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BRIEF REPORT

High Prevalence of Ceftriaxone-Resistant *Neisseria gonorrhoeae* in Hanoi, Vietnam, 2023–2024

Jolein Gyonne Elise Laumen,[1] Vu N. Hieu,[2,3] Pham Hong Nhung,[2,3,4] Koen Vandelannoote,[1] Tam Thi Nguyen,[5] Trang T. Nguyen,[5] Diep T. N. Nguyen,[5] Thomas Kesteman,[5,6] H. Rogier van Doorn,[5,6] Tran M. Chau,[2,3] and Paul C. Adamson[7]

[1]Bacterial Phylogenomics Group, Pasteur Institute of Cambodia, Phnom Penh, Cambodia; [2]Department of Microbiology, Hanoi Medical University, Hanoi, Vietnam; [3]Department of Microbiology and Parasitology, Hanoi Medical University Hospital, Hanoi, Vietnam; [4]Department of Microbiology, Bach Mai Hospital, Hanoi, Vietnam; [5]Oxford University Clinical Research Unit, Hanoi, Vietnam; [6]Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom; and [7]Division of Infectious Diseases, School of Medicine, University of California, Los Angeles, California, USA

Ceftriaxone resistance in *Neisseria gonorrhoeae* is an escalating global health concern, threatening the efficacy of empiric treatment. In the Asia-Pacific region, a rapid surge in ceftriaxone resistance has been observed, primarily associated with the *penA-60.001* allele. This study, conducted in Hanoi, Vietnam between 2023 and 2024, analyzed 352 *N. gonorrhoeae* isolates and found a 27% prevalence of ceftriaxone resistance. Whole-genome sequencing identified that resistance was predominantly driven by the mosaic *penA-237.001* allele, with a smaller contribution from *penA-60.001*. The high prevalence of resistance and the emergence of new alleles underscore the urgent threat to ceftriaxone as an empiric treatment option.

Keywords. gonorrhea; ceftriaxone; antimicrobial resistance; whole-genome sequencing; Asia, Southeastern.

Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is an urgent global health threat. Ceftriaxone, the last effective option for empiric gonococcal treatment, is facing increasing resistance. *N. gonorrhoeae* isolates with ceftriaxone minimum inhibitory concentration (MIC) greater than 0.125 mg/L are defined as resistant (European Committee on Antimicrobial Susceptibility Testing [EUCAST] breakpoints, version 14.0). Recent studies document a significant rise in ceftriaxone-resistant *N. gonorrhoeae* strains in the Asia-Pacific region, with a prevalence exceeding 20% in many settings [1, 2]. In Vietnam, resistance increased markedly from just 1% during the 2011–2019 period to approximately 27% in 2023 [3–5]. This underscores the immediate threat to ceftriaxone's continued efficacy in the region. Since 2015, the FC428 strain carrying the *penA-60.001* allele, first reported in Japan, has spread internationally [6]. More recently, ceftriaxone-resistant strains in the United States [7], United Kingdom [8], and Europe [9] have been linked to the *penA-60.001* allele, with several cases associated with travel to the Asia-Pacific region, including Vietnam. Additionally, reports from Cambodia suggest the prevalence of the *penA-60.001* allele was more extensive in the region than previously thought [1]. This report describes the prevalence and molecular epidemiology of ceftriaxone-resistant *N. gonorrhoeae* strains in Hanoi, Vietnam isolated between 2023 and 2024.

METHODS

From 1 January 2023 to 27 June 2024, all *N. gonorrhoeae* isolates collected through routine laboratory procedures at Hanoi Medical University (HMU) Hospital (Hanoi, Vietnam) underwent antibiotic susceptibility testing. Culture specimens were obtained from patients with genital discharge who sought care at the outpatient department; anyone can attend the HMU outpatient clinics. MICs for ceftriaxone, azithromycin, and cefixime were determined using E-tests (bioMérieux) and interpreted according to the EUCAST breakpoints (version 14.0). All isolates with ceftriaxone MICs ≥0.125 mg/L (the World Health Organization [WHO] alert-level) were selected for cefixime and azithromycin MIC determination and for whole-genome sequencing (WGS) as part of this study. Isolates underwent DNA extraction (Qiagen DNeasy Kit), library preparation with the Nextera XT Library Preparation kit, and WGS on an Illumina MiSeq system using Miseq Reagent Kit version 3 (Illumina). Additional laboratory methods are included in Supplementary Material 1.

