

Isolation and Identification of *Streptococcus pyogenes* that Isolated from Different Clinical Sources in Al-Najaf Province

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ABSTRACT

Background: *Streptococcus* species are associated with many bacterial diseases in both humans and animals. Arthritis, neonatal sepsis, meningitis, and pneumonia are some examples of diseases in humans, while in animals they mainly cause mastitis. **Aim:** this study aimed to identify *S. pyogenes* in clinical sources and detect its abundance. **Method:** collect samples such as throat swabs. Specimens were collected 20 oral swaps obtained from patients whose ages are (6-60) years both nostalgic suffer from tonsillitis, coughing, and oral sore for the period from December to January 2023, then diagnose it by routinely methods, blood hemolysis. **Results:** The results showed that bacteria *Streptococcus pyogenes* is the most abundant among samples 70%. **Conclusion:** conclude that the *Streptococcus pyogenes* is highly in abundance percentage and causing oral inflammation due to its active virulence factors.

Keywords: *Streptococcus* species, *S. pyogenes* , Oral sore, Oral swaps.

Article Information

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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 [1,2].

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 [3].

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 [4].

Streptococcus species are associated with many bacterial diseases in both humans and animals. Arthritis, neonatal sepsis, meningitis, and pneumonia are some examples of diseases in humans, while in animals they mainly cause mastitis [5]. *S. pyogenes* is differentiated as group A streptococcus (GAS) as it contains N-acetyl glucosamine linked to rhamnose polymer [6]. *S. pyogenes* is capable of infecting humans, mainly through adhesion and colonization of the host mucosal surface epithelial cells of the upper respiratory tract [7]. Virulence factors are equally distributed within *S. pyogenes* ; some are encoded by chromosomes, while others depend on the presence of mobile genetic elements. Confirmation of their presence or absence is considered as a simple clinical diagnosis method [8].

M protein is the most analyzed virulence factor which can be used in the serotype classification of *S. Hindawi International Journal of Microbiology* Volume 2020, contribute to GAS pathogenicity based on their immune stimulatory activity. SAg gene distribution has been used as a method for the detection of genomic heterogeneity, the correlation between gene contents, and the determination of clinical manifestation [9].

MATERIALS AND METHODS

Specimens' collection

Depending on the suspected source of infection, you can collect specimens such as throat swabs. Specimens were collected 20 samples obtained from patients whose ages are (6-60) years both nostalgic suffer from tonsillitis, coughing, and oral sore for the period from December to January 2023, then it culture on appropriate media and follow sterile technique to inoculate the media with the sample.

Diagnosis

Growth on conventional media, such as nutrient agar, blood agar, and other selective media then, one of the bacterial colonies was fixed by the flame on the slide, and then it was staining and examine under light microscope. The media preparation of *Streptococcus* can vary depending on the specific species and purpose of the culture.

Nutrient agar is a basic culture medium commonly used for the culture of non-fastidious microorganisms, and for quality control and checking purity prior to biochemical or serological testing and is an ideal medium for the demonstration and teaching purposes as it allows more prolonged survival of cultures at ambient temperature without the risk of overgrowth that might occur with more nutritious mediums.

Blood agar is a differential medium that can be used to identify different *Streptococcus* species based on their hemolytic patterns. Then

observe the growth on the agar plates for any growth of *Streptococcus* colonies.

The catalase test

facilitates the detection of this enzyme in bacteria. It is essential for differentiating catalase-positive *Micrococcaceae* from catalase-negative *Streptococcaceae*. While it is primarily useful in differentiating between genera, it is also valuable in speciation of certain gram positives.

RESULTS AND DISCUSSION

This research was conducted in the Bacteriology Laboratory of the Medical Laboratory Techniques Department. This study aims to isolate and diagnose *Streptococcus* bacteria. Samples (20) were collected from patients in Al-Najaf governorate suffering from tonsillitis, coughing, or various oral ulcers. The samples were cultured on a normal nutrient medium, then the samples were purified and any growth other than bacteria was excluded. Then, we cultured the samples on selective medium for *Streptococcus* bacteria. Bacteria were diagnosed by microscopic examination, biochemical assays, and selective media. The results showed that bacteria *Streptococcus pyogenes* is the most visible among samples by 70%. The results of collecting samples and data explaining as following: 9 Coughing 45%, 7 Tonsillitis 35%, 4 Oral sore 20%, 11 Males 55%, 9 Females, 45% .

