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Prevalence and Antibiotic Susceptibility Pattern of *Streptococcus pyogenes* Isolated from Aljanad University Students, Taiz City, Yemen

Dr. Najeeb Kaid Nasser Al-Shorgani
Department of Applied Microbiology, Faculty of Applied Science, Taiz University – Yemen
nagnas2010@gmail.com

Abeer R. Al-Yosofi **Ehsan M. Al-Sharaby**
Entesar A. Al-Shormani **Randa T. Al-Mekhlafi**
Sara A. A-Sharaby **Somia A. Al-Odaini**
Worda T. Al-Kadasy **Yazid G. Al-Bokari**

Department of Medical Laboratory Sciences
Faculty of Medical Sciences, Aljanad University of Science and Technology – Yemen

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مدى انتشار ونمط اختبارات حساسية المضادات الحيوية لبكتيريا ستربتوكوكس بيوجينيس المعزولة من طلبة جامعة الجند، مدينة تعز، اليمن

د/ نجيب قائد الشّرجاني
قسم الميكروبيولوجي، كلية العلوم التطبيقية
جامعة تعز – اليمن

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قسم المختبرات الطبية، كلية العلوم الطبية
جامعة الجند للعلوم والتكنولوجيا – اليمن

الملخص

بكتيريا *ستربتوكوكس بيوجينيس*، هي بكتيريا موجبة الجرام كروية الشكل تتجمع خلاياها في سلاسل. هذه البكتيريا تسبب أمراض عديدة للإنسان تتراوح من أمراض جلدية سطحية إلى أمراض جهازية مهددة للحياة. هدفت هذه الدراسة إلى تقدير معدل انتشار حاملي هذه البكتيريا بين طلبة جامعة الجند في تعز بالإضافة إلى معرفة نمط إختبارات حساسية المضادات الحيوية لعزلات هذه البكتيريا المعزولة من حاملي هذه البكتيريا. تم جمع 200 عينة مسحات من الحلق لطلبة جامعة الجند الذين تتراوح أعمارهم بين 19–24 سنة. تم تزريع العينات وإجراء الإختبارات الميكروبيولوجية والبيوكيميائية لتشخيص البكتيريا المعزولة ومن ثم إجراء إختبارات حساسية بكتيريا *ستربتوكوكس بيوجينيس* المعزولة تجاه المضادات الحيوية. أظهرت النتائج أن معدل الانتشار لبكتيريا *ستربتوكوكس بيوجينيس* بين الطلبة كانت 4,5%. وجد أيضاً أن معدل الانتشار بين الإناث كانت 55,6% وهي نسبة أكثر بقليل من تلك التي وجدت لدى الذكور (44,4%). في اختبارات حساسية المضادات الحيوية لعزلات بكتيريا *ستربتوكوكس بيوجينيس*، تبين أن هذه العزلات كانت عالية الحساسية لمضاد أزيثروميسين (100%)، وكانت الحساسية لمضاد سبروفلاكساسين بنسبة 88,8%. على النقيض، وجد أن هذه العزلات كانت عالية المقاومة لمضاد لينكومايسين بنسبة 89,9%، البنسلين بنسبة 88,8%، وكذلك مضاد أمبيسلين بنسبة 66,7%. ظهور أنماط مقاومة لعزلات بكتيريا *ستربتوكوكس بيوجينيس* ضد المضادات الحيوية الأكثر شيوعاً واستخداماً لعلاج الإصابات الناتجة عنها يجب أن يدق ناقوس الخطر وينبه الأطباء لزيادة الصرامة وتحري الدقة عند وصف المضادات الحيوية للعلاج وذلك بهدف تقليل أو إبطاء معدل زيادة مقاومة البكتيريا ضد المضادات الحيوية.

الكلمات المفتاحية: ستربتوكوكس بيوجينيس، معدل الانتشار، نمط اختبارات حساسية المضادات الحيوية.

