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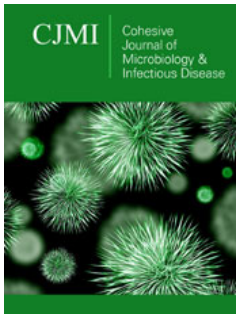

In Vitro Antimicrobial Susceptibility Testing of Azithromycin Against Bacteria Isolated from Patients with Respiratory Tract Infection

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Abstract

Background: Azithromycin is an amoxicillin antibiotic that has been widely used in the treatment of respiratory tract infections due to its broad-spectrum activity, favorable pharmacokinetics and immunomodulatory properties.

Objective: To evaluate the *in vitro* sensitivity of common respiratory tract pathogens to Azithromycin.

Methodology: This is a prospective cross-sectional study conducted in Shendi City, Sudan, from January to February 2025, at the Microbiology Laboratory, Faculty of Medical Laboratory Sciences at Shendi University. A total of 30 sputum samples were collected, from which fourteen strains of pathogenic gram-positive bacteria and two strains of pathogenic gram-negative bacteria were isolated and identified using Gram stain and biochemical tests.

Results: Of the 30 clinical specimens, *Staphylococcus aureus* was confirmed in 6 (60%), *Streptococcus pyogenes* in 2 (20%), *Klebsiella pneumoniae* in 1 (10%) and *Pseudomonas aeruginosa* in 1 (10%). Azithromycin demonstrated remarkable antimicrobial activity against all gram-positive bacteria and one of the gram-negative bacteria (*Pseudomonas aeruginosa*), which showed intermediate sensitivity.

Conclusion: The findings of this study indicate that Azithromycin can be used as an antibacterial agent against gram-positive bacterial strains.

Keywords: Antimicrobial; Azithromycin; Gram-positive bacteria; Respiratory tract infection

Introduction

Respiratory Tract Infections (RTIs) are among the leading causes of morbidity and mortality worldwide, significantly burdening healthcare systems [1]. These infections are often caused by bacterial pathogens, such as *Streptococcus pneumoniae*, Haemophilus influenzae and Moraxella catarrhalis, which are commonly associated with both upper and lower respiratory tract infections [2]. The widespread and often inappropriate use of antibiotics has led to the emergence of antimicrobial resistance, complicating the management of Respiratory Tract Infections (RTIs) and reducing the efficacy of commonly used antibiotics [3]. Is amacrolide antibiotic, has been widely utilized in the treatment of (RTIs) due to its broad-spectrum activity, favorable pharmacokinetics, and immunomodulatory properties [4]. However, increasing resistance to azithromycin among respiratory pathogens poses a challenge to the continued effectiveness of this medication. Periodic surveillance of local antimicrobial susceptibility patterns is essential to guide empirical therapy and ensure optimal clinical

outcomes [5]. Although generally bacteriostatic, azithromycin is bactericidal against *S. pyogenes*, *S. pneumonia* and Haemophilus influenzae. Resistance appears to correlate with the amount of macrolide use within a community, as evidenced by a decrease in erythromycin resistance among group A streptococci associated with a nationwide decrease in macrolide use [6]. Azithromycin was developed for oral treatment of bacterial infections of the upper and lower respiratory tract caused by organisms such as *S. pneumoniae* and *S. pyogenes*, skin and skin structure infections caused by *S. aureus* and *S. pyogenes* [7]. In patients who are allergic to penicillins, erythromycin has been thought to be the primary treatment for Gram-positive streptococcal infections. Nonetheless, there have been reports of elevated erythromycin resistance in *S. pyogenes* isolates in a number of global locations [8,9]. The prevalence of resistance to other beta-lactams and other classes of antimicrobial agents has increased globally since *S. pneumoniae* developed penicillin resistance in the 1960s. This has led to clinical issues when treating infections caused by this microorganism and highlighted the need for alternative therapeutic approaches [10]. *In vitro* antimicrobial susceptibility testing of azithromycin for bacteria isolated from respiratory tract infections is crucial for guiding effective treatment, especially with rising resistance trends. It ensures appropriate empirical therapy, prevents treatment failures and supports personalized medicine.

