Scientific Research Publishing

Advances in Microbiology, 2022, 12, 500-510
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Molecular Identification of *Streptococcus pyogenes* in Isolates from Children with Pharyngitis, Gezira State, Sudan 2022

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How to cite this paper: Balla, M.M., Mergani, A., Medani, M.E.A.M.E. and Abakar, A.D. (2022) Molecular Identification of *Streptococcus pyogenes* in Isolates from Children with Pharyngitis, Gezira State, Sudan 2022. *Advances in Microbiology*, **12**, 500-510.
https://doi.org/10.4236/aim.2022.128034

Received: July 12, 2022
Accepted: August 23, 2022
Published: August 26, 2022

Abstract

Background: *Streptococcus pyogenes* (Group A streptococcus) is an important Gram-positive human pathogen affected the upper respiratory tract, such as the tonsils and pharynx, and is also induces post-infection diseases such as rheumatic fever and glomerulonephritis. This study aim to isolate *Streptococcus pyogenes* from children with pharyngitis and to evaluate the molecular identification of *S. pyogenes* compared with conventional methods. **Methods:** A cross sectional study was conducted on total of 200 throat swab samples which were collected from children with pharyngitis referred to Wad medani Pediatric Teaching Hospital and Wad medani ENT hospital from January to November 2021. Demographic and clinical data were collected by questionnaire. Throat swabs were tested with the standard microbiological techniques to isolated Group A streptococcus (GAS). Antimicrobial susceptibility testing was performed to all GAS isolates using the Kirby Bauer disk diffusion method according to clinical laboratory standard institute (CLSI) guidelines. Additionally, PCR was used to identify Spy 1258 gene of isolated bacteria. **Results:** From all throat swab samples screened, 51 isolates (25.5%) were identified as GAS. Antibiotic susceptibility testing revealed that all the GAS isolates were sensitive to Penicillin and Azithromycin. Sensitivity to Erythromycin, Gentamicin, Clarithromycin, Amoxicillin and Cephalexin were 88.2%, 86.3%, 45.1%, 41.2%, 13.7%, respectively. Based on PCR identification of Spy 1258 gene the percentage of isolated bacteria was 21%. **Conclusion:** The rate of isolated *Streptococcus pyogenes* was 25.5% by conventional methods and 21% by PCR. The bacteria were sensitive to Penicillin and Azithromycin. The

Spy 1258 gene was specific for detection of *Streptococcus pyogenes*.

Keywords

Antimicrobial Sensitivity Test, Pharyngitis, Spy 1258, *Streptococcus pyogenes*, Sudan

1. Introduction

Streptococcus species are associated with many human diseases. Neonatal sepsis, meningitis, arthritis, and pneumonia are some examples of these diseases. Group A Streptococcus (GAS) is a gram-positive cocci bacteria that appear in chains and produces small white to grey colonies with a clear zone of *β*-hemolysis on blood agar, rare strains are not hemolytic. GAS can be subdivided into more than 100 serotypes by the M-protein antigen that is found on the cell surface and by fimbriae. The GAS cell is a complicated structure, the cell is covered with a hyaluronic acid capsule that makes the colonies mucoid or water drop appearance in rapidly dividing strains. The cell surface and the hyaluronic capsular layer are microscopic hair-like fimbriae which enable adherence of GAS to epithelial cells and extracellular matrix proteins [1]. GAS causes mild infectious diseases such as skin infections and pharyngitis but is also able to cause severe, life-threatening invasive diseases such as streptococcal toxic shock syndrome or necrotizing fasciitis. Recurrent GAS infections may induce autoimmune diseases including rheumatic fever, rheumatic heart disease and acute post-streptococcal glomerulonephritis [2]. *Streptococcus pyogenes* contain N-acetyl glucosamine linked to rhamnose polymer that is characterized as group A streptococcus. *Streptococcus pyogenes* is able of infecting humans through adhesion and colonization of the host mucosal surface epithelial cells of the upper respiratory tract. The infection rate is more than 600 million infections annually resulting in more than 500,000 deaths a year [3].

