Mesopo. Environ. j., Special Issue E :58-66 , 2018
ISSN 2410-2598
proceeding of 2nd International conference of science and Art
University of Babylon and Liverpool John Moores
University, UK
Mesopotemia Environmental journal
journal homepage:www.bumej.com

Molecular Study of Some Virulence Factors in *streptococcus pyogens*

Zainab N Nabat[1] **Abdalnabi J Abid[2]** **Mohammad A Jabur[3]**

[1] Babylon Technical institute/ AL-Furat AL Awsat Technical University, 51015 Babylon, Iraq

[2]Collage Of Science for women /Department Of Biology/ / University Of Babylon

[3]Collage of Science /Department Of Biology/ / University Of Babylon

Corresponding author: zainabnasser1989@gmail.com

To cite this article:

Nabat Z. N., Abdalnabi J Abid and Jabur M. A., Molecular Study of Some Virulence Factors in *streptococcus pyogens* *Mesop. environ. j*., 2018, Special Issue E.;58-66.

Abstract

The study revealed the presence of certain gene in some isolate of bacteria, including the gene *SpeB* gene that gave large proportions in the study isolates 12 out of 37 at a rate of (32.4%), while in case of *SpeJ* gene 7 isolates out of 37(18.9 %) and *SmeZ* gene that gave 10 isolates out of 37at a rate of (27%), moreover *SpeA* gene the proportion of its existence appears in a few isolate 4 out of 37isolateat a rate of (19%). PCR assay was performed to identify the presence of some genes related to pathogenicity in bacteria responsible for Tonsillitis

Keywords: Tonsillitis, Streptococcus pyogens,PCR, Super antigen

Introduction

The expression *SpeA*, *SpeJ*, and *SmeZ* during experimental *S. pyogenes* pharyngitis, and *SmeZ* particular appears to mirror acute inflammation in tonsillitis [1]. Streptococcal pyrogenic exotoxin b (*SpeB*) gene was also used as a target for the detection of *S. pyogenes*[2]. Many extracellular virulence factors are also produced by GAS[3] .including several superantigens (SAgs), such as Streptococcal pyrogenic exotoxins. This Gram-positive bacterium produces a variety of exotoxins, known as streptococcal pyrogenic exotoxins (SPEs), which are believed to be involved in pathogenicity or virulence. These proteins are also known as superantigens [4].

Material and Methods

Sample collection and identification

This study includes a total of 100 swabs from patient's tonsillitis who admitted to Hilla General teaching hospital and Al-Noor hospital for children during the period from October 2013 to April 2014. Swabs were collected from patient tonsils by disposable transport media sterile. Swabs were taken and close it until transported to laboratory. Microbiological analysis was done and the organisms were identified by direct Gram staining, culture methods on Nutrient Agar, Blood Agar, MaCConkey agar, ,Simmon Citrate Medium and Eosin Methylene Blue (EMB) Agar at 37 for 42h**.** And different biochemical tests like catalase test, oxidase, indole, , Simmon Citrate, coagulase, voges-proskauer (VP), methyl red (MR),capsule test , motility test ,Bacitracin sensivity test, Urease and were performed for the diagnosis of *Streptococcus pyogenes*

DNA Extraction

A single colony of *Streptococcus pyogens*, which had been incubated overnight, transfer to sterile Brain heart infusion Broth and incubated at 37 ◦C for 24 hour. The DNA extracted using the genomic DNA purification kit Supplemented by the manufacturing Company.

PCR assay:

A PCR (Polymerase Chain Reaction) is performed in order to make a large number of copies of a gene. Otherwise, the quantity of DNA is insufficient and cannot be used for other methods of testing. A PCR is performed on an automated cycler, which heats and cools the tubes with the reaction mixture in a very short time. PCR is performed for 30-40 cycles, in three major steps [5].