Prevalence and Antibiotic Susceptibility Pattern of *Streptococcus pyogenes* Isolated from Aljanad University Students, Taiz City, Yemen

Dr. Najeeb Kaid Nasser Al-Shorgani1
Department of Applied Microbiology, Faculty of Applied Science, Taiz University – Yemen

Abeer R. Al-Yosofi **Ehsan M. Al-Sharaby**
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Department of Medical Laboratory Sciences, Faculty of Medical Sciences, Aljanad University of Science and Technology – Yemen

Abstract

Group A streptococci (GAS), also known as *Streptococcus pyogenes*, is a Gram-positive spherical bacterium arranged in chains. GAS causes various infections for humans ranging from superficial skin infections to fatal sever systemic infections. The aim of this study was to estimate the prevalence rate of GAS carriers among students of Aljanad University in Taiz city, Yemen, and to determine the antibiotic susceptibility patterns of GAS isolates against the commonly prescribed antibiotics for treatment of GAS infections. This study is a community based cross-sectional study design in which a 200 university students aged 19–24 years were participated. A total of 200 throat swab specimens were collected from the participants and then subjected for culturing and identification of GAS isolates followed by antimicrobial susceptibility testes. The results revealed that the prevalence rate of GAS carriers among the population group was 4.5 %. The prevalence of GAS among females was 55.6%, which was slightly higher than that found in males (44.4%), with no significant differences ($P > 0.05$). The antimicrobial susceptibility profiles revealed that the isolated GAS showed high sensitivity to azithromycin (100%) and ciprofloxacin (88.8%). In contrast, GAS isolates were highly resistant to lincomycin (89.9%), penicillin (88.8%) and ampicillin (66.7%). The high resistance patterns of GAS isolates to the common prescribed antibiotics should warn physicians to restrict the prescription of antibiotics to slow down the rising of bacterial resistance rate against antibiotics in the future.

Keywords: Group A streptococci, Prevalence rate, Antibiotic Susceptibility Pattern.

Introduction

Streptococcus pyogenes, commonly known as a Group A *Streptococcus* (GAS), is spherical Gram-positive β-hemolytic, arranged in chains bacterium (Jasim, *et al.*, 2021). GAS is a significant human pathogen responsible for a variety of human infections ranging from superficial infections to severe systemic complications as well as post-streptococcal syndromes. It causes mild human diseases such as pharyngitis, tonsillitis (strep throat), erysipelas, cellulitis, impetigo and necrotizing fasciitis (Ralph & Carapetis, 2013). GAS also can cause invasive life-threatening diseases, such as sepsis, puerperal sepsis, pneumonia, osteomyelitis and toxic shock syndrome. GAS infections can cause serious consequences, if not treated promptly, such as acute rheumatic fever and acute glomerulonephritis (Walker *et al.*, 2014).

The worldwide burden of GAS infections is taken in concern, which involved for both public health and economic stability. According to the World Health Organization (WHO) estimation in 2005, the global GAS infections prevalence is about 18.1 million cases and the annual new severe GAS infections cases are 1.78 million. The WHO report also estimated that the worldwide annual death cases in developing countries caused by GAS infections are more than 517,000 deaths and GAS infections are the ninth infections causing human deaths in the world (WHO, 2005).

GAS infection such as pharyngitis can infect people of all ages but most commonly infects children between the ages of 5 to 15 years old, mainly in the winter months. It can spread through direct contact with an infected person or by contact with respiratory droplets of coughing or sneezing (Renner *et al.*, 2012). Prevention measures of GAS infections can be conducted by applying good hand washing, good respiratory hygiene and avoiding sharing personal items.

Numerous studies have reported GAS prevalence and the patterns of antibiotic susceptibility in schoolchildren (Elnaim *et al.*, 2023; Tadesse *et al.*, 2023). However, the prevalence of GAS among university students is not well investigated, and this gap in knowledge affects outbreak management and prevention strategies. In university settings, the crowding and close contact conditions of students and social interactions poses a significant public health concern, which can ease the transmission of GAS bacterium, leading to spread of infections that may have serious health complications.