The result of (*Streptococcus pyogenes*) Circular, pinpoint colonies of the size 0-5 to 1 mm in diameter; light yellow colored with low convex elevation; matt surface in virulent strains but glossy surface are seen in non-virulent strains; mucoid colonies in the case of capsule production.

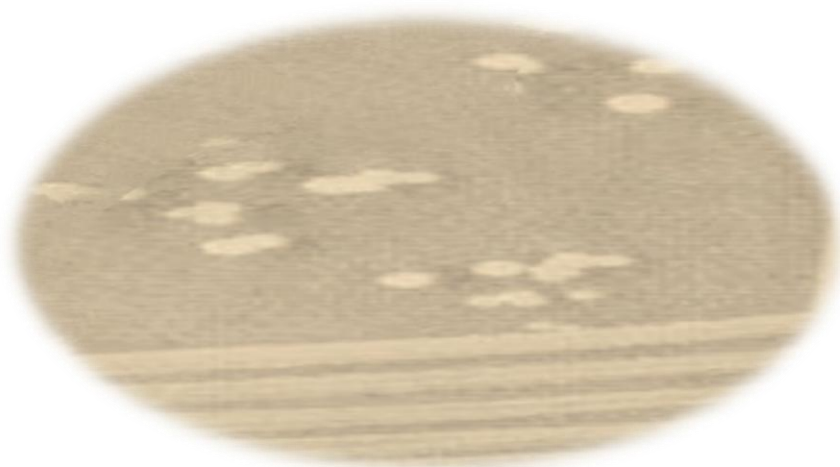
The colonies may exhibit different hemolytic patterns such as alpha, beta, or gamma hemolysis, which can help identify the species of *Streptococcus*. In broth, the growth of *Streptococcus* can be observed by turbidity or by examining a Gram-stained smear under a microscope.

Table 1: Samples collection and data of patients

	Age/ years	Gender	Disease/ symptoms
Sample / oral swap	14	Male	Tonsillitis
	13	Male	Tonsillitis
	12	Male	Tonsillitis
	12	Male	Tonsillitis
	10	Male	Tonsillitis
	10	Male	Tonsillitis
	13	Female	Tonsillitis
	20	Female	Coughing
	22	Female	Coughing
	25	Female	Coughing
	32	Female	Coughing
	29	Female	Coughing
	45	Male	Coughing
	55	Male	Oral sore
	6	Female	Oral sore
	7	Female	Coughing
	10	Male	Oral sore
	15	Female	Oral sore
	17	Male	Coughing
	19	Male	Coughing
Total number of samples	20	11 Males 55% 9 Females 45%	7 Tonsillitis 35% 9Coughing 45% 4 Oral sore 20%

The appearance of *Streptococcus* on nutrient agar media

Streptococcal colonies vary in color from gray to whitish and usually glisten. Often, dry colonies are observed. Encapsulated strains may appear mucoid. Many variations exist among Lancefield groups [5].

**Figure (1): The appearance of *Streptococcus* on nutrient agar media.**

The appearance of *Streptococcus* under light microscope

Gram Stain: Positive. Morphology: Spherical, ovoid, or cocci shaped. Often occur in pairs or chains when grown in liquid media. They are sometimes elongated in the axis of the chain to form a lancelet shape. Size: 0.5-2.0 micrometers in diameter. Motility: Usually no motility occurs. Capsules: Encapsulation occurs often. Spores: None [6].

