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Molecular Detection for Some Virulence Factors of Salmonella typhi from Iraqi Patients

Menan Abdul-Razaq Abdul-Khadum[1], Ali Jaffar Saleem[2], Amal Aziz Karim[3]

[1] *College of Education for Pure Science- Diyala University, Iraq.*
Email: menan.abdulrazaq@uodiyala.eduo.iq
[2] *College of Education for Pure Science- Diyala University, Iraq.*
Email: alijaffar3@yahoo.com
[3] *Middle Technical University, College of Health and medical Technology, Research unite, Iraq.*
Email: amal.aziz68@yahoo.com

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Abstract

In current study, 29 clinical isolates Salmonella typhi were collected from different Private laboratories in Diyala. In addtion of phenotypic method, all isolates diagnosed as S. typhi by genotypic method was done by Polymerase Chain Reaction (PCR) technique to detection 16SrRNA gene. And detection of adhesion factors associated genes that encoding for cdtB, tviA, fimA and orfL by using specific primers which showed the prevalence in percentage 100% for all genes.

Keywords

Salmoella typhi ,16SrRNA, cdtB, tviA, fimA and orfL genes, Virulence Factors genes.

The genus salmonella is one of the genera of the intestinal bacterial family and includes about 2600 serotypes, salmonella is a gram stain-negative bacterium, classified into two types S. enterica and S. bongori (ALJobouri , 2019). They move by peritrichous flagella, are non-spore-forming , contain pilli (Grimont et al., 2000). Salmonella grows at optimum pH between 6,5-7.5 and temperature is 37C,also it can grow at pH between 3-9.5 and temperature between 3-52C(ALJobouri, 2019).

Possessing special Salmonella invasion proteins such as SipB and SipA these proteins cause death of phagocytic cells . Many genes of virulence factors for salmonella are clustered in certain chromosomal regions known as Salmonella pathogenicity islands (SPIs) (Santos et al.,2003).SPIs are chromosomal site containing virulence genes ,it's found only pathogenic bacteria and it's located either on chromosomes or plasmid surrounded by repeated sequences and differs in contain of G/C content in relation to the surrounding area (AL-Muhannak ,2020) .

Recent international statistics indicate that typhoid fever contributes to 21.6-26.9 million cases of illness and 21,600 deaths annually (WHO, 2018; Wong et al., 2019).

S.typhi has combination of properties that make it an effective pathogen, as it has many Virulence factors ,that play an important role at different stages of infection ,some of which are necessary for entry and survival inside and outside cells, including H and Vi antigens ,Lipopolysaccharide ,endotoxin and siderophores (Abbas,2012).

tviA gene encodes a regulatory protein that plays an important role in regulating the expression of antigen Vi ,flagella, and a number of genes necessary for host invasion (Santander et al., 2008). This gene located on pathogenicity island of salmonella SPI-7 , which has a capacity of 134Mb. The synthesis and transport

proteins of the Vi capsular polysaccharide of S.typhi are encoded by viaB Operon (Zhang et al.,2018).

cdtB gene is one of the most important newly known invasion factors for S.typhi, which represents a completely wide range of other intestinal pathogens , cytotoxicity entails the so-called DNA Damage response (DDR), which is characterized by irreparable cell damage and, consequently, cell death by apoptosis (AL-Oqaili, 2019).Typhoid toxin (A2B5) contains two active subunits connected by a disulfide bridge (cdtB and pltA) connected with five subunits (pentameric pltB) of auxiliary memory aid - memory, secreted from S.typhi only an intracellular and intracellular vacuole containing salmonella (SCV) (Song et al., 2013).

fimA gene encodes the main unit of the first type of helminth , there is a significant similarity in the nucleotide sequence of the fimA gene of E.coli and Klebsiella pneumonia but they are completely heterogeneous(COHEN,1996). The genes fimA, fimI, fimC, fimD, fimH, fimF are assembled in the form of a single operon located under the control of the catalyst fimA encodes for a protein involved in biosynthesis and the synthesis of aliphates (Kolenda, 2019). orfL gene was diagnosed in Salmonella Pathogenicity Island 4 (SPI-4) and is involved in adhesion, spontaneous transport and colonization. orfL gene is essential for survival inside macrophages and possibly carries a system involved in the excretion of toxins (LEGBA et al., 2017).