Furthermore, the increasing rate of antibiotic resistance among GAS strains poses a challenge for the available effective treatment procedures, necessitating an extensive understanding of the current antibiotic susceptibility profiles of GAS. GAS prevalence and its antibiotic susceptibility patterns is vary geographically. GAS strains show universal sensitivity to penicillin and other β-lactams group, which make them the first-choice antibiotics for successful treatment of GAS infections (Michos *et al.*, 2009; Abd El-Ghany *et al.*, 2015; Meletis *et al.*, 2023). However, a variable high rate resistance of GAS to macrolides, particularly erythromycin was reported in Portugal (35.8%) (Melo-Cristino & Fernandes, 1999), Spain (23.5%) (Alós *et al.*, 2000) and Germany (12.8%) (Arvand *et al.*, 2000), but lower resistant rates were found in Austria (4.7%) and Hungary (3.7%) (Gattringer *et al.*, 2004). A considerable tetracycline resistance by GAS strains was also reported, with rates more than 40% in some previous studies (Melo-Cristino & Fernandes 1999; Meletis *et al.*, 2023).

In addition, the PROTEKT antimicrobial surveillance program revealed that GAS resistance to macrolides varied significantly by geographical region as well as almost 10% of GAS isolates were resistant to erythromycin A. Whereas, GAS resistance to β-lactams and fluoroquinolones was not identified (Cantón *et al.*, 2002). Continuous monitoring is also crucial to monitor developing antimicrobial resistance patterns and to ensure effective management of GAS infections (Cantón *et al.*, 2002: Meletis *et al.*, 2023). The increase of GAS resistance to tetracyclines and macrolide in certain geographical regions proposes a need for routine antibiotic susceptibility testing to aid for careful antibiotic selection for treatment (Melo-Cristino & Fernandes 1999: Meletis *et al.*, 2023).

This study aimed to investigate the prevalence of *S. pyogenes* (GAS) carriers among university students and to determine the antibiotic susceptibility profiles of GAS isolates from students, with particular attention to resistance against commonly prescribed antibiotics. In addition, the study tried to identify the possible association between socio-demographic, healthy factors and some habits (such as sex, smoking, chewing khat, removal of tonsils and the frequent utilizing of antibiotics) and the prevalence of GAS among the university student population. This study will contribute to the understanding of GAS epidemiology in a university setting and providing

insight into the development of effective prevention strategies to mitigate the spread of this pathogen. Moreover, data from this study can be vital for guiding empirical antibiotic therapy as well as informing future antibiotic stewardship efforts.

Materials & Methods

Study design

A cross-sectional study was performed to determine the prevalence and antibiotic susceptibility pattern of *Streptococcus pyogenes* among students of Aljanad University. The study involved the collection of throat swab specimens from students within a defined age range from 19–24 years old.

Throat swab specimen collection

Throat swabs were collected from university' students using sterile swabs. The specimens were collected carefully to minimize contamination by oral microflora.

Data collection

The required information were collected by filling a questionnaire during the specimen collection. The demographic and socio-economic characterizations such as gender, age, physical level, area of residence, symptoms, health history, previous sore throat infection, rheumatic fever or other related infections were recorded.

Culture and identification

The collected throat swabs specimens were cultured immediately on blood agar medium and incubated at 37°C with 5% CO_2 in a candle jar. After overnight incubation, the grown bacterial colonies that showed β-hemolysis on blood agar plates were considered and further identified using Gram stain, catalase test, and bacitracin test. The bacterial isolates that appeared microscopically as Gram positive and cocci arranged in chains, catalase positive and showed sensitive to bacitracin antibiotic were identified presumptively as *S. pyogenes*.

