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Different Laboratory Techniques of *Streptococcus pyogenes* Isolated from Inflammatory Secretions of Skin

Hassanain Jwad Abidalhussein[1], HebatAllah Mohammed Khudair[2], Aseel Abdulridha Shilba[3], Zahraa AbdulAdheem Almaiyaly[4]

[1]Dentistry Collage, Kerbala University, Iraq. hesinen.j@uokerbala.edu.iq
[2]Dentistry College, Karbala University, Karbala, Iraq. hebatallah.k@uokerbala.edu.iq
[3]Dentistry College, Karbala University, Karbala, Iraq. aseel.shilba@uokerbala.edu.iq
[4]Dentistry College, Karbala University, Karbala, Iraq. zahraa.abduladheem@uokerbala.edu.iq

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Abstract

Background: *Streptococcus pyogenes* is a well-known human pathogen that causes a variety of illnesses, from simple skin infections to more serious invasive problems. The prevention of sequelae such as rheumatic fever and post-streptococcal glomerulonephritis is dependent on an accurate diagnosis.

Objective: Our main objective is to thoroughly study S. pyogenes in pus-leaking skin infections, by using methods like PCR amplification. These advanced techniques will help us to gain new insights into the prevalence and significance of S. pyogenes in this particular infection, thereby enhancing our current understanding of the subject.

Methods: We studied sixty grown-up patients with exudative skin problems. We collected samples by swabbing fluid from their inflamed areas and putting it on special plates. Then we used the boiling method to isolate the DNA and amplify the Spy 1258 gene with PCR.

Results: Thirty-six (or sixty percent) of the sixty samples tested under culture were S. pyogenes positive. S. pyogenes' presence in these samples was further evidenced by the production of a unique DNA fragment with a length of 407 base pairs using PCR amplification.

Conclusion: Apart from underlining the need of using reliable diagnostic instruments, the study reveals that S. pyogenes is rather common in exudative skin disorders. Correct antibiotic treatment resulting from precision and timely diagnosis is essential to avoid major adverse effects. Treatment approaches will be improved by further investigation on the genetic variety of S. pyogenes and its antibiotic resistance.

Keywords: ***Streptococcus pyogenes***, Skin Infections, PCR Amplification, Diagnosis, Antibiotic Treatment.

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Introduction

Streptococcus pyogenes, a well-known bacterial illness that only affects humans, causes a range of symptoms. Poor S. pyogenes infection treatment, among other postinfection effects, can cause severe rheumatic fever and post-streptococcal glomerulonephritis. It also leads to two invasive illnesses linked to great morbidity and death: toxic shock syndrome and necrotizing fasciitis. [1].

Gram-positive, coagulase-negative, and catalase-negative bacteria, as well as streptococci, can form pairs or chains. According to blood agar test findings, they fall under either hemolytic (green hemolysis), beta-hemolytic (full red cell destruction), or gamma-hemolytic (no hemolysis). *Streptococcus pyogenes* is type A beta-hemolytic streptococci; type B is Streptococcus agalactiae. [1].

Pathogenesis of *Streptococcus pyogenes* Infections

Streptococcus pyogenes is a gram-positive bacterium that lacks catalase and oxidase enzymes, and exhibits β-hemolysis. This bacterium, which exhibits facultative anaerobic respiration, develops minute colonies on blood agar plates and thrives optimally in an atmosphere containing 5-10% carbon dioxide. Lancefield serology distinguishes group A streptococci (GAS) from other kinds. The S. pyogenes type A antigen is a polysaccharide consisting of a polymer backbone made up of N-acetylglucosamine and rhamnose. The predominant surface protein present on the cell walls of S. pyogenes is referred to as M protein. The categorization of GAS strains is determined on the antigenic properties of the M protein. More than 80 unique protein serotypes have been found. S. pyogenes is classified into Class I or Class II based on the presence of postinfectious sequelae. Rheumatic fever is caused by Class I strains of bacteria, whereas acute glomerulonephritis is caused by Class II strains. [2].

Streptococcus pyogenes inhabits the vaginal mucosa, anus, and throat. S. pyogenes infections are transmissible. The illness can be transmitted by airborne droplets, direct contact with nasal discharge or contaminated surfaces or objects, contact with infected sores, and consumption of contaminated food. GAS strains have the ability to penetrate the skin through abrasions and skin wounds, resulting in the development of cellulitis or erysipelas. [3] Minor injuries can lead to the development of myositis and necrotizing fasciitis, while GAS can induce toxic shock syndrome. Infections of the uterus and vagina with S. pyogenes can lead to sepsis. Skin lesions are the primary risk factor for major infections caused by S. pyogenes. Military posts, hospitals, densely populated places, and educational institutions—all of which support effective group communication and the unexpected rise of streptococci bacteria [4].

