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Brief communication

Whole-genome characterization of two azithromycin-resistant *Neisseria gonorrhoeae* ST1600 isolates from Busan, South Korea

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Abstract

Azithromycin-resistant *Neisseria gonorrhoeae* poses a threat to the efficacy of gonorrhea treatment. We report the whole-genome characterization of two azithromycin-resistant *N. gonorrhoeae* ST1600 isolates collected in Busan, South Korea, in 2018 and 2019. Both isolates showed moderate azithromycin resistance (minimum inhibitory concentration 32 μg/mL) mediated by the 23S rRNA C2611T mutation in all four alleles and the -35A deletion in the *mtrR* promoter, while harboring distinct non-mosaic *penA* alleles without reduced susceptibility to extended-spectrum cephalosporins. Multilocus sequence typing classified both isolates as ST1600, which is related to ST7363. *N. gonorrhoeae* multi-antigen sequence typing assigned the 2018 isolate to ST16190, whereas the 2019 isolate represented a novel NG-MAST type closely related to ST16190 (differing by a single SNP in *porB*). Taken together, these data suggest a limited local transmission cluster with short-term persistence and microevolution, rather than widespread sustained clonal dissemination. Continued phenotypic and genomic surveillance is needed to monitor azithromycin resistance in *N. gonorrhoeae* and inform national treatment strategies.

Keywords: Azithromycin; Drug resistance, bacterial; Gonorrhea; *Neisseria gonorrhoeae*; Whole genome sequencing

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Until 2006, gonorrhea was South Korea's most prevalent bacterial sexually transmitted infection, but its incidence has since declined, primarily affecting individuals in their twenties [1]. In contrast, rates in the United States have increased, particularly among individuals aged 15–24 years [2]. Globally, ciprofloxacin resistance remains high, azithromycin resistance is increasing, and susceptibility to ceftriaxone is declining [3]. This trend has made treatment increasingly challenging and has complicated the selection of appropriate treatment regimens for gonorrhea.

Since 2014, we have analyzed *Neisseria gonorrhoeae* isolates from 35 hospitals in South Korea, and in 2018 and 2019, identified two strains with moderate azithromycin resistance. Azithromycin resistance and decreased susceptibility in *N. gonorrhoeae* have previously been reported in South Korea [4]. However,

detailed whole-genome characterization of such isolates remains limited. Here, we report the genomic characterization of these two isolates in the context of evolving gonococcal treatment guidelines. These isolates represent, to our knowledge, the first azithromycin-resistant *N. gonorrhoeae* ST1600 strains reported in South Korea, and their *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) profiles differ from those of the azithromycin-resistant NG-MAST ST4207 and ST6762 lineages with elevated azithromycin minimum inhibitory concentrations (MICs) previously described in Japan [5].

We characterized these isolates as follows:

Specimens were inoculated onto modified Thayer-Martin medium and identified by MALDI-TOF mass spectrometry (Bruker) and biochemical testing using Vitek NHI cards (bioMérieux). Azithromycin susceptibility was assessed by the agar dilution method using GC II agar supplemented with 1% IsoVitaleX, according to the Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines [6,7]. Sequencing libraries were prepared from 50 ng of extracted DNA using the Twist Library Preparation EF Kit (Twist Bioscience), according to the manufacturer's instructions. Paired-end sequencing was performed on a NovaSeq 6000 system (Illumina) using 2 × 150 bp reads. Library quantity and fragment size distribution were assessed using Qubit fluorometric assays (Thermo Fisher Scientific) and the Agilent 4200 TapeStation system (Agilent Technologies). FASTQ files were de novo assembled using Unicycler v0.4.0, and the resulting assemblies were evaluated for sequencing depth and standard assembly quality metrics before downstream analyses. Assembled FASTA files were analyzed using Pathogenwatch and the PubMLST database to determine NG-MAST and multilocus sequence typing (MLST) profiles, *penA* and 23S rRNA variants, and antimicrobial resistance determinants. Antimicrobial resistance determinants were identified based on curated *N. gonorrhoeae* reference schemes within Pathogenwatch and PubMLST and were confirmed when sequence identity and coverage relative to reference alleles were ≥ 95% and ≥ 90%, respectively.