Antibiotic susceptibility testing of isolated GAS bacteria

Antibiotic susceptibility tests on the identified *S. pyogenes* (GAS) isolates were conducted by disk paper diffusion method (Kirby–Bauer disk paper diffusion) according to CLSI. The tested antibiotics for antibiotic susceptibility tests were chosen as they are the most prescribed antibiotics for

treatment of GAS infections in Yemen. These antibiotics were penicillin, ampicillin, amoxicillin, azithromycin, erythromycin, cefotaxime, ciprofloxacin, tetracycline, and lincomycin.

Statistical analysis

The data were analyzed statistically using one-way analysis of variance (ANOVA) in Statistical Package for Social Sciences (SPSS 20) analytical tool.

Results & Discussion

Isolation of GAS bacteria from throat swab specimens

A total of 200 throat swab specimens were collected and processed for isolation and determination of *Streptococcus pyogenes* using cultural methods and biochemical tests. According to the cultural growth characteristics on Blood Agar medium, among all the collected throat swab specimens, only 38 specimens gave positive culture for β-hemolytic streptococci which accounts for 19.0%, and 13 specimens showed α – hemolytic streptococci which represents 6.5%.

The majority of the grown bacterial cultures on blood agar medium, which account 136 specimens, showed γ-hemolytic streptococci (68.0%). The remaining cultures grown on blood agar medium were non streptococci bacteria which representing 13 specimens (6.5%) of the total throat isolates (Figure 1).

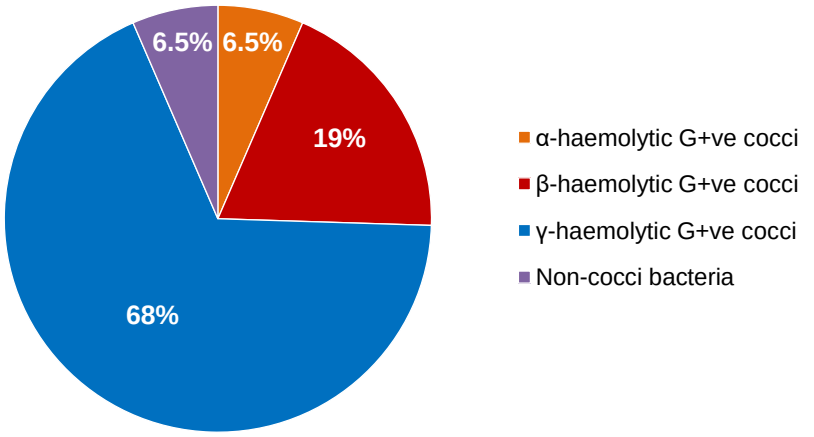

Figure (1) The growth characteristics of isolated bacteria on Blood Agar medium from throat swab specimens.

Identification of isolated GAS bacteria

Bacterial isolates from throat swab specimens that showed β-hemolytic, Gram–positive reaction and their morphology were cocci arranged in chins were considered for further investigation using biochemical tests such as catalase enzyme activity and bacitracin antibiotic tests.

The catalase test indicated that some of β-hemolytic Gram-positive cocci isolates had a negative catalase activity, which accounts for 9 isolates, while the positive isolates for catalase test were 27 isolates (71.05%). Bacterial isolates that were β-hemolytic Gram–positive, cocci in chains, and positive for catalase test were further identified and confirmed using the bacitracin disk susceptibility test. Among the 27 isolates, only 9 isolates (33.3%) were sensitive to the bacitracin disk paper, while the others were resistant to bacitracin antibiotic. Bacterial isolates that showed β-hemolytic on blood agar, Gram–positive, cocci arranged in chains, positive for catalase and sensitive to bacitracin disk were considered presumptively as group A streptococci (GAS).

Prevalence of group A streptococci (GAS)

Out of the 200 participants tested for GAS, only nine participants were found to have been colonized with GAS bacteria as GAS carriers. Therefore, the prevalence rate of GAS carriers in this study was 4.5%.