Epidemiology of *Streptococcus pyogenes* Infections

The frequency of *Streptococcus pyogenes* (S. pyogenes) infections influences global health; clinical symptoms define this variation. The prevalence of major diseases connected to GAS infections had dropped by the middle of the 20th century. Conversely, major GAS infections started to resurface by the end of the 1980s. Over the last twenty years, both suppurative and non-suppurative effects have become more prevalent. Factors such as virulence and drug resistance can help explain this rise. While highly developed nations have fewer GAS infections, underdeveloped nations have a greater prevalence of rheumatic heart disease (RHD) and more RHD-related mortality. [5]

With 1.78 million new cases annually, severe S. pyogenes infections affect 18.1 million people globally. Rheumatic heart disease (RHD) affects at least 15.6 million people globally; it causes 282,000 new cases and about 233,000 deaths annually. *Streptococcus pyogenes* causes 5% to 20% of adult cases and 15% to 30% of children's pharyngitis infections in the US. From January to April, there is a rise in the severity of invasive skin infections caused by GAS, which is linked to the host's susceptibility to acquiring a serious infection. [6]

Diagnostic Techniques for *Streptococcus pyogenes*

Group A streptococcus (GAS), also known as *Streptococcus pyogenes*, is the leading cause of pharyngitis in children and adolescents. Clinicians should evaluate the risk of GAS pharyngitis using both clinical and epidemiological data. The Infectious Disease Society of America (IDSA) recommends that the rapid antigen detection test (RADT) be used as the first-line intervention to assist clinicians in diagnosing GAS pharyngitis. To minimize complications, throat cultures should be

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collected from children with negative RADT results. Combining RADT with established clinical criteria, such as the modified Centor score or the Fever PAIN score, can help save money on unnecessary testing and medicines. [7].

A throat culture is regarded the gold standard for identifying GAS; nevertheless, it is costly and can cause therapeutic delays. To distinguish S. pyogenes from other beta-hemolytic streptococci, the pyrrolidinyl arylamidase activity (PYR) test is used. This test also evaluates if the bacteria have the enzyme pyrrolidinyl aminopeptidase. [7].

Anti-DNase B (ADB) and anti-streptolysin O (ASO) titers are utilized for diagnosing post-streptococcal sequelae and indicating a previous streptococcal infection. [7].

Treatment and Management of *Streptococcus pyogenes* Infections

Since penicillins do not demonstrate resistance to the germs, they are the most often used antibiotics to treat *Streptococcus pyogenes* infections. Ten days of therapy is the advised period for pharyngitis to guarantee total eradication and avoid complications, including rheumatic fever. You can administer cephalosporins—like cephalexin—in place of mild penicillin allergies, but they can encourage antibiotic resistance. Macrolides and lincosamides are used for severe penicillin allergies, but resistance to macrolides is increasingly reported[8].

Resistance mechanisms to other antibiotic classes, such as macrolides and tetracyclines, have been observed, and monitoring these patterns is essential for guiding appropriate antibiotic choices. Adjunctive therapies include intravenous immunoglobulin (IVIG), which provides neutralizing antibodies against streptococcal superantigens, reducing inflammation and improving outcomes in patients with severe toxin-mediated diseases[9]. Anti-inflammatory agents, such as corticosteroids, can be used in cases of severe inflammation to reduce systemic inflammatory responses and improve clinical outcomes but must be balanced against potential immunosuppression[10].

Currently, there is no licensed vaccine for *Streptococcus pyogenes*, but research is ongoing on vaccine candidates targeting various virulence factors. A successful vaccine would be a significant advancement in preventing S. pyogenes infections and their sequelae. Infection control measures in healthcare settings and communities, including proper hand hygiene, respiratory etiquette, and isolation of infected individuals, are crucial for preventing the spread of S. pyogenes[11].

Equipment and Materials

The following equipment and materials were utilized in this study:

- **Equipment**: Autoclave, Incubator, Oven (Memmert, Germany), Refrigerator (Beko, Korea), Water bath, Light microscope, Camera Lucida, Thermocycler (Bio-Rad, USA), UV-visible spectrophotometer (Shimadzu, Japan), Vortex mixer (Thermolyne, USA).
- **Consumables**: Nutrient agar (500g BD), Sterile petri dishes, Conical flask, Glass Cylindrical, Micropipette, Distilled water, 70% Ethanol alcohol, Wire loop, Cotton, Gram stain, Slides, Swabs, Transport media, Agarose (Conda, USA), Crystal violet powder (BDH, England), Trise EDTA (TE), DNA Ladder 100 bp (Promega, USA), DNA loading dye (Promega, USA), Ethidium Bromide Solution Bio (Basic, Canada).