1-Denaturation
2-Annealing
3-Extension

Primers:

Molecular assay in this study include 4gene .each one have specific nucleotide and product size in table (1)

Primer name	Product size (pb)	Sequences	References
SpeA F	576	′5AGGTAGACTTCAATTTGGCTTGTGT3′	6
SpeA R		′5GGGTGACCCTGTTACTCACG3′	
SpeJ F	247	′5 CTATGGTGGAATTACACC′3	7
SpeJ R		′5 CATGTTTATTGCCATTGATCGC′	

SmeZ F *SmeZ* R	391	′5TAACTCCTGAAAAGAGGC3′ 5′CATTGGTTCTTCTTGATAAG 3′	8
SpeB F *SpeB* R	423	′5GTAGCAACACATCCTGTAGCTGCA3′ ′5AGGTGCAC GAAGCG CAG AAG ATAT3 ′	9

The reaction mixture:

Amplification of DNA was carried out in a final volume of 20µl containing as in table [2].

(Table2): Protocols of PCR reaction mixture volumes

NO	Content s of reaction mixtures	Volume
1	Master mix	5 µl
2	Forward primer	2.5 µl
3	Reveres primer	2.5 µl
4	DNA template	5 µl
5	Nuclease free water	5 µl

Thermal reaction Cycling Conditions

Reaction processes for each gene in thermal cycle was differing in type of gene .Specificity and reference recommendation are as follows:

1-*SpeA*

Table (3) Thermal cycling condition

steps	Temperature	Time	NO. of cycles
1- Initial denaturation	95 °C	3min	1
2-Denaturation	95 °C	15sec	3
3-Anneling	60 °C	20sec	40
4-Elongation	72 °C	2min	2
5-Final elongation	72 °C	7min	2
6-Hold	4 °C	α	1

2-SpeJ

Table (4) Thermal cycling condition

steps	Temperature	Time	NO .of cycles
1- Initial denaturation	95 °C	10min	1
2- Denaturation	94 °C	30 s	3
3- Annealing	50 °C	30s	35
4- Elongation	72 °C	60	2
5- Final elongation	72 °C	2min	2
6- Hold	4 °C	α	1

3-Smez

Table (5) Thermal cycling condition

steps	Temperature	Time	NO. of cycles
1- Initial denaturation	94 °C	5min	1
2- Denaturation	94 °C	30sec	3
3- Annealing	50 °C	Sec30	30
4- Elongation	72 °C	60 sec	2
5- Final elongation	72 °C	7min	2
6-Hold	4 °C	α	1

4-*SpeB*

Table (6) Thermal cycling condition

steps	Temperature	Time	NO. of cycles
1- Initial denaturation	94 °C	3min	1
2- Denaturation	94 °C	7s	3
3- Annealing	55 °C	10s	25
4- Elongation	72 °C	5s	2
5- Final elongation	72 °C	3min	2
6-Hold	4 °C	α	1

Agarose Gel Electrophoresis

Agarose gel was prepared by dissolving 1gm of Agarose powder in 100 ml of TBE buffer (pH 8.0) in boiling water bath, allowed to cool to 50 °C, and ethidium bromide at concentration of 0.5 gm/ml was added. The Agarose poured kindly in equilibrated gel tray earlier set with two combs fixed in the end and in the middle, and the two ends of gel tray were sealed. The Agarose allowed solidifying at room temperature for 30 min. The combs and the seal were removed gently from the tray. The comb made wells used for loading DNA samples.5 µL of amplified PCR product were loaded to the Agarose gel wells followed by DNA marker (ladder) to one of the wells. The gel tray was fixed in electrophoresis chamber and TBE buffer was added to the chamber until covered the surface of the gel. The electric current was used at 70 volt for 2 hrs. UV transilluminator was used at 320 or 336 nm for the observation of DNA bands, and the gel was photographed using Egraph .UV transilluminator system [10].

Statistical Analysis

In this study analysis results are statistically calculated according to Factorial experimental with completely randomized design by LS.D,. *P* value of <0.05 [11].

