Isolation and Identification of *Streptococcus pyogenes* from Patients and Objects in Hospital Environment in Thi-Qar city in Iraq

Adyan Nafee Abbas¹, Qasim Hassan Wida²

¹Research Scholar, College of Science, University of Thi-Qar, Iraq, ²Professor, Biology

Department, College of Nursing, University of Thi-Qar, Iraq

Abstract

This study aimed to screen *Streptococcus pyogenes* in the hospital and investigate the incidence of this bacteria. The genotypes of the *S. pyogenes* in the study samples will be determined using 16Sr RNA and some virulence factors (emm, scpA, speA genes). The majority of the samples (74.1%) were isolated from patients at the emergency room and (25.9%) were from hospital environment (objects). The current study showed that the total of 215 patients with pharyngitis were included 118 (55%) males higher than 97 (45%) females. The patients’ ages ranged from 2 to 62 years, which were divided into four categories. The majority of patients 65 (30.1%) of patients from 3 to 6 years old, 54 (25%) of patients less than 3 years, 51 (23.6%) from 7 to 11 years old, and 46 (21.3%) more than 12 years. Six isolates sent for sequencing were after that submission in NCBI-GenBank database. The results of nucleotide sequence alignment of the isolates revealed that there are multiple point mutations, four of them appeared as a single nucleotide polymorphism (SNP) and two appeared in duplicate. Polymorphic sites (Mutations) of 6 *S. pyogenes* isolates.

Keywords: GAS, 16S rRNA, Pharyngitis, Streptococcus pyogenes

Introduction

(Group A Streptococcus (GAS) is a gram-positive bacterium that grows in pairs or chains and causes complete, or B-hemolysis when cultured on sheep blood agar (¹). *Streptococcus pyogenes* is an important extracellular species of gram -positive, spherical and non-spore species, *S. pyogenes* is a facultative anaerobic bacterium, which is present in chain form. This bacterium is a conditional and opportunistic pathogen, which is always in search for suitable environment to cause a disease, this bacterium built colonies in the skin or throat and causes a wide range of complexes (²,³). The most common site of GAS infection is the oropharynx, and this presence is usually considered a prerequisite for the development of rheumatic fever, the leading cause of preventable childhood heart disease in the developing world, while GAS is mainly an extracellular pathogen, data over the past decade have shown that the organism can invade epithelial cells and live within them, GAS proliferation in the oropharynx triggers the signs and symptoms of pharyngitis (⁴). A number of virulence factors that contribute to the infectious process are created by GAS. One of the major virulence factors is the M protein, encoded by the *emm* gene, which promotes evasion of phagocytic killing (⁵). In *S. pyogenes* pathogenicity M protein shows its effect by facilitating adhesion, providing opsonophagocytosis resistance and contributing to the overall burden of GAS infections (⁶). Up to 11 different secreted superantigens can be produced by the human pathogen *Streptococcus pyogenes* that contribute to the features of cytokine-induced toxic shock.
during lethal, invasive infections such as necrotizing fasciitis. However, invasive diseases, manifested as pharyngitis, tonsillitis and childhood exanthem scarlet fever, are rare compared with symptomatic non-invasive disease that occurs in the nasopharynx, indeed, the throat and tonsils constitute the largest source of *S. pyogenes* in human populations carriage (7).

**Materials and Methods**

(Two hundred and ninety samples collected from hospital environment (75) and throat of patients (215) suffering from acute pharyngo-tonsillitis based on the symptoms and which the diagnosis by physicians with age range from 2 years to 60 years. The samples were collected in AL-Nassiriyah City. Selected samples were labeled and cultivated on blood agar and azide blood agar for 24 hours or 48 hours for better growth at 37°C in incubator. Any *S. pyogenes* suspected from samples make subculture on blood agar. Depending on morphological features of the B-haemolytic colonies and microscopically examination with gram’ stain, the pure cultures were prepared for biochemical tests and API-20 Strep to distinguish *S. pyogenes*. Polymerase Chain Reaction technique used for the amplification of specific genes belonging to *streptococcus* that is suspected to be present in both human pus. PCR products for 16S rRNA were sequenced by Macrogen Company (South Korea).

The samples were sent through Iraq biotechnology and according to the requirements of the company including 15μl for each forward and reverse the PCR products and 50 μl (10pmoles/μl) for each forward and reverse prime. Each samples was labeled with a number and name identical to the number that sent to company and samples were sent in a cool box containing cool gel pack.

