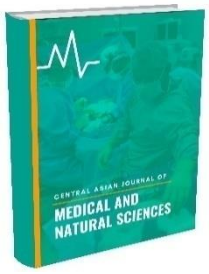
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 03 | May-Jun 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Prevalence of Multidrug Resistance in Streptococcus Pyogenes Isolates from Different Clinical Sources in Al-Diwaniyah City

1. **Huda Refaat Ibraheem**
2. **Ghaidaa Jihadi Mohammed**

Received 2nd Mar 2023,
Accepted 3rd Apr 2023,
Online 8th May 2023

[1,2] Department of Biology, College of Science, University of Al-Qadisiyah, Al- Diwaniayh , Iraq

Abstract: Background: Streptococcus pyogenes also known as group A Streptococci (GAS) is an important pathogen for human that causes a wide variety of diseases. Despite the fact that antibiotics are effective to treat GAS infections, antibiotic resistance particularly to macrolides is increasing in a number of countries causes an increase in mortality, which has become a global public health concern.

Aim: Determine the prevalence of multidrug resistance in Streptococcus pyogenes isolates from different clinical sources in Al-Diwaniyah city.

Method : The study included the collection of (125) samples from different clinical source(Throat swabs, tonsils, urine ,burns) from patients in Al-Diwaniyah teaching hospital and Burns' hospital in Al-Diwaniyah city, during the period from 15-8-2022 to 1-12-2023 .The patients were with different ages ranging from 6-50 years, and from both sexes. The identification of bacterial isolates were achieved by morphological characteristics of bacterial colonies on selective media , result of Bacitracin sensitivity test ,Gram stain, and biochemical tests and confirmed the diagnosis by Vitek system.

Results : A total of 20 (16.0%) isolates were identified as S.pyogenes from (125) clinical samples that collected from different sources, that distributed by 1 (5.0%)from Throat Swab ,and 19 (95.0%) from Tonsils samples , and No isolate detected from urine and burns samples . The results of antibiotic sensitivity test showed all isolates were sensitive to Ciprofloxacin and Vancomycin, on the other hand, 1(5.0%), 1(5.0%), 3(14.0%), 2(9.0%), 4(18.0%), 5(23.0%), 15(68.0%), 21(95.0%) isolates were resistance for Azithromycin,

Clarithromycin, Erythromycin, Clindamycin, Levofloxacin, Chloramphenicol, Tetracycline, Penicillin, Oxacillin, respectively. Based on the multidrug-resistance (MDR) profile, a total of 3 out of 22 (14.0%) Streptococcus pyogenes isolates were resistant to 3 or more of antibiotics.

Conclusion : This study revealed that Streptococcus pyogenes mostly isolated from tonsils. Isolates showed high levels of resistance to most of the commercially available antibiotics. A high percentage of MDR among commonly isolated bacteria were found in this study would be a serious, alarming issue.

Key words: Multidrug resistance, S. pyogenes, Pharyngitis.

Introduction

Streptococcus pyogenes (S.pyogenes) is a species of Gram-positive, aerotolerant bacteria in the genus Streptococcus. These bacteria are extracellular, non-motile and non-sporing cocci that tend to link in chains. They are clinically important for humans, as they are an infrequent, but usually pathogenic, part of the skin microbiota that can cause Group A streptococcal infection. S. pyogenes is the predominant species harboring the Lancefield group A antigen, and is often called group A Streptococcus (GAS)(1) . It causes a wide range of diseases in humans and has evolved a slew of virulence mechanisms to evade host defenses (2, 3) .It is frequently carried asymptomatically and causes superficial infections such as impetigo and pharyngitis(4). In rare cases, GAS enters the bloodstream and deeper tissues, causing invasive infections such as bacteremia, necrotizing fasciitis, and streptococcal toxic shock syndrome. Repeated infections can lead to post-infection sequelae such acute post-streptococcal glomerulonephritis (PSGN) and acute rheumatic fever (ARF). GAS has a significant impact on human health, infecting over 600 million people per year and causing over 500,000 deaths (5).

