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# Isolation and Amplification of Emm Gene *Streptococcus Pyogenes* Isolated from Different Ages and Different Clinical Samples

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**Abstract**

A total of 250 samples were collected from patients returning to outpatient clinics in Al-Diwaniyah And for different clinical cases and different age groups and for both gender for the period from the third of September 2020 to the end of December of 2021, and the study included the use of polymerase chain reaction to detect some of the virulence genes of *emm* gene From and ten isolates belonging to *StreptococcusPyogenes* were obtained, where the results showed that all of them contain 100% of the aforementioned genes.

**Keywords:** *Streptococcus Pyogenes*, emm,.Virulence factors

## INTRODUCTION

microorganisms, including Streptococcus. The pathogen is streptococcus (twisted berry). The term refers to bacteria that are found in pairs or clusters. Chains resembling a bead loop Streptococci are bacteria that cause infections. are gram-positive, anaerobic microorganisms facultative anaerobic and capable of withstanding both Cocci that thrive in anaerobic and microaerobic environments –type, the diameter of 0.5-1.0 mas well as the age group *S.pyogenes* causes 18 million cases of serious infections per year, resulting in 517.000 deaths [1]. This common and adaptable bacterium is responsible for a host of human diseases illness, be generalized from the most typical clinical manifestations Pharyngitis, cellulite, and impetigo are examples of infections. scarlet fever, which can be fatal on rare occasions toxic shock caused by streptococcal bacteria(STSS), puerperal sepsis, and postpartum sepsis Infection complications, such as acute glamerulonephritis[2] , as well as rheumatic fever The colonization of the upper respiratory tract has been effective.

The first stage of S.*pyogenes* disease is a penetration of the human mucosa or skin. Epithelia adhere to several textures.M-protein, Pili, Lipoteichoic acid, and Fibronectin-binding proteins]8[ have all been qualified.M protein antigen, which is found on Streptococcus group A will colonize the bacterial surface. Protein- M can be subdivided into over 100 serotypes[3]. fimbriae on the bacterial surface that protrude from the cell's edge A The use of the molecular mechanism has been adopted by Using the Polymerase Chain Reaction (PCR) and The *emm* gene, which is a novel gene, was sequenced using DNA[4].

The virulence factor (M-protein) is encoded by this gene، The Mprotein activates host activity by taking into account the key virulence factors aiding in the removal of bacteria from the host immune system reactionwhen it comes to adhesion to human epithelial cells and phagocytosis suppression [5.] M-protein is a protein that is found in the cell wall. Via its binding to the *emm* gene, it is synthesized. fibrinogen and complement regulatory proteins It is a protein that prevents complement-mediated damage.[6].opsonization It has become clear that horizontal gene expression is an issue. shift (HGT) also plays a major role in Creating genetic diversity in streptococci is not straightforward. Any people are inherently transformable.*S.pyogenes*, in

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particular. To the greatest extent possible, The findings of the studies have been verified.[5]. [6]. features for the *emm* gene family. Many of which code for the M protein and Mike proteins, which have binding properties for a diverse variety of human proteins and play important roles in the immune response involving fibrinogen. Albumin, IgA, and IgG Fc are all examples of complement factor H. complement C4-domains, plasminogen, and domains a protein that binds. The *emm* genes are found in a specific area of the genome. The virregulon chromosome is surrounded by a regulatory gene (*mga*) [7].

**Material and method**

This study was conducted on ten isolates of Diagnosis of pyogenic streptococcus Two hundred and fifty swab samples were collected from the throat with tonselitis. Urine and skin private clinics in Al-Diwaniyah, During the period from the third of September( 2020) until the end of February( 2021). All isolates It has been recognized as *S.pyogens* On a hemolytic colony and bacitracin In addition to the diagnosis, an sensitivity test is performed .A Vitek 2technique was used to confirm this. Detection of the target *emm* gene using DNA extraction and pregnancy (PCR)The ten samples of genomic DNA were analysed.Isolates of the bacteria *S.pyogenes* Isolates that are brand new At Trypton Soy Agar, we remained overnight.She uses 5% of the blood from sheep.A genomic DNA processor was used to extractionDNA.USA Geneaid (purification kit)According to the firm's extract New colonies are supported by the company.The brain was inoculated with *S.pyogenes*bacteria.The broth is injected into the heart brain and left to incubate for 24 hours.The genomic DNA from A is then removed at 37 degrees Celsius.Infuse the brothwith new brains and hearts.The recommendations can be found above. The polymerase chain follows after that The *emm* gene was amplified using this interaction, The primers for this study were designed in Canada by IDT. It is shown in the following table( 1) according to the method of Nagano][8and his group (2003) and as follows ,Then measure the purity،The purity of nucleic acids can be measured by a nanodrop and by the two wavelengths (260/ 280 nm).