Earlier studies reported that the prevalence rates of GAS varied depending on geographical location, socio-economic factors, and diagnostic methods. In developed countries, GAS prevalence in pharyngitis ranges from 5–20%, while in developing countries, it can reach 30% (Kebede *et al.,* 2021; Muthanna *et al* 2021; Tadesse *et al.*, 2023). In addition, age is another important factor affecting the GAS prevalence, and it was found that children are being more susceptible to GAS infections than adults (Yildiz *et al.*, 2023).

The prevalence rate of GAS bacteria among the healthy people was found to be 6.8% in Poland (Bura *et al.* 2016) and 4.3% in Canada (Palla *et al*. 2012). In Yemen, a recent study has reported a lower prevalence rate of GAS isolates in cases of sore throat infection as 1.7% (Al-aomary *et al*., 2023), while Edrees & Anbar (2021) reported higher GAS prevalence (39.03%) among schoolchildren in Sana'a city, Yemen. Other studies, such as that conducted in Afghanistan have reported a higher prevalence rate of 23.5% among

university students (Noori, *et al*., 2020) and the GAS prevalence rate among children and adults was 21% in Canada (Forward, *et al*., 2006).

Our study has resulted in nine confirmed cases colonized by GAS bacterium from university students' population, which represent 4.5%. It is possible that prevalence rate of GAS among the study population (university students) is due to the busy nature of the university environment, which may be a contributing factor to the distribution of GAS infection.

Prevalence of GAS according to gender of the participants

In this study, the gender of participates was taken in concern and then the number of the investigated males and females was equal, 100 specimens for each gender type. The prevalence of GAS colonization among male participants was 44.4% while the GAS prevalence among females was slightly higher as 55.6%. Statistical analysis of GAS prevalence according to participant's gender clearly showed that there is no significant effect (p >0.05).

A similar finding by Shikasho *et al*. (2019), who reported that 58.3% of females and 41.7% of males were found to be colonized by GAS bacterium. Elnaim *et al*. (2023), found that the GAS carrier percentage in females was 43.4% and 38.8% of males.

On the other hand, other studies have demonstrated that the percentage of male's carriers of GAS is higher than that of females (Palla *et* al., 2012). Otori *et al*. (2019) reported that the prevalence of GAS in females was slightly higher than males. In another study conducted by Prajapati *et al.* (2012) reported that the GAS prevalence among asymptomatic schoolchildren aged between 10–12 years was found to be 10.8% and the prevalence rate was higher among boys (12.0%) compared to girls (10.1%). In this study, we found that there is no clear association between sex and prevalence of GAS bacteria among the studied population group.

Prevalence of GAS in association with some factors

The correlation and prevalence of GAS carriers with personal some habits and related factors were investigated to determine the impact or possible correlation of these factors on GAS prevalence. These factors including the effect of surgical removal of tonsils (tonsillectomy), khat chewing comparing to no khat chewing habit, smoking and no smoking, and the frequent or rare use of antibiotics. Figure 2 summarized the association of some factors and GAS prevalence.

Results in this study, showed that students who are frequently chewing khat had a higher prevalence of GAS comparing to non-chewing khat students as 66.6% and 33.4%, respectively. This observation can be explained as that khat acts as a drying agent in the mouth, and creating a suitable environment for bacterial and fungal growth. This finding is in agreement with the study conducted by Al-hebshi *et al*. (2005). Statistical analysis of GAS prevalence in association to khat chowing clearly revealed that there is a significant effect (p = 0.03).

Regarding to smoking, this study found that non-smokers' participants are more susceptible to GAS colonization in their throat compared to smokers (Figure 2). Although the fact that smoke has harmful chemicals that can inflame and irritate the throat's lining tissues and weaken the immune system, smokers are less vulnerable to GAS infections as reported by Hong *et al.* (2004). Statistical analysis of GAS prevalence according to smoke clearly showed that there is a significant effect (p <0.01). In addition, this study exhibited that there is a clear relationship between khat chewing and smoking and the prevalence of GAS bacteria.

