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Streptococcal Pyrogenic Exotoxin Genes SpeA and SpeB in Isolates of *Streptococcus pyogenes* from Children with Pharyngitis, Gezira State, Sudan

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Abstract

Background: *Streptococcus pyogenes* (group A streptococcus, GAS) is an important human bacterial pathogen. This organism possesses many virulence factors, Streptococcal pyrogenic exotoxinone of these. **Aim:** Detection of Streptococcal pyrogenic exotoxin SpeA and SpeB in isolated *Streptococcus pyogenes.* **Methods:** Tow hundred throat swab samples were collected from children with pharyngitis referred to Pediatric Teaching hospital and ENT hospital Wad medani, Sudan, from January to November 2021. The questionnaire was filled out to collect clinical and demographic data. Throat swabs were collected and processed with the standard microbiological procedure to isolate *Streptococcus pyogenes*. Antimicrobial susceptibility testing was done on all GAS isolates using the Kirby Bauer disk diffusion method according to clinical laboratory standard institute (CLSI) guidelines. Detection of Spy 1258 gene and Streptococcal pyrogenic exotoxins SpeA and SpeB were done by using Multiplex PCR. **Results:** Amongst the Tow hundred collected samples fifty-one isolates (25.5%) were identified as *S. pyogenes*. Antibiotic susceptibility testing showed that all the GAS isolates were sensitive to Azithromycin and Penicillin. Sensitivity to Erythromycin, Gentamicin, Clarithromycin, Amoxicillin and Cephalexin were 88.2%, 86.3%, 45.1%, 41.2%, 13.7%, respectively. SpeA was detected in 17 (33.3%) and SpeB in 48 (94.1%). **Conclusion:** Streptococcal pyrogenic exotoxin genes SpeA and SpeB were detected in 17 (33.3%) and 48 (94.1%) respectively of *Streptococcus pyogenes*

isolates.

Keywords

Streptococcus pyogenes, Streptococcal Pyrogenic Exotoxin Genes, Sudan, SpeA, SpeB

1. Introduction

Streptococcus pyogenes (Group A Streptococci GAS) cause a diverse of human diseases, from uncomplicated superficial infections of the respiratory tract and skin to severe invasive diseases associated with high morbidity and mortality. Many virulence factors of GAS have been identified, including bacterial surface proteins, secreted streptolysins, hyaluronic acid capsule hyaluronidase, streptokinase and DNase [1], and among the major virulence factors of GAS are the secreted Streptococcal pyrogenic exotoxins (Spe), which act as superantigens (SAg) due to their ability to interact with the host major histocompatibility complex (MHC) class II molecules and with the changeable region of the T-cell receptor β-chain without previous processing by antigen-presenting cells. This interaction ends up in the activation of an oversized range of T-cells. Eleven distinct SAgs are known in GAS all of them sharing a standard macromolecule fold and therefore, the same target receptors on host cells: 3 chromosomally encoded (SpeG, SpeJ, and SMEZ) and eight encoded on temperate phages (Spe A, Spe C, Spe H, Spe I, Spe K, Spe L, Spe M, and SSA). Spe B and Spe F are each encoded on the microorganism body. Multiple forms are recognized in Spe A, Spe C, Spe G, SSA, and SmeZ that will be related to variations in super-antigenic activity and in substance properties [2]. The severity of GAS infections depends on multiple host and microorganism factors. The infective properties of GAS strains square measure typically coupled to the assembly of virulence factors like toxins, proteases or DNases and toxins gifted within the explicit GAS strain are often the predictor of its invasiveness [3].