Acute pharyngitis/tonsillitis is defined as inflammation of posterior pharynx and tonsils. Most cases of acute pharyngitis resolve without antibiotic treatment. Many viruses and bacteria can cause acute pharyngitis; however, *Streptococcus pyogenes* (also known as Lancefield group A, *β*-hemolytic streptococci) is the most common bacterial cause of pharyngitis and only causative agent that requires an etiologic diagnosis and specific treatment. *S. pyogenes* is of major clinical importance because it can cause post-infection systemic complications which occur after 1 - 3 weeks of the pharynx infection. *S. pyogenes* is responsible for 5% to 30% of cases of acute pharyngitis, and it is more common in children between 5 years and 15 years than in adults. In addition to group A streptococci several strains of bacteria can cause acute pharyngitis such as group C streptococci and group G streptococci. Common symptoms of acute pharyngitis are sore throat and fever with or without tonsillar erythema, swelling, exudate, or ulcerations. In streptococcal infections, symptom onset is usually sudden and

includes sore throat, chills, malaise, fever, headache, tender and enlarged anterior cervical lymph nodes, and pharyngeal or tonsillar exudate. Scarlatina form rash and Palatal petechiae are highly specific, but rarely found. Cough, conjunctivitis, coryza and diarrhea are uncommon in streptococcal infection, and their presence suggests a viral etiology. Viruses cause approximately 75% of pharyngitis [4].

Treatment of GAS infections naturally depends on the use of convenient antibiotics. GAS remains globally sensitive to penicillin, while antibiotics such as cephalosporins, macrolides, and clindamycin are also used clinically. In some regions of the world, GAS resistance to antibiotics such as clindamycin and macrolides has become an increasing worry and epidemiological wakefulness is required to ensure that treatment matches the antibiotic sensitivity of GAS strains [5]. This study aims to isolate *Streptococcus pyogenes* from children with pharyngitis and to evaluate the molecular identification of *S. pyogenes* compared with conventional methods.

2. Methods

This study was cross sectional Laboratory based. Samples were collected from children attending Wad Madani Pediatric Teaching Hospital and Wad Madani Ear, Nose and Throat (ENT) hospital with symptoms of Pharyngitis, ages from 5 to 17 years from January to November 2021. Culture, identification and sensitivity test were done in Medical Laboratory of University of Gezira and molecular detection of *Streptococcus pyogenes* using PCR and Genetics study were done in Molecular Biology Department-National Institute of Cancer, Wad Madani, Sudan. Exclusion criteria included Children with prior antibiotic therapy in less than 7 days and respiratory tract symptoms such as rhinorrhea or nasal congestion.

2.1. Ethical Approval

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

2.2. Sample Size

$$N = \frac{Z^2 \cdot pq}{d^2} \quad (1)$$

N = Sample size.

Z = the standard normal deviation at 95% confidences level (set at 1.96).

p = is $1 - q$.

q = the proportion in the target population estimated to have a particular characteristic or disease.

d = the degree of accuracy/accepted margin.

According to the mentioned statistical calculation, this study included 200 samples.

2.3. Sample Collection

Throat swabs were collected by sterile swab and care was taken not to swab the Cheeks, tongues, lips or other areas of the mouth of children at the age from 5 - 17 years suffering from pharyngitis. Informed consent was taken from parents. Each selected child was subjected to a brief focused medical history and physical examination. And questionnaire was filled.

2.4. Throat Swabs Culture and Identification of Bacteria

The throat swabs were inoculated on 5% sheep blood agar plates and incubated at 37˚C for 24 h in a candle jar, which can provide an atmosphere of 5% - 10% CO_2. Culture plates negative for beta-haemolytic colonies were incubated for additional 24 hours. Identification of GAS isolates was made based on the standard microbiological techniques which include beta-hemolytic activity on sheep blood agar, small colony characteristics, Gram positive cocci (Streptococci), catalase production negative, 0.04-U bacitracin disc susceptible and PYR test positive. For other bacteria Different identification methods were followed including cultural, morphological, microscopic, and biochemical tests.

2.5. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using the standard disk diffusion method on Mueller Hinton agar with 5% sheep blood, incubated overnight at 37˚C in 5% - 10% CO_2 according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. The commercial antibiotic discs were used to determine the susceptibility of isolates to penicillin (10 U), Erythromycin (15 µg), Amoxicillin (10 mcg), Azithromycin (15 µg), Clarithromycin (15 µg), Gentamicin (10 mcg), and Cephalexin (30 mcg). The strains were designated sensitive, intermediately sensitive or resistant, according to CLSI guidelines.

2.6. PCR Detection of *Streptococcus pyogenes*

2.6.1. Primer Design of Spy 1258 Gene

Molecular detection of *Streptococcus pyogenes* by Polymerase Chain Reaction (PCR) amplification of Spy 1258 gene, primers were designed according to (Table 1).

