



RESEARCH ARTICLE

Spread of Some Virulence Factors of Streptococcus Pyogenes Isolated From Children Tonsillitis In Kirkuk City

Heba Hilal ^{1*}, Dr Raghad Saad ²^{1,2} University of Tikret, Tikrit, Iraq**ARTICLE INFO**

Received: Apr 24, 2024

Accepted: Jun 30, 2024

Keywords

Pyogenes
Serotypes
Health risk assessment
Chronic carrier in Kirkuk city

ABSTRACT

Streptococcus pyogenes a common pathogen that causes infectious illnesses in children is also known as Lancefield group A streptococcus or GAS. In the process of figuring out which carbohydrates were involved in the Lancefield Group typing, Lancefield also isolated a protein that is a significant immunogen of GAS. The hypervariable region (HVR) of the N-terminal domain of the M protein contains the majority of its variants, and serotyping is dependent on antibodies that target this area.

***Corresponding Author:**

heba.hela@st.tu.edu.iq

INTRODUCTION

Streptococcus pyogenes a common pathogen that causes infectious illnesses in children is also known as Lancefield group A streptococcus or GAS. It is the cause of up to 15 to 30 percent of pediatric cases of acute tonsillitis that happen in the age range of 5 to 15 years. It can result in both suppurative and non-suppurative illnesses, including glomerulonephritis, erysipelas, suppurative tonsillitis, scarlet fever, and rheumatic fever (1).

Streptococcus pyogenes, is a species of aero-tolerant, Gram-positive bacterium. These extracellular bacteria are composed of non-spore forming, non-motile cocci that have a tendency to form chains. Since they are an uncommon but typically pathogenic component of the skin microbiota that can result in Group A streptococcal infection, Known as group A Streptococcus (GAS), *S. pyogenes* is the main species that carries the Lancefield group A antigen(2).

Rarely, GAS can penetrate deeper tissues and the bloodstream, leading to invasive infections such streptococcal toxic shock syndrome, necrotizing fasciitis, and bacteremia. Acute rheumatic fever (ARF) and acute post-streptococcal glomerulonephritis (PSGN) are two post-infection sequelae that can result from repeated infections. GAS affects more than 600 million people annually and results in over 500,000 fatalities, which has a substantial negative influence on human health(3).

The clinical symptoms of *S. pyogenes* are caused by a variety of virulence factors. Hyaluronic acid-based bacterial capsules offer defense against phagocytosis. M protein, lipoteichoic acid, and protein

F are responsible for the attachment of the bacteria to host cells. Additionally, *S. pyogenes* produces exotoxins, such as an erythrogenic (pyrogenic) toxin that causes toxic shock syndrome and the scarlet fever rash.

In the process of figuring out which carbohydrates were involved in the Lancefield Group typing, Lancefield also isolated a protein that is a significant immunogen of GAS. She then used this protein to type different strains of GAS. This protein was named the M protein by her upon finding that a non-typable strain had less mucoidity. She then organized the initial GAS strain collection and classification based on the expression of the M protein (5).

The hypervariable region (HVR) of the N-terminal domain of the M protein contains the majority of its variants, and serotyping is dependent on antibodies that target this area (5).

MATERIALS AND METHODS

Sample collection and culture methods

The study included the collection of (215) samples from acute and chronic tonsillitis children aged from (2 to 12 years) in Kirkuk city (Kirkuk and alnasr hospital) during the period from (15-10-2023 to 1-2-2024) these sample included (15) sample is control. Tonsils samples were taken from the inside of the tonsils by cotton swab in the cases acute tonsillitis, and in the cases chronic tonsillitis swabs were taken from the inside of the tonsils after tonsillectomy, and by sterilizing the extracted tonsils by washing them with normal saline and then with alcohol at a concentration of 70%, and opening them with a sterile scalpel and a smear of the fibrosis found in the tissue using a disposable transport media swab. Cotton swabs containing the transport medium were used during collection to ensure the vitality of the isolates and its survival for the longest period. Samples were taken from patients after get their consent and recording their data of age and gender. The collected samples were planted directly on Azide blood agar medium, blood agar medium, and β -Selective *Streptococcus* agar medium containing 5 % fresh blood and incubated under 5- 10% CO₂ for 24 hours at 37°C

