Molecular investigation of virulence factors genes in *streptococcus pyogenes* by PCR

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Abstract

This study was designed to detect some genes associated with important virulence factors in *streptococcus pyogenes* . A total of 200 throat swabs were collected from patients suffering from pharyngitis from both sex and age from (1 - 15) years. carried out from ENT unit in Al-Hilla General Teaching Hospital and Al Noor Hospital during the period from January 2018 to December 2018 , Out of the 200 samples only 177 samples showed positive bacterial culture ,No growth was seen in other 23 samples ,The results indicate that the rate of *Streptococcus pyogenes* isolated from patient with pharyngitis is 30 (15%), others bacterial growth 147 (73%), and no growth 23 (12%).

Molecular detection of virulence factor genes was done like M protein (*emm*) gene , the result shows that these genes were detected in all isolates bacteria (100%) with molecular length (914 bp) , *SpeA* gene was carried by using specific primer and it was found that (3.3%) isolates give positive result for this gene with amplicon (576 bp) , *SpeB* gene the result shows that (100%) isolates contain the gene with molecular length (952 bp) , *SpeC* gene also study by using specific primer at molecular length (405 bp) and the result s show that (75%) give positive results to this gene , the *mac* gene in all *S.pyogenes* isolates the results show that (30%) give positive , results to this gene with molecular length (389 bp) , *scpA* gene in all isolates the result shows that 17 isolates (56.6%) give positive result to this gene with amplicon size (622 bp)

Keywords: *PCR ; streptococcus pyogenes ; virulence factors*

Introduction

Streptococcus pyogenes, commonly known as group A streptococcus (GAS) is a fermentative, facultative anaerobe, nonmotile, nonspore-forming gram-positive coccus, which occurs in chains or pairs, having a diameter of 0.5-1.0 μm. GAS are beta haemolytic streptococci. They require an enriched medium containing blood to grow [(1)]. The group A streptococci are fastidious organisms that have complex growth requirements. A highly nutritious growth medium that provides optimal growth GAS is generally grown on agar media supplemented with blood [(2)]. Pharyngitis, or commonly known as sore throat or strep throat, is the most common manifestation of infection with *Streptococcus pyogenes* (GAS) Infection with this bacterium is diagnosed in 20 to 40% of pharyngitis cases in children and in 5 to 15% in adults [(3)].M protein considered one of the most important virulence factors, the M protein promotes host interactions and adherence to human epithelial cells; specially helping bacteria to escape from host immune response by inhibition of phagocytosis [(4)].Other important virulence factors include the streptococcal superantigens (SAgs). SAgs are bacterial toxins which bind to major histocompatibility complex class II and T-cell receptors [(5)].

SpeB plays a role in the pathogenesis of *S. pyogenes* infections by Destruction of host defense system proteins and cleavage of GAS surface proteins may help the bacterium to escape immune clearance, invade the deeper tissues, and disseminate from the primary infection site [(6)].SPE-C was the first streptococcal SAg SPE-C binds to the polymorphic MHC class II β-chain with the formation of a tetravalent zinc complex that includes three residues within the C-terminal domain of SPE-C [(7)].Mac-2 is a related IgG endopeptidase that prevents the recognition of IgG bound to *S. pyogenes* by competitively blocking

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IgG from recognition by Fc receptors on host cells ,Given that IdeS/Mac have homologs across group A streptococcal strains [8]. Streptococcal C5a Protease ScpA, is a cell-bound peptidase anchored to the cell wall by sortase A ,The streptococcal C5a protease (SCP) is expressed on the surface of all serotypes of *S. pyogenes* and most human isolates of groups B, C, and G streptococci, where it specifically destroys C5a. The enzyme also binds fibronectin and functions as a low level invasion for *S. pyogenes*, group B streptococci and group G streptococci [9].

Materials & Method

This study included 200 patients (aged 1-15years) collected from throat swab who admitted to Al-Hilla Teaching Hospital, during a period extending from January 2018 to desember 2018. The specimens were collected from patients with pharyngitis to detect *Streptococcus pyogenes* by bacteriological analysis and vitek 2 system in a proper way to avoid any possible contamination

Detection of virulence Genes by PCR technique

DNA extraction and purification: This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid, (UK). The suspension containing DNA was stored at-20 C until used as a template for PCR. **Primer Sequences:** The primer sequences and PCR conditions that are used in the study[10].

Results and Discussion

Isolation of *streptococcus pyogenes* :

In this study, a total of 200 throat swab were collected from patients suffering from pharyngitis from both sex with age (1 - 15) years. carried out from ENT unit in Al-Hilla General Teaching Hospital and Al Noor Hospital during the period from January 2018 to december 2018 .

