Acknowledgements

We thank B. Dragsten for performing the PFGE.

Transparency declaration

B. Söderquist has served as a consultant to Pfizer AB, Sweden.

References

1. Berglund C, Mölling P, Sjöberg L, Söderquist B. Predominance of staphylococcal cassette chromosome *mec* (SCC*mec*) type IV among methicillin-resistant *Staphylococcus aureus* (MRSA) in a Swedish county and presence of unknown SCC*mec* types with Panton–Valentine leukocidin genes. *Clin Microbiol Infect* 2005; 11: 447–456.
2. Berglund C, Söderquist B. The origin of a methicillin-resistant *Staphylococcus aureus* (MRSA) at a neonatal ward in Sweden—possible horizontal transfer of a staphylococcal cassette chromosome *mec* between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*. *Clin Microbiol Infect* 2008; 14: 1048–1056.
3. Okuma K, Iwakawa K, Turnidge JD *et al.* Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002; 40: 4289–4294.
4. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol* 1991; 29: 2240–2244.
5. Hussain Z, Stoakes L, Massey V *et al.* Correlation of oxacillin MIC with *mecA* gene carriage in coagulase-negative staphylococci. *J Clin Microbiol* 2000; 38: 752–754.
6. Swenson JM, Tenover FC. Cefoxitin disk study group. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. *J Clin Microbiol* 2005; 43: 3818–3823.
7. Souza Antunes AL, Secchi C, Reiter KC, Rodrigues Perez LR, Peixoto de Freitas AL, Alves d'Azevedo P. Evaluation of oxacillin and cefoxitin disks for detection of resistance in coagulase negative staphylococci. *Mem Inst Oswaldo Cruz* 2007; 102: 719–723.
8. Higashide M, Kuroda M, Ohkawa S, Ohta T. Evaluation of a cefoxitin disk diffusion test for the detection of *mecA*-positive methicillin-resistant *Staphylococcus saprophyticus*. *Int J Antimicrob Agents* 2006; 27: 500–504.
9. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS project. *J Antimicrob Chemother* 2003; 51: 69–76.
10. Widerström M, Wiström J, Ferry S, Karlsson C, Monsen T. Molecular epidemiology of *Staphylococcus saprophyticus* isolated from women with uncomplicated community-acquired urinary tract infection. *J Clin Microbiol* 2007; 45: 1561–1564.
11. Wisplinghoff H, Rosato AE, Enright MC, Noto M, Craig W, Archer GL. Related clones containing SCC*mec* type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob Agents Chemother* 2003; 47: 3574–3579.
12. Miragaia M, Couto I, de Lencastre H. Genetic diversity among methicillin-resistant *Staphylococcus epidermidis* (MRSE). *Microb Drug Resist* 2005; 11: 83–93.
13. Mombach Pinheiro Machado AB, Reiter KC, Paiva RM, Barth AL. Distribution of staphylococcal cassette chromosome *mec* (SCC*mec*) types I, II, III and IV in coagulase-negative staphylococci from patients attending a tertiary hospital in southern Brazil. *J Med Microbiol* 2007; 56: 1328–1333.
14. Hanssen AM, Kjeldsen G, Sollid JU. Local variants of staphylococcal cassette chromosome *mec* in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci: evidence of horizontal gene transfer? *Antimicrob Agents Chemother* 2004; 48: 285–296.
15. Higashide M, Kuroda M, Omura CT *et al.* Methicillin-resistant *Staphylococcus saprophyticus* carrying staphylococcal cassette chromosome *mec* (SCC*mec*) have emerged in urogenital tract infections. *Antimicrob Agents Chemother* 2008; 52: 2061–2068.

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

A. Skoczynska[1,2], C. Ruckly[1], E. Hong[1] and M-K. Taha[1]

1) *Neisseria Unit, Institute Pasteur, Paris, France and* 2) *National Reference Centre for Bacterial Meningitis, National Medicines Institute, Warsaw, Poland*

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*

Original Submission: 17 March 2008; **Revised Submission:** 27 August 2008; **Accepted:** 2 September 2008
Editor: A. Sundsfjord
Article published online: 18 May 2009

Clin Microbiol Infect 2009; **15:** 1178–1181
10.1111/j.1469-0691.2009.02783.x

Corresponding author and reprint requests: A. Skoczynska, National Reference Centre for Bacterial Meningitis, National Medicines Institute, Chelmska 30/34, 00-725 Warsaw, Poland
E-mail: skoczek@cls.edu.pl

