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Original Research Article

Molecular Characterization of *Salmonella Typhi* Isolated from Typhoidial Humans

Adil Abead Hassuny* Iman Jawad Kadhim Akbal Harby Kadhim
Al-Mussaiab Technical College , Al-Furat Al-Awsat Technical University
Al-Mussaiab , Hilla , IRAQ

*E-mail:adil_abead@yahoo.com

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Abstract

Present research work was carried out for detection and molecular characterization of *Salmonella Typhi* isolated from Blood plasma of person severing from Typhoid fever using biochemical test, and detection of virulence gene by using of polymerase chain reaction (PCR) techniques. A total of 12 clinical samples were collected from patients. The isolated strains were also investigated for antibiotic susceptibility patterns as a control measure. Microbiological and biochemical investigations revealed the presence of *Salmonella typhi* from 12 samples. PCR analysis confirmed these presence of *fliC* (Phage-1 flagellin gene), *via*B (*Vi* antigen gene) and *SipA* genes. Antibiotic susceptibility test was carried out among the isolates against 12 antimicrobial agents. Results showed that 100 % resistance to only ampicillin and 100 % sensitivity to carbenicillin, chloramphenicol, clindamycin, gentamycin, kanamycin, and tetracycline.

Key words: *Salmonella typhi*, Antibiotic Susceptibility, PCR , Vi

الخلاصة

تم في هذه الدراسة الكشف والتوصيف الجزيئي لبكتريا السالمونيلا المعويه من نوع المسبب للتيفوئيد المعزوله من الانسان المصاب بحمى التيفوئيد باستخدام الاختبارات البايوكميائيه ,النمط المظهري والعينات المسؤوله عن ضراوة البكتريا المعتمده على تقنيه تفاعل البلمره المتسلسل (PCR). تم جمع ١٢ عينه سريريه من المرضى. كذلك تم فحص السلالات المعزوله لاختبار الحساسيه للمضادات الحيويه. وقد اظهرت الفحوصات البايوكيمائيه والحياتيه وجود ١٢ عينه تحوي بكتريا السالمونيلا المسببه للتيفوئيد. اكد تحليل تفاعل البلمره المتسلسل وجود الجينات *FliC* ,*ViaB* و *SipA*. وقد اظهر اختبار الحساسيه للمضادات الحياتيه مابين ١٢ نوع من المضادات الحياتيه بين هذه العزلات وجود نسبه ١٠٠% مقاومه فقط للمضاد الامبسلين ووجد نسبه ١٠٠% حساسه لمضادات كاربينسيلين, كلورام فينيكول, كليندومايسين, جنتامايسين, كانامايسين وتتراسايكلين .

مفاتيح الكلمات ، بكتريا التيفوئيد، الحساسية للمضادات الحيوية، تفاعل البلمرة المتسلسل، المستضد Vi

Introduction

Salmonella is a genus of zoonotic bacteria that is enable of causing disease in a deferent type of species. In humans, infection is occur via intake or ingestion food or water contaminated by bacteria this include three types of infection associated with *Salmonella* gastroenteritis, systemic disease and a carrier state [1].

The movement of bacteria is regulated by sensory networks including chemotaxis proteins embedded in the cell surface, which recognize specific attractant and repellant molecules and signal via the CheA-CheW transmitter complex most *Salmonella* have two distinct flagellin genes *fliC* and *fljB*, but express only one at a time [2, 3].This process, known as phase variation, is

present in four subspecies of *Salmonella enterica* (including subspecies *enterica* serovars) and is absent from *Salmonella bongori* [4].

On the other hand Phase variation may be a mechanism of avoiding cellular immunity, the *FliC* has been shown to be a target antigen for *Salmonella* specific T-cells in a murine model while the ends of the flagellin proteins are conserved, variation within the center of flagellin genes generates distinct flagellar antigens [5].

A handful of *Salmonella enterica* serovars, including *Typhi* but not *Paratyphi* A, express a Vi polysaccharide capsule Vi is also expressed by some strains of *Citrobacter freundii* but has not been detected in any other species [6].

Vi expression is regulated by two loci *viaA* and *viaB* which are separated on the chromosome, *viaA* is present in non-Vi strains, but *viaB* is specific to strains capable of expressing Vi [7]. Vi expression is important for virulence in humans and expressing *Typhi* strains are more resistant to innate immune defenses (complement-mediated killing and phagocytosis) can inhibit inflammatory responses in human intestinal epithelial cell lines upon infection with *Typhi* Infection caused by *Salmonella enterica* is the second most common cause of bacterial gastroenteritis food poisoning in the developed world [8, 9].

The secretion from the bacterial cell, the *SipA,* protein are thought to form a complex in the eukaryotic membrane that is required for translocation of the remaining effectors into the host cell cytoplasm [10].

