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Assessing the Risk of Laboratory-Acquired Meningococcal Disease

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***Neisseria meningitidis* is infrequently reported as a laboratory-acquired infection. Prompted by two cases in the United States in 2000, we assessed this risk among laboratorians. We identified cases of meningococcal disease that were possibly acquired or suspected of being acquired in a laboratory by placing an information request on e-mail discussion groups of infectious disease, microbiology, and infection control professional organizations. A probable case of laboratory-acquired meningococcal disease was defined as illness meeting the case definition for meningococcal disease in a laboratorian who had occupational exposure to an *N. meningitidis* isolate of the same serogroup within 14 days of illness onset. Sixteen cases of probable laboratory-acquired meningococcal disease occurring worldwide between 1985 and 2001 were identified, including six U.S. cases between 1996 and 2000. Nine cases (56%) were serogroup B; seven (44%) were serogroup C. Eight cases (50%) were fatal. All cases occurred among clinical microbiologists. In 15 cases (94%), isolate manipulation was performed without respiratory protection. We estimated that an average of three microbiologists are exposed to the 3,000 meningococcal isolates seen in U.S. laboratories yearly and calculated an attack rate of 13/100,000 microbiologists between 1996 and 2001, compared to 0.2/100,000 among U.S. adults in general. The rate and case/fatality ratio of meningococcal disease among microbiologists are higher than those in the general U.S. population. Specific risk factors for laboratory-acquired infection are likely associated with exposure to droplets or aerosols containing *N. meningitidis*. Prevention should focus on the implementation of class II biological safety cabinets or additional respiratory protection during manipulation of suspected meningococcal isolates.**

Reports of cases of invasive disease caused by *Neisseria meningitidis* infection acquired in the laboratory setting have appeared in the literature for many years (1, 3, 13, 15–17, 20). However, a systematic evaluation of the risk of meningococcal disease among clinical microbiologists and an assessment of the potential laboratory procedures that might predispose technicians to infection have not previously been undertaken.

In 2000, the Centers for Disease Control and Prevention (CDC) was notified of two cases of fatal meningococcal disease in laboratorians (5). Both laboratorians had handled isolates of *N. meningitidis* within 10 days of the onset of their illness, and epidemiologic data suggested that both cases were acquired in the laboratory setting. Subsequent testing at state public health laboratories and at the CDC using pulsed-field gel electrophoresis and multilocus enzyme electrophoresis indicated that, in both cases, at least one isolate handled by the laboratorians and the isolates infecting the laboratorians were indistinguishable.

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Prompted by these two cases, we conducted an investigation to identify additional previously unreported cases of laboratory-acquired meningococcal disease and to identify laboratory activities that might predispose technicians to infection.

Methods. Between 1 August and 10 November 2000, we placed a request for information regarding all cases of suspected laboratory-acquired meningococcal disease occurring since 1985. This request was posted on several selected e-mail discussion groups (i.e., listservs) distributed to members of infectious disease, microbiology, and infection control professional organizations, including ClinMicroNet, the Emerging Infections Network of the Infectious Diseases Society of America, and the Program for Monitoring Emerging Diseases. In addition, the same posting was placed on the home pages of the Internet websites of the CDC, the American Society for Microbiology, the Infectious Diseases Society of America, the College of American Pathologists, and the Association of Public Health Laboratories. Responders were asked to contact the authors by e-mail or by telephone. We requested information on case description, onset of illness, method of diagnosis, outcome of illness, and laboratory procedures performed within 14 days of illness onset. A probable case of laboratory-acquired meningococcal disease was defined as an illness in a laboratorian meeting the case definition for confirmed or probable

meningococcal disease (6) who had occupational exposure in the form of handling an *N. meningitidis* isolate or specimen within the 14 days prior to onset of illness and who had illness from a serogroup that matched the potential source isolate.

Since a reliable estimate of the number of microbiologists in the United States was unavailable, we estimated the number of microbiologists at risk. Each year in the United States, approximately 3,000 isolates of invasive *N. meningitidis* are reported (7). On the basis of standard practices used for isolation and identification of *N. meningitidis*, each of the clinical samples and isolates was handled by an average of three microbiologists during the course of the investigation, resulting in an estimated 9,000 microbiologists exposed per year. We used this estimate as a basis for calculating the attack rate. The attack rate was then compared to age-specific data on meningococcal disease for the 30- to 59-year-old age group (the likely age range for laboratorians), and 95% confidence intervals were calculated using StatXact-Turbo, release 5.0 (Cytel Software Corp., Cambridge, MA), assuming a normal distribution. Hence, the rate of infection for the five years from 1996 to 2000 was calculated as follows:

$$\frac{6 \text{ cases in the United States}}{(3{,}000 \text{ isolates} \times 3 \text{ microbiologists} \times 5 \text{ yr})} = \frac{x}{100{,}000}$$

where x represents the estimated number of cases per 100,000 persons (see below).

