**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 S5. The traditional spectinomycin resistance gene (16S rRNA) was wild type. Despite this exceedingly rare finding, spectinomycin available for treatment of cephalosporin-resistant urogenital gonorrhea would be very valuable.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Resulta,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (resistance)c</td>
<td>&gt;1,024 (R)</td>
</tr>
<tr>
<td>SPT</td>
<td>0.016 (S)</td>
</tr>
<tr>
<td>CFM</td>
<td>&lt;0.016 (S)</td>
</tr>
<tr>
<td>AZM</td>
<td>0.125 (S)</td>
</tr>
<tr>
<td>CIP</td>
<td>0.125 (I)</td>
</tr>
<tr>
<td>Serovar</td>
<td>Byvu</td>
</tr>
<tr>
<td>NG-MAST</td>
<td>ST918</td>
</tr>
<tr>
<td>16S rRNA gene</td>
<td>WT</td>
</tr>
<tr>
<td>Ribosomal protein 5S</td>
<td>Deletion of valine (amino acid 25d) and an alteration of lysine to glutamic acid (amino acid 26d)</td>
</tr>
</tbody>
</table>

For MIC data, Etest was used and only whole MIC dilutions are presented. NG-MAST, Neisseria gonorrhoeae multiantigen sequence typing; SPT, spectinomycin; CFM, ceftriaxone; AZM, azithromycin; CIP, ciprofloxacin.

a ST, sequence type; WT, wild type.

b 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).

c Escherichia coli (GenBank accession no. AAC58100) numbering that corresponds to amino acids 27 and 28, respectively, in the ribosomal protein S5 of *Neisseria gonorrhoeae*.

d 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).

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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, \(1,024/1,024\) g/ml), intermediate susceptibility to ciprofloxacin, and susceptibility to cefixime,

![FIG 1](http://aac.asm.org/)

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treating also pharyngeal gonorrhea and inhibiting resistance de-
appropriately in a dual antimicrobial treatment regimen, effectively
anogenital gonorrhea and for the rare patients who cannot toler-
and sequenced with the primers 5S-F (5′-TGGCAAAAACATGA
AATTGAAG-3′) and 5S-R (5′-GCCATGTTAACCCTCCAAA-
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-am-
plified 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 spectinom-
cyin 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 S5). High-
level MIC-verified spectinomycin resistance in N. gonor-
rhoeae 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,
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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 S5 (Fig. 1). The amino acids 19 to 33
in the N terminus of the ribosomal protein S5 form a loop struc-
ture which nonspecifically binds to helix 34 of 16S rRNA, and this
loop is also involved in the binding of spectinomycin to the ribo-
some 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 Pas-
teurella multocida, deletion of the conserved lysine at position 23,
which interacts directly with 16S rRNA (31), and deletion ofphe-
nylalanine 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 con-
served 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 (mu-
tated ribosomal protein S5). Nevertheless, resistance to spectino-
mycin is exceedingly rare globally, spectinomycin is an effective
alternative for treatment of urogenital gonorrhea, and spectino-
mycin 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. gonor-
rhoeae rpsE allele has been assigned the GenBank/EMBL/DDBJ
accession number KC311362.

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