Illumina paired reads were preprocessed with Trimmomatic, and then de novo assembled into contigs using Shovill. The pyngoST tool assigned multilocus sequence types (MLST) and AMR genotypes (NG-STAR). AMR genes and point mutations were identified using the organism-specific mode of AMRFinderPlus.

Trimmed reads were mapped to a reference genome (FA1090) and variants were called using the Snippy pipeline. A core single-nucleotide polymorphism (SNP) alignment was

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Correspondence: Paul Adamson, MD, MPH, Division of Infectious Diseases, School of Medicine, University of California, Los Angeles, 911 Broxton Avenue, Suite 301, Los Angeles, CA 90024 (PAdamson@mednet.ucla.edu); Tran Minh Chau, MD PhD, Microbiology Department, Hanoi Medical University, No. 1 Ton That Tung Street, Dong Da District, Hanoi 10000, Vietnam (tranminhchau@hmu.edu.vn).

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produced with snippy-core, combining all positions with an SNP in at least 1 of the isolates in the studied panel. This multiple sequence alignment of core SNPs was then filtered down by excluding all variation detected in repetitive, recombinant (detected with Gubbins), and prophage (detected with PHASTER) regions to obtain an alignment of purely vertically acquired mutations. IQtree was used to infer maximum likelihood (ML) phylogenetic trees with the *ModelFinder Plus* with ascertainment bias correction model, which incorporates SNP ascertainment bias correction and uses ModelFinder to automatically pick the best-fit substitution model. Branch support was evaluated with 10 000 bootstrap replicates. Median joining haplotype networks of clades of interest were inferred with PopART. Details of the bioinformatics methods and references are in Supplementary Material 1. Data are available under BioProject PRJNA1161034, and isolate run accession numbers for individual samples in Supplementary Material 2.

The study was approved by the HMU Institutional Review Board (1155/GCN-HMUIRB).

RESULTS

In total, there were 365 *N. gonorrhoeae* isolates collected during the study period, nearly all (98.9%) were from the outpatient urology department; 13 were nonviable or unavailable for additional testing and were excluded from subsequent analysis. Of the 352 available isolates, 26.7% (94/352) were ceftriaxone resistant (MICs >0.125 mg/L) and 29.5% (104/352) had WHO alert values (MICs ≥0.125 mg/L); a ceftriaxone MIC distribution plot is shown in Supplementary Figure 3. Among the 104 isolates with MICs ≥0.125 mg/L, all were from urethral swabs from men for evaluation of urethritis with genital discharge; the median age was 27 years (interquartile range [IQR], 21–33 years). These 104 isolates were all resistant to cefixime but susceptible to azithromycin. The MICs for ceftriaxone, cefixime, and azithromycin for the 104 isolates can be found in Supplementary Material 2.

Of 104 *N. gonorrhoeae* isolates with ceftriaxone MICs ≥0.125 mg/L, 95 underwent WGS; 9 were excluded due to insufficient amounts of extracted DNA. Five isolates with an average read depth <30 were excluded from further analyses. Detailed metadata, phenotypic antimicrobial susceptibility, and WGS quality metrics are shown in Supplementary Material 2. Genotyping revealed 13 distinct MLST sequence types, comprising 12 known STs and 1 new ST with a novel combination of previously reported alleles. ST1901 was the most prevalent, found in 64 isolates (71.1%), followed by ST13871 in 9 isolates (10%), and ST7822 in 4 isolates (5.6%) (Supplementary Material 3).

The majority of isolates carried the *penA-237.001* allele (76%, n = 68). Phylogenomic analysis showed that *penA-237.001* isolates were found in 3 distinct clades (Figure 1). One major clade (clade 1) contained a total of 70 isolates, of which 66 harbored the *penA-237.001* allele. In contrast, the 2 smaller clades included clade 3 with 4 isolates, of which only 1 carried the *penA-237.001* allele, and clade 4 with 3 isolates, with 1 carrying the *penA-237.001* allele. Median SNP distances between these 3 clades were 390 (clade 1–3), 424 (clade 1–4), and 522 SNPs (clade 3–4), suggesting 3 separate events of allele acquisition across these distinct genetic backgrounds (Supplementary Table 2 and Supplementary Figure 1).

