https://doi.org/10.47108/jidhealth.Vol7.Iss6.374
Alghrairi AH, Bunyan IA, Journal of Ideas in Health (2024); 7(6):1187-1191
Journal of Ideas in Health

Biofilm formation and virulence factors in streptococcus pyogenes isolated from pharyngitis patients: implications for diagnosis and treatment

Alyaa Hashim Alghrairi[1*], Ilham A. Bunyan[2]

Abstract

Background: Streptococcus, including Streptococcus pyogenes, causes infections from mild to severe. Understanding biofilm formation and virulence is crucial due to high mortality. This study aims to assess the biofilm production capabilities of different Streptococcus species isolated from patients with suspected pharyngitis. Specifically, it examines the correlation between biofilm formation and the bacterial virulence of Streptococcus pyogenes.

Methods: A total of 100 throat swabs were collected and cultured on selective media, specifically 5% defibrinated sheep blood agar and azide blood agar. Identification of isolates was achieved using the Vitek2 System and confirmed through 16S rRNA gene amplification and sequencing, utilizing specific primers. The nucleotide sequences were compared to reference databases to ensure accurate identification. Biofilm production was evaluated using the Congo Red Agar method and the Microtiter Plate Test, categorizing isolates based on their production capacity.

Results: The results revealed a high bacterial growth rate of 89.0%, with Streptococcus pyogenes being the most prevalent isolate (28.6%), followed by Streptococcus pneumoniae and Streptococcus viridans. Significant variations in biofilm production were observed, with Streptococcus pyogenes exhibiting a strong correlation with significant biofilm formation, suggesting its potential enhanced virulence and increased resistance to treatments.

Conclusion: The findings highlight the critical role of biofilm formation in the virulence of Streptococcus pyogenes and underscore the need for integrating biochemical, molecular, and phenotypic methods for accurate bacterial identification and understanding of their pathogenic mechanisms. The use of molecular methods such as 16S rRNA sequencing provides a robust framework for the identification and characterization of these pathogens, contributing essential insights into the clinical implications of bacterial biofilm formation and informing improved management strategies against Streptococcus-related infections.

Keywords: 16s rRNA typing, Pharyngitis, VITEK 2 system, Streptococcus, Biofilm production, Iraq

Correspondence: Alyaa Hashim Alghrairi (aliaa.h@uodiyala.edu.iq)
[1]Department of Microbiology, College of Medicine, University of Babylon, Babylon, Iraq

How to cite: Alghrairi A, Bunyan I. Molecular identification and typing of streptococcus isolates from sore throat patients using 16S rRNA sequencing. J Ideas Health. 2024 Dec. 31;7(6): 1187-1191. https://doi.org/10.47108/jidhealth.Vol7.Iss6.374

Article Info: (**Original Research**)
Received: 08 September 2024
Revised: 26 September 2024
Accepted: 27 December 2024
Published: 31 December 2024

Journal Home page: https://www.jidhealth.com

e ISSN: 2645-9248

Background

The Streptococcus genus covers distinct Gram-positive bacteria that all have a spherical microscopic appearance. Their classifier is primarily determined by the antigens on the cell wall's surface [1]. The members of this genus cause purulent infections that can range in severity from minor throat infections to meningitis, abscesses, bacteremia, pneumonia, and streptococcal toxic shock syndrome. They have been found in the mucosal membranes of the mouth, upper respiratory tract, and lower genital tract. The agents that cause these infections are also members of this group of bacteria. Millions of deaths worldwide are attributed to Streptococcus pneumoniae, Streptococcus pyogenes, and Streptococcus agalactiae due to their virulence and detrimental effects on human health [2]. The members of this genus cause purulent infections that can range in severity from minor throat infections to meningitis, abscesses, bacteremia, pneumonia, and streptococcal toxic shock syndrome. They are frequently observed in the mucosal membranes of the mouth, upper respiratory tract, and lower genital tract. The agents that cause these infections are also members of this group of bacteria. Millions of fatalities nationwide are related to the pneumonia-causing bacteria Streptococcus pyogenes, and Streptococcus agalactiae due to their virulence and adverse impacts on human health [3,4]. Over the past ten years, the application of sequencing-based molecular diagnostics has grown in complexity as a means of addressing the shortcomings of culture-

