

Isolation and Identification of *Salmonella typhi* from Clinical Samples with Molecular Detection of O-antigen Encoded Genes

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Abstract

Treatment of *S. typhi* is difficult as compared to treatment of acute infection. Antibiotic susceptibility test carried against *S. typhi* by using 6 kinds of antibiotics from different classes, their results showed that all isolates were high resistance to Ampicillin (99%), Gentamicin (98%), Amikacin (79%) and less resistances to Trimethoprim (55%), Imipenem (60%) and Ceftriaxone (66%). The present study focused on the molecular detection of Wzx flippase and Wzy polymerase genes in some *Salmonella typhi* isolates, Samples were collected from typhoid patients by classical lab work. Antibiotics susceptibility was investigated using the disc diffusion method. The DNA and molecular Wzx flippase, Wzy polymerase were implemented using specific primers. The results showed that there was 33.33% had Wzy gene. The Wzx gene did not observe in any *Salmonella* isolates. The present study concluded that there was an importance of the genetic diversity of O-antigen encoded genes included Wzx and Wzy, which may be effective in typhoid diagnosis and treatment.

Keywords: Molecular Identification, Wzx flippase, Wzy polymerase genes salmonella typhi

عزل وتشخيص *S. Typhi* من العينات السريرية مع الكشف الجزيئي للجينات المشفرة للمستضد O هدى جاسم محمد*¹، سيماء حسن شلال* و هديل علي غجيري**

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الخلاصة

أن العلاج بالمضادات الحيوية من التيفوئيد صعب مقارنة بعلاج العدوى الحادة. تم تحديد المضادات الحيوية ضد *S. typhi* باستخدام 6 أنواع من المضادات الحيوية من فئات مختلفة، وأظهرت نتائجها أن جميع العزلات كانت ذات مقاومة عالية للأمبيسلين (99%)، الجنتاميسين (98%)، أميكاسين (79%) وأقل مقاومة Trimethoprim (55%)، إيميبينيم (60%)، سيفترياكسون (66%). ركزت الدراسة الحالية أيضا على الكشف الجزيئي لجينات Wzx flippase و Wzy polymerase في بعض عزلات السالمونيلا التيفية، وتم جمع العينات من مرضى التيفوئيد عن طريق العمل المختبري التقليدي. تم فحص حساسية المضادات الحيوية باستخدام طريقة الانتشار القرصي. تمت دراسة وجود Wzx flippase الجزيئي، Wzy polymerase باستخدام بادئات محددة. وأظهرت النتائج أن 33.33% لديهم جين Wzy. لم يلاحظ جين Wzx في أي من عزلات السالمونيلا. استنتجت الدراسة الحالية أن هناك تنوعاً جينياً مهماً في الجينات المشفرة لمستضد O المتضمنة Wzx و Wzy والتي قد يكون لها تأثير في تشخيص التيفوئيد وأنواع العلاج. الكلمات المفتاحية: التشخيص الجزيئي، جينات Wzx، Wzy، بكتريا التيفوئيد

Introduction

Salmonella typhi is an enterobacteriaceae member responsible for typhoid fever, its Gram negative and serological positive to lipopolysaccharide antigens O9 and O12 in addition to polysaccharide capsular antigen Vi^(1,2). The diversity of antigen –O in bacterial strains regarding to the genetic polymorphisms in the rfp cluster that coded the enzymes involved in the synthesis of construction of O-antigen⁽³⁾, these genes included the enzymes that contributed in sugar synthesis, transferase for construction sugar and O subunit, and genes encoded proteins involved in construction O antigen via assembly subunits, these genes included wxz and wzy that encoded to O-antigen transporter or flippase and O-antigen polymerase respectively^(4,5,6). The Wzx proteins in *Salmonella enterica* the O-antigen transport mechanism is dependent on the Wzx proteins⁽⁸⁾. The O-antigen variability which

basis on the *shigella* serotypes schemes, there are 12 O-antigen clusters includes (2, 10, 12, 23, 25, 26, 32-34, 66, 75 and 76) these clusters located between rep and aqpZ genes on chromosomes^(4,9). Bacteria: The *Salmonella typhi* isolates were collected from patients suffering from typhoid fever who attended to the hospital centers. After clinical and serological diagnostic tests, collected samples using conventional methods by using blood agar, XLD agar and SSA agar The antibiotic susceptibility detected using disc diffusion method (Ampicillin, Gentamicin, Amikacin, Trimethoprim, Imipenem and Ceftriaxone). The multi-resistance isolates were used for molecular detection of Wzx flippase and Wzy polymerase genes.

