Molecular characterization of a ceftriaxone-resistant *Neisseria gonorrhoeae* strain found in Switzerland

Konrad Egli (✉ konrad.egli@risch.ch)

CLM Dr Risch <https://orcid.org/0000-0003-3029-4428>

Anna Roditscheff

Labormedizinisches Zentrum Dr Risch

Ursula Flückiger

Hirslanden Klinik Aarau

Martin Risch

Labormedizinisches Zentrum Dr Risch

Lorenz Risch

Labormedizinisches Zentrum Dr Risch

Thomas Bodmer

LMZ Dr Risch

Case report

Keywords: *Neisseria gonorrhoeae*, phenotypic resistance, mutations of target genes, ceftriaxone resistance.

Posted Date: December 22nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-131740/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Annals of Clinical Microbiology and Antimicrobials on August 6th, 2021. See the published version at <https://doi.org/10.1186/s12941-021-00456-5>.

Abstract

Background: Resistance of *Neisseria gonorrhoeae* to ceftriaxone is very rare in Switzerland. The underlying genotype is suspected to be novel. Generally, resistance in *Neisseria gonorrhoeae* (Ng) involves a comprehensive set of genes with many different mutations causing resistance to different β -lactams and fluoroquinolones.

Case presentation: A patient had a positive result with specific PCR for Ng. We routinely start culturing for all clinical specimens with a positive result with NG-PCR. In this particular case, we isolated such a strain with resistance to ceftriaxone in Switzerland. A total of 7 different genes in this strain were partially sequenced for comparison with phenotypic susceptibility testing. Interestingly, two different mutations in the *porinB* gene were observed, and data on this gene are limited. Information on the identified allele type of the *penA* gene is very limited as well. Three different mutations of *parC* and *gyrA* that correlate with ciprofloxacin resistance were found.

Conclusion: The combined results for all genes show the appearance of new mutations in central Europe either due to worldwide spread or the emergence of new genetic combinations.

Background

The increased resistance of *Neisseria gonorrhoeae* (Ng) isolates hinders the treatment of Ng infections. Decreased susceptibility of Ng to cefixime and ceftriaxone has been reported in Switzerland; however, very few clinical specimens have an MIC ≥ 0.25 [1], which is above the EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoint of 0.125 mg/l [2]. Ceftriaxone resistance in other European countries is also rare [3][4]. There are few reports on the molecular characterization of different resistance genes/mechanisms [3][5][6][7][8][9] from different continents. Here, we describe the molecular characterization of a strain found in Switzerland with an unusual combination of mutations in relevant genes.

Case Presentation

In December 2019, the patient presented himself with a painful glans penis, showing signs of local inflammation, urethral discharge and inguinal lymphadenopathy in an emergency room of a Swiss hospital. One week prior of symptoms development, he had protected intercourse and unprotected oral sex with a female partner, of whom the health status is unknown. Furthermore, it is not known, if he had a history of sexually transmitted infections, nor if he was treated priorly with antibiotics. Based on his clinical symptoms, *Chlamydia trachomatis* (Ct)-, Human Immunodeficiency Virus (HIV)-, *Treponema pallidum* (Tp)-, and Ng infection were included in the differential diagnosis. An empirical antibiotic treatment was started with 100 mg oral doxycycline two times a day for seven days. Prior to treatment, a urethral swab was sampled to check for Ct and Ng, using the multiplex real time cobas® CT/NG assay on the Cobas 6800 system (Roche Molecular Diagnostics, Pleasanton, CA, USA). We do not know whether

the antibiotic therapy was adjusted, according to current treatment guidelines for Ng infections or a test of cure was performed, since the patient is currently lost for follow-up.

Screening for Ng was performed by PCR. Clinical specimens with a positive PCR were subsequently cultured using “Chocolate agar PolyViteX VCAT3” and “Columbia agar + 5% sheep blood” (both Biomerieux, Switzerland). In this case, a urethral swab was cultured and tested against different antibiotics (see Table 1). Susceptibility testing was performed according EUCAST 2019 [2]. Due to resistance to ceftriaxone and ciprofloxacin, different relevant genes were sequenced (see Table 2).

