Study Characteristics of Streptococcus Pyogenes Isolation From Pharyngitis in Children

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Abstract

Background: Streptococcus pyogenes is a common cause of bacterial pharyngitis in children. Although distinguishing between viral and bacterial pharyngitis solely on the basis of signs and symptoms can be difficult, culture-based diagnosis and study characteristics are crucial to avert potentially fatal outcomes. Therefore, the purpose of this investigation was to ascertain the occurrence of S. pyogenes using a culture approach that followed a biochemical test and a PCR experiment that targeted the 16S rRNA, sepl, and spek genes. By logging and evaluating the results, the PCR assay's sensitivity, specificity, positive, and negative predictive values were established in relation to the culture method. Methods: Between 2022 and 2023, a total of 170 throat swabs were taken from pharyngitis patients who were referred to Fallujah General Teaching Hospital and AL Hussein Teaching Hospital for children in Iraq ages 2 to 10. The identification of S. pyogenes using biochemical testing, 16S rRNA, and multiplex polymerase chain reaction (multiplex PCR) for the detection of virulence factor genes (SpeL and SpeK genes). Results: This study included a total of 170 children with acute pharyngitis. Of these, 75 (44.11%) were culture optimistic for S. pyogenes, a biochemical test and 16SrRNA based on the Multiplex PCR examination presented, sepl 9 (12%)and spek 6 (8%) genes were noticed in 10.51% and 8.55%, respectively, of the isolates. Conclusion: The study aimed to identify risk factors for S. pyogenes infection among children in a large clinical trial in Iraq.

Introduction

Group A Streptococcus or Streptococcus pyogenes, is a gram-positive bacterium that causes a range of infectious diseases. Necrotizing fasciitis, meningitis, bacteremia, and rheumatic heart disease (RHD) are examples of invasive infections [1-3]. The more general word "pharyngitis" refers to acute inflammation of the tonsils, pharynx, or both throughout this review, Throat pain is the most typical sign of pharyngitis.
While viruses are the most common cause of pharyngitis, *S. pyogenes*, also referred to as group A β-haemolytic *Streptococcus* (GAS), is the most frequently found bacterium during acute pharyngitis [4,5]. Every year, an estimated 450 million children globally are thought to get GAS pharyngitis [6].

While most cases are benign and resolve on their own in a week, others are suppurative. Acute glomerulonephritis, Sydenham's chorea, scarlet fever, and an autoimmune neuropsychiatric disorder linked to group A *Streptococci*, retropharyngeal abscess, peritonsillar cellulitis or abscess, sinusitis, acute otitis media, and mastoiditis are examples of non-suppurative post-streptococcal diseases that can arise [7]. Clinical conditions associated with *S. pyogenes* included scarlet fever, acute rheumatic fever, glomerulonephritis, sepsis, necrotizing fasciitis, meningitis, streptococcal toxic shock syndrome, impetigo, and acute pharyngitis [8]. An estimated 100 million people annually contract a serious *S. pyogenes* infection, which also causes 660,000 invasive infections, 616 million instances of pharyngitis, and 163,000 deaths annually, according to data from 2009 to 2014 [9]. *S. pyogenes* was identified in youngsters suffering from acute pharyngitis in African nations. For instance, in Nigeria [10], Egypt [11], Kenya [12], Jimma, Ethiopia [13], and other countries, the frequency was as high as 66.7%, 28%, 2.3%, and 11.3%, respectively. The most common bacterial cause of pharyngitis today is *S. pyogenes*, which initially affects school-age children between the ages of 5 and 15 [14].