The results from many studies recommend that the genetic background of the host may play a vital role in invasive illness conditions [4]. Superantigens contribute to GAS pathogenicity supported by their immune stimulatory activity [5]. Superantigens distribution has been used as a technique for the detection of genomic heterogeneousness, the correlation between gene contents and therefore, the determination of clinical manifestation [6]. Their biological toxicity and environmental stability have resulted in some superantigens being classified as chosen agents of the terrorist act [7]. The Spe toxins measure contributes to the pathologic process of severe invasive unwellness and has additionally been involved in reaction disorders [8]. The streptococcal pyrogenic toxin B (SpeB), is the predominantly secreted cysteine protease of GAS acid enzyme of GAS. SpeB degrades host protein like human extracellular matrix, immunoglobulins and

complement elements. Destruction of each host and microorganism proteins makes SpeB the key virulence consider GAS pathologic process. Though many lines of proof have shown that SpeB is a very important virulence factor of GAS, its role in bacterial infection remains contentious [1].

2. Methods

A cross-sectional study was done and samples were collected from children attending Pediatric Teaching Hospital and (ENT) hospital Wad medani, Sudan with symptoms of Pharyngitis, ages 5 to 17 years from January to November 2021. Exclusion criteria included Children with respiratory tract symptoms such as rhinorrhea or nasal congestion and prior antibiotic therapy in less than 7 days.

2.1. Ethical Approval

The study was approved by the Ethics Committee of the Ministry of Health, Gezira State.

2.2. Isolation of Bacteria

Throat swabs were inoculated on 5% sheep blood agar plates and incubated in 5% - 10% CO_2 at 37˚C for 24 h. Identification of GAS isolates was made based on beta-hemolytic activity on sheep blood agar, small colony characteristics, Gram stain positive cocci (Streptococci), catalase production negative, 0.04-U bacitracin disc susceptiblility and PYR test were positive.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial sensitivity testing was done using the standard disk diffusion method on Mueller Hinton agar supplemented with 5% sheep blood, incubated overnight in 5% - 10% CO_2 at 37˚C. The commercial antibiotic discs were used to determine the susceptibility of isolates to penicillin (10 U), Azithromycin (15 µg), Clarithromycin (15 µg), Erythromycin (15 µg), Amoxicillin (10 mcg), Gentamicin (10 mcg), and Cephalexin (30 mcg).

2.4. PCR Amplification of SpeA and SpeB

Streptococcal pyrogenic exotoxin genes were detected by using the Multiplex PCR amplification method. The target genes included Streptococcal Pyrogenic exotoxin genes SpeA and SpeB. Spy 1258 was used as an internal control.

2.4.1. DNA Extraction

DNA was isolated from *Streptococcus pyogenes* using standard laboratory protocol. DNA was extracted by Boiling method, *S. pyogenes* isolates were placed in 500 µL of Trise EDTA (TE) buffer and frozen in −70˚C, after thawing 150 µL of this suspension was taken and heated at 95˚C for 30 minute, centrifuge was used and spin at 14,000 rpm for 5 minute at 25˚C and the supernatant was taken into

a new sterilized tube.

2.4.2. Primer Design

Identification of SpeA and SpeB genes by Polymerase Chain Reaction (PCR) was done by using primers designed according to Table 1.

2.4.3. PCR Mix

The standard PCR reaction mixture used in the amplification of the DNA target was contained in a total volume of 14.4 µL in 0.5 mL Eppendorf tube, containing 3 µL DNA, 5 µL PCR master mix, 0.6 µL of Forward and Reverse primer of SpeA, 0.3 µL of Forward and Reverse primer of SpeB. 0.3 µL of Forward and Reverse primer of SPY 1258 and 4 µL PCR water.

2.4.4. PCR Amplification

Amplification reactions were performed for 35 cycles using PCR program shown in Table 2.

2.4.5. Gel Electrophoresis

Amplicons were visualized on 1.5% Agarose gel by Electrophoresis; the PCR products were electrophoresed through agarose gel with current 120 V for about 30 min. Gels are photographed under UV light.

2.4.6. Statistical Analysis

Statistical analysis was done by SPSS statistical software. Participants' demographic and clinical characteristics were described by using descriptive statistics.