The results indicate that the rate of *Streptococcus pyogenes* isolated from a patient with pharyngitis is (15%), others bacterial growth (73%), and no growth (12%).as shown in table(1)

Table (1): Numbers and Percentages of *Streptococcus pyogenes*

Isolates From patients with pharyngitis

bacterial growth	Numbers of bacterial growth	Percentages %
Streptococcus pyogenes	30	15%
Other bacteria	147	73%
No growth	23	12%
Total	200	100%

The low percentage of *Streptococcus pyogenes* may be due to normal flora that found in the pharynx and other bacteria that cause secondary infection , there compete pathogenic bacteria in nutrient in culture media

No growth was seen in other 23 samples which indicate the presence of other microorganisms that may be cultured with difficulties such as viruses , fungi and other agents or because of the misuse of antibiotics that cause the disappearance of the bacteria. Antibiotic treatment is recognized as an effective means to reduce transmission of the organism particularly for respiratory and cutaneous infections [11].

Genetic detection of Virulence factors of *Streptococcus pyogenes* by PCR

Molecular detection of (*emm* gene)

By using specific two primers for detection of M protein (*emm*) gene , the result shows that these genes were detected in all isolates bacteria (100%) with molecular length (914 bp) when compared with allelic ladder as shown in figure (1)

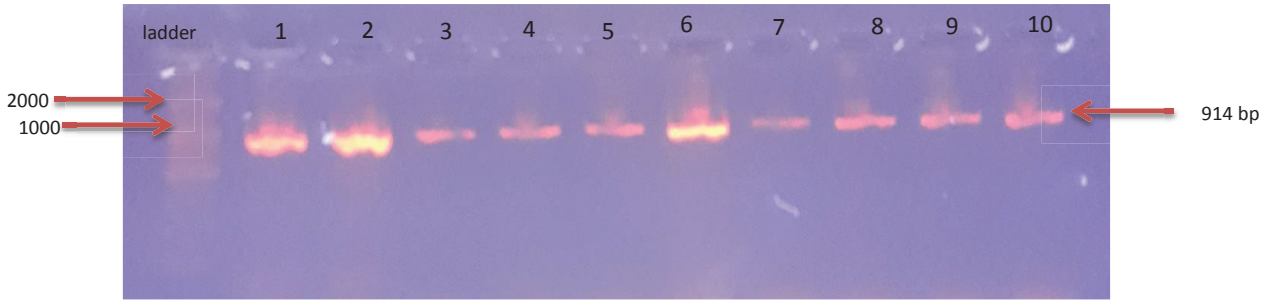

Figure (1) agarose gel electrophoresis at 70 volts for 50 min. for *emm* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 914 bp.

Khosravi *et al*, (2016) found that the *emm* gene detects in all isolates of GAS isolates isolated from throat samples in children with sore throat , and found that the types of *emm* gene different according to the types of diseases[12].

Molecular detections of pyrogenic exotoxins (*SpeB* ,*SpeC*)

In this study two primers were used to detect the SpeB gene and the result show that (100%) isolates contain the gene with molecular length (952 bp) when compared with allelic ladder as shown in figure (2)

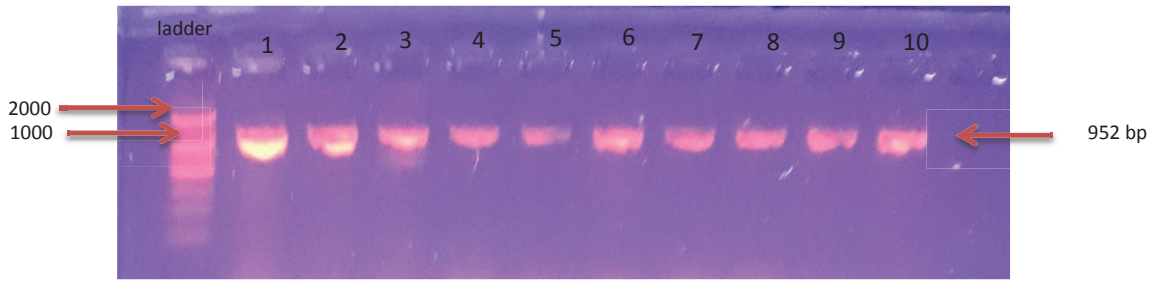

Figure (2) agarose gel electrophoresis at 70 volts for 50 min. for *speB* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 952 bp.

