Detection of Tn*916* Conferring Tetracycline Resistance in Clinical Isolates of *Streptococcus pyogenes*

Hameed M. Jasim[1], Abbas Aboud Farhan[2], Hanan Raheem Hassooni[3], Adil Hassan, Alhusseiny[4]

[1]Professor, College of Biotechnology, Al-Nahrain University, Baghdad- Iraq, [2]Professor, Faculty of Education for Pure Science, Diyala University, Diyala- Iraq, [3]Lecturer (Ph.D), Faculty of Education for Pure Science, Diyala University, Diyala- Iraq, [4]Professor, Faculty of Medicine, Diyala University, Diyala –Iraq

Abstract

Background: During the past twenty years, tetracycline resistance average has increased in *Streptococcus pyogenes* in many countries. Pneumococcal resistance to erythromycin and tetracycline is associated with the insertion of the *erm*(B) into the transposons of the Tn916 family. To get datum that may be beneficial in resolving the diffusion of antimicrobial resistance, therefore, we can be specified antibiotic resistant genes and their corporation with mobile genetic elements. This study was proceed to explore the genetic regulation of Tn*916*-carrying *tet*(M) in clinical isolates of *S.pyogenes* . **Methods:** A twenty two of *S.pyogenes* isolates were assemble from patients suffering from upper respiratory infection, and the susceptibility of these isolates to tetracycline antibiotics was examined. Molecular detection of Tn*916* was carried out by employ certain primers to amplify *tet*(M) gene in each isolates.

Results: The results appeared that the resistance of the tetracycline group was 68.1%, 54.5%, 36.3% and 31.8% for minocycline, tetracycline, oxytetracycline and doxycycline, respectively. Genetic analysis showed that Tn*916* was detected in eight of the *S.pyogenes* clinical isolates resistant to tetracycline. **Conclusions:** Our findings suggest that clinical isolates of *S. pyogenes* harboring a copy(s) of Tn*916* conferring tetracycline resistance. One possible explanation for resistance to tetracycline in these isolates is due to *tet* gene, which was most likely located on Tn*916*.

Keywords: *Tn916, S.pyogenes, Transposable elements, Antibiotic resistance* .

Introduction

Streptococcus pyogenes is the major human morbid connected with topical or systemic invasion and post-streptococcal immunologic disorders [1]. These bacteria colonize the throat or skin and cause several purulent infections [2,3]. In addition, it may be stimulating autoimmune diseases [4]. Increased levels of antibiotic resistance were reported in *S.pyogenes* in many countries, particularly among groups includes (MLSB, β-lactams, Aminoglycosides, Tetracyclines and Sulfonamides) causing a problem of treatment failure in patients with these bacteria [5]. In *S.pyogenes*, the prevalence of *tet*(M) may be dissected by this gene that is carried by "conjugative transposons" like Tn*916* or by composite construction like Tn*3701*, which can in full swing translocate from chromosome to anther [6,7]. Tn*916* family, is a prototype of "conjugative transposons" widely dispersed in gram-positive streptococci, Tn*916* was first detected in chromosomal DNA of *Enterococcus faecalis* strain DS16 [8]. All transposable elements relationship to this family include the tetracycline resistance determinant *tet*(M), either solo such as Tn*916* and Tn*5397*, or linked with another genes

Corresponding Auother:
Hanan Raheem Hassooni
The research was completed at Baqubeh Teaching Hospital and Beirut Medical Center in Beirut-Lebanon
hanan6319@gmail.com

such as Tn*6002*,Tn*6003*,Tn*3872*, and Tn*1545* [9]. Based on the above, to the importance of Tn*916* in pervasion tetracycline resistance among bacterial, this study was strived to find the presence of transposable element in *S.pyogenes*.

Material and Methods:

Bacterial Isolates Identification:

Swab was collected from adults and children complain from upper respiratory infection Diyala, Baghdad and Erbil -Iraq, and Beirut Medical Center in BeirutLebanon for the time from January/2018 to April/2019. These samples were cultured on β-Selective Streptococcus agar medium (β-SSA), and the growing colonies were identified later by colonial morphology, gram staining, and biochemical tests (catalase test, blood hemolysis test, and bacitracin sensitivity test), furthermore bacterial isolates suspected to be *S.pyogenes* were identified by using Vitek-2 system and finally by molecular method.

Susceptibility testing:

Susceptibility of the versus Tetracycline, Minocycline, Doxycycline, and Oxytetracycline was checked, by virtue of to the disk diffusion by the Kirby-Bauer

method [10].