Identification of *Streptococcus pyogenes*

Morphological and Cultural Characteristics

Each primary positive culture had a single colony, which was identified using its morphological characteristics (colony size, shape, color, and kind of pigments, translucency, edge and elevation)

Biochemical identification *Streptococcus pyogenes*

Capsule test, camp test, bacitracin susceptibility, oxidase and catalase were carried on bacterial isolates.

Molecular detection of *Streptococcus pyogenes*

Molecular detection of *Streptococcus pyogenes* by Polymerase Chain Reaction (PCR) amplification of 16S rRNA, M1, M3, M4 & M12 gene, primers were showed in (Table 1).

Table 1: The Housekeeping gene PCR primers with their nucleotide sequence and product size(10)

Gene name	Primer Name	Sequence (5'-3')		Product Size
16sRNA	16sRNA	F	GAGTTTGATCCTGGCTACGG	480bps
		R	GGCACGTAGTTAGCCGTCCC	
M Protein	M1	F	CTTGACGCAACAATCCCGC	578bps
		R	CGAAGGCTTTGACGACTTGC	

DNA Extraction

Bacterial genomic DNA was extracted from bacterial isolates by using (Presto™ Mini gDNA Bacteria Kit) and done according to company instructions

Gel Electrophoresis

Amplicons were visualized on 1.5% Agarose gel by Electrophoresis. The PCR products were electrophoresed through agarose gel with current 5 V/CM² for about 45 min. Gels are photographed under UV light.

Statistical Analysis

Statistical analysis was done by SPSS statistical software. Participants' demographic and clinical characteristic were described by using descriptive statistics.

p-value less than 0.05 taken as statistically significant at 95% confidence level.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of the Al-Nasr Hospital of Kirkuk city (No.).

RESULTS AND DISCUSSION

Type of tonsillitis correlated to age groups

A total of 215 clinical samples (200 patients , 15 control) are collected, 200 samples from patients with tonsillitis depending on the diagnosis of specialist doctor and additionally to many information taken from the patients questionnaire (Appendix1), and many of them were taken antibiotic before swab taken about period not exceed three days, they are distributed according to gender 110 male and 90 female and ages range from 2 to 12 years.

Results of present study showed that there is significant differences ($p < 0.05$) among age groups with acute and chronic infections. Based on age groups only, 6-9 years scored highest percentage (40%) followed by 2-5 years (36,7%), and 10-12 years scored lowest percentage (23.3%). Regarding to age groups with infection types, 6-9 years scored highest percentage in patients with chronic infection (30%), 10-12 years scored lowest percentage in acute infection (3.3%), table (1). In contrast, results study showed no significant different ($p > 0.05$) between patients with acute and chronic tonsillitis and genders.

Table 2: Type of tonsillitis correlated to age groups

Age groups	Acute (n=20)		Chronic (n=40)		Total		
	n	%	n	%	n	%	
2-5 years	12	20%	10	16.7%	22	36.7%	
6-9	6	10%	18	30%	24	40%	
10-12	2	3.3%	12	20%	14	23.3%	
P value	0.07						
Gender	Males	10	17%	18	30%	28	46.7%
	Females	10	17%	22	36%	32	53.3%
P value	0.1						

The present study shows that 2-5 years scored highest frequency in acute tonsillitis because it can be associated with increased activity of children at this age, which gives a greater chance of exposure to infection than other ages (6). Al-Tameemi *et al.* showed that tonsillitis is highly prevalence in peoples <18 years than adults, and these results matched with present study (7). Tonsillitis is referred as an acute infection of the palatine tonsils in the study of Norton and Myers, which mentioned that it is more common in <18 years than adults which supported present study results (8). Besides it may identical to the study from Nasiriyah in Iraq, in which patients of <10 years was common ages infected with tonsillitis (9).

