administered in Australia. This finding is supported by other studies in The Netherlands and Finland that demonstrated increasing genetic diversity. However, it is unclear whether the genetic changes have led to the increased outbreaks.

The author (Poland, 2012) reminds us that in the U.S., whole-cell pertussis vaccines were used exclusively until 1997. Then, acellular vaccines were recommended for children. Only in 2006 was the use of Tdap also recommended for adolescents and adults. Initially, this vaccine was recommended only for persons up to age 65, and only recently were all age restrictions dropped. The most recent guidelines include pregnant women after 20 weeks of gestation.

Poland (2012) argues that the continuous changes and adaptations of guidelines may have confused practitioners and patients alike, and resulted in inadequate population vaccine coverage.

Poland (2012) concludes that the current vaccines are safe and provide relatively good short-term protection, but appear to require intermittent boosting. This is important because pertussis is not just a childhood disease and immunity is not life-long.


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### Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biosafety problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

**N. meningitidis as a Cause of Laboratory-acquired Infections: The Sequel**

In 2007, 31 cases of meningococcal laboratory-acquired infections (LAI) that resulted in 11 fatalities were reviewed in this column (Byers, 2007). News reports of another fatal LAI has prompted this second column on *N. meningitidis*. A laboratory worker described by his coworkers as “careful” started feeling ill about 2 hours after leaving work on a Friday; he was dead 17 hours later. The previous week, he had worked with *N. meningitidis* serotype B; the laboratory was working on developing a vaccine for this pathogen. According to press reports, California OSHA has closed the laboratory and is investigating the circumstances that led to this LAI (Fabervor, 2012). Federal OSHA issues “serious citations” when there is “substantial probability that death or serious physical harm could result from a hazard about which the employer knew or should have known.” Three serious citations were issued in this case, for the employer’s failure to:

1. require use a biosafety cabinet when performing work with a viable culture of *N. meningitidis*,
2. provide training on the signs and symptoms of illness as a result of exposure to a viable culture, and
3. offer vaccines to staff potentially exposed to *N. meningitidis* (DOL, 2013).

Since meningococcal meningitis is contagious to close contacts, prophylactic antibiotics were provided to personal and laboratory contacts, as well as the potentially exposed healthcare providers. No secondary infections resulted from this LAI (Moise, 2012).

In the 2007 review article, it was noted that, in all 31 cases of meningococcal LAI, all of the infected staff conducted routine microbiological procedures on the open bench (e.g., making a suspension with a cotton swab, performing catalase assays or a Gram stain, subculturing, aspirating blood culture bottles, testing for antibiotic sensitivities, adding formalin to a inactivate a culture). No known exposures, spills, or breaches in the laboratory’s standard procedures were reported or suspected in 30 cases. An anecdotal report of a “mishap” exists for only 1 case. Thirty of the individuals infected by *N. meningitidis* were in good health; one had a cold. In every case, only the microbiologist who manipulated the patient isolates was infected; this indicates transmission from droplet contact with mucous membranes, either directly or through hand contamination. All of the microbiologists were experienced; some handled *N. meningitidis* cultures routinely. None of the individuals had been immunized. For more details and references on these cases, please refer to Byers (2007).

*N. meningitidis* is a stealthy pathogen; its pathogenesis is still not completely understood. In the general population, individuals contract it from the respiratory droplets or oral secretions of an asymptomatic carrier. On average, in non-epidemic conditions, 10% of the human population
asymptomatically carries *N. meningitidis* in their nasopharynx (Shoen, 2007). When humans are crowded together, the rate of carriage, or colonization, of the nasopharynx increases. A study in a university in the United Kingdom found that the rate of carriage for students in their first year of dormitory life rose from 23.2% in late September to 55.7% by December (Ala’Aldeen, 2011). Studies from several countries on military recruits also show high percentages of carriage (WHO, 2011).

