Molecular profile of *scpA and sdaB* virulence genes in *Streptococcus pyogenes* isolated from pharyngitis

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الخلاصه

تعد المكورات السبحية القيحية (مجموعة ا) بكتريا ذات امراضية مهمة وتسبب العديد من الامراض للإنسان. اجريت الدراسة الحالية في مستشفى الحبوبي التعليمي-قسم الاذن والانف والحنجرة في محافظة ذي قار جنوب العراق للفترة من تشرين الاول 2015 الى نيسان 2016. تم جمع 210 مسحه من مرضى يعانون من اخماج الحنجرة واظهرت نمو البكتريا السبحية 152 (73.3%) من مجموع العينات المزروعة. كذلك تم اجراء الفحص الجزيئي لجينات الضراوة (*scpA* و *sdaB*) للبكتريا المعزولة باستخدام تفاعل البلمرة المتعدد وبادئات نوعية وتسجيل تسلسلات للجينات المستهدفة ومقارنتها مع تسلسلات الجينات الموجودة في بنك الجينات العالمي (BLAST-NCBI Genbank). كذلك تم تسجيل 6 سلالات محلية في بنك الجينات العالمي تحت ارقام اعتماد MF49318-MF497323. واظهرت نتائج اختبار مطابقة التسلسلات المعزولة محليا مع التسلسلات الموجودة في بنك الجينات العالمي بنسبة تراوحت بين 96-99 %. كذلك تم تحديد العلاقة التطورية لشجرة تحديد الصفات المظهرية مع ما مسجل عالميا. تفرز المكورات السبحية القيحية العديد من السموم الخارجية والتي لها دور كبير في اتلاف مباشر لانسجة المضيف او من خلال تحفيز افراز الحركيات الخلوية.

Abstract

Group A Streptococci (GAS) or *Streptococcus pyogenes* is an important pathogen which causes a wide-ranging of diseases for human. This study was carried out in Ear Nose Throat (ENT) department in Al–Habboby Teaching Hospital, Thi-Qar province, south of Iraq during the period from October 2015 to April 2016. Two hundred and ten swabs were collected from patients infected with pharyngitis. 152 (72.3 %) showed positive growth with *S. pyogenes*. GAS isolates were subjected to detect two virulence genes (*scpA* and *sdaB*) by conventional PCR technique using specific primer pairs and DNA sequencing analysis. The sequencing of PCR products produced from bacterial DNA showed significant alignments identities (96-99%) to the *S. pyogenes* which are located in BLAST-NCBI Genbank. The six sequences of *Streptococcus pyogenes scpA* and *sdaB* genes determined in this study have been deposited in the GenBank under the accession numbers MF49318-MF497323. Phylogenetic analysis of *S. pyogenes* based upon the neighbour-joining of partial *scpA* and *sdaB* gene sequences showed that these sequences were derived from Streptococcal genes. In addition, *S. pyogenes* can produce several exotoxins that have the potential to damage the host tissues either directly or through the stimulation of cytokine production.

Key words: **gene sequences, pharyngitis**, **phylogenetic tree, *Streptococcus pyogenes***

Introduction

Streptococcus pyogenes also known as group A Streptococci (GAS) and is an important pathogen for human that causes a wide variety of diseases. Infections caused by Streptococci range from localized sore throat infection such as tonsillitis or pharyngitis, to aggressive infections such as bacteremia, cellulitis, puerperal sepsis, meningitis, pneumonia and Streptococcal toxic shock syndrome (1). The majority of these diseases are found in kids less than seven years of age. From the mid of the 1980s, expanding report quantities of which have been describing the serious diseases caused by *S. pyogenes* (2). Borek *et al.* (3) referred to the recognition of harmfulness elements discharged by GAS strains can be valuable to decide pathogenic capability of the microorganisms and to screen quickly as a phenotypic technique. The seriousness of the GAS strain contaminations relies on upon other bacterial diseases and host highlights. The pathogenic components of the microscopic organisms are frequently identified with the delivering of virulence

factors (4). *ScpA* (C5a peptidase) gene produces by GAS specifically to cleave the human serum chemotaxin at leukocyte binding site. *ScpA* also acts as virulence factor by retarding the influx of inflammatory cells and clearance of Streptococci during the first few hours after infection (5). In addition, *sdaB* is also a major nuclease and a possible virulence factor in *S. pyogenes*. A virulence factor represses the transcription of the other adjacent gene, encoding streptodornase B (*sdaB* also called DNase B), which is a secreted nuclease (6). GAS genomes are known for their plasticity and genomic variation, namely due to horizontal gene transfer between bacterial cells, and the presence of prophages. All of these features can confer virulence and resistance capabilities to GAS strains, in addition to influencing the regulation of existing genetic elements a fact that further necessitates a broader look at the bacterial genome, rather than a localized one (7). Mutations can lead to strong upregulation of much virulence associated genes encoding the hyaluronic acid capsule synthesis, streptolysin O, streptococcal inhibitor of complement, interleukin-8 protease and DNase *sda*1 which allows *S. pyogenes* to escape killing by neutrophils through the degradation of the DNA-based neutrophil extracellular traps (1,6). The aim of this study was to improve the knowledge about *S. pyogenes* outbreaks, and to monitor emerging antibiotic resistances. In addition, comparative genomic sequencing analysis and phylogenetic tree generating, allows for an epidemiological discrimination of closely related bacterial isolates.

