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# *Neisseria gonorrhoeae* Strain with High-Level Resistance to Spectinomycin Due to a Novel Resistance Mechanism (Mutated Ribosomal Protein S5) Verified in Norway

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**Gonorrhea may become untreatable, and new treatment options are essential. Verified resistance to spectinomycin is exceedingly rare. However, we describe a high-level spectinomycin-resistant (MIC, >1,024 μg/ml) *Neisseria gonorrhoeae* strain from Norway with a novel resistance mechanism. The resistance determinant was a deletion of codon 27 (valine) and a K28E alteration in the ribosomal protein 5S. The traditional spectinomycin resistance gene (16S rRNA) was wild type. Despite this exceedingly rare finding, spectinomycin available for treatment of ceftriaxone-resistant urogenital gonorrhea would be very valuable.**

*Neisseria gonorrhoeae* has developed resistance to all antimicrobials previously recommended for first-line empirical treatment of gonorrhea (1–5). In recent years, *in vitro* resistance and treatment failures with the currently recommended extended-spectrum cephalosporins (cefixime and ceftriaxone), the last remaining options for empirical antimicrobial monotherapy, have been verified (6–14). Gonorrhea may become untreatable, particularly in settings where dual antimicrobial therapy is not feasible or affordable (1, 3–5, 8, 11, 15). From a global public health perspective, an effective antimicrobial monotherapy remains crucial. Gentamicin (16–18), solithromycin (19), and ertapenem (20) have been suggested; however, none of these appear to be an effective long-term solution for single antimicrobial therapy of gonorrhea.

Nevertheless, spectinomycin remains an effective option for treatment, with the exception of pharyngeal gonorrhea (4, 21–25). Verified resistance to spectinomycin is exceedingly rare worldwide, e.g., in the U.S. Gonococcal Isolate Surveillance Project (GISP), established in 1986 (http://www.cdc.gov/std/gisp), and the European Gonococcal Antimicrobial Surveillance Programme (EURO-GASP), initiated in 2004 (26, 27), only five spectinomycin-resistant isolates (all between 1988 and 1990) and no isolates, respectively, have been identified. Unfortunately, spectinomycin is not available in many settings worldwide (4, 8, 24, 25, 28), and, in some settings, resistance emerged when it was widely used as a first-line drug in the 1980s (29).

Spectinomycin inhibits protein translation by binding to the bacterial 30S ribosomal subunit; i.e., it interacts directly with 16S rRNA and inhibits the elongation factor G (EF-G)-catalyzed translocation of the peptidyl-tRNA from the A site to the P site during polypeptide elongation (30, 31). The interaction with 16S rRNA is in helix 34, close to the base-paired nucleotides G1064-C1192 (31–33). In bacterial species, spectinomycin resistance has resulted from production of adenyltransferases that inactivate the drug, alterations of specific amino acids in loop 2 of 30S ribosomal protein S5 (encoded by *rpsE*), and mutations in the spectinomycin binding region of helix 34 encompassing the cross-linked positions 1063 to 1066 and 1190 to 1193 (*Escherichia coli* numbering) in 16S rRNA (30, 33–48). In *N. gonorrhoeae*, only a single nucleotide polymorphism (SNP), C1192U transition, in 16S rRNA has been verified to result in high-level spectinomycin resistance (39, 49).

**TABLE 1** Phenotypic and genetic characteristics of a *Neisseria gonorrhoeae* strain with high-level resistance to spectinomycin identified in Norway

| Characteristic[a] | Result[b] |
|---|---|
| MIC (resistance)[c] | |
| SPT | >1,024 (R) |
| CRO | 0.016 (S) |
| CFM | <0.016 (S) |
| AZM | 0.125 (S) |
| CIP | 0.125 (I) |
| Serovar | Byvu |
| NG-MAST | ST918 |
| 16S rRNA gene | WT |
| Ribosomal protein 5S | Deletion of valine (amino acid 25[d]) and an alteration of lysine to glutamic acid (amino acid 26[d]) |

