SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: 10.36346/sarjpm.2024.v05i01.001

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Antimicrobial Activity of Azithromycin and Erythromycin against Streptococcus Pyogenes Isolated from Sore Throat Patients in Shendi, Sudan

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Article History: | Received: 26.11.2023 | Accepted: 01.01.2024 | Published: 03.01.2024 |

Abstract: ***Background***: *Streptococci* is considered one of the predominant flora colonizing the respiratory tract of humans. The group A *Streptococci* (GAS) causes the broadest range of diseases that can lead to the asymptomatic carriage, superficial infection of the upper respiratory tract mainly throat infection. ***Objectives***: The study was carried out to assess the antimicrobial activity of azithromycin and erythromycin against *Streptococcus pyogenes* (Group A) isolated from sore throat patients. ***Methods***: Sixty-one throat swab samples from both sexes were collected randomly from different clinics in Shendi, Sudan from patients with clinical findings suggestive of throat infection between August to November 2021. *Streptococcus pyogenes* were isolated by standard cultural techniques and identified by using Gram stain and biochemical tests. Also, the antimicrobial activity of Azithromycin and Erythromycin were assessed using the disc diffusion method. ***Result***: 19 throat swab samples (31%) out of 61 had S. pyogenes growth, whereas 42 (69%) did not. Of the patients, 12 (63.2% of them) were men, and 7, 36.8%, were women. The ages of the infected patients ranged from 1 to 10 years old in 2 (5.3%) cases, 11 to 20 years old in 2 (10.5%), 21 to 30 years old in 15 (78.9%), and 41 to 50 years old in 2 (5.3%) cases. In contrast to the other 5 (26.3%), 14 of them (73.7%) had recurring throat infections. Out of the 19 *S. pyogenes* isolates that tested positive, only 12 (63.6%) were susceptible to azithromycin and just 7 (36.8%) were resistant. 13 (68.4%) of the 19 *S. pyogenes* positive isolates were erythromycin sensitive, whereas 6 (31.6%) were resistant. ***Conclusion***: Azithromycin and erythromycin are more sensitive to *S. pyogenes*, which indicates less excessive usage of these antibiotics in Shendi. Streptococcal infections in the respiratory tract are challenging to treat, and selecting an antibiotic treatment involves numerous considerations. Any isolated strain's susceptibility to antibiotics should be assessed because this is the only way to ensure quick and successful treatment. In order to improve public health, antibiotic therapy should be accompanied by adequate preventive measures, such as training nursing staff to prevent as many nosocomial infections as possible, educating the general public about the importance of hygiene and encouraging them to stop self-medicating and fostering closer scientific collaboration between clinicians and microbiologists.

Keywords: Group A Streptococci, glomerulonephritis, Penicillin, Throat infections.

INTRODUCTION

Streptococci are considered part of the predominant flora colonizing the respiratory tract of humans [1]. *Streptococcus pyogenes* is an obligate human pathogen that causes major human morbidity and mortality worldwide. School-age children (5-15 years) are considered the major reservoir of group A beta-hemolytic Streptococci [2]. Group A Streptococci (GAS) causes the broadest range of diseases that can lead to asymptomatic carriage, superficial infection of the upper respiratory tract (pharyngitis) and skin (impetigo or pyoderma), or invasive disease (bacteremia or focal infection such as osteomyelitis, pneumonia, and meningitis) [2]. GAS also has the potential to release exotoxins, resulting in scarlet fever or streptococcal toxic shock syndrome, and to top it all off this is one of the few

Citation: Leila Mohamed A. Abdelgader, Thekra Mohamed M. Abdo, Ghanem Mohammed Mahjaf, Tibyan Abd Almajed Altaher, Mosab Nouraldein Mohammed Hamad (2024). Antimicrobial Activity of Azithromycin and Erythromycin against Streptococcus Pyogenes Isolated from Sore Throat Patients in Shendi, Sudan. *SAR J Pathol Microbiol*, 5(1), 1-7.

organisms that unequivocally cause autoimmune disease acute rheumatic fever (ARF) and acute post-streptococcal glomerulonephritis (APSGN). These last two manifestations can, in turn, lead to chronic sequelae, rheumatic heart disease (RHD), which may follow ARF [2]. Many European nations have shown high rates of macrolide resistance in *Streptococcus pyogenes* [3].