Rustamova and Samieva, suggested some reasons for their acute tonsillitis study on children in schools; such low immunity in the children, overcrowded class rooms and poor ventilation inside (10).

In chronic tonsillitis also young aged patient was at high prevalence about (30%) followed by child's (2-5 years) about (36.7%) which was close percentage to the young aged. In addition, this is the school age in which children mix and communicate with one another in the classroom without ignoring the role of transportation within the family (11)

The present findings are in contrast to those determined by Mattila *et al.* who found that chronic tonsillitis is more common in person over 20 but less common in those under 10 years of age (12), since most patients younger than 10 years old are examined by pediatricians rather than ENT specialists, they may have missed the diagnosis in the previous study.

Bacterial isolation and Identification

Isolation of bacteria

A total of 200 clinical samples were obtained from patients. All samples were grown on blood agar and Azide blood agar and incubated aerobically at 37 °C for 24 hours. About 60 (30%) [40 chronic (20%) and 20 acute (10%)] of the samples showed positive for *Strep. pyogenes* growth, Other bacteria that causes tonsillitis also isolated like Group G *Streptococcus* 10 (5%), *H. influenza* 8(4%), *Staphylococcus aureus* and *E.coli* 5(2.5%) for both, *Corynebacterium diphtheroid* 4(2%) and *Klebsiella pneumonia* 2(1%).

whereas 111 (55.5%) showed no growth (Figure 4-1), which might be attributed to antibiotic treatment or the presence of other types of causative agents, such as viruses and fungi, which may need specialized diagnostic tests.

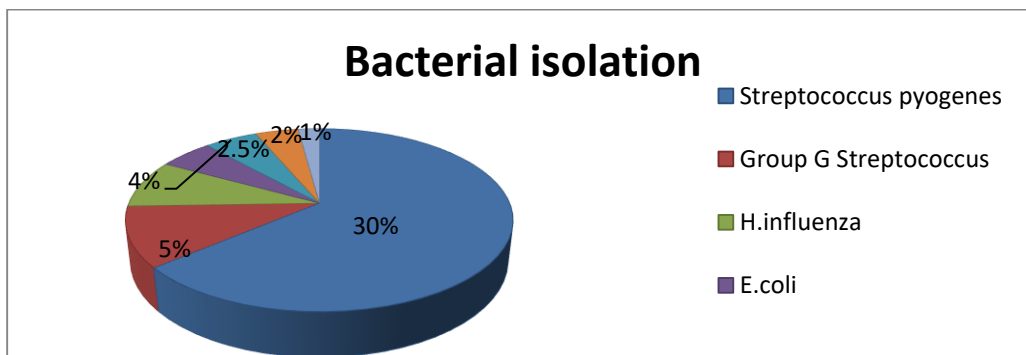


Figure (1): Bacterial growth proportion in clinical specimens

The study carried out in AL –Diwaniyah province taken 300 samples from tonsillitis patients showed 165 positive bacterial growth and 135 with no growth (13). The study carried out in Hilla city indicate that the results of bacteriological culture show 197 positive growth and 15 no growth from 212 samples, Gram-positive include *Streptococcus pyogens* (20.2%), *Staphylococcus aureus* (19.3%),

Streptococcus pneumoniae (15.3%) and *Streptococcus viridans* (11.2%). *Streptococcus pyogenes* show high percentage of isolate 20.2%. While Gram negative include, *H. influenzae* (17.1%), *K. pneumoniae* (8.1%), *P. aeruginosa* (2.03), *E. coli* (3.6%). *H. influenzae* show high percentage of isolate (17.1%) while *E. coli* shows low percentage of isolates (3.6%). The cause for not growing is probably because patients even take antibiotics before diagnosis or cause tonsillitis of the virus (6).

