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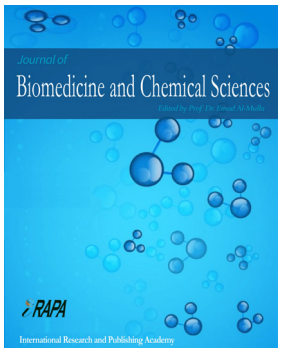

Expression of MacAB-TolC and MdfA Efflux Pumps in Associated with Multi Drug Resistant *Salmonella Enterica* Serovar Typhi Isolates

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ABSTRACT

Salmonella Typhi is the cause of typhoid fever. Together, Salmonella Typhi and *Salmonella* serovar Paratyphi A are the major agents of enteric fever. Typhoid fever affects millions of individuals each year in low-income nations. Human infection severity is determined by infectious doses and organism pathogenicity. Bacterial membrane and periplasm transporter proteins construct this barrier. These proteins remove antimicrobials, organic solvents, and toxic heavy metals from bacterial cells. Overexpression of the macAB-TolC genes induces macrolide resistance in Salmonella enterica. Salmonella pathogenicity is reduced in mice by macAB-TolC gene deletion. Due to its ability to expel structurally unrelated drugs. MdfA, a single cytoplasmic efflux protein, exports antibiotics when overexpressed.

INTRODUCTION

Salmonellaenterica serotype Typhi is responsible for typhoid fever and has been a burden on developing nations for generations. In 1829, Pierre Louis was the first to coin the term “typhoid fever” after identifying lesions in the abdominal lymph nodes of patients who had died from “gastric fever”[1]. *Salmonella enterica* serotype Typhi is a Gram-negative, rod-shaped, flagellated bacterium whose only reservoir is the human body. The bacterium is serologically positive for lipopolysaccharide antigens O9 and O12 as well as the distinct polysaccharide capsular antigen -Vi [2]. *Salmonella enterica* serotype Typhi is usually contracted by ingestion of food or water that is contaminated with the excrements of those that carry the organism and must survive the gastric pH barrier in the stomach prior to adherence in the small intestine. An infectious dose of *Salmonella* Typhi in healthy individual’s ranges between 1000 and 1 million organisms but can be related to the host’s defense mechanisms [3]. The incubation period of typhoid fever is typically 6–30 days. Untreated, it has a mortality rate of 12%–30% [4].

Patients will typically present after a 7 to14 day asymptomatic period after initial inoculation with *Salmonella* Typhi. Following the initial

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asymptomatic period, patients will present with an influenza-like illness with associated fever [5]. Multi-drug resistance typhoid traditionally describes resistance to all the first line of antibiotics suggested by WHO: ampicillin, trimethoprim, sulfamethoxazole, and chloramphenicol [6]. Approximately 1% to 5% of patients will become chronic carriers of *Salmonella enterica* serotype Typhi despite adequate antimicrobial therapy [7]. One of the problems of *S.* Typhi treatment it's emerging of a fluoroquinolone and cephalosporins-resistant *S.* Typhi strain which possesses the ability to transform from MDR (strains exhibits resistance to all first-generation antibiotics) to XDR when the bacteria gaining a plasmid in suitable factors [8]. The *S.* Typhi strains were stratified depending on uniform international standards to MDR, when is strain non-susceptible to a minimum one agent in more than three antibacterial classes, and XDR if strain remains sensitive to only 1 or 2 antibacterial classes [9]. Resistant *Salmonella* strains are commonly found in food animal sources [10].

Mismanagement of antimicrobial agents for treatment in humans and animals and the use of growth promoters in livestock have promoted antimicrobial resistance in *Salmonellae* [11]. In March 2018, CDC began enhanced surveillance for ceftriaxone-resistant Typhi in response to an ongoing outbreak of XDR typhoid fever in Pakistan [12]. All microorganisms, with a few exceptions, have highly conserved DNA sequences in their genome that are transcribed and translated to efflux pumps. Efflux pumps are capable of moving a variety of toxic compounds out of cells, such as antibiotics. This active efflux mechanism is responsible for various types of resistance to bacterial pathogens within bacterial species, the most concerning being antibiotic resistance because microorganisms can have adapted efflux pumps to divert toxins out of the cytoplasm and into extracellular media [13].

Bacterial efflux transporters are classified into five major super families, based on their amino acid sequence and the energy source used to export their substrates[14], efflux pump *Mac*AB-TolC Belongs to a family the ATP-binding cassette superfamily (ABC) .The macrolide-specific pump *Mac*AB is the only ABC-type drug efflux pump in *S. enterica*. that overexpression of macAB confers resistance to macrolides [15]. *Mac*AB-TolC efflux pump, which actively extrudes macrolides and polypeptide virulence factors powered by the AT-Pase *Mac*B and participates in the secretion of enterotoxin TII in *Escherichia coli* [16]. MdfA efflux pump Belongs to a family the Major facilitator superfamily (MFS). MdfA exists as a single cytoplasmic efflux protein and exports chloramphenicol, doxorubicin, norfloxacin and tetracycline [17]. The aim of this study is to estimate the correlation between gene expression level of *Mac*AB-TolC and *Mdf*A efflux pumps genes in *Salmonella* Typhi with MDR and XDR phenotypes .