Methods

Patients

A total of 60 adult patients presenting with exudative skin infections were enrolled in this study. The cohort comprised 30 male and 30 female patients, all of whom were treated at the dermatology

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clinic. Ethical approval was obtained from the institutional review board, and informed consent was secured from each participant.

- *Preparation of Blood Agar*

1. Media Preparation:
    - Nutrient agar (1.5%) was prepared by dissolving 500g of the medium in 1000 ml of distilled water. The mixture was sterilized in a conical flask using an autoclave.
    - After autoclaving, 50 ml of sterile, defibrinated sheep blood was aseptically added to the nutrient agar. The mixture was gently mixed to avoid bubble formation.
2. Plate Preparation:
    - The prepared blood agar was dispensed aseptically into 60 sterile petri dishes, each containing approximately 15 ml of the medium.
    - The plates were allowed to solidify at room temperature before being stored at 4°C in sealed plastic bags to maintain moisture. The blood agar plates were viable for use for up to four weeks.

- *Bacterial Growth on Culture Media*

3. Sample Collection:
    - Swabs were taken from the inflammatory secretions of skin infections in the 60 patients.
    - The swabs were streaked onto the previously prepared blood agar plates.
4. Incubation and Observation:
    - The inoculated plates were incubated at 37°C for 24 hours.
    - Post-incubation, the plates were examined for bacterial growth. Out of the 60 samples, 36 showed positive growth for *Streptococcus pyogenes*, while 24 were negative (Figure 1).

- *PCR Detection of Streptococcus pyogenes*

1. DNA Extraction:
    - The boiling method was employed for DNA extraction. *Streptococcus pyogenes* isolates were suspended in 500 µl of Trise EDTA (TE) solution and frozen at -70°C.
    - Subsequently, 150 µl of the suspension was heated at 95°C for 30 minutes. The mixture was centrifuged at 14,000 rpm for 5 minutes, and the supernatant was transferred to a new sterile tube.
2. PCR Amplification:
    - PCR was conducted to amplify the Spy 1258 gene using the primers listed in Table 1. The sequences for the primers were as follows:

Sense: 5'-AAAGACCGCCTTAACCACCT-3'
Antisense: 5'-TGGCAAGGTAAACTTCTAAAGCA-3'

    - The PCR conditions are detailed in Table 2, with the amplification reactions run for 40 cycles. The conditions were as follows:

Steps	Temperature (˚C)	Time (minutes)
Initial denaturation	94	3
Denaturation	94	1
Annealing	58	45
Elongation	72	45
Final elongation	72	3

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Results

Bacterial Growth and Colony Characteristics

At 37°C *Streptococcus pyogenes*, a bacteria able to survive both with and without oxygen, flourishes. It needs a nutrient-dense medium like blood and serum if it is to expand. In this work, inflammatory skin secretions were grown on blood agar. This allowed different kinds of colonies—including plain colonies, mucoid colonies, and β-hemolytic zones—to be established. The categorization of these colonies was much influenced by the developing environment and hyaluronidase production. Among the several virulence elements the bacteria produce are bacteriocins, hemolysin O, streptokinase, NADH enzyme, hyaluronidase, and M protein. These elements influence its pathogenicity.

Sample Analysis

A total of 60 samples were collected from adult patients with exudative skin infections (30 male and 30 female). After culturing the samples on blood agar and incubating them at 37°C for 24 hours, 36 samples tested positive for S. pyogenes, while 24 samples were negative (Figure 1).

