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Original Article

Integron-Associated Antibiotic Resistance in *Salmonella typhi*

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Abstract

Salmonella enteric serovar Typhi (*S.* typhi) and paratyphi (*S*. paratyphi) bacteria exclusively found in humans, cause typhoid fever, an acute, and possibly deadly systemic infection. Typhoid fever is caused by a species of rod-shaped, Gram-negative Enterobacteriaceae called *S. typhi*. The present study aimed to examine the *intI* gene and investigate the possible relation between this gene and multi-drug resistance in *S*. typhi. A total of 30 blood samples were obtained from patients who were suspicious of typhoid fever using the direct strategy of inoculation. Each specimen was injected into a culture of a selective medium, such as XLD and SS agar, and then incubated at 37°C for 24 h. The genomic DNA was extracted through a boiling process. Tris-EDTA was used to suspend bacterial colonies cultured on MacConkey agar plates. The suspension of bacterial colonies was centrifuged for 5 min at 8000×g and for 20 min at -20°C which lyses the organisms and extracts the DNA from the buffer. The supernatant is then transferred to a fresh Eppendorf tube. Gel electrophoresis was carried out utilizing a UV transilluminator. The *intI* gene for *S.* typhi was found using a PCR test. The antibiotic sensitivity testing showed that the *S.* typhi isolates were classed as multi-resistant. These results were confirmed using the polymerase chain reaction (PCR) technique using *intI* gene where twenty specimens isolated from typhoid patients were positive for *S.* typhi.

Keywords: *Integron* gene, Multidrug resistance, *S.* typhi

1. Introduction

Typhoid fever refers to a severe and systemic illness resulting from an infection with the bacteria *Salmonella enteric* serovar typhi (*S.* typhi). Enteric fevers, such as typhoid fever, are a significant public health issue in developing countries and are defined as a systemic sickness marked by fever, stomach ache, as well as non-specific symptoms, such as nausea and vomiting. In some cases of typhoid, a rash known as "rose spots" appears on the belly and breast that resemble small (1/4 inch) red dots. *S.* typhi is more frequent in children than adults; however, it can live for years in feces and spread to others. Typhoid can induce serious gastrointestinal bleeding, intestinal perforation, renal failure, and peritonitis (1). *S.* Typhi represents an anaerobic, non-spore producing rod bacillus that is 2-3 m long and 0.4-0.6-0.6 m in diameter. This bacteria does not ferment the lactose (2). The Enterobacteriaceae family includes the enteric bacillus. The lipopolysaccharide antigens (O9 and O12), protein flagellar antigen, and polysaccharide capsular antigen Vi serological procedure (Widal test) are serologically positive for identifying *S.* typhi (3). Infected people's lymphatic tissues, including the small intestine, liver, spleen, and bloodstream, are infected by the multi-organ pathogen *S.* typhi. No natural host has been found for this obligate parasite. A temperature range of 7°C-45°C is required for optimum development, and the ideal range is between 35℃ and 37°C (3). The growth of *S.* typhi, a facultative anaerobe, is somewhat

lower in nitrogen than in air. It may also grow at 8℃ to 11°C in the presence of 20% to 50% carbon dioxide, and the ideal pH for growth is 7-7.5. It should be noted that *S.* typhi may survive in the environment for weeks or months without a host. In addition to a minimal medium, most *S.* typhi strains require one or more amino acids and vitamins. These are primarily involved in the transfer of drug-resistant genes and are linked to mobile DNA elements. Mobile integrons have been shown to have a role in the propagation of drug-resistant genes. Class 1, 2, and 3 of mobile integrons, which have been linked to multiple-drug resistance (MDR), are among the 'historical' classes. Integrons of class 1 include several different resistance gene cassettes (4), while *aadA* which encodes streptomycin-spectinomycin resistance appears in the majority of them. Two additional mobile integron classes (class 4 and class 5) have been discovered in *Vibrio* spp. (5).

This study was designed to find the *intI* gene in *S.* typhi in the Iraqi population and explore the possible link between this gene and MDR in *S.* typhi.

2. Materials and Methods

2.1. Collection and Identification of Bacterial Specimens

Between December 2020 and September 2021, 30 samples were obtained from patients who had clinical symptoms of typhoid fever using the direct strategy of inoculation. Each specimen was injected into a culture of a selective medium, such as XLD and SS agar, and then incubated at 37°C for 24 h (6).