[a] For MIC data, Etest was used and only whole MIC dilutions are presented. NG-MAST, *Neisseria gonorrhoeae* multiantigen sequence typing; SPT, spectinomycin; CRO, ceftriaxone; CFM, cefixime; AZM, azithromycin; CIP, ciprofloxacin.
[b] ST, sequence type; WT, wild type.
[c] Susceptibility (S), intermediate susceptibility (I), and resistance (R) were determined based on the interpretative criteria stated by the Clinical and Laboratory Standards Institute (CLSI) (M100-S22).
[d] *Escherichia coli* (GenBank accession no. AAA58100) numbering that corresponds to amino acids 27 and 28, respectively, in the ribosomal protein 5S of *Neisseria gonorrhoeae*.

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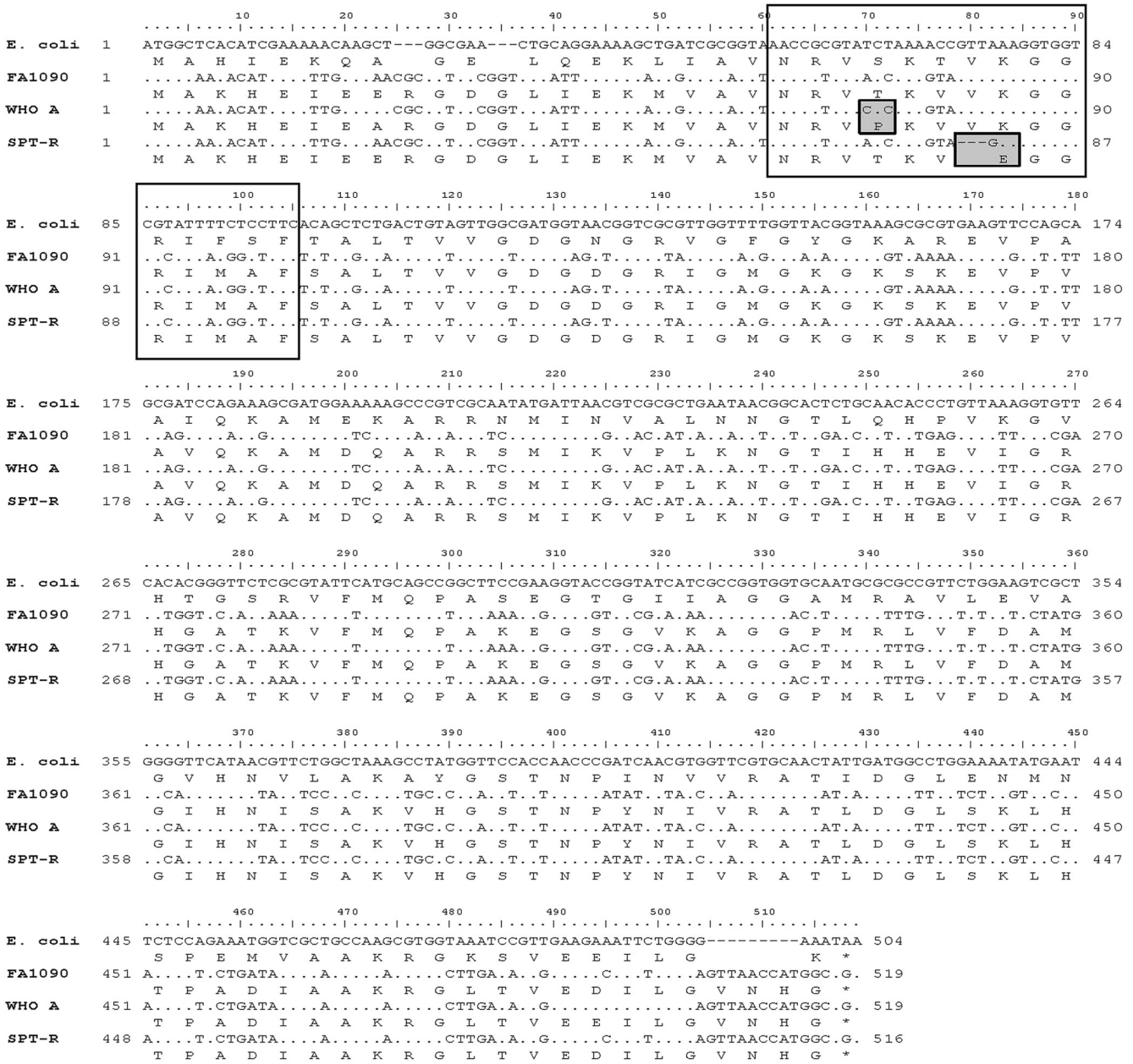