The strains carried are classed into 13 serological groups based on their capsule antigens; unencapsulated strains are also found but cannot be classified by serotyping. Ninety percent of meningococcal disease is caused by serogroups A, B, C, W135, and Y (Yazdankhah, 2004). Harboring this pathogen in the nasopharynx results in some level of immunity, since antibodies against the specific strains carried can be documented in healthy individuals. This immunity does not prevent colonization, but it apparently helps to prevent the colonizing bacteria from breaking through the mucosal barrier and accessing the bloodstream.

Within the general population, the groups with the lowest rate of colonization, or carriage, of *N. meningitidis* strains are the most susceptible to meningococcal disease. The highest rates of fatality are in the young, particularly infants. When studies of the nasopharyngeal carriage rate are broken into age groups, the rate varies. Less than 3% of children under age 4 carry *N. meningitidis*; the rate is 24%-37% in the age group 15-25 years, and the rate drops as the age increases (Yazdankhah, 2004).

Susceptibility to disease also increases when mucosa are compromised. The dusty winds of sub-Saharan Africa, coupled with respiratory infections in participants, are key factors in the rates of meningococcal transmission during the Hajj (WHO, 2012). Active or passive exposure to smoke also increases susceptibility to infection by *N. meningitidis* (Yazdankah, 2004). Individuals who are asplenic, or have blood complement deficiencies, hypogammaglobulinemia, lack of IgA, and some chronic kidney diseases are also particularly vulnerable to meningococcal infection (Cramer, 2012).

Colonization of the nasopharynx with *N. meningitidis* is not limited to social exposures; this can also be a result of laboratory exposure. This was documented in a technician who worked in a laboratory studying *N. meningitidis* and received routine medical surveillance including monthly nasopharyngeal cultures. Over a 6-year period, the monthly throat cultures from this technician working with *N. meningitidis* were always negative. In the 7th year of work in the same lab, his throat swab grew approximately 50 colony forming units (cfu) of *N. meningitidis*. The organisms were identified as a nalidixic-resistant strain identical to a serogroup C disease isolate he worked with in the laboratory called FAM20. The isolate from the laboratory worker was named LW1. Both FAM 20 and LW1 were sensitive to 1 microgram Rifampin. The technician was immediately treated with the standard dose of rifampin (600 mg twice a day orally for 2 days), and his throat culture cleared. The following two routine monthly throat cultures were again negative. Then, for the next 7 months, all throat cultures were positive—yielding 500 cfu/ml to 10^3 cfu/ml. The isolates, labeled LW2-8, were now resistant to 100 micrograms of rifampin. Changes in the bacterial capsule proteins, as well as the laboratory worker’s antibodies, were carefully analyzed in this study and described as a demonstration of the ability of *N. meningitidis* to adapt to selective pressure. The publication also noted that, fortunately, the laboratory worker was protected against invasive disease by his vaccination, and colonization with the invasive strain FAM 20 did not result in the development of disease in this technician (Woods, 1990).

The factors that allow the bacteria to breach the mucosal barrier and cause disease are the focus of a number of genomic research studies. Insights into pathogenesis were inadvertently provided when a researcher became infected; the exposure was attributed to a malfunctioning biosafety cabinet (Omer, 2011). Bacterial meningitis progresses quickly; she was ill one night and hospitalized the next day with classic symptoms of meningitis: fever; a rash characteristic of meningococcal infection (purpuric lesions); and a stiff neck. Pulsed-field gel electrophoresis showed that the isolate from her blood culture (called Z5463BC) was identical to the fourth subculture of Z5463, serogroup A, an invasive strain that she had worked with the previous week. Blood samples taken during her treatment were frozen to allow studies of the *in vivo* changes in the bacteria since *N. meningitidis* is a pathogen that can evade the immune system with highly variable capsule proteins. Despite the low number of bacterial cell divisions within the researcher, estimated to be about 25, a comparison of the laboratory fourth subculture and the LAI blood culture showed a number of changes. Z5463BC differed from the fourth subculture of Z5463 in that it used a different hemoglobin-linked iron receptor and showed genomic changes in areas that regulate adhesive properties and endotoxin production. This ability to adapt rapidly to selective pressure also demonstrates the complexity of *N. meningitidis* pathogenesis. Fortunately, 5 days of antibiotic therapy were effective and the scientist recovered fully.