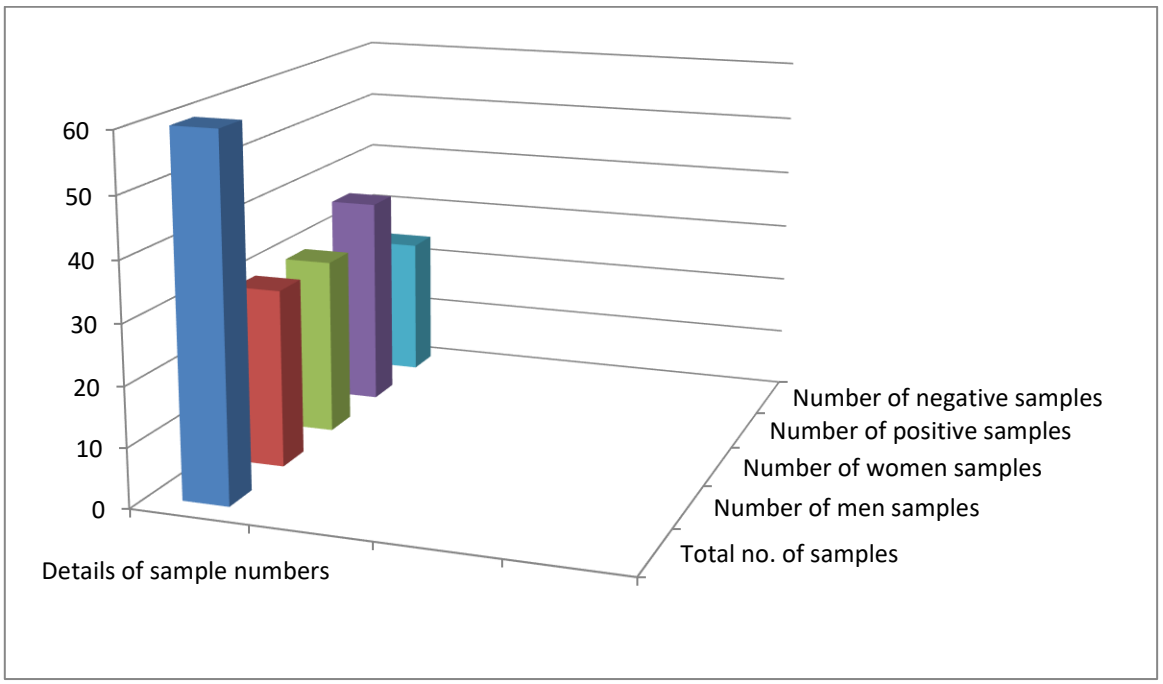

Figure 1: The schematic representation to show collected samples of skin inflammatory secretions to observe *Streptococcus pyogenes*, which be cultured on blood agar.

- *PCR Detection of Streptococcus pyogenes*

The presence of S. pyogenes in the skin inflammation samples was confirmed by PCR amplification of the Spy 1258 gene, producing a specific PCR product of 407 bp. This molecular detection underscores the prevalence of S. pyogenes in the inflammatory secretions from skin infections (Figure 2).

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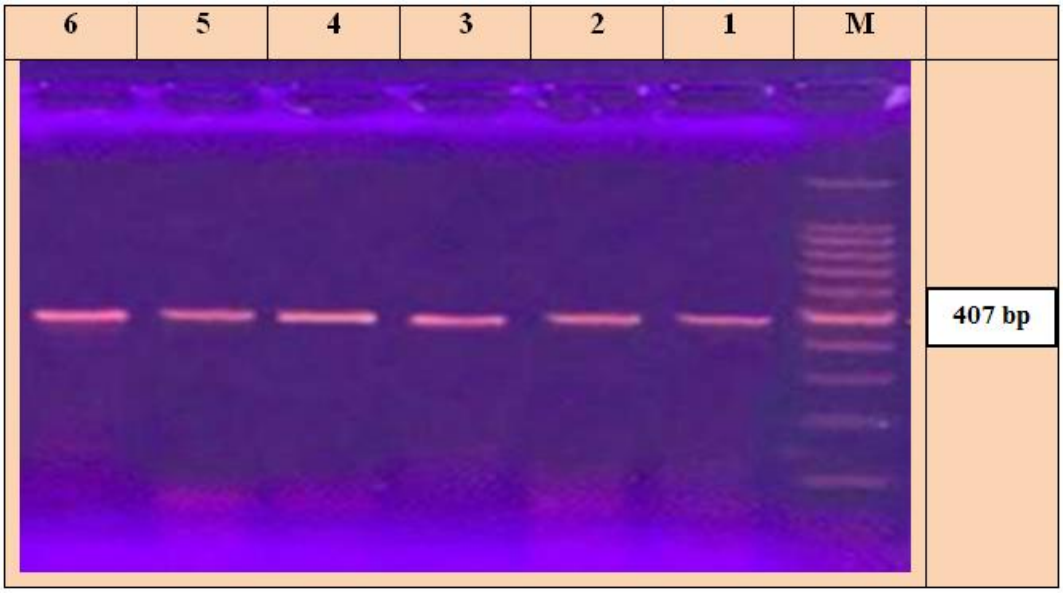

Figure 2: Agarose gel electrophoresis specific PCR product for 409bp of *Spy 1258 gene*.