**FIG 1** Nucleotide and amino acid alignment of the *rpsE* genes and the corresponding amino acid sequences of ribosomal protein 5S in *Escherichia coli* (GenBank accession no. AAA58100), the international genome-sequenced *N. gonorrhoeae* reference strain FA1090 (GenBank accession no. AE004969.1), *N. gonorrhoeae* reference strain WHO A (low-level spectinomycin resistance; MIC, 128 μg/ml), and the *N. gonorrhoeae* strain with high-level spectinomycin resistance (MIC, >1,024 μg/ml) identified in Norway (SPT-R). The transparent boxes indicate the amino acids 19 to 33 in the N terminus of the ribosomal protein 5S that form a loop structure (loop 2) which nonspecifically binds to helix 34 of 16S rRNA and is within 50 nm of the spectinomycin-binding site. Amino acid alterations at this loop can disrupt the binding of spectinomycin to the ribosome, which results in spectinomycin resistance (31, 37, 42, 43). The shaded boxes indicate the mutations causing low-level spectinomycin resistance and high-level spectinomycin resistance in WHO A and SPT-R, respectively.

This study describes an exceedingly rare *N. gonorrhoeae* strain with high-level spectinomycin resistance, due to a novel resistance mechanism (mutated ribosomal protein S5), identified in Norway.

The strain (SPT-R) was isolated in 2010 from a 32-year-old Norwegian man who had sex with men (MSM) after unprotected anal intercourse in Oslo, Norway, with an anonymous, untraceable Norwegian man. The patient had proctitis, and *porA* pseudogene PCR (50) and selective culture of rectal specimens were positive for gonococci. The patient was administered ceftriaxone (250 mg once intramuscularly). Five weeks later, the patient returned with resolved symptoms and *porA* pseudogene PCR (50) of a rectal sample was negative.

SPT-R was species confirmed using an oxidase test, microscopy (Gram staining), a sugar utilization test, and a Phadebact GC monoclonal test (Bactus AB, Solna, Sweden). SPT-R showed high-level resistance to spectinomycin (MIC, >1,024 μg/ml), intermediate susceptibility to ciprofloxacin, and susceptibility to cefixime,

ceftriaxone, and azithromycin, and it was assigned to serovar Byvu and *N. gonorrhoeae* multiantigen sequence type (NG-MAST) ST918 (49) (Table 1).

For elucidation of the spectinomycin resistance determinant, the full-length 16S rRNA gene (1,545 bp) was sequenced (51), which surprisingly revealed a wild-type gene. Consequently, the full-length *rpsE* gene (519 bp) was PCR amplified and sequenced with the primers 5S-F (5′-TGGCAAAACATGAAATTGAAG-3′) and 5S-R (5′-GCCATGGTTAACTCCCAAAA-3′), which were designed based on the genome sequence of the spectinomycin-susceptible gonococcal reference strain FA1090 (GenBank accession no. AE004969.1). Compared to *rpsE* genes in FA1090, the eight 2008 WHO gonococcal reference strains (49), and the old *N. gonorrhoeae* reference strain WHO A (a strain with low-level spectinomycin resistance), *rpsE* in SPT-R contained a deletion of nucleotides 79 to 81 (whole codon 27 encoding valine) and an A82G transition resulting in the amino acid alteration K28E (lysine to glutamic acid) in the ribosomal protein S5, which correspond to amino acids 25 and 26, respectively, in *Escherichia coli* (GenBank accession no. AAA58100). A nucleotide blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) did not find these alterations in any *rpsE* from gonococci or other bacterial species. Notable, WHO A contained a T22P amino acid alteration (*E. coli* numbering) compared to the spectinomycin-susceptible reference strains (Fig. 1). A transformation experiment (8, 52), using purified PCR-amplified full-length *rpsE* transformed to WHO M (49), verified that the *rpsE* alleles in SPT-R and WHO A resulted in high-level and low-level spectinomycin resistance, respectively. The spectinomycin MICs of the transformants increased to donor levels for both SPT-R (from 16 μg/ml to 1,024 μg/ml) and WHO A (from 16 μg/ml to 128 μg/ml). Both transformants contained full-length *rpsE* allele from the donor (identical sequence to *rpsE* in SPT-R and WHO A, respectively) and no changes in, e.g., the 16S rRNA gene sequence.