DNA isolated from a pure culture (with the QIAamp DNA Mini Kit from Qiagen, Switzerland) was amplified by primers based on sequences from two internet sites, NGSAR and PubMLST (see Table 2) and obtained from Microsynth (Switzerland). Sanger sequencing of the purified PCR products was performed at Microsynth (Switzerland) according to their instructions.

Table 1

Reported susceptibility data (MIC) of culture (according to EUCAST 2019) as well as corresponding classes of antibiotics and/or target genes.

Substance	MIC	Reported phenotype	Antimicrobial/target gene*	Remarks
Ceftriaxone	0.25 mg/l	resistant	β -Lactam <i>penA, porinB, ponA, bla</i>	0.38: value from LMZ Dr Risch Group 0.25: value from IMM (UZH) MIC breakpoint: S $\leq$ 0.125 mg/l, R > 0.125 mg/l
Penicillin	0.5 mg/l	intermediate	<i>PorinB</i>	
Ciprofloxacin	12.0 mg/l	resistant	Fluorchinolone <i>gyrA</i> and <i>parC</i>	MIC breakpoint: S $\leq$ 0.03 mg/l, R > 0.06 mg/l
Azithromycin	0.5 mg/l		Makrolide <i>23S rDNA</i>	Azithromycin is always used in conjunction with another effective agent. ECOFF: 1 mg/l
Tetracycline	0.38 mg/l	susceptible	Tetracycline <i>rpsJ, mtrR, penB,</i> <i>TetM</i> -encoding plasmids	MIC breakpoint: S $\leq$ 0.5 mg/l, R > 1.0 mg/l
Ceftriaxone testing was performed at the LMZ Dr Risch group as well as at the Institute of Medical Microbiology (IMM) of the University of Zurich (UZH) for confirmation. ECOFF is the “epidemiological cut-off value”. *More details on target genes are provided in reviews of the literature [10, 11].				

Table 2
Overview of observed sequences based on NGSTAR and PubMLST.

Locus	Contig	Allele -type	Length (bp)	Start position	End position	Comments
penA*	<i>penA</i>	148	607	na	na	<i>penA</i> type 148 NonMosaic; A517G
NG_ponA	<i>ponA</i>	1	75	218	292	Mutation: L421P
NG_porB	<i>porinB</i>	55	30	116	145	Mutations: G120N, A121G
proNEIS1635	<i>mtr</i>	3	66	146	211	Adenosine deletion in promoter
NG_gyrA	<i>gyrA</i>	7	264	121	384	Mutations: S91F, D95A
NG_parC	<i>parC</i>	3	332	1	332	Mutation: S87R
NG_23S	23SrDNA	100	567	33	599	Wild type
*Analysis of penA was performed with Ng Star Allele Query (https://ngstar.canada.ca/alleles/query?lang=en)						

All other sequence queries were performed with PubMLST “Sequence query - Neisseria profile/sequence definitions” using “all loci” (https://pubmlst.org/bigdb?db=pubmlst_neisseria_seqdef&page=sequenceQuery). The sequences were analyzed together, and the results of this analysis are shown in the table. na: No information available.

Discussion And Conclusions

Penicillin and extended-spectrum cephalosporin resistance has been associated with mutations and recombination within the *penA*, *porinB*, and *ponA* genes and the presence of *bla* and involves a complex interaction [12].

Interestingly, resistance to penicillin was intermediate, although there were two different mutations in the *porinB* gene (cephinase testing was negative). Unfortunately, there is no additional information on allele type 55 at NG-STAR (regarding origin, MICs or epidemiological data). Furthermore, the mutations G120N and A121G are available only in combination with other mutations in all other allele types but not as single mutations (based on information from NG-STAR). The combination of G120N and A121G in Switzerland has also not been previously reported in publications with extensive datasets [13].