Table 1. Sequence of primer sets used for PCR amplification of spy 1258 gene.

Gene	Primer name	Primer Sequence	Product length
Spy 1258	Spy 1258 (F)	5' AAAGACCGCCTTAACCACCT 3'	407 bp
	Spy 1258 (R)	5' TGCCAAGGTAAACTTCTAAAGCA 3'	

2.6.2. DNA Extraction

Deoxyribonucleic acid (DNA) was extracted from strains of *Streptococcus pyogenes* by Boiling method, after thawing of *S. pyogenes* isolates which placed in

500 µL of Trise EDTA (TE) buffer and frozen at −70˚C, 150 µL of this suspension was taken and heated at 95˚C for 30 minutes, centrifuge was used and spin at 14,000 rpm for 5 minutes and the supernatant was taken into new sterilized tube.

2.6.3. PCR Amplification of Spy 1258 Gene

Amplification reactions were performed for 40 cycles using PCR program shown in (Table 2).

Table 2. PCR program of amplification of spy 1258 gene.

	Temperature	Time
Initial Denaturation	94˚C	3 min.
Denaturation	94˚C	1 min.
Annealing	58˚C	45 sec.
Elongation	72˚C	45 sec.
Final Elongation	72˚C	3 min.

2.6.4. 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.

2.7. Statistical Analysis

Statistical analysis was done by SPSS statistical software. Participants' demographic and clinical characteristic were described by using descriptive statistics. *p*-value less than 0.05 taken as statistically significant at 95% confidence level.

3. Results

3.1. Social Demographic Data

In this study a total of 200 throat swabs were collected from Pharyngitis from January to November 2021. Females accounted 126 (63%) and males were 74 (37%). The most common infected age group was 5 - 7 years representing 68 (34%) followed by age group from 11 - 13 and the age group 14 - 17 is less infected by pharyngitis 36 (18%) (Figure 1).

And urban residents were 62% when the rural was 38%. Pharyngitis decrease during the age, the age from 14 to 17 is the less infection and especially in male more than female.

3.2. Symptoms and Clinical Presentation

Enlarged and Red Tonsils and fever were the most dominant symptoms which represent 100% and 81% respectively and the less dominant symptoms are vomiting and abdominal pain which are 6.5% (Figure 2).

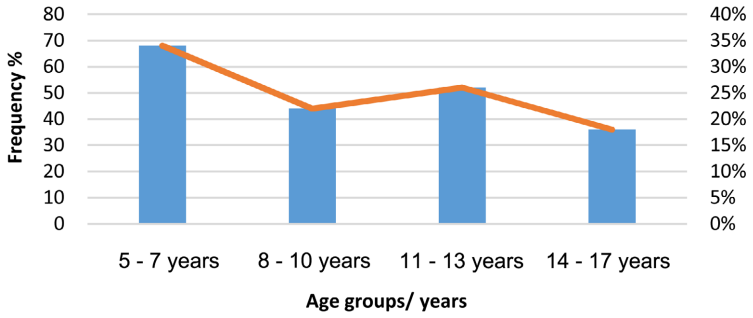

Figure 1. Age groups/years.

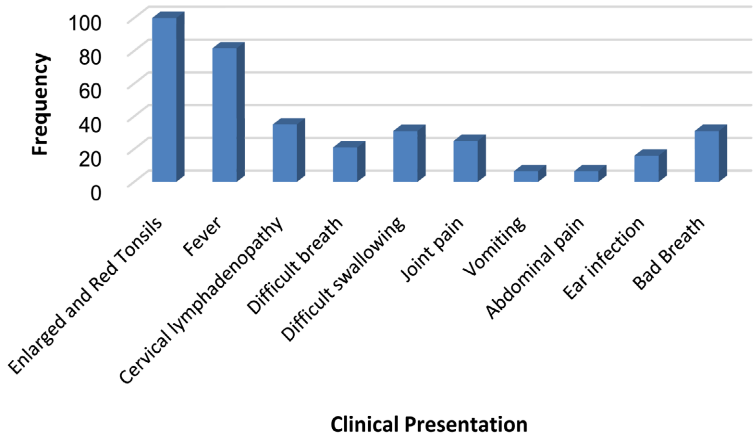

Figure 2. Clinical presentation.