Materials and Methods

Ethical approval: This research was approved by the Science College Ethics Committee, Thi-Qar University, Thi-Qar Province, Iraq.

Samples collection: Two hundred and ten swabs were collected from patients infected with pharyngitis whom admitted to Ear Nose Throat (ENT) department in Al–Habboby Teaching Hospital in Nasiriyah City, Thi-Qar province, south of Iraq during the period from October 2015 to April 2016. Swabs were collected from patients by disposable transport media and directly transported to the laboratory for diagnosis.

Bacterial Identification: *Streptococcus pyogenes* identification was performed depending on cultural characteristic, colony morphology, β-hemolysis on blood agar, and Bacitracin susceptibility (8). Mast Strep Kit was used for differentiation between of *S. pyogenes* (group A Streptococci) and other groups to Streptococci. It has a rapid slide latex agglutination test that was performed according to the instruction of the manufacturing company (Mast, United Kingdom). Finally, bacterial diagnosis was confirmed by API system (BioMerieux, France). The antibiotic susceptibility was performed by using disk diffusion method on Mueller Hinton agar. The resistance profile of the bacteria was recognized through the inhibition zone of their antibiotic items (9).

Detection of *scpA*and *sdaB* genes by Polymerase Chain Reaction: *S. pyogenes* isolates were subjected to the detection of the two targeted genes by conventional PCR technique using specific primer pairs (Table 1). The amplification was conducted in a Thermal Cycler (ESCO, India) with the programs described by Borek *et al*. (3) for the selected genes.

DNA sequencing: Six PCR products of *S. pyogenes*, distributed to three *scpA* and three *sdaB* 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 SHKH1, SHKH2, SHKH3, SHKH4, SHKH5 and SHKH6 were edited, aligned, and compared with the reference sequences using BioEdit sequence Alignment Editor Software Version 7.1 (DNASTAR, USA) (10). A phylogenetic

tree for each gene sequence was constructed by using MEGA7 software (11).

Statistical Analysis: The results of the present study were statistically analyzed by using SPSS program version 16. *P* value below or equal to 0.05 was considered statically significant.

Table 1: Primer sequences of *scpA*, and *sdaB* virulence genes of *S. pyogenes*.

Gene	Primer sequence (5′-3′)	Product size (bp)	Reference
scpA	F:GCTCGGTTACCTCACTTGTCC R: CAATAGCAGCAAACAAGTCACC	622	(3)
sdaB	F: TATAGCGCATGCCGCCTTTT R: TGATGGCGCAAGCAAGTACC	440	

Results

Out of 210 pharyngeal swabs, 152 (72.3%) showed positive growth with *Streptococcus pyogenes*. The resistance to antibiotic showed variable evidences against bacterial isolates, 37 (24.4 %) of isolates showed resistance to all antibiotic medications which were used in the treatment of pharyngitis, while 115 (75.6 %) of bacterial isolates showed excellent sensitivity to some antibiotics such as Amikacin, Augmentin, Azithromycin and Ceftriaxone.

Detection of virulence genes using PCR: The PCR amplifications of the *scpA*and *sdaB* genetic primers successfully showed a single band with extracted DNA. The presence of clear band indicates the presence of the specific gene. Eighty two isolates (53.9 %) of *S. pyogenes* were positive with *scpA* gene while *sdaB* gene gave positive results in 70 (46.1 %) isolates with product sizes of 622 and 440 bp, respectively (Figure 1).

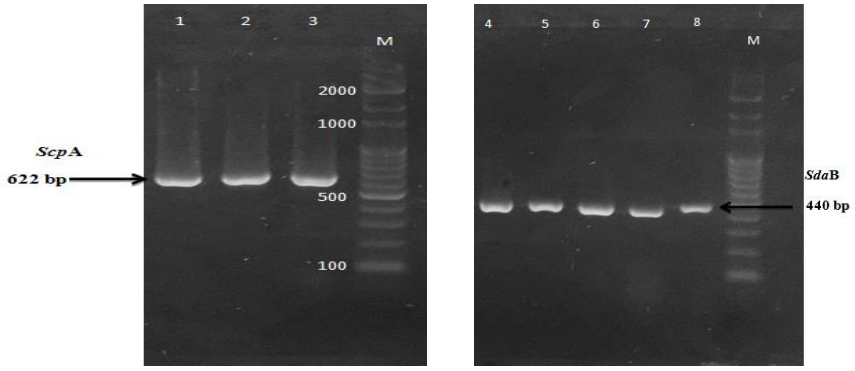

Figure 1: Agarose gel electrophoresis (1 %) of PCR amplification of *Streptococcus pyogenes scpA*gene (A) at product size 622 bp and *sda*B gene (B) at product size 440 bp with 100-2000 ladder.