In addition to the two cases prompting the request for information, we identified 14 previously unreported cases of probably laboratory-acquired meningococcal disease occurring worldwide from 1985 to 2001; six of these cases occurred in the United States in the previous 5 years (1996 to 2000). Of the 16 cases, 12 (75%) were female; for the 5 cases in which age was available, the median age was 45 years; data on race were not available.

Of these 16 previously unreported cases, 9 (56%) were due to *N. meningitidis* serogroup B, and 7 (44%) were due to serogroup C (Table 1). Eight cases (50%) were fatal, three of which were from serogroup B and five of which were from serogroup C. Case/fatality ratios were higher for serogroup C, but not significantly (serogroup C, 71%; serogroup B, 33%; $P = 0.16$). In the 10 cases for which data were available, there was a median of 4 days (range, 2 to 10 days) between handling of the source isolate and onset of symptoms. Procedures that were performed on the 16 source isolates included examining petri solid medium plates (50%), subculturing isolates (50%), and performing serogroup identification at the laboratory bench (38%). In 15 (94%) of the 16 cases, the laboratorian reportedly did not perform procedures within a biosafety cabinet or employ the use of additional respiratory protection from droplets and aerosols. All 16 cases occurred among workers in the microbiology section of the laboratory; no cases were identified among workers in hematology, chemistry, or pathology.

To address issues of different ascertainment between U.S. cases and non-U.S. cases, an analysis of U.S. cases alone was performed; results were not substantially different from those that included non-U.S. cases. The source isolates from five of the six recent U.S. cases were from either blood or cerebrospinal fluid (CSF); the source of the sixth isolate was unable to be definitively determined but was most likely CSF or middle-ear fluid.

TABLE 1. Years of occurrence, ages, sex, identified serogroups of *N. meningitidis*, and outcomes in 16 cases of probably laboratory-acquired meningococcal disease from 1985 to 2000

Case	Yr	Age of patient	Sex[c]	Serogroup	Outcome
1	1985			B	
2[a]	1985		F	C	Fatal
3[a]	1987		F	B	Fatal
4	1989		F	B	Fatal
5	1991	46	F	B	Fatal
6	1991		F	C	Fatal
7[a]	1992		M	B	Survived
8[a]	1995		M	B	Survived
9[a]	1997	40	M	B	Survived
10	1997		F	B	Survived
11	1998	45	F	B	Survived
12[a]	1999		F	C	Survived
13[a]	1999		F	C	Survived
14[a]	2000	35	M	C	Fatal
15[a]	2000	52	F	C	Fatal
16[a]	2000		F	C	Fatal
17[b]	2002	50	F	C	Survived
18[b]	2002	21	M	A	Survived
19[b]	2002	65	F	C	Survived

[a] U.S. cases, included in analysis.
[b] Identified following conclusion of study.
[c] F, female; M, male.

For the interval from 1996 to 2000, using U.S. cases alone, we calculated a U.S. attack rate (see above for details) of 13 per 100,000 population (95% confidence interval, 5 to 29/100,000), compared with approximately 0.3/100,000 population among U.S. adults aged 30 to 59 (6). If the three cases occurring in 2000 were excluded from this estimate, the attack rate would be 7 per 100,000 (95% confidence interval, 1 to 19/100,000).

Data from 2002. In 2002, after the study was completed, an additional three cases of probably laboratory-acquired meningococcal disease were reported to the CDC. Two patients were female; two cases were due to serogroup C and one to A. The source specimen was blood in all cases. None was fatal. In the two cases in which data were available, subculturing and Gram stain preparation were performed on an open benchtop, and respiratory protection in the form of a splash guard was used. Including the three cases from 2002, our results yielded an attack rate of 20 per 100,000 population.

Conclusions. The results of this analysis suggest that, although the absolute risk for disease remains low (12), laboratory-acquired meningococcal disease represents a significant occupational hazard to clinical microbiologists. In addition to case reports which have appeared in the literature over the years, this informal request for information identified 14 previously unreported cases of probably laboratory-acquired meningococcal disease occurring worldwide, including six occurring in the United States in the past 5 years. Even if the two cases prompting the evaluation and a third identified prior to posting of the listservs are excluded from analysis, the attack rate among microbiologists is far greater than for the general population of comparable age range. Cases of laboratory-ac-

quired meningococcal disease may have been underreported; alternatively, the incidence of laboratory-acquired disease may have increased. In addition, the case/fatality rate of 50% seen among survey cases is substantially higher than that observed among community-acquired cases (2, 18). This may be explained by ascertainment bias due to underreporting of mild cases of disease. However, an alternative possibility is that the conditions in which meningococcus is found in the laboratory environment are contributing factors, as clinical microbiologists routinely work with highly virulent strains and high concentrations of organisms. There were several limitations to this type of assessment, including the necessity of access to Internet services and e-mail, the informal method of data collection, and the significant problems of recall bias in cases occurring many years ago. This study, however, succeeded in detecting several previously unreported cases and suggests that, even if the true incidence is underestimated, laboratory-acquired meningococcal disease represents a significant risk to U.S. microbiologists.