The 2018 strain exhibited an azithromycin MIC of 32 μg/mL and resistance to penicillin G, tetracycline, and ciprofloxacin, while remaining susceptible to ceftriaxone and spectinomycin (Table 1). It carried a non-mosaic *penA*-109.001 allele with an A502V mutation, a -35A deletion in the *mtrR* promoter, and a C2611T mutation in all four 23S rRNA alleles. It was classified as ST1600 by MLST, and NG-MAST assigned it to ST16190 based on the *porB* 9414 and *tbpB* 831 alleles. The 2019 strain had a similar antimicrobial resistance pattern, with an azithromycin MIC of 32 μg/mL, but carried a non-mosaic *penA*-120.001 allele. It differed from the 2018 strain in the *porB* 9414 allele by a single-nucleotide polymorphism (A169G), yielding a novel NG-MAST type with the same *tbpB* 831 allele and MLST ST1600.

Table 1. Characteristics of two azithromycin-resistant *Neisseria gonorrhoeae* isolates

Characteristics	Year of isolation	
	2018	2019
Area of isolation (city)	Busan	Busan
Antimicrobial susceptibility (MIC (μg/mL) and interpretation)		
Azithromycin	32, R	32, R
Ciprofloxacin	32, R	16, R
Spectinomycin	32, S	16, S
Tetracycline	2, R	4, R
Penicillin G	2, R	2, R
Cefixime	0.06, S	0.03, S
Ceftriaxone	0.06, S	0.03, S
Resistance determinants		
23S rRNA	C2611T mutation (4 alleles)	C2611T mutation (4 alleles)
mtrR promoter	-35A deletion	-35A deletion
Epidemiology		
NG-MAST	ST16190	ST16190[a)]
MLST	ST1600	ST1600
penA genotype	109.001	120.001

[a)]A169G single-nucleotide polymorphism in *porB* 9414 and an identical *tbpB* allele 831.
Abbreviations: MIC, minimum inhibitory concentration; R, resistant; S, susceptible; NG-MAST, *Neisseria gonorrhoeae* multi-antigen sequence typing; MLST, multilocus sequence typing.

Two isolates collected in Busan, South Korea, in 2018 and 2019 shared a common molecular resistance profile characterized by moderate azithromycin MICs of 32 μg/mL and C2611T substitutions in all four 23S rRNA alleles, together with a -35A deletion in the *mtrR* promoter. This combination of target-site modification and enhanced efflux is consistent with previously described mechanisms of moderate azithromycin resistance in *N. gonorrhoeae*, rather than the high-level azithromycin resistance typically associated with 23S rRNA A2059G mutations [8].

Both isolates belonged to MLST ST1600 (126-39-67-78-148-153-65), a lineage closely related to ST7363 (59-39-67-78-148-153-65) and previously associated with reduced susceptibility to extended-spectrum cephalosporins in East Asia [9]. However, no increase in ceftriaxone MIC was observed, supporting previous observations that antimicrobial susceptibility phenotypes within this lineage are determined primarily by specific resistance determinants, such as *penA* alleles, rather than by MLST background alone.

NG-MAST analysis identified the two isolates as ST16190 and a closely related ST16190 variant, suggesting sporadic emergence or recent recombination rather than clonal dissemination. Similar low-to-moderate azithromycin resistance profiles associated with C2611T and *mtrR* alterations have been reported in South Africa and South America [8,10]. In contrast, repeated detection of identical NG-MAST or *N. gonorrhoeae* sequence typing for antimicrobial resistance types has been used to characterize clonal expansion in high-level resistance lineages reported from Japan [11]. Together, the nearly identical resistance determinants, shared MLST ST1600 background, and common geographic origin in Busan support a local epidemiological link and microevolution.

N. gonorrhoeae has developed resistance to all first-line antimicrobials previously used for the treatment

of gonorrhea, with high-level azithromycin resistance (MIC ≥ 256 μg/mL) reported in several countries [12]. Current WHO and national guidelines recommend ceftriaxone-based regimens as the mainstay of treatment and generally discourage routine dual therapy with azithromycin because of rising macrolide resistance [13]. In South Korea, ceftriaxone remains the preferred agent, with spectinomycin or gentamicin plus high-dose azithromycin reserved for situations in which cephalosporins cannot be used [1]. The emergence of azithromycin-resistant *N. gonorrhoeae*, as observed in our two isolates, is consistent with these evolving recommendations and underscores the need for cautious use of azithromycin-containing regimens in settings where macrolide resistance is increasing.