Table 1. Sequence of primers used for Multiplex PCR Amplification of SpeA, SpeB and Spy1258 genes.

Gene	Primer name	Primer Sequence	Product length
SpeA	SpeA-F	5'-CCAAGCCAACTTCACAGATC3'	309 bp
	SpeA-R	5'-CCCTTCATGATTTGTTACCCC3'	
SpeB	SpeB-F	5'-GTGGAGTCTCTGACGGCTTC3'	191 bp
	SpeB-R	5'-GTGTTTTCGGCACAAAAGGT3'	
SPY1258	SPY1258-F	5'-AAAGACCGCCTTAACCACCT3'	407 bp
	SPY1258-R	5'-TGCCAAGGTAAACTTCTAAAGCA3'	

Table 2. PCR program.

	Temperature	Time
Initial Denaturation	94˚C	3 min.
Denaturation	94˚C	1 min.
Annealing	53˚C	1 min.
Elongation	72˚C	1 min.
Final Elongation	72˚C	3 min.

P-value less than 0.05 taken as statistically significant at 95% confidence level.

3. Results

In this study, a total of 200 throat swabs were collected from Pharyngitis children from January to November 2021. Females accounted 126 (63%) and males were 74 (37%). The rate of *Streptococcus pyogenes* was 25.5% (51/200) which has been identified by using culture and biochemical tests. Total number of males infected by *S. pyogenes* was 13 (25.5%) and females were 38 (74.5%).

3.1. Antimicrobial Susceptibility Testing

The *S. pyogenes* were sensitive to Penicillin and Azithromycin. Sensitivity to Erythromycin, Gentamicin, Clarithromycin, Amoxicillin, Cephalexin were 88.2%, 86.3%, 45.1%, 41.2%, 13.7%, respectively.

Resistance of Cephalexin was 70.6%; there was no significant association between the patients having SpeA and SpeB genes and resistance of bacteria to Cephalexin in Table 3 and Table 4.

3.2. Detection of Streptococcal Pyrogenic Exotoxins

Streptococcal pyrogenic exotoxin A (SpeA) was detected in 17 samples (33.3%) and SpeB in 48 samples (94.1%) of *streptococcus pyogenes* isolates (Table 5). Figure 1 shows the result of Multiplex PCR of Spy 1258, SpeA and SpeB genes.

There was a significant association with P-value between the patients having SpeA gene and the presence of cervical lymphadenopathy (Table 6). And there

Table 3. SpeA and cephalexin resistance.

		Cephalexin		P-Value	O.R	C.I (95%)
		Sensitive	Resistance			
SpeA	Absence	10 (76.9%)	24 (63.2%)			
	Presence	3 (23.1%)	14 (36.8%)	0.57	0.9	0.6 - 1.2

Table 4. SpeB and cephalexin resistance.

		Cephalexin		P-Value	O.R	C.I (95%)
		Sensitive	Resistance			
SpeB	Absence	1 (7.7%)	2 (5.3%)			
	Presence	12 (92.3%)	36 (94.7%)	1.0	0.89	0.93 - 2.0

Table 5. Distribution of Streptococcal pyrogenic exotoxin genes.

Gene	Frequency	Percentage
SpeA	17	33.3%
SpeB	48	94.1%
Total	51	100%

Table 6. Correlation between SpeA and cervical lymphadenopathy.

		SpeA		P-Value	O.R	C.I (95%)
		Absence	Presence			
Cervical lymphadenopathy	Absence	10 (29.4%)	11 (64.7%)			
	Presence	24 (70.6%)	6 (35.3%)	**0.035**	**2.6**	**1.1 - 5.9**

Table 7. Correlation between SpeB and fever.