Despite the fact that antibiotics are effective and widely used to treat GAS infections, antibiotic resistance particularly to macrolides, is increasing in a number of countries (6, 7). Antibiotic resistance causes an increase in mortality, which has become a global public health concern (8,9). So, this study aimed to determine the prevalence of multidrug resistance in Streptococcus pyogenes isolates from different clinical sources in Al-Diwaniyah city.

Methodology

Collection of samples

The study included the collection of (125) samples from different clinical cases in Al-Diwaniyah Teaching hospital and burns hospital in Al-Diwaniyah city during the period from 15-8-2022 to 1-12-2023 .These samples included(55)from patients with chronic and acute tonsillitis from the auditors of the Ear, Nose and Throat Division, tonsils samples were taken by passing a sterile cotton swab on the tonsils by the specialist, 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 7%, and opening them with a sterile scalpel and a smear of the fibrosis found in the

tissue using a disposable transport media swab; (35) samples from throat swabs; 10 urine samples from patients infected with urinary tract infections ;and 25 abscesses samples from the burns' patients at the burns hospital . 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 age of participant patients were (6-50 years) ,and from both sexes. 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 bacterial isolates

The identification of bacterial isolates were achieved by bacteriological methods including morphological characteristics of bacterial colonies on agar(Azide blood agar medium, blood agar medium, and β-Selective Streptococcus agar), result of Bacitracin sensitivity test ,Gram stain, and biochemical tests. Gram stain was conducted for all positive cultures, all the isolates were diagnosed by traditional biochemical tests and confirmed by automatic test (Vitek system).

Antibiotic susceptibility test

The isolates were tested for antimicrobial susceptibility to 11 antibiotics using the agar disc diffusion method on Mueller–Hinton agar (Mast, UK) following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI , 2022). The 11 antibiotics tested were as follows: Clarithromycin (5 µg), Erythromycin (10 µg),Azithromycin (15 µg), Tetracycline (10 µg), Clindamycin (10 µg),Levofloxacin (5 µg), Ciprofloxacin (10 µg), Vancomycin (30 µg), Chloramphenicol (10 µg) Penicillin (10 µg), Oxacillin (5 µg). These antibiotics are representative of the major classes of antimicrobial drugs important to both veterinary and human medicine. Isolates were classified as susceptible, moderately resistant, and resistant using breakpoints specified by the CLSI.

Results

Bacterial Isolation and Biochemical Identification

The culture of different clinical samples(125) on selective media showed that the bacterial agent were detected in 22 (17.6%) samples as shown in figure (1).

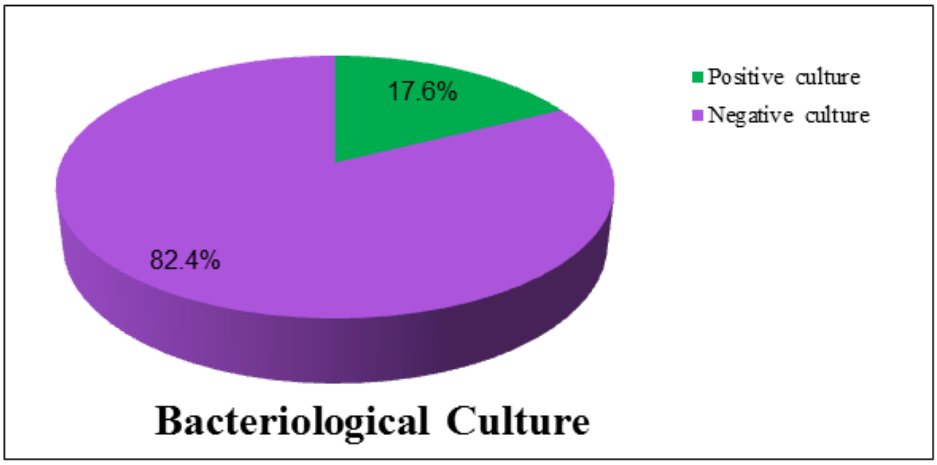


Figure (1): Percentages of Bacterial culture from clinical samples.

