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Serogroup A *Neisseria meningitidis* with Reduced Susceptibility to Ciprofloxacin

To the Editor: Reduced susceptibility to ciprofloxacin of *Neisseria meningitidis* has been reported with increasing frequency since 1992, mainly because of mutations in the quinolone resistance determining regions (QRDRs) of the gyrase and topoisomerase IV genes (*I*,*2*). Reduced fluoroquinolone susceptibility due to gyrase A mutations in serogroup A strains has previously been reported from a 2005 outbreak in Delhi, India (*I*). We describe 2 clinical isolates of serogroup A *N. meningitidis* with reduced ciprofloxacin susceptibility that were recognized in March 2003 and April 2006 in Israel, a country with low incidence of invasive meningo-

coccal disease (<2/100,000/laboratory-confirmed cases/year) in which this serogroup accounts for <2% of cases (data from the National Center for Meningococci, Tel Hashomer, Israel).

The 2 isolates in question (M12/03 and M24/06; suffixes denote year of isolation) were compared with 2 fully susceptible strains, M44/01 and M23/00 (online Appendix Table, available from www.cdc.gov/EID/content/14/10/1667-appT.htm). MICs were measured by Etest (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) supplemented with 5% sheep blood. Demographic information was obtained from the Israel Ministry of Health Department of Epidemiology.

Chromosomal DNA was isolated by using the NucleoBond kit (Macherey-Nagel, Düren, Germany). The location of the QRDR in gyrase and topoisomerase IV genes was based upon prior studies in meningococci (online Appendix Table) and on the complete sequence of strain *N. meningitidis* Z2491 (serogroup A; GenBank accession no. NC_003116). We amplified and sequenced extended regions encompassing the QRDRs by using the upstream and downstream primer pairs in *gyrA* (522 bases) 5'-GTTCCGCGTCAAATATGCT-3', 5'-CCGAAATTGACGGTTTCTTC-3'; *gyrB* (649 bases) 5'-GGTTTGACC TGCGTGTGTC-3', 5'-CGGCTGG GCGATATAGATG-3'; *parC* (635 bases) 5'-CACTATGGTTTGCCGT TTTG-3', 5'-ATTTCCGACAACAG CAATTC-3'; and *parE* (610 bases) 5'-GGACAGGATGGCGATTTTG-3', 5'-CGTCAGCAACTTCATCAACC-3'. PCR was performed by using *Taq* DNA polymerase (New England BioLabs, Beverly, MA, USA). DNA sequencing was performed using the ABI PRISM 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Screening for plasmid-mediated quinolone resistance genes was carried out by multiplex PCR amplification of *qnrA*, *qnrB*, and *qnrS* as

previously described (3). Multilocus sequence typing (MLST) was carried out by using the primers, protocols, and databases available from the Neisseria MLST website (<http://pubmlst.org/neisseria>) (4).

The online appendix Table shows our results and condenses previously published findings. The ciprofloxacin MIC for M24/06 was 42- to 125-fold higher than for susceptible strains and consistently 2-fold higher than that for M12/03. We have not referred to our isolates as resistant, because M12/03 would be categorized as "intermediate" by Clinical Laboratory Standards Institute breakpoints (5). The extended QRDRs in *gyrA* and *parC* of M44/01 (susceptible) were identical to those of *N. meningitidis* Z2491. M24/06 and M12/03 had a Thr91Ile mutation in *gyrA*. M24/06 also had Asn103Asp, Ile111Val, and Val120Ile mutations in *gyrA* (online Appendix Table; 1). In M12/03, an Ala78Val mutation was found in *gyrA*, and new mutations Ile474Leu and Thr365Ala were found in *gyrB* and *parE*, respectively. No *parC* mutations were found.

