

Resistance Mechanisms and Molecular Epidemiology of *Neisseria gonorrhoeae* with Low Susceptibility/Resistance to Azithromycin or Cephalosporins

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Purpose: To investigate the resistance patterns of *Neisseria gonorrhoeae* strains with low susceptibility or resistance to azithromycin or ceftriaxone in the Wenzhou region from 2021 to 2023, as well as their resistance to other antibiotics and their *Neisseria gonorrhoeae* multi-antigen sequencing and typing (NG-MAST) genotypes.

Patients and Methods: A total of 212 *Neisseria gonorrhoeae* isolates collected from 2021 to 2023 were subjected to agar dilution testing to determine the minimum inhibitory concentrations (MIC) for penicillin, tetracycline, ciprofloxacin, azithromycin, ceftriaxone, and Daquanmycin. Identification of Penicillinase -Producing *Neisseria gonorrhoeae* (PPNG) Strains Mediated by Plasmids Using the Paper Strip Acidity Assay and perform *Neisseria gonorrhoeae* multi-antigen sequencing typing (NG-MAST).

Results: Among the 212 *Neisseria gonorrhoeae* isolates tested, 27 (12.74%) exhibited azithromycin resistance. Eight strains (3.77%) showed low susceptibility or resistance to ceftriaxone. Among the 27 azithromycin-resistant *Neisseria gonorrhoeae* strains, the detection rates for PPNG and plasmid-mediated highly tetracycline-resistant *Neisseria gonorrhoeae* (TRNG) were 81.48% (22/27) and 33.33% (9/27), respectively. NG-MAST typing revealed that the 27 azithromycin-resistant strains primarily included ST5061 (n = 7), ST1866 (n = 2), ST4183 (n = 2), ST14504 (n = 1), ST9659 (n = 1), and ST12196 (n = 1). All 8 ceftriaxone low-susceptibility/resistant strains were ST1866 (n=3).

Conclusion: The prevalence of low susceptibility/resistance to ceftriaxone among *Neisseria gonorrhoeae* strains in Wenzhou remains low, but long-term surveillance is still necessary, particularly among young and middle-aged adults. NG-MAST typing can serve as a molecular biological marker for monitoring gonococcal resistance.

Plain Language Summary:

1. This study detected low-sensitivity/drug-resistant strains resistant to azithromycin and cephalosporins for the first time. This study was the first report of ST typing in this region, which is of great significance to the study of drug-resistant gonococcus in this region.
2. This study also showed that only one strain was resistant to both azithromycin and ceftriaxone, which was the first detection of azithromycin and cephalosporins low sensitivity/drug resistance strains in this region, which is of great significance.
3. Our findings provide a scientific basis for rational and optimized antibiotic use in clinical practice, helping to delay the emergence of resistant bacteria and combat the threat of antibiotic resistance.

Keywords: *Neisseria gonorrhoeae*, azithromycin, ceftriaxone, NG-MAST, genotype

Introduction

As one of the sentinel sites for gonococcal antibiotic resistance surveillance in Zhejiang Province, In recent years, amid the high prevalence of drug-resistant *Neisseria gonorrhoeae*, clinical reliance and misuse of cephalosporin antibiotics have led to reduced susceptibility to these agents, particularly with emerging cases of ceftriaxone treatment failure.^{1,2} Consequently, an increasing number of gonorrhea treatment guidelines now recommend combined therapy with cephalosporins and azithromycin.³ In this study, we conducted susceptibility testing on *Neisseria gonorrhoeae* isolates, screened for strains exhibiting low susceptibility or resistance to azithromycin or cephalosporins, and performed epidemiological analysis and *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) to gain preliminary insights into the epidemiological characteristics and genotyping of azithromycin- or cephalosporin-resistant/low-susceptibility strains in Wenzhou.