A total of 20 (16.0%) isolates were identified as S.pyogenes from (125) clinical samples that collected from different sources, by Gram stain (Figure 2), traditional biochemical tests(Table 1) and Vitek

system; that distributed by 1 (5.0%)from Throat Swab ,and 19 (95.0%) from Tonsils samples , and No isolate detected from urine and burns samples as shown in table (2).

Table (1) : Results of Gram stain and Biochemical tests of S.pyogenes isolates

Test Bacteria	Gram stain	Oxidase	Catalase	Coagulase	Capsule	CAMP test
Streptococcus pyogenes	Gram positive cocci	Positive	Positive	Negative	Positive	Negative

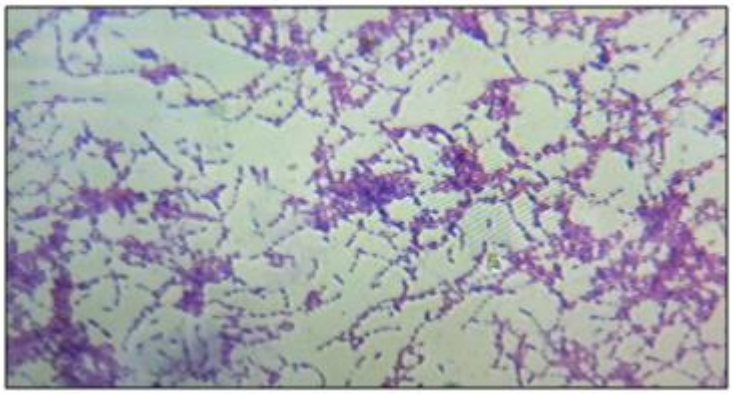


Figure (2): The shape of S.pyogenes under light microscope(100X)

Table (2): Frequency distribution of *S.pyogenes* from different clinical samples

Characteristic	Total number	*S. pyogenes* n=20	*P*
Clinical samples			
Throat Swab, *n* (%)	35 (28 %)	1 (2.8%)	0.001 ¥ HS
Tonsils, *n* (%)	55 (44 %)	19 (34.54%)	
Urine, *n* (%)	10 (8 %)	0	
Burns, *n* (%)	25 (20 %)	0	
Total	125(100)	20(16%)	

n: number of cases; ¥: Chi-square test; S: significant at P > 0.05

Bacitracin Sensitivity Test

The positive bacterial culture of S. pyogenes tested for bacitracin sensitivity and the results was shown in figure (3 A&B). Streptococcus pyogenes (GAS) is inhibited by a small amount of bacitracin (0.04 U) in the disk. After an overnight incubation at 35°C in 5% CO2, a zone of inhibition surrounds the disc, indicating the strain's susceptibility.