		Fever		P-Value	O.R	C.I (95%)
		Absence	Presence			
SpeB	Absence	0 (0.0%)	3 (20.0%)			
	Presence	36 (100.0%)	12 (80.0%)	0.035	4.0	2.5 - 6.5

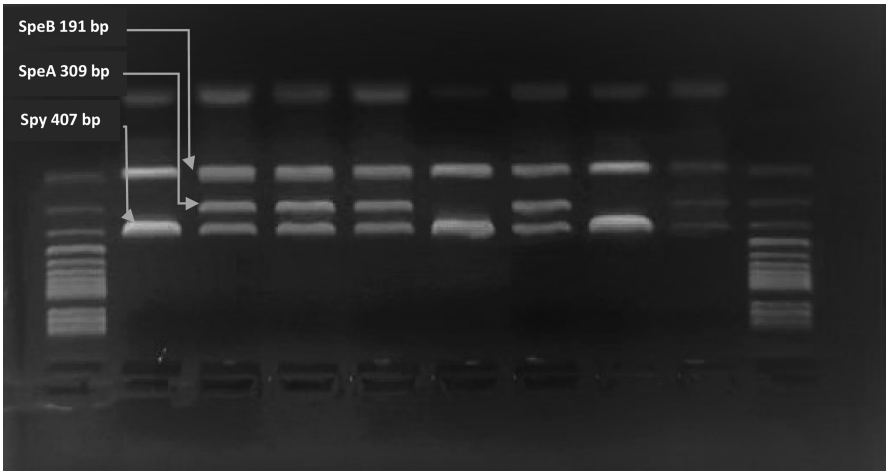

Figure 1. Multiplex PCR of Spy 1258, SpeA and SpeB genes.

was a significant association between the patients having SpeB gene and the presence of fever was detected. Patients having this gene prospect get a fever by about 4 times (Table 7) more than patients who haven't this gene.

4. Discussion

The outcome of GAS infection is not only strain related but is also related to a combination of several factors, such as exotoxin production and host immunity. Bacterial characteristics may also play a pathogenic and important role in the severity of streptococcal infections [9].

Streptococcus pyogenes is sensitive to penicillin; different studies worldwide showed that like [10] study from Iran, [11] study from Senegal, [12] study from Ethiopia, [13] study from China and our study also showed that which confirms the penicillin is still the drug of choice for the treatment of GAS pharyngitis.

Resistance to antimicrobial to *Streptococcus pyogenes* is variable and the emergence of drug resistance among streptococci to macrolides (erythromycin

and clarithromycin) is widely reported [14]. Our study showed the resistance to Clarithromycin was 33.3% similar to [10], showing resistance 33.9% and the resistance to Erythromycin was 7.8% agree with 9.7% of [15] study and 6.9% of [9] study. And also show 52.9% resistance to amoxicillin, whereas the study of [5] from Egypt showed 81% sensitivity to amoxicillin.

The Superantigen SpeA was found in 33.3% of this study and SpeA was 36.8% in the study of [5]. Also, this result is similar to [13] study which showed the percentage of SpeA gene was 34.34%. A study of [9] showed a lower percentage of SpeA (17.2%)

SpeB gene was detected in 94.1% of *Streptococcus pyogenes* isolates and showed 100% specificity agrees with [16] study that showed 100% sensitivity and 100% specificity. Among the toxin genes that are thought to be chromosomally encoded, SpeB was found in all isolates [17]. This finding differs markedly from that reported in [9] which showed 72.4% of SpeB. And up-close to [17] showed the presence of SpeB was 100% in their isolated *Streptococcus pyogenes,* also reporting the SAg and antibiotic resistance genes appeared to be associated with the emm type. The streptococcal pyrogenic exotoxin B gene was associated with pyrogenicity, T-lymphocyte mitogenicity, and the ability to increase susceptibility to endotoxic shock in individuals infected with group A streptococcus [18]. Our study showed there was a significant association between the patients having SpeB gene and fever. SpeA was detected in 33.3% of *streptococcus pyogenes* isolates. [4] Reported the SpeA gene was found in a majority (40% - 90%) of *S. pyogenes* isolates from the USA associated with invasive disease and STSS, but only in a minority (15% - 20%) of isolates from noninvasive diseases. A high frequency of SpeA (80%) was found in STSS isolates collected in Australia. In [9] study isolates from patients with pharyngotonsillitis, the frequencies were 17.2% for SpeA, 72.4% for SpeB. And in [19] the incidence of SpeA, SpeB and SpeF were 5 (83%), 56 (933%) and 53 (883%), respectively.