Other study carried out in Basrah showed 148 microbial isolates from 64 sample, bacteria were (95.37%) *Staphylococcus* (*S. aureus* and *S. epidermidis*), *Klebsiella* spp 8.5%, *Streptococcus* spp 7%, *Enterobacter* spp 6.3%. and 4.9% for *Pseudomonas* spp.(14)

Molecular identification of *Strep. pyogenes*

In order to confirm the results of biochemical test, the molecular diagnosis of *Strep. pyogenes* isolates were performed using specific primer for 16S rRNA gene by PCR technique for all *Strep. pyogenes* isolates. The result showed that 95% of isolates from chronic and 80% of isolates from acute tonsillitis gave a positive result as a single DNA band of PCR product with molecular base of 480bp (Figure 2).

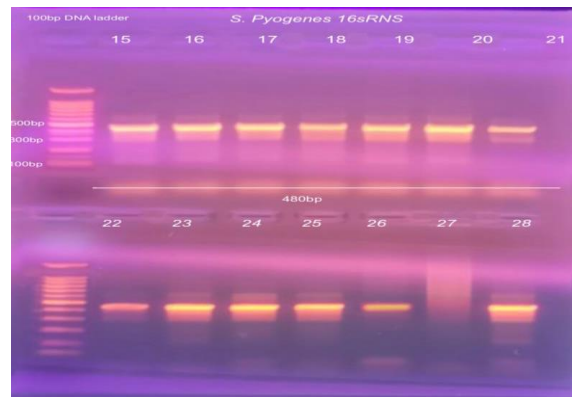


Figure (2): Conventional PCR for detection of 16S rRNA gene (bp), in *Streptococcus pyogenes* isolates. PCR product the band size 480 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm². 1x TBE buffer for 45 minutes. N: DNA ladder (100).

The result of current study was close to the finding of Abbas *et al.* (15) in which investigate that 83.3% of *Strep. pyogenes* was positive for 16sRNS in chronic infections. Also agreed with Altun *et al.*(16). *Strep. pyogenes* was identified using 16S rRNA in study carried out in Baghdad, the result showed that 50% of *Strep. pyogenes* carried 16S rRNA in acute infections. The absence of identification genes in the remaining *S. pyogenes* strains may be due to the genetic variations (17).

Molecular detection of some *Strep. pyogenes* serotypes

Detection of M1 serotype of *Strep. pyogenes*

Detection of M1 *Strep. pyogenes* isolates, the bacterial DNA amplified for this gene using PCR technique by used specific primers (Table 1), and the optimum condition to amplified this gene in PCR. The M1 protein confirmed by agarose gel electrophoresis, were the amplification to reveal a product of 578bp. The result showed that 12(30 %) of chronic and 5(25%) of acute isolates carried M1 protein, Figure (3).

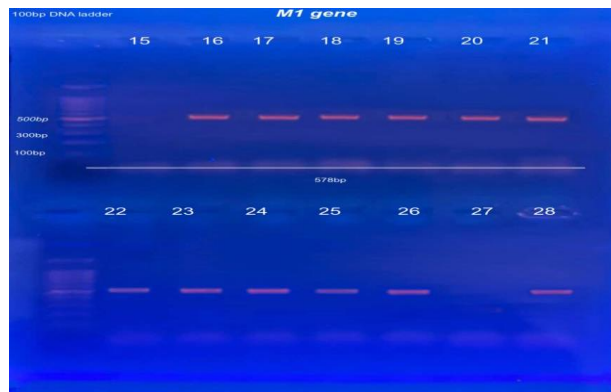


Figure (3): Conventional PCR for detection of M1 gene (bp), in *Streptococcus pyogenes* isolates. PCR product the band size 578 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm². 1x TBE buffer for 45 minutes. N: DNA ladder (100).