Herein, we describe a high-level spectinomycin-resistant (MIC, >1,024 μg/ml) gonococcal strain from Norway with a novel resistance mechanism (mutated ribosomal protein 5S). High-level MIC-verified spectinomycin resistance in *N. gonorrhoeae* has been exceedingly rare globally (2, 4, 24-27; http://www.cdc.gov/std/gisp). This may reflect its rare use in most settings; however, in some settings it has been relatively frequently used, and it is surprising that international spread of spectinomycin-resistant successful gonococcal clones has never been documented. This may indicate that, at least in many gonococcal clones, the characteristic spectinomycin resistance SNP in 16S rRNA (C1192U), and possibly other resistance determinants, results not only in high-level spectinomycin resistance but also in a decreased biological fitness, limiting the further spread of the resistant clone. A project addressing this issue is in progress. Recently, the first three extensively drug-resistant (XDR) (4) gonococcal strains with high-level ceftriaxone resistance were reported, and all those strains were susceptible to spectinomycin (8, 11, 53). Based on this fact and because spectinomycin resistance is exceedingly rare globally, it would be very valuable to have spectinomycin available worldwide for treatment of ceftriaxone-resistant anogenital gonorrhea and for the rare patients who cannot tolerate cephalosporins (4, 21-23, 25). Spectinomycin might also be appropriate in a dual antimicrobial treatment regimen, effectively treating also pharyngeal gonorrhea and inhibiting resistance development.

This study also verified a novel resistance determinant for gonococcal high-level spectinomycin resistance, i.e., a deletion of amino acid 25 and a K26E amino acid alteration (*E. coli* numbering) in the ribosomal protein 5S (Fig. 1). The amino acids 19 to 33 in the N terminus of the ribosomal protein 5S form a loop structure which nonspecifically binds to helix 34 of 16S rRNA, and this loop is also involved in the binding of spectinomycin to the ribosome and spectinomycin resistance (37). For example, in *E. coli*, mutations at amino acid positions 20 to 22 (31, 37, 38, 42, 43) and a G28D mutation (43) result in spectinomycin resistance. In the present study, a T22P alteration in WHO A was also verified to result in low-level spectinomycin resistance in gonococci. In *Pasteurella multocida*, deletion of the conserved lysine at position 23, which interacts directly with 16S rRNA (31), and deletion of phenylalanine at position 33 accompanied by a Ser32Ile alteration result in spectinomycin resistance (42). Likely, the deletion of valine (amino acid 25) accompanied by the alteration of the conserved lysine at position 26, which is proposed to interact with 16S rRNA (31), to glutamic acid (K26E) in SPT-R (Fig. 1) disrupts the binding to 16S rRNA and spectinomycin that results in high-level resistance to spectinomycin in gonococci.

In conclusion, this study describes an *N. gonorrhoeae* strain with verified high-level resistance to spectinomycin (MIC, >1,024 μg/ml) due to a novel spectinomycin resistance mechanism (mutated ribosomal protein 5S). Nevertheless, resistance to spectinomycin is exceedingly rare globally, spectinomycin is an effective alternative for treatment of urogenital gonorrhea, and spectinomycin should be available worldwide, in particular for emergent cases of multidrug resistance, including clinical resistance to cefixime and ceftriaxone.

**Nucleotide sequence accession number.** The novel *N. gonorrhoeae rpsE* allele has been assigned the GenBank/EMBL/DDBJ accession number KC311362.

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