3.3. Isolation of *Streptococcus pyogenes*

In this study, the rate of *Streptococcus pyogenes* was 25.5% (51/200) which has been identified by Conventional methods using culture and biochemical tests. Total number of males infected by *S. pyogenes* was 13 (25.5%) and females were 38 (74.5%). The age group from 5 - 7 years were the most infected group with *S. pyogenes* 17 (33.4%) and infection decreased throw the age in male and increased again in female from in age groups 13 - 15 years and 15 - 17 years (Figure 3).

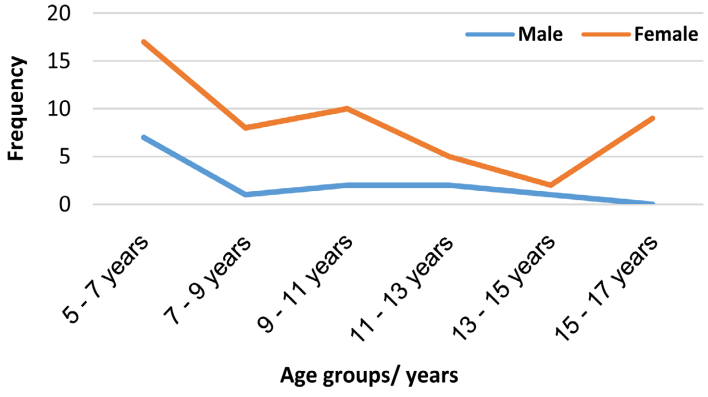

Figure 3. Distribution of gender and age groups infected by *S. pyogenes*.

3.4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing for all *S. pyogenes* isolates was performed using Clinical and Laboratory Standard Institute guidelines CLSI by Kirby–Bauer disk diffusion method, the bacteria 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.

3.5. Bacterial Isolates

The bacterial isolates include *Streptococcus pyogenes* (25.5%), Staphylococcus aureus (31.5%), other streptococci (11%) and coagulase negative staphylococci (5%) and no growth was detected in (27%) of total 200 samples.

3.6. Detection of *S. pyogenes* Using Spy 1258 Gene

PCR detection of Spy 1258 gene which has 407 bp Product length (Figure 4) showed 42 samples positive (82.4%) of 51 *S. pyogenes* samples were detected by culture and biochemical test and 9 samples were negative (17.6%). The results showed the percentage of *S. pyogenes* was 21% by using PCR detection of Spy 1258 gene and 25.5% by using conventional methods (Table 3).

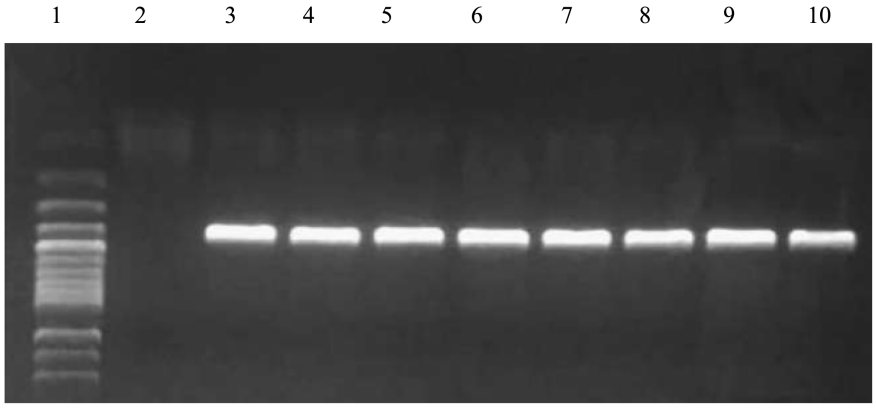

Figure 4. Detection of *S. pyogenes* Spy 1258 gene. Lane 1 DNA ladder, lane 2 Negative control, lanes 3 to 10 *S. pyogenes* Spy 1258 gene.

Table 3. Detection of S. pyogenes by culture method and spy 1258 gene.

Detection Method	Frequency	Percentage
Culture method	51	25.5%
Spy 1258	42	21%
Total	200	100%

3.7. Specificity of Spy 1258 Gene

The study showed the Spy 1258 gene specific for *S. pyogenes* only when tested for other species included Staphylococcus aureus, Coagulase negative staphylococci, other streptococci, *E. coli* and human DNA (Figure 5).

