV*, *Cat2*, *cmlA*, *ant (3")-la*, *tet A*, *tet B* genes were prevalent in low rates. On the other hand, there were no any prevalence in *bla CMY-2*, *Cat3 and strA-strB* genes (Table 7). And the results proved that *S. typhi* strains isolated from inpatients were carried antimicrobials resistance associated-genes more than those isolated from outpatients ($p<0.05$) (Table 7, Fig. 13). This results are in agreement with some previous studies[2,3,47,48].

Production of extended-spectrum-lactamases enzymes lead to increase resistance to 3rd generation cephalosporins, is an ever increasing problem and is a cause of serious concern worldwide. These enzymes have been detected in many Gram-negative bacteria especially in the Enterobacteriaceae family including different serovars of *Salmonella enterica*[47-49]. In Iraq, Chloramphenicol was used as the first line to treatment typhoid fever caused by *S. typhi* and *S. paratyphi*, this recurrent use of the same antimicrobial led to limiting its therapeutic value, therefore, chloramphenicol associated-genes were high prevalence in this study.

In South African, Zishiri *et al.*[7] reported that the *pse-1* gene was prevalent with percentage 50% and *tet A* gene was 70%, *tet B*, *sul 1* and *sul 2* genes were exhibited 60% while *ant (3")-la* gene was prevalent with percentage 80%. The *pse-1* gene is responsible for resistance to β-lactamase was detected in 46.15% of the total *S. typhi* strains found to be resistant to ampicillin and amoxicillin. Llanes *et al.*[50] proved that resistance to β-lactam is due to the production of the *pse-1* enzyme. Glenn *et al.*[51] reported that *Salmonella* was carried the *pse-1* gene and it is one of the most prevalent β-lactamases. One of the most important aminoglycoside resistance association-genes is the *ant (3")-la* and it has been detected in many pathogenic bacteria such as *K. pneumoniae* and *p.aeruginosa* but there is no enough information about the prevalence of this gene in *Salmonella*, especially in Iraq. In this study there was only one *S.typhi* strain was carried this gene. In this study, none of *S. typhi* strains were found to be positive for Extended-spectrum beta-lactamases by a Double-disk synergy test.

This result is quite agreement with Elumalai *et al.*[2], who suggested a possible reason for this result is that *bla-TEM* gene don't has the ability to hydrolysis 3rd and 4th generation cephalosporins while has the ability to hydrolysis penicillins and may be 1st generation cephalosporins, therefore which could not be detected by double-disk synergy test.

Overproduction and mutation in beta-lactamases is due to the continuous exposure of bacterial strains to different types of β-lactams and lead to enables these bacteria to resist antimicrobials duo to hydrolysis different β-lactam antibiotics such as penicillins and cephalosporins[2,52].

The *blaTEM* genes are found mainly in clinical isolates of *K. pneumoniae* and *E. coli*. The first *blaTEM* variant with increased activity against extended spectrum cephalosporins was *blaTEM*-3. Th e *BlaSHV*-2 isolated from *K. pneumoniae* in 1983, showed transferable resistance to cephalosporins such as cefotaxime[53]. Pathogenic bacteria that cause different nosocomial infections such as *K. pneumoniae*, *P. aeruginosa* and *S. aureus* have long been known to produce β-lactamase[13]. These enzymes have spread to other bacteria such as *S. typhi* and *S. paratyphi* that previously lacked to these enzymes[54]. In this study, *tetA* and *tetB* genes were detected in 48.71 and 28.20%, respectively, in total isolates. The mechanism of energy-dependent membrane-associated efflux proteins is encoded by *tetA* and *tetB* genes[55]. But Nde and Logue[56] suggested that there were another mechanisms restraint against tetracycline such as enzymatic inactivation and ribosomal protection may be including in this study. In this study, by phenotypic test a total of 39 *S. typhi* strains were resistant to streptomycin with percentage 23.07% but the identity of the gene *strA-strB* was not found, this may be due there are many genes other than *strA-strB* responsible for streptomycin resistant and it is very difficult to cover all antimicrobials association- genes in the present study. Also, in the current study, there was no fully compatibility between phenotypic and molecular in antimicrobials resistance results.

