RELATION OF CLASS 1 INTEGRON GENE WITH MULTI-DRUG RESISTANCE SALMONELLA TYPI ISOLATES

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ABSTRACT

This study aimed to detect the intI gene and its relationship with multi-drug resistance of Salmonella typhi isolates. The results of the antibiotic sensitivity test showed that the S. typhi isolates were highly resistant to the antibiotic; the results of PCR technique for intI gene amplification show that there are where 14/30 S. typhi isolated had positive results of amplification.

Keyword: S. typhi, MDR, intI gene

INTRODUCTION

Typhoid fever is an acute, potentially fatal systemic illness caused by Salmonella enterica serovar typhi and paratyphi, pathogens only specific to humans. S. typhi is a genus of rod-shaped gram-negative enterobacteriaceae that cause typhoid fever (Ryan and Ray, 2004). Drugs have been used to treat typhoid for 60 years ago and chloramphenicol was the first introduce of this purpose in 1984, later ampicillin was introduced followed by Co-trimoxazole. The three mentioned were called the first line antityphoidal drugs. On emergence of resistance against these first line drugs with additional resistance to streptomycin, and tetracyclines have been reported in many developing countries, such strains have been termed multi-drug resistant (MDR) (Rowe et al., 1997).

Bacterial activity to antimicrobial agents is an interesting worldwide problem with regard to treatment of infectious diseases. Either the colonel spread of an epidemic strain or through independent acquisition of the resistance genes on plasmids, transposons or integrons usually associated with antibiotic resistance (Martínez et al., 2007). Naturally occurring gene expression elements called “integrons” is one of the mobile genetic elements which can carry genes of resistance to different antibiotics, which contain integrase gene, two conserve areas of sll and intl, and one variable area of gene cassettes (Mirmnejad et al., 2013, Ibraheem and Al-Ardhi, 2017).

MATERIALS AND METHODS

Table-1: Primers used in this study

Specimen’s collection and bacterial identification: A blood, stool and urine using as a source of 30 samples using in this study with clinical suspicion of typhoid fever who attended different hospitals during the period from December 2015 to September 2016 in Al-Najaf provenance according to ethical approval of ministry of Iraqi health, continuous high-grade fever with median temperature - 38°C were using, and constitutional symptoms. The median duration of illness at consultation was 6 days (range 6 – 18 days). Each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood, XLD and SS agar, then inoculated at 37°C for 18-24 hours (Cheesbrough, 2010).

DNA Extraction: Genomic DNA was extracted by using a commercial extraction system (Genomic DNA Promega Kit).

Molecular Identification: Detection of DNA has been done by using gel electrophoresis in the presence of UV transilluminator. The PCR assay was performed to detect the (intI) gene for S. typhi shown in table (2). This primer was produced by Alpha DNA Company, Canada as in table (1). Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. It run at 80v for 1.5h. A single band was observed at the bands were photographed using gel documentation system (Cleaver, UK). A 100 bp ladder (Bioneer, Korea) was used to measure the molecular size of amplified products (Levy et al., 2008).

<table>
<thead>
<tr>
<th>Primer Type</th>
<th>Primer Target</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>intI</td>
<td>Int1</td>
<td>F-ATCATCCTGCTGTAGAAGACGTCGG R-GTCAAGGTTTCTGGACCAGTTGC</td>
<td>892</td>
<td>(Rosser and Young, 999)</td>
</tr>
</tbody>
</table>
Table 2: PCR program of intI1primer that apply in the thermocycler

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial denaturation</th>
<th>No. of cycles</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>intI1</td>
<td>96 C° for 2 min</td>
<td>27</td>
<td>96C° for 15 sec</td>
<td>55C° for 30 sec</td>
<td>72 C° for 1 min</td>
<td>72 C° for 7 min</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
This study was conducted on 30 specimens from blood typhoid, stool of suspected patients during the period from December 2015 to September 2016.

The clinical diagnosis depended on the specialist physician diagnostic. The results showed that class 1 integron gene was detected in 14 / 30 of S. typhi isolates as in figure 1. There have been a number of reports describing the prevalence of class 1 integrons within gram-negative clinical isolates Jones et al., (1997) and huge work on integrons in S. typhi has been reported (Al-Sanouriet al., 2008).