This study aimed for detection and molecular characterization of *Salmonella Typhi* Isolated from Typhoidial Humans.

Materials and Methods

Sample collection

Blood specimens were obtained aseptically before the antibiotic therapy from 12 patients with typhoidial fever from various hospitals. Blood samples were transported in an ice cold container and immediately processed for microbial investigation, therefore, it is essential to reappraise the antibiotic sensitivity pattern of the isolates periodically.

Detection & molecular characterization of *Salmonella Typhi* isolated from human blood samples suffering from typhoid fever has been carried out phenotypically by biochemical tests and molecular characterization tools.

Present study also determines the antibiotic susceptibility pattern of the *Salmonella Typhi* strains and their prevalence towards the multi-drug resistance for epidemiological study.

Isolation and identification

A volume of three to five milliliters of venous blood was inoculated into 30 mL of brain heart infusion broth. A minimum blood-to-broth ratio of 1 to 10 was maintained. Blood culture broths were incubated at 37° C for 7 days. All tubes were examined daily and if any visible growth was observed were then streaked on sheep blood agar followed by streaking on xylose lysine deoxycholate (XLD) agar plates and incubated at 37° C for 24 hr. Bacterial colonies were purified based on the size, shape, color on XLD agar and patterns of haemolysis on blood agar and were subjected to Gram staining. Bacterial isolates were identified by standard biochemical tests; motility test, citrate utilization, methyl red and Voges Proskauer test, hydrogen sulfide production, fermentation of mannitol, arabinose, sorbitol, lactose, sucrose and glucose [11].

Antimicrobial Susceptibility Test

Antibiotic diffusion test (the Kirby-Bauer susceptibility test) .

1- It was performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on brain heart infusion plates to 5 ml of broth; this culture was then incubated for 2 hours to produce a bacterial suspension of moderate turbidity. A sterile

swab was used to obtain an inoculum from the standardized culture, this inoculum was then swapped on Mueller – Hinton plate and left to dry.

2- The antibiotic discs were placed on the surface of the medium at evenly spared intervals with flamed forceps or a disc applicator, incubation was usually for an overnight at 37^{o}C.

3- Inhibition zones were measured using a caliper, zone size was compared to standard zones (from the CLSI, 2010) to determine the susceptibility or resistance of organism to each antibiotic [12].

DNA extraction for Gram negative bacteria:

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Promega, USA).

Detection of some virulence genes by PCR:

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in table (1). The primers includes *fliC, viaB* and *sipA* genes, Each 25µl of PCR reaction contained 2.5µl of each upstream and downstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of master mix. The PCR amplification product were visualized by electrophoresis on 1% agarose gels for 45min at 70v. The size of the amplicons were determined by comparison to the 100 bp allelic ladder (Promega, USA).

Table 1: Primers sequences and PCR conditions

Gene	Primer sequence (5′-3′)	product (bp)	PCR conditions
FliC	ATGGCACAAGTCATTAATACAAACAGC CTGTCGCTGGTTGACCCAGAATAATGTG	587	94ºC 2min 1x 94ºC 1min 54ºC 1min 30x 72ºC 1min 72ºC 10min 1x
viaB	TT ATT TCA GCA TAA GGA G CTT CCA TAC CAC TTT CCG	738	94ºC 2min 1x 94ºC 1min 55ºC 1min 30x 72ºC 1min 72ºC 10min 1x
sipA	CGGCTTCACATTCACAA CGGGCTCTTTCGTTCA	354	94ºC 2min 1x 94ºC 1min 55ºC 1min 30x 72ºC 1min 72ºC 10min 1x

Results

Isolates were subjected to produce a virulent genes (*fliC, viaB* and *sipA*). Molecular detection of *Salmonella fliC, viaB* and *sipA* was done by using specific PCR primer. The results showed that all of investigated isolates contained this genes, as shown in figure (1),(2), and (3):