MATERIALS & METHODS

This cross-sectional study, from January 2021 to July 2022, 350 blood and stool samples were taken from patients with enteric fever at Imamain Kadhimain Medical City Hospital, clinics, and private laboratories, which was carried out in Iraq. Bacteria were cultured on traditional media as well as on special media (HiCrome™ Salmonella Agar). Confirm determination and antibiotic sensitivity tests were performed using the automated VITEC-Compact 2 system.Conventional PCR was performed to identify the *fliC* gene by using specific primers. In addition, *fliC* was sequenced using the Sanger method after being submitted to the Microgen company.

Gene expression of efflux pumps was performed using a quantitative real-time polymerase chain reaction of *macB, macA, tol*Cand *Mdf*Agenes. This study included 350 samples were collected for this study, and 50 samples (21 blood and 29 stool) were laboratory diagnosed with Salmonella serovar Typhi. These samples were taken from outpatients and admitted patients who had symptoms like fever, diarrhea, headache, rash, fatigue, and abdominal pain. They were obtained from Al-Imamein Al-Kadhimein Medical City Hospital and other local laboratories in Baghdad from January 2021 to July 2022. Patients were divided into two group according to susceptibility to antibiotics, patients with sensitive phenotypes to antibiotics and patients' group with resistance phenotypes to antibiotics. The questionnaire was conducted by patient age, sex, medications, and the existence of other diseases such as bacterial and viral infections that cause fever and diarrhea.

This study approved by the ethical Committee of the College of Medicine-Al-Nahrain University. The subjects enrolled in this study provided five ml of blood and stool samples, which were collected using disposable syringes., then the whole blood was placed into blood culture bottles (BacT/ALERT FA or Brain heart infusion broth) and mixed gently, then the incubation is done in the incubator for automated blood culture until the reading of the culture result by used bacterial culture media (usual and specific media). According to the stool sample,

a small part of the specimen was taken and cultured on selenite and tetrathionate broth media, incubated for 24 hours at 37 ◦ C. Then the bacterial growth from broth were cultured on XLD, SS agar, and Hi-chrome Salmonella agars incubating for 24 hours to diagnose the type of bacteria and sensitivity to antibiotics used the VITEK device. Gene expression of efflux pumps was performed using a quantitative real-time polymerase chain reaction of *macB, macA, tol*Cand *Mdf*Agenes. Amplification primers and it is sequences were used in this study are listed in table (1 & 2)

Table 1
Primers and it is sequences that used in conventional-PCR and Phylogenetic tree

Target gene		Sequence ("5-------3")	Size of amplification (bp)	Ref
Fli C	F	TTAACGCAGTAAAGAGAG	1450	18, 19
	R	ATGGCACAAGTCATTAATAC		

Table 2
Primers and it is sequences that used in Real Time-PCR

Target gene		Sequence ("5-------3")
Tol C	F	CAGACCGATCAGCAAACCTTGA
	R	GCCTGTGTATAGGAAAGAACGTCAA
Mac A	F	CGCGCCAGCAGCAGTTA
	R	CGCCGCGGTATCCAGAT
Mac B	F	ACAGCAGCAGCGTGTCAGTATT
	R	TCGGCTCATCTGCCAGAATC
Mdf A	F	GGCTGGCCGATTATGATTGGT
	R	CGCGTGCGATGAGATAACC
16Rrna (Rf)	F	CCACACTGGGACTGAGACAC
	R	CCACTCCCGCTAACGTTCTT

Rf (reference gene).

Statistical analysis of this cross-sectional study performed with the statistical package for social sciences (SPSS) 20.0. Numerical data were described as mean and standard deviation. Categorical data were described as count and percentage. Chi-square test used to estimate the association between variables. The lower level of accepted statistically significant difference is bellow or equal to 0.05.