Integrons are mobile genetic elements that could be important in the dissemination and accumulation of resistance genes in bacteria. Integrons are usually located within transposons or conjugative plasmids (Mazel, 2006). In a previous study, 65% of isolates harbored class 1 integron (Bashir et al., 2015), whereas in another study class 1 integron was found in 49% of uropathogenic isolates (Ajiboye et al., 2009).

Figure 1: PCR amplification products of S. typhi isolates that amplified with intI1 gene primers with product 892 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1 to 7) show positive results with intI1 gene.

Multi-drug Resistance of Salmonella typhi isolates: This study focused on the presence of multi-drug resistant (MDR) in the S. typhi isolates. Multidrug resistant (MDR) phenotype was defined for isolates that are resistant to a minimum of 3 classes of antibiotics. By present definition of MDR, 19/30 (63.3%) of isolates were confirmed as MDR, 10/30(33.3%) of them were resistant to four classes of antibiotics and the remaining 9/30(30%) of them were resistance to three classes of antibiotics. 11/30(36.6%) of isolates were non MDR as in figure 2.

Figure 2: Frequency of MDR S. typhi isolates

There has been a worldwide increase in MDR typhoid caused by microorganisms resistant to multiple antimicrobial agents. Several factors are responsible, particularly the selection pressure exerted by misuse of antimicrobial agents which resulted in the emergence of resistant microorganisms. The emergence of MDR S. typhi that was resistant to all the first-line drugs was sporadic, but the first documented outbreaks were in Malaysia (Ling and Chang, 1984). Since then MDR strains have spread throughout Southeast Asia and China, where they have become endemic. These strains caused infectious outbreaks in Vietnam (Hoaet al.,1998), Middle East (Rowe et al.,1999), Pakistan (Shanahan et al., 2000) and Africa (Kariukiet al., 2000). Chloramphenicol resistance was associated with high-molecular-weight, self-transferable IncHI plasmids. S. typhi were also resistant to sulfonamides, tetracycline, and streptomycin, but initially Amoxicillin and Trimethoprim–Sulfamethoxazole remained effective alternative drugs. IncHI plasmids encoded the resistance genes. The spread resulted from the clonal dissemination of indivi-
dual multidrug-resistant *S. typhi* strains or from transfer of the plasmid to multiple *S. typhimurium* strains (Thong et al., 2000).

In most cases resistance to Chloramphenicol, Ampicillin and Trimethoprim/Sulfamethoxazole is transferable on plasmids (Parkhill et al., 2001). During 1990’s quinolones especially ciprofloxacin became the drug of choice for treatment of MDR typhoid but sporadic cases as well as epidemic spread of resistance to even these drugs were reported within a few years after their introduction. *S. typhi* strains with reduced susceptibility to fluoroquinolones are now emerging as a major problem in Asian countries. More than half were also resistant to Chloramphenicol, Ampicillin, and Trimethoprim. Most infections were observed in patients with a recent history of travel to India and Pakistan. Quinolone resistance is frequently mediated by single point mutations in the quinolone-resistance determining region of the *gyrA* gene, characteristically occurring at position 83 of the DNA gyrase enzyme (changing serine to phenylalanine) and position 87 (changing aspartate to tyrosine or glycine) (Threlfall and Ward, 2001).

The emergence and continual increase in the multiple drug resistance, early determination of drug resistance pattern along with timely diagnosis of typhoid has become a matter of vital importance. The frequency of resistance in *S. typhi* has increased dramatically, presumably due to the extensive use of antimicrobial agents which has also led to the emergence of multidrug-resistant (MDR) strains. There has been an increasing concern about the prevalence of MDR *S. typhi* strains that are susceptible to Chloramphenicol, Ampicillin, and Trimethoprim (Parkhill et al., 2001).