The antimicrobials resistant associated-genes were also detected in some susceptible and resistant isolates, this might be due to that some antimicrobial-resistant genes are silent in *S. typhi* strains under some circumstances. These silent genes can spread to other bacteria under continuous use of antimicrobials. Finally, the main recommendations in this study are: Avoid recurrent use the same antimicrobials such as ampicillin and chloramphenicol to treated typhoid fever, study the prevalence of other antimicrobials resistance genes in *S. typhi* strains and make more precautionary measures to different bacterial infections especially in hospitals.

CONCLUSION

Chloramphenicol and ampicillin resistance associated-genes (*floR*, *Cat1* and *pse-1*) were more prevalent in *S. typhi* strains isolated from blood of inpatients with typhoid fever more than those isolated from blood of outpatients.

SIGNIFICANCE STATEMENTS

This study discovers that most *S. typhi* strains isolated from hospital patients became highly resistant to antimicrobials especially against chloramphenicol and ampicillin. This study will help the researcher to use the ceftriaxone to treat patients with typhoid fever and create a good image about resistance associated-genes in clinical *S. typhi* strains. On the other hand, the results of this study proved that *S. typhi* strains isolated from hospitals patients were more virulent than those isolated from non-hospital patients.

ACKNOWLEDGMENT

We are grateful to staff of Medical Laboratory in Al-Najaf Hospital for providing the clinical samples.

REFERENCES

1. Lugito, N.P.H. and Cucunawangsih, 2017. Antimicrobial resistance of *Salmonella enterica* serovars typhi and paratyphi isolates from a general hospital in Karawaci, Tangerang, Indonesia: A five-year review. Int. J. Microbiol. 10.1155/2017/6215136.
2. Elumalai, S., G. Muthu, R.E.M. Selvam and S. Ramesh, 2014. Detection of TEM-, SHV- and CTX-M-type β-lactamase production among clinical isolates of *Salmonella* species. J. Med. Microbiol., 63: 962-967.
3. Adesiji, Y.O., V.K. Deekshit and I. Karunasagar, 2014. Antimicrobial resistant genes associated with *Salmonella* spp. isolated from human, poultry and seafood sources. Food Sci. Nutr., 2: 436-442.
4. Dutta, S., S. Das, U. Mitra, P. Jain and I. Roy *et al*., 2014. Antimicrobial resistance, virulence profiles and molecular subtypes of *Salmonella enterica serovars* Typhi and Paratyphi a blood isolates from Kolkata, India during 2009-2013. PLoS One, Vol. 9.
5. Das, S., S. Samajpati, U. Ray, I. Roy and S. Dutta, 2017. Antimicrobial resistance and molecular subtypes of *Salmonella enterica serovar* Typhi isolates from Kolkata, India over a 15 years period 1998-2012. Int. J. Med. Microbiol., 307: 28-36.
6. Aljanaby, A.A.J., 2013. Antibacterial activity of an aqueous extract of *Petroselinum crispum* leaves against pathogenic bacteria isolated from patients with burns infections in Al-Najaf governorate, Iraq. Res. Chem. Intermed., 39: 3709-3714.
7. Zishiri, O.T., N. Mkhize and S. Mukaratirwa, 2016. Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort J. Vet. Res., Vol. 83.
8. Munir, T., M. Lodhi, J.K. Ansari, S. Andleeb and M. Ahmed, 2016. Extended spectrum beta lactamase producing cephalosporin resistant *Salmonella* Typhi, reported from Rawalpindi, Pakistan. J. Pak. Med. Assoc., 66: 1035-1036.
9. Trongjit, S., S. Angkititrakul, R.E. Tuttle, J. Poungseree, P. Padungtod and R. Chuanchuen, 2017. Prevalence and antimicrobial resistance in *Salmonella enterica* isolated from broiler chickens, pigs and meat products in Thailand-Cambodia border provinces. Microbiol. Immunol., 61: 23-33.
10. Collee, J.G., B.P. Marmion, A.G. Fraser and A. Simmons, 1999. Mackie and McCartney Practical Medical Microbiology. 14 Edn., Churchill Livingstone, New York, pp: 263-298.
11. MacFaddin, J.F., 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd Edn., Lippincott Williams & Wilkins, London, UK., Pages: 912.
12. CLSI., 2014. Performance Standards for Antimicrobial Susceptibility Testing. 22nd Edn., Clinical and Laboratory Standards Institute, USA., ISBN: 9781562388973, Pages: 226.
13. Aljanaby, A.A.J. and Q.M.H. Alfaham, 2017. Phenotypic and molecular characterization of some virulence factors in multidrug resistance escherichia coli isolated from different clinical infections in Iraq. Am. J. Biochem. Mol. Biol., 7: 65-78.
14. Bacci, C., E. Boni, I. Alpigiani, E. Lanzoni, S. Bonardi and F. Brindani, 2012. Phenotypic and genotypic features of antibiotic resistance in *Salmonella enterica* isolated from chicken meat and chicken and quail carcasses. Int. J. Food Microbiol., 160: 16-23.
15. Ensor, V.M., W. Jamal, V.O. Rotimi, J.T. Evans and P.M. Hawkey, 2009. Predominance of CTX-M-15 extended spectrum β-lactamases in diverse *Escherichia coli* and *Klebsiella pneumoniae* from hospital and community patients in Kuwait. Int. J. Antimicrob. Agents, 33: 487-489.
16. Alcaine, S.D., S.S. Sukhnanand, L.D. Warnick, W.L. Su, P. McGann, P. McDonough and M. Wiedmann, 2005. Ceftiofur-resistant *Salmonella* strains isolated from dairy farms represent multiple widely distributed subtypes that evolved by independent horizontal gene transfer. Antimicrob. Agents Chemother., 49: 4061-4067.
17. De Vito, D., R. Monno, F. Nuccio, M. Legretto and M. Oliva *et al*., 2015. Diffusion and persistence of multidrug resistant *Salmonella typhimurium* strains phage type DT120 in Southern Italy. BioMed Res. Int., Vol. 2015.