The *ponA* (penicillin binding-protein 1, PBP1) mutation L421P also seems to be a widespread mutation [5] in Switzerland [13]. According to NG-STAR, this mutation has been observed in Ng with elevated

cephalosporin MICs, although the L421P mutation has not been shown to cause resistance in transformation experiments [7].

NG-STAR does not provide additional information on *penA* allele-type 148 NonMosaic with the A517G mutation (regarding origin, MICs or epidemiological data). The most recent literature only confirms the dependence of A517G on MICs for extended-spectrum cephalosporins (ceftriaxone and cefixime) [12]. For *penA* (penicillin binding-protein 2, PBP2), diverse sequence variations were observed (i.e., 363 entries for allele types in the NG-STAR database).

The deletion of an adenine in the *mtr* promoter region may reduce susceptibility to different drugs and is also widespread [5, 13].

The elevated MIC for ciprofloxacin was due to 2 different mutations of the *gyrA* gene as well as 1 mutation of the *parC* gene. The combination of *gyrA* (S91F, D95A) and *parC* (S87R) mutations was previously observed in locations other than Switzerland [5, 13, 14]. Two strains with the same combination of *gyrA* and *parC* found in Switzerland [13] had a lower MIC (of 1.5 and 3 mg/l) than the strain in this study (Table 1).

Azithromycin is below the ECOFF and is consistent with the wild-type allele. Due to phenotypic susceptibility towards tetracycline, we did not additionally sequence the corresponding genes *tetM* and *rpsJ* [11, 15].

It remains unclear whether this particular strain was imported from abroad or evolved due to the selective pressure of applied antibiotics or patient noncompliance.

The observed combination of mutations is, to the best of our knowledge, at least very unusual (since it has not been mentioned in different recent publications, e.g., [5, 12, 13, 16, 17]) and has not been previously described in Switzerland. Resistance mechanisms remain complex due to the many combination possibilities [12]. Therefore, our findings contribute to a more extensive knowledge of Ng phenotypes and genotypes.

Declarations

Ethics approval and consent to participate

We have written consent of patient for publication of data for case presentation.

Consent for publication

All authors agreed for publication of submitted version.

Availability of data and material

All relevant data are mentioned in the manuscript.

Competing interests

There are no competing interests.

Funding

There are no external fundings.

Authors' contributions (according to CRediT author statement)

Konrad Egli: Conceptualization, Methodology (molecular biology), Resources, Validation, Investigation, Writing - Original Draft, Visualization

Anna Roditscheff: Conceptualization, Methodology (phenotypic susceptibility testing), Resources, Validation, Writing - Review & Editing, Visualization

Ursula Flückiger: Resources (patient informations)

Martin Risch: Funding acquisition

Lorenz Risch: Funding acquisition

Thomas Bodmer: Conceptualization, Project administration

Acknowledgements

The work of the technicians from the microbiology laboratory is gratefully acknowledged.

References

1. Kovari H, de Melo Oliveira MDG, Hauser P, Lächli S, Meyer J, Weber R, et al. Decreased susceptibility of *Neisseria gonorrhoeae* isolates from Switzerland to Cefixime and Ceftriaxone: Antimicrobial susceptibility data from 1990 and 2000 to 2012. *BMC Infect Dis.* 2013;13:1–5.
2. European Committee on Antimicrobial Susceptibility Testing. Testing Breakpoint tables for interpretation of MICs and zone diameters. 2020;0–77.
3. Eyre DW, Town K, Street T, Barker L, Sanderson N, Cole MJ, et al. Detection in the United Kingdom of the *Neisseria gonorrhoeae* FC428 clone, with ceftriaxone resistance and intermediate resistance to azithromycin, october to december 2018. *Eurosurveillance.* 2019;24.
4. Cole MJ, Quinten C, Jacobsson S, Day M, Amato-Gauci AJ, Woodford N, et al. The European gonococcal antimicrobial surveillance programme (Euro-GASP) appropriately reflects the antimicrobial resistance situation for *Neisseria gonorrhoeae* in the European Union/European Economic Area. *BMC Infect Dis. BMC Infectious Diseases;* 2019;19:1–12.