Materials and Methods

General Materials

From 2012 to 2023, a tertiary hospital in Wenzhou diagnosed 212 cases of gonorrhea in its outpatient department. Strains were confirmed through Gram staining, culture, and Bruker mass spectrometry identification. Patient ages ranged from 15 to 63 years old, with 195 male samples (91.98%) and 17 female samples (8.02%). By age group: ≤20 years: 24 cases (11.32%) 21–30 years: 91 cases (42.92%) 31–40 years: 60 cases (28.30%) 41–50 years: 20 cases (9.43%) >50 years: 17 cases (8.03%).

Antibiotic Susceptibility Testing

Determine the MIC values for azithromycin, ciprofloxacin, spectinomycin, ceftriaxone, cefuroxime, tetracycline, and penicillin using the agar dilution method recommended by the World Health Organization (WHO) Western Pacific Region *Neisseria gonorrhoeae* Antimicrobial Resistance Surveillance Program. Strains with a tetracycline MIC ≥ 16 mg/L are classified as TRNG strains. PPNG was determined using the paper disc acidity test, with known β-lactamase-positive (E strain) and -negative (D strain) standard strains as controls. The three WHO gonococcal standard strains (G, J, L) were provided by the Reference Laboratory of the Center for STD Control, Chinese Center for Disease Control and Prevention (CDC).

Screening of Drug-Resistant *Neisseria gonorrhoeae* Strains

According to the CDC guidelines for gonococcal antimicrobial susceptibility testing,⁴ strains resistant to azithromycin (AZ-R, MIC ≥ 1 mg/L) and those with low susceptibility to ceftriaxone (MIC ≥ 1.25 mg/L) were identified.

DNA Extraction and Purification

The OMEGA DNA extraction kit was selected for DNA extraction and purification of the experimental strains.

PCR Amplification and Sequencing

PCR amplification of the *porB* and *tbpB* genes was performed using primers from Table 1.^{5,6} Amplified products were identified via 2% agarose gel electrophoresis and subsequently sent to Shanghai Shengsong Biotechnology for sequencing. The *porB* and *tbpB* sequences from both sets of strains were input into the NG-MAST database (<http://www.ng->

Table 1 Primer Information

Target Gene	Primer Sequences (5'→3')	Length (bp)
<i>porB</i>	<i>porB</i> -F:CAAGAAGACCTCGGCAA <i>porB</i> -R:CCGACAACCACTTGGT	737
<i>tbpB</i>	<i>TbpB</i> -F:CGTTGTCGGCAGCGCGAAAAC <i>TbpB</i> -R:TTCATCGGTGCGCTCGCCTTG	589

[mast.net](#)) for comparative analysis. The corresponding gene sequences were obtained, combined into sequence numbers, and re-input into the database to derive the corresponding sequence types (STs).^{7,8}

Statistical Methods

Data entry, aggregation, and organization were performed using Excel software. Count data are expressed as n and %.

Results

Epidemiological Characteristics

Patients with drug-resistant strains ranged in age from 16 to 60 years. Males accounted for 33 cases (94.29%) and females for 2 cases (5.71%). Among these, 12 cases (34.29%) were ≤ 20 years of age, 10 cases (28.57%) were 21–30 years, 9 cases (25.71%) were 31–40 years, 2 cases (5.71%) were 41–50 years, and 2 cases (5.71%) were > 50 years; thus, young and middle-aged adults constituted the majority. Male patients most commonly presented with urethral discharge (Table 2).

Antibiotic Susceptibility Test Results

A total of 212 *Neisseria gonorrhoeae* isolates were collected. Twenty-seven isolates were resistant to azithromycin (resistance rate 12.74%, 27/212), and eight showed reduced susceptibility or resistance to ceftriaxone (3.77%, 8/212). One isolate was co-resistant to azithromycin and ceftriaxone. Among the 27 azithromycin-resistant isolates, PPNG and TRNG were detected in 81.48% (22/27) and 33.33% (9/27), respectively. Among the eight ceftriaxone-non-susceptible isolates, PPNG and TRNG were found in 62.5% (5/8) and 12.5% (1/8), respectively (Table 3); resistance patterns to other antibiotics are summarized in Table 4.