Materials and Methods

This cross-sectional study was conducted in Shendi Locality, River Nile State, Sudan. Shendi is a historic town situated approximately 150km northeast of Khartoum on the east bank of the Nile and 45km southwest of the ancient city of Meroe. As the center of the Ja'aliin tribe and a traditional trading hub, the locality hosts multiple hospitals and clinical centers. Its principal suburb, Al-Matamma, lies on the west bank and connects to Northwest Sudan via a major trade route across the Bayuda Desert. The study population comprised patients presenting with symptoms of Respiratory Tract Infection (RTI). Inclusion criteria required participants to be admitted with RTI, while those under antimicrobial treatment were excluded. A total of thirty sputum samples (No=30) were collected from eligible patients. Data were obtained using a structured questionnaire documenting all study variables.

Collection of the specimens

Sputum was collected in sterile containers.

Cultivation of the specimens

Culture media, such as Chocolate agar, are used for the identification and isolation of clinical isolates.

Interpretation of cultural growth

The plates were observed for any bacterial colonies to grow significantly. The bacteria were well isolated and then identified by colonial morphology, Gram stain, and biochemical tests.

Preparation of bacterial suspension

Clinical isolates were isolated from different samples and subculture. Ten ml of normal saline was distributed in test tubes and sterilized in an autoclave at 121 °C for 15 minutes. A loopful of purified bacterium was inoculated into sterile normal saline. Inoculum density was compared with the McFarland standard solution.

Kirby-Bauer Disc Diffusion Method

The disk diffusion method (Kirby-Bauer technique) is a standardized approach for Antimicrobial Susceptibility Testing (AST). It involves applying an antibiotic. Impregnated disks to an agar plate inoculated with the test organism, and measuring inhibition zones are measured to determine susceptibility. Mueller-Hinton Agar (MHA) is the preferred medium due to its reproducibility and compliance with international guidelines [11].

Ethical consideration

Permission was given by the College Ethical Committee of SHENDI UNIVERSITY and Hospitals. Participants have been notified, and no coercion of any sort has been done and any information that may disclose the participant's identity was not kept in consideration.

Data Collection and Analysis

A self-administered questionnaire was used and supported with coding numbers to facilitate the sorting of data. Data were entered, checked and analyzed using Microsoft Excel 2007. The final results were presented as frequencies and percentages.

Results

(Table 1-Table 7).

Table 1: The distribution of clinical specimens according to gender.

Gender	Frequency	Percentage
Male	16	53%
Female	14	47%
Total	30	100%

Table 2: The distribution of clinical specimens according to age.

Age	Frequency	Percentage
18-30	20	67%
31-50	7	23%
51-70	3	10%
Total	30	100%

Table 3: The distribution of clinical specimens according to smoker.

Gender	Frequency	Percentage
Smoker	7	23%
Non-smoker	23	77%
Total	30	100%

Table 4: Distribution of clinical specimens according to the type of smoking.

Type of smoking	Frequency	Percentage
Cigarettes	4	57%
Hookah	2	29%
Cigarettes and Hookah	1	14%
Total	7	100%

Table 5: Distribution of clinical specimens according to the rural-urban.

City	Frequency	Percentage
Khartoum	13	43%
Shendi	10	33%
Atbara	5	17%
Al-damir	2	7%
Total	30	100%

Table 6: Susceptibility of Gram-positive and Gram-negative bacteria to azithromycin.

Bacteria	Mean of Zone	p-value
Gram positive	26.75	0.69
Gram negative	22	

Table 7: Susceptibility of any type of Gram-positive and Gram-negative bacteria to azithromycin.