Amplification experiments:

Amplification of *tet*(M) gene of Tn*916* was performed by using specific primer

O6: 5'-GGTACTTGAAAAGAACGGGAG-3' and TETM11: 5'

TTCACCTTAGTATTTTTCCACTG-3'[11,12]. The dehydrated primer was solving in distilled water to reach a concentration of 10 picomole/ µl. The amplification conditions began with primary and secondary denaturation at 95 °C, the first continued for 5 minutes, and the second continued for 30 seconds during which 30 cycles of denaturation were made. The other stage of amplification was the annealing process at 61 °C, which took only 30 seconds; the last stage was the initial and final extension process at 72 °C. The first took one minute, and the final one lasted for 7 minutes. Then PCR products were run by use electrophoreses technique.

Results and Discussion

Ninety-three bacterial isolates were obtained from clinical samples collected from pharyngitis, tonsillitis, and otitis cases from patients of different age groups and gender. All samples were cultured on β- selective *Streptococcus* agar and blood agar base supplemented with 5% of fresh human blood for determining the agricultural characteristics of colonies, then identified by using Vitek-2 system. Results of isolation showed that 93 bacterial isolate were characterized, among them 22 were identified as *S. pyogenes*. The prevalence of these isolates among clinical isolates is shown in figure (1).

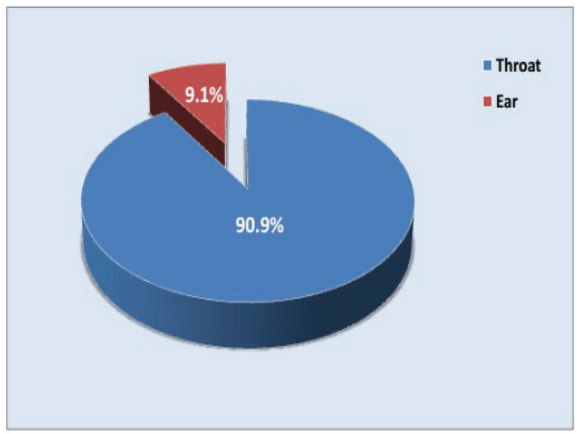

Figure (1): Prevalence of *S.pyogenes* among clinical sources

Susceptibility of *S.pyogenes* isolates to tetracycline's antibiotics was determined.

The results illustrated in figure (2) that resistance in the tetracycline group was 68.1%, 54.5%, 36.3% and 31.8% for minocycline, tetracycline, oxytetracycline and doxycycline, respectively. These resistance is consequent to the presence of tetracycline resistance gene *tet*(M) carried by Tn*916*, which mediates tetracycline resistance by the tetracycline-minocycline or *tet*(M) gene coding for tetracycline inactivating enzyme play a role in bacterial resistance to tetracycline, and that's explains the tetracycline resistant phenotype in these isolates. However, one isolate symbolized H1 was sensitive to tetracycline, which may refer that this transposon harboring a silent copy of *tet* (M) as mentioned by Montanari *et al.,* [13]. Resistant isolates may presented *tet* (M) promoter that resulted in an increase

in the gene transcription thus causing a higher level of resistance as mentioned by El Moujaber *et al*., 14.

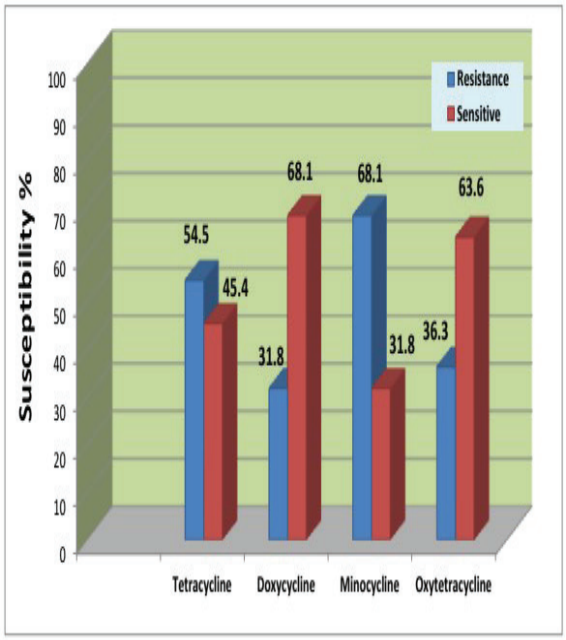

Figure (2): Percentage of tetracycline resistance among *S.pyogenes* isolates

International, many studies have indicated to rise in antibiotic resistance rate between *S.pyogenes* in tetracycline group [15]. It seems clear that resistance significantly varies between geographical regions and time period variation, as well as, the increased use of antimicrobials, especially when misdiagnosing the disease, using unnecessary antimicrobials, or due to the irrational use of antimicrobials, as well as irregular consumption 12, 16.