Table 2 Epidemiological Characteristics of Drug-Resistant Gonorrhea Cases, n (%)

	Azithromycin-Resistant Strains (n = 27)	Ceftriaxone-Low Susceptibility/Resistant strains (n = 8)	Total (n = 35)	Sensitive Strains (n = 177)
Gender				
Male	25 (92.59)	8 (100)	33 (94.29)	162 (91.53)
Female	2 (7.41)	0 (0.00)	2 (5.71)	15 (8.47)
Age (years)				
≤ 20 years old	9 (33.33)	3 (37.50)	12 (34.29)	12 (6.78)
21–30 years old	7 (25.93)	3 (37.50)	10 (28.58)	81 (45.76)
31–40 years old	7 (25.93)	2 (25.00)	9 (25.71)	51 (28.81)
41–50 years old	2 (7.41)	0 (0.00)	2 (5.71)	18 (10.17)
> 50 years old	2 (7.41)	0 (0.00)	2 (5.71)	15 (8.48)
Clinical Diagnosis				
Urethritis	18 (66.67)	6 (75.00)	24 (68.57)	130 (73.45)
Balanitis	7 (25.92)	2 (25.00)	9 (25.72)	20 (11.30)
Cervicitis	2 (7.41)	0 (0.00)	2 (5.71)	2 (1.13)
Vaginitis	0 (0.00)	0 (0.00)	0 (0.00)	12 (6.78)
Other	0 (0.00)	0 (0.00)	0 (0.00)	13 (7.34)

Table 3 PPNG and TRNG Detection Rates, n (%)

	Azithromycin-Resistant Strains (n = 27)	Ceftriaxone Low Susceptibility/Resistant strains (n = 8)
PPNG	22 (81.48%)	5 (62.50)
TRNG	9 (33.33%)	1 (12.50)

Table 4 Resistance Profiles to Other Antibiotics, n (%)

Antibiotics	Azithromycin-Resistant Strains (n = 27)	Ceftriaxone Low Susceptibility/Resistant strains (n = 8)
Ciprofloxacin	27 (100)	8 (100)
Spectinomycin	0 (0)	0 (0)
Tetracycline	27 (100)	7 (87.5)
Penicillin	24 (88.89)	8 (100)

Ng-Mast

The 27 azithromycin-resistant isolates comprised ST5061 (n = 7), ST1866 (n = 2), ST4183 (n=2), ST14504 (n = 1), ST9659 (n = 1), and ST12196 (n = 1), indicating high diversity without a dominant sequence type. All eight ceftriaxone-resistant or reduced-susceptibility isolates belonged to ST1866 (n = 8).

Discussion

The age range of drug-resistant patients in this region is 16 to 60 years, with males accounting for 94.29% of cases. The predominant age group is 21–40 years, representing 54.28% of cases. The significantly higher number of male cases compared to females is associated with the low screening rate among female gonorrhea patients due to their often mild or absent symptoms. Health authorities in this region should intensify gonococcal screening among women. Additionally, the region has a substantial migrant population, with higher incidence among sexually active individuals aged 21–40, consistent with reported findings,⁹ indicating sexual transmission as the primary route. Given the significant proportion of adolescents—who experience rapid physical development but receive limited sexual education in China—their limited awareness of self-protection calls for enhanced public-education campaigns.