Results

SpeA: The *SpeA* gene considered virulence factor gene for *Streptococcus pyogen*es and used for identification *Streptococcus pyogenes* and molecular size for *SpeA* is 576pb. The study shows that *SpeA* gene the proportion of its existence appears in a few isolate 4 out of 37at a rate of (10.8%), as in figure (1)

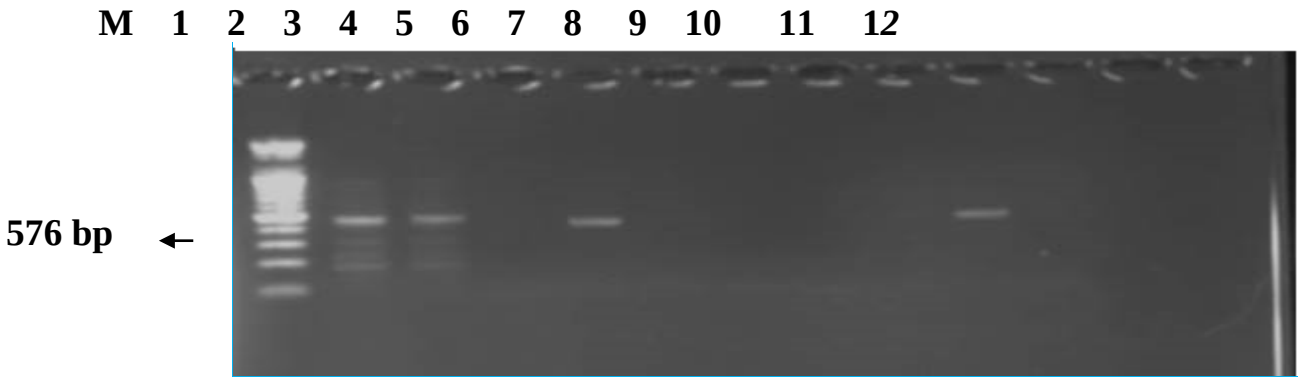

Figure (1) Agarose Gel Electrophoresis of 576bp PCR products of *SpeA* gene, lane M represent 1000bp molecular marker,Lane 1, 2, 4, 9 represent sample no. of positive results while lane3 ,5,6,7,8 ,10,11 and 12 represent sample no. of negative results.

SpeJ

The *SpeJ* gene considered as virulence factor gene for *S. pyogen*es and used for identification *S.pyogenes* and molecular size for 247bp.Positive results for amplification. This gene is about presence 7 isolates out of 37 at a rate of (18.9%), as in figure (2)

Mesopotamia Environmental Journal
Mesop. environ. j. 2018, Special Issue E.;58-66
ISSN 2410-2598
(proceeding of 2nd International conference of science and Art –University of Babylon and Liverpool John Moores University, UK).

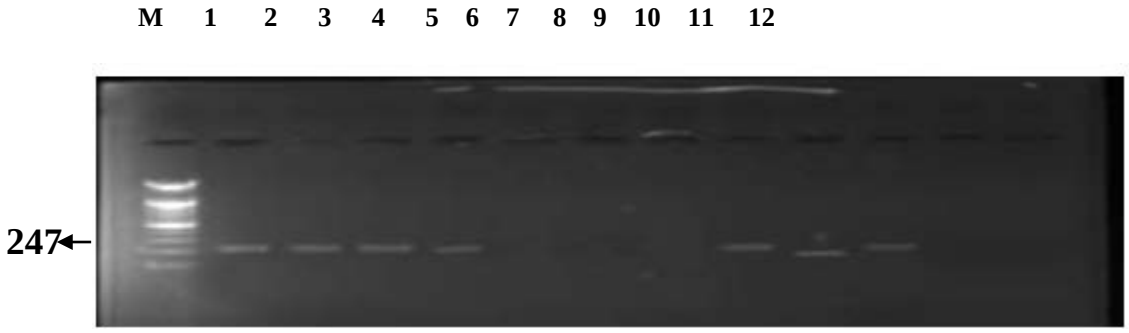

Figure (2) Agarose Gel Electrophoresis of 247bp PCR products of *SpeJ* gene, lane M represent 1000bp molecular marker, lane,1,2, 3, 4, 8,9,10 represent sample no. of positive results while 5,6,7 ,11 and 12 represent sample no. of negative results.