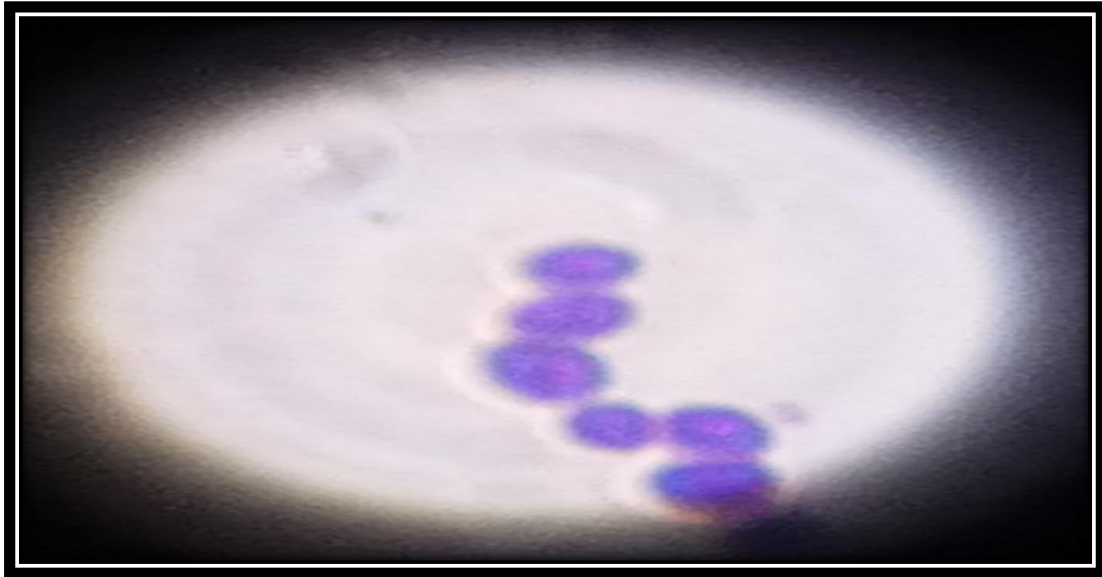


Figure (2): The appearance of *Streptococcus. pyogen* under light microscope power 100X, staining by set of Gram stain.

The appearance of *Streptococcus pyogenes* on selective media

These results agree with [7,8 and 9].

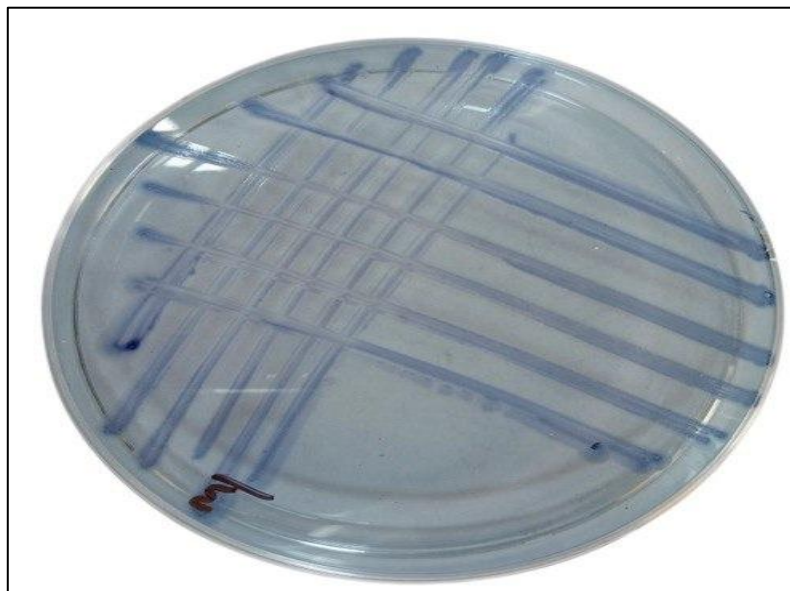


Figure (3): Demonstrates the appearance of *Streptococcus pyogenes* on selective media.

The appearance of *Streptococcus pyogenes* s on blood agar media

Streptococci are gram-positive, catalase-negative, coagulase-negative cocci that occur in pairs or chains. They are divided into three groups by the type of hemolysis on blood agar: beta-hemolytic (complete lysis of red cells), a hemolytic (green hemolysis), and gamma-hemolytic (no hemolysis). Beta-hemolytic *streptococci* are characterized as group A *streptococci* (*Streptococcus pyogenes*) and group B *streptococci* (*Streptococcus agalactiae*). These results agree with [10,11].

