Capsule

Felix K. Gmuender
Basler & Hofmann Singapore Pte Ltd., Singapore

What’s new, what’s hot, what’s timely? If you don’t have time to search the Internet for the latest developments that might impact your work environment, you just might find some of that information in this “Capsule” column. Please e-mail any comments or suggestions to felix.gmuender@bh.com.sg or to Co-Editor Barbara Johnson at barbara.johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Evaluation of the Disinfection Efficacy of a Novel Steam Vapor System, Protective Efficacy of mRNA Vaccines Against Influenza A Virus Infection, Emerging Disease or Diagnosis, and Pertussis Outbreaks and Pertussis Vaccines

Biofilms on Environmental Surfaces: Evaluation of the Disinfection Efficacy of a Novel Steam Vapor System

Bacteria attached to surfaces as biofilms or aggregates of single cells can form a reservoir of pathogens in healthcare, educational, and food-service settings, as reported by Song et al. (2012). Biofilms are known to protect the bacteria from antimicrobial agents and commonly used decontamination techniques. Song et al. (2012) compared a traditional decontamination method (sodium hypochlorite) with a novel steam disinfection system developed by Advanced Vapor Technologies (AVT, Seattle, WA). AVT has experimented with a new method of steam generation by using superheated low-moisture steam and using tap water’s impurities (mineral salts) to enhance the effect of steam on a surface (Song et al., 2012). Biofilms of four bacterial strains (Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, and Staphylococcus aureus) were grown with a standardized method (ASTM 2562-07) on 10 mm coupons made of polycarbonate, rubber, stainless steel, and ceramic. The initial biomasses on the coupons (N0) were in the range of 7.4 x 10^4 (E. coli) to 1.6 x 10^5 (S. aureus). A commercially available steam vapor device (MondoVap 2400, AVT, Seattle, WA) was fitted with a 14 x 14 x 14 cm triangular cotton terrycloth towel affixed to the device’s cleaning head. Ordinary tap water was used for cleaning. The steam disinfection efficacy was expressed as a log reduction (log_10 N/N0) vs. treatment time in seconds. Steam cleaning was compared with chemical disinfection of the coupons with 10 ppm sodium hypochlorite for 10 minutes. The results showed that a 3-second steam treatment killed all biofilms on all surfaces with more than 99.95% efficacy, and complete killing was observed after 10 seconds. Treatment of the surfaces with 10 ppm sodium hypochlorite resulted in a 2-log reduction of viable cells after 10 minutes. Song et al. (2012) conclude that the steam vapor system is effective in killing biofilms on experimental surfaces and shows potential to control biofilms.


Protective Efficacy of In Vitro Synthesized, Specific mRNA Vaccines Against Influenza A Virus Infection

Petsch et al. (2012) have developed and tested an mRNA influenza vaccine on mice, ferrets, and pigs. The authors explain that mRNA-based vaccines offer substantial improvements over conventional vaccines: Genetic vector vaccines can be rapidly developed and tested, are heat-stable, and can, at least in mice, induce long-lived immunity. The vaccine production is rapid and highly scalable. For example, during the 2009 H1N1 pandemic, an mRNA vaccine could have been provided 6-8 weeks after the influenza virus sequence was published. Petsch et al. (2012) describe that an mRNA-based vaccine consists of an RNA segment that encodes for the antigen of interest, and 5' and 3' untranslated regions that serve to increase the efficacy of translation and the intracellular stability of the RNA molecule. The vaccine formula includes protamine (small arginine-rich nuclear proteins) to increase the stability and activity of the vaccine. Petsch et al. (2012) claim that successful mRNA immunization against infectious diseases has not been reported. However, their mRNA vaccines against full-length hemagglutinin of H1N1, H3N2, and H5N1 viruses induce protective efficacy in mice, ferrets, and domestic pigs. In young and old mice, immediate full protection was seen after a single application of the vaccine, a finding that is relevant for the protection of children and the elderly. A second application was necessary for lifetime protection in older mice. In contrast to mice, ferrets and pigs showed a rapid decrease of protection after the first application; booster vaccinations were required to reach serological results required for vaccine licensure. The authors (Petsch et al., 2012) conclude that their mRNA platform “opens attractive perspectives for a broad range of pathogens.”

Emerging Disease or Diagnosis?