**Results**

(The total number of patients (215) who have symptoms were included 118 (55%) males and 97 (45%) females, where it was significant difference between them depending on frequency of different ages in males and females. The age ranged from 2 to 62 years old in a total 215 patients. The typical appearance of *S. pyogenes* colonies is dome – shaped with a smooth or moist surface and clear margins on blood agar after 24 hours of incubation at 37°C, and the most important feature is the hemolysis zone around the colonies. They display a white –greyish colour and have diameter of > 0.5 mm as shown in figure (1-A). Out of 61 beta haemolytic *Streptococci* isolates tested, 30 (49.2%) were bacitracin sensitive, the appearance of *Streptococcus pyogenes* produces inhibition zone around the disc on the blood agar, following 24 h of incubation under anaerobic conditions as shown in figure (1-B).

![Figure 1](image)

Figure (1): (A) : Hemolysis of *S.pyogenes* ; (B): Bacitracin disc test
A total of twenty five isolates of *S.pyogenes* which identified by conventional methods such as: morphology of colonies, microscopic examination, biochemical tests and API 20 strep. Were subjected to DNA extraction and PCR assay for presence of *16S rRNA* gene. The results demonstrated that 25 (83.3%) of the isolates had *16SrRNA* gene with band 1500pb, as in figure (2). Twenty four isolates gave positive for each *emm* gene, *speA, scpA* gene as shown in figure (3), (4) and (5).

Figure (2): Conventional PCR for detection of 16S rRNA gene (bp), in *Streptococcus pyogenes* isolates.

Figure (3): Conventional PCR for detection of (*emm*) gene.

Figure (4): Conventional PCR for detection of (*speA*) gene.
Figure (5): Conventional PCR for detection of (scpA) gene.

The results of nucleotide sequence alignment of the isolates revealed that there are multiple point mutations, four of them appeared as a single nucleotide polymorphism (SNP) and two appeared in duplicate, where a substitution of the nucleotide G instead of A occurred at site 262 as shown in, and AIX of G at site 849 of the sample gene segment with MW0425850 Accession number, also the nucleotide G was replaced by the A at site 363 of the links with MW0425846, there are double mutations that occurred at site 199 and 200 of sample MW0425847 where the nucleotides are CC instead of TT, and the same mutation was seen at site .

Discussion

*S. pyogenes* has remained a significant human pathogen for centuries, it causes a wide variety of infections in humans, which vary from mild upper respiratory and skin infections to non-suppurative sequelae like Acute Rheumatic Fever and Rheumatic Heart Disease (8). The majority of patients were range (2-63 years) these results are similar to a study reported by (9), and also similar to another study reported by (10). The Samples were from (97) females (45%) and (118) males (55%), the males were predominant more than female, these results are agreement with results of study conducted by (11-13) and disagree with studies showed by (14, 15), as shown in these result there is no big difference in the sex of patients. The results of the present study displayed that the occurrence of *S. pyogenes* was 25/290 isolates (6.8%), and this result disagree with a study in Thi-qar revealed by (16) who documented a high emergence of isolates from patients with tonsillitis, but similar to a study revealed by (17). Nosocomial transmission of GAS infection into patients in the hospital environment has been described in the medical literature, the range of transmission has been limited to small numbers of health care workers (18). The current study revealed that is no presence of *Streptococcus spp.*, especially *S. pyogenes* and this result disagree with a study reported by (19) who showed that the rate of Streptococcus species is (2.75) and a high rate of *Staphylococcus aureus* (41.28). The results appeared 25 of the isolates were positive for 16S rRNA and 24 were positive for emm gene, these results are similar to a study reported by (6), who showed a similar DNA sequences of the 16S rRNA gene region of GAS isolates. The isolates appeared genetically close according to their sequence in the tree, as they are different in terms of the common ancestor, so we see them in the tree in the form singleton. Two isolates appeared in common with the ancestor, due to the presence of a high genetic similarity that appeared between them. In general, the isolates of the study were performed in the tree by their participation with one of the common ancestors, but it confirms the genetic changes that occurred in the isolates of the study, where a genetic bond was found with the isolates taken from the database NCBI. Phylogenetic tree analysis based on the partial 16S ribosomal RNA gene sequence that used for confirmative detection.
of *Streptococcus pyogenes* isolates, the analysis involved 6 nucleotide sequences, include (MW425845, MW425846, MW425847, MW425848, MW425849, MW425850). The resulting sequence were compared with worldwide reference sequence through NCBI BLAST, showed there is closely identical relationship between them (CP043530.1, CP049800.1, CP049799.1, CP047120.1, CP036531.1, CP031635.1) which were an isolates from USA (NCBI base). Sequence were aligned and edited using blast aligner.

**Conclusions**

High genetic variations were detected in *S. pyogenes* isolates. These genetics variations may make *S. pyogenes* more virulent and resistant to antibiotics.

**Ethical Clearance** : Taken from University of Thi-Qareethical committee

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**Conflict of Interest** : Nil

**References**


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