based diagnostics. In recent years, there has been an increase in the availability of a particular tool that is based on the amplification and sequencing of the 16S ribosomal RNA (rRNA) gene, along with improvements in turnaround time. Depending on the assay's design, the variable and conserved portions of the 16S rRNA gene, which is found in all bacteria, allow for the identification of most of them down to the genus or species level. Studies in the literature have revealed that, depending on the region used for sequencing, species-level identification is possible in 65%–91% of cases [5]. It is necessary to carry out Multilocus Sequence Analysis (MLSA) on many housekeeping genes. However, from a practical point of view, a more straightforward method for the initial identification of a species is required, especially in cases where the extracted DNA is insufficient for MLSA or does not respond with all of the typing primers [6]. Clinical laboratories first employed 16S rRNA gene polymerase chain reaction (PCR) and Sanger sequencing (16S rRNA PCR/sequencing) to detect isolates that were difficult to identify by phenotypic means [7]. In recent times, 16S rRNA PCR/sequencing has been applied directly to clinical specimens, particularly for the purpose of identifying bacteria that are challenging to grow or those that are rendered non-cultivable by antibiotic therapy [8]. Biofilm formation is thought to be an important GAS virulence factor, and many GAS strains can form biofilms in vitro [9] Numerous hypotheses explaining antibiotic treatment failure have been proposed, including biofilm formation [10]. The biofilm phenotype provides an increased survival advantage, enabling bacteria to persist and resist both host immune defenses and antimicrobial treatment [11-13]. However, biofilm formation has consistently been studied on abiotic (plastic or glass) surfaces that do not represent or mimic the mucosal surfaces occupied by GAS during colonization and infection. Biofilms formed on abiotic surfaces produce an extracellular matrix (ECM) composed primarily of DNA and proteins (14), and the ability of GAS strains to auto-aggregate in culture has been found to correlate with their ability to form biofilms (15). It has therefore been proposed that auto aggregation as well as attachment to structures on biological surfaces may play a role in GAS biofilm formation in vivo [9].

Methods

Participants and collection of specimens

This cross-sectional study involved the isolation and analysis of Streptococcus spp. from clinical specimens. A total of 100 samples were collected from patients diagnosed with streptococcal infections at Baquba Teaching Hospital, Diyala, Iraq during the period from September 2023 to April 2024. The ages of the patients ranged between 10 and 65 years, with a gender distribution of 45 males and 55 females (45% and 55% respectively). The 100 throat swabs containing clear Amies gel in transport culture medium (Copan™ Throat Swab, Copan, Italy) were collected from patients with suspected Streptococcus-induced pharyngitis and transported to the laboratory under appropriate conditions.

Isolation and Identification

On a 5% defibrinated sheep blood agar plate (BBLTM Blood Agar Base, BD, USA), each specimen was streaked. The plates were kept in anaerobic surroundings at 37 °C with 5% CO2 in a candle jar (Candle Jar System™, VWR, USA) using an incubator (Incubator™, Memmert, Germany). For selectively isolating Streptococcus spp. after incubation the presumptive colonies were further subcultures on azide blood agar (Azide Blood Agar™, HiMedia, India) to ensure purity.

Bacterial strains

The cultures purified were tentatively identified on the basis Vitek2 System. Colony DNA extraction was performed using the Taco™ DNA Extraction Kit (Taco, Taiwan) for further analysis.