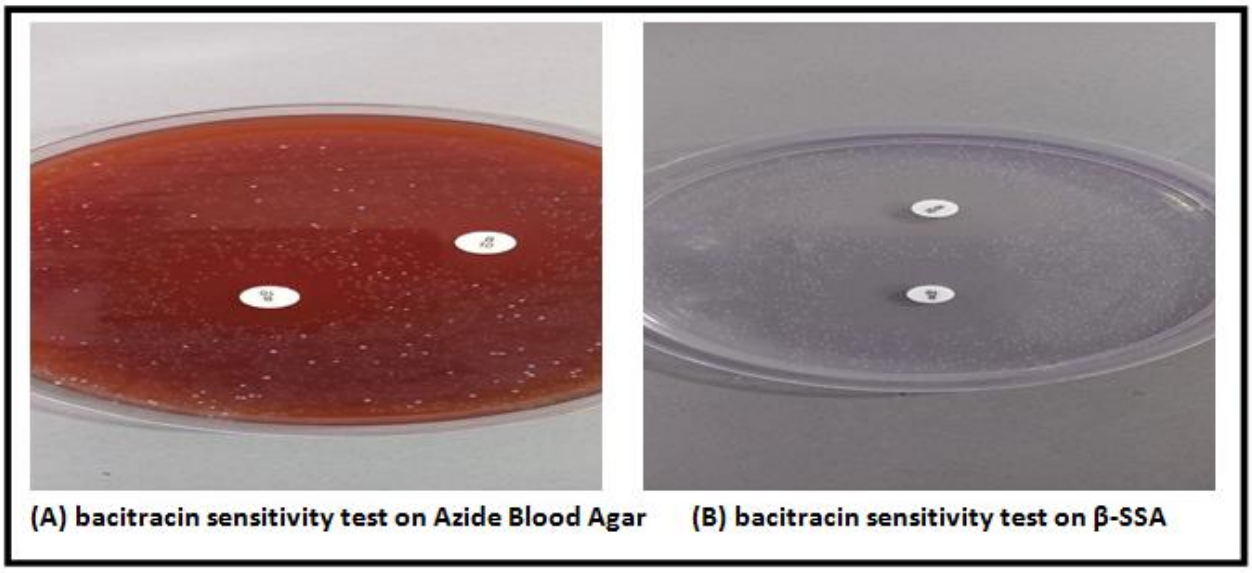


Figure (4): Results of bacitracin sensitivity test ;where β-SSA: β-Selective Streptococcus Agar Medium.

Antibiotic susceptibility of S.pyogenes isolates

The Antibiogram testing was performed with selected antibiotics that is commonly recommended by (CLSI., 2022). The rates of sensitivity and resistance to antibiotics by S.pyogenes isolates are shown in table 3, and as indicated that bacterial isolates varied in their susceptibility to the antibiotics, where all isolates were sensitive to Ciprofloxacin and Vancomycin. On the other hand, 1(5.0%), 1(5.0%), 3(14.0%), 2(9.0%), 4(18.0%), 5(23.0%), 15(68.0%), 21(95.0%) isolates were resistance for Azithromycin, Clarithromycin, Erythromycin, Clindamycin, Levofloxacin, Chloramphenicol, Tetracycline, Penicillin, Oxacillin, respectively. Based on the multidrug-resistance (MDR) profile, a total of 3 out of 22 (14.0%) Streptococcus pyogenes isolates were resistant to 3 or more of antibiotics as shown in table (4).

Table (3): Sensitive Testing of S. pyogenes Isolates with selected Antibiotics

Types of Antibiotics	Interpretation			
	Resistance	**Intermediate**	**Sensitive**	***P***
S. pyogenes				
Ciprofloxacin	0 (0%)	0 (0%)	22 (100%)	**0.001 † HS**
Vancomycin	0 (0%)	0 (0%)	22(100%)	
Azithromycin	1(5.0%)	2 (9.0%)	19 (86.0%)	
Clarithromycin	1(5.0%)	2 (9.0%)	19 (86.0%)	
Erythromycin	1(5.0%)	2 (9.0%)	19 (86.0%)	
Clindamycin	3 (14.0%)	0 (0%)	19 (86.0%)	
Levofloxacin	2 (9.0%)	1 (5.0%)	19 (86.0%)	
Chloramphenicol	4 (18.0%)	1 (5.0%)	17 (77.0%)	
Tetracycline	5 (23.0%)	4 (18.0%)	13 (59.0%)	
Penicillin	15 (68.0%)	2 (9.0%)	5 (23.0%)	
Oxacillin	21 (95.0%)	1 (5.0%)	0 (0%)	

SD: standard deviation; †:a nova; HS: Highly significant at P ≤ 0.001

Table (4): Results of Antibiotics susceptibility patterns of S. pyogenes.

Sample	MDR n (%)	XDR n (%)	PDR n (%)
S. pyogenes			
S. pyogenes n=22	3 (15.0%)	2 (10.0%)	0 (0%)
P value	0.142 NS		

. n: number of cases; X2 : Chi square ; NS: not significant at P ≤ 0.05.

Discussion

This study revealed that Streptococcus pyogenes mostly isolated from tonsils. Tonsillitis is a common bacterial disease caused by β-hemolytic streptococci group A, Adeno and Epstein-Barr viruses spread in most part of the world causes inflammation of tonsils (10).