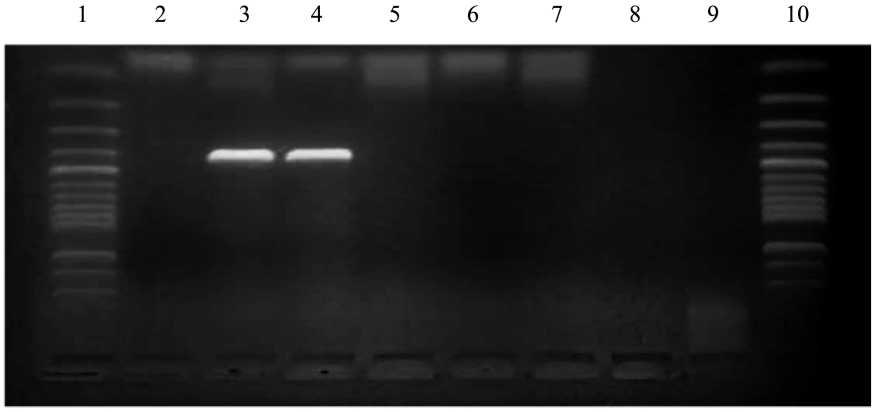

Figure 5. Specifity of spy 1258. Lane 1& 10 DNA ladder, lane 2 Negative control, lane 3 and 4 Spy 1258 (407 bp), lane 5 Staph. aureus, lane 6 Coagulase negative Staphylococci, lane 7 other streptococci, lane 8 *E. coli* and lane 9 Human DNA.

4. Discussion

Group A Streptococcus (GAS) is the most important bacterial cause of pharyngitis, children from 5 - 15 years are most infected, *Streptococcus pyogenes* is responsible for 20% - 30% of sore throat in children [6]. In this study the females were more commonly infected with pharyngitis than males (63%) which agree with [7] study showing the female infection more than males (57%). Isolation of *Streptococcus pyogenes* was 25.5% this is high result compared with 11.3% reported in Ethiopia [7], 12% in Turkey [8]. The study of [9] shows 24.1% isolation percentage near to this study and agrees with 26% isolate of [10] study. Our result has a lower rate than [11] and [12] studies which show 30% and 46% isolates of *Streptococcus pyogenes*.

Streptococcus pyogenes is sensitive to penicillin; different studies worldwide showed that like [11] study from Iran, [13] study from Senegal, [14] study from Ethiopia, [15] study from China and our study also showed that which confirm the penicillin is still the drug of choice for the treatment of GAS pharyngitis.

Antibiotics such as cephalosporins, macrolides, and clindamycin are also used clinically. In some regions of the world, GAS resistance to antibiotics such as clindamycin and macrolides has become an increasing worry and epidemiological wakefulness is required to ensure that treatment matches the antibiotic sensitivity of GAS strains [5]. Resistance among streptococci to macrolides (erythromycin and clarithromycin) are widely reported [16]. Our study showed the resistance of Clarithromycin was 33.3% similar to [11] showing resistance 33.9% and the resistance to Erythromycin was 7.8% agree with 9.7% of [17] study and 6.9% of [18] study. And also show 52.9% resistance to amoxicillin where study of [19] from Egypt showed 81% sensitive to amoxicillin.

The Spy 1258 is the most widely common primer used for molecular detection of *S. pyogenes* [20], this study detects 82.4% of bacterial isolates, in [12] study from Iraq the percentage of detection by Spy 1258 was 61%. [21] In a comparative study (Identification of *Streptococcus* pyogenes—Phenotypic Tests vs Mo-

lecular Assay (Spy 1258 PCR)) showed the percentage *Streptococcus pyogenes* contain Spy 1258 gene was 85.9%. This study showed 100% Specifity of Spy 1258 agree with [10] study which also showed 100% Specifity and 87% sensitivity and [22] showed that Spy 1258 gene was specific for *S. pyogenes* only, but not from other species of the genus Streptococcus and common bacteria. And this result disagrees with [5] study showed all *S. pyogenes* isolates contain Spy 1258 genes and [21] study's conclusion to use Spy 1258 for the best results of identification. [20] study from Sudan showed that the low sensitivity of Spy 1258 primer and the variability in *S. pyogenes* genome sequence necessitate developing new primers according to the environmental and geographical distribution of *S. pyogenes* isolates.

Limitation of the study includes the Corona pandemic and its impact on various aspects, such as the difficulty of providing research materials.

5. Conclusion

The rate of *Streptococcus pyogenes* was 25.5% by conventional methods and 21% by using Spy 1258 gene. The bacteria were sensitive to Penicillin and Azithromycin. And the Spy 1258 gene is specific for detection of *Streptococcus pyogenes*.

Conflicts of Interest

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

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