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′-gttttcccagtcacgacgttgtaTGCGGCTCGCCGCCTTGTCCTG-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

TABLE 1. Characteristics of and results for *Neisseria meningitidis* isolates used in the present study

Isolate	Year	Source	Phenotype	ST	CC	Rif E-test	Rif AD	Rif+ T80	RpoB	MtrR
20427	2003	RT	NG:15:P1.6	1136	NA	0.38	0.25	0.12	–[a]	Y48H
20500	2003	RT	NG:15:P1.6	1136	NA	0.38	0.12	0.06	–	wt
20605	2003	Blood	C:2a:P1.5,2	11	11	0.38	0.12	0.06	–	Δ79-81
20642	2003	RT	C:NT:NST	1031	334	0.38	0.25	0.25	–	Stop 54
20704	2003	RT	NG:1:P1.12	6119	NA	0.38	0.25	0.12	–	No PCR product
20782	2003	RT	NG:15:P1.6	1136	NA	0.38	0.25	0.12	–	wt
20796	2003	Blood	B:NT:P1.9	571	41/44	0.5	0.0075	≤0.0037	–	A77T
20938	2003	RT	NG:NT:P1.7	53	53	0.38	0.12	0.12	–	A77T
21221	2003	Urethra	NG:15:P1.6	1136	NA	0.38	0.06	0.06	–	M1L
21696	2004	Rectum	NG:15:P1.6	1136	NA	0.38	0.12	0.06	–	wt
21788	2004	RT	NG:15:P1.6	6095	NA	0.38	0.06	0.03	–	wt
21814	2004	RT	NG:15:P1.6	1136	NA	0.38	0.12	0.12	–	wt
22092	2004	RT	X:15:P1.6	1136	NA	0.38	0.06	0.03	–	wt
22107	2004	RT	X:15:NST	1136	NA	0.5	0.25	0.12	–	Stop 114
22137	2004	RT	W135:NT:NST	184	22	1	0.25	0.12	–	Stop 85
22167	2005	CSF	B:NT:P1.4	41	41/44	0.5	0.25	0.25	G560S	wt
22179	2005	RT	NG:15:P1.6	1136	NA	0.38	0.25	0.12	–	wt
22290	2005	RT	NG:15:P1.6	198	198	0.5	0.12	0.12	–	A77T
22399	2005	RT	NG:15:P1.6	1136	NA	0.5	0.25	0.12	–	wt
22578	2005	RT	NG:15:P1.6	1136	NA	0.38	0.06	0.03	–	wt
22676	2005	RT	NG:NT:NST	571	41/44	0.38	0.12	0.12	–	A77T
22703	2005	RT	NG:15:P1.7	2154	NA	0.38	0.12	0.12	–	No PCR product
22740	2005	Rectum	NG:NT:P1.6	6120	NA	0.5	0.12	0.12	–	A29V
22750	2005	CSF	C:2a:P1.5	11	11	0.38	0.015	0.015	–	wt
22751	2005	Blood	C:2a:P1.5	11	11	0.38	0.015	0.015	–	wt
23616	2006	Blood	A:4:NST	7	5	0.38	0.12	0.12	–	wt[b]
23859	2007	RT	NG:15:P1.6	6096	NA	0.5	0.12	0.12	–	Stop 106
20957	2003	CSF	C:2a:P1.5	11	11	>32	512	256	S557F	wt
22093	2004	Blood	B:NT:NST	749	32	>32	64	64	H552N	F51C
22330	2005	CSF	B:14:P1.7,16	32	32	>32	1024	256	H552Y	wt
22342	2005	CSF	B:14:P1.7,16	32	32	>32	1024	256	H552Y	wt
22637	2005	Blood	C:2a:P1.5,2	11	11	>32	>1024	256	H552R	wt
23001	2005	CSF	B:NT:P1.4	6005	41/44	>32	512	256	H552Y	wt
23269	2006	Blood	C:NT:P1.5,2	11	11	>32	256	32	H552Y	G45D
24089	2007	Blood	C:2a:P1.1,7	6048	11	>32	256	256	S548F	wt
21335	2004	Blood	B:4:P1.10	6094	41/44	0.012	≤0.0037	0.0075	–	wt
23026	2006	Blood	C:NT:P1.5,2	11	11	0.008	≤0.0037	≤0.0037	–	wt
23036	2006	Blood	E29:NT:P1.2	60	60	0.008	≤0.0037	0.0075	–	wt
23047	2006	CSF	B:4:P1.10	32	32	0.006	≤0.0037	≤0.0037	–	wt
23051	2006	CSF	B:1:NST	1946	461	0.032	≤0.0037	0.03	–	A29T
23100	2006	Blood	B:1:NST	207	41/44	0.008	≤0.0037	0.0075	–	L47R
23135	2006	CSF	A:4:P1.9	4789	5	0.064	0.03	0.06	–	A77T
23142	2006	CSF	Y:14:NST	23	23	0.003	≤0.0037	≤0.0037	–	wt
23198	2006	Blood	W135:NT:NST	22	22	0.008	≤0.0037	0.0075	–	wt
23248	2006	Blood	B:2b:NST	8	8	0.008	≤0.0037	≤0.0037	–	E78K, G83E, A86T
23280	2006	blood	B:14:P1.7,16	32	32	0.008	0.015	0.06	–	wt
23318	2006	CSF	B:15:NST	6359	41/44	0.012	≤0.0037	0.0075	–	wt
23324	2006	Blood	C:2a:P1.5,2	1026	11	0.008	≤0.0037	0.0075	–	wt
23343	2006	CSF	C:2b:P1.5,2	8	8	0.023	0.015	0.06	–	E78K, G83E, A86T
23352	2006	Blood	B:14:NST	492	269	0.008	≤0.0037	0.0075	–	wt
23390	2006	CSF	B:NT:P1.9	6122	NA	0.032	≤0.0037	0.0075	–	E78K, G83E, A86T
23396	2006	CSF	B:NT:P1.13	6121	NA	0.012	≤0.0037	0.0075	–	wt
23441	2006	Blood	NG:15:P1.6	823	198	0.032	0.0075	0.015	–	A77T
23575	2006	CSF	B:1:P1.6	6360	461	0.125	0.06	0.06	–	A29T