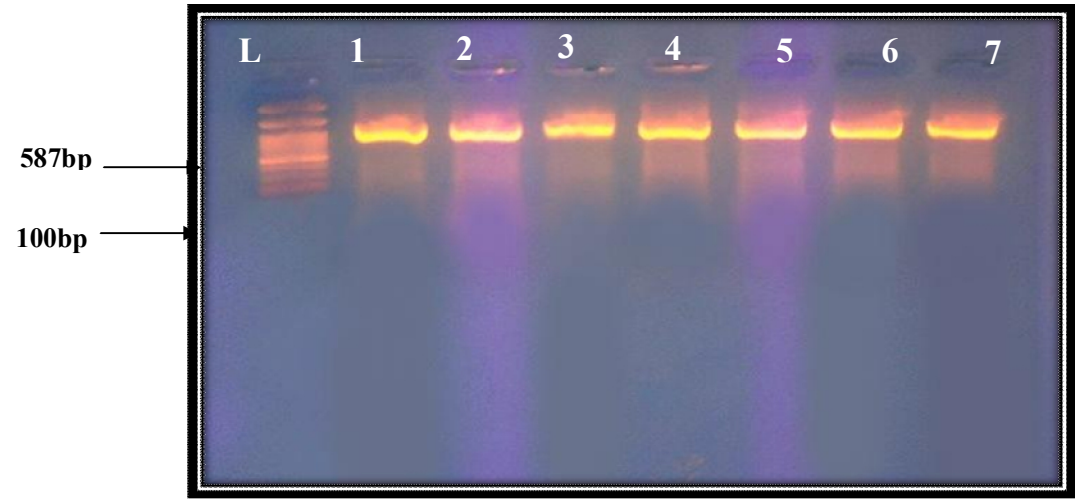

Figure 1: Gel electrophoresis of PCR product of *fliC* (1,2,3,4,5,6,7) isolates with positive result for *fliC*. L= ladder (1500-100). The electric current was allowed at 70 volt for 30 min.

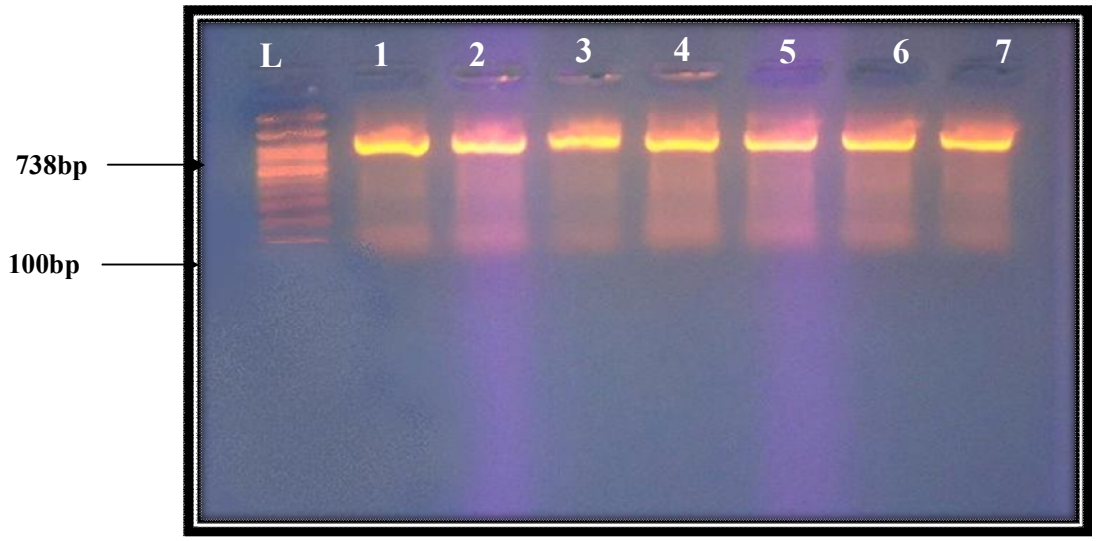

Figure 2: Gel electrophoresis of PCR product of *viaB* (1,2,3,4,5,6,7) isolates with positive result for *viaB*. L= ladder(1500-100). The electric current was allowed at 70 volt for 30 min.

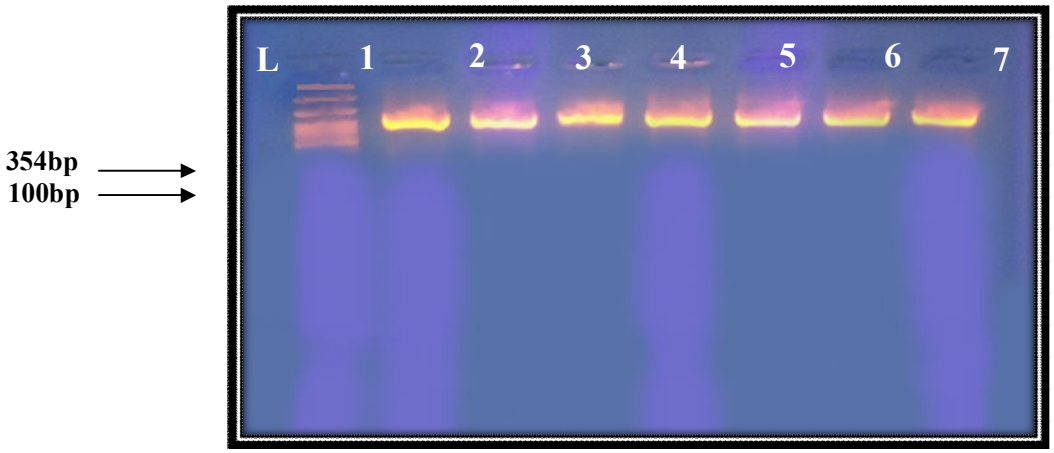

Figure 3: Gel electrophoresis of PCR product of *sipA* (1,2,3,4,5,6,7)isolates with positive result for *sipA*. L= ladder(1500-100). The electric current was allowed at 70 volt for 30 min.