In conclusion, we describe two azithromycin-resistant *N. gonorrhoeae* ST1600 isolates from Busan, South Korea, with moderate azithromycin MICs (32 μg/mL) mediated by the 23S rRNA C2611T mutation and the -35A deletion in the *mtrR* promoter. Their high genetic similarity but distinct NG-MAST types suggest local microevolution and sporadic emergence rather than sustained clonal spread. As Busan is a major urban center with notable domestic and international connectivity, continued integrated phenotypic and genomic surveillance is warranted to detect emerging resistant gonococcal lineages and inform national treatment strategies.

Ethics statement

The research did not provide demographic information to the patient. Following internal sentinel surveillance processes, all gonococcal isolates were grown and stored. This research was also given an exemption from the ethics approval requirement because it performed as one of the projects of the national sentinel surveillance program supported by the Korean Centers for Disease Control and Prevention.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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None.

Data availability

The whole-genome shotgun assembly has been deposited in DDBJ/ENA/GenBank under accession number JBYPZC000000000 (BioProject PRJNA1469664; BioSample SAMN60371742).

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Authors' contributions

Conceptualization: Lee H; Methodology: Seo YH; Investigation: Roh KH; Supervision: Lee K; Writing - Original Draft: Seo YH, Liu C; Writing - Review & Editing: Roh KH, Lee H, Lee K

References

1. Yang HJ, Lee HM, Lee SJ, Choi JB, Bae S, Jung JH, et al. 2023 Korean Association of Urogenital Tract Infection and Inflammation guidelines for gonococcal infection. Investig Clin Urol 2024;65:1–8.
2. Pollock ED, Clay PA, Kreisel KM, Spicknall IH. Estimated incidence and prevalence of gonorrhea in the United States, 2006–2019. Sex Transm Dis 2023;50:188–95.
3. Unemo M, Lahra MM, Escher M, Eremin S, Cole MJ, Galarza P, et al. WHO global antimicrobial resistance surveillance for *Neisseria gonorrhoeae* 2017–18: a retrospective observational study. Lancet Microbe 2021;2:e627–36.
4. Lee H, Unemo M, Kim HJ, Seo Y, Lee K, Chong Y. Emergence of decreased susceptibility and resistance to extended-spectrum cephalosporins in *Neisseria gonorrhoeae* in Korea. J Antimicrob Chemother 2015;70:2536–42.
5. Miura M, Shigemura K, Osawa K, Nakanishi N, Nomoto R, Onishi R, et al. Genetic characteristics of azithromycin-resistant *Neisseria gonorrhoeae* collected in Hyogo, Japan during 2015–2019. J Med Microbiol 2022;71:10.1099/jmm.0.001533.
6. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 31st ed. CLSI supplement M100. Wayne, PA: CLSI; 2021.
7. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021. https://www.eucast.org [Online] (last visited on 8 April 2026).
8. Müller EE, Gumede LYE, Maseko DV, Mahlangu MP, Venter JME, Da Costa Dias B, et al. Emergence of high-level azithromycin-resistant *Neisseria gonorrhoeae* causing male urethritis in Johannesburg, South Africa, 2021. Sex Health 2024;21:SH23143.
9. Peng JP, Yin YP, Chen SC, Yang J, Dai XQ, Zheng HP, et al. A whole-genome sequencing analysis of *Neisseria gonorrhoeae* isolates in China: an observational study. EClinicalMedicine 2019;7:47–54.
10. Gianecini RA, Poklepovich T, Golparian D, Cuenca N, Tuduri E, Unemo M, et al. Genomic epidemiology of azithromycin-nonsusceptible *Neisseria gonorrhoeae*, Argentina, 2005–2019. Emerg Infect Dis 2021;27:2369–78.
11. Shimuta K, Lee K, Yasuda M, Furubayashi K, Uchida C, Nakayama SI, et al. Characterization of 2 *Neisseria gonorrhoeae* strains with high-level azithromycin resistance isolated in 2015 and 2018 in Japan. Sex Transm Dis 2021;48:e85–7.
12. Unemo M and Nicholas RA. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. Future Microbiol 2012;7:1401–22.
13. World Health Organization. Updated recommendations for the treatment of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Treponema pallidum* (syphilis), and new recommendations on syphilis testing and partner services. Geneva: World Health Organization; 2024.