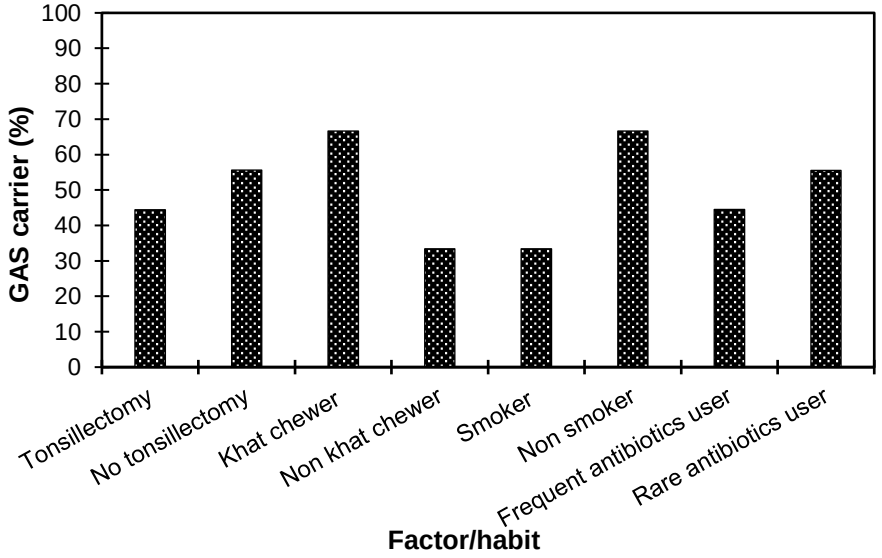

Figure (2) Prevalence of GAS according to some related factors and habits.

According to the results presented in his study, students without a prior tonsillectomy had a higher prevalence rate of GAS bacteria (55.6%) compared to those who had previous tonsillectomy (44.4%) as shown in

Figure 2. This may be attributed to repeated antibiotic therapy for recurrent sore throat infections caused by GAS, which may result in the emergence of bacterial resistance.

Moreover, this study also showed that students who use antibiotics less frequently for prophylaxis have slightly higher percentage of GAS prevalence compared to those who use antibiotics more frequently. This could be due to incomplete treatment by the proper antibiotic or failure to follow the prescribed treatment regimen.

Prevalence of symptoms in GAS carriers

The presence of sore throat symptoms and signs associated with positive GAS carriers are presented in Figure 3. In this study, the most common symptoms of GAS sore throat infection in GAS carriers were headache (77%), watery eyes and rhinitis (66%), fever (55%), arthralgia (55%) and throat congestion (44%). Pharyngeal exudates, joint pain, and cough represented for 44% of symptoms. While, gastrointestinal symptoms such as vomiting, abdominal pain and swallowing pain accounted for 33%. In addition, symptoms such as redness in tonsils, follicular tonsils and hoarseness of voice appeared in 33% of GAS carriers.