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PCR experiment

Bacterial DNA was extracted from all strains using the appropriate kit. The molecular detection of Wzx flippase and Wzy polymerase genes were implemented using the following primers (5).

wzx (F): CCG GGT TTC GAT TTG TGA AGG TTG,

(R): CAC AAC AGC CAC TAC TAG GCA GAA

wzy (F): GAA ATT ATG CCA TCT TGG CGA GCG

(R): CAT GTG AAG CCT GAA GGC AAA CTC

The following conditions were used for PCR. 5 min 94°C, 35 cycles consist of 30 s 94°C, 30 s 59°C, 30 s 72°C, and 10 min at 72°C for both primers in mono-plex reactions (10). PCR products were visualized in 1% agarose, TBE 0.5 X for 1 hour, 70 V and 20 mA under UV trans-illuminator.

Results

Bacterial Isolation: strains of S. typhi were recovered from blood samples of outpatients confirmed by conventional method plus Vitek identification (Table1)

Table 1. Bacterial isolation during the study

| Sample No. | Bacteria | Widal test | Conventional and Vitak |
|------------|------------------|------------|------------------------|
| 1 | Salmonella typhi | + | + |
| 2 | Salmonella typhi | + | + |
| 3 | Salmonella typhi | + | + |
| 4 | Salmonella typhi | + | + |
| 5 | Salmonella typhi | + | + |
| 6 | Salmonella typhi | + | + |
| 7 | Salmonella typhi | + | + |
| 8 | Salmonella typhi | + | + |
| 9 | Salmonella typhi | + | + |

Antibiotic susceptibility test

The results of the present study show that, there were 9 isolates were multi-resistance to antibiotics in different manner, Figure1.

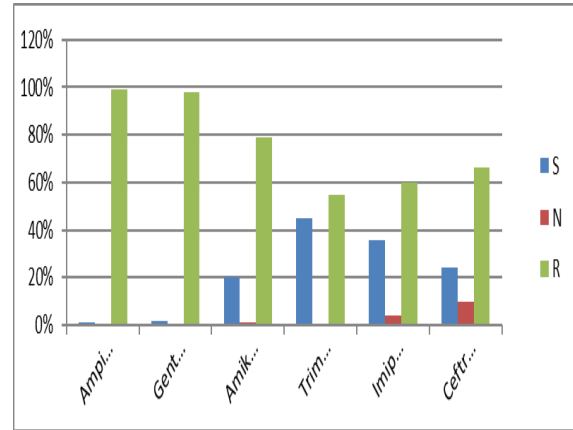


Figure 1. Multi drug resistances Salmonella typhi isolates (n=9)

Molecular detection of Wzx and Wzy genes

Genomic DNA was extracted from bacterial cells using a DNA extract kit. Figure2.

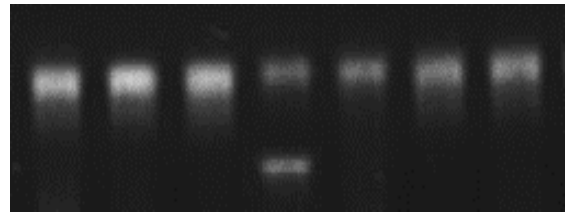


Figure 2. Gel electrophoresis 2% of DNA extracted from Salmonella typhi isolates

Polymerase chain reaction were applied for detection of Wzx and Wzy genes

The molecular detection of Wzx and Wzy were showed that some isolates of S. typhi had wzy gene while no isolates detected that have wxz gene. Figure 3.