Methods And Materials

Sample collection and identification

During the period from September 2021 to December 2021, 75 blood specimens were collected at suspected outpatient's patients for typhoid fever in Diyala .The specimens were transplanted on MacConkey ,Blood agars also on XLD and S-S agars ,that incubated at temperature of 37c for 24-48 hours, diagnosed on phenotypic, cultural characteristics ,microscopy with gram stain and biochemical tests: catalase and imvic (Macfaddin ,2000), as well as Molecular detection by 16SrRNA gene.

Molecular Identification

DNA extraction from *S.typhi* isolates by Genomic DNA extraction kit (ABIO, USA). DNA preparation was then analyzed via electrophoresis thereof 1.5% agarose gel. PCR be used to amplify the entire sequences of the genes studied in this research. The specific primers (Macrogen, Korea) utilized for the expansion to these genes were shown in (table1).

PCR mixture was prepared by adding 12.5µl of GoTaq®Green master Mix (2X) promega,2µl template DNA, 1µl from each forward and reverse primers with final concentration 1 poml/µl, finally volume was completed to 25µl by adding nuclease free water. PCR condition illustrated in Table (2), stained with ethidium bromide and visualized by transilluminator.

Table (1): Primers and amplified PCR products used in study

Gene	Primer sequences (5'- 3')	size(bp)	Reference
16SrRNA	F:CCTGGACAAAGACTGACGCT R: CGCTTCTCTTTGTATGCGCC	523	Al- Oqaili,2019
fimA	F: GTGAGCGGCGGTACTATTC R: TAAAGGTGGCGTCGGCATT	451	
cdtB	F:TAAGTGGTACTGCCGGTGTG R:GTAGGTGCGAGTACGGCTAC	508	
tviA	F: GTTATTTCAGCATAAGGAG R: ACTTGTCCGTGTTTTACTC	599	Liaquat et al. 2018)(
orfL	F: GGAGTATCGATAAAGATGTT R: GCGCGTAACGTCAGAATCA	323	(Albanwawy and Abdul-Lateef,2021)

Table (2): PCR condition to genes used in study

Amplified gene	Initial denaturation	No.of cycles	Denaturation	Annealing	Elongation	Final extension
16SrRNA	5min. at 95C	30	30sec.at95C°	30sec.at58C°	30sec.72C°	7min.at72C
fimA	5min.at95C°	30	30sec.at95C°	30sec.at57C°	30sec.72C°	7min.at72C
cdtB	5min.at 95C°	30	30sec.at95C°	30sec.at58C°	30sec.72C°	7min.at72C
TviA	5min.at 95C°	30	30sec.at95C°	30sec.at 55C	30sec.72C°	7min.at72C
orfL	5min.at 95C°	30	30sec.at95C°	30sec.at58C°	30sec.72C°	7min.at72C

Results and Discussions

Identification of S. typhi

Bacteria isolates have been diagnosed in two ways: the first is phenotypic by microscopic ,it's gram negative and cultural characteristics (on XLD agar showed red colorwith or without black center for H_2S production ,and on S-S agar showed pale yellow for not fermentation lactose with a black center for producing H_2S), in addition of biochemical tests, the second is genotypic by polymerase chain reaction (PCR).

The results of the current study showed 29 (38%) isolation of *S.typhi* depending on phenotypic and culture characteristics. Also genetically diagnosed by *16SrRNA* gene and the isolates of *S.typhi* possessed the *16S rRNA* gene 100%. (Fig.1).