SmeZ

The *SmeZ* gene considered virulence factor gene for *S. pyogene*s and used for identification *S. pyogenes* and molecular size for 391pb.Positive results for amplification this gene that gave large proportions in the study isolates 10 out of 37 at a rate of (27 %), as in figure(3).

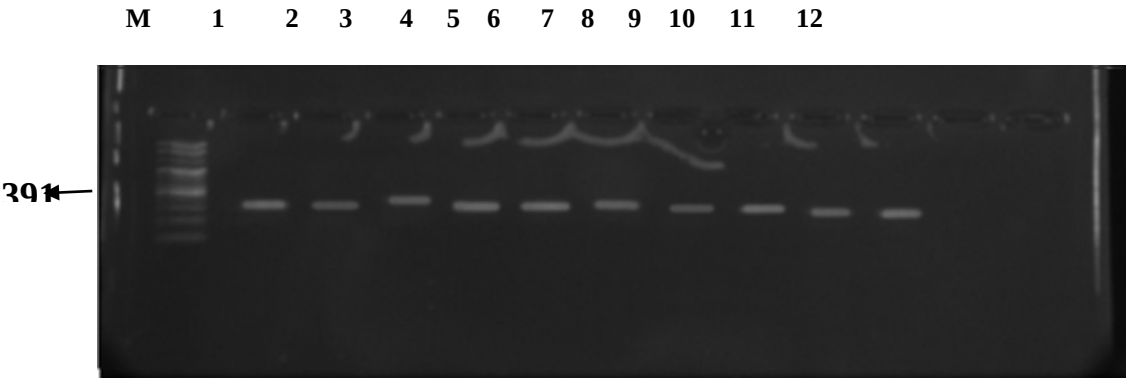

Figure (3) Agarose Gel Electrophoresis of391bp PCR products of *SmeZ* gene, lane M represent 1000bp molecular marker, 1, 2, 3, 4, 5,6, 7,8,9,10 represent sample no. of positive results while 11 and 12 represent sample no. of negative results.

SpeB

The *SpeB* gene considered virulence factor gene for *S.pyogene*s and used for diagnosis *S. pyogenes* and molecular size for 423.Positive results for amplification of this gene that gave large proportions in the study isolates 12 out of 37 at a rate of (32%), as in figure (4)

Mesopotamia Environmental Journal
Mesop. environ. j. 2018, Special Issue E.;58-66
ISSN 2410-2598
(proceeding of 2nd International conference of science and Art –University of Babylon and Liverpool John Moores University, UK).

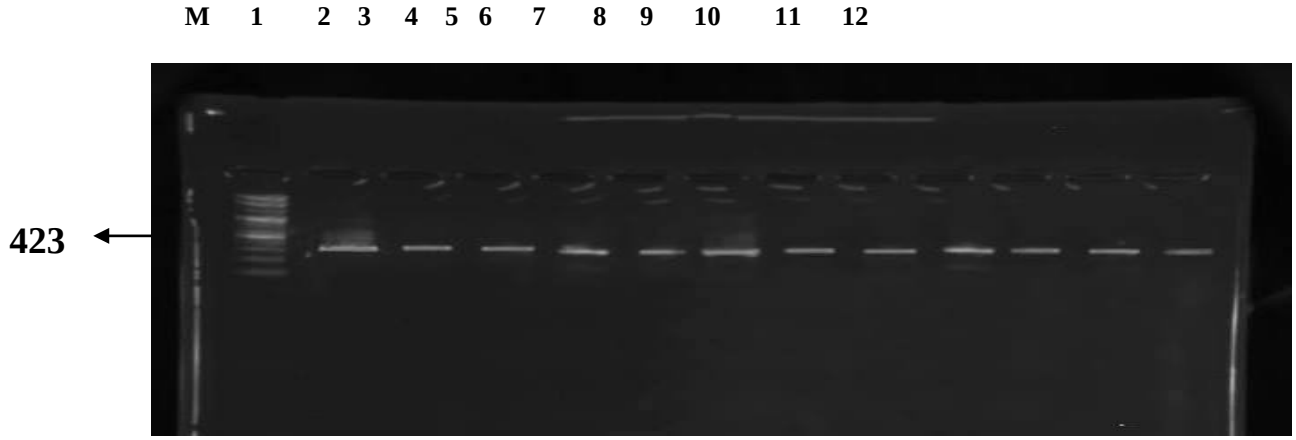

Figure (4) Agarose Gel Electrophoresis of423bp PCR products of *SpeB* gene, lane M represent 1000bp molecular marker, lane, 1, 2, 3, 4, 5,6, 7,8,9,10,11,12 represent sample no. of positive results.