18. Ma, M., H. Wang, Y. Yu, D. Zhang and S. Liu, 2007. Detection of antimicrobial resistance genes of pathogenic *Salmonella* from Swine with DNA microarray. J. Vet. Diagn Invest., 19: 161-167.
19. Tamang, M.D., J.Y. Oh, S.Y. Seol, H.Y. Kang and J.C. Lee *et al*., 2007. Emergence of multidrug-resistant *Salmonella enterica* serovar Typhi associated with a class 1 integron carrying the *dfrA7* gene cassette in Nepal. Int. J. Antimicrobial Agents, 30: 330-335.
20. Naheed, A., P.K. Ram, W.A. Brooks, M.A. Hossain and M.B. Parsons *et al*., 2010. Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh. Int. J. Infect. Dis., 14: e93-e99.
21. Ahmed, D., M.A. Nahid, A.B. Sami, F. Halim and N. Akter *et al*., 2017. Bacterial etiology of bloodstream infections and antimicrobial resistance in Dhaka, Bangladesh, 2005-2014. Antimicrobial Resist. Infect. Control, Vol. 6.
22. Hakanen, A., P. Kotilainen, J. Jalava, A. Siitonen and P. Huovinen, 1999. Detection of decreased fluoroquinolone susceptibility in *Salmonellas* and validation of nalidixic acid screening test. J. Clin. Microbiol., 37: 3572-3577.
23. Ackers, M.L., N.D. Puhr, R.V. Tauxe and E.D. Mintz, 2000. Laboratory-based surveillance of *Salmonella serotype* Typhi infections in the United States: Antimicrobial resistance on the rise. JAMA., 283: 2668-2673.
24. Munir, T., M. Lodhi, T. Butt and K.A. Karamat, 2001. Incidence and multidrugresistance of *Salmonella* typhoid in Bahawalpur area. Pak. Armed Forces Med. J., 51: 10-13.
25. Threlfall, E.J. and L.R. Ward, 2001. Decreased susceptibility to ciprofloxacin in *Salmonella enterica serotype* typhi, United Kingdom. Emerg. Infect. Dis., 7: 448-450.
26. Nadeem, M., N. Ali, H. Achkzai and I. Ahmed, 2002. A profile of Enteric fever in adults at Quetta. Pak. J. Pathol., 13: 12-17.
27. Rahman, B.A., M.O. Wasfy, M.A. Maksoud, N. Hanna, E. Dueger and B. House, 2014. Multi drug resistance and reduced susceptibility to ciprofloxacin among *Salmonella enterica serovar* Typhi isolates from the Middle East and Central Asia. New Microbes New Infect., 2: 88-92.
28. Roy, J.S., L. Saikia, M. Medhi and D. Tassa, 2016. Epidemiological investigation of an outbreak of typhoid fever in Jorhat town of Assam, India. Indian J. Med. Res., 144: 592-596.
29. Fashae, K., F. Ogunsola, F.M. Aarestrup and R.S. Hendriksen, 2010. Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. J. Infect. Dev. Countries, 4: 484-494.
30. Misra, R.N., K.S. Bawa, S.K. Magu, S. Bhandari, A. Nagendra and P.K. Menon, 2005. Outbreak of multi-drug resistant *Salmonella* Typhi enteric fever in Mumbai Garrison. Med. J. Armed Forces India, 61: 148-150.