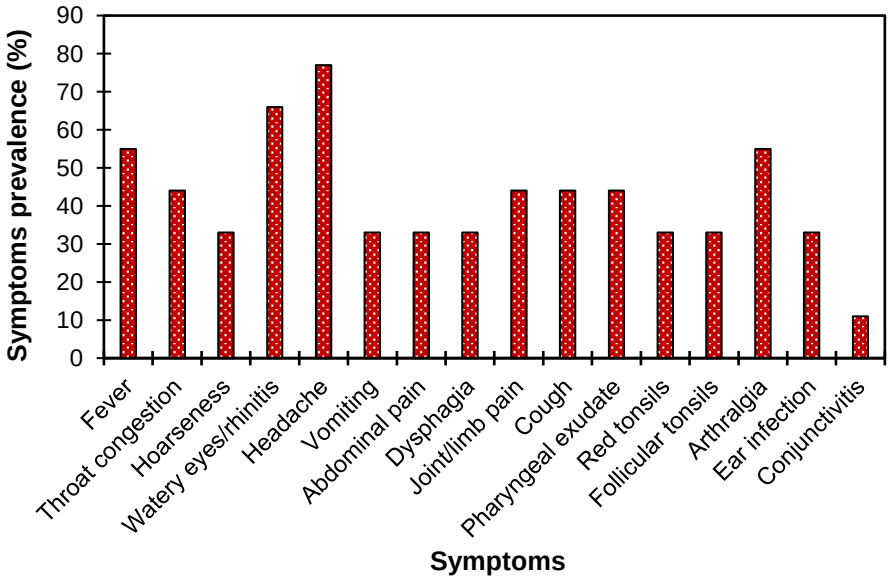

Figure (3) Prevalence of symptoms in student's carriers with GAS.

Several previous studies have discussed the association between definite clinical symptoms of sore throat caused by GAS. It was reported that GAS sore throat infection is commonly associated with some signs and symptoms including high fever, headache, erythema with or without exudates of tonsils or pharyngeal exudates, enlarged cervical lymph nodes, dysphagia, vomiting and abdominal pain (Shulman 1989; Reed *et al.*, 1990; Dajani *et al.*, 1995; Nandi *et al.*, 2002).

Wald *et al.* (1998) found that there is a correlation between GAS pharyngitis infection symptoms, characterized by presence of fever, redness, enlargement or exudate of the throat or tonsil, and positive culture for GAS in 72% of children aged between 5–15 years old.

Clinical signs and symptoms associated with sore throat infection by GAS are used for diagnosis and predicting GAS. However, many earlier studies reported variable clinical symptoms for predicting GAS infection. The variability of these symptoms is mainly depend on various GAS strains, immunity status of different populations and patients age (Bassili *et al.*, 2002).

Antibacterial susceptibility patterns of isolated GAS

The current study involved antibiotic susceptibility testing of GAS isolates against 9 common used antibiotics using disk paper diffusion method. The used antibiotics were penicillin (10 µg), ampicillin (10 µg), amoxicillin (20 µg), azithromycin (15 µg), erythromycin (15 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), and lincomycin (10 µg). Antibacterial susceptibility profiles of GAS isolates are given in Figure 4.

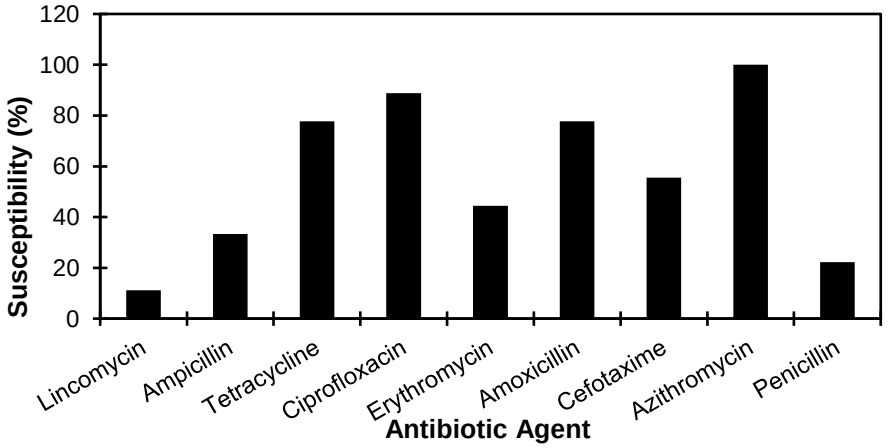

Figure (4) Antibiotic susceptibility pattern for GAS isolates.

