

Molecular Characterization of genes
spy1258 and *emm* of *Streptococcus*
pyogenes Isolated from
Pharyngotonsillitis patients

Summary

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Pharyngotonsillitis is one of the most common diseases, which is an inflammation of the pharynx and tonsils (palatine tonsils). *Streptococcus pyogenes* is the most common bacterial cause of pharyngotonsillitis, which is a prevalent human-specific bacterial pathogen capable of causing a broad spectrum of illnesses, ranging from mild localized infection to life-threatening invasive disease. Key symptoms of streptococcal infection include high fever, swollen and reddened tonsils, often with white patches or pus, and enlarged lymph nodes in the neck. If left untreated, the infection can lead to rheumatic fever and glomerulonephritis.

The study involved the collection of 212 throat swabs from patients suffering from tonsillitis and pharyngitis (aged 2-70 years) of both genders, who attended the Women and Children Hospital as well as Al-Hussain Teaching Hospital between December 2023 and April 2024. The samples were collected using a sterile cotton swab containing transport medium, and then these samples were transferred to the microbiology laboratory and immediately placed in brain-heart infusion broth with 5% blood.

In a total of 212 samples, 39 samples were identified as β -hemolysis, which represents 18.3% depending on the standard biochemical tests, and 173 (81.6%) represent other bacteria. Out of 39 β – hemolytic Streptococci are isolates, 8(20.5%) *S. pyogenes* were detected by bacteriological methods and confirmed by *spy1258* gene.

The susceptibility of 8 isolates of *S. pyogenes* was performed using eight antibiotics and examined by using the disc diffusion method on Muller-Hinton agar, and the results showed that all isolates were resistant (100%) to the Ampicillin, on the

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other hand, (37.5), (75.0%), (12.5%), (50.0%), (25.0%), (12.5%), and (37.5%) isolates were resistant to Azithromycin, Erythromycin, Clindamycin, Tetracyclin, Vancomycine, Chloramphenicol, and Levofloxacin respectively.

Polymerase chain reaction results confirmed the presence of the *emm* gene in only 4 isolates (50%) out of 8 isolates while all 8 isolates had the specific *spy1258* gene. The analysis of the *emm* gene sequences with a size of (907) base pairs revealed high levels of nucleotide sequence similarity between the local isolates studied and their global counterparts registered in the National Center for Biotechnology Information (NCBI) database. A phylogenetic analysis illustrate the evolutionary relationships among different isolates of *S. pyogenes* based on the *emm* gene. The phylogenetic tree showed genetic proximity between the local and reference isolates, forming cladding. This suggests that they share a recent common ancestor or have evolved in similar genetic ways. It may also indicate that they were exposed to similar environmental factors or evolutionary pressures.

The analysis identified that the M protein produced by the *emm* gene has three conserved domains, which is indicative of its roles in environmental sensing and chromosomal stability. These results imply that the M protein influences cellular functions as well as the organism's ability to adapt to mechanical stressors, hence adding to *S. pyogenes* virulence and adaptability.