In a multi-variate analysis, drug resistance was associated independently with higher bacteremias, this suggests that the multidrug-resistant phenotype may be associated with virulence in *S. typhi*. MDR *S. typhi* harbors a variety of plasmids, but those of the *incH1* incompatibility type appear to be specifically common. In particular, resistance to chloramphenicol, ampicillin, trimethoprim, sulfonamides and tetracycline is often encoded by plasmids belonging to the incompatibility complex group *incH1*. A possible explanation for their common occurrence is a transmission potential related to plasmids that is enhanced compared to that of drug sensitive strains (Thong et al., 2000). The increase of MDR isolates in the present study may be the results of uncontrolled antibiotic use in medicine over the last several years. The careless antibiotics usage, without antibiotic sensitivity testing, is the most important factor promoting the emergence of MDR, which cause selection and dissemination of antibiotic resistant pathogens in clinical medicine. The increased resistance to antibiotics may be due to lack of proper policy to antibiotics usage and transfer of resistance genes by transportation tools such as plasmids, bacteriophage and integrons (Hall and Collis, 2005).

**Relationships between Salmonella typhi MDR with integron class I:** The association between integrons and antibiotics resistance were investigated. The current study showed that the presence of an integron was significantly associated with 19 (63.3%) MDR isolates. The detected genes encode (integrases) of class 1 integron was in 10(33.3%) from MDR isolates. The present study revealed the strong association between integron class 1 and resistance to Ampicillin, Pipercillin, Amikacin, Gentamyacin, Trimethoprim, Trimethoprim/ Trimethoprim/ Sulphamethoxazole and Chloromphenicol. A 90% of the *MDR S. typhi* isolates contained Class1 integrons indicative of their high frequency of occurrence in *S. typhi*. Class1-like integrons have been shown to carry from one to four antibiotic resistance genes. The integron-carrying isolates are usually multi resistant, whereas the integron-lacking isolates are more susceptible to antibiotics. In previous studies (Miko et al., 2005).

A strong association of presence of class1 integrons and resistance to antibiotics has been demonstrated and attributed in part to the existence of resistance genes (*aadA* for streptomycin and spectinomycin, *aadB* for gentamicin and kanamycin, *beta-lactamase* for ampicillin) within these integrons (Miko et al., 2005).

Studies of selected clinical bacterial populations have shown that 59-75% of drug-resistant isolates contain class 1 integrons (Jones et al., 1997). Multidrug resistance has been associated with classical mobile genetic elements (i.e., transposons and plasmids). However, in recent years, a novel group of DNA elements able to incorporate antimicrobial resistance genes cassettes by a site specific recombination event have been identified in gram negative bacteria. These elements are termed integrons (Hall and Collis, 2005).

Class 1 integrons are closely related to antimicrobial resistance and MDR in *Enterobacteriaceae*. There are a variety of gene cassettes in the variable region of int1 that encode resistance to sulfonamides and aminoglycosides (Raet et al., 2006). The role of integrons in the capture and expression of antimicrobial drug resistance related genes in Gram-negative clinical bacteria is now well established. Class 1 integrons possess two conserved segments separated by a variable region.
which includes integrated antimicrobial resistance genes or gene cassettes with unknown functions (Recchia and Hall, 1997). The 3’ conserved segment of class 1 integron is characterized by the qacEAl and sulI genes, which impart resistance to disinfectants and sulfonamides, respectively. This segment was amplified in all class 1 integron positive *S. typhi* isolates in our study.

Further characterization of the integrons revealed that all contained a >700 bp insert in the variable region. DNA sequencing showed the inserted DNA to comprise of dfrA7 gene that encodes resistant to trimethoprim, the most prevalent resistance gene cassette after aminoglycoside resistance gene cassette aadA1. The sulI gene was associated with class 1 integrons. The presence of the integrons must not be undervalued since two reports have already described multidrug-resistant *S. typhistrains* harboring integrons with up to six drug resistance genes (Palet al., 2003). Furthermore, integrons can acquire additional resistance gene cassettes such as *veb*-1 or *bla*VIM, leading to serious problems in the management of these infections (Al-Sanouriet al., 2008).

**Conclusion**

Conclusion of present study of PCR technique for *intI* gene amplification show that there are where 14/30 *S. typhi* isolated had positive results of amplification while the ratio of integron relation to multiple-resistance isolates was 10 (33.3%).

**References**


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