Molecular detection of Isolates

Using the gene-specific primers 16S rRNA F and 16S rRNA R [16]. (Table1) and an annealing temperature of 57 °C, the 1,350 bp gene was amplified by PCR. The sequence of 16S rRNA was recorded in the NCBI database. Each isolated strain has gained its accession number with the coordinate species belonged to. To verify the existence of a band, PCR products were seen under ultraviolet light on a 1% agarose gel stained with ethidium bromide. In this study, the biofilm-forming ability of bacterial isolates from patients with sore throats was assessed using two methods: the Congo Red Agar method and the Microtiter Plate Test. The Congo Red Agar method involved inoculating a medium supplemented with sucrose and Congo red, where positive results were indicated by black colonies with a dry crystalline consistency. The Microtiter Plate Test quantitatively evaluated biofilm production by measuring the optical density after crystal violet staining, with results categorized into four levels of biofilm production—non-biofilm, weak, moderate, and strong producers—based on the cut-off optical density determined from negative controls.

Table 1: Oligonucleotides used in this study

Target	Primer	Primer Sequence 5′ ⟶ ⟶ ′3	Product size (bp)
16S rRNA	F1 R1	5’-ATGAAAAATTACTTATCTTTTGGGATGT-3’ 5’- TTATTTGTCGTTAGGGTTATCAGG-3’	1,350

Results

Bacterial Isolation and Growth

Out of one hundred samples subjected to culturing on blood agar with 5% defibrinated sheep blood, bacterial growth was observed in eighty-nine percent of the samples, while eleven percent showed no bacterial growth. Additionally, the selective medium azide blood agar was employed to isolate Gram-positive bacteria, specifically targeting the Streptococcus genus. Growth in this Selective medium was confirmed for 35 (35%) samples that exhibited a bacterial presence. To ensure accurate identification, bacterial isolates were subjected to the Vitek2 System, with S. pyogenes (10 isolates, 28.6%) being the highest among other species, followed by a closely equal percentage (20%) for both S. pneumoniae and S. viridans. The system’s results showed only 4 (11.4%) aligned with the expected profiles for S. mitis. To further confirm bacterial identification, the Vitek2 System was

employed, which produced consistent results with the selective media. This alignment between phenotypic and biochemical identification systems supports the reliability of the culturing methods used [17, 18]. This result is in agreement with previous studies, which identified bacteria isolated from throat swabs using VITEK 2. The results showed that S. pyogenes had 9 isolates (10.71%), S. sanguinis 8 isolates (9.52%), S. mitis, S. parasanguinis 7 isolates (8.33%), S. pneumoniae, S. salivarius 6 isolates (7.14%), S. oralis 5 isolates (5.95%), and finally S. agalactiae 4 isolates (4.76%) with significant differences indicated by a p-value of 0.00001*. The use of azide blood agar allowed for the effective isolation of Gram-positive bacteria, specifically Streptococcus species, by inhibiting the growth of Gram-negative organisms, as reported in various studies [21, 22]. The high rate of bacterial growth (eighty-nine percent) suggests that the sample collection and culturing conditions were optimal. The absence of growth in eleven percent of samples may be due to the lack of viable Gram-positive organisms in those cases or possibly due to suboptimal conditions for bacterial growth in specific samples. As a further step in identification, DNA was extracted from the mostly identical bacterial isolates (17 out of 35, 48%). The 16S rRNA gene was successfully amplified using PCR with specific primers, and sequencing confirmed the identity of the bacteria by the presence of (1323 bp) bands (Figure 1). This method is widely used for bacterial identification.