Clade 1 was primarily composed of *penA-237.001* isolates (n = 65) but also included a notable subclade of 4 *penA-265.001* isolates. Within this subclade, 3 different sequence types were identified (ST1901, ST18077, and ST7822), while 97% of the *penA-237.001* isolates in the clade were assigned to ST1901. The median SNP distance within clade 1 was 17 (IQR, 8–33), suggesting the clade represents a local clonal expansion. Sixty-nine isolates closely clustered with strain F92; 8 differed by only 12 SNPs, indicating a close genetic relationship [10] (Figure 1). Clade 2, the second largest clade, consisted of 12 *penA-60.001* isolates that clustered closely with reference strain WHO R (ST1903). Within this clade, 9 isolates were assigned ST13871, 2 had ST17523, and 1 represented a novel sequence type (Figure 1 and Supplementary Figure 2).

Among the ceftriaxone-resistant isolates, 7 different mosaic *penA* alleles, including 1 novel mosaic *penA* allele, were identified (Supplementary Material 3). Median ceftriaxone MICs were similar between isolates carrying *penA-60.001* (0.44 mg/L; IQR, 0.38–0.5) and *penA-237.001* (0.25 mg/L; IQR, 0.19–0.5) ($P = .073$, Mann-Whitney U test; Supplementary Figure 4). All the mosaic *penA* alleles featured the A311V and N513Y mutations, which are linked to phenotypic extended-spectrum cephalosporin resistance. Additionally, 96.7% (87/90) of the isolates harbored G120 and/or A121 *porB1b* mutations. Eighty-four isolates (93.3%) had a single base-pair deletion in the promoter region of *mtrR*, which negatively regulates the expression of the mtrCDE efflux pump. Four isolates had mutations in the mtrR structural gene itself (3 A39T and 1 G45D), all of which diminish repression and result in increased efflux pump activity. Details of AMR associated determinants are in Supplementary Material 3.

Only 3 patients returned for evaluation after treatment. Two patients did not have symptoms, and additional cultures were not performed. The third patient returned 6 weeks after the initial visit with urethral discharge and a repeat culture grew *N. gonorrhoeae*. Genome sequence reads of the 2 isolates (VN-HA-24-00014 and VN-HA-24-00023) were cross-mapped against each other's de novo assembled contigs, revealing zero SNPs across the 2.2-Mb bacterial chromosome. There were no other isolates in the dataset that were identical to this one (Figure 1*D*).

DISCUSSION

Our report describes the alarmingly high prevalence of *N. gonorrhoeae* isolates with ceftriaxone resistance (27%) in Hanoi,