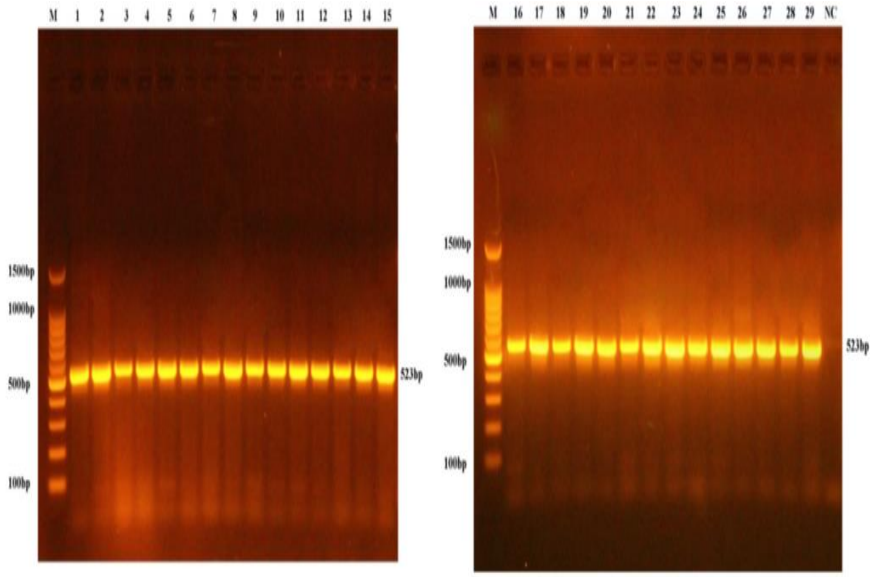

Fig.1: Agaros gel electrophoresis (1.5 agaros,100 V for 1h ,for *16SrRNA* gene, DNA ladder (100-1500 pb).

This gene contains conserved regions that overlap with variable regions ,there are 9 variable regions, that are useful in determining genus and orders bacteria because 16SrRNA is present in all bacterial species and also has little variable(Suardana,2014 ;Srinivasan et al ,2015).

The results of our study some virulence factors *orfL, cdtB*, *tviA* and *fimA genes*, it was found that isolates carried all genes rate 100%.

orfL gene is encoded into the adhesion protein , which helps in the adhesion and survival of bacteria inside phagocytic cells, and the bacteria also have a secretion mechanism that regulates the secretion of toxins by stimulating the programmed death of host cells and thus promotes the pathogenicity of *S.typhi* (Gassama-sow et al.,2006 ;Ramatle et al.,2022).Fig.2

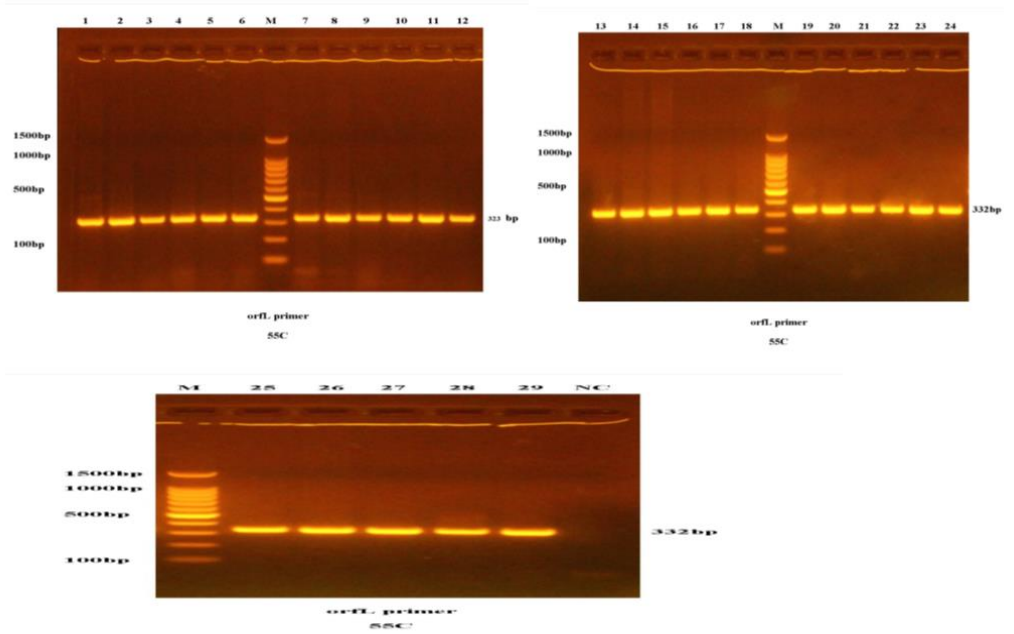

Fig.2: Agaros gel electrophoresis (1.5 agaros,100 V for 1h,for *orfL* gene, DNA ladder (100-1500 pb).