Hytonen *et al ,(*2001) found that the *SpeB* gene is carried by all strains of *S.pyogenes* , but the degree of expression varies from strain to strain , and *SpeB* has been considered to produce only in a secreted form . the expression of *SpeB* is controlled by the multiple gene activator *mga* [13].Also in this study the *SpeC* gene also study by using specific primer at molecular length (405 bp) and the result s show that (75%) give positive results to this gene when compared with allelic ladder as shown in figure (3)

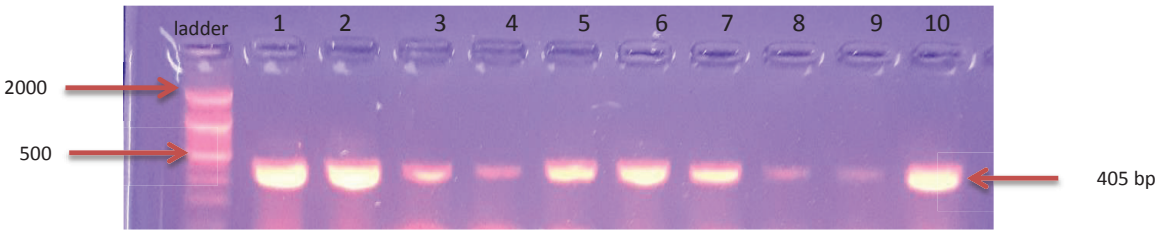

Figure (3) agarose gel electrophoresis at 70 volts for 50 min. for *speC* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 405 bp.

In this result it was found that *SpeB* is more prevalence than other gene *SpeC* this may due to that this gene coded by chromosome and *SpeB* is the key virulence factors in GAS pathogenesis .

Also the distribution in the prevalence these genes may attributed to site of infection or may the isolates contain other genes responsible for exotoxin , which it was found that there are (11) gene responsible for *Streptococcus pyogenes* exotoxin

Complement membrane attack complex (Mac) gene detected by PCR

Specific primer was used to amplify the *mac* gene in all *S.pyogenes* isolates the results show that (30%) give positive , results to this gene with molecular length (389 bp) when compared with an allelic ladder as shown in figure (4)

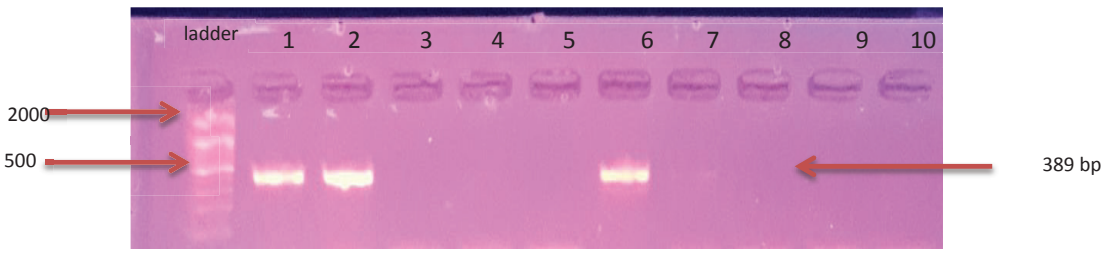

Figure (4) agarose gel electrophoresis at 70 volts for 50 min. for *mac* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 389 bp.

The different the percentage of this gene may be due to the bacterial contain other gene encoded for complement factor degradation other than mac like Endos and C5a peptidase .

Molecular detection of scpA (streptococcal C5a peptidase) by PCR .

DNA was extracted from (30) *streptococcus pyogenes* isolates , PCR was carried outusing these DNA from the amplification of specific primer (scpA) after the gel electrophoresis , the result shows that 17 isolates (56.6%) give positive result to this gene with amplicon size (622) when compared with allelic ladder as shown in figure (5)

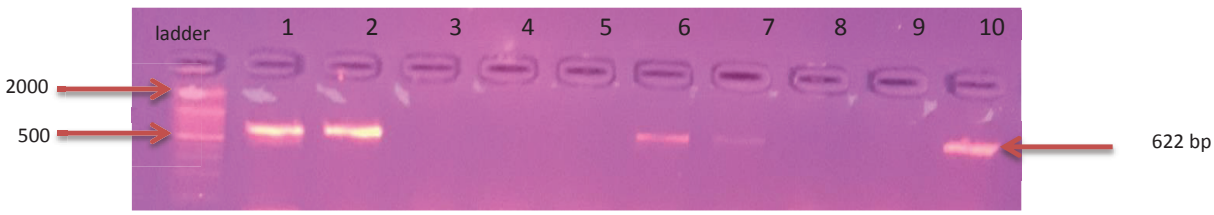

Figure (5) agarose gel electrophoresis at 70 volts for 50 min. for *scpA* gene in *S. pyogenes*. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 622 bp.

scpA decreases the rate of GAS clearance by inhibiting chemotactic recruitment of phagocytic cells to the site of infection , also shown to promote Fn independent GAS invasion of the human epithelial cell [(14)]. The negative results may belong to that the isolates contain another gene responsible for protease gene like (speB and SPYCEP) or gene is non-functional gene.

Conclusion

Humans are the only reservoir for GAS. It is most common among children 5 through 15 years of age ,presence of superantigen genes within the S. pyogenes genome suggests that they do play a significant role in S. pyogenes disease , high prevalence of *emm ,speB, sdaB* gene in *streptococcus pyogenes* isolates .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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