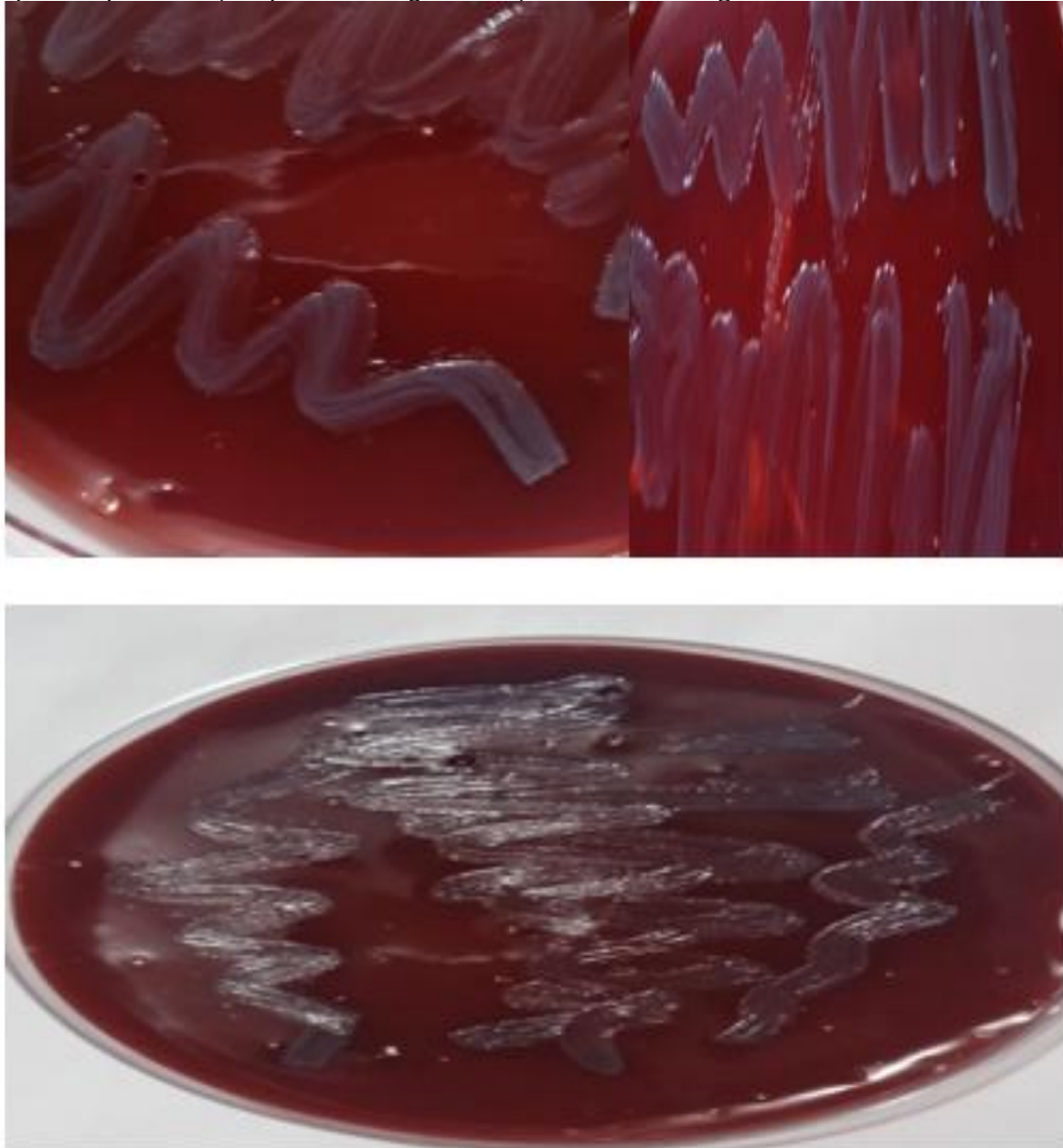


Figure (4): The appearance of *S. pyogenes* on blood agar media, show the beta hemolytic, complete hemolysis of RBCs.

The results indicate that tonsillitis was the most frequently recorded symptom among patients, followed by coughing, while oral sores showed the lowest prevalence.