Discussion

In this study the results revealed found that *SpeA* gene isolates appears in a few numbers 4 out of 37 at a rate of (10.8%) positive results and molecular weight 576pb while 8 negative results. These results when compared to other studies are agreement with [12].who found ratio of *SpeA* in tonsillitis patients was (12.7%). (13) Reported that the presence of *SpeA* showed a high association (81%) with scarlet fever strains, a lower association (18.4%) with pharyngitis isolates. [14] found the *SpeA* gene has been identified previously as a virulence factor associated with invasive disease. In this study, the results were show that *SpeJ* gene isolates were 7 out of 37at a rate (19.8%) positive result and molecular size 247pb while 5 isolates negative results. This study is in agreements with [15]. Who found *SpeJ* gene isolates (25.37%) but disagreements with [16].found *SpeJ* in tonsillitis patients was (84%). [17] found *SpeJ* increased in *S.pyogens* pharyngitis. In this study found *SmeZ* gene isolates 10 out of 37 at a rate (27%) positive result and molecular size 391pb this result agreements with [7]showed *SmeZ* gene isolate is(35%).disagreements with(18). Found *SmeZ* isolates 85% it may differ with this study because *SmeZ* have 23 alleles [13]. [1] Found *SmeZ* gene increased in patients Tonsillitis. While *SpeB* gene is virulence factor used for diagnosis of *Streptococcus pyogens* results found 12 isolates out of 37 at rate (32.4%) positive result and molecular size 423pb. This study is agreement with [16].who found *SpeB* isolates is (35%) in contrast with [19].who found *SpeB* isolates (69%).

Conclusions

The molecular study showed that *SpeB* gene that most prevalent in *Streptococcus pyogens* isolated from tonsils inflammation compared to other genes isolated.

Acknowledgment

The preferred spelling of the word "acknowledgment" in America is without an "e" after the "g". Avoid the stilted expression, "One of us (R. B. G.) thanks . . ." Instead, try "R. B. G. thanks". Put sponsor acknowledgments in the unnumbered footnote on the first page.

References

[1]Virtaneva,K.; Stephen ,F. P.; Morag, R. ;Graham, R .M.; Ireland.; Claire A.; Johnson, R. M. .;Claire A.; Johnson, S. M.; Ricklefs, I .;BabaLarye, D.; Parkins R. A.; Romero, G.; John R.; Bailey, J.;Corn, D .J.; Gardner M. J.; Parnell, and James M. M). Longitudinal analysis of the group A Streptococcus transcriptome in experimental pharyngitis in cynomolgus macaques. J.Annu. Rev. Med . Vol .102 No. (25):9014-9019. 2005

[2]-Louie, M.; Louie, L. and Simor, A.E.. The role DNA amplification technology in the diagnosis of infectious disease.J. Cand.Med.Asso.Vol. 63 No.(3):301-309. 2000

[3]Bisno, A.L.; Brito, M.O. and Collins ,C.M. Molecular basis of group A streptococcal virulence. J.Clin. Infect. Dis .3: 191–200. 2006.