Discussion

Streptococcus pyogenes is a bacterial pathogen that causes various symptoms, from localized infections to life-threatening invasive infections. Ineffective treatment can lead to complications like acute rheumatic fever and post-streptococcal glomerulonephritis, necrotizing fasciitis, and toxic shock syndrome, causing high morbidity and death[12].

Our study aimed to investigate by both culture techniques and PCR amplification the presence of *Streptococcus pyogenes* in exudative skin infections. Our results show a high frequency of S. pyogenes in these diseases, which emphasizes the need for a correct diagnosis and quick treatment to minimize consequences.

Through techniques S. Pyogenes was detected in thirty six out of sixty samples collected from individuals, with skin infections. This is consistent with earlier studies, notably those by Carapetis et al., which revealed that S. pyogenes is a common pathogen in skin illnesses like erysipelas and impetigo. [6]. The great frequency of the pathogen emphasizes its involvement in the genesis of skin diseases and the need of accurate diagnosis methods.

More proof that the positive samples had S. pyogenes was found in the 407-bp product that was made by PCR amplification aimed at the Spy 1258. We confirmed the accuracy of our results and the results of the culture approach by sensitively and deliberately using PCR to identify S. pyogenes. This combination approach proves essential for efficient patient care and raises the accuracy of the diagnosis.

Our results agree with previous studies on the frequency of S. pyogenes in skin disorders. For example, Hall et al. found in their patient sample the same frequency of impetigo and cellulitis. The similarity of our results with previous studies shows the validity of our diagnostic techniques particularly the efficacy of PCR in bacterial detection generally [13] and the need of our work in the present therapeutic environment. This connection verifies the techniques applied and indicates the continuous therapeutic relevance of S. pyogenes in dermatological diseases [14].

Clinical Implications

The great frequency of S. pyogenes in exudative skin infections highlights the need of meticulous diagnosis techniques in dermatology clinics. Correct identification of the organism and focused antibiotic treatment are crucial to prevent the infection from spreading and maybe causing systemic

effects. Early management is essential to lower tissue damage and the risk of invasive infections as S. pyogenes is known to be pathogenic and to have virulence factors include hemolysins, streptokinase, and M protein. Two effects that can be prevented with fast and correct treatment include rheumatic fever and post-streptococcal glomerulonephritis.

Limitations

Our study has several limitations that should be acknowledged:

1. **Sample Size and Single Center:** The small sample size of the study and exclusive focus on one facility might limit its relevance to a more general population. To validate the results and provide a more complete knowledge of the epidemiology of S. pyogenes in skin infections, additional study with larger, multicentric groups of patients is required. This will raise the dependability and statistical power of the conclusions.

2. **Potential for False Positives in PCR:** PCR has a high level of specificity; nonetheless, it is critical to recognize the risk of false positive findings caused by contamination or the presence of non-viable bacteria. The study proposes using both culture techniques and molecular approaches to improve diagnostic accuracy. Strict laboratory practices must be followed to avoid contamination and to perform confirmation tests as needed.

3. **Study Duration and Follow-Up:** The study's cross-sectional approach, which overlooks the temporal aspects of S. pyogenes infections, emphasizes the need for longitudinal research with follow-up to understand the pathogen's persistence and recurrence in skin diseases.

Further research is required to comprehend the composition of the S. Pyogenes strain responsible, for skin issues. This will enhance treatment protocols and aid in identifying the root cause. The development of vaccines and therapies hinges on understanding how the immune system responds to S. Pyogenes infections. Future investigations should focus on identifying antibiotic strains of S. Pyogenes exploring variability and virulence factors and conducting epidemiological studies to determine the prevalence across various locations and populations; thereby bolstering immune defenses. These studies could uncover risk factors for diseases. Provide valuable insights, for public health initiatives.

Conclusion

The research highlights the importance of diagnosing *Streptococcus pyogenes* in skin conditions, with pus discharge. Among 60 patients 36 were identified with S. Pyogenes using PCR testing and culture methods. It is advised to use antibiotics to reduce risks like fever and post streptococcal glomerulonephritis. Accuracy is key in diagnosis despite challenges like sample sizes and potential false positives from PCR tests. Maintaining approaches is essential. Future studies focusing on variations, antibiotic resistance, interactions between hosts and pathogens and epidemiological research will play a role, in effectively managing S. Pyogenes infections.

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