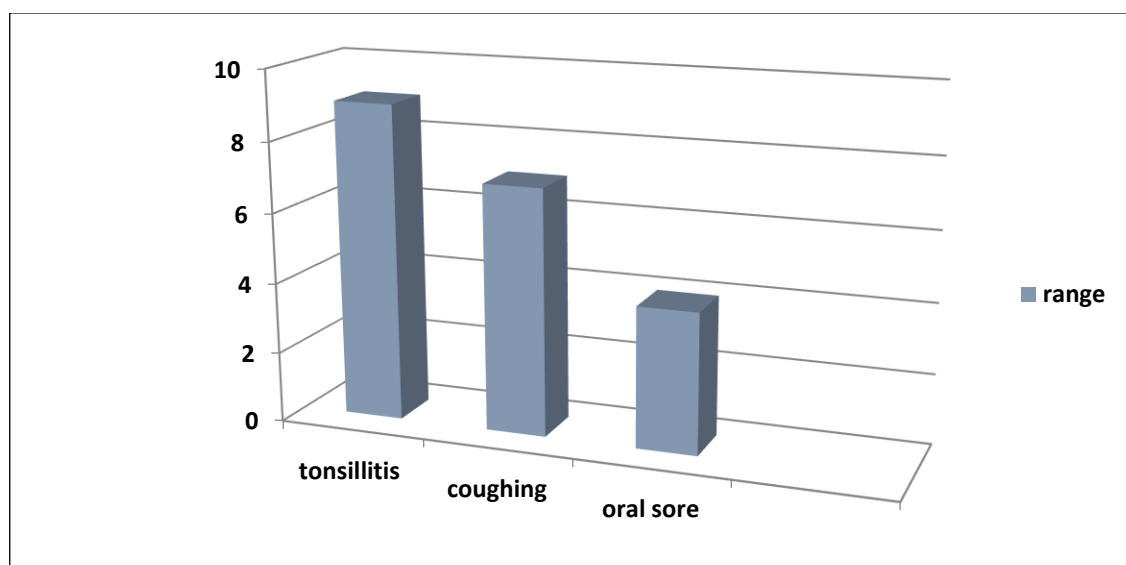


Figure (5): Demonstrates the appearance percentage of tonsillitis, coughing, and oral sore, in patients samples.

The results showed that bacteria *Streptococcus pyogenes* is the most abundant among samples 70%, we conclude that the is highly in abundance percentage and causing oral inflammation due to its active virulence factors.

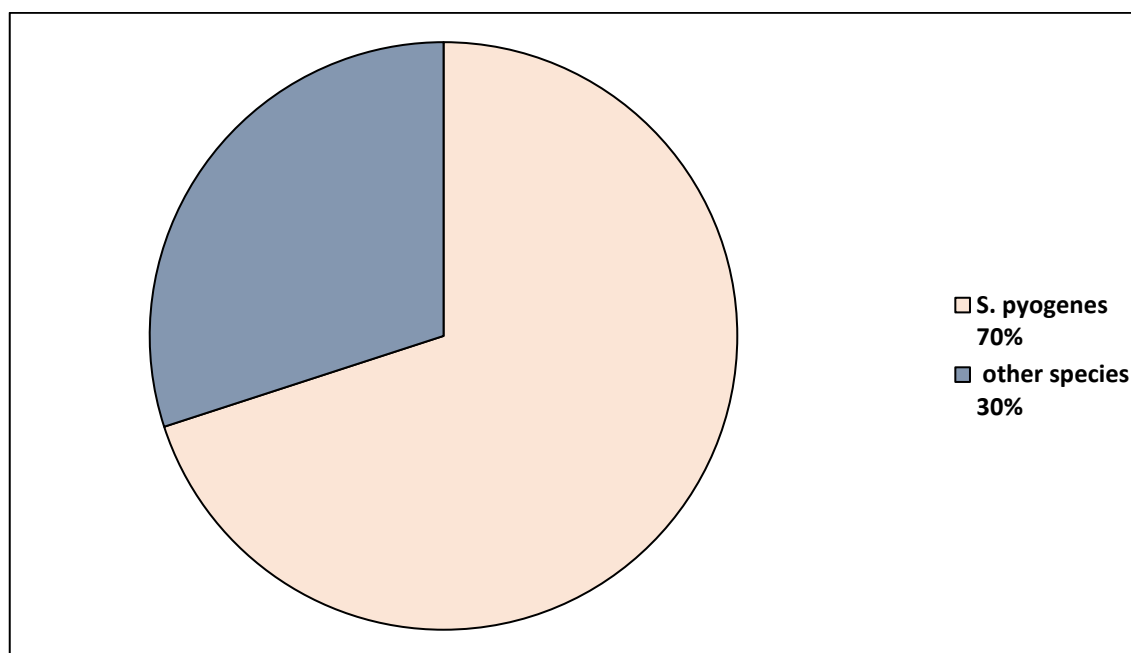


Figure (6): demonstrates the appearance percentage of *S. pyogenes* to others species of *Streptococcus*.

CONCLUSION

Conclude that the *Streptococcus pyogenes* is highly in abundance percentage and causing oral inflammation due to its active virulence factors. Early diagnosis and treatment are crucial on account of the potential severity of disease, which may require intensive supportive care in some cases.

RECOMMENDATION

We recommend conducting as many researches as possible on oral ulcers and using modern technologies for diagnosis because it is a contemporary disease and unresolved treatment and prevention.

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