Antibiotic susceptibility test

In the present study, all the 12 (100 %) isolates were subjected to *in vitro* susceptibility test by disc diffusion method resistant to ampicillin, moderately sensitive to Nalidixic acid and Nitrofurantoin and sensitive to Carbenicillin, Chloramphenicol, Clindamycin, Gentamycin, Kanamycin and Tetracycline. However, (80.25 %) isolates were also found resistance to cefuroxime, while (66.75 %) isolates were found resistant to Penicillin-G and Cephalothin. The remaining (18.75 %) were moderately sensitive to Cefuroxime and (31.25 %) isolates were moderately sensitive to Penicillin-G and Cephalothin.

Discussion

Gastroenteritis caused by *Salmonella enterica* is a significant problem, particularly in areas of high population density and with poor sanitation and close proximity of livestock and human habitation. The presence of SPI-1-deficient strains of *Salmonella enterica* serovars Senftenberg and Litchfield in various environmental reservoirs including livestock feed and marine environments has been reported in the literature [13].

The characterization of serovar *Typhi* for expression of Vi capsular polysaccharide is necessary to define the role of Vi in the pathogenesis and epidemiology of typhoid fever. Serovar *Typhi* lacking Vi capsular polysaccharide antigen has been known and reported worldwide for several decades [14]. Non typhoidal serovars of *Salmonella enterica* rank second as the cause of food-borne disease, Intestinal pathogenesis of non typhoidal *Salmonella* infection has been modeled in several hosts using multiple *Salmonella enterica* serovars.

Recently, molecular evidence of the loss of Vi antigen has suggested that Vi-negative strains can be derived by the excision of SPI or by a spontaneous base change in the *viaB* operon . It has been postulated that after long-term storage or repeated culturing on laboratory media Vi-negative strains would predominate [15]. Based on animal model infections and epidemiological investigations, it is widely accepted that SPI-1-mediated intestinal epithelial invasion is essential for *Salmonella* induced enter colitis and diarrhea.

Typhoid is a common disease in world and, using stored cultures and blood samples from typhoid patients, a two-pronged strategy was adopted to investigate whether serovar *Typhi* that is unable to express Vi could be detected in this region without culture and storage [16].

sipA caused intestinal lesions of reduced severity, such as necrosis of the upper mucosa surrounded by a dense neutrophilic infiltrate and moderate lymphoid depletion in lymphoid follicles. In addition, lymphoid depletion was observed in the germinal centers of mesenteric lymph nodes [17].

All the 12 patients were diagnosed typhoid positive from the fifth to eighth days of onset of disease and the attack rate (87.5 %) was significantly higher among the people below 30 years old. Very similar to the present study, higher frequency of detection of typhoid cases from the patients of less than 30 years old [18]. All the isolated bacteria produced pink coloured and black centered colonies on XLD plates and were positive for mannitol, l-arabinose, sorbitol, glucose fermentation, methyl red test, indole test, H2S production, citrate utilization, motility, oxidase test and urease activity. The microbiological investigation confirmed the tentative isolation of *S. enterica* serovar *Typhi* [19].

All isolates (100 %) were classified as biotype IV for fermenting l-arabinose but not xylose. This biotyping have added data to the epidemiological based classification system according to their fermentation ability of sugars and based on other biochemical properties.

The result of antibiotic susceptibility test revealed that isolates of *S. typhi* were 100 % resistant to ampicillin, 81.25 % to cefuroxime and 68.75 % resistant to penicillin-G and cephalothin respectively. This study confirmed the association of virulent strains of *Salmonella* typhi in the occurrence of the typhoidial fever in humans. It is suggested from the present study that PCR technique could be a useful, high through put and rapid diagnostic tool for the detection of *Salmonella typhi* and could be employed by the diagnostic laboratories or clinics for the clinical diagnosis of typhoidial fever from patients. The use of only 12 antibiotics for susceptibility test, present findings helped to know the current status of typhoidial fever.

Although chloramphenicol and other antibiotics showed 100 % sensitivity, still continuous evaluation of sensitivity resistance pattern of *S. typhi* isolates is necessary to make rational use of antibiotics in the management of typhoidial fever in future [20].

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