31. Brooks, W.A., A. Hossain, D. Goswami, A.T. Sharmeen and K. Nahar *et al*., 2005. Bacteremic typhoid fever in children in an urban slum, Bangladesh. Emerg. Infect. Dis., 11: 326-329.
32. Singh, L. and M.P. Cariappa, 2016. Blood culture isolates and antibiogram of *Salmonella*: Experience of a tertiary care hospital. Med. J. Armed Forces India, 72: 281-284.
33. Ali, A., H.A. Ali, F.H. Shah, A. Zahid, H. Aslam and B. Javed, 2017. Pattern of antimicrobial drug resistance of *Salmonella* Typhi and Paratyphi A in a teaching hospital in Islamabad. J. Pak. Med. Assoc., 67: 375-379.
34. Dimitrov, T., E.E. Udo, O. Albaksami, S. Al-Shehab, A. Kilani, M. Shehab and A. Al-Nakkas, 2007. Clinical and microbiological investigations of typhoid fever in an infectious disease hospital in Kuwait. J. Med. Microbiol., 56: 538-544.
35. El-Tayeb, M.A., A.S. Ibrahim, A.A. Al-Salamah, K.S. Almaary and Y.B. Elbadawi, 2017. Prevalence, serotyping and antimicrobials resistance mechanism of *Salmonella enterica* isolated from clinical and environmental samples in Saudi Arabia. Brazil. J. Microbiol., 8382: 30320-30322.
36. Rotimi, V.O., W. Jamal, T. Pal, A. Sovenned and M.J. Albert, 2008. Emergence of CTX-M-15 type extended-spectrum β-lactamase-producing *Salmonella* spp. in Kuwait and the United Arab Emirates. J. Med. Microbiol., 57: 881-886.
37. Burke, L., K.L. Hopkins, D. Meunier, E. de Pinna, D. Fitzgerald-Hughes, H. Humphreys and N. Woodford, 2014. Resistance to third-generation cephalosporins in human non-typhoidal *Salmonella enterica* isolates from England and Wales, 2010-12. J. Antimicrobial Chemother., 69: 977-981.
38. Ceyssens, P.J., W. Mattheus, R. Vanhoof and S. Bertrand, 2015. Trends in serotype distribution and antimicrobial susceptibility in *Salmonella enterica* isolates from humans in Belgium, 2009 to 2013. Antimicrobial Agents Chemother., 59: 544-552.
39. Thong, K.L., Z.A. Bhutta and T. Pang, 2000. Multidrug-resistant strains of *Salmonella enterica* serotype *typhi* are genetically homogenous and coexist with antibiotic-sensitive strains as distinct, independent clones. Int. J. Infect. Dis., 4: 194-197.
40. Afzal, A., Y. Sarwar, A. Ali, A. Maqbool, M. Salman, M.A. Habeeb and A. Haque, 2013. Molecular evaluation of drug resistance in clinical isolates of *Salmonella enterica* serovar *Typhi* from Pakistan. J. Infect. Dev. Count., 7: 929-940.
41. Hassing, R.J., G.A. Menezes, W. van Pelt, P.L. Petit, P.J. van Genderen and W.H. Goessens, 2011. Analysis of mechanisms involved in reduced susceptibility to ciprofloxacin in *Salmonella enterica* serotypes Typhi and Paratyphi A isolates from travellers to Southeast Asia. Int. J. Antimicrob. Agents, 37: 240-243.