RESULTS & DISCUSSION

This current study included the collection of 350 blood and stool samples, but only 50 samples were positive for *Salmonella serovar* Typhi growth, 21(42%) samples were from blood, and 29 (58%) samples were stools.In the present study, disease occurrence was highest (28%) among age groups 21–30 years, followed by age group 31–40 years (18%), while the lowest incidence (8%) of disease was among age groups >51 years. As demonstrated in Table (3)

Table 3
Distribution of the age among study group

Age groups	Number	percentage	Total
≤ 10years	8	16%	100 %
11-20years	7	14%	
21-30years	14	28%	
31-40years	9	18%	
41-50years	8	16%	
>51years	4	8%	

The frequency of male participants was found to be 28 (56%), which was greater than the frequency of

female participant, which was found to be 22 (44%). The antibiotic resistance testing in this current study, the bacterial isolates showed complete resistance (100%) against Cefazolin and Ceftriaxone. Whereas low resistance levels were shown against Ticarcillin-Clavulanic Acid (37.5%), followed by Tobramycin (33.3%), Amikacin (16%), Gentamicin (14%), Minocycline (12.1%), and Trimethoprim-Sulfamethoxazole (4.1%). In contrast, Pipracillin-Tazobactam, Imipenem, Meropenem, Cefoxitin, and Ertapenem were associated with complete sensitivity against all bacterial isolates. Among the 50 Salmonella enterica serovar Typhi isolates investigated, 22 isolates were extensively drug-resistant (XDR), 22 isolates were multi-drug resistant (MDR); in contrast, 6 isolates were associated with the sensitive pattern. As demonstrated in Table (4).

According to the laboratory isolation of *Salmonella enterica* serovar Typhi isolates, stool and blood samples were cultivated on MacConKey (colonies were colorless due to the lack of lactose fermentation), *HiCrome™ Salmonella Agar* (light purple colonies) Salmonella-Shigella (SS) agars (black-centered colonies), and Xylose lysine deoxycholate (XLD) (Red colonies with black centers). As illustrated in Figure (1).

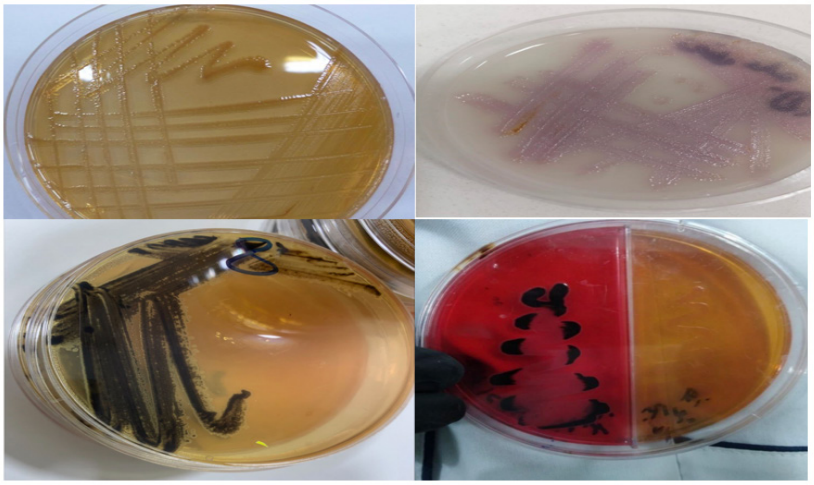
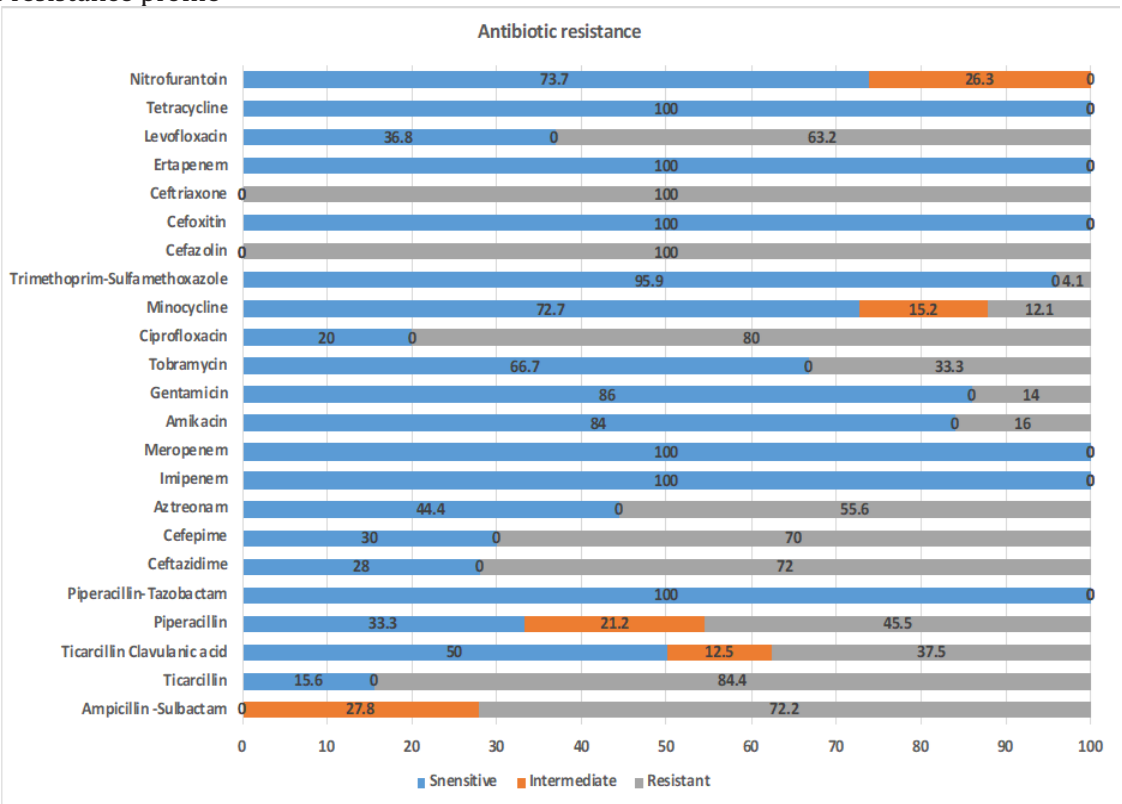