The M protein is of great importance as a vital landmark and can be used in the diagnosis of pathogenic bacterial strains, there are specific regions on the M protein that are common antigens, so their interaction with host proteins (humans) can cause autoimmune diseases such as respiratory, cardiac and skin infections (13).

M1 was the most commonly identified serotype (39.5%) of *Strep. pyogenes* in chronic infection study of Helal *et al.*(18). A similar observation has been documented in a previous study in the Spain (19).

The authors revealed that 1,852,442-bp sequence of an M1 strain of *Streptococcus pyogenes*, a Gram-positive pathogen, has been determined and contains 1,752 predicted protein-encoding genes. Approximately one-third of these genes have no identifiable function, with the remainder falling into previously characterized categories of known microbial function. Consistent with the observation that *S. pyogenes* is responsible for a wider variety of human disease than any other bacterial species, more than 40 putative virulence-associated genes have been identified. Additional genes have been identified that encode proteins likely associated with microbial “molecular mimicry” of host characteristics and involved in rheumatic fever or acute glomerulonephritis (20).

M1 organisms also commonly cause pharyngitis. For reasons that are unknown, M1 isolates and organisms expressing other M serologic types can undergo rapid temporal variation in disease frequency and severity (21).

CONCLUSION

Core of tonsils resemble a store for many bacteria. In present study, different genera have appeared. The 16S rRNA gene was identical gene for *S. pyogenes* in chronic infection, and used as useful tool for tonsillitis GAS rapid method to diagnosis.

ACKNOWLEDGEMENTS

To My Country

To My University

To My Department

To Dr.Mohammed Hamed Yassen fo help me.

Conflict of interests

The study is aimed to isolate and diagnosis of streptococcus pyogenes from chronic and acute tonsillitis and molecular studied of bacterial serotypes

