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RESEARCH ARTICLE

Molecular Study of *spy*1258 and *smeZ* genes in Group A Streptococcal Tonsillitis

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Abstract

Streptococcus pyogenes **was the most common bacterial causes of tonsillitis. The detection of virulence factors of this pathogen can be used to determine pathogenic potential as a rapid screening method. A total of 109 isolates (46%) showed positive culture for *S. pyogenes*, these isolates recovered from 235 swabs were collected from tonsillitis patients in ENT department in Al–Habboby Teaching Hospital, Thi-Qar province, Iraq. *S. pyogenes* isolates exposed to detect the specific gene (*spy*1258)and one of the virulence factors were *sme*Z gene by PCR technique and DNA sequencing analysis. The PCR results recorded that 61% and 50% of isolates harbor *spy*1258 and *smeZ* genes, respectively. The sequencing of PCR products showed significant alignments identities (94-100%) to the *S. pyogenes* for both genes which are located in BLAST-NCBI Genbank. The four PCR products of both genes were registered in Genbank under the named as (ZKD1 Spy-like gene; ZKD2 Spy-like gene; ZKD3 SmeZ-like gene and ZKD4 SmeZ-like gene). The results of Multiple sequence alignment analysis recorded that C > T and T > C polymorphism for *sme*Z gene.**

Keywords: *S. pyogenes*, tonsillitis, *spy*1258, *sme*Z, gene sequences.

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INTRODUCTION

Group A *streptococcus*, *S. pyogenes* (GAS) was a common human bacteria responsible for a wide range of diseases, from restricted throat infections like pharyngitis, tonsillitis, to hostile infections as streptococcal toxic shock syndrome, sepsis, bacteraemia, and necrotizing fasciitis[1]. Even though' the *S. pyogenes* was repeatedly present extra cellulary in the host cells. Hertze *et al*.,[2] demonstrated that this bacteria might exist in intracellularly and evade the killing by in cooperation the human macrophages and /or neutrophils[3] .

The austerity of GAS infections were hinged on various factors of bacteria and host. Also, the pathogenic possessions of *S. pyogenes* isolates were allied to creation of the multiple virulence factors like DNases, proteases, toxins, and other toxins which present in goal bacteria (Group A *streptococcus*) might be the forecaster of its invasiveness[4] . Huge group of toxins coded by *sme*Z, *speM*, *speC*, *spe*Igenes is involved in systemic toxicity[5] .

The complex pathogenicity of GAS were related to its ability to production of numerous virulence factors such as Streptococcal mitogenic exotoxin Z (*smeZ*) was stimulated the immune system, and its linked with different disease like scarlet fever, acute rheumatic fever, and toxic shock syndrome[6].

'The polymerase chain reaction (PCR) technique directing to transcriptional regulator genes supplied the rapid and dependable manner for detection of a pathogenic microbes[7]. The transcriptional regulators were dedicated a DNA binding proteins that performance a critical role in guiding the gene expression inside microbes aimed at the adaptation and endurance in diverse environment. A *spy*1258 gene was one of reputed transcriptional regulator gene (TetR/AcrR family) that was precise for GAS and could be used as a marker for the detection of this bacteria[8] .

Liu *et al*.,[8] described *spy*1258 gene that is exceptionally lay out in isolates of *S. pyogenes*. The using of PCR technique of *spy*1258 gene assisted an amplification of DNA that extracted from GAS only, but never from the other *Streptococcus* species and public bacteria. The main goal of this study was to detect the GAS, to establish the sensitivity of *spy*1258 gene to identification of *S. pyogenes*, to determine the occurrence of *smeZ* gene in this bacteria. Furthermore, to comparative genomic sequencing analysis permits for an epidemiological investigation of closely associated bacterial isolates.

MATERIALS AND METHODS

Ethical approval

This research was approved by the Medicine College Ethics Committee, Thi-Qar University, Thi-Qar Province, Iraq.

Bacterial isolates

Two hundred and thirty five swabs were collected from patients infected with tonsillitis whom admitted to Ear Nose Throat (ENT) unit in AL-Habbuby Teaching Hospital of Thi-Qar province, during the period from November, 2015to May, 2016 by moistened sterile swabs with normal saline, these swabs directly inoculated on bloodagar (LAB/ United Kingdom) and incubated at 37°C for 24 hour.