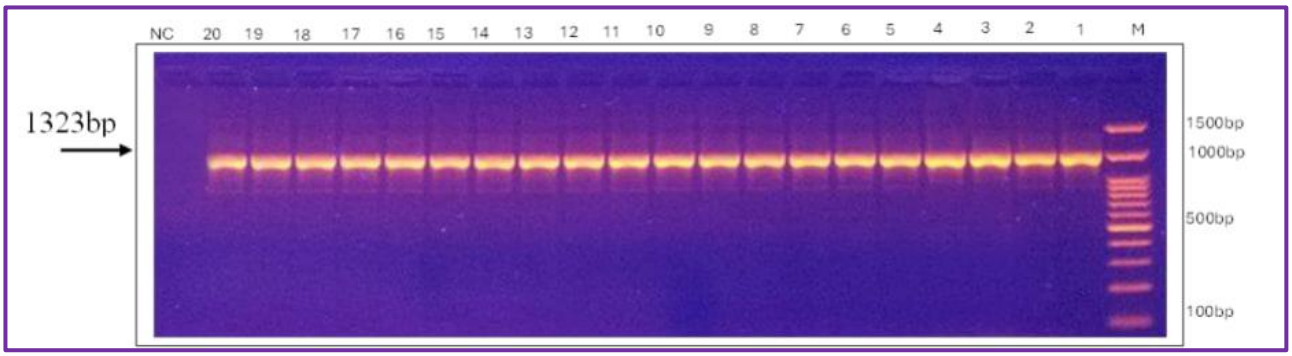

Figure 1: Analysis of PCR products by 1% agarose gel electrophoresis. Gels were visualized by staining with ethidium bromide of PCR-amplified 16S rRNA gene (1323 bp). The results of the molecular analysis were fully consistent with those obtained from the Vitek2 System and the selective culturing process. Each isolated strain has gained its accession number with the corresponding species.

Table 2: Accession Numbers for Streptococcus Strains

Species	Strain	Accession Number
Streptococcus pyogenes	AIBHM1	PQ288985
Streptococcus pyogenes	AIBHM2	PQ288986
Streptococcus pyogenes	AIBHM3	PQ288987
Streptococcus pyogenes	AIBHM4	PQ288988
Streptococcus pyogenes	AIBHM5	PQ288989
Streptococcus pyogenes	AIBHM6	PQ288990
Streptococcus pyogenes	AIBHM9	PQ288993
Streptococcus pyogenes	AIBHM10	PQ288994
Streptococcus pyogenes	AIBHM11	PQ288995
Streptococcus pyogenes	AIBHM12	PQ288996
Streptococcus pyogenes	AIBHM13	PQ288997
Streptococcus pyogenes	AIBHM14	PQ289000
Streptococcus dysgalactiae	AIBHM17	PQ289001
Streptococcus dysgalactiae	AIBHM18	PQ289002
Streptococcus dysgalactiae	AIBHM19	PQ289003
Streptococcus dysgalactiae	AIBHM20	PQ289004

Molecular analysis using 16S rRNA sequencing added an additional layer of confirmation, as the results from PCR amplification and sequencing were in complete agreement with both the selective media and the Vitek2 System. This consistency across different methodologies demonstrates the robustness of the approach taken in this study. The findings have key advantages in the field of diagnostics regarding pathogen diagnosis, where the quality and time taken to identify causative agents of disease are fundamental for management. Subsequent work should seek to broaden the scope of this approach to more infections and patient populations and tackle the challenge of Optimizing culture in non-growing bacterial specimens. Additionally, the diagnosis of the patient at the hospital level, using this integrated method of laboratory engineering, shows a tendency to improve clinical outcomes and management. Biofilm

Production Statistical Analysis

The analysis of biofilm production revealed significant differences among the isolates, as summarized in Table 3. The data indicates significant differences among the biofilm production categories. Specifically, the P-value for weak biofilm producers (0.0006) and strong biofilm producers (0.0225) highlights their significant departure from the expected values, even at a 0.01 significance level. These findings emphasize the importance of categorizing biofilm production, as they reveal varying capabilities among the isolates, which can be critical for understanding their impact on clinical outcomes. The analysis of biofilm production in this study highlighted significant variations among the isolated strains of Streptococcus pyogenes, which aligns with findings from recent research in this area. Specifically, a total of nine isolates were categorized as non-biofilm producers, exhibiting characteristics consistent with what is noted in previous literature where non-biofilm forming strains show limited virulence potential and are less likely to persist in clinical contexts. The statistics for this group indicated no significant deviation from expected frequencies, suggesting that these isolates may follow a predictable pattern regarding biofilm production. In contrast, the categorization of nineteen isolates as weak biofilm producers presented a notable divergence from expected values, resulting in a statistically significant P-value of 0.0006. This finding is particularly important, as recent studies have indicated that weak biofilm formation may be linked to

