Acknowledgments

We thank Teresa Howard and David Anderson for kindly providing anti-HEV kits.

This work was supported by a short-term PhD program training fellowship (to M.C.M.V.) from the International Center of Genetic Engineering and Biotechnology.

María de la Caridad Montalvo Villalba,* Licel de los Angeles Rodríguez Lay,* Vivek Chandra,† Marité Bello Corredor,* Susel Sariego Frometa,* Aidonis Gutierrez Moreno,* and Shahid Jameel†

*Institute for Tropical Medicine "Pedro Kourí," Havana, Cuba; and †International Center for Genetic Engineering and Biotechnology, New Delhi, India

DOI: 10.3201/eid1408.080049

References

1. Emerson SU, Purcell RH. Hepatitis E virus. Rev Med Virol. 2003;13:145–54. DOI: 10.1002/rmv.384
2. Lemos G, Jameel S, Pande S, Rivera L, Rodríguez L, Gavilondo JV. Hepatitis E virus in Cuba. J Clin Virol. 2000;16:71–5. DOI: 10.1016/S1386-6532(99)00062-1
3. Gambel JM, Drabick JJ, Seriwatana J, Innis BL. Seroprevalence of hepatitis E virus among United Nation Mission in Haiti (UNMIH) peacekeepers, 1995. Am J Trop Med Hyg. 1998;58:731–6.
4. Schlauder GG, Desai SM, Zanetti AR, Tassopoulus NC, Mushawar IK. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. J Med Virol. 1999;57:243–51. DOI: 10.1002/(SICI)1096-9071(199903)57:3< 243::AID-JMV6>3.0.CO;2-R
5. Zhai L, Dai X, Meng J. Hepatitis E virus genotyping based on full-length genome and partial genomic regions. Virus Res. 2006;120:57–69. DOI: 10.1016/j.virusres.2006.01.013
6. Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinojara Y, et al. Hepatitis E virus transmission from wild boar meat. Emerg Infect Dis. 2005;11:1958–60.
7. Jameel S, Zafrullah M, Charla YK, Dilawari J. Reevaluation of a North India isolate of hepatitis E virus based on the full-length genomic sequence obtained following long RT-PCR. Virus Res. 2002;86:53–8. DOI: 10.1016/S0168-1702(02)00052-7
8. Huang CC, Nguyen D, Fernandez J, Jun KY, Fry KE, Bradley DW, et al. Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). Virology. 1992;191:550–8. DOI: 10.1016/0042-6822(92)90230-M
9. Maila HT, Bowyer SM, Swanepoel R. Identification of a new strain of hepatitis E virus from an outbreak in Namibia in 1995. J Gen Virol. 2004;85:89–95. DOI: 10.1099/vir.0.19587-0
10. Buisson Y, Grandadam M, Nicand E, Cheval P, van Cuyck-Gandre H, Innis B, et al. Identification of a novel hepatitis E virus in Nigeria. J Gen Virol. 2000;81:903–9.

Address for correspondence: Shahid Jameel, Virology Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi, India; email: shahid@icgeb.res.in

Ciprofloxacin Resistance in *Neisseria meningitidis*, France

To the Editor: Infections with *Neisseria meningitidis* may occur as outbreaks or epidemics. Consequently, chemoprophylaxis for contacts is generally recommended. Ciprofloxacin is frequently used in adults in a convenient 1-dose regimen (*1*). Resistance to this antimicrobial drug in *N. meningitidis* is rare (MIC>0.06 mg/L) and has been reported only in sporadic cases in Greece, France, Australia, Spain, Argentina and Hong Kong Special Administrative Region, People's Republic of China (*2–5*). However, recent reports have described ciprofloxacin-resistant (Cip-R) serogroup A meningococci from 2 outbreaks in Delhi, India, (*6*) and a cluster of 3 serogroup B meningococci in the United States (*7*). This information is of concern because of the high epidemic potential of serogroup A isolates, lack of vaccine against serogroup B meningococci, and possible horizontal Cip-R gene transfer to other meningococcal isolates.