[4]-Darenberg ,J.; Luca-Harari ,B.; Jasir, A.; Sandgren ,A.; Pettersson, H.; Schalen ,C.; Norgren M.; Romanus ,V.; Norrby-Teglund ,A.and Normark ,B.H. Molecular and clinical characteristics of invasive group A streptococcal infection in Sweden.j. Clin .Infect .Dis . 45:450-458. 2007

[5]-Jordan, R.C.K.; Daniels ,T.E.;Greenspan ,J.S and Regezi, J.A..Advanced diagnostic methods in oral and maxillofacial pathology Part 1:molecular method. J.Oral Maxillofac. Pathol .92(6): 650-669. 2001

[6]-Anna,I.; Brook,K .; Obszanska,W. H. and Izabela ,S..Detection of *Streptococcus pyogenes* virulence factors by multiplex PCR.j. Bioscience .3(6):5 -10. 2012

[7]-Robert,C.;Susan,R.;Travis,G.;Margie,D.;Jonathan,C.;Roy,R.B. and Nigle,C. Superantigen genes in group Astreptococcal isolates and their relationship with emm types . J .Med. Microbiol. 57:1238-1246.2008.

[8]-Schmitz, F.J,.;Beyer, A.; Charpentier, E.; Normark ,B.H.; Schade, MandFluit,A.C (2003).Toxin-gene profile heterogeneity among endemic invasive European group A streptococcal isolates. J. Infect. Dis. 188:1578-1586.

[9]- Naglaa, F. G.; Wesam ,H. A. and Mohammed, S.H.). *SpeB* Gene as an Early Specific Genetic Marker for Diagnosis of Group A Beta-Hemolytic *Streptococcus* (GABHS): Considerable virulence factor .J. Egyptian. Med .Microbiol. 23(1):99-108. 2014

[10]-Bartlett, J. S. and Stirling D.. PCR Protocols: Methods in Molecular and Biology. 2th. Humana Press Inc. Totowa. NJ. 1998

[11]-Sorlie, D.(1995).Medical biostatistics and epidomiology. Examination and board review. 1ed. Norwalk, Connecticut, Appleton and Lange. : 47-83.

[12]-Wang, X.; Zhang, Y. and Yang, X.O. (2012). Transcription of Il17 and Il17f is controlled by conserved noncoding sequence.j. Immunity. 36(2): 23–31.

[13]-Tyler, S.D.; Johnson, W.M.; Huang, J.C.; Ashton, F.E.; Wang, G.; Low, D.E. and Rozee, K.R. Streptococcal erythrogenic toxin genes. J. Clinical. Microbiol.30: 3127–3133. 1992

[14]-Beres ,S.B.; Richter ,E.W.; Nagiec, M.J.; Sumby, P.; Porcella, S.F.; DeLeo ,F.R and Musser ,J.M. Molecular genetics anatomy of inter and intra serotype variation in the human bacterial pathogen group A Streptococcus .J. Med. Microbial. 103: 7059–7064. 2006

[15]-Rivera, A.; Rebollo, M.and Miro ,E. .Superantigens gene profile, emm type and antibiotic resistance genes among group A streptococcal isolates from Barcelona, Spain. J. Med .Microbial. 55: 1115–1123. 2006

[16]-Broudy, T. B.; Pancholi, V. and Fischetti, V. A. Induction of lysogenic bacteriophages and phage-associated toxin from group *Streptococci* during culture with human pharyngeal cells.J. Infect. Immu. 69: 1440–1443. 2001

[17]-Linda, M.; Anna ,E and Mari, N. Super antigen gene profile diversity among clinical group A streptococcal isolates .j.Biomed .Laborat .Sci .45:236–244. 2008

[18]-Commons,R.;Rogers,S.;Gooding,T.;Danchin,M.;Carapetis,J.;Robin,N.;Browne,R.;and Curtis,N.Superantigens in group A streptococcal isolates and their relation with emm types .J.Med .Microbiol.8: 1246-1256. 2008

[19]-Dmitrieva ,N.F.; Trofimov, D.I.u.;Eshchina,A.S.; Riapis ,L.A.;Pavlova, O.G.; Petrova ,T.V.;Skorkina ,I.u.A.; Gerasimov ,A.N.;Alekseev,L.P.; Zhuravlev ,M.V.and Briko, N.I.Frequency of genes *SpeA*, *SpeB*, and *SpeC* in *Streptococcus pyogenes* strains and the identification of the infective agent by polymerase chain reaction .j. Microbiol .Epidemiol .Immunobiol. (5):3-6. 2002