Fig. 1. Salmonella enterica serovarTyphion different culture media

Table 4
Antibiotic resistance profile



Genes expression among bacterial isolates

The efflux-pump gene expression among bacterial isolates during the current study was variable. According to the macB gene, it was expressed in 20 (40% of the isolates), the macA gene was expressed in 36 (52% of the isolates), and the tolC gene was expressed in 32 (64% of the isolates). In addition, the *Mdf*A gene was expressed in zero of the all isolates. As shown in Table (5).

Table 5
The efflux pump gene expression among bacterial isolates

Genes	Expressed N0 (%)	Not expressed NO (%)
macB	20 (40%)	30 (60%)
macA	26 (52%)	24 (48%)
tolC	32 (64%)	18 (36%)
MdfA	(0 %)	(0 %)

The expression of the gene was successfully detected by using a molecular technique, quantitative real-time PCR (qRT-PCR), with specific primers. The RT-PCR results of the *mac*A, *mac*Band MdfAgenesare illustrated in Figures (2), (3) and.

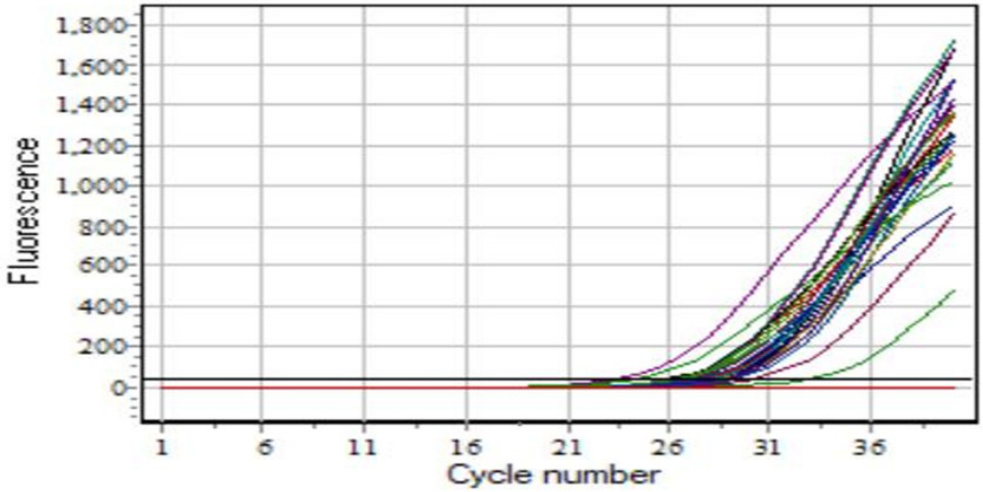


Fig. 2. RT-PCR results of *mac*Agene expression

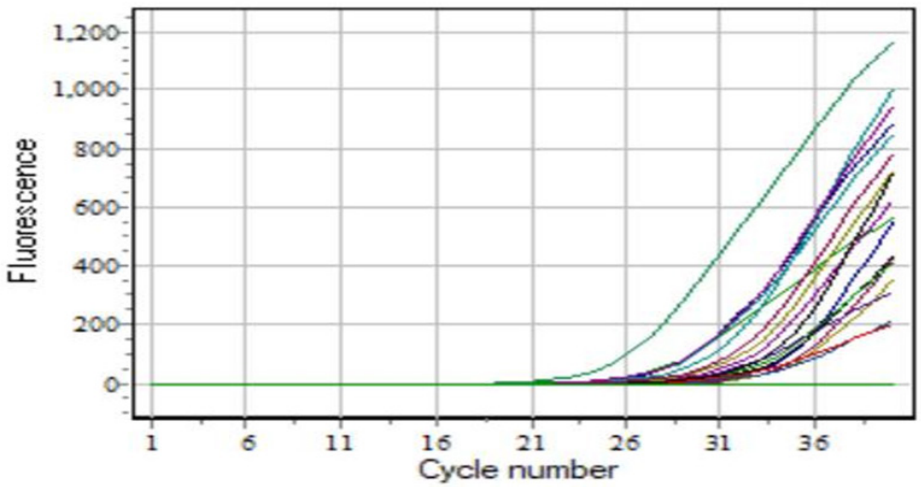


Fig. 3. RT-PCR results of *mac*Bgene expression

Note: There was no expression of the *mdfA* gene among bacterial isolates

According to the relationship between gene expression and resistance pattern of bacterial isolates during the study. The *macA, macB*, and *tolC* genes expression was high among XDR-isolates 16 (61.5 %), 12 (60 %), and 17 (53.1%), respectively, compared with other patterns, while the no expression genes were high among MDR-isolates 15 (62.5 %), 16 (53.3 %), and 10 (55.6 %), respectively.As illustrated in Table (6).