Identification of *S. pyogenes*

S. pyogenes was identified depending on morphological properties on culture media, biochemical tests and Bacitracin susceptibility using 0.04 units Bacitracin discs (Bio analyse/ Turkey) [9,10]. *S. pyogenes* diagnosis was confirmed by API system (BioMerieux / France). Lastly, to serological identification of *S. pyogenes* by used the Mast *Streptococcus* kit (Mast/United Kingdom), it is a rapid latex slide agglutination for identification of Streptococci of 'Lancefield groups A,B,C,F and G, according to the instructions of the manufactured company.

Preparation of bacterial DNA

Completely *S. pyogenes* isolates were inoculated on Brain Heart Infusion broth (LAB/ United Kingdom) and incubated for 24h at 37°C. 'The DNA of bacteria extracted from a fresh culture in a Brain Heart Infusion broth by using DNA Bacteria plus kit (Geneaid / Korea) according to the manufacturers instructions'.

Polymerase Chain Reaction diagnosis of *spy*1258 and *sme*Z genes

The specific primer pairs of *spy*1258 gene as following: forward : 5'-AAAGACCG CCTTAACCACCT-3' and reverse: 5'-TGGCAAGGTAAA CTTCTAAAGCA-3'[8], whereas for *sme*Z gene: forward: '5'-TTTCTCGTCCTGTGTTTGGA-3' and reverse : 5' TTCCAATCAAATGGGACGGAGAACA-3'.

The PCR cycling program of *spy*1258 gene: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 20 seconds, annealing at 55°C for 20 seconds, extension at 72°C for 45 seconds and final extension for 2 minutes after the last cycle[11] , while for *smeZ* gene was set at 95°C for 5 minutes followed by 30cycles of 94°C for 30 second, 58°C for 30seconds, and 72°C for 1 minute, and final extension at 72°C for 5 minutes'[12] .

The visualization of PCR products was showed in 2% agarose gelelectrophoresis and the attendance of a 407bp and 246bp band as a positive result for *spy*1258 and *sme*Z genes, respectively.

DNA sequencing

Seven PCR products of *S. pyogenes* distributed to three for *spy*1258 and four to *sme*Z genes, were selected for sequencing and forward and reverse primers for each gene were sent to the laboratory to be sequenced (Macrogen, Korea). Basic Local Alignment Search Tool analysis (BLAST) was lead to blast algorithm (www.ncbi.nlm.nih.gov/ BLAST). The sample sequences designated as (ZKD1, ZKD2, ZKD3, ZKD4, ZKD5, ZKD6 and ZKD7) for both genes were edited, aligned, and compared with the reference sequences using Bio Edit sequence Alignment Editor Software Version 7.1 (DNASTAR, USA)[13] (Hall, 1999). A phylogenetic tree for each gene sequence was constructed by using MEGA7 software[14] .

RESULTS AND DISCUSSION

The results of the present study displayed that the occurrence of *S. pyogenes* was 109/235 isolates (46%), *S. pyogenes* was one of recurrent bacterial agent of tonsillitis. Simon,[15] ; and Beye *et al.*,[16] recorded that *S. pyogenes* was an important pathogen causes of tonsillitis, cutaneous and systemic infections.

The current results incorporated with results of [17] showed that *S. pyogenes* and *S. aureus* were caused tonsillitis and pharyngitis and revealed 35.42% as positive culture for *S. pyogenes*, while the frequency of *S. pyogenes* was higher than the results of[18] recorded that the most agents of tonsillitis were *S. pyogenes* with a percentages of 20.2 %.

The frequency of *spy*1258 gene in *S. pyogenes* isolates was 61%, and the size of this gene was approximately 407 bp, Fig. (1). *spy*1258 was definite gene only for GAS and used for identification of this species. Dunne *et al*.,[19] revealed that the s*py*1258 gene was the major gene targets of *S. pyogenes*. Also[7] recorded that this gene was specific 'for GAS and could be involved in species to specific conservation or adaptation'. On other hand, the current results agreed with studies performed by[8] and[20] , those studies showed that *spy*1258 gene was specific for *S. pyogenes* only, but not from other species of the genus *Streptococcus* and common bacteria.

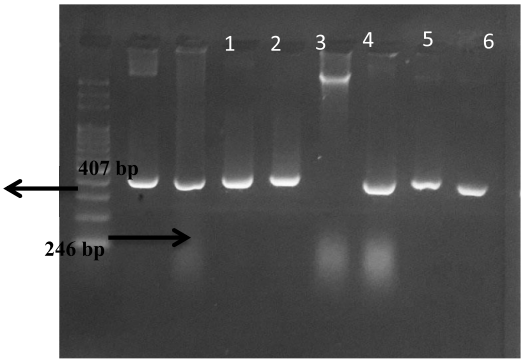

Fig. 1. Agarose gel electrophoresis of *spy*1258gene amplification,M: ladder, 1-4, 6-8: positive results, 5: negative results.