Experimental work was conducted in the *Neisseria* Unit of the Institut Pasteur in Paris. We screened all clinical *N. meningitidis* isolates received at the French National Reference Center for Meningococci in Paris since 1999 for ciprofloxacin resistance. Of these isolates, 4,900 were from France and 246 were from African countries (Burkina Faso, Cameroon, Central African Republic, Côte d'Ivoire, Madagascar, Niger, Rwanda, Senegal, and Tunisia). Only 3 isolates tested were resistant to ciprofloxacin (MICs = 0.19 mg/L), and all were isolated from cases of invasive disease in France.

Two serogroup A, serotype 4, serosubtype P1.9, Cip-R isolates belonged to different sequence types (STs), ST-7 (Cip-R1) and ST-4789 (Cip-R2), although they belonged to the same clonal complex (ST-5/subgroup III). Cip-R1, which showed decreased susceptibility to penicillin, was isolated in 2004 from the blood of a 7-year-old girl. This isolate was most likely imported from Africa. Cip-R2 was isolated from the cerebrospinal fluid of a 77-year-old man who had arrived in France from India in 2006. The ST of this isolate (ST-4789) is the same as the ST of isolates from an outbreak in Bangladesh and similar to isolates from an outbreak in India (*6*; http://neisseria.org/nm/typing/mlstdb). Cip-R3 (serogroup W-135, nontypeable, subtype P1.5), which was isolated from blood and cerebrospinal fluid of an 82-year-old woman in 2006, belonged to a new ST (ST-6361). Current ciprofloxacin resistance has not been documented among invasive W-135 meningococcal isolates since 2 W-135 resistant meningococci were isolated from sputum samples of elderly patients in Spain (*3*).

To investigate the mechanism of resistance in the isolates, fragments of *gyrA* (847 bp) and *parC* (822 bp) genes were amplified by using primers gyrA-1F (5′-gttttcccagtcacgacgttgtaATGACCGACGCAACCATCCGCCAC-3′) and gyrA-1R (5′-ttgtgagcggataacaatttcCCAGCTTGGCTTTGTTGACCTGATAG-3′), and parC-1F (5′-gttttcccagtcacgacgttgtaATGAATACGCAAGCGCACGCCCCA-3′) and parC-1R (5′-ttgtgagcggataacaatttcGGAATTGGCGTTCGGCGGCAGCTC-3′), respectively (sequences in lower case letters are adaptors for universal forward and reverse sequences were added for sequencing after amplification). Primers used for amplification of the *parE* gene were as described (*8*).

Sequencing of fragments of *gyrA*, *parC*, and *parE* genes showed a mutation in the *gyrA* gene in the 3 Cip-R isolates resulting in a Thr91 → Ile substitution. Cip-R1 also showed additional alterations of Asn103 → Asp, Ile111 → Val, and Val120 → Ile, which were described for meningococcal isolates (*3*). Sequences of *parC* and *parE* genes were the same as in a ciprofloxacin-susceptible isolate tested. The association of the Cip-R phenotype with mutations in *gyrA* was confirmed by transformation into the susceptible isolate by using appropriate PCR products (*9*). In addition to the common Thr91 → Ile substitution, the 3 Cip-R isolates were distinguishable by additional *gyrA* alterations or phenotypic and genotypic characteristics. This finding suggests independent events and argues against clonal expansion of Cip-R meningococci.

Serogroup A meningococcal isolates in France are rare and mostly imported. Lack of detection of ciprofloxacin resistance among African isolates tested in this study may be caused by the relatively low number of these isolates (n = 246). Therefore, surveillance of antimicrobial drug susceptibility of meningococcal isolates should be enhanced by using molecular approaches that can also be used as nonculture techniques. This molecular approach will be useful in countries with limited access to classic microbiologic culture–based methods. Reports of invasive cases caused by W-135 Cip-R meningococci should alert physicians who use quinolones to treat respiratory infections in elderly persons. This age group is affected most often by invasive meningococcal pneumonia and 54.5% of such cases are caused by W-135 meningococci (*10*).