Table 6
The relationship among gene expression and resistance pattern of bacterial isolates

		Resistance			p value
		Sensitive	MDR	XDR	
macA	Not expressed	3	15	6	0.025*
		12.50%	62.50%	25.00%	
	Expressed	3	7	16	
		11.50%	26.90%	61.50%	
macB	Not expressed	4	16	10	0.171NS
		13.30%	53.30%	33.30%	
	Expressed	2	6	12	
		10.00%	30.00%	60.00%	
tolC	Not expressed	3	10	5	0.218NS
		16.70%	55.60%	27.80%	
	Expressed	3	12	17	
		9.40%	37.50%	53.10%	

Association of *macA* Gene Expression and Antibiotics Resistance level

The resistance levels against Ampicillin-Sulbactam, Ticarcillin, Cefepime, Amikacin, Trimethoprim-Sulfamethoxazole, and Tobramycin were higher among isolates without gene *macA* expression than those with expression. While the resistance level against Ticarcillin, Clavulanic acid, Piperacillin, Ceftazidine, Aztreonam, Gentamicin, and Levofloxacin was higher among isolates with gene expression than those without. As shown in table (7).

Table 7
Association between resistance level and *mac*Agene expression

Antibiotic types	macA		P value
	Not expressed	Expressed	
Ampicillin -Sulbactam	80.00%	62.50%	0.410NS
Ticarcillin	85.70%	83.30%	0.854 NS
Ticarcillin Clavuanic acid	28.60%	44.40%	0.655 NS
Piperacillin	40.00%	50.00%	0.754 NS
Piperacillin-Tazobactam	0.00%	0.00%	.
Ceftazidine	66.70%	76.90%	0.420 NS
Cefepime	70.80%	69.20%	0.902 NS
Aztreonam	47.60%	62.50%	0.316 NS
Imipenem	0.00%	0.00%	.
Meropenem	0.00%	0.00%	.
Amikacin	16.70%	15.40%	0.902 NS
Gentamicin	12.50%	15.40%	0.769 NS
Tobramycin	38.90%	28.60%	0.496 NS
Ciprofloxacin	66.70%	92.30%	0.294 NS
Minocycline	20.00%	5.60%	0.929 NS
Trimethoprim-Sulfamethoxazole	4.30%	3.80%	0.912 NS
Cefazolin	100.00%	100.00%	.
Cefoxitin	0.00%	0.00%	.
Ceftriaxone	100.00%	100.00%	.
Ertapenem	0.00%	0.00%	
Levofloxacin	50.00%	72.70%	0.311 NS
Titracycline	0.00%	0.00%	
Nitrofurantoin	0.00%	0.00%	

The phylogenetic tree during the current study was constructed based on the observation of the nucleic acid variation in the fliC gene of the salmonella enterica serovar isolated from blood and stool of patients suffering from enteric fever (Figure4), and all positions containing gaps and missing data were eliminated automatically by MEGA X software. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model.

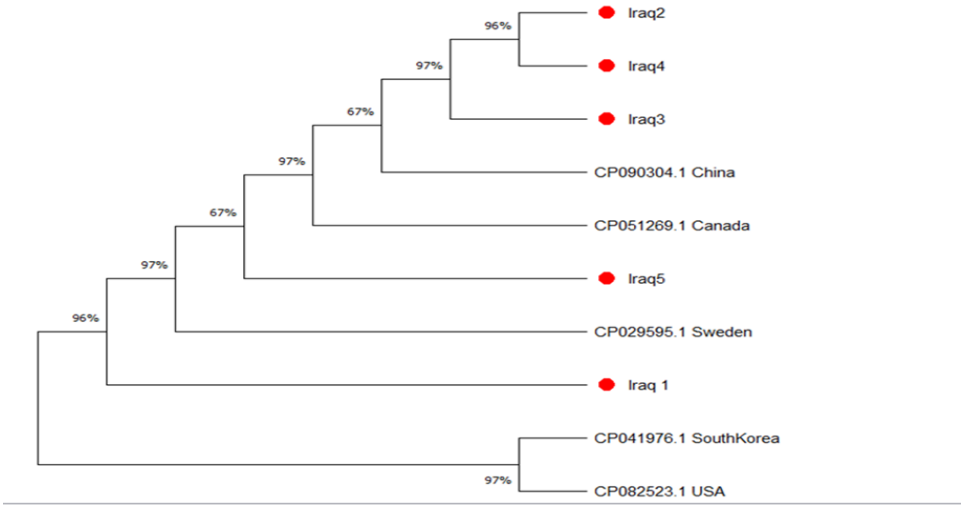


Fig. 4. Molecular analysis of phylogenetic tree of *salmonella enterica*serovar Typhi based on *fli*C gene