Pharyngitis, impetigo, cellulitis, scarlet fever, puerperal sepsis, bacteremia, pneumonia, streptococcal toxic shock syndrome (STSS), necrotizing fasciitis, and endocarditis are only a few of the numerous human illnesses that GAS can cause, both benign and serious. Acute post streptococcal glomerulonephritis (APSGN), acute rheumatic fever (ARF), and rheumatic heart disease (RHD) are a few other significant postinfectious immune-mediated illnesses that can be brought on by GAS infection [4–8]. Global disease burden data from the World Health Organization (WHO) place GAS as the ninth most common infectious cause of human mortality, with the majority of deaths predominantly occurring in developing nations due to invasive infections and RHD [4-9]. In the middle of the 20th century, several studies had found a decrease in the prevalence of GAS illness in developed nations [10–13]. Although there have been numerous cases of substantial ARF [14, 15]. APSGN [16, 17]. GAS invasive illness [13–20].

Puerperal sepsis [21–23], and scarlet fever [24, 25], outbreaks throughout the past 50 years. Penicillin is thedrug of choice for the treatment of *Streptococcus pyogenes* infection. However, for patients sensitive to b-lactam antibiotics, and when these drugs fail, macrolides are often the recommended substitute. Penicillin resistance has not yet been described in *S. pyogenes*, but resistance to erythromycin and related antibiotics hasbeen widely reported [26]. Erythromycin is bacteriostatic and inhibits the protein synthesis drug with a wide spectrum of activity [27]. Azithromycin is an antibiotic useful for the treatment of bacterial infections. It is derived from erythromycin, with a methyl-substituted nitrogen atom incorporated into the lactone ring, thus making the lactone ring 15-membered [28]. Rational use of antibiotics can decrease the adverse effects of antibiotics as well as decrease the costs of therapy but more importantly, decreased antibiotic usage will prevent the rise of drug-resistant bacteria, which is now a growing problem worldwide [29]. Health authorities have been strongly encouraging physicians to decrease the prescribing of antibiotics to treat common upper respiratory tract infections because antibiotic usage does not significantly reduce recovery time for these viral illnesses [29]. Most studies show no difference in the improvement of symptoms between those treated with antibiotics right away and those with delayed prescriptions [30].

Similar to wealthy nations, research has been started in Dakar to track the evolution of S. pyogenes resistance to present antibiotics. The particular goals of this study are to identify S. pyogenes isolates from respiratory tract infections and to analyze how they respond to the frequently used antibiotics azithromycin and erythromycin.

MATERIALS AND METHODS

Study Design: This was a prospective cross-sectional st udy.

Study Area: The study was conducted in River Nile Sta te, Shendi locality, Northern Sudan.

Study Duration: This study was done between Augusts to November 2021.

Study Population: Individuals with clinical findings su ggestive of throat infection were enrolled in this study fr om both sexes with varying ages.

Sample Size: A total of sixty-one throat swab samples were collected from patients who suffered from throat in fections during the period of study.

Data Collection: A structured questionnaire was used f or the collection of the data.

Collection of Samples

The patient sat facing a light source. While the tongue is kept down with a tongue depressor, a sterile co tton-wool swab was rubbed vigorously over each tonsil and the back wall of the pharynx. Care was taken not to touch the tongue or buccal surfaces. Within two hours o f collection, the swabs were delivered to the laboratory [31].

Culture of Swab Sample

The swab was rubbed over one-quarter of a blo od agar plate, and the rest of the plates were streaked wi th a sterile wire loop. Then the plate was incubated anae robically at 37˚c overnight.

Sample Identification

The plates were examined for any significant g rowth of beta-hemolytic bacteria. The isolated bacteria were then identified by Gram stain and biochemical test s.