42. Fey, P.D., T.J. Safranek, M.E. Rupp, E.F. Dunne and E. Ribot *et al*., 2000. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. N. Engl. J. Med., 432: 1242-1249.
43. Gebreves, W.A. and C. Altier, 2002. Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolates from swine. J. Clin. Microbiol., 40: 2813-2822.
44. Guerra, B., S. Soto, R. Helmuth and M.C. Mendoza, 2002. Characterization of a self-transferable plasmid from *Salmonella enterica* serotype Typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. Antimicrobial Agents Chemother., 46: 2977-2981.
45. Arduino, S.M., P.H. Roy, G.A. Jacoby, B.E. Orman, S.A. Pineiro and D. Centron, 2002. $bla_{CTX-M-2}$ is located in an unusual class 1 integron (In35) which includes Orf513. Antimicrobial Agents Chemother., 46: 2303-2306.
46. Di Conza, J., J.A. Ayala, P. Power, M. Mollerach and G. Gutkind, 2002. Novel class 1 integron (InS21) carrying $bla_{CTX-M-2}$ in *Salmonella enterica* serovar infantis. Antimicrobial Agents Chemother., 46: 2257-2261.
47. Al Naiemi, N., B. Zwart, M.C. Rijnsburger, R. Roosendaal and Y.J. Debets-Ossenkopp *et al*., 2008. Extended-spectrum-β-lactamase production in a *Salmonella enterica* serotype Typhi strain from the Philippines. J. Clin. Microbiol., 46: 2794-2795.
48. Harrois, D., S. Breurec, A. Seck, A. Delaune and S. Le Hello *et al*., 2014. Prevalence and characterization of extended spectrum β lactamase producing clinical *Salmonella enterica* isolates in Dakar, Senegal, from 1999 to 2009. Clin. Microbiol. Infect., 20: 109-116.
49. Pokharel, B.M., J. Koirala, R.K. Dahal, S.K. Mishra, P.K. Khadga and N.R. Tuladhar, 2006. Multidrug-resistant and extended-spectrum β-lactamase (ESBL)-producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: Surveillance of resistance and a search for newer alternatives. Int. J. Infect. Dis., 10: 434-438.
50. Llanes, C., V. Kirchgesner and P. Plesiat, 1999. Propagation of TEM-and PSE-type β-lactamases among amoxicillin-resistant *Salmonella* spp. isolated in France. Antimicrobial Agents Chemother., 43: 2430-2436.
51. Glenn, L.M., R.L. Lindsey, J.F. Frank, R.J. Meinersmann, M.D. Englen, P.J. Fedorka-Cray and J.G. Frye, 2011. Analysis of antimicrobial resistance genes detected in multidrug-resistant *Salmonella enterica* serovar Typhimurium isolated from food animals. Microbial Drug Resist., 17: 407-418.
52. Eliopoulos, G.M. and K. Bush, 2001. New β-lactamases in gram-negative bacteria: Diversity and impact on the selection of antimicrobial therapy. Clin. Infect. Dis., 32: 1085-1089.
53. Gupta, V., 2007. An update on newer β-lactamases. Indian J. Med. Res., 126: 417-427.
54. Jacoby, G.A. and L.S. Munoz-Price, 2005. The new β-lactamases. N. Engl. J. Med., 352: 380-391.
55. Chopra, I. and M. Robert, 2001. Tetracycline antibiotics: Mode of action, applications, molecular biology and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev., 65: 232-260.
56. Nde, C.W and C.M. Logue, 2008. Characterization of antimicrobial susceptibility and virulence genes of *Salmonella serovars* collected at a commercial turkey processing plant. J. Applied Microbiol., 104: 215-223.