Previous reports identified chromosomal mutations in *N. meningitidis* (online Appendix Table). M24/06 and M12/03 possess the same Thr91Ile mutation in *gyrA* as a 2002 serogroup B isolate from Spain (online Appendix Table) that had a similar increase in ciprofloxacin MIC (0.12 mg/L). The Thr91Ile mutation is homologous with the Ser83Leu mutation in *gyrA* of *Escherichia coli* that is responsible for a 60-fold increase in ciprofloxacin MICs (6). Further mutations in a primary target enzyme (gyrase) have been associated with additional 2-fold increases in the MIC of ciprofloxacin (7). The level of resistance observed in M24/06 might suggest additional mechanisms. An efflux pump mechanism is unlikely; we showed no reduction in MICs in the presence of reserpine (online Appendix Table) and this organism was fully susceptible to penicillin,

tetracycline, erythromycin, and Triton X-100 (data not shown). This finding suggests the absence of an efflux pump encoded by a mutated *mtrRCDE* (8). Neither M24/06 nor M12/03 had plasmid-mediated genes *qnr* genes or elevated kanamycin MICs, suggesting the presence of *aac(6')-Ib-cr*. Both of these genes can confer low-level quinolone-resistance and facilitate the emergence of higher level resistance (9) (data not shown).

MLST showed that M24/06 and M12/03 did not derive from a single clone after selection of the T91I mutation. M12/03 was sequence type (ST) 2 and was isolated from a recent immigrant from Russia, which is the origin of most ST 2 strains deposited in the Neisseria MLST database (29/34 records; 85%). M24/06 was ST4789 in the ST5 clonal complex, isolated from a person who had immigrated many years previously from Romania. ST4789 has been encountered only once previously, in Dhaka, Bangladesh.

Disease associated with serogroup A *N. meningitidis* has been extremely unusual in Israel (10) and has remained rare. This serogroup comprised only 9 (1.9%) of all 463 isolates submitted during 1997–2006 (data from the National Center for Meningococci).

The isolates described in our study confirm that serogroup A should be added to the list of meningococci with the potential for reduced fluoroquinolone susceptibility and raise the question why they have appeared in a region with particularly low serogroup A meningococcal disease incidence while frequently encountered serogroups have remained fully susceptible. The importance of continuous monitoring for reduced ciprofloxacin susceptibility in these more prevalent serogroups has been emphasized by the recent replacement of rifampin by ciprofloxacin as the preferred agent for chemoprophylaxis of meningococcal disease in adults in Israel.

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Identification of All Dengue Serotypes in Nepal

To the Editor: Nepal is situated on the southern slopes of the Himalayas, surrounded by India on 3 sides and China to the north. Nepal's altitude ranges from 8,848 m in the Himalayas to 90 m in the Terai, the southern, low, flatland bordering India. Nepal is a disease-endemic area for many vector-borne diseases, including malaria, kala-azar, Japanese encephalitis, and lymphatic filariasis. Because of the porous border between Nepal and India, social, cultural, and economic activities in cross-border areas are common.

Dengue is an emerging disease in Nepal; presumably transmission is moving north from India into the Terai (1–5). The first report of dengue virus isolation or RNA (serotype 2 with nucleotide homology closest to a dengue virus type 2 isolate from India) was in 2008 involving a Japanese patient returning from Nepal in October 2004 (5). Entomologic investigations from the 1980s showed *Aedes albopictus* in the Terai plains, but *Ae. aegypti* has not been previously reported.

After Indian outbreaks now known to include all 4 dengue serotypes (6), a team from the Epidemiology and Disease Control Division,

Kathmandu, investigated suspected cases of dengue fever during September–October 2006 in Banke, the district bordering Uttar Pradesh, India. The team collected blood samples from persons in Banke and, subsequently, from persons in a number of other districts and sent them to the National Public Health Laboratory in Kathmandu or the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand for analysis with ELISA, reverse transcription–PCR, (RT-PCR), or both.

Case definitions for dengue fever were adopted based on World Health Organization guidelines (7). Blood samples were obtained from patients with an acute febrile illness of 2–7 days' duration and with ≥ 2 of the following manifestations: headache, retro-orbital pain, muscular or joint pain, and rash. If laboratory tests were positive, cases were confirmed. Results were confirmed by ELISA performed at the Armed Forces Research Institute of Medical Sciences as previously described (8). Positive results were immunoglobulin (Ig) M ≥ 40 units or IgG ≥ 100 units. RT-PCR was performed by extracting RNA from 140 μ L of each serum sample using QIAGEN Viral RNA Extraction Kit per manufacturer's instructions (QIAGEN, Germantown, MD, USA). RT-PCR and nested PCR were conducted according to the Lanciotti protocol (9) with the following modifications. Reverse transcriptase from avian myeloblastosis virus (Promega, Madison, WI, USA) was used in the first round RT-PCR. The concentrations of the primers used in the RT-PCR and nested reactions were reduced from 50 pmol to 12.5 pmol per reaction, and the number of nested PCR amplification cycles was increased to 25.