The present study was disagreed with results of[21,22] displayed that all *S.pyogenes* isolates contains *spy*1258 gene which isolated from patients with tonsillitis, while[19] recorded that 21 isolates from 24 *S. pyogenes* had this gene, also the *spy*1258 gene had lesser sensitivity to detection three isolates of *S. pyogenes*.

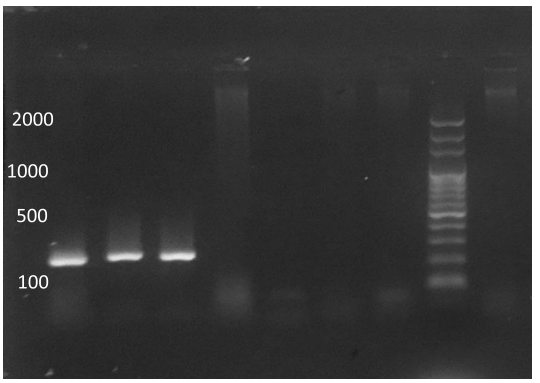

Fig. 2. Agarose gel electrophoresis of *smeZ*gene amplification,M: ladder, 1-3: positive results, 4-7: negative results.

The existence of *sme*Z gene was 50% in *S. pyogenes* isolates, Fig. (2) showed the size of this gene which was approximately 246 bp. *smeZ* was as virulence determinants implicated in the 'initiation of the systemic toxicity which linked with fierceness of diseases and severe infections caused by *S. pyogenes*, a *sme*Z gene was the most exhibiting an effective super antigen, contributed imperative role, and coded to highly mitogenic proteins produced by numerous isolates of *S. pyogenes*[23]. The recent data was difference from results of the study performed by[24] documented that 79% of invasive isolates had this gene.

The changed distribution of *sme*Z gene may be associated with transferred this gene by different elements through chromosomal DNA. Furthermore, Schmitz *et al*.,[25] showed the distribution rate of *smeZ*, *speA*, *speC*, *speH*, *speJ* and *ssa* genes were associated with movable elements, and the *smeZ* allele was found in 95.8% strains. The results of this study were a low emergence than the results of the study by[26] recorded that all invasive strains harbored *sme*Z gene.

In the same field, the results of current data disagreed with the study of [27] documented a high emergence of isolates had this gene (99%).

'The DNA sequences of specific gene (*spy*1258) was only presented in *S. pyogenes* and absent in other bacterial species'. Numerous studies had used the *spy*1258 for the fast detection of GAS that recovered from countless clinical samples'. Also the results of study performed by[21] recorded that *spy*1258 gene was sensitive and specific for *S. pyogenes*, and this gene found in completely isolates of GAS (100%), while [19] revealed that the sensitivity of *spy*1258 gene by using of qPCR technique for direct detection of GAS isolated from throat[19] . The study validated by[28] Zhao *et al*., (2015) documented that the *spy*1258

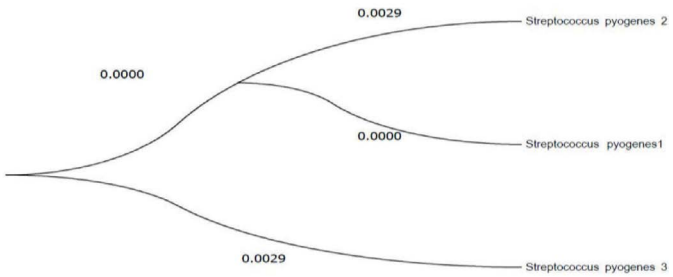

Fig. 3. The evolutionary relationships of *S. pyogenes*, phylogeny tree of the *sme*Z gene inferred through distance based analysis using Tamura-Nei distance estimates of aligned nucleotide sequences resulting from the PCR sequence data.

