

Genetic Assessment of Antibiotic Resistance in *Salmonella Enteric* a Serovar Typhi in Kirkuk Province

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ABSTRACT

Around fifty isolates of *Salmonella enterica* serovar Typhi were isolated from blood specimens of patients referring to several hospitals in Kirkuk province, Iraq. The results revealed that all isolates developed resistance to trimethoprim-sulfamethoxazole and chloramphenicol. However, neither *sul2* nor *tem* genes were detected. Moreover, only ten isolates were positive for *catP*. Our data suggested participation of other genes or mechanisms allow these multidrug isolates to resist the antibiotics in question.

Keywords: Antibiotic Resistance, *Salmonella Enteric*, Typhi.

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INTRODUCTION

Salmonella enterica serovar Typhi, the causative agent of enteric fever, is considered as one of the highly resistant pathogens.^{1,2} It has been reported that typhoid fever causes 26 million cases worldwide annually. Nevertheless, this infection is endemic in a wide range of countries, and if not treated correctly, the mortality rate may increase dramatically.¹

In developing countries, this pathogen has been reported as a major health problem.³ Treatment with appropriate antimicrobial drugs faced some failure due to ongoing plasmid-mediated resistance to some antibiotics, principally in southern and southeast Asia.⁴

Multidrug resistance (MDR) in typhoid is used to designate combined resistance to chloramphenicol, co-trimoxazole (trimethoprim-sulfamethoxazole), and ampicillin. These antibiotics are commonly known as first-line antimicrobials.¹ The incidence of MDR to trimethoprim, amoxicillin, streptomycin, chloramphenicol, tetracycline, and sulfonamides in *S. Typhi* has been increasing and MDR strains were responsible for plentiful epidemics in Asia.⁵ MDR *S. Typhi* has been reported from different parts of world such as Pakistan,⁶ India,⁷ Bangladesh,⁸ Canada,⁹ and Iraq.^{10,11}

To the best of our knowledge, Limited data is available on the prevalence of genes responsible for antibiotic resistance in Kirkuk province, Iraq. Consequently, the current study aimed to investigate the antimicrobial resistance at the genetic level in local isolates of *S. Typhi*.

MATERIALS AND METHODS

Isolation and Identification

Fifty isolates of *S. Typhi* were isolated from blood specimens obtained from patients visiting Kirkuk Hospitals, Iraq, using conventional cultural, morphological, and Biochemical tests depending on Harley *et al.*¹² Besides, VITEK 2 compact system (bioMérieux, France) system was performed to confirm the results of identification.

Antibiotic susceptibility

The susceptibility of the Fifty *S. Typhi* isolates towards eight antibiotics (ciprofloxacin, imipenem, ceftriaxone, azithromycin, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and tetracycline) were determined by means of Kirby-Bauer technique.¹³ An isolate was interpreted as resistant, intermediate resistant, or susceptible following the breakpoints of CLSI.¹⁴ *Escherichia coli* (*E. coli* ATCC 25922) was used as a quality control strain.

Amplification of, *catP*, *sul2* and *tem* genes in multiplex PCR

Multiplex PCR assay targeting *catP*, *sul2*, and *tem*, was performed using primers listed in Table 1.

The reaction mixture contained 10 µL of master mix (Eurofin, USA), 1 µL of 10 pmol of each primer, 2 µL of a template. Amplification protocol was performed with T100 thermal cycler (Bio-rad, USA) as follows: 1 cycle of 94°C

Table 1: Primers sequences used in study

Primer name	Primer sequences (5'–3')	Targeted gene	Antibiotic		Amplicon size (bp)	Reference
ASRCF	ATGGAGAAAAAATCACTGG	<i>catP</i>	Chloramphenicol		623	15
ASRCR	AATTCATTAAGCATTCTGCCGAC					
sulF	GATGAAGTCAGCTCCACCT	<i>sul2</i>	Trimethoprim-		707	16
sulR	GCACGAGTGGGTTACATCGA		sulfamethoxazole			
AF	GGTCCTCCGATCGTTGTCAG	<i>Tem</i>	Ampicillin		311	17
A R	TACCCTCATACTTATCCACCA					

for 4 minutes, 30 cycles each at 94°C (1.5 min), 45°C (1 min) and 72°C (2 min) followed by 5 min at 72°C.¹⁵ The PCR amplicons were electrophoresed in 2% agarose gel at 5V/cm for 1 hour stained with ethidium bromide and observed under UV transilluminator.

RESULTS AND DISCUSSION

It is well recognized that multidrug resistance is highly associated with the severity of typhoid. This linkage has long been ascribed to unsuitable initial treatment and accordingly promotes the prognosis of the disease.^{8,19}

All isolates were susceptible to ceftriaxone, imipenem, and azithromycin. While a total of 43 isolates were resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Likewise, 31 isolates were resistant to tetracycline. Upon that, *catP*, *sult2*, and *tem* related with resistance to the chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin, respectively, were assayed.

Although all isolates developed resistance to trimethoprim-sulfamethoxazole and chloramphenicol, *sul2* and *tem* were not detected in all of them. While *catP* were detected in only ten isolates (Figure 1). These results highly emphasize the contribution of other genes or posttranslational mechanisms enables these isolates to have resistance against antibiotics under investigation.

Huovinen demonstrated that bacterial resistance to TMP and sulfonamides is mediated by the following 5 main mechanisms: (a) efflux pumps and/or the permeability barrier, (b) natural insensitivity of target enzymes, (c) configurational alteration in target enzymes, (d) mutational or recombinational

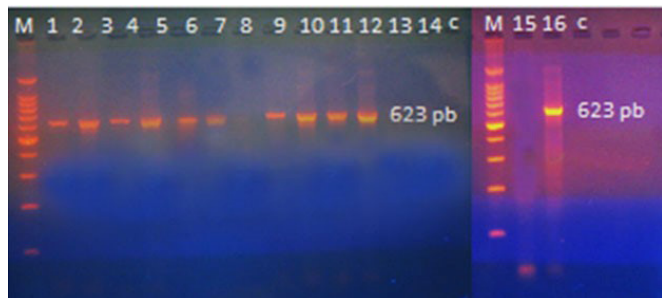


Figure 1: Analysis of the genomic DNA of *Salmonella enterica* serovar Typhi isolates shows a presence of *catP* (623 pb) separated on agarose gel (0.8%) at 5 V/cm for 1 hour, stained with ethidium bromide and visualized on a UV transilluminator documentation system. Lane M, 100 bp DNA ladder, Lane 1-14: DNA of different *S. Typhi* isolates. Lane C, Negative control (had all PCR mixture including water instead of DNA template).

changes in the target enzymes, and (e) acquired resistance by drug-resistant target enzymes.²⁰

In *Salmonella*, *bla_{TEM}* confers the β -lactamase resistance.²¹ Interestingly, none of the testes isolates carried this gene. Such finding highly signifies the role of other genes or mechanisms in resistance towards antibiotics under investigation.

In conclusion, the Iraqi *S. Typhi* are Multidrug-resistant isolates owing to their resistance to various genes and resistance mechanisms.

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