2.2. Molecular Identification

Genomic DNA was extracted using the boiling process. Tris-EDTA was used to suspend bacterial colonies cultured on MacConkey agar plates. The suspension of bacterial colonies was centrifuged for 5 min at 8000×g and for 20 min at -20°C to lyses the organisms and extract the DNA from the buffer. The supernatant was then transferred to a fresh Eppendorf tube. Gel electrophoresis was carried out utilizing a UV transilluminator. The (*intI*) gene for *S.* typhi was found using a PCR test (Table 1). This primer is mentioned in table 2. Electrophoresis with 1% agarose gel electrophoresis was used to accurately assess the sizes of PCR products. Ethidium bromide (Sigma, USA) was added to the gel and ran for 1.5 h at 80 volts. A single band was visible at the proper point on an ultraviolet light transilluminator and bands were shot using a gel documentation system (Cleaver, UK). The molecular weights of amplified productions were measured using a 100bp ladder (Bioneer, Korea) (7).

2.3. Statistical Analysis

DNASIS software (Hitachi Software Engineering Co., Ltd) was used for data analysis.

Table 1. Thermocycler-based PCR procedure using intI primers

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
intI1	"96 Cº for 2 min"	27	"96Cº for 15 sec"	"55Cº for 30 sec"	"72 Cº for 3min"	"72 Cº for 7min"

Table 2. Primers used in the present study

Primer Type	Primer Target	Primer sequence (5'-3')	Amplicon size (bp)	Reference
intI1	Int1	"**F**-ATCATCGTCGTAGAGACGTCGG **R**-GTCAAGGTTCTGGACCAGTTGC"	892	(8)

3. Results and Discussion

3.1. Morphological Characterization

Microscopical and biochemical tests and culture morphology were initially used to identify bacterial isolates obtained from clinical samples. It was possible to identify the cultural origins of *S.* typhi based on the colonial morphology of these isolates. *S.* typhi could not ferment lactose sugar and formed pink colonies with black cores on XLD agar. However, the colonies formed on blood agar seemed to be non-hemolytic smooth white colonies, while the colonies grown on MacConkey agar were pale and smooth (Figure 1). Under the microscope, *S.* typhi seemed to be Gram-negative bacilli.

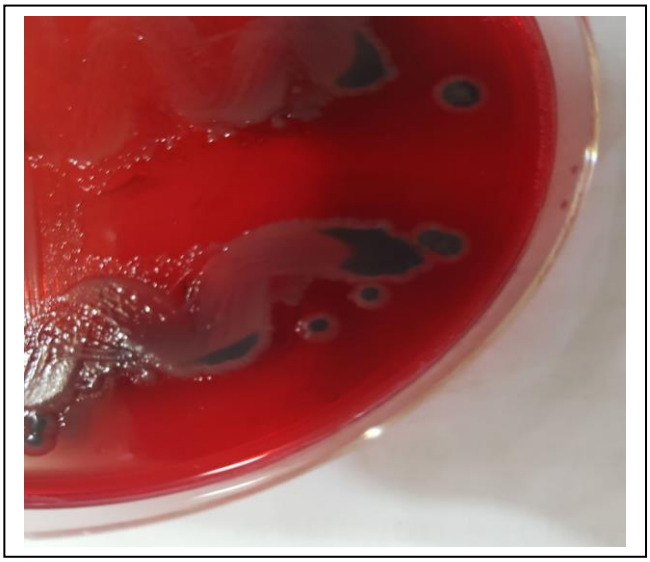

Figure 1. Growth of *S.* typhi on XLD agar medium

As shown in table 3, biochemical assays were used to identify *S.* typhi isolates. Isolates tested negative for oxidase activity, urease production, and Simmon's citrate utilization but positive for H2S production and catalase activity. On triple sugar iron agar (TSI), the *S.* typhi isolates produced hydrogen sulphide but did not produce any gas. Both the butt and the slant turned yellow and crimson in triple-sugar iron slants, suggesting that only glucose was fermented and no acid was produced in the butt. The morphology and culture results were all the same (9).

Table 3. Biochemical features of *S.* typhi

Test	Result
"Oxidase test"	-
"Simmons Citrate"	-
"H2S production"	+
"Urea hydrolysis"	-
"Catalase test"	+

In total, 30 isolates were provisionally identified as *S.* typhi after a thorough bacteriological analysis based on morphological, cultural, and biochemical testing. Eventually, 47 biochemical tests and a negative control well on GN-ID cards were completed using the automated VITEK-2 compact system. Only 30 blood samples were found to contain *S.* typhi, with ID message confidence levels ranging from excellent to excellent (probability percentages ranging from 95 to 99).