Careful review of the laboratory practices revealed no problems other than a biosafety cabinet malfunction. However, there was a breach in the occupational health program. The article states that the researcher had been vaccinated against groups A and C at the initiation of her work with *N. meningitidis* with a vaccine considered effective for 3 years; but she had not received a booster during her 5-year career (Omer, 2011). The Advisory Committee on Immunization Practices (ACIP) recommends re-vaccination with conjugate vaccines (CDC, 2007).

Once *N. meningitidis* does breach the epithelial barrier, meningitis is not the only possible outcome. *N. meningitidis* may cause sepsicaemia with the vascular damage so substantial that limb amputation may be required. This type of LAI occurred in a PhD microbiologist who began work in a meningitidis antibody testing laboratory in 2005. After just
Vaccination as a preventive measure has a proven 30-year track record in reducing meningitis epidemics, including those in the “meningitis belt” of sub-Saharan Africa (WHO, 2011). Selection of an appropriate vaccine depends on the geographic area; travelers are advised to seek destination- and outbreak-specific vaccination advice (Cramer, 2012). Vaccines were initially developed using capsular components as antigens. This strategy can be used with serogroups A, C, W, and Y. To further enhance the immune response, capsular antigens can be conjugated to a protein. Tetavalent A, C, Y, and W135 conjugate vaccines have been licensed since 2005 for use in children and adults in Canada, the United States, and Europe (WHO, 2011). Unfortunately, the same strategy cannot be used with serogroup B; the capsular antigens are too similar to the adhesion factors in healthy human nervous tissue. Vaccines using the outer membrane proteins of serogroup B were developed in Cuba, New Zealand, and Norway, but they were strain-specific for specific epidemics (WHO, 2011). Currently new vaccines against serogroup B are undergoing regulatory review; these promise protection against all, or most, of the serogroup B strains. Research has also yielded a relatively inexpensive vaccine against serogroup A, developed specifically for the African “meningitis belt.” This new vaccine is not dependent on cold storage to maintain effectiveness, and not only generates a sustained immune response but also reduces the carriage rate, which should reduce transmission (Meningitis Vaccine Project).

Minimizing the risk of LAI requires the collaboration of occupational health and biosafety professionals as well as laboratory directors. Working together, we can stress the advisability of vaccination for clinical and research laboratory workers routinely exposed to N. meningitidis and the close supervision of students. The problems are illustrated in the LAI case study of a 21-year-old summer student in a laboratory working with N. meningitidis. The student told the Principal Investigator in the laboratory that he had been vaccinated. After a 5-day illness, including fever, headache, and increasing confusion, the student was hospitalized and he assured his physician that he had received meningococcal vaccine. On the second day of hospitalization, PCR of the spinal fluid indicated N. meningitidis serogroup A as the source of the undergraduate student’s illness. At that point, the student denied vaccination and admitted to subculturing five isolates of serogroup A out on the bench. Fortunately, he recovered completely (Kessler, 2007).

There is no national or international registry that records critical information about LAI exposures, and publications vary in the level of detail provided. For example, a Promed email reported a case of meningococcal meningitis in a graduate student and that fingerprinting confirmed that the infection was laboratory-acquired, but did not include the serogroup, vaccination status, or potential exposure, if known (PROMED, 2009). However, the data accumulated are sufficient to stress compliance with the CDC
recommendations to prevent additional LAI with *N. meningitidis*. (CDC, 2009; Sevjar, 2005).

To summarize:
1. Perform all procedures with samples from sterile sites (blood, cerebrospinal fluid, inner ear fluid) in the biosafety cabinet.
2. Wear gloves, remove gloves when work with these samples is completed, and wash hands.
3. Remind microbiologists that LAI have occurred from procedures not generally considered aerosol-producing.
4. Offer staff immunization with tetravalent conjugate vaccine and consultation about boosters.
5. Offer post-exposure prophylaxis if *N. meningitidis* is manipulated outside of the biosafety cabinet.

**References**


