Detection of Tn554 carrying *erm*(A) gene in a clinical isolates of *S. pyogenes*

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**Abstract**

**Background:**
This study was conducted to investigate the genetic organization of *erm*(A)-carrying Tn554 in clinical isolates of *Streptococcus pyogenes*. Tn554 is a communicable sequence to multiple plasmid sites, it contains *erm*(A) gene and has the ability to bind the resistance of MLS and spectinomycin. Over the past two decades, erythromycin resistance rates have increased in *S. pyogenes* in many countries. To obtain information that may be useful in solving the spread of antimicrobial resistance, antibiotic-resistant genes can be identified as well as their association with mobile genetic elements.

**Material and Methods:**
A total of 22 isolates of *S. pyogenes* were obtained from 93 bacterial isolates. These isolates were examined by detecting their antibiotic susceptibility against two different antibiotics groups (Macrolides and Tetracycline) then genomic DNA was extracted from each isolate for detection Tn554 by using specific primers to amplify *erm*(A) gene carried by this transposable element.

**Results:**
Results showed that there is a high level of resistance to erythromycin, (90.9%), then to Minocycline (68.1%), Tetracycline (54.5%), Azithromycin and Clarithromycin (36.3%), Doxycycline (31.8%) and then to Oxytetracycline (27.2%). Results also showed that 13 of *S. pyogenes* isolates were harboring Tn554 transposable element carrying *erm*(A) gene. On the other hand, results showed that there are another isolates resistant to erythromycin that may possess a chromosomal or plasmid copy of the erythromycin resistance gene, or maybe the resistance was caused by another structural erythromycin gene carried by other type of transposable elements rather than Tn554.

**Conclusion:**
Our findings suggest that the isolates of *S. pyogenes* are harboring chromosomal copy of Tn554 conferring erythromycin resistance. One possible explanation for the presence of genes at different isolates is due to *erm* gene, which was most likely located on Tn554.

**Keywords:** Tn554, Erythromycin resistance, *Streptococcus pyogenes*, *erm*(A)gene


**Introduction:**

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Streptococcus pyogenes is the main human pathogen linked with local or systemic infestation such as pharyngitis, necrotizing fasciitis, impetigo and streptococcal toxic shock syndrome \[^{[1,2]}\]. Besides, S. pyogenes may stimulate autoimmune diseases like rheumatic fever, acute post-streptococcal glomerulonephritis and rheumatic heart disease \[^{[3,4]}\]. The development of antimicrobial resistance shows a major challenge for the management of infections worldwide \[^{1}\]. When penicillin use is inappropriate among patients with \(\beta\)-lactam allergy, macrolides represent the best solution for treating S. pyogenes \[^{5}\].

The main reason for the emergence of resistance and transmission of drug-resistant strain is the excessive use of antimicrobials \[^{6}\]. Since the 1990s, the global increase in resistance to erythromycin in the S. pyogenes has led researchers around the world to conduct more epidemiological and molecular studies, which have helped to identify mechanisms, determinants and genetic components that cause resistance \[^{7,8}\]. Tn554 contain \(\text{erm}(A)\) gene which has the ability to bind the resistance of MLS and spectinomycin \[^{9}\]. The first discovery of this transposon was in S. pyogenes and was assigned as \(\text{erm}\) (TR) at that time \[^{10}\]. According to the importance of Tn554 in spreading erythromycin resistance between bacterial isolates, this study was aimed to explore the presence of Tn554 in S. pyogenes isolated.

Material and Methods:

Bacterial Isolates:
Clinical samples were collected from patients (adults and children) complain from tonsillitis, pharyngitis and otitis media, who attends the Consulting clinic at Baqubah Teaching Hospital, Al-Batoul Teaching Hospital, Private clinics in Diyala governorate. To ensure the viability of pathogens, samples were sent directly to the microbiology in a sterile transport medium. Several factors have been relied upon in this study to identify S. pyogenes, including colonial morphology, gram staining, and biochemical tests by using Vitek 2 system.

Susceptibility testing:
Susceptibility of S. pyogenes isolates against Macrolides group (Erythromycin, Azithromycin, and Clarithromycin) and Tetracycline group (Tetracycline, Minocycline, Doxycycline, and Oxytetracycline) was examined on \(\beta\)- selective S. pyogenes agar with the addition of 5% of fresh blood according to the standard disc diffusion method, these antibiotics were supplied by Bioanalyse (Turkey).

Amplification experiments:
Amplification of chromosomal DNA was performed by using specific primer \(\text{III}_8\): 5'-GCATGACATAAACCTTCA -3' and \(\text{III}_{10}\): 5'-AGGTATATAATGAAACAGA -3', which targeting \(\text{erm}\) (TR)or \(\text{erm}(A)\)gene \[^{11}\]. This primer was provided inyophilized form and was dissolved in sterilized distilled water to give a final concentration of 10 picomole/\(\mu\)l. PCR master mix supplied by Intron, Korea. The optimum conditions for amplification of Tn554 were described in table (1).

Table (1): The optimum conditions for amplification of Tn554

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After PCR amplification, agarose gel electrophoresis (2%) was used to detect the amplified products. Aliquot of 5 µl of pure DNA solutions were mixed with 2 µl of 6x loading dye, then DNA samples were loaded into the wells. DNA bands were visualized under a UV-light transilluminator.