This current study included the collection of 350 blood and stool samples, but only 50 samples were positive for *Salmonella* ser. Typhi growth including (42%) samples from blood, and (58%) samples from stools. The presence of Salmonella Typhi in both feces and blood is consistent with the presence of typhoid fever. A variety of factors can contribute to the difficulty to isolate the organism from blood, including insufficient laboratory culture media, recent antibiotic medication, insufficient blood volume, incubation conditions, time of blood collection, and collection duration.

The frequency of male participants was found to be 28 (56%), which was greater than the frequency of female participants which was 22 (44%).These findings were comparable to those reported in other research carried out in Iraq in 2019 at Hilla City that include one hundred patients suffering from typhoid fever and found that males appeared to be more infected than females [20]. Also, these findings are consistent with the findings of earlier research conducted in AL-Musaib [21] in 2022, researchers in Iraq performed an epidemiological study that included 312 people who were diagnosed with typhoid illness. It was also reported that men were infected with typhoid fever at a rate of 57.7%, whereas only 42.3% of females were confirmed to have the illness [22].

The majority of men may have been outside, which suggests that they may have been considered food eaters, food handlers, or patients who had interacted with other patients. There is a possibility that a higher risk of infection in this group is due to increased exposure of men to potentially contaminated water and food outside of the house. In last years, the development of antibiotic resistance to the primary antibiotics used to treat the disease has rendered therapy useless and extremely costly [23]. To establish which treatments are most likely to be successful in killing the bacteria that causes typhoid fever, it is required to do antibiotic susceptibility testing on a regular basis [24].

In the current investigation, 50 Salmonella enterica serovar Typhi were tested for antibiotic susceptibility to 23 different antibiotics. The bacterial isolates demonstrated varying resistance to the Cephalosporin family, with total resistance to Cefazolin and Ceftriaxone (100%), and a significant level of resistance to Ceftazidime (72%). All isolates, on the other hand, were entirely sensitive to Cefoxitin (100%). This finding contradicts a prior Iraqi investigation, which found minimal resistance to Ceftriaxone and Cefazolin, moderate resistance to Ceftazidime, and complete resistance to Cefoxitin [25]. In another Iraqi investigation, Salman et al, 2021 discovered that all bacterial isolates shown moderate resistance to the third and fourth generations of Cephalosporins [26]. Furthermore, this conclusion contradicted the findings of the study of Yang et al., 2020. That reported that

whole salmonella species were sensitive to Cetazidime [27].

The current study showed a high resistance level against Ticarcillin (84.4%) and Ampicillin-Sulbactam (72.2%), with moderate resistance to Piperacillin (45.5%). and low resistance against Ticarcillin-Clavulanic Acid (37.5%). In contrast, all bacterial isolates were complete sensitive to Pipracillin-Tazobactam (100%). The Iraqi study was in agreement with the current study as it found high resistance frequency to Ampicillin-Sulbactam, moderate resistance to Piperacillin, and low resistance to Ticarcillin-Clavulanic Acid, but it was disagreed with the current study as it found moderate and low resistance to Ticarcillin and Pipracillin-Tazobactam, respectively [28]. Piperacillin is often used in conjunction with tazobactam, which increases the effectiveness of piperacillin treatment by blocking the enzymes responsible for beta-lactamase production in a variety of species.

The findings of the current research are in consistent with those of an earlier study conducted in Iraq, which found that piperacillin-tazobactam was sensitive against 96.5% of the bacterial strains [29]. The findings of the current study is similar with a study that was carried out by Perera et al [30]. Ticarcillin and clavulanic acid are a common treatment for gram-negative bacteria, and they are often used together [31]. Ciprofloxacin and Levofloxacin are the two fluoroquinolones that have been demonstrated to have a proportion of resistance that is 80% and 63.2%, respectively. In agreement with the present study, another study conducted in Pakistan also found high resistance to Ciprofloxacin [32]. In a similar manner conducted in Al-Dewanya, Iraq, moderate and low resistance to Ciprofloxacin and Levofloxacin, respectively, were demonstrated [33]. Prior studies found a growing percentage of bacterial isolates that were resistant to the antibiotic Ciprofloxacin [34, 35].