3.2. Antimicrobial Susceptibility of *S.* typhi Isolates

The findings of an antibiotic sensitivity test on *S.* typhi isolates are presented in table 4. The results revealed that *S.* typhi had high resistance (100%) to ampicillin, chloramphenicol, cefotaxime, clindamycin, gentamycin, nalidixic acid, penicillin, tetracycline, trimethoprim, and vancomycin (Figure 2).

In Brazil, Cortez, Carvalho (10) discovered that 78% of isolates of *S.* typhi were ampicillin resistant. According to Bacci, Boni (11), 33.3% of Salmonella strains isolated from blood in Italy were ampicillin resistant.

Table 4. Antibiotic sensitivity pattern of *S.* typhi isolates

	Antibiotic	Diameters of inhibition	Resiste isolate of *S. typhi*
1	Ampicilin	0	30%
2	Chloromphenicol	0	30%
3	Cefotoxime	0	30%
4	Clindamycin	0	30%
5	Gentamycin	0	30%
6	Nalidixic acid	0	30%
7	Pencillin	0	30%
8	Tetracycline	0	30%
9	Trimethoprim	0	30%
10	Vancomycin	0	30%

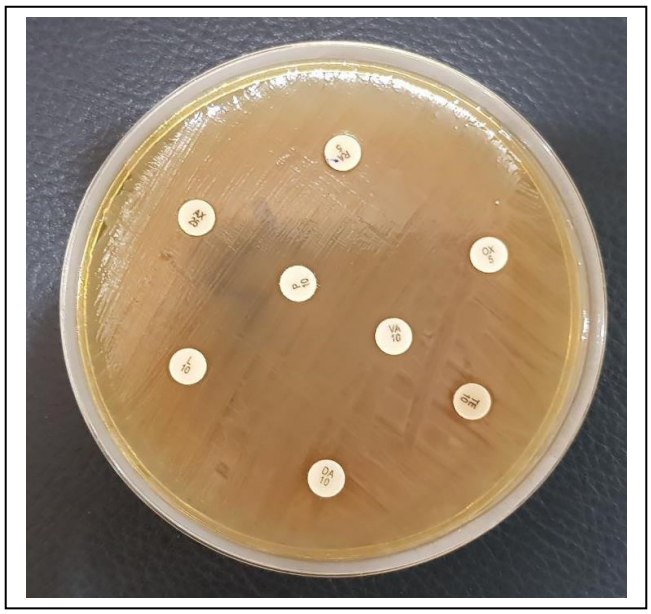

Figure 2. Sensitivity pattern of *S*. typhi to some antibiotics

As previously stated, none of the *S.* typhi strains were resistant to chloramphenicol (12). The sensitivity to chloramphenicol is owing, in part, to the antimicrobial's dual role as a growth stimulant and therapeutic agent. When the susceptibility of chloramphenicol to isolates from both sources fluctuates, the low resistance rate may be a result of the restricted usage of antibiotics due to concerns over their severe side effects which increases its efficacy (13). Several investigations have shown that *salmonella* is very sensitive to tetracyclines (13), which is consistent with the findings of this study.

In the antimicrobial susceptibility test, all 22 isolates were found to be multi-resistant, with 100% resistance to quinolones, sulfonamides, and tetracycline. Only 36.4% and 81.8% of isolates were resistant to aminoglycosides and cephalosporins, respectively. According to the World Health Organization (14), quinolones, third-generation cephalosporins, penicillin, and monobactams are among the most significant antimicrobials used in human medicine (13).

Trimethoprim resistance was found in roughly 98.6% of *S.* typhi isolates in this study. As previously reported, the prevalence of *Salmonella* isolates resistant to ampicillin, chloramphenicol, and cotrimoxazole has increased significantly.

According to a study conducted on the Malawi-Mozambique border, 100% of *S.* typhi isolates were resistant to ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim (15). Ampicillin and cotrimoxazole were effective against 76% of Ugandan S. typhi, whereas chloramphenicol was only effective against 85.9% of the bacteria in this study. Cefotaxime was the only drug that entirely wiped off *S.* typhi. Fluoroquinolone susceptibility is critical for the treatment of resistant cases (16). Cefoxitin, colistin, imipenem, meropenem, and tigecycline were all effective against *S.* typhi isolates. Since the isolates are already resistant to a wide spectrum of cephalosporins, cefoxitin is classified as extended-spectrum cephalosporins and may have no impact.