In Canada, the number of GAS infections increased from 2.4 cases per 100,000 people in 2003 to 5.24 cases in 2015 [15]. According to reports, the incidence rate of GAS disease in the UK is 2.9 cases per 100,000 people annually [16]. Socioeconomically disadvantaged communities tend to have higher rates of GAS infections and related complications [17,18]. This covers both emerging nations and the economically disadvantaged citizens of affluent nations [19, 20]. Common among school-age children, this infection peaks in the winter and early spring and is mainly transmitted by direct contact with the saliva or nasal secretions of sick individuals in crowded environments [22-24]. Diagnostics can be made using laboratory techniques, such as the gold standard tonsil culture, molecular testing, or the rapid antigen detection test (RADT), which has a 95–96% specificity and an 85–86% sensitivity in children [25, 26]. Regarding whether a culture is necessary for the diagnosis of *S. pyogenes* and whether a prescription for antibiotics is necessary for therapy, guidelines from different countries differ considerably. The distribution of virulence factors varies among *S. pyogenes* strains. Some are linked to the presence of mobile genetic elements, and some are encoded chromosomally. The profile of virulence factors that a specific strain encodes is one of its properties; determining whether these factors are present or absent can be a straightforward diagnostic technique in clinical diagnostics [27]. We have recently created a technique that broadens the scope of previous approaches. The set of four low-volume multiplex PCR procedures detects two GAS virulence factors concurrently, in addition to superantigens (*sepl* and *spek* genes). The current study aims to identify 16S rRNA of *Streptococcus pyogenes* and detect *sepl* and *spek* virulence factor genes as targets in *S. pyogenes* for multiplex polymerase chain reaction.

**Materials and Procedures**

**Study design**

The Comprehensive children hospital in the Iraqi city of Karbala served as the site of the research. The pediatric hospital serves almost two million residents of Karbala and the neighboring areas. From January 5, 2021, to September 17, 2021, a cross-sectional study was carried out in the pediatric hospital located in Karbala. The study was hospital-based.
1. **Isolation and identification of *S. pyogenes***

Every research participant had one pharynx swab sample taken with a sterile cotton swab over the tonsils and posterior pharynx for culture. The sample was taken from the afflicted area, where a piece of cotton was wrapped three times over the secretions, tonsils, and pharyngitis. And transported to the microbiology laboratory at Wraith Al-Anbiyaa, Faculty of Medicine, within 4 hours. *S. pyogenes* isolates that were presumptively identified were collected from the different hospitals and patient centers that formed part of the study. Isolates were added to 5% sheep blood agar plates, which were then left to incubate at 37°C for the entire night. This catalase is negative. *Streptococcus* species were used in the bacitracin test. Thus, a bacitracin disk was placed on inoculated 5% sheep blood agar, and colony suspension was prepared using normal saline matched with 0.5 McFarland standards from newly developed 24-hour colonies. Any inhibition was thus shown to be bacitracin sensitive for *S. pyogenes* and biochemical tests, as well as types of enzymatic reactions, after a 24-hour incubation period at 37°C [28, 29].

2. **Extraction of DNA from *S. pyogenes* on pharynx swabs**

The water boiling procedure was used to extract the bacterial DNA template. To sum up, the bacteria were injected into LB liquid medium and allowed to thrive over night at 37°C. A 1.5 mL EP tube was filled with one milliliter of bacterial culture, centrifuged for two minutes at 12,000 RPM, and the supernatant was disposed of subsequently, the sediments were again suspended in 1000 μL of water, centrifuged using the same parameters, and the supernatant was disposed of. To lysate the thallus, add 500 μL of water and boil for 10 to 15 minutes at 95°C. Centrifuged at 13,500 rpm for 10 minutes at 20°C after freezing for 10 to 15 minutes. After being extracted into a new EP tube, the genomic DNA supernatant was kept at -20°C. The following *S. pyogenes* 16S rRNA, (sepl and spke) genes were amplified by PCR using sterile water as the blank control and *S. pyogenes* ATCC CP003901. In the current work, the 16S rRNA gene sequences from the NCBI that belong to the genus *Streptococcus* were analyzed.

3. **Detection *S. pyogenes* genes by Polymerase Chain Reaction Assay**

The *S. pyogenes* isolates were cultivated for a whole night on LB broth (Thermo-Fisher Sentific). Genes encoding for virulence factors were found using the PCR method (Table 1). The master mix PCR, primers, and thermal cycler system (Australia) were utilized for the PCRs. For PCR, the 405-bp 16S rRNA gene (GenBank accession number: 2353759) was selected as the GBS primer [32]. The primers' forward and reverse sequences were (Table 1). The control positive isolates were supplied by the Iraqi Karbala University of Medical Sciences.