Indirect Gram's Stain

On a dry clean clearly labeled slide, the smear was made by transferring a loop full of colony emulsifie d into a drop of normal saline to make a thin smear, the slide was left for drying and then fixed with heat. The sl ide was flooded with crystal violet stain (basic stain) for 30-60 seconds, rapidly the slide was washed with clean tap water, and the slide was covered with Lugol's iodin e solution 23 (mordant), for another 30-60 seconds, repe at washing step, the slide was decolorized rapidly with a cetone alcohol for 10 seconds, repeat the washing proce ss, finally, the slide was covered with safranin solution f or 2 minutes, then was washed with clean tap water, and

left to dry and examined for morphological appearance and arrangement with oil immersion 100x.

Biochemical Tests

Catalase test Catalase enzyme is produced by the bacteria to break down hydrogen peroxide (H2O2) into H2O and O2. 1-2 ml 3% hydrogen peroxide solution (H2O2) was placed into on test tube, using a sterile wooden stick, several colonies of bacterial growth were taken and immersed in the hydrogen peroxide solution, and the result was observed for immediate bubbling.

Bacitracin sensitivity test this test was used for presumptive identification and distinction of *S. pyogenes* from other beta-hemolytic Streptococcus, a zone of inhibition greater than10 mm around bacitracin disk (0.04 IU) considered susceptible and confirms the presence of *S. pyogenes.*

Antimicrobial Drug Susceptibility Testing
Disc Diffusion Technique

Colonies of similar appearance to the test organism were emulsified in a small volume of sterile saline and matched the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight growth culture. A sterile loop (4 mm diameter) was used to apply a loop full of the test organism suspension to the center of the sensitivity testing plate. The sterile dry cotton wool swab was used to spread the inoculums evenly across the Blood agar plate and then the inoculation was allowed to dry for a few minutes with the Petri dish lid in place. Finally, by using sterile forceps or a needle mounted in the holder, the Azithromycin disc and Erythromycin disc were placed within 30 minutes. The plate was incubated aerobically at 37˚c overnight. The test was read after checking bacterial growth (read zone) [32].

Ethical Considerations

The study proposal got approval from the University of Shendi, Faculty of Graduate Studies and the Faculty of Medical Laboratory Sciences. Verbal consent was taken from all participants or their guardians before being enrolled in the study. All participants were informed about the research's importance and all of them accepted to be part of it.

Data Analysis

Data were analyzed by using the statistical package for social sciences (SPSS) computer program Version19, with Independent T-tests and Correlation analysis.

RESULTS

Table 1: Distribution of study group according to age

Age	*Frequency*	*Percent %*
1-10	6	9.8%
11-20	9	14.8%
21-30	37	60.7%
31-40	4	6.6%
41-50	5	8.2%
Total	**61**	**100.0**

Table 2: Distribution of bacteria within study group

Isolated organisms	*Frequency*	*Percent%*
S. pyogenes	19	31.1%
S. pneumoniae	3	4.9%
S. aureus	28	45.9%
Other β haemolytic, streptococci	4	6.6%
No growth	7	11.5%
Total	**61**	**100.0%**

Table 3: Distribution of the group infected with *S. pyogenes* according to age

Age	*Frequency*	*Percent %*
1-10	1	5.3%
11-20	2	10.5%
21-30	15	78.9%
31-40	1	5.3%
41-50	1	5.3%
Total	**19**	**100.0**

Table 4: Sensitivity of *Streptococcus Pyogenes* to Azithromycin

Susceptibility patterns	***Frequency***	***Percent %***
Sensitive	12	63.2%
Resistant	7	36.8%
Total	**19**	**100.0%**

Table 5: Sensitivity of *Streptococcus pyogenes* to Erythromycin

Susceptibility patterns	***Frequency***	***Percent %***
Sensitive	13	68.4%
Resistant	6	31.6%
Total	**19**	**100.0%**

Table 6: Correlation of Azithromycin and Erythromycin with Age, gender, and History of recurrent infection:

Variables	***P. value***	
	Azithromycin	***Erythromycin***
Age	0.399	0.505
Gender	0.526	0.622
History of recurrent infection	0.634	0.480