RT, respiratory tract; CSF, cerebrospinal fluid; NG, non-groupable isolate; NT, non-typeable isolate; NST, non-subtypeable isolate; ST, sequence type; CC, clonal complex; NA, not assigned to a known clonal complex; Rif, rifampicin MIC (mg/L); AD, agar dilution; Rif+T80, rifampicin MIC in the presence of Tween80 (mg/L).
[a]No mutations around the position 552; wt, wild-type sequence found as the most common sequence present among Rif-susceptible isolates.
[b]Isolate with G to A transition within the *mtr*R promoter region.

isolates, as had been previously suggested [12,14,15]. However, further studies are required to explore other molecular mechanisms of control of the *mtrCDE*-encoded efflux pump.

The rifampicin breakpoints established by the EUCAST and the CLSI differ (http://www.srga.org/eucastwt/MICTAB/MICmiscellaneous.html) [16]. In addition, a wide range of breakpoints, in the range 0.25–5 mg/L, is used by laboratories that are members of the European Monitoring Group of Meningococci [17]; see also M. K. Taha (unpublished data). During a recent multicentre study, rifampicin was one of the antibiotics for which agreement between the E-test and the agar dilution method, and among laboratories, was the weakest [18]. The data obtained in the present study suggest that the Rif-I and Rif-S isolates, differentiated by the E-test method, may represent a single group. Consequently, we suggest the clinical breakpoint of 1.0 mg/L for rifampicin in *N. meningitidis* for both the agar dilution and E-test method [16]. This MIC is lower than the concentration of 5.3 mg/L reached by rifampicin in epithelial lining fluid 2–5 h after a single dose of 600 mg [19]. The proposed value differs from the EUCAST clinical breakpoint

(0.25 mg/L), which appears to be too low for the E-test method routinely employed in many laboratories. It is necessary to emphasize that the efficacy of eradication is not 100%, and that chemoprophylaxis failures were reported even for fully susceptible isolates [20].

The suggested breakpoint should now be challenged using a large collection of varied meningococcal isolates from different countries spanning many years.

Acknowledgements

We thank J.-M. Alonso for generous support for this project and S. Murchan for editing the English language. Some data included in the paper were presented as an oral presentation at the 9th European Monitoring Group on Meningococci (EMGM) Meeting, Rome, 30 May to 1 June 2007.