Serum specimens were obtained from 70 suspected case-patients from 16 districts from October 13 through December 3, 2006; 25 confirmed cases (13 by ELISA, 10 by RT-PCR, and 2 by both tests) came from 9 districts

(Table). The average age was 29 years (range 5–65 years); 80% of the case-patients were men. Three patients had a history of travel to India, but clusters of dengue fever cases reported in October (Banke and Dang districts) indicated local transmission was occurring among patients with no travel history. The Terai districts accounted for 80% of cases. Entomologic collections done indoors and outside at 5 different sites reporting suspected cases identified *Ae. albopictus* and *Ae. aegypti* in all 5 districts.

These clinical and laboratory test results confirmed the presence of all 4 dengue serotypes. Notably, patients from the Dang district had no travel history outside the Dang valley. Because *Aedes* spp. have been identified in Dang, the data strongly suggest the existence of an endemic cycle of dengue. Underreporting is expected in the absence of diagnostic facilities at the field level. It is unclear whether the predominance of male patients is indicative of greater outdoor as opposed to indoor transmission. Of note, *Ae. albopictus* has been found in the country since the 1980s; in this study, we found *Ae. aegypti* in Nepal. Men typically wear short-sleeved clothes due to hot and humid conditions and, therefore, are frequently exposed to mosquito bites. However, men may also access the healthcare system more frequently. The ages of case-patients point to a relative lack of dengue immunity among the older population, and this finding is consistent with a new introduction of dengue. Because dengue hemorrhagic fever appears when >1 serotype becomes endemic to an area (10), the presence of all 4 serotypes portends the emergence of more severe dengue disease in Nepal.

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Appendix Table. Activity of ciprofloxacin against serogroup A *Neisseria meningitidis* with reduced and full susceptibility to fluoroquinolones and comparison of mechanisms associated with elevated MICs reported in *N. meningitidis*

Location (isolates)	Serogroup	Source*	Ciprofloxacin MIC, mg/L	<i>gyrA</i> mutation	<i>parC</i> mutation	<i>gyrB</i> mutation	<i>parE</i> mutation
France 1996–98†	B	STD clinic	0.125	Asp-95→Gly	None		
Australia 1998‡	C	Blood, CSF	0.25	Asp-95→Asn	None		
Spain 2002§	B	CSF	0.12	Thr-91→Ile	None		
Argentina 2002¶	Y	CSF	0.12	None	None	None	None
Argentina 2002¶	B	Blood, CSF	0.06	Asp-95→Asn	None		
Spain 2007#**	Nongroupable	Sputum	0.25	Asp-95→Asn Asn-103→Asp Ile-111→Val Val-120→Ile Thr-91→Ile Asn-103→Asp Ile-111→Val Val-120→Ile	None		His-495→Asn
India 2005 (1)	A (12 outbreak isolates)			Thr-91→Ile Asn-103→Asp Ile-111→Val Val-120→Ile			
USA 2007–08 (2)	B (3 cases)	CSF	0.19	Thr-91→Ile			
Israel 2001 (M44/01)††	A	Blood	0.001	None	None	None	None
Israel 2000 (M23/00)	A	Blood	0.003	None	None		
Israel 2003 (M12/03)	A	Blood, CSF	0.064	Thr-91→Ile Ala-78→Val	None	Ile-474→Leu	Thr-365→Ala
Israel 2006 (M24/06)	A	Blood	0.125	Thr-91→Ile Asn-103→Asp Ile-111→Val Val-120→Ile	None	None	None

*STD, sexually transmitted disease; CSF, cerebrospinal fluid.

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**Nine additional strains reported; shown is a strain with mutations in *gyrA* similar to those found in isolate in present study.

††Identifiers of the National Center for Meningococci.