The results of *cdtB* gene were completely identical to the results of the study of researcher AL-Oqaili (2019) in Babylon and researcher Liaquat et al. (2018) in Pakistan. While the results of the study of researcher Al-Khafaji (2022) in AL- Najaf were not consistent with the results of the current study, where the presence of the gene was 57%. The *cdtB* gene encodes the cytolytic distending toxin protein subunit B, which contains 269 amino acids (NCBI-a). It is believed that typhoid poison contributes to the development of chronic typhoid fever and causes the appearance of symptoms (Al-Khafaji , 2022). Studies have indicated that typhoid toxin is responsible for the onset of symptoms and the transformation of the infection from acute to chronic (Johnson et al., 2018). Host cell death is due to the presence of typhoid toxin , which can be encoded by the *cdtB* gene (Al-Khafaji ,2022).Fig.3

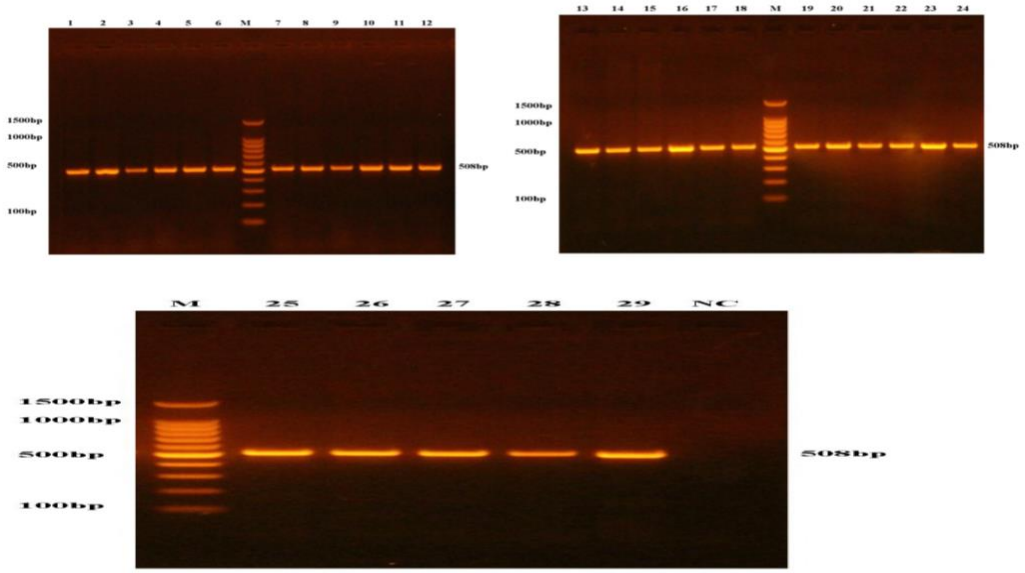

Fig.3: Agaros gel electrophoresis (1.5 agaros,100 V for 1h,for *cdtB* gene, DNA ladder (100-1500 pb).