Detection of Tn*916*

Tn*916* is a conjugative transposon (or integrative conjugative element ICE) which was originally isolated from *Enterococcus faecalis* DS16 [8]. All transposable elements relationship to this family include the tetracycline resistance determinant *tet*(M), either solo such as Tn*916* and Tn*5397*, or linked with another resistance genes such as Tn*6002*, Tn*6003*, Tn*3872*, and Tn*1545* [9].

In this study, Tn*916* was detected in the clinical isolates of *S.pyogenes* by amplification of genomic DNA using specific primer (O6/TETM11), this primer targeting *tet* (M) gene carried by Tn*916*. Results explain in figure (3) appeared that there is an amplified product of 620bp shown the being Tn*916* in eight isolates (36.3%) of *S.pyogenes*. All of these isolates are resistant to tetracycline. These findings are similar to those results detects Tn*916* in *S.pyogenes* using the same primers [17]. Resistant isolates may presented *tet*(M) promoter that resulted in an increase in the gene transcription thus causing a higher level of resistance [14]. The results indicated that there was an isolation (symbolized H1) which was sensitive to tetracycline even though it contained a copy of Tn916 which indicates that this copy may be silent.

Moreover, results appeared that there are other eight isolates shown resistant for tetracycline, but they don't have Tn*916* which indicate that may be a chromosomal or plasmid copy of the tetracycline resistance gene, or cause by to another thematic tetracycline gene carried by other form.

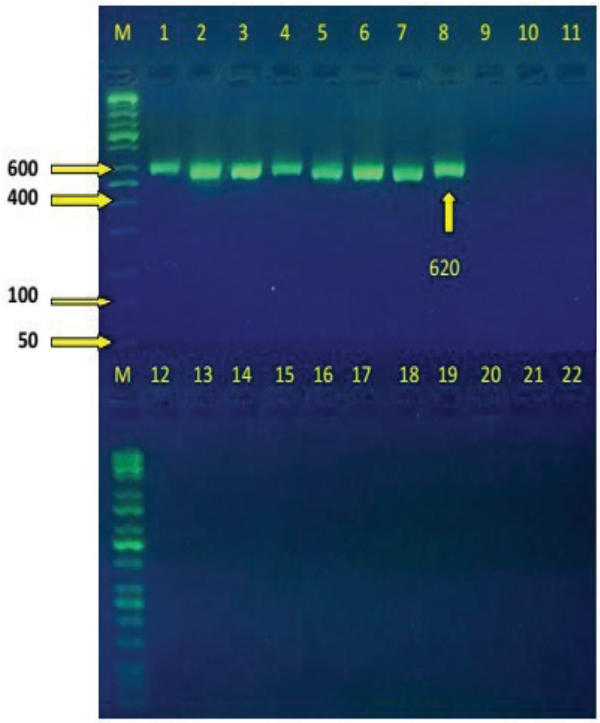

Figure (3): Detection of Tn*916* conferring tetracycline resistance

Prevalence of Tn*916* among clinical isolates of *S.pyogenes* was illustrated in figure (4). Tn*916* was prevalent in 7 out of 8 (87.5%) throat culture isolates and 1of 8 (12.5%) ear isolates. From these data, we conclude that Tn*916* represents a specific element, which is more likely to occur in clinical *S.pyogenes* isolates.

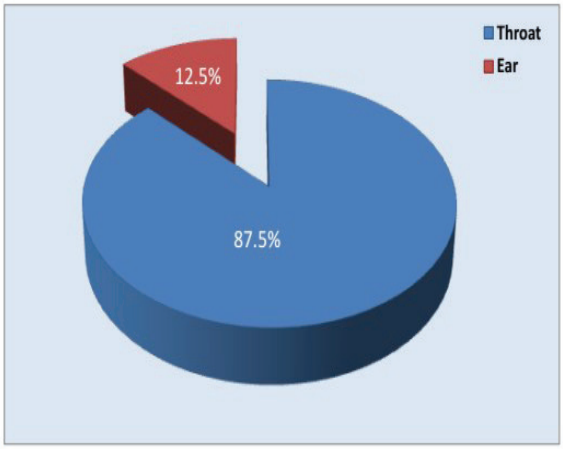

Figure (4): Clinical sources of isolates and percentage of Tn*916*

Declarations:

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Conflict of Interest: The authors declare that there is no conflict of interest.

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Ethics approval and consent to participate:

Approval for the research was obtained after implementing the protocol recommended by the specialized committee in the Diyala Health Department and according to the official letter issued in 2018.

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