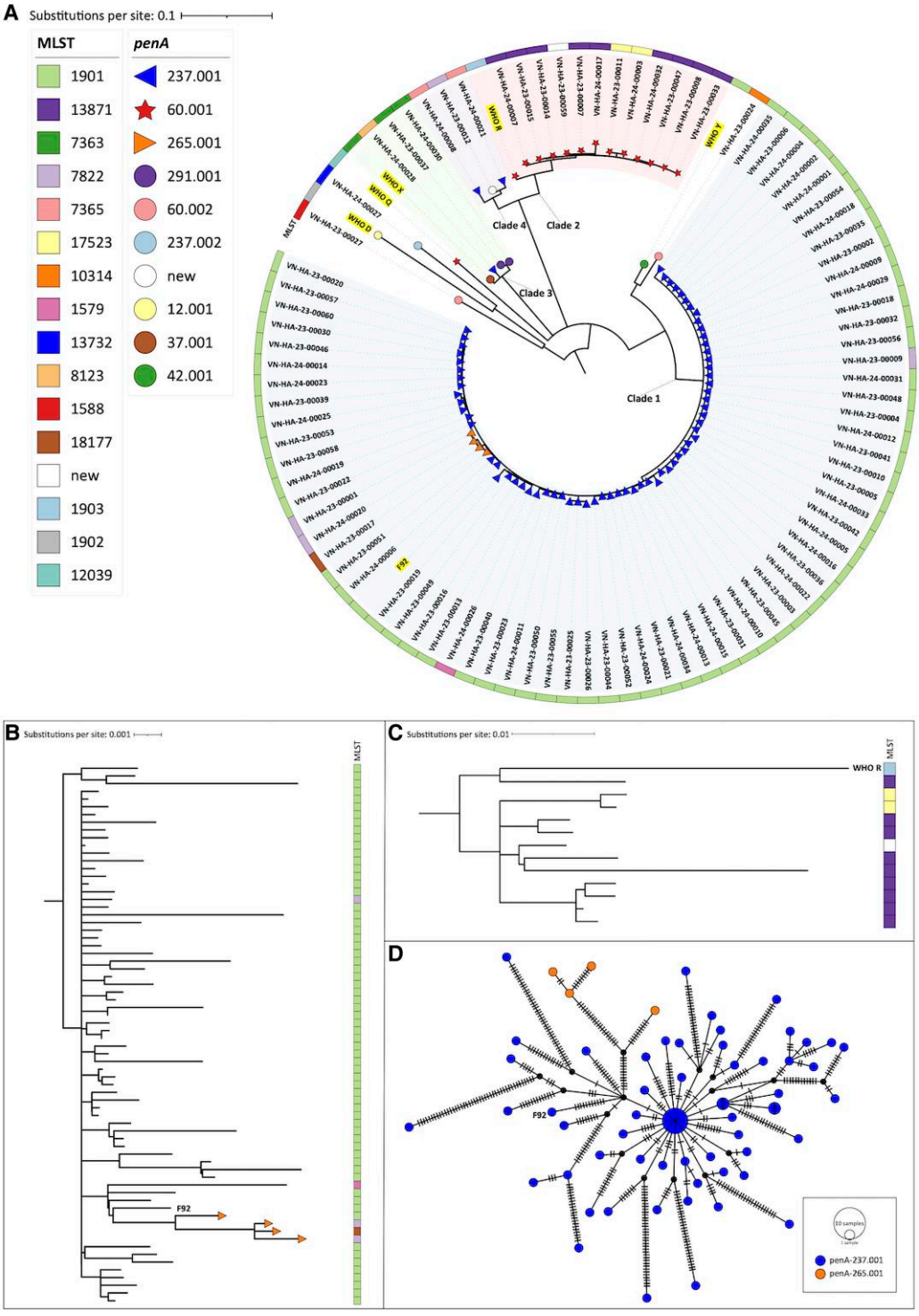

Figure 1. Phylogenetic relatedness of 90 *Neisseria gonorrhoeae* isolates from Hanoi, Vietnam with ceftriaxone alert values (minimum inhibitory concentrations ≥ 0.125 mg/L). *A*, ML tree (midpoint rooted) of 90 isolates collected at Hanoi Medical University Hospital between January 2023 and June 2024. In addition, 6 well-characterized *N. gonorrhoeae* reference isolates were included in this panel: 5 WHO reference strains, WHO D, WHO Q (2018, UK), WHO R (FC428, 2015, Japan), WHO X (H041, 2009, Japan), and WHO Y (F89, 2010, France), and 1 ceftriaxone-resistant strain (F92) linked to Vietnam (2022, France). The ML tree is based on 2088 SNP differences detected across the whole core genome of the isolate panel and was visualized using iTOL version 6.9.1. MLST are annotated on the ring around the ML tree and *penA* alleles are depicted as symbols on the branch leaves. Separate ML phylogenetic trees of isolates belonging to clade 1 (*B*) based on 706 core SNPs and clade 2 (*C*) based on 367 core SNPs are depicted to provide higher granularity. *D*, A median joining tree of clade 1. Each circle represents a unique genome, and the size of the circle is proportional to the number of isolates sharing that genome. Hatch marks on the edges connecting the genomes represent the number of SNPs between genomes. Node color corresponds to *penA* alleles. Abbreviations: ML, maximum likelihood; MLST, multilocus sequence type; SNP, single-nucleotide polymorphism; WHO, World Health Organization.