Compared to the results of Al-Oqaili's (2019) study in Babylon, she showed that the presence of the *tviA* gene in *S. typhi* was 100%, so it matches the results of the current study, while the results of the study of researcher Al-Khafaji (2022) in AL- Najaf, where the percentage of the presence of the gene was 42.7%, were not consistent with the results of the study. In another study by Baker et al. (2005) and Liaquat et al. (2018) in Pakistan, where the presence of this gene was 15% and 89%, respectively, where the results were not consistent with the results of the current study..Fig.4

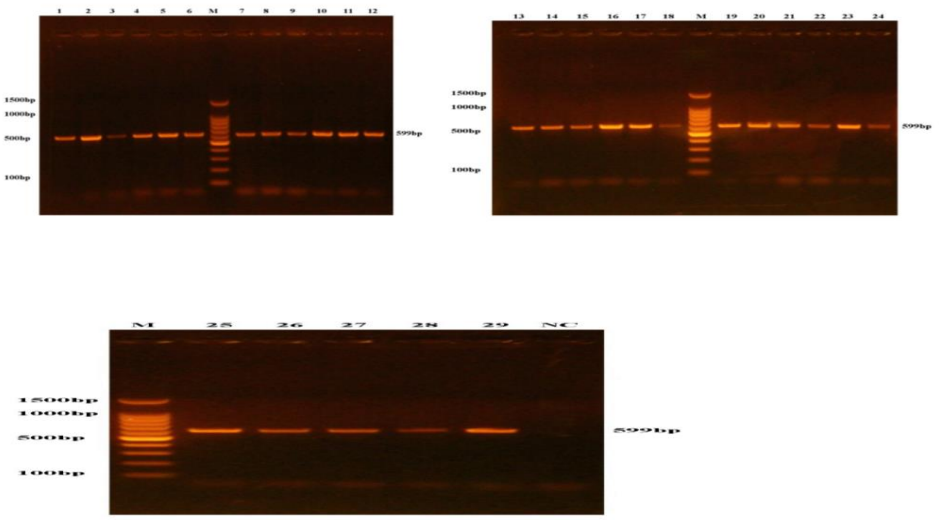

Fig.4: Agaros gel electrophoresis (1.5 agaros,100 V for 1h,for *tviA* gene, DNA ladder (100-1500 pb)

The Vi Capsular polysaccharide of salmonella bacteria, which is the cause of typhoid infection, is important in the occurrence of infection and virulence, its biosynthesis mechanism is encoded within the ViaB site, as it consists of 10 genes involved in the expression of the *tviA* gene, polymer construction (tviB-tviE) and localization of the cell surface of the VexA-VexE capsule (Albanwawy and Abdul-Lateef,2021). The *tviA* gene encodes a capsule synthesis regulatory protein of 179 amino acids (NCBI-b).

The results of *fimA* Al-Oqaili's (2019) study in Babylon did not correspond to the results, as the presence of the gene was 84.21%. The *fimA* gene encodes for the filament type I protein type - 1 fimbrial protein which contains 184 amino acids(NCBI-c). Cilia or fimbriae filaments are located on the surface of the S-cell. typhi play an important role in the process of adhesion and invasion of host cells (Khazaal, 2019).Fig.5

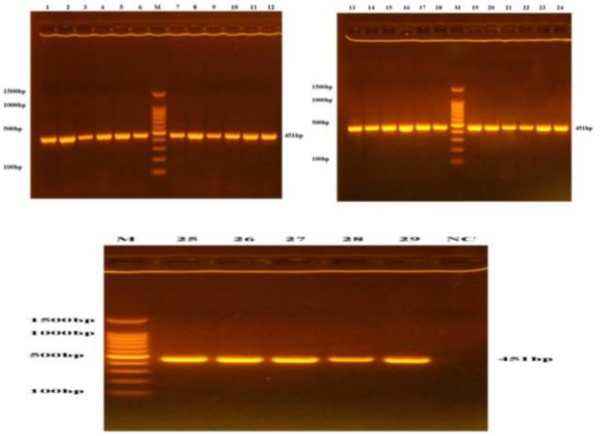

Fig.5: Agaros gel electrophoresis (1.5 agaros,100 V for 1h,for *fimA* gene, DNA ladder (100-1500 pb).

Conclusion

The local isolates in the study possess all the genes of Virulence Factors that increase the pathogenicity of bacteria ,and these genes encode adhesion proteins that are associated with the process of Biofilm formation ,as adhesion is the first stage of biofilm formation ,thus increasing the pathogenicity of bacteria through resistance to the host body's immune defenses and resistance to antibiotics.

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