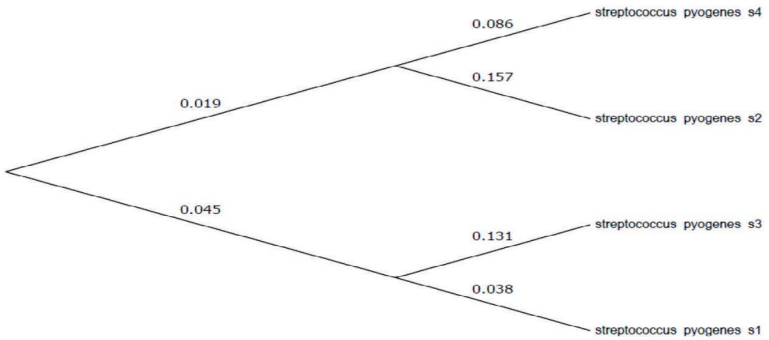

Fig. 4. The evolutionary relationships of *S. pyogenes*, phylogeny tree of the *spy*1258 gene was showedvia distance depended on analysis by Tamura-Nei distance estimates of allied nucleotide sequences consequent from the PCR sequence data.

gene used for accurate and precise identification of GAS strains,

Phylogenetic tree of *S. pyogenes* based on the neighbour-linking of part*spy*1258 and *sme*Z genes sequences presented those sequences were resulted from genes of *Streptococcus* (Fig 3 and 4).

To classify any organism such as bacteria used the phylogenetic analysis method[29] showed the evolutionary history was inferred via the neighbor joining process. The best tree with the sum of branch-length = 0.47675007 was revealed (above the branches), while Maximum Composite Likelihood process was performed to compute the an evolutionary distances and in the units of the numeral of base substitutions of each site[30].

On the other hand, Babbar *et al.*,[31] reported that the more examinations of phenotypic features and determination of bacterial species shed extra light on the microbial diversity like *S. pyogenes* and expanding the understanding the infections of this microbe, and its diagnosis.

The analysis including four nucleotide sequences. Codon locations involved were 1st + 2nd + 3rd + Noncoding. All vague positions were detached for each sequence pair, there were a entire of 220 locations in the ending dataset, and the evolutionary analyses were directed in MEGA7[32].

The sequencing of PCR products produced for *spy*1258 gene showed significant alignments identities (100%) to *Streptococcus pyogenes* strain NCTC12696 genome assembly, chromosome: 1 Sequence ID: LS483332.1 which are located in BLAST-NCBI Genbank. While the alignments identities for *sme*Z gene was (94-100%) to *S. pyogenes* (ID: LS483384.1, LS483330.1 and

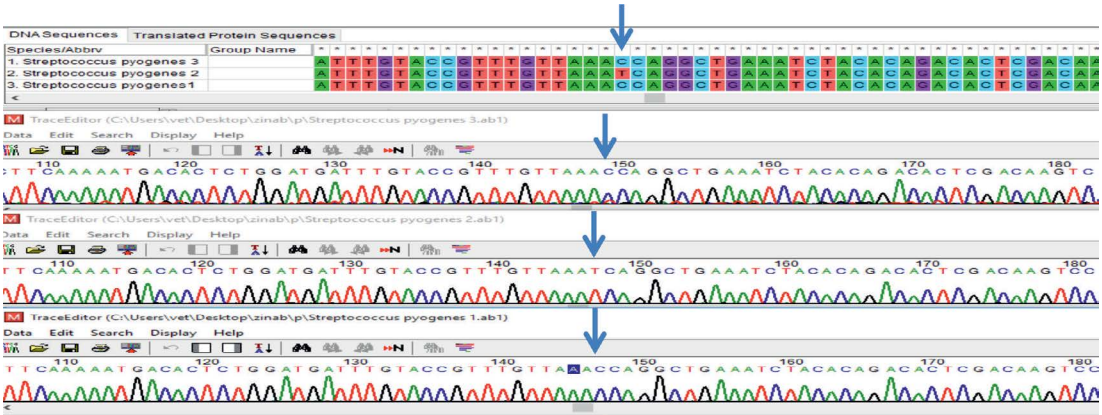

Fig. 5. Multiple sequence alignment analysis show C>T polymorphism in sample two for *sme*Z gene of *S. pyogenes*

Fig. 6. Multiple sequence alignment analysis show T>C polymorphism in sample one for *sme*Z gene of *S. pyogenes*

LS483394.1) which are located in BLAST-NCBI Genbank.

From the results of Multiple sequence alignment analysis, showed C > T polymorphism in sample two of smeZ gene, Fig (5). While recorded T > C polymorphism in sample one, Fig (6). This variation in nitrogen base may be related for differentiation the product of current gene (*sme*Z) which may be related to increased pathogenicity of this microorganism that harbored in it.

CONCLUSION

The *spy* 1258 gene was identical gene for *S. pyogenes*, and used as useful tool for tonsillitis GAS rapid method to diagnosis.

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CONFLICTS OF INTEREST

The author declares that there are no conflict of interest.

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