**Table (1): The DNA primers that were used in this study**

| Primer | | Primer sequence (5′-3′) | Amplicon |
|---|---|---|---|
| ***emm*** | **F** | **TATTCGCTTAGAAAATTAA** | **914 bp** |
| | **R** | **GCAAGTTCTTCAGCTTGTTT** | |

In a final volume of 50 µl working solution, a traditional Polymerase chain reaction was carried out with 25 µl master mix and 3 µl of each one primer, 5 µl of template DNA, and 14 µl of water that hasn't been deionized table(2)

**Table (2): The components of the Monoplex PCR master mix and their sizes**

| No | Mixture Contents | Volume (µl) |
|---|---|---|
| **1.** | **Master Mix** | **25** |
| **2.** | **Forward Primer** | **3** |
| **3.** | **Reverse Primer** | **3** |
| **4.** | **Template DNA** | **5** |
| **5.** | **Nuclase -Free Water** | **14** |
| **Total** | | **50** |

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PCR terminology is used with the specific prefixes Initial denaturation at 95 ° C for five minutes, one cycle Followed by thirty cycles of denaturation at 95 ° C Half a minute annealing at 63 ° C for half a minute, 30 cycles as well At 72 ° C for 1 min, 30 cycles and final extension at 72 ° C for 5 min, 1 cycle (Table 3).

**Table (3): Conditions used in the PCR thermal amplifier**

| PCR Step | Repeat cycle | Temperature | Time |
|---|---|---|---|
| Initial denaturation | **1** | **95°C** | **5 min.** |
| Denaturation | **30** | **95°C** | **30 sec.** |
| Annealing | | **63°C,** | **30 sec.** |
| Extension | | **72°C** | **1 min.** |
| Final extension | **1** | **72°C** | **5 min.** |
| Hold | **-** | **4°C** | **Forever** |

The result was after cycling the reactions at 4 Cappeared at base (914 bp)for the gene (*emm*).

**Gel electrophoresis:**

Agarose gel, electrophoresis for the PCR product of (*emm* ) gene, The PCR products were run for120min and 60 V /1% Agarose gel. Agarose gel Containing 0.5mg/ml fluorescent ethidium bromide pigmentation Results were recorded by using a U.V transilluminator, As a size marker, an l00bp DNA ladder was used.

**RESULTS & DISCUSSION:**

The results showed that all of the *S.pyogenes*isolates contained the *emm* gene, meaning the percentage was 100% for the ten isolates,As in Figure (1)