A.S. was supported by a Marie Curie Intra-European Fellowship (no. 23188) within the Sixth European Community Framework Program.

Anna Skoczynska,*† Jean-Michel Alonso,* and Muhamed-Kheir Taha*

*Institut Pasteur, Paris, France; and †National Medicines Institute, Warsaw, Poland

DOI: 10.3201/eid1408.080040

References

1. Bilukha OO, Rosenstein N; National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). Prevention and control of meningococcal disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2005;54(RR-7):1–21.
2. Shultz TR, Tapsall JW, White PA, Newton PJ. An invasive isolate of *Neisseria meningitidis* showing decreased susceptibility to quinolones. Antimicrob Agents Chemother. 2000;44:1116. DOI: 10.1128/AAC.44.4.1116-1116.2000
3. Enriquez R, Abad R, Salcedo C, Perez S, Vazquez JA. Fluoroquinolone resistance in *Neisseria meningitidis* in Spain. J Antimicrob Chemother. 2008;61:286–90. DOI: 10.1093/jac/dkm452
4. Corso A, Faccone D, Miranda M, Rodriguez M, Regueira M, Carranza C, et al. Emergence of *Neisseria meningitidis* with decreased susceptibility to ciprofloxacin in Argentina. J Antimicrob Chemother. 2005;55:596–7. DOI: 10.1093/jac/dki048
5. Chu YW, Cheung TK, Tung V, Tiu F, Lo J, Lam R, et al. A blood isolate of *Neisseria meningitidis* showing reduced susceptibility to quinolones in Hong Kong. Int J Antimicrob Agents. 2007;30:94–5. DOI: 10.1016/j.ijantimicag.2006.11.028
6. Singhal S, Purnapatre KP, Kalia V, Dube S, Nair D, Deb M, et al. Ciprofloxacin-resistant *Neisseria meningitidis*, Delhi, India. Emerg Infect Dis. 2007;13:1614–6.
7. Centers for Disease Control and Prevention. Emergence of fluoroquinolone-resistant *Neisseria meningitidis*—Minnesota and North Dakota, 2007–2008. MMWR Morb Mortal Wkly Rep. 2008;57:173–5.
8. Lindbäck E, Rahman M, Jalal S, Wretlind B. Mutations in *gyrA, gyrB, parC*, and *parE* in quinolone-resistant strains of *Neisseria gonorrhoeae.* APMIS. 2002;110:651–7. DOI: 10.1034/j.1600-0463.2002.1100909.x
9. Antignac A, Kriz P, Tzanakaki G, Alonso JM, Taha MK. Polymorphism of *Neisseria meningitidis penA* gene associated with reduced susceptibility to penicillin. J Antimicrob Chemother. 2001;47:285–96. DOI: 10.1093/jac/47.3.285
10. Vienne P, Ducos-Galand M, Guiyoule A, Pires R, Giorgini D, Taha MK, et al. The role of particular strains of *Neisseria meningitidis* in meningococcal arthritis, pericarditis, and pneumonia. Clin Infect Dis. 2003;37:1639–42. DOI: 10.1086/379719

Address for correspondence: Anna Skoczynska, National Reference Centre for Bacterial Meningitis, National Medicines Institute, Chelmska 30/34, Warsaw, Poland; email: skoczek@cls.edu.pl

Rare *Cryptosporidium hominis* Subtype Associated with Aquatic Center Use

To the Editor: Cryptosporidiosis is the most frequently reported gastrointestinal illness in outbreaks associated with treated (disinfected) recreational water venues in the United States (*1*). In 2003, an increased number of cryptosporidiosis cases occurred in the Tri-Cities area of the Lower Mainland region (near Vancouver), in British Columbia, Canada. Although all cases were associated with the use of a community aquatic