Meningococci may be present in patient specimens, including blood, CSF, and pharyngeal exudates. All cases identified in this inquiry occurred among microbiologists and not among workers in other areas of the clinical laboratory. This suggests that exposure to isolates of *N. meningitidis*, and not patient samples, represents the increased risk for infection. In addition, all isolates were derived from sterile sites; none of the microbiologists identified were working with isolates obtained from pharyngeal or respiratory secretions, suggesting that such pharyngeal isolates represent a lower risk, presumably due to their lower pathogenicity.

This request for information did not identify definitive risk factors for laboratory-acquired meningococcal infection; however, in 15 of the 16 cases, procedures performed on the meningococcal isolates were carried out on a laboratory benchtop, outside of a biosafety cabinet, and without the use of splash guards or other forms of protection from droplets. All three cases identified in 2002 performed procedures on cultured isolates on a laboratory benchtop; in addition, two of these patients performed procedures using a standard splash guard. *N. meningitidis* is classified as a biosafety level 2 organism (8), and current guidelines recommend that a biosafety cabinet be used for mechanical manipulation of samples that carry with them a significant risk of droplet formation or aerosolization (9, 14); such procedures, as outlined, include "centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs" (8). In addition, the frequent contact with organisms in high concentrations, as seen in the research setting, has been recognized as representing an increased risk, and such research and industrial workers are recommended to perform manipulations within a biosafety cabinet (8, 9, 14). A recent assessment in the United Kingdom found a similar increased risk among microbiologists who prepared concentrated suspensions of *N. meningitidis* outside of a biosafety cabinet (4). However, the cases identified in this study were not identified as performing manipulations such as those which have been associated with a high risk of droplet or aerosol formation. Rather, they were manipulating isolates in a manner which has not previously been identified as representing a high risk of droplet or aerosol formation, such as transferring cultures with an inoculating loop; the risk of droplet or aerosol formation during these routine activities is less well understood.

Although the exact mechanism of transmission of *N. meningitidis* in the laboratory setting remains unclear, the route of natural infection with the organism and the risk associated with manipulation of invasive isolates on an open laboratory benchtop suggest that exposure to droplets or aerosols of *N. meningitidis* is the most likely risk factor. On this basis, and until further data are available, microbiologists should perform manipulations of sterile-site isolates within a class II biosafety cabinet. The utility of alternative methods of protection from droplets and aerosols, such as splash guards and masks, needs further assessment. However, the identification of two cases in which such facial protection was employed suggests that adequate protection is not always afforded by these modalities. If a biosafety cabinet or other means of protection is unavailable, manipulation of these isolates should be minimized, and workers should consider sending specimens to laboratories possessing this equipment. Biosafety cabinets are not uniformly available, and the frequency of any one laboratory encountering invasive isolates of meningococcus is low; however, the elevated risk and high case/fatality ratio mandate consideration of increased precautions despite the relative inconvenience. Further studies are needed to better outline the risks of routine manipulation of invasive meningococcal isolates.

Current guidelines recommend that research and industrial laboratory scientists who are exposed routinely to *N. meningitidis* in solutions that may be aerosolized should consider vaccination (9, 10, 11). While primary prevention of laboratory-acquired meningococcal disease should focus on laboratory safety, laboratory leaders and individual microbiologists in clinical laboratories will also need to make informed decisions regarding vaccination. The vaccine currently available in the United States includes serogroups A, C, Y, and W-135; it decreases but does not eliminate the risk of infection since it is less than 100% effective, and it does not provide protection against serogroup B, which caused one-half of the laboratory-acquired cases in this survey. In addition, the vaccine has a duration of efficacy of approximately 5 years (19), which would necessitate repeated doses of vaccine in many laboratorians during the course of their careers. However, new meningococcal vaccines are under development and may prompt a reassessment of the current vaccine strategy for laboratorians.

Laboratorians with percutaneous exposure to an invasive *N. meningitidis* isolate from a sterile site should receive treatment with penicillin; those with known mucosal exposure should receive antimicrobial chemoprophylaxis (11). Microbiologists who manipulate invasive *N. meningitidis* isolates in a manner that could induce droplet or aerosol formation (including plating, subculturing, and serogrouping) on an open benchtop and in the absence of effective protection from droplets or aerosols should also consider antimicrobial chemoprophylaxis.

Continuing surveillance for cases of laboratory-acquired meningococcal disease is necessary to determine incidence, as well as specific laboratory procedures representing risk factors. In addition, basic research is needed to further define the risks involved in the routine manipulation of isolates of meningo-

coccus and other potentially fatal infectious agents, regardless of current biosafety level status. The combination of increased awareness of the risk among laboratorians, increased focus on laboratory safety, and more-effective vaccines will be important in protecting scientists who work with *N. meningitidis* in clinical laboratories.

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