5. Gianecini RA, Golparian D, Zittermann S, Litvik A, Gonzalez S, Oviedo C, et al. Genome-based epidemiology and antimicrobial resistance determinants of *Neisseria gonorrhoeae* isolates with decreased susceptibility and resistance to extended-spectrum cephalosporins in Argentina in 2011-16. *J Antimicrob Chemother.* 2019;74:1551–9.
6. Ferreira WA. Genotyping of the Resistance Determinant of *Neisseria Gonorrhoeae* with Reduced Susceptibility to Ceftriaxone in Manaus-AMBrazil. *SOJ Microbiol Infect Dis.* 2017;5:1–3.
7. W. Demczuk, S. Sidhu, M. Unemo, D. M. Whiley, V. G. Allen, J. R. Dillon, M. Cole, C. Seah, E. Trembizki, D. L. Trees, E. N. Kersh, A. J. Abrams, H. J. C. de Vries, A. P. van Dam, I. Medina, A. Bharat, M. R. Mulvey, G. Van Domselaar IM. *Neisseria gonorrhoeae* Sequence Typing for Antimicrobial Resistance , a Novel Antimicrobial Resistance Multilocus. *J Clin Microbiol.* 2017;55:1454–68.
8. Karunakaran R, Sam IC. Mutations in *gyrA* and *parC* in ciprofloxacin-resistant *Neisseria gonorrhoeae* in Kuala Lumpur, Malaysia [4]. *J Antimicrob Chemother.* 2007;59:803–4.
9. Liao M, Gu WM, Yang Y, Dillon JAR. Analysis of mutations in multiple loci of *Neisseria gonorrhoeae* isolates reveals effects of PIB, PBP2 and MtrR on reduced susceptibility to ceftriaxone. *J Antimicrob Chemother.* 2011;66:1016–23.
10. Rubin DHF, Ross JDC, Grad YH. The frontiers of addressing antibiotic resistance in *Neisseria gonorrhoeae*. Elsevier Inc.; 2020;
11. Unemo M, Shafer M. Antimicrobial Resistance in *Neisseria gonorrhoeae* in the 21st Century : Past , Evolution , and Future. 2014;27:587–613.
12. Demczuk W, Martin I, Sawatzky P, Allen V, Lefebvre B, Hoang L, et al. Equations to predict antimicrobial MICs in *Neisseria gonorrhoeae* using molecular antimicrobial resistance determinants. *Antimicrob Agents Chemother.* 2020;64:1–11.
13. Endimiani A, Guilarte YN, Tinguely R, Hirzberger L, Selvini S, Lupo A, et al. Characterization of *Neisseria gonorrhoeae* isolates detected in Switzerland (1998-2012): Emergence of multidrug-resistant clones less susceptible to cephalosporins. *BMC Infect Dis.* 2014;14.
14. Golparian D, Rose L, Lynam A, Mohamed A, Bercot B, Ohnishi M, et al. Multidrug-resistant *Neisseria gonorrhoeae* isolate, belonging to the internationally spreading japanese FC428 clone, with ceftriaxone resistance and intermediate resistance to azithromycin, Ireland, August 2018. *Eurosurveillance.* 2018;23:1–4.
15. Młynarczyk-bonikowska B, Kujawa M, Malejczyk M, Młynarczyk G, Majewski S. Plasmid-mediated resistance to tetracyclines among *Neisseria gonorrhoeae* strains isolated in Poland between 2012 and 2013. 2016;475–9.
16. Thakur SD, Levett PN, Horsman GB, Dillon JAR. Association of *neisseria gonorrhoeae* genogroups and specific PBP2/MtrR/PorB mutation patterns with susceptibility to penicillin in a susceptible gonococcal population. *J Antimicrob Chemother.* 2018;73:2682–6.
17. Jamaludin N, Gedye K, Collins-Emerson J, Benschop J, Nulsen M. Phenotypic and Genotypic Characterization of *Neisseria gonorrhoeae* Isolates from New Zealand with Reduced Susceptibility to Ceftriaxone. *Microb Drug Resist.* 2019;25:1003–11.