increased persistence and resistance to treatment, facilitating the organism's survival in host environments. Furthermore, the five isolates identified as moderate biofilm producers mirrored trends observed in earlier reports that link moderate levels of biofilm formation to adaptive responses in stressed environments. The strong biofilm producers—two isolates in this analysis—also exhibited a significant departure from the expected values (P = 0.0225), emphasizing their potential pathogenicity. This observation is supported by evidence indicating that robust biofilm formation is associated with higher virulence in S. pyogenes, particularly in invasive infections. Additionally, the consistent observation of different biofilm capacities across emm types highlight the relevance of understanding biofilm formation in epidemiological patterns related to specific strains. The results of this analysis contribute to understanding the clinical implications of biofilm formation in S. pyogenes infections. Variations in biofilm production capabilities can significantly impact the pathogenic potential of different isolates, influencing their ability to cause chronic infections and enhance resistance to antibiotic therapies. Understanding these phenotypic characteristics can aid in predicting clinical outcomes and tailoring therapeutic strategies to combat biofilm-associated infections in patients.

Table 3: Biofilm Production Statistical Analysis.

Category	Count	Observed Values	Expected Values	P-Value
Non-biofilm producer (OD ≤ ODc)	9	9	8.75	0.9332
Weak biofilm producer (ODc < OD ≤ 2 x ODc)	19	19	8.75	0.0006
Moderate biofilm producer (2 x ODc < OD ≤ 4 x ODc)	5	5	8.75	0.2048
Strong biofilm producer (4 x ODc < OD)	2	2	8.75	0.0225

Discussion

The findings from this study provide significant insights into the bacterial isolation and identification processes, particularly concerning Streptococcus species. The high rate of bacterial growth observed (89%) suggests that the sample collection and culturing conditions were optimal, which is consistent with previous studies that reported similar success rates in isolating Streptococcus species from clinical samples. The absence of growth in 11% of samples may indicate either a lack of viable Gram-positive organisms or suboptimal growth conditions, which aligns with findings in the literature regarding the challenges of culturing certain bacterial species [21, 22]. The use of the Vitek2 System for bacterial identification proved effective, as it yielded results consistent with those obtained from selective media. This corroboration between phenotypic and biochemical identification methods supports the reliability of the culturing techniques employed in this study [17, 18]. Furthermore, the molecular analysis using 16S rRNA sequencing provided an additional layer of confirmation, reinforcing the robustness of the identification methods utilized. The integration of these methodologies enhances the diagnostic capabilities in clinical microbiology, particularly in identifying pathogens responsible for infections. The analysis of biofilm production revealed significant differences among the isolates, highlighting the varying capabilities of Streptococcus pyogenes strains to form biofilms. The categorization of isolates into non-biofilm, weak, moderate, and strong producers is crucial for understanding their pathogenic potential. Previous research has indicated that biofilm formation is associated with increased virulence and resistance to treatment, particularly in chronic infections [25, 26]. The significant P-values obtained for weak and strong biofilm producers in this study further emphasize the clinical relevance of these findings, suggesting that biofilm production may play a critical role in the persistence and treatment resistance of these bacterial strains. Moreover, the observed variations in biofilm production capabilities among different isolates can inform therapeutic strategies. Understanding the phenotypic characteristics of these strains can aid in predicting clinical outcomes and tailoring treatment approaches to effectively manage biofilm-associated infections. Future research should aim to expand the scope of these findings by exploring biofilm production in a broader range of infections and patient populations, as well as optimizing culture techniques for non-growing bacterial specimens.