Transparency Declaration

A. Skoczynska was supported by a Marie Curie Intra-European Fellowship (No. 23188) within the Sixth European Community Framework Programme. All authors: no conflict of interest.

References

1. Yagupsky P, Ashkenazi S, Block C. Rifampicin-resistant meningococci causing invasive disease and failure of chemoprophylaxis. *Lancet* 1993; 341: 1152–1153.
2. Abadi FJ, Carter PE, Cash P, Pennington TH. Rifampin resistance in *Neisseria meningitidis* due to alterations in membrane permeability. *Antimicrob Agents Chemother* 1996; 40: 646–651.
3. Carter PE, Abadi FJ, Yakubu DE, Pennington TH. Molecular characterization of rifampin-resistant *Neisseria meningitidis*. *Antimicrob Agents Chemother* 1994; 38: 1256–1261.
4. Abdillahi H, Poolman JT. *Neisseria meningitidis* group B serosubtyping using monoclonal antibodies in whole-cell ELISA. *Microb Pathog* 1988; 4: 27–32.
5. Fox AJ, Taha MK, Vogel U. Standardized nonculture techniques recommended for European reference laboratories. *FEMS Microbiol Rev* 2007; 31: 84–88.
6. Maiden MC, Bygraves JA, Feil E *et al.* Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA* 1998; 95: 3140–3145.
7. Taha MK, Zarantonelli ML, Ruckly C, Giorgini D, Alonso JM. Rifampin-resistant *Neisseria meningitidis*. *Emerg Infect Dis* 2006; 12: 859–860.
8. Nolte O, Muller M, Reitz S, Ledig S, Ehrhard I, Sonntag HG. Description of new mutations in the *rpoB* gene in rifampicin-resistant *Neisseria meningitidis* selected in vitro in a stepwise manner. *J Med Microbiol* 2003; 52: 1077–1081.
9. Telenti A, Imboden P, Marchesi F *et al.* Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* 1993; 341: 647–650.
10. Severinov K, Soushko M, Goldfarb A, Nikiforov V. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the beta subunit of Escherichia coli RNA polymerase. *J Biol Chem* 1993; 268: 14820–14825.
11. Maiden MC. Population genetics of a transformable bacterium: the influence of horizontal genetic exchange on the biology of *Neisseria meningitidis*. *FEMS Microbiol Lett* 1993; 112: 243–250.
12. Pan W, Spratt BG. Regulation of the permeability of the gonococcal cell envelope by the mtr system. *Mol Microbiol* 1994; 11: 769–775.
13. Hui J, Gordon N, Kajioka R. Permeability barrier to rifampin in mycobacteria. *Antimicrob Agents Chemother* 1977; 11: 773–779.
14. Hagman KE, Pan W, Spratt BG, Balthazar JT, Judd RC, Shafer WM. Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the mtrRCDE efflux system. *Microbiology* 1995; 141: 611–622.
15. Rouquette-Loughlin CE, Balthazar JT, Hill SA, Shafer WM. Modulation of the mtrCDE-encoded efflux pump gene complex of *Neisseria meningitidis* due to a Correia element insertion sequence. *Mol Microbiol* 2004; 54: 731–741.
16. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement*. CLSI document M100-S15. Wayne, PA: CLSI, 2005.
17. Block C. Antibiotic susceptibility testing. In: Pollard AJ, Maiden MCJ, eds, *Meningococcal disease. Methods and protocols*. Totowa, NJ: Humana Press, Inc., 2001; 89–106.
18. Vazquez JA, Arreaza L, Block C *et al.* Interlaboratory comparison of agar dilution and Etest methods for determining the MICs of antibiotics used in management of *Neisseria meningitidis* infections. *Antimicrob Agents Chemother* 2003; 47: 3430–3434.
19. Ziglam HM, Baldwin DR, Daniels I, Andrew JM, Finch RG. Rifampicin concentrations in bronchial mucosa, epithelial lining fluid, alveolar macrophages and serum following a single 600 mg oral dose in patients undergoing fibre-optic bronchoscopy. *J Antimicrob Chemother* 2002; 50: 1011–1015.
20. Tsakris A, Trakatelli C, Souliou E, Sofianou D, Ntoutsou K, Antoniadis A. Failures of rifampicin and ciprofloxacin to eradicate a susceptible meningococcal isolate from a close contact of a fatal case. *Infection* 2001; 29: 293–294.