Contrary to the results of the present study, a different study conducted in Ethiopia found that bacterial isolates were susceptible to the Ciprofloxacin [36]. Ciprofloxacin is an effective alternative antibiotic for the treatment of Salmonella spp. Reduced ciprofloxacin sensitivity, particularly in developing countries, poses a significant danger of rendering typhoid fever treatment ineffective. Iraq, a Middle Eastern country, experienced the largest reduction in sensitivity to Ciprofloxacin [37]. Imipenem, Meropenem, and Ertapenem belong the Carbapenem antibiotics family, during the current study all strains of bacteria were completely sensitive to Carbapenem. This result was in accordance with other study was also found sensitivity to Carbapenem [38].

In addition, there was moderate resistance to Azetreonam and low resistance to Trimethoprim-Sulfamethoxazole and Minocycline during the current study. In a similar vein, a study conducted in Indonesia showed resistance to Trimethoprim-sulfamethoxazole [39]. Trimethoprim-sulfamethoxazole, on the other hand, was shown to have a high degree of sensitivity in a Nepalese study [40]. For over thirty years, Minocycline has been considered an effective antimicrobial [41]. In agreement with these results, a study from South Korea reported the resistance against Minocycline was showed by a few strains of Salmonella spp. [42]. These differences among many studies may be attributed to the origin of bacterial isolates and the misuse of antibiotics without a medical prescription, especially in developing countries.

A multidrug-resistant organism (MDR) is one that has evolved resistance to at least one medication from three or more distinct antibiotic families. An organism must be resistant to at least one antibiotic agent from the majority of known antibiotic families to be called extensively drug-resistant (XDR) [43]. In the current study, 50 *Salmonella ser.* Typhi isolates were obtained from stool and blood. During the current study, a high frequency of isolates was extensively drug-resistant (XDR) 22 (44%), and multi-drug resistant (MDR) 22 (44%). According to this finding, an Iraqi previous study discovered that 80.4% of bacterial isolates were resistant to at least one agent from more than three antibiotic groups; these isolates were classified as MDR, while 19.6% of isolates were resistant to at least six antibiotic groups and were classified as XDR [44]. In line with this conclusion, a recent Iraqi investigation in Baghdad found that 90% of bacterial isolates were MDR [45].

Furthermore, in a comparable study with 777 bacterial isolates, it was discovered that 80% of the isolates were MDR, whereas 53% were XDR [46]. On the other hand, this contradicts the findings of an Indian study, which discovered that only a small percentage of bacterial isolates were MDR [47]. In addition, in contrast to the current study, another investigation was undertaken in Nepal that screened for the incidence of MDR among Salmonellase. Typhi isolates and discovered that there were no MDR isolates [48]. It is possible that the high prevalence of MDR and XDR *Salmonella*ser. Typhi isolates is due to overuse of antibiotics without visiting a doctor, which is common in developing countries, particularly Iraq.

The expression level of Multi-drug resistance efflux pump genes was evaluated using quantitative Real-Time polymerase chain reaction during this study. This is the first study to look at the expression levels of these genes in *Salmonella* ser.

Typhi isolated from typhoid fever patients without using any stimulations. During the current study, and in regard to the ATP-binding cassette (ABC) efflux pump family, the Macrolide-specific pump gene expression was determined. The *macB* gene was expressed in 20 (40% of isolates), while the *macA* gene was expressed in 36 (52% of the isolates). There is no study evaluated the *macA* and *macB* gene expression in *Salmonella enterica* serovar Typhi. The previous study conducted in 2019 included 20 *K. pneumoniae* isolates with variable resistance to Eravacycline. This study revealed that the overexpression of *macA* or *macB* was detected in 12 of 20 variable eravacycline-resistant isolates compared to the reference strain [49]. Mitchev, Nireshni, et al. in 2022 was found that *macA* and *macB* gene expression was high in Azithromycin-resistant *Neisseria gonorrhea* isolates in comparison to non-resistant isolates [50]. TolC is a protein that is recognized for being the shared outer-membrane channel protein.

This protein has the ability to make selections about the exit routes that are taken by antibacterial medicines and virulence proteins [51]. One of *Salmonella*'s main facilitator superfamily-multidrug resistance (MFS-MDR) pumps is called mdfA. It is a cytoplasmic efflux protein that is present in *S.* Typhimurium and exports a wide variety of antibiotic kinds [52]. during the study, the tolC gene was expressed in 32 (64% of the isolates). In contrast, there was no expression of mdfA gene among bacterial isolates. Previous study was examined the tolC and mdfA gene expression in 47 clinical Ciprofloxacin resistant *E.* coli and found the overexpression of these genes was determined in 33 isolates [53].

The current study is the first to examine the expression levels of these genes in *Salmonella* ser. Typhi, and the expression of these MacAB-TolC and MdfA efflux pumpswas assessed without the use of any stimulation. The occurrence of expression of these genes among the bacterial isolates is because the obtaining of these isolates were from patients undergoing treatment, and this is the reason behind this expression, as is the lack of gene expression of *mdf*A among the isolates. Since *mdf*A is a single cytoplasmic efflux protein that plays an important role in the export of chloramphenicol, doxorubicin, norfloxacin, and tetracycline, it may be that all 50 patients enrolled during the current study who did not use these antibiotics in the treatment of typhoid fever were the cause of the non-expression of a *mdf*A gene.