Conclusion

This study establishes the benefit of culture methods with that of 16S rRNA sequencing in diagnosing Streptococcus species in patients with sore throats. Our results suggest that although the Vitek2 system can provide reliable diagnostic results, 16S rRNA sequencing offers more rapid and accurate methods for diagnosis that go beyond what is traditionally applied. The good level of agreement obtained between the molecular biological method and the culture method emphasizes the effectiveness of this dual approach strategy.

Abbreviation

rRNA: Ribosomal RNA; MLSA: Multilocus Sequence Analysis; ECM: Extracellular Matrix; PCR: Polymerase Chain Reaction

Declaration

Acknowledgment

None

Funding

The author received no financial support for the research, authorship, and/or publication of this article.

Availability of data and materials

Data will be available by emailing aliaa.h@uodiyala.edu.iq

Authors' contributions

Alyaa Hashim Alghrairi (AHA) participated in the design of the study, data collection, analysis, interpretation, and writing the

final draft. Ilham A. Bunyan (IAB) participated in supervising, analysis, and interpretation. The authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

We conducted the research following the Declaration of Helsinki. The protocol was granted by the scientific ethical committee in the College of Medicine, Babylon University, Iraq [Ref. No. issued on 1st November 2023]. Moreover, written consent was taken from patients, and privacy was ensured for all participants.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

Author Details

[1]Department of Microbiology, College of Medicine, University of Babylon, Babylon, Iraq.