DNA sequencing: Amplicons of the tow virulence genes (*scpA* and *sdaB*) were sequenced in both directions using the specific primers described above. Six selected *S. pyogenes* isolates were submitted to DNA sequencing for *scpA* and *sdaB* genes. The sequences of *Streptococcus pyogenes scpA* and *sdaB* genes determined in this study have been deposited in the GenBank (http://www.ncbi.nlm.nih.gov/genbank/)

under the six accession numbers MF49318-MF497323. The sequencing of PCR products produced from bacterial DNA showed significant alignments identities (96-99 %) to the *S. pyogenes* which are located in BLAST-NCBI Genbank. Phylogenetic analysis of *S. pyogenes* based upon the neighbour-joining of partial *scpA* and *sdaB* gene sequences showed that these sequences were derived from Streptococcal genes (Figure 2).

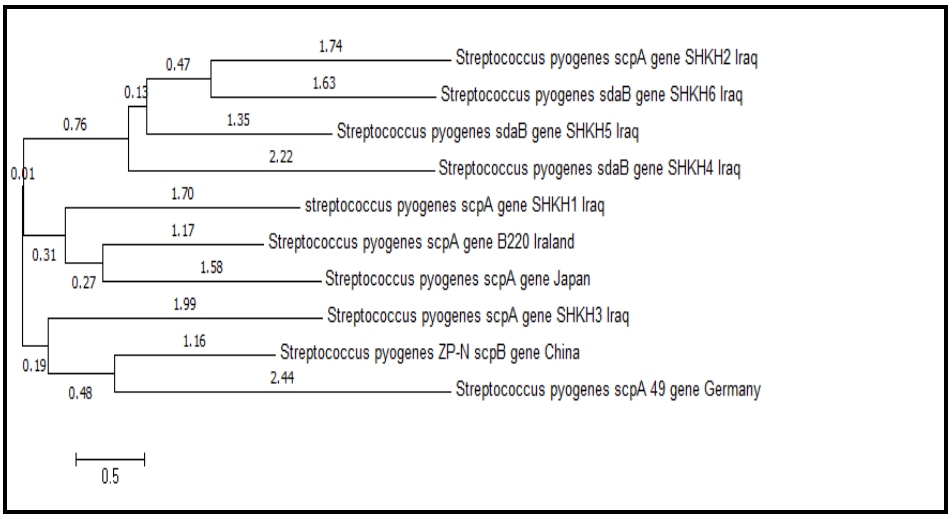

Figure 2: The evolutionary relationships of *S. pyogenes*, phylogeny tree of the *scpA*and *sda*B virulence genes was inferred by distance based analysis using Tamura-Nei distance estimates of aligned nucleotide sequences derived from the PCR sequence data.

Discussion

The results of the present study showed 152 positive growths (72.3%) from 210 throat swabs were taken from patients suffered from pharyngitis. *Streptococcus pyogenes* is the common frequent bacterial agent of pharyngitis and is also may cause a variety of cutaneous and systemic infections. However, the incidence of *S. pyogenes* pharyngitis has reduced significantly since the introduction of antibiotics (12). In addition, the results showed an excellent sensitivity (75.6%) of the isolates against antibiotic medications. Except some of antibiotics, *S. pyogenes* has continued susceptible *in vitro* to antibacterial agents since the 1940s. In fact, even if antibiotics resistance is common with β-lactam group in clinical practice, no acquired mechanism of resistance has been described to date (13)

Sixty operational taxonomic units (OTUs) as a complete or partial gene in NCBI-BLAST were closely related at the similarity 96 to 99% to the partial sequencing of *scpA* virulence gene, while seventy OTUs complete or partial gene were related at high similarity 98 to 99% to the partial sequencing of *sdaB* virulence gene. Whole bacterial genome analysis of *S. pyogenes* revealed gene size 1.83 M bp with G-C content of 38.5 % and the both values falling in the general ranges for the microorganism (7). The phylogenetic analysis using the *scpA* and *sdaB* genes classified as local strains (SHKH1 to SHKH6). In addition, the isolates were positive for *scpA* or *sdaB*, indicating that the isolates were having virulence genes and frequently present among pathogenic bacteria. The amplicon sequences were up to 99% identical to sequences of the other *S. pyogenes* genes have been recorded in the

NCBI website such as *scpA*49 gene from Germany, B220 gene from Ireland and ZP-N *scp*B gene from China and *scpA* gene from Japan. In this cluster, the *scpA* acts as C5a peptidase. In addition, *S. pyogenes* can produce several exotoxins that have the potential to damage the host tissues either directly or through the stimulation of cytokine production (14). On the other hand, Babbar *et al*. (15) reported that the more examinations of phenotypic characteristics and species determination shed extra light on the variety of the pathogens such as *Streptococcus pyogenes* and increasing our understanding of their infections and improving their diagnosis. **In conclusion**, in spite of modern advancements in medicine, the disease caused by S. pyogenes remains a very actual problem, particularly in developing countries. In addition, antibiotic resistance is another burden for treatment the Streptococcal diseases. Local sequencing of Streptococcal isolates were deposited and recorded as new sequences in the global gene bank. The high genomic DNA similarity of the species adds to the method of the identification process.

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