3.3. Multidrug Resistance of *S.* typhi Isolates

The MDR isolates are those that are resistant to at least three different antibiotics. According to the current criteria of MDR, 30 (100%) isolates were verified as MDR and were resistant to five antibiotic classes. Microorganisms resistant to various antimicrobial drugs caused an upsurge in the MDR typhoid all over the world. Several causes are to blame in this regard, including selection pressure caused by antibiotic abuse, which leads to the evolution of resistant microbes (17).

Resistance to chloramphenicol has been associated with high-molecular-weight and self-transferable incHI1 plasmids. *S.* typhi was resistant to sulfonamides, tetracycline, and streptomycin as well, although amoxicillin and trimethoprim-sulfamethoxazole were originally viable substitutions. Resistant genes were coded via IncHI plasmids. Individual multidrug-resistant *S.* typhi strains were clonally propagated, or the plasmid was transferred to several *S.* typhi strains, resulting in the spread (18).

Detection of drug resistance patterns, as well as quick typhoid diagnosis, has become increasingly important as the prevalence of MDR continues to

grow. Antimicrobial medicines, which have resulted in the emergence of MDR strains, as well as widespread usage of antimicrobials, can be blamed for the development of antibiotic-resistant *S.* typhi isolates. The incidence of MDR *S.* typhi isolates that are resistant to chloramphenicol, ampicillin, and trimethoprim has been a growing cause for concern (19). In a multivariate analysis, the MDR phenotype of *S.* typhi was found to be associated with an increased risk of bacteremia. Many other plasmids have been found in MDR *S.* typhi; however, the incHI1 incompatibility type plasmid appears to be the most common of all. Resistance to chloramphenicol, ampicillin, trimethoprim, sulfonamides, and tetracycline is frequently encoded by incompatibility complex group incHI1 plasmid.

The stronger transmission potential of plasmids, compared to drug-resistant strains, may account for their widespread frequency (18). The surge of MDR isolates in the current study might be the result of unregulated antibiotic usage during the previous few years. Although numerous variables contribute to the selection and development of antibiotic-resistant bacteria in clinical practice, the most significant variable is the blind administration of antibiotics. Bacterial resistance may be on the rise due to inadequate antibiotic policies, as well as the transfer of resistant genes via such vehicles as plasmids and bacteriophages (20).

3.4. Molecular Study of Integron

As shown in figure 3, the class 1 integron gene was found in 20 out of 30 *S.* typhi isolates, which confirms the findings of the study performed by AL-Kraety and Al-Ammar (21). The class1 integron gene was found in 14 out of 30 *S.* typhi isolates. Numerous investigations have been conducted to determine the prevalence of class 1 integrons in clinical isolates of Gram-negative bacteria. Jones and Peters (22) discussed considerable research on integrons in *S.* typhi (23).

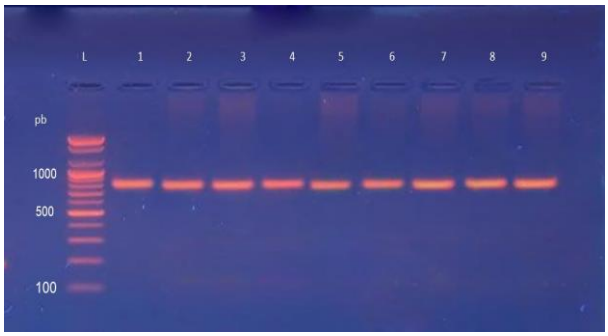

Figure 3. PCR results of *S.* typhi isolates amplified using intI1 gene primers, having a product size of 892 bp

The mobility of genetic pieces, known as integrons, may increase the spread and accumulation of resistant genes in bacteria. Transposons and conjugative plasmids are common places for integrons to be found (5). A uropathogenic isolate with class 1 integron was found in 49% of samples in one investigation (24), whereas class 1 integron was found in 65% of those samples (25).

Eventually, the results revealed that *S.* typhi had high resistance (100%) to ampicillin, chloramphenicol, cefotaxime, clindamycin, gentamycin, nalidixic acid, penicillin, tetracycline, trimethoprim, and vancomycin. The Class1 integron gene was found in 20 out of 30 *S.* typhi isolates.

Authors' Contribution

Study concept and design: Z. M. J.
Acquisition of data: Z. M. J.
Analysis and interpretation of data: Z. M. J.
Drafting of the manuscript: F. M. O.
Critical revision of the manuscript for important intellectual content: F. M. O.
Statistical analysis: S. N.
Administrative, technical, and material support: S. N.

Ethics

The study was approved by the ethics committees at Al-Qasim Green University, Al Qasim, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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