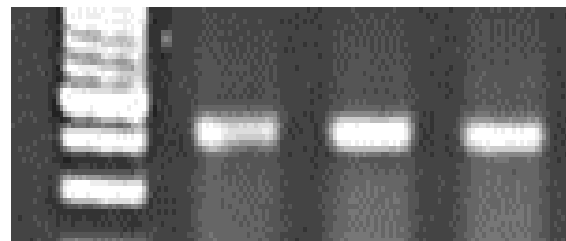
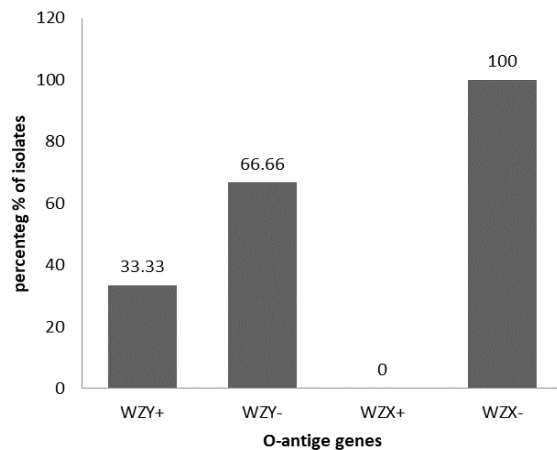


Figure 3. PCR product of Salmonella typhi isolates carrying wzy gene with molecular weight of 451bp visualized on 1% agarose (conditions).

Percentage of Wzx gene was 33.33% from all samples while the percentage of presence of Wzy was 0%. Table 2 and Figure 4.

Table 2. Presence of Wzy and Wzx gene in different isolates of Salmonella typhi.

| Sample No. | Bacteria | Wzy gene | Wzx gene |
|------------|-------------------------|----------|----------|
| 1 | <i>Salmonella typhi</i> | + | - |
| 2 | <i>Salmonella typhi</i> | - | - |
| 3 | <i>Salmonella typhi</i> | - | - |
| 4 | <i>Salmonella typhi</i> | + | - |
| 5 | <i>Salmonella typhi</i> | - | - |
| 6 | <i>Salmonella typhi</i> | + | - |
| 7 | <i>Salmonella typhi</i> | - | - |
| 8 | <i>Salmonella typhi</i> | - | - |
| 9 | <i>Salmonella typhi</i> | - | - |

**Figure 4. The percentage of Wzy and Wzx genes in Salmonella typhi.**

Discussion

In the present research, the resistance to common antibiotics used in the treatment of *S. typhi* was tested and appeared that all the persisting isolates of *S. typhi* obtained from patients who suffered typhoid fever showed high resistance to gentamycin, ampicillin, amikacin, and ceftriaxone; these results agree with Anduaem et al. and Al-aarajy et al. ^(11, 12) they were found that all isolates were resistant to four or more classes of antibiotics as antimicrobials.

The present study implemented to identify Wzx and Wzy genes in *Salmonella typhi* isolates that have an important role in typhoid diagnosis via O-antigen, although of the low sensitivity and specificity of Widal test in typhoid diagnosis, it is still used in some labs ⁽¹¹⁾, on the other hands the false negative results ^(13, 14) may be associated with genetic diversity of O-antigen genes which formed diversity in O-antigen proteins. The evidence found that according to The Kauffmann-White scheme there are 46 somatic O antigens of *Salmonella*, thus the diversity of O-antigens encoded genes can be used in the PCR development test to molecular serotypes identification.

The negative results in the present study of Wzx gene and in Wxy (66.66%) maybe because of genetic diversity in target loci such as duplication, formation of the pseudogene, deletion, and insertion of the bacteriophage elements that occur ubiquitously through serogroups ⁽¹⁵⁾. The present finding needs more investigation to detect the genetic diversity of O-antigen in *S. typhi* which may have an important role in serotypes identification and development of new molecular tests for typhoid fever diagnosis. The detection of these genes in *S. typhi* was poor in the last decades, Yara et al ⁽¹³⁾ in a study dealing with the pangenome of *Salmonella* O-Antigens, they found high metabolic and genetic differences within and across O-antigen groups in *Salmonella* strains. In Iraq, there were high multi-resistance bacterial isolates in the last decade which contributed to the health problems of diseases infection and this need to focused efforts for developing a new method for diagnosis and treatments based on the new finding like O-antigen encoded genes.

Conclusion

The analysis of the drug susceptibilities of the isolates was observed that all isolates were multidrug-resistant (MDR). Besides, *Salmonella typhi* is shown to persist chronically within the patients and molecular methods are more rapidly than the conventional method. The present study concluded that there was an importance of the genetic diversity of O-antigen encoded genes including Wzx and Wzy which may be effective in typhoid diagnosis and treatment types.

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