Relationship between the efflux pumps gene expression and developing of MDR and XDR *Salmonella enterica* serovar Typhi. The efflux pump gene expression in *Salmonella enterica* serovar Typhi (*macA, macB,* and *tolC*)which included during the current study was associated with multi-drug resistance (26.9%,30%, and 37.5, respectively), and extensive-drug resistance (44.8%, 38.5%, 61.5, 60%, and 53.1, respectively) bacterial isolates. In Gram-negative bacteria, the development of multidrug resistance may be caused by regulatory alterations that lead to increased production of chromosomally encoded efflux pumps [54]. Fang et al, was exposed MDR-*Klebsiella pneumonia* isolates to synthetic efflux pumps inhibitors results in decreased in the minimal inhibitory concentration of these isolates [55]. These results suggested that efflux pumps system contributed in the developed resistance to wide spectrum of antibiotics as well as emergence of the MDR and XDR phenotypes among *Enterobacteriace.*

During the current study the association between *macA* and *macB* gene expression with resistance to variety types of antibiotics was examined and the study found that the there was no statistically significant correlation between the expression of the both *macA* and *macB* genes and different forms of antibiotic resistance. It was discovered that *mac*B and its periplasmic adaptor protein *mac*A conferred resistance to macrolide medications in a strain missing the main RND efflux pump a*cr*AB during an analysis of *E. coli* transporter genes [56]. Additional research has shown that expression of *macAB* results in a rise in *E. coli's* ability to resist bacitracin and colistin [57]. Have been studies conducted on the function of MacAB-TolC in various gram-negative organisms, the bacterium *Stenotrophomonas maltophila* MacAB is able to impart resistance to a wide range of polymyxins, aminoglycosides, as well as macrolides [58]. Mutations within the *macAB* promoter of *Neisseria gonorrhea* may improve the pathogen's resistance to macrolides [59].

Flagellin is the only protein found in a cell's flagellum that forms the flagellar filament. Serological tests have revealed that each strain is connected with many strains of Salmonella. The amino acid sequence within flagellin has been partitioned into four domains of the flagellin component, which have been structurally identified within the filament structure, based on data from biochemistry, immunology, and structural research [60]. Samples of amplified PCR products for the *fliC* gene of *Salmonella* ser. Typhi were isolated from patients with enteric fever and sent for

direct sequencing using Sanger's sequencing method. For a complete detection of *Salmonella*ser. Typhi, the alignment information for the *fliC* gene was analyzed to see the distribution of the similarity with NCBI information, which gives the nearest national strains to the Iraqi strains and their identity level [61].

The distribution of the Iraqi strains (see Figure 5) in the strains number 2, 4, and 3 were significantly like to the national strains from China and Canada although the strains number 2 and 4 are come from same ancestor, whereas, the strain number 3 come from different ancestor. While the distribution of Iraqi strain number 5 was significantly like to the national strains from Sweden. In addition, the distribution of the Iraqi strains in the strain number 1 was significantly like to the national strains from South Korea and USA.The current study considered the first study performed the phylogenetic tree of *Salmonella* ser. Typhi based on the *fliC* gene only. Another Iraqi study constructed the phylogenetic tree of *Salmonella enterica* utilizing *16S rRNA*, *avrtA*, and *spvC* gene sequencing. This study concluded that the phylogenetic analysis demonstrates that Iraqi strains of *Salmonella* are extremely comparable, and they possess the identical sequence of the 16S *rRNA* gene compared to national *Salmonella* strains. On the other hand, the bases of the *avrA* and *spvC* genes do not share any similarities [62].

The bacterial growth positive in stool samples was higher than blood samples, this indicate that stool sample considered the best among different samples particularly blood for isolation o*f Salmonella enterica* serovar Typhi from patients with enteric fever.The disease occurrence was higher in younger age group than child and old age groups, in addition, its frequency was higher among males than females, and these results indicated that typhoid fever disease considered one of more common outdoor diseases in Iraqi community.

There were higher resistance levels against different types of antibiotics among bacterial isolates this make it one of difficult treatment bacterial infections. The emergence of multi-drug resistance and extensive-drug resistance among bacterial isolates was high, lead to make challenge issue for the health settings. The expression of efflux pumps genes (*acrA, acrB, macA, macB*, and *tolC*) among bacterial isolates indicate that these efflux pumps genes play important role in pathogenesis and resistance process of bacteria. The efflux pumps genes expression in reality paly important role in emergence of MDR and XDR isolates.

Competing Interest

The authors had no competing interests.

CONCLUSION

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