Antimicrobial resistance is popular problem worldwide and the consuming of antimicrobials has been identified as a driving force for the development and transmission of antimicrobial resistance in bacteria. Increasing of antimicrobial resistance in S. pyogenes is a global problem, although resistance significantly varies between geographical regions (11).

In present study, the susceptibility test of S. pyogenes strains showed that Ciprofloxacin have a high activity where 20 (100%) of the isolates were susceptible. This result correspond with a study which showed the susceptibility of S. pyogenes to ciprofloxacin was 84.9% (12).

Vancomycin was also very active against S. pyogenes with susceptibility rate 100%. Our results in agreement with results of a study done in Baghdad (13) which reported the sensitivity rate for Vancomycin was 96.3%. Other study consistence with present results ,a study found that most common pathogens isolated from acute tonsillitis are Streptococci and staphylococci and according to antibiotic susceptibility founded that Vancomycin showed high sensitivity (100%)(14). The high sensitivity of Vancomycin may be due to its mechanism of inhibiting cell wall synthesis by binding to the building blocks of peptidoglycan. than the results of a study which found the sensitivity of isolate to Vancomycin was (56%)(15).

The present study observed good activity of macrolid antibiotic, the percentage of susceptibility of S. pyogenes to Erythromycin, Clarithromycin and Azithromycin were 85% for each one. The present result is dramatically different from other studies that has been reported in Iraq which showed high resistance to macrolid antibiotic (16,17). But the present results consistence with study in the United States which reported the resistance ratio of S. pyogenes isolates to erythromycin was 6.8%, Azithromycin 6.9% and Clarithromycin 6.6%(18). Also agree with a study carried out in Turkey (19) which found that the resistance rate of S. pyogenes to erythromycin was 9%. In china investigate the sensitive rate of S.pyogenes to erythromycin was 9.5% while the resistance rate was 88.9%(20).also found that S. pyogenes isolated from core of tonsillectomy of patients recorded 80% resistance to Erythromycin(21).

The present results also showed that chloramphenicol have high activity with 16 (80.0%) of the isolates were susceptible, and only 3 (15%) of the isolates were resistant to chloramphenicol. This result was similar to the results study which obtained (82.1%) f the isolates was sensitive(22).

The activity of clindamycin on S. pyogenes was good where 17 (85.0%) of isolates were susceptible to it and only 3 (15.0%) isolates were resistance. The present result was in agreement with a study which

also show low rates of resistance to Clindamycin(18). The present results also consistence study that found the sensitivity to Clindamycin was (90%)(23).

In the present study, Levofloxacin showed a good activity where 17 (85.0%) of isolates were susceptible and only 2 (10%) were resistance, and this result was in agreement with results of study which show 69% of isolates were sensitive for Levofloxacin. Based on results, Levofloxacin may play an effective alternative choice for treatment of patients infected with S. pyogenes infections in case of resistance to macrolides antibiotic(16).

The present study observed the weak activity of Beta-lactams antibiotic against S. pyogenes, where the Oxacillin showed 100% of the isolates were resistant to it and only 23% susceptible isolates of S. pyogenes to the penicillin with 68% resistant isolates. According to the susceptibility testing, this results agrees with the results of study which reported resistance of S. pyogenes to Ampicillin(24). The results of current study were slightly close to the results of study which reported that isolates of S. pyogenes were resist to Penicillin 100%(25). But the present result disagree with that reported in a local study(17).

Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid. β -lactam antibiotics are bactericidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls (26).

Therefore, the present study show 3 (15.0%) isolates of S. pyogenes were MDR. Whereas, 2 (10.0%) isolates of S. pyogenes were found as XDR, and no PDR was observed in this study, table (4). However, the present study revealed that several of the isolates were considered multiple antibiotics resistant. Multidrug-resistant (MDR) was considered in isolates with resistance to at least one agent in three or more antibiotic categories (27). Infections with these multiple resistance isolates may be associated with increased morbidity and mortality, which can attributed to limited effective antibiotic choices (28).