DISCUSSION

The management of infections globally is currently faced with a significant issue due to the advent of antibiotic resistance [33]. It is good knowledge that intake of antibiotics plays a significant role in the spread of bacterial strains that are resistant to antibiotics. More information about the resistance-consumption link in the Shendi population is required. Macrolides (erythromycin, azithromycin) are second-line treatments for Streptococcus pyogenes infections and are frequently used to treat respiratory infections. However, there is an increase in macrolide resistance in S. pyogenes in several nations [34-40], which has been linked to the use—or overuse—of macrolides. Azithromycin, a long-acting macrolide that is taken once daily, has also been reported to select resistance more efficiently than macrolides taken three times daily (erythromycin) or twice daily (clarithromycin and roxithromycin) [41-43]. Penicillin is still effective against Group A streptococci, thus erythromycin or other macrolides are recommended for patients who are allergic to it [44]. However, more recently, macrolide resistance has been seen to rise in a number of nations [45-49]. Recent increases in the prevalence of S. pyogenes clinical isolates that are resistant to antibiotics highlight the necessity for ongoing monitoring of antimicrobial resistance patterns. In our study, the majority of the infected patients, 14 (73.70%), had recurrent throat infections. A total of 61 throat swab samples were randomly collected from patients with clinical findings suggestive of throat infection from both sexes at various clinics; 33 (54.1%) of the patients were males and 28 (45.9%) were females. and the Erythromycin was a higher rate of resistance than azithromycin. Worldwide, reports on the prevalence of macrolide (erythromycin, azithromycin) resistance vary from one country to the next. Portugal, Belgium, Spain, and Italy all exhibited higher resistance rates than Portugal (27%) [50-53]. While most European nations have documented widespread Macrolide (erythromycin, Azithromycin) resistance, the US has primarily observed low-level resistance [54]. Recent investigations, however, have indicated that resistance rates have risen to 6-7%, with pockets of higher resistance occurring at varying intervals between 10-20% [55-57]. The usage of Macrolides (erythromycin, azithromycin) has increased nationally in tandem with this rise in resistance [58]. At our facility, erythromycin and azithromycin prescriptions climbed by 11% and 15%, respectively, between 2002 and 2004 for both inpatients and outpatients. The bacteria that may be responsible for sore throat symptoms and probable suppurative and non-suppurative sequelae are the ones that antibiotics aim to kill. Successful bacterial eradication may encourage quicker healing and the avoidance of secondary problems. However, not all episodes of sore throat are caused by bacteria, and certain bacteria may be resistant to antibiotics, which could reduce the intervention's overall effectiveness. It is debatable whether or not to recommend antibiotics for sore throats. The problem is significant since the disease is widespread and variations in prescribing have a significant impact on costs. Additionally, higher prescribing raises patient attendance rates.

CONCLUSIONS

Azithromycin and erythromycin are more sensitive to *S. pyogenes*, which indicates less excessive usage of these antibiotics in Shendi. Streptococcal infections in the respiratory tract are challenging to treat, and selecting an antibiotic treatment involves numerous considerations. Any isolated strain's susceptibility to antibiotics should be assessed because this is the only way to ensure quick and successful treatment. In order to improve public health, antibiotic therapy should be accompanied by adequate preventive measures, such as training nursing staff to prevent as many nosocomial infections as possible, educating the general public about the importance of hygiene and encouraging them to stop self-medicating and fostering closer scientific collaboration between clinicians and microbiologists.

Recommendations

It is advised to measure the minimum bactericidal and inhibitory concentrations of azithromycin and erythromycin against Streptococcus pyogenes. Antibiotic purchases from pharmacies without a prescription ought to be against the law. It is strongly advised to utilize antibacterial medications as directed. To survey the antibacterial medication resistance of a wide spectrum of dangerous bacteria, more thorough work should be conducted on a regular basis. Further research using control strains is also necessary.

Acknowledgments

The authors are thankful to the Department of Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan, for their support duri ng the period of study.

Sources of Funding

There was no specific grant for this research from any funding organization in the public, private, or nonprofit sectors

Conflict of Interest

Authors have declared that no competing interests exist.

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