**Results and Discussion:**

Results indicated in table (2) showed that the bacterial isolates of *S. pyogenes* were highly resistant to erythromycin (90.9%), then to Azithromycin and Clarithromycin (36.3%) in macrolides group. While were highly resistant to Minocycline (68.1%), then to Tetracycline (54.5%), Doxycycline (31.8%) and then to Oxytetracycline (27.2%) in tetracyclines group. In recent years, treatment for *S. pyogenes* has become difficult owing to the global rise in the prevalence of antibiotic resistance, particularly against first-line antibiotics such as erythromycin and penicillin [13]. Increasing of antimicrobial resistance in *S. pyogenes* that causes infectious diseases is a global problem, although resistance significantly varies between geographical regions [12].
Table (2): Pattern of antibiotic susceptibility of *S. pyogenes* to different groups of antibiotics

<table>
<thead>
<tr>
<th>Isolate symbol</th>
<th>Macrolides</th>
<th>Tetracycline</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>AZM</td>
<td>CLR</td>
</tr>
<tr>
<td>H1</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>H2</td>
<td>S</td>
<td>S</td>
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<tr>
<td>H3</td>
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<td>H4</td>
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<td>H5</td>
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<td>H6</td>
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<td>H21</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>H22</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>

AZM: Azithromycin; CLR: Clarithromycin; E: Erythromycin; TE: Tetracycline; DO: Doxycycline; MI: Minocycline; T: Oxytetracycline; R: Resistance; S: Sensitive

In a local study, *S. pyogenes* resistance to erythromycin was 33.33%, while tetracycline was 52.38% [14]. Globally, most studies have shown a frighteningly high increase in antibiotic resistance ratios between *S. pyogenes* within the macrolide group, particularly erythromycin, and the tetracycline group. A study conducted in Spain between 1986 and 1997 showed that the percentage of resistance to erythromycin in Madrid in 1987 was 0.0%, then the percentage increased in 1989, 1992 and 1997 to
3.0%, 8.1%, 31.8%, respectively \cite{15}. Also, another a study carried out in Turkey found that from a total of 127 isolates of the \textit{S. pyogenes}, the resistance to erythromycin was 9% and tetracycline 18\% \cite{16}.

Furthermore, results indicated in the table (2) showed that the multi-drug resistant was spread among the isolates of \textit{S. pyogenes} as they give a different resistant pattern to antibiotics of different groups. Results also showed that the isolate \textit{S. pyogenes} H6 was resistant to all antibiotics used in this study (100\%), while the isolate H14 was resistant to six antibiotics (85.7\%), then the isolates (H3, H8,H15, H19, and H22) were resistant to five antibiotics (71.4\%). Common bacterial pathogens can be resistant to all known antimicrobial agents; \textit{Streptococcus} has special genetic elements structures called conjugative transposons. These structures can transport large-range of resistance genes and have the ability to capture other resistance elements to form composite structures allowing them to spread multi drug resistance between different bacteria \cite{12}.

To identify the resistance gene and resistance determinants a PCR-based approach by using specific primers (\textit{III}_8/\textit{III}_{10}). Results illustrated in figure (1) showed an amplified product of 342 bp appeared after electrophoresis on agarose gel (2\%), refer to the presence of this transposon in many bacterial isolates of \textit{S. pyogenes}. This result was agrees with Weber \textit{et al.} \cite{11} who detect Tn554 in \textit{S. pyogenes} after amplification of genomic DNA using the same primers. This transposable element was detected in 13 isolates of \textit{S. pyogenes} out of the total isolates (22 isolate). All these isolates (H2, H3, H4, H5, H6, H7, H8, H11, H12, H13, H14, H15, and H16) were resistant to erythromycin as indicated in table (2). Tn554 carries \textit{erm}(A) gene coding for erythromycin resistance, this result explains the erythromycin resistant phenotype in these isolates. In \textit{S. pyogenes}, drug resistance determinants such as that encoding erythromycin resistance usually are located on conjugative transposon that inserted into the host chromosome rather than on plasmids \cite{17}.

![Figure (1): Amplification product for Tn554 conferring erythromycin resistance in S. pyogenes isolates after electrophoresis on agarose gel (2\%) for 90min.](image)

Lane M: Ladder marker; Lane (1-22): Bacterial isolates

Furthermore, results indicated in table (2) showed that there are other seven isolates (H1, H9, H17, H18, H19, H21 and H22) were resistant to erythromycin that may possess a chromosomal or plasmid
copy of the erythromycin resistance gene, or may be caused by another structural erythromycin gene carried by other type of transposon found in these isolates. Moreover, results showed that there are two isolates of *S. pyogenes* (H10 and H20) were sensitive to erythromycin among the total isolates because they give negative PCR product and were unable to grow on enrichment medium containing this antibiotic. Antibiotic resistance is caused by methylation or RNA modification, which usually carries cross resistance to the macrolides-lincosamide streptogramins B (MLSB), the sensitive bacteria become resistant to macrolide, lincosamides and streptogenes B, this type of resistance is associated with *erm(A)* gene encoding methyl transferase and modifying the target site of macrolides and lincosamides in 23S ribosomal RNA. This explains the emergence of resistance patterns in this group of bacteria.

**Declarations:**

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**Ethics approval and consent to participate:**

The research was approved after the adoption of the protocol by the Center of Training and Human Resource Development, Diyala Province Health Directorate, Ministry of Health, Iraq (Ref: official letter No. 303 issued in 21st January 2018). Confidentiality was assured with signed informed consent.

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