Conclusion

This study revealed that Streptococcus pyogenes mostly isolated from tonsils. In this study, the isolates were highly resistant to mostly used antibiotics. which is considered a serious issue affecting public health. In Iraq, like in many other countries, antibiotics are readily available from the pharmacy desk. Alternatively, pharmacists prescribe medications to patients just based on their external symptoms, causing the intake of wrong antibiotic and/or over- or underdosage. Moreover, in majority of cases, patients do not complete the prescribed course of antibiotics. This causes patients to be at the hospital harboring resistant strains. These strains may cause endogenous or exogenous infections in other patients. The higher prevalence of resistance to antimicrobial agents in this environment could be due to wide- spread, indiscriminate use of antibiotics. The formulation and implementation of a national drug policy by Iraqi governments are fundamental to ensure rational drug use.

References

1. Kimberlin, D.W.; Brady, M.T.; Jackson, M.A.(2015) Group A Streptococcal Infections; American Academy of Pediatrics: Itasca, IL, USA, pp. 732–744.
2. Walker, M. J.; Barnett, T. C.; McArthur, J. D.; Cole, J. N.; Gillen,C.M.; Henningham, A. (2014). Disease manifestations and pathogenic mechanisms of group A streptococcus. J Clinical Microbiology. 27 (2). pp: 264-301

3. Abbas, A. and Wida, Q.H., (2021). Isolation and Identification of Streptococcus pyogenes from Patients and Objects in Hospital Environment in Thi-Qarcity in Iraq. Indian Journal of Forensic Medicine & Toxicology, 15(4).
4. Soderholm AT, Barnett TC, Sweet MJ, Walker MJ. (2017). Group A streptococcal pharyngitis: immune responses involved in bacterial clearance and GAS-associated immunopathologies. J Leukoc Biol doi:10.1189/jlb.4MR0617-227RR.
5. Soderholm, A., (2018). The Group A Streptococcus M1 T1 clone post-transcriptionally modifies innate immune signaling to promote infection. Doctor of Philosophy. The University of Queensland. Australia.
6. Lu, B., Fang, Y., Fan, Y., Chen, X., Wang, J., Zeng, J., Li, Y., Zhang, Z., Huang, L., Li, H. and Li, D., (2017). High prevalence of macrolide-resistance and molecular characterization of Streptococcus pyogenes isolates circulating in China from 2009 to 2016. Frontiers in microbiology, 8, p.1052.
7. Bhardwaj, N.; Mathur, P.; Behera, B.; Mathur, K.; Kapil, A. and Misra, M. C. (2018). Antimicrobial resistance in beta-haemolytic streptococci in India: a four-year study. Indian J. Med. Res. 147, 81–87. doi: 10.4103/ijmr.IJMR_1517_16.
8. Wajima, T., Chiba, N., Morozumi, M., Shouji, M., Sunaoshi, K., Sugita, K., Tajima, T., Ubukata, K. and GAS Surveillance Study Group, (2014). Prevalence of macrolide resistance among group A streptococci isolated from pharyngotonsillitis. Microbial Drug Resistance, 20(5), pp.431-435.
9. Silva-Costa, C.; Friães, A.; Ramirez, M., and Melo-Cristino, J. (2015). Macrolideresistant Streptococcus pyogenes: prevalence and treatment strategies. Expert Rev. Anti Infect. Ther. 13,615-628.doi;10.1586-14787210.2015.1023292.
10. Muthanna A., Salim H.S., Hamat R. A., Shamsuddin N.H. and Zakariah, S. Z., (2018). Clinical Screening Tools to Diagnose Group A Streptococcal Pharyngo-tonsillitis in Primary Care Clinics to Improve Prescribing Habits. Malays J Med Sci. 25(6): 6–21.
11. Ferretti J., Stevens D. and Fischetti V. (2016). Streptococcus pyogenes Basic Biology to Clinical Manifestations, editer book, University of Oklahoma Health Sciences Center.1050 page.
12. Singla, V., Mandal, S., Sharma, P., Anand, S. and Tomar, S.K., 2018. Antibiotic susceptibility profile of Pediococcus spp. from diverse sources. 3 Biotech, 8, pp.1-17.
13. Mahdi, A.Z., Hassan, J.H. and Jebur, K.S., (2017). Antibiotic Susceptibility of Streptococcus pyogenes Isolated from Otitis Media and Tonsilitis among Children Patients. Int. J. Curr. Microbiol. App. Sci, 6(8), pp.998-1004.
14. Ingale M.H. and Mahajan G.D. (2018). Evaluation of antibiotic sensitivity pattern in acute tonsillitis. Int J Otorhinolaryngol Head Neck Surg. 4(5):1162-1166.
15. Khosravi, P., Rezvani, A. and Wiewiora, A., (2016). The impact of technology on older adults' social isolation. Computers in Human Behavior, 63, pp.594-603.
16. Khalaf N.Z., (2020). Master Thesis on the Study of the Diagnostic and Molecular study of Streptococcus Pyogenes causing Pharyngitis in childhood of some region in Anbar Governorate. College of science. University of Anbar.
17. Ali H., Dhahi M. and Abd A. (2015). Molecular screening for erythromycin resistance genes in Streptococcus pyogenes isolated from Iraqi patients with tonsilo-pharyngites. African Journal of Biotechnology, 14(28): 2244-2250.