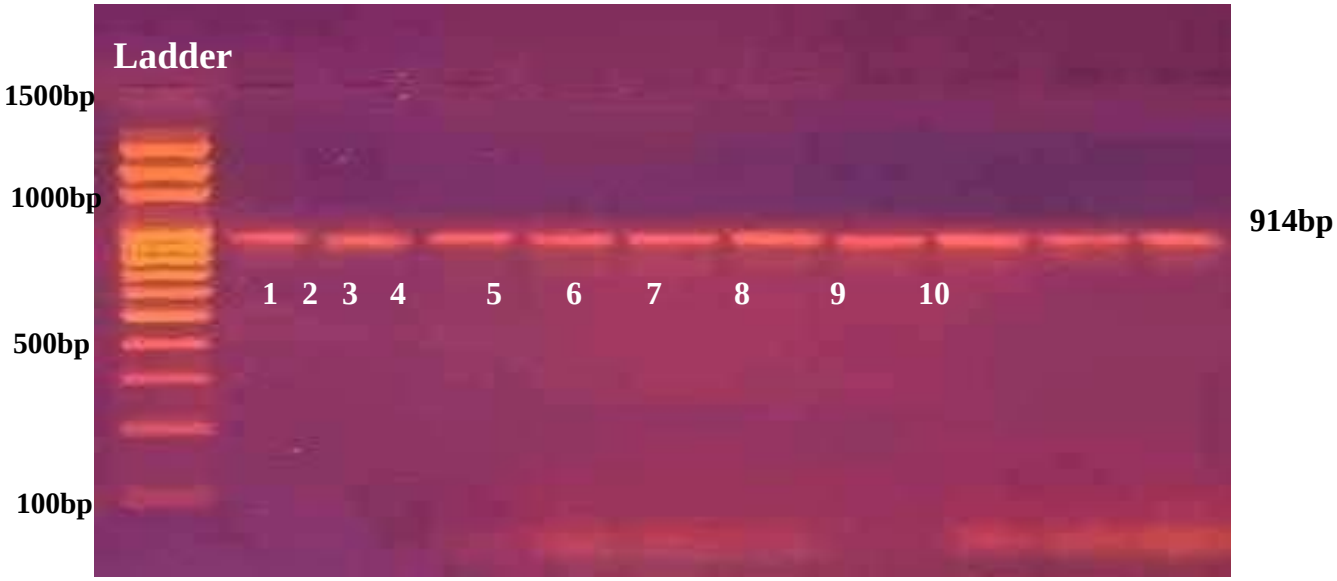


Figure (1) the electrophoresis of the Agarose gel that shows the results of the polymerization of the pathogen gene M protein (*emm* ) of *S. pyogenes* from 1-10 represent bacterial isolates

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The results of this study were not consistent with the study conducted by KHALAF9][for the year (2020), which obtained a rate of 61.5% in Anbar Nearly close to what was recorded%8.95 of what was recorded by the analyst was very similar to what was recorded Koutouzi(2015)[10]The researcher Arêas(2014)[11]received 94.5% percent.The percentage reached in this study is the highest among the previous percentages

**References**

1. Krzyściak, W., Pluskwa, K. K., Jurczak, A., and Kościelniak, D. (2013). The pathogenicity of the Streptococcus genus. European Journal of Clinical Microbiology & Infectious Diseases, 32(11), 1361-1376.
2. Parks, T., Barrett, L., and Jones, N. (2015). Invasive streptococcal disease: a review for clinicians. British medical bulletin, 115(1).
3. Facklam, R. (2002). What happened to the streptococci: overview of taxonomic and nomenclature changes. Clinical microbiology reviews, 15(4), 613-630.
4. Ralph AP, Carapetis JR. 2013. Group A streptococcal diseases and the global burden. Curr. Top. Microbiol. Immunol. 368:1–27.
5. Levering, J., Musters ,M.W., Bekker , M., fiedler , T., Bellomo , D., de vos, M.W., Hugennholtz , J., Kreikemeyer , B., Kummer, U., &Teusink,B. (2011). Role of phosphate in the central
6. metabolism of to lactic acid bacteria – a comparative system biology approach . FEBJ.Al-Kareem, F. E. A., Abbas, A. K., and Hussein, M. A. (2014). Comparative study of the Antibody Responses to *Streptococcuspyogenes* between school Children carriers and patients with Tonsillitis. Iraqi Journal of Science, 55(2), 403-410.
7. Cho, K. H., and Caparon, M. G. (2008). tRNA modification by GidA/MnmE is necessary for *Streptococcuspyogenes* virulence: a new strategy to make live attenuated strains. Infection and immunity, 76(7), 3176-3186.
8. Nagano, N., Shibata, N., Saitou, Y., Nagano, Y.and Arakawa, Y. (2003). Nosocomial outbreak of infections by Proteus mirabilis that produces extended-spectrum CTX-M-2 type b-lactamase. J. Clin. Microbiol., 41(12): 5530–5536
9. Khalaf, N. Z., Najeeb, L. M., & Abdul-Jalil, A. A. (2020). Molecular Study of spy1258 gene in Streptococcus Pyogenes Isolated from Pharyngitis Patients in Fallujah City. Medico Legal Update, 20(1), 585-590.
10. Arêas, G. P., Schuab, R. B., Neves, F. P., & Barros, R. R. (2014). Antimicrobial susceptibility patterns, emm type distribution and genetic diversity of Streptococcus pyogenes recovered in Brazil. Memórias do InstitutoOswaldo Cruz, 109(7), 935-939.
11. Koutouzi, F., Tsakris, A., Chatzichristou, P., Koutouzis, E., Daikos, G. L., Kirikou, E., ... &Michos, A. (2015). Streptococcus pyogenesemm types and clusters during a 7-year period (2007 to 2013) in pharyngeal and nonpharyngealpediatric isolates. Journal of clinical microbiology, 53(7).