Currently, ceftriaxone is clinically recommended as the first-line drug for gonorrhea treatment in China.¹⁰ In this region, the ceftriaxone low sensitivity/resistance rate was 3.77%, indicating a concerning resistance trend. This finding is comparable to the 4.4% ceftriaxone low sensitivity/resistance rate reported in the Chinese Gonococcal Antibiotic Sensitivity Project.¹¹ This rate is lower than the 13.00% prevalence of reduced ceftriaxone susceptibility reported by Liang Huanxi¹² in Panyu District in 2022. This suggests that untreatable gonococcal infections and their reproductive complications could become a major public health threat in this region. Research indicates macrolide antibiotics readily induce gonococcal resistance.¹³ This study shows local gonococcal resistance to azithromycin at 12.74%, comparable to rates in Guangzhou, Nanjing, and Changsha.¹⁴ The WHO stipulates that if antibiotic resistance to gonococci reaches $\geq 5\%$, that antibiotic must be prohibited for empirical use.¹⁵ This indicates that monotherapy with azithromycin is no longer appropriate for routine treatment for gonorrhea patients in this region. In recent years, the emergence of highly resistant strains to ceftriaxone has led to new treatment guidelines recommending combination therapy with azithromycin plus ceftriaxone or other third-generation cephalosporins.¹³ This approach not only produces favorable synergistic effects but also effectively mitigates the rapid rise in resistance. This study also revealed that only one strain exhibited simultaneous resistance to azithromycin and ceftriaxone. Nevertheless, statistics from other regions in China, such as Guangzhou, indicate an increasing trend in strains simultaneously resistant to azithromycin and exhibiting low susceptibility/resistance to cephalosporins.¹⁶ This may be attributable to the widespread availability of pharmacies in China, where patients often obtain antibiotics without prescriptions for self-treatment. The improper and repeated use of antibiotics can easily lead to drug resistance. Additionally, gonorrhea is prone to recurrent infections, which may result from untreated or inadequately treated partners spreading drug-resistant strains. Research indicates that individuals with recurrent gonorrhea infections can cause a significant number of gonorrhea cases. Patients with recurrent *Neisseria gonorrhoeae* infections serve as major sources of transmission for gonorrhea. Prioritizing the prevention and control of recurrent infections can reduce the incidence of gonorrhea. Enhanced surveillance of local gonococcal resistance, particularly focusing on strains exhibiting simultaneous resistance to azithromycin and low susceptibility/resistance to cephalosporins, will provide more effective drug guidance for clinical management of gonorrhea.

Neisseria gonorrhoeae is the sexually transmitted pathogen most prone to developing antibiotic resistance. Strains resistant or minimally susceptible to azithromycin or ceftriaxone exhibit severe resistance to other antibiotics. The detection rates of azithromycin-resistant gonococci (PPNG and TRNG) were 81.48% and 33.33%, respectively. Among 8 ceftriaxone-resistant/low-susceptibility *Neisseria gonorrhoeae* isolates, PPNG and TRNG detection rates were 62.5% and 12.5%, respectively, exceeding provincial rates,¹⁷ indicating this region has become a high-prevalence area for PPNG and TRNG. The average chromosomally mediated penicillin resistance rate exceeded 88.89%. Ciprofloxacin resistance was 100%, while spectinomycin maintained high susceptibility, confirming its continued suitability as a first-line agent for gonococcal infections in this region.

NG-MAST typing was first proposed by Martin et al⁸ in 2004 as a gonococcal sequence typing method developed in parallel with advances in sequencing technology, characterized by simplicity and rapidity, was developed in parallel with advances in sequencing technology. Azithromycin-resistant strains predominantly belonged to ST5061, while ceftriaxone-resistant strains were primarily ST1866, consistent with findings from similar domestic studies.¹⁸ Continuous surveillance of local resistance patterns and molecular types will provide evidence-based guidance for antibiotic selection.

Ethics Approval

This study was reviewed and approved by the Ethics Committee of Wenzhou Hospital of Integrated Traditional Chinese and Western Medicine (NO. 2025-L074). This study complies with the Declaration of Helsinki. Informed consent was waived due to the retrospective nature of the study.

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Disclosure

The authors report no conflicts of interest in this work.

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