5. Conclusion

Streptococcal pyrogenic exotoxin genes SpeA and SpeB were detected in 17 (33.3%) and 48 (94.1%) respectively of *Streptococcus pyogenes* isolates.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Chuan, C.-N., and Wu, J.-J. (2008) Effects of Streptococcal Pyrogenic Exotoxin B on Pathogenesis of *Streptococcus pyogenes*. *Journal of the Formosan Medical Association*, **107**, 677-685. https://doi.org/10.1016/S0929-6646(08)60112-6

[2] Friães, A., Pinto, F.R., Silva-Costa, C., Ramirez, M. and Melo-Cristino, J. (2013) Superantigen Gene Complement of *Streptococcus pyogenes*—Relationship with Other Typing Methods and Short-Term Stability. *European Journal of Clinical Microbi-*

ology & Infectious Diseases, **32**, 115-125.
https://doi.org/10.1007/s10096-012-1726-3

[3] Borek, A.L., Obszańska, K., Hryniewicz, W. and Sitkiewicz, I. (2012) Detection of *Streptococcus pyogenes* Virulence Factors by Multiplex PCR. *Virulence*, **3**, 529-533. https://doi.org/10.4161/viru.21540

[4] Proft, T. and Fraser, J.D. (2016) Streptococcal Superantigens: Biological Properties and Potential Role in Disease. In: Ferretti, J.J., Stevens, D.L. and Fischetti, V.A., Eds., *Streptococcus pyogenes*: *Basic Biology to Clinical Manifestations*, University of Oklahoma Health Sciences Center, Oklahoma City.

[5] Helal, Z.M., Rizk, D.E., Adel El-Sokkary, M.M. and Hassan, R. (2020) Prevalence and Characterization of *Streptococcus pyogenes* Clinical Isolates from Different Hospitals and Clinics in Mansoura. *International Journal of Microbiology*, 2020, Article ID: 5814945. https://doi.org/10.1155/2020/5814945

[6] Chang, H.S., Shen, X.Z., Huang, G.Y., Fu, Z., Zheng, Y.J., Wang, L.B., Li, C.R., Liu, L., Shen, Y. and Liu, X.R. and Yang, Y.H. (2011) Molecular Analysis of *Streptococcus pyogenes* Strains Isolated from Chinese Children with Pharyngitis. *Diagnostic Microbiology and Infectious Disease*, **69**, 117-122. https://doi.org/10.1016/j.diagmicrobio.2010.09.011

[7] Spaulding, A.R., Salgado-Pabón, W., Kohler, P.L., Horswill, A.R., Leung, D.Y. and Schlievert, P.M. (2013) Staphylococcal and Streptococcal Superantigen Exotoxins. *Clinical Microbiology Reviews*, **26**, 422-447. https://doi.org/10.1128/CMR.00104-12

[8] Kalia, A. and Bessen, D.E. (2003) Presence of Streptococcal Pyrogenic Exotoxin A and C Genes in Human Isolates of Group G Streptococci. *FEMS Microbiology Letters*, **219**, 291-295. https://doi.org/10.1016/S0378-1097(03)00022-3

[9] Wu, P.-C., Lo, W.-T., Chen, S.-J. and Wang, C.-C. (2014) Molecular Characterization of Group A Streptococcal Isolates Causing Scarlet Fever and Pharyngitis among Young Children: A Retrospective Study from a Northern Taiwan Medical Center. *Journal of Microbiology, Immunology and Infection*, **47**, 304-310. https://doi.org/10.1016/j.jmii.2013.02.007