Bacteria	Mean of Zone
Staphylococcus aureus	26.5
Streptococcus pyogenes	27.5
Klebsiella pneumoniae	11
Pseudomonas aeruginosa	33

Discussion

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains and this has become a major cause of the failure of the treatment of infectious diseases [12]. The sensitivity of bacteria to antibiotics varies from place to place due to several factors, even within the same country or between different hospitals. 30 sputum samples were collected and many bacteria were isolated, like Staphylococcus and which represents 75% of the total Gram-positive bacteria isolated. And *Streptococcus pyogenes*, which represents 25% of the total gram-positive bacteria isolated, *Staphylococcus* and *Streptococcus pyogenes* together represent 80% of the total bacteria isolated. The remaining 20% of the total isolated bacteria were gram-negative bacteria, which included *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Klebsiella pneumoniae* represents 50% of the total Gram-negative bacteria and *Pseudomonas aeruginosa* also represents 50% of the remaining Gram-negative bacteria. Gram-positive bacteria are the most susceptible to Azithromycin, which shows a mean zone of 26.75mm and gram-negative bacteria are less susceptible to Azithromycin, which shows a mean zone of 22mm. This study is consistent with the study by Javed which found that *Staphylococcus aureus* and *Streptococcus pyogenes* showed higher susceptibility to Azithromycin (mean zones: 25-28mm) compared to gram-negative bacteria like *Klebsiella pneumonia* (mean zone: 10-12mm) [11], Some studies found lower susceptibility in Gram-positive bacteria Mishra reported that *S. aureus* had a mean zone of 22mm (compared to my result 26.5mm), suggesting variability in resistance patterns based on geographic location [13]. In Gram-positive bacteria isolated *Streptococcus pyogenes* is the most susceptible to Azithromycin than *S. aureus*. *Streptococcus pyogenes* shows a mean zone of 27.5mm and *Staphylococcus aureus* shows a mean zone of 26.5mm. Altamimi's studies show that *Streptococcus pyogenes* was more susceptible than *Staphylococcus aureus*. He reported that *S. pyogenes* had a mean inhibition zone of 28mm, while *S. aureus* showed 25mm [14] like my observation that *Streptococcus pyogenes* is slightly more sensitive. In gram-negative bacteria isolated *Pseudomonas aeruginosa* is susceptible to Azithromycin, which shows intermediate susceptibility with a mean zone of 33mm, and in *Klebsiella pneumoniae*, there is no susceptibility to Azithromycin, and it shows a resistant pattern, it is mean zone of 11mm. the research of Khan observed that Pseudomonas aeruginosa had a mean zone of 30-32mm, whereas *Klebsiella pneumoniae* was resistant (mean zone: 10mm) [15]. In Ahmed's studies, *Klebsiella pneumonia* showed slight susceptibility, noting that some *Klebsiella pneumonia* strains had a mean inhibition zone of 15mm, indicating possible strain-dependent differences [16]. *Pseudomonas* resistance was higher in some reports. Patel found that *Pseudomonas* had a mean zone of 25mm, suggesting regional resistance variations [17]. Other studies agree with us by Leila Mohamed A. Abdelgader and her colleagues in 2024 found that 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 [18]. Adequate preventive measures, such as teaching nursing staff to avoid as many nosocomial infections as possible, educating the public about the value of hygiene and motivating them to quit self-medicating and promoting closer scientific collaboration between clinicians and microbiologists, should be implemented in conjunction with antibiotic therapy to improve public health [18].

Conclusion

This study demonstrates that Azithromycin exhibits greater efficacy against gram-positive bacteria (such as *Staphylococcus aureus* and *Streptococcus pyogenes*) compared to gram-negative bacteria (including *Klebsiella spp.* and *Pseudomonas spp.*). *Streptococcus pyogenes* was found to be slightly more sensitive than *Staphylococcus aureus*, while *Klebsiella* showed notable resistance. Although some variations in susceptibility reported by other studies exist-potentially attributable to differences in bacterial strains or geographic locations-the overall findings consistently support Azithromycin being more effective for treating infections caused by gram-positive bacteria.

Recommendations

A. Test More Bacterial Strains: Check different strains of *Klebsiella* and *Pseudomonas* to see if some respond better to Azithromycin.

B. Compare Different Regions: Study bacteria from different areas to understand why some show resistance while others don't.

C. Use Other Antibiotics for Comparison: Test if other drugs work better against resistant bacteria like *Klebsiella*.

D. Study Resistance Mechanisms: Find out why Gram-negative bacteria resist Azithromycin more than Gram-positive ones.

E. Monitor Changing Resistance: Regularly test bacteria over time to track if they become more or less resistant to Azithromycin.

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Consent

The patient's written consent has been collected.

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Conflict of Interest

The authors have declared that no competing interests exist.

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