The following are the parameters for multiplex PCR cycling: The heating process involves an initial one-minute heating at 95°C, followed by 25 cycles of denaturation (30 seconds at 95°C), annealing (30 seconds at 53°C), extension (30 seconds at 72°C), and final extension (2 minutes after the last cycle). Ultimately, an electrophoresis on a 1.5 percent (w/v) agarose gel in 1X TAE buffer (1st Base, Malaysia) was used to separate the isolated DNA from the organisms for 50 minutes at 100 V. The buffer has a pH of 8.0, 40 mM Tris-acetate, and 1 mM EDTA. Fermentas Gene Ruler TM 1 kb and 100 bp were utilized, and Maestro safe TM Nucleic Acid (V-Bio Science, Malaysia) was used for pre-staining the gel [33, 34].
Table 1: primer sequences of S. pyogenes for used in PCR

<table>
<thead>
<tr>
<th>Detected virulence factor</th>
<th>Primer sequence</th>
<th>Size of the PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16srRNA-F</td>
<td>F-AAAAAGACCGCCTTAACCACCT</td>
<td>407 bp</td>
</tr>
<tr>
<td>16srRNA-R</td>
<td>R-TGGCAAGGTAAACTCCTAAGCA</td>
<td></td>
</tr>
<tr>
<td>sepl gene-F</td>
<td>F- CCTGAGCCGTGAAATCCCCA</td>
<td>657</td>
</tr>
<tr>
<td>sepl gene-R</td>
<td>R-ACACCGAATTGTCGTTTGGT</td>
<td></td>
</tr>
<tr>
<td>SpeK gene-F</td>
<td>F-CCTTGTGTGTGTATCGCTTG</td>
<td>568</td>
</tr>
<tr>
<td>Spek gene-R</td>
<td>R-TTGCTGTCxxxxCATCAACT</td>
<td></td>
</tr>
</tbody>
</table>

Analytical Statistics
SPSS was used to perform this analysis (Version 22.0). The categorical variables were displayed using percentages. A statistically significant result was defined as a p-value of less than 0.001.

Results

1. Rate of S. pyogenes prevalence
For this study, 170 pediatric patients in all were included. Of those, 75 (44.11%) had culture-positive findings for S. pyogenes, making up the majority. The age range of the study participants was 5 to 10 years old, with a mean age of 7.5, a median age of 8.0, and a standard deviation of 3.6.

2. Identification of S. pyogenes by biochemical tests and 16SrRNA sequencing
All S. pyogenes indol, oxidase, and methyl red tests were positive. All S. pyogenes were negative for the catalase and citrate tests. The results for the enzymatic reactions of the S. pyogenes isolation hyaluronidase, arginine dehydratase, neuraminidase, and acetoin production were 30 (40%), 25 (33.33%), 21 (28%), and 16 (21.33%), respectively. In the present study, 50% of the fried rice samples were positive for S. pyogenes the 45 presumed S. pyogenes isolates were identified and confirmed using biochemical tests, and 16S rRNA partial sequences would generate amplicons of 407 bp in size (Figure 1).

Table 2: Enzymatic Reactions of the Streptococcus pyogenes isolation from clinical swabbing

<table>
<thead>
<tr>
<th>Types of Enzymatic Reactions</th>
<th>Strains no. of Streptococcus pyogenes</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Hyaluronidase</td>
<td>SPN1,SPN3,SPN6,SPN7,SPN9,SPN10 ,SPN23,SPN24,SPN25,SPN29 ,SPN11,SPN13,SPN15,SPN16,SPN18,SPN19,SPN21,SPN22,SPN27,SPN28,SPN30 ,SPN2,SPN4,SPN26,SPN5,SPN8,SPN12,SPN14,SPN17,SPN20</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dehydratase</td>
<td>SP N1,SPN3,SPN6,SPN7,SPN9,SPN10 SPN17,SPN2,SPN4,SPN26,SPN5,SPN8,SPN12,SPN14,SPN11,SPN13,SPN15,SPN16,SPN18,SPN19,SPN21,SPN22,SPN27,SPN28,SPN30</td>
<td>+</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>SPN11,SPN13,SPN15,SPN16,SPN18,SPN19,SPN21,SPN22,SPN27,SPN28,SPN30 SP N1,SPN3,SPN6,SPN7,SPN9,SPN10 SPN11,SPN13,SPN15,SPN16,SPN18,SPN19,SPN21,SPN22,SPN27,SPN28,SPN30</td>
<td>+</td>
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*SPN: Streptococcus pyogenes isolation
3. Detection of pyrogenic exotoxin genes in S. Pyogenes isolates:

A total of 75 (24%) isolates of *S. pyogenes* were identified after biochemical and multiplex PCR methods. A total of the obtained *S. pyogenes* harbored the pyrogenic exotoxin genes (*spek* & *sepl*): 6 (8%) and 9 (12%) would generate amplicons of 568 and 657 bp in size respectively, shown in (Figures 2 and 3).
Discussion

*Streptococcal pyogenes* is one of the most common bacterial infections among children aged between 5 and 10 years old. It has the most occurrence in the primary years of school and will slowly decrease in the elderly [35]. The study by [36] Thirteen (4.3%) of the forty-three β-hemolytic streptococci isolates had *S. pyogenes* identified. The phenotypic characteristics of the *S. pyogenes* isolates were used to identify them. PCR testing was performed on all 396 isolates, and 72 of them confirmed positive: 27 isolates (6.8%) are confirmed to have an ideal biochemical result for *S. pyogenes*; 45 isolates [36]. According to the findings by [37], *S. pyogenes* was found to be GABH (3.07%), whereas the remaining 252 (96.92%) group of bacteria were found to be different bacteria from all 260 study samples. The study's findings aligned with those of [38], who reported that gender had no effect on the prevalence rate of infection. However, the results of this study disagreed with those of [39], who reported that females were more responsive to infection than males were, with a ratio of 3:1.

In order to develop techniques for their identification, the current work intends to investigate the intrinsic properties of *16S rRNA* gene sequences in several *Streptococcus* species. Using several sample sequences, a phylogenetic framework was built. Significance sequence identification and restriction enzyme analysis came next [40]. 35 schoolchildren (12.2%) with a 95% confidence interval [19–27.8] had throat swabs that confirmed *S. pyogenes* infection. The percentage of *S. pyogenes* colonization in kids who were in the age ranges of 5–8 years old, 9–12 years old, 13–15 years old, and those who live with a mother who works were, respectively, 12 (17.1%), 18 (10.0%), 5 (13.5%), 8 (17.0%), and 26 (16%) [41], in the current investigation, we evaluated several variables that might raise the rate at which *S. pyogenes* colonizes an area. 23 (16.4%) of them had female children, which was 2.21 times more compared to male children in terms of colonization by *S. pyogenes* (p = 0.013). Turkey [42], and Ethiopia [43] all observed similar results. This may be brought on by the way society views female children or by their frequent interactions with other people when helping their mother with everyday duties. A youngster living in a rural area had a three-times higher chance of having *S. pyogenes* carriage (p = 0.002). This result is consistent with what the report discovered in Uganda, in the district of Wakiso [44]. While the colonization rate of *S. pyogenes* among previously sick children in the current study was high (12.8%), it was not statistically significant (p > 0.05).

Figure 3: Amplicons of *sepl* gene detected in *Streptococcus pyogenes* strains by multiplex polymerase chain reaction (PCR) technique. M: 1 kb ladder; Lane 1: Positive control; Lane 2-7: *S. pyogenes* isolates.
Ethical Clearance:
The ministries of health, education, the environment, and science have given their approval to the study by the by the Ethical Committee for Scientific Study in Iraq. Possibility of Conflict of Interest:
According to the authors, they have no conflicting interests.

Conclusion
The utilization of Multiplex PCR for viral detection at a large pediatric clinic improved the percentage of correctly diagnosing S. pyogenes pharyngitis, according to the results. Compared to the current treatment paradigm, which includes reflex culture and 16SrRNA, this reduced the prescription of needless antibiotics while the patient was in the clinic. As recommended by the American Society for Microbiology, clinics should revamp their office routine to incorporate Multiplex PCR testing. Additionally, PCR-based S. pyogenes pharyngitis testing may produce results similar to those of gold standard laboratory testing, despite the fact that it is not currently recommended in treatment guidelines. By including PCR-based techniques in practice guidelines, providers may be better able to comprehend the benefits of these techniques and encourage their use.

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