References

1. Creti R. Have group A and B streptococcal infections become neglected diseases in Europe? Springer; 2017. p. 1063-4.
2. Karpay S, Sarada C, Kondu D, Pavuluri P, Gadepalli R, Naresh B. Evaluation of biochemical parameters in acute myocardial infarction and angina patients. J Ideas Health. 2022 May 14;5(2):664-668. doi: 10.47108/jidhealth.vol5.iss2.213.
3. Slotved H-C, Hoffmann S. The epidemiology of invasive group B Streptococcus in Denmark from 2005 to 2018. Front Public Health. 2020;8:40. doi: 10.3389/fpubh.2020.00040.
4. Stevens DL, Bryant AE. Streptococcus pyogenes Impetigo, Erysipelas, and Cellulitis. 2022.
5. Patel JB. 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Mol Diagn. 2001;6:313-21. doi: 10.1385/MD:6:4:313.
6. Gonzales-Siles L, Karlsson R, Schmidt P, Salvà-Serra F, Jaén-Luchoro D, Skovbjerg S, et al. A pangenome approach for discerning species-unique gene markers for identifications of Streptococcus pneumoniae and Streptococcus pseudopneumoniae. Front Cell Infect Microbiol. 2020;10:222. doi: 10.3389/fcimb.2020.00222.
7. Church DL, Cerutti L, Gürtler A, Griener T, Zelazny A, Emler S. Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clin Microbiol Rev. 2020;33(4):e00053-19. doi: 10.1128/CMR.00053-19.
8. Bador J, Nicolas B, Chapuis A, Varin V, Dullier-Taillefumier N, de Curraize C, et al. 16S rRNA PCR on clinical specimens: impact on diagnosis and therapeutic management. Med Mal Infect. 2020;50(1):63-73. doi: 10.1016/j.medmal.2020.01.001.
9. Fiedler T, Köller T, Kreikemeyer B. Streptococcus pyogenes biofilms—formation, biology, and clinical relevance. Front Cell Infect Microbiol. 2015;5:15. doi: 10.3389/fcimb.2015.00015.
10. Lembke C, Podbielski A, Hidalgo-Grass C, Jonas L, Hanski E, Kreikemeyer B. Characterization of biofilm formation by clinically relevant serotypes of group A streptococci. Appl Environ Microbiol. 2006;72(4):2864-75. doi: 10.1128/AEM.72.4.2864-2875.2006.
11. Baldassarri L, Creti R, Recchia S, Imperi M, Facinelli B, Giovanetti E, et al. Therapeutic failures of antibiotics used to treat macrolide-susceptible Streptococcus pyogenes infections may be due to biofilm formation. J Clin Microbiol. 2006;44(8):2721-7. doi: 10.1128/JCM.00561-06.
12. Conley J, Olson ME, Cook LS, Ceri H, Phan V, Davies HD. Biofilm formation by group A streptococci: is there a relationship with treatment failure? J Clin Microbiol. 2003;41(9):4043-8. doi: 10.1128/JCM.41.9.4043-4048.2003.
13. Marks LR, Reddinger RM, Hakansson AP. Biofilm formation enhances fomite survival of Streptococcus pneumoniae and Streptococcus pyogenes. Infect Immun. 2014;82(3):1141-6. doi: 10.1128/IAI.01310-13.
14. Speziale P, Geoghegan JA. Biofilm formation by staphylococci and streptococci: structural, functional, and regulatory aspects and implications for pathogenesis. Front Media SA. 2015; p. 31. doi: 10.3389/978-2-88919-555-1.
15. Matysik A, Kline KA. Streptococcus pyogenes capsule promotes microcolony-independent biofilm formation. J Bacteriol. 2019;201(18):e00052-19. doi: 10.1128/JB.00052-19.
16. Luo F, Lizano S, Banik S, Zhang H, Bessen DE. Role of Mga in group A streptococcal infection at the skin epithelium. Microb Pathog. 2008;45(3):217-24. doi: 10.1016/j.micpath.2008.04.008.
17. Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, et al. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One. 2015;10(2):e0117617. doi: 10.1371/journal.pone.0117617.
18. Alim-Marvasti A. Non-invasive thrombectomy: magnetized antibodies in reperfusion of thromboses. J Ideas Health. 2020 May 17;3(1):135-137. doi: 10.47108/jidhealth.vol3.iss1.46.
19. Al Bulushi IM, Al Kharousi ZS, Rahman MS. Vitek: a platform for a better understanding of microbes. In: Techniques to measure food safety and quality: microbial, chemical, and sensory. 2021:117-36.
20. Radi AQ, Hammadi AA, HM AA. Detection of Streptococcus pyogenes from Clinical Isolates in Iraqi Community. 2022.
21. Snyder ML, Lichstein HC. Sodium azide as an inhibiting substance for gram-negative bacteria. J Infect Dis. 1940;67(2):113-5. doi: 10.1093/infdis/67.2.113.
22. Mallmann W. A new yardstick for measuring sewage pollution. Sewage Works J. 1940;875-8.
23. Rizal NSM, Neoh H-M, Ramli R, Hanafiah A, Samat MNA, Tan TL, et al. Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: perspectives from a middle-income country. Diagnostics. 2020;10(10):1-15. doi: 10.3390/diagnostics10100874.
24. Peker N, Garcia-Croes S, Dijkhuizen B, Wiersma HH, van Zanten E, Wisselink G, et al. A comparison of three different bioinformatics analyses of the 16S–23S rRNA encoding region for bacterial identification. Front Microbiol. 2019;10:620. doi: 10.3389/fmicb.2019.00620.
25. Skutlaberg DH, Wiker HG, Mylvaganam H, IS Group, Norrby-Teglund A, Skrede S. Consistent biofilm formation by Streptococcus pyogenes emm 1 isolated from patients with necrotizing soft tissue infections. Front Microbiol. 2022;13:822243. doi: 10.3389/fmicb.2022.822243.
26. Alves-Barroco C, Paquete-Ferreira J, Santos-Silva T, Fernandes AR. Singularities of pyogenic streptococcal biofilms–from formation to health implication. Front Microbiol. 2020;11:584947. doi: 10.3389/fmicb.2020.584947.
27. Gómez-Mejia A, Orlietti M, Tarnutzer A, Mairpady Shambat S, Zinkernagel AS. Inhibition of Streptococcus pyogenes biofilm by Lactiplantibacillus plantarum and Lacticaseibacillus rhamnosus. mSphere. 2024; e00430-24. doi: 10.1128/msphere.00430-24.
28. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals. 2023;16(11):1615. doi: 10.3390/ph16111615.