Vietnam from January 2023 through June 2024. Genomic analyses demonstrated that ceftriaxone resistance was primarily driven by the mosaic *penA-237.001* allele, accounting for 76% of resistant strains, and to a lesser extent by the *penA-60.001* allele. These findings are similar to the 2023 Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) surveillance report, which also found *penA-237.001* to be prevalent in both Hanoi and Ho Chi Minh City, while *penA-60.001* was notably more common in Ho Chi Minh City [11]. While the EGASP-Vietnam report contains separate data, the studies are complementary and reinforce the findings of ceftriaxone resistance. Our report provides more recent data spanning all of 2023 through June 2024 and includes 352 isolates from 1 hospital in Hanoi, whereas the EGASP report included data from sexually transmitted infection hospitals in Ho Chi Minh City (n = 101) and Hanoi (n = 153) in 2023. Nevertheless, the data highlight the geographical variations in resistance and transmission patterns between northern and southern Vietnam, which are approximately 1000 km apart, while also aligning with data from neighboring Cambodia, where *penA-60.001* dominates among resistant isolates [1]. This report expands on our prior work [5], adding 2024 isolates and WGS analysis, to provide a broader, updated view of the emerging ceftriaxone-resistance in Vietnam.

Interestingly, *penA-60.001* isolates from this study were predominantly associated with ST13871, a sequence type that likely evolved from strain FC428 (WHO R). A prior report from Vietnam using isolates from 2019–2020 detected the FC428 subclone with ST13871 in and around Ho Chi Minh City [12]. The persistence of this continuously evolving sequence type in Vietnam years after its initial detection underscores its successful adaptation and ongoing local transmission as part of a globally circulating lineage.

The majority of isolates harbored mosaic *penA-237.001*, which was first reported in 2022 from 2 female patients in France and the United Kingdom, 1 of whom had a partner who recently traveled to Vietnam (strain F92) [8, 10]. Additionally, we identified 4 isolates with *penA-265.001* that were closely related to *penA-237.001* isolates. This allele was previously only reported in the Vietnam EGASP data [11]; in the NG-STAR database, it was linked to a male patient in Norway infected in Asia in May 2023. These instances are the only known reports of *penA-237.001* and *penA-265.001*, suggesting a potential local introduction.

Our findings underscore the threat to ceftriaxone as first-line treatment for gonorrhea in the region, highlighting the need for updated treatment guidelines and new therapeutic agents. The 2024 WHO gonococcal treatment guidelines now recommend 1 g ceftriaxone intramuscularly once as first-line monotherapy, an increase from the 2016 recommendation of 250 mg ceftriaxone combined with azithromycin dual therapy [13]. Our data support the recommendations for using a higher dose of ceftriaxone empirically in settings with a high prevalence of ceftriaxone resistance. However, the recommended 1-g dose of ceftriaxone was primarily determined by pharmacodynamic data. Clinical trials directly comparing 500 mg to 1 g of ceftriaxone in these settings have not been performed, but could help determine the optimal dose needed to treat infections while minimizing side effects and further development of resistance. One limitation of our study is the use of routine microbiology laboratory data, which lacks clinical data, including patient demographics, behaviors, treatments, and follow-up. This includes tests-of-cure, which are not done routinely but could provide important data on possible treatment failures. Our report includes 1 person who returned after treatment and had a positive culture; genomic data of the 2 isolates showed exact genetic similarity, suggesting either reinfection from the same partner or a possible treatment failure. Improved detection and management of treatment failures are needed, as tests-of-cure are not routinely collected in most clinical settings.