REFERENCES

- 1-Koutouzi F, Tsakris A, Chatzichristou P, Koutouzis E, Daikos GL, Kirikou E, Petropoulou N, Syriopoulou V, Michos A. *Streptococcus pyogenes* emm types and clusters during a 7-year period (2007 to 2013) in pharyngeal and nonpharyngeal pediatric isolates. *J Clin Microbiol.*;2015;53(7):2015–21.
- 2-Kimberlin, D. W., Brady, M. T., Jackson, M. A., Long, S. S., & American Academy of Pediatrics. Group A streptococcal infections. *Red Book: 2015 Report of the Committee on Infectious Diseases*;2015; 732.
- 3-Soderholm, A. T., Barnett, T. C., Sweet, M. J., & Walker, M. J. (2018). Group A streptococcal pharyngitis: Immune responses involved in bacterial clearance and GAS-associated immunopathologies. *Journal of leukocyte biology*, 2018;103(2): 193-213.
4. Kanwal S, Vaitla P. *Streptococcus Pyogenes*. [Updated 2023 Jul 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK554528/>
5. Wajima, T., Chiba, N., Morozumi, M., Shouji, M., Sunaoshi, K., Sugita, K., et al. Prevalence of macrolide resistance among group A streptococci isolated from pharyngotonsillitis. *Microb. Drug Resist.*2014; 20:431–435.
6. Nabat ZN, ALateef BA, Hussain IM. Bacteriological and Immunological Study of Patients with Tonsillitis in Hila City. *Iraqi j of biotechnology* .2019;18(2).
7. Al-Tameemi K A K H, Hraishawi R M, & Aaiz F L. Isolation, Identification and Evaluation of Resistance of Bacterial Isolates from Patients with Tonsillitis. *Annals of Tropical Medicine and Public Health*.2020; 23: 23-1017.
8. Norton L, Myers A. The treatment of streptococcal tonsillitis/pharyngitis in young children. *World Journal of Otorhinolaryngology - Head and Neck Surgery*. 2021 Jul;7(3):161–5.
9. Dakhil BR, Hamim SS. Antibiotic susceptibility of *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from Pharyngitis and Tonsillitis patients in Nasiriyah City, Iraq. *World Journal of Pharmaceutical Sciences*. 2016;4(4):14-19.
10. Rustamova GR, Samieva GU. DIAGNOSIS AND TREATMENT OF CHRONIC TONSILLITIS IN CHILDREN AT THE PRESENT STAGE.(LITERATURE REVIEW). *British Medical Journal*.2022; 2(1).
11. U S, Rao PN, G R AR, V M. EFFECT OF KUMARABHARANA RASA ON CHRONIC TONSILLITIS IN CHILDREN: A PILOT CLINICAL STUDY. *International Journal of Research in Ayurveda and Pharmacy*. 2013 Apr 28;4(2):153–7.
12. Mattila PS, Olli Tahkokallio, Jussi Tarkkanen, Janne Pitkaniemi, Karvonen M, Jaakko Tuomilehto. Causes of Tonsillar Disease and Frequency of Tonsillectomy Operations. 2001 Jan 1;127(1):37–7.
13. Wajid A R & Abd AL-Mayahi F S. Molecular Identification of *Streptococcus pyogenes* isolated from tonsillitis in AL-Diwaniyah province.2023;12(3):2230-5807.
14. Al-Tameemi K A K H, Hraishawi R M, & Aaiz F L. Isolation, Identification and Evaluation of Resistance of Bacterial Isolates from Patients with Tonsillitis. *Annals of Tropical Medicine and Public Health*.2020; 23: 23-1017.
15. Abbas A & Wida Q H. Isolation and Identification of *Streptococcus pyogenes* from Patients and Objects in Hospital Environment in Thi-Qarcity in Iraq. *Indian Journal of Forensic Medicine & Toxicology*,2021; 15(4): 216-221.
16. Altun M &Yapıcı B. M. Detection of Group A Beta Hemolytic Streptococci Species, emm, and Exotoxin Genes Isolated from Patients with Tonsillopharyngitis. *Current : .2064-2070 2020.;77(9)microbiology*
17. Ali H N, Dhahi M A & Abd A K H. Molecular screening for erythromycin resistance genes in *Streptococcus pyogenes* isolated from Iraqi patients with tonsilo-pharyngites. *African Journal of Biotechnology*,2015; 14(28): 2244-2250.

18. Helal, Z. M., Rizk, D. E., Hassan, R., & Adel El-Sokkary, M. M. (2020). Prevalence and characterization of Streptococcus pyogenes clinical isolates from different hospitals and clinics in Mansoura. *International Journal of Microbiology*, 2020, 1-11.
19. González-Abad M J, Sanz M A. Invasive Streptococcus pyogenes infections (2011–2018): EMM-type and clinical presentation. *Anales de Pediatría (English Edition)*, 2020; 92(6): 351-358.
20. Ferretti J. J., McShan, W. M., Ajdic, D., Savic, D. J., Savic, G., Lyon, K., ... & McLaughlin, R. (2001). Complete genome sequence of an M1 strain of Streptococcus pyogenes. *Proceedings of the National Academy of Sciences*, 2001; 98(8): 4658-4663.
21. Xu T & Zhang W. Streptococcus pyogenes. In *Molecular Medical Microbiology* (pp. 705-753). Academic Press. 2024.
22. Jam, F. A., Singh, S. K. G., Ng, B., & Aziz, N. (2018). The interactive effect of uncertainty avoidance cultural values and leadership styles on open service innovation: A look at malaysian healthcare sector. *International Journal of Business and Administrative Studies*, 4(5), 208-223.
23. Kanval, N., Ihsan, H., Irum, S., & Ambreen, I. (2024). Human Capital Formation, Foreign Direct Investment Inflows, and Economic Growth: A Way Forward to Achieve Sustainable Development. *Journal of Management Practices, Humanities and Social Sciences*, 8(3), 48-61.