

Acknowledgements

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Transparency declaration

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Molecular characterization of resistance to rifampicin in clinical isolates of *Neisseria meningitidis*

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Abstract

Among 3904 meningococcal isolates collected between October 2002 and June 2007 by the French Meningococcal Reference Centre, eight (0.20%) were resistant to rifampicin (Rif-R; MIC >1 mg/L) and 27 (0.69%) were intermediate-resistant to rifampicin (Rif-I; MICs between 0.38 mg/L and 1 mg/L) according to the E-test method. The MICs determined by agar dilution were lower, eliminating the E-test intermediate category. All Rif-R isolates had mutations in the *rpoB* gene, resulting in substitutions at or near amino acid position 552, which were absent in non-resistant isolates. These data suggest that a rifampicin clinical breakpoint of 1.0 mg/L should be adopted for *Neisseria meningitidis*.

Keywords: Meningococcus, *mtrR*, resistance mechanisms, rifampicin, *rpoB*

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Rifampicin is one of the antibiotics of choice for chemoprophylaxis of meningococcal disease, but its use may be hindered by contraindications or by the emergence of resistant strains [1–3]. However, the characterization of rifampicin resistance in *Neisseria meningitidis* remains problematic because there is no consensus concerning the breakpoints. We aimed to characterize meningococcal isolates with different rifampicin MIC values in order to better define and characterize meningococcal resistance to this antibiotic.

For the present study, we considered all isolates ($n = 3904$) received by the French Meningococcal Reference Centre between 15 October 2002 and 30 June 2007, including 2812 invasive and 1092 non-invasive isolates. We selected all isolates ($n = 35$) with rifampicin MICs >0.25 mg/L as determined by the E-test method (AB Biodisk, Solna, Sweden), which is the breakpoint value for this antibiotic according to the Committee of the Antibiogram of the French Society for Microbiology (<http://www.sfm.asso.fr>). The isolates were further divided into two groups: (i) MICs >1 mg/L, highly resistant to rifampicin (Rif-R; $n = 8$) and (ii) MICs between 0.38 mg/L and 1 mg/L, intermediate-resistant (Rif-I; $n = 27$). All Rif-R isolates and six Rif-I were invasive. The Rif-R isolates seem to be very rare because they account for only 0.28% of all invasive meningococci collected during the study. Nineteen rifampicin-susceptible (Rif-S; MIC ≤ 0.25 mg/L) invasive isolates were randomly chosen as controls. Serotyping and characterization by multilocus sequence typing (MLST) and *porA* typing revealed a heterogeneity of the selected isolates [4–6] (<http://pubmlst.org>, <http://neisseria.org>) (Table 1).

High levels of resistance to rifampicin (MIC >32 mg/L) correlated with point mutations in the *rpoB* gene encoding the β subunit of RNA polymerase [2,3,7,8]. Therefore, a fragment of the *rpoB* gene (encoding amino acids 435–644) was amplified using primers as previously described [7]. All Rif-R isolates (MIC >32 mg/L) belonged to serogroups B or C and several sequence types. They showed mutations, absent in non-resistant isolates, resulting in the substitutions at or near position 552, which are known to confer resistance to rifampicin (Table 1) [2,3,7,8]. The mutation H552R was detected for the first time in a clinical meningococcal isolate. Previously, this has been reported

only in *N. meningitidis* and *Escherichia coli* mutants generated *in vitro* and in clinical *Mycobacterium tuberculosis* isolates [3,9,10].

Most of the Rif-R isolates (six of eight) in the present study were detected after a previous round of chemoprophylaxis with rifampicin, where the corresponding index isolates were Rif-S according to phenotypic methods and/or *rpoB* sequencing. It is noteworthy that four different mutations were detected, suggesting independent events occurring in the four Rif-R isolates belonging to the same genetic lineage (the clonal complex ST-11).

One Rif-I isolate had mutation G560S, which did not confer decreased susceptibility/resistance to rifampicin upon transformation of a susceptible isolate with the appropriate PCR products; however, a mutation in the corresponding position has been reported previously in a rifampicin-resistant strain of *E. coli* [10]. The other *rpoB* alterations that were detected in Rif-S and Rif-I isolates may correspond to polymorphic sites that characterize genes in *N. meningitidis* [11].

Other mechanisms may be involved in resistance to rifampicin [2,12,13]. In *Neisseria gonorrhoeae*, resistance to diverse hydrophobic agents (including Triton X, rifampicin and erythromycin) is associated with mutations in the *mtrR* gene or its promoter region, encoding a transcriptional repressor (MtrR) of the efflux pump genes for MtrCDE, found in both pathogenic *Neisseria* spp. [12,14,15]. Agar dilution was used according to the guidelines of the CLSI, in addition to the E-test method, to test susceptibility to rifampicin (Sigma-Aldrich Chemie), rifampicin with 0.05% Tween 80 (Merck), Triton X (Merck), and erythromycin (Sigma-Aldrich Chemie) [16]. All MICs of rifampicin for the Rif-I isolates, as determined by the E-test method, were lower, ranging from <0.0037 to 0.25 mg/L by the agar dilution method.

MICs of rifampicin combined with 0.05% Tween 80 (added to determine whether low permeability of the cell membrane could be responsible for the higher MICs of rifampicin) were generally the same as, or up to three dilutions lower than, MICs of rifampicin [2]. There were no significant differences in the MICs of Triton X and erythromycin among the Rif-R, Rif-I and Rif-S isolates (data not shown).

Next, a segment of the *mtrR* gene with its promoter region was amplified using primers *mtrF* (5'-gtttccagctcagcgttgtaTGCGGCTCGCCGCTTGTCCTG-3') and *mtrR* (5'-ttgtgagcggataacaatttcGCTTGCGGGCAATGGCGATAACGG-3') with the universal adaptors (in lower case) added for sequencing. Analysis of the sequence alignment suggested no correlation between resistance to rifampicin and alterations in the *mtrR* gene and its promoter in meningococcal

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