Our data also demonstrate the extent to which non–*penA-60.001* alleles contribute to ceftriaxone resistance in Vietnam, highlighting the importance of local surveillance, which can inform local and international epidemiology. Molecular tests to detect mutations associated with ceftriaxone resistance can be important tools for providing timely resistance data, but there are challenges to using allele-specific molecular tests, such as ones detecting *penA-60.001*, as there might be cross-reactivity with non–*penA-60.001* alleles [5, 14]. While WGS is ideal, the high costs remain prohibitive in many low-resource settings. Thus, it is crucial to develop new molecular tests that can detect a broad range of resistance mutations, are allele-agnostic, and are easy to implement in resource-limited settings. These tests are essential for timely identification of resistant strains, guiding effective treatment, and preventing further spread.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. V. N. H., P. H. N., T. M. C., and P. C. A. designed the study. V. N. H., P. H. N., and T. M. C. performed laboratory investigations and data validation. J. G. E. L., K. V., Tam T. N., Trang T. N., and D. T. N. N. carried out the genomic sequencing, data validation, and bioinformatics analyses. J. G. E. L. wrote the first draft of the manuscript. P. C. A. obtained

funding and provided study supervision. All authors critically reviewed and revised the manuscript, and approved the final submitted version.

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References

1. Ouk V, Say HL, Virak M, et al. World Health Organization enhanced gonococcal antimicrobial surveillance programme, Cambodia, 2023. Emerg Infect Dis **2024**; 30: 1493–5.
2. Zhu X, Xi Y, Gong X, Chen S. Ceftriaxone-resistant gonorrhea—China, 2022. MMWR Morb Mortal Wkly Rep **2024**; 73:255–9.
3. Lan PT, Golparian D, Ringlander J, Van Hung L, Van Thuong N, Unemo M. Genomic analysis and antimicrobial resistance of *Neisseria gonorrhoeae* isolates from Vietnam in 2011 and 2015–16. J Antimicrob Chemother **2020**; 75: 1432–8.
4. Adamson PC, Van Le H, Le HHL, Le GM, Nguyen TV, Klausner JD. Trends in antimicrobial resistance in *Neisseria gonorrhoeae* in Hanoi, Vietnam, 2017–2019. BMC Infect Dis **2020**; 20:809.
5. Adamson PC, Hieu VN, Nhung PH, Whiley DM, Chau TM. Ceftriaxone resistance in *Neisseria gonorrhoeae* associated with the *penA-60.001* allele in Hanoi, Viet Nam. Lancet Infect Dis **2024**; 24:e351–2.
6. Lahra MM, Martin I, Demczuk W, et al. Cooperative recognition of internationally disseminated ceftriaxone-resistant *Neisseria gonorrhoeae* strain. Emerg Infect Dis **2018**; 24:735–40.
7. Reimche JL, Pham CD, Joseph SJ, et al. Novel strain of multidrug non-susceptible *Neisseria gonorrhoeae* in the USA. Lancet Infect Dis **2024**; 24:e149–51.
8. Day M, Pitt R, Mody N, et al. Detection of 10 cases of ceftriaxone-resistant *Neisseria gonorrhoeae* in the United Kingdom, December 2021 to June 2022. Euro Surveill **2022**; 27:2200803.
9. Maubaret C, Caméléna F, Mrimèche M, et al. Two cases of extensively drug-resistant (XDR) *Neisseria gonorrhoeae* infection combining ceftriaxone-resistance and high-level azithromycin resistance, France, November 2022 and May 2023. Euro Surveill **2023**; 28:2300456.
10. Berçot B, Caméléna F, Mérimèche M, et al. Ceftriaxone-resistant, multidrug-resistant *Neisseria gonorrhoeae* with a novel mosaic *penA-237.001* gene, France, June 2022. Euro Surveill **2022**; 27:2200899.
11. Lan PT, Nguyen HT, Golparian D, et al. The WHO enhanced gonococcal antimicrobial surveillance programme (EGASP) identifies high levels of ceftriaxone resistance across Vietnam, 2023. Lancet Reg Health West Pac **2024**; 48:101125.
12. Trinh TM, Nguyen TT, Le TV, et al. *Neisseria gonorrhoeae* FC428 subclone, Vietnam, 2019–2020. Emerg Infect Dis **2022**; 28:432–5.
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, Switzerland, 2024. https://www.who.int/publications/i/item/9789240090767. Accessed 19 December 2024.
14. Shimuta K, Igawa G, Yasuda M, Deguchi T, Nakayama SI, Ohnishi M. A real-time PCR assay for detecting a *penA* mutation associated with ceftriaxone resistance in *Neisseria gonorrhoeae*. J Glob Antimicrob Resist **2019**; 19:46–9.