[10] Sayyahfar, S., Fahimzad, A., Naddaf, A. and Tavassoli, S. (2015) Antibiotic Susceptibility Evaluation of Group A Streptococcus Isolated from Children with Pharyngitis: A Study from Iran. *Infection & Chemotherapy*, **47**, 225-230. https://doi.org/10.3947/ic.2015.47.4.225

[11] Camara, M., Dieng, A. and Boye, C.S.B. (2013) Antibiotic Susceptibility of *Streptococcus pyogenes* Isolated from Respiratory Tract Infections in Dakar, Senegal. *Microbiology Insights*, **6**, 71-75. https://doi.org/10.4137/MBI.S12996

[12] Kebede, D., Admas, A. and Mekonnen, D. (2021) Prevalence and Antibiotics Susceptibility Profiles of *Streptococcus pyogenes* among Pediatric Patients with Acute Pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Microbiology*, **21**, Article number: 135. https://doi.org/10.1186/s12866-021-02196-0

[13] Li, H.X., Zhou, L., Zhao, Y., Ma, L.J., Liu, X.Y. and Hu, J. (2020) Molecular Epidemiology and Antimicrobial Resistance of Group A Streptococcus Recovered from Patients in Beijing, China. *BMC Infectious Diseases*, **20**, Article number: 507. https://doi.org/10.1186/s12879-020-05241-x

[14] Sunaoshi, K., Murayama, S.Y., Adachi, K., Yagoshi, M., Okuzumi, K., Chiba, N., Morozumi, M. and Ubukata, K. (2010) Molecular Emm Genotyping and Antibiotic Susceptibility of *Streptococcus dysgalactiae* Subsp. *equisimilis* Isolated from Invasive and Non-Invasive Infections. *Journal of Medical Microbiology*, **59**, 82-88.

https://doi.org/10.1099/jmm.0.013201-0

[15] Ali, H.N., Dhahi, M.A.R. and Abd, A.K.H. (2015) Molecular Screening for Erythromycin Resistance Genes in *Streptococcus pyogenes* Isolated from Iraqi Patients with Tonsilo-Pharyngites. *African Journal of Biotechnology*, **14**, 2251-2257. https://doi.org/10.5897/AJB2014.14365

[16] Dunne, E.M., Marshall, J.L., Baker, C.A., Manning, J., Gonis, G., Danchin, M.H., Smeesters, P.R., Satzke, C. and Steer, A.C. (2013) Detection of Group a Streptococcal Pharyngitis by Quantitative PCR. *BMC Infectious Diseases*, **13**, Article number: 312. https://doi.org/10.1186/1471-2334-13-312

[17] Rivera, A., Rebollo, M., Miro, E., Mateo, M., Navarro, F., Gurguí, M., Mirelis, B. and Coll, P. (2006) Superantigen Gene Profile, Emm Type and Antibiotic Resistance Genes among Group A Streptococcal Isolates from Barcelona, Spain. *Journal of Medical Microbiology*, **55**, 1115-1123. https://doi.org/10.1099/jmm.0.46481-0

[18] Louie, L., Simor, A.E., Louie, M., Mcgeer, A. and Low, D.E. (1998) Diagnosis of Group A Streptococcal Necrotizing Fasciitis by Using PCR to Amplify the Streptococcal Pyrogenic Exotoxin B Gene. *Journal of Clinical Microbiology*, **36**, 1769-1771. https://doi.org/10.1128/JCM.36.6.1769-1771.1998

[19] Nandi, S., Chakraborti, A., Bakshi, D.K., Rani, A., Kumar, R. and Ganguly, N.K. (2002) Association of Pyrogenic Exotoxin Genes with Pharyngitis and Rheumatic Fever/Rheumatic Heart Disease among Indian Isolates of *Streptococcus pyogenes*. *Letters in Applied Microbiology*, **35**, 237-241. https://doi.org/10.1046/j.1472-765X.2002.01176.x