The results of this study revealed that the isolated GAS bacteria demonstrated a high resistance rate towards three of the tested antibiotics, namely lincomycin (88.9%), penicillin (77.7%), and ampicillin (66.3%). Notably, it was found that 100% of GAS isolates bacteria showed extreme sensitivity to azithromycin and 88.8% of GAS isolates were susceptible to ciprofloxacin (Figure 4).

Antibiotic susceptibility patterns of GAS isolates against other tested antibiotics in this study exhibited various degrees of resistance and susceptibility. In case of erythromycin, as a macrolide antibiotic, 66.6% of GAS isolates showed resistance while a moderate resistance of GAS isolates was observed to cefotaxime antibiotic as 45%. The susceptibility patterns of GAS showed that 77.7% of GAS isolates were sensitive to both tetracycline and amoxicillin.

Earlier study reported that azithromycin and ciprofloxacin were the most effective antibiotics against GAS bacteria, and this could be potentially due to that physicians not prescribing them frequently to treat GAS infections (Khademi *et al.,* 2021), and this finding is strongly agree with our study.

The resistant pattern of GAS to ampicillin and erythromycin that observed in this study is almost comparable to the outcomes of an earlier study conducted in Nepal (Prajapati *et al.,* 2012). In contract, Bassili *et al.* (2002) reported that GAS isolates exhibited high sensitivity to penicillin and erythromycin antibiotics.

To date, GAS bacterium has continued universally susceptible to penicillins and other β-lactam antibiotics due to lack of β-lactamase enzyme production by GAS bacterium (Tanyi *et al.*, 2022). Hence, penicillin remains the first-line drug of choice for sore throat or sever invasive infections caused by GAS (Michos *et al.,* 2009). In contrast, Telmesani & Ghazi (2002) reported that 62% of GAS isolates were resistant to penicillin among children aged between 1–12 years infected with acute pharyngitis in Saudi Arabia. Similarly, Mahdi *et al*. (2017) found that the GAS isolates showed high resistant rate to penicillin as 59.6%.

The current study indicated a high level of resistance to penicillin, ampicillin, and lincomycin, and these antibiotics are frequently prescribed for treatment of GAS infections in Yemen. A recent study conducted in Yemen

found that GAS isolates from school-aged children was highly resistant to penicillin (85.71%) and ampicillin (80%) (Edrees & Anbar, 2021), which supported our finding. A similar trend of GAS resistance patterns to lincomycin, penicillin and ampicillin has been reported in previous studies conducted by Otori, *et al*. (2019) and Khademi, *et al.* (2021).

While there are several antibiotics are available for treatment of GAS infections, it is crucial to use them judiciously and appropriately as indiscriminate usage can lead to a serious global health issue. Prescription of antibiotics by clinicians without proper supervision exposes community to the unnecessary use of antibiotics and contributes to the rise of antibiotic resistance. The overuse of antibiotics drugs and their availability for purchase without a prescription in pharmacies, as well as the prescribing of incorrect or unnecessary antibiotics, may contribute to the emergence of bacterial resistance against the current antibiotics.

Conclusion

The obtained results in this study revealed that the prevalence rate of GAS carriers among students of Aljanad University was 4.5 %. The incidence rate of GAS bacteria among females was slightly higher than males as 66.6% and 44.4%, respectively. GAS isolates showed high resistant to penicillin, ampicillin and lincomycin. In contrast, GAS isolates showed high sensitivity to azithromycin and ciprofloxacin. The increase of resistance of GAS isolates towards some antibiotics such as erythromycin demand the use of proper and effective alternatives for the management and treatment of GAS infections. Therefore, extensive investigation is required to understand epidemiological factors that facilitate antibiotic–resistance distribution and develop strengthen control and preventive measure strategies. Standard precaution such as adequate and frequent-hand washing and respiratory hygiene as well as avoiding overcrowded setting are important to prevent and control the transmission of GAS infections. Surveillance the use and the rational of antimicrobial drugs prescription are essential to slow down the bacterial resistance against the current antimicrobial drugs.

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