18. Richter, S.S., Heilmann, K.P., Beekmann, S.E., Miller, N.J., Miller, A.L., Rice, C.L., Doern, C.D., Reid, S.D. and Doern, G.V., (2005). Macrolide-resistant Streptococcus pyogenes in the United States, 2002–2003. Clinical infectious diseases, 41(5), pp.599-608.

19. Dundar D., Sayan M. and Tamer G. (2010). Macrolide and tetracycline resistance and emm type distribution of Streptococcus pyogenes isolates recovered from Turkish patients, Microbial Drug Resistance.16(4):279–284.

20. Hua, C.Z., Yu, H., Xu, H.M., Yang, L.H., Lin, A.W., Lyu, Q., Lu, H.P., Xu, Z.W., Gao, W., Chen, X.J. and Wang, C.Q., (2019). A multi-center clinical investigation on invasive Streptococcus pyogenes infection in China, 2010–2017. BMC pediatrics, 19, pp.1-6.

21. Elsherif A.M., Abdelrahman Y.O., Mostafa A.H., Nahla M.E., (2011). Discrepancy between tonsillar surface and core culture in children with chronic tonsillitis and incidence of post tonsillectomy bacteraemia. AAMJ. 9: 171-182.

22. Camara M., Dieng A., and Boye C.S.B. (2013). Antibiotic susceptibility of Streptococcus pyogenes isolated from respiratory tract infections in dakar, senegal. Microbiology insights, 6. MBI-S12996.

23. Al Momani, W., Khatatbeh, M. and Altaany, Z., 2019. Antibiotic susceptibility of bacterial pathogens recovered from the hand and mobile phones of university students. Germs, 9(1), p.9.

24. Okonko, I.O., Soleye, F.A., Amusan, T.A., Ogun, A.A., Ogunnusi, T.A., Ejembi, J., Egun, O.C. and Onajobi, B.I.,(2009). Incidence of multi-drug resistance (MDR) organisms in Abeokuta, Southwestern Nigeria. Global journal of pharmacology, 3(2), pp.69-80.

25. Al-Saimary I.E. (2011). Antibiogram and multidrug resistance patterns of Staph. aureus (MDRSA) associated with postoperative wound infections in Basra- Iraq. Med. Pract. Rev. 2 (6): 66-72.

26. Elander R.P., (2003). Industrial production of beta-lactam antibiotics. Appl Microbiol Biotechnol. 61(5-6):385-92.

27. Horcajada, J.P., Montero, M., Oliver, A., Sorlí, L., Luque, S., Gómez-Zorrilla, S., Benito, N. and Grau, S., (2019). Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clinical microbiology reviews, 32(4), pp.e00031-

28. Buehrle, D.J., Shields, R.K., Shah, N., Shoff, C. and Sheridan, K., (2017). Risk factors associated with outpatient parenteral antibiotic therapy program failure among intravenous drug users. In Open forum infectious diseases (Vol. 4, No. 3). Oxford University Press.