Outbreaks of highly contagious Ebola, Marburg, and Lassa virus in 2012 in central Africa raised once more worries about possible epidemics of these hemorrhagic fevers as reported by Gire et al. (2012). The “emerging disease concept” was based on the appearance “out of nowhere” of these pathogens in the middle of the 20th century. Gire et al. (2012) puts the hypothesis to the test that “emerging diagnostics” could explain the rise of hemorrhagic fevers caused by these viruses. In fact, epidemiologic and genetic analysis of Lassa and Ebola fever indicates that these viruses have circulated in central Africa for a long time. The authors explain that emerging pathogens fall into two categories: zoonotic microorganisms that are transmitted from animal reservoirs to humans and existing but rare human pathogens that spread quickly from a so far confined reservoir or develop higher pathogenicity. The sudden appearance can be associated with human intrusion into animal habitats, changing socioeconomic conditions, increased mobility, and genetic changes in the microorganisms. Under these circumstances, the concept of emerging diagnostics seems not to fit. Gire et al. (2012) theorize that hemorrhagic fever viruses are rated as rare because tools to routinely detect them are not available. Because hemorrhagic fevers often have nonspecific symptoms, clinical diagnosis is difficult. The paper mentions that serological surveys indicate widespread exposure to Lassa and Ebola viruses in certain parts of sub-Saharan Africa. Seroprevalence for Lassa virus, for example, can be as high as 54.9% in parts of Sierra Leone and Guinea, and 21.3% in Nigeria. These figures show that the diseases are endemic. Health officials conclude that in West Africa between 100,000 and 300,000 people become infected every year. Seroprevalence of Ebola viruses also points to widespread exposure. The actual prevalence may be higher, because antibody concentrations in the blood may wane over time, or lower because some tests tend to produce false positives. The evolutionary history of Lassa virus is about 500 years old, when it possibly split from other arenaviruses. Ebola virus appears to have evolved from Marburg virus about 10,000 years ago (Gire et al., 2012). Contrary to common perception, subclinical infections do exist, meaning that there is natural human immunity. Typically, fatality rates among hospitalized cases range between 12% and 78% for Lassa fever, and 42% to 88% for Ebola fever (Gire et al., 2012), but during Ebola outbreaks, many cases are also asymptomatic. The possibility that “emerging diseases” are really ancient and widespread raises the possibility that exposure is more common, and the agents are very likely circulating—undetected—in the population. Gire et al. (2012) conclude that with the right detection tools and resources, a public health strategy to better detect, monitor, and characterize these diseases should be formulated to protect the affected communities from outbreaks.


Pertussis Outbreaks and Pertussis Vaccines: New Insights, New Concerns, New Recommendations?

The U.S. and many other industrialized countries are still affected by large-scale pertussis outbreaks (Poland, 2012). The size and scale of the outbreaks noted over the last 3 years are astounding; they are larger in scope than any in the last 50 years. In California alone, at least 10 children died of whooping cough. In 2012, another eight children died in the U.S. This raises the question about the possible root causes. The following list of likely reasons is quoted from Poland (2012):

• Secondary vaccine failure (i.e., waning immunity)
• Possible skewing of pertussis immune responses in children due to the use of the acellular pertussis vaccine (DTap) in early childhood
• Possible vaccine-resistant B. pertussis strains
• Inadequate and confusing Tdap immunization recommendations and guidelines, resulting in underuse of the vaccine
• Lack of awareness of the need for tetanus–diphtheria–acellular pertussis (Tdap), specifically among adults, and anti-vaccine sentiment, leading to inadequate pertussis vaccine coverage levels in the population (i.e., lack of herd immunity)
• Unprecedented population mixing on a global scale (i.e., opportunity for exposure)
• High transmissibility of Bordetella pertussis

Although all factors contribute to the cases and epidemics, Poland (2012) focuses on the first four factors as the most interesting ones.

Current information suggests that waning vaccine immunity plays a relevant role when acellular pertussis vaccines (DTap or Tdap) or secondary vaccines are used (Poland, 2012). The immunity induced with Tdap is less durable as compared to that induced by whole-cell vaccines. Data from a study in California indicate that protection can wane within 5 years after the last booster vaccination. A report from Australia showed that disease rates were higher among children who had received the acellular pertussis vaccine as compared with those who were immunized with the whole-cell preparation.

An issue that requires more research is the possible skewing of the immune response. The acellular vaccines contain a much smaller range of antigens and induce high titers of antibodies to each of the few components. In contrast, the complex whole-cell vaccines induce lower titers of antibodies against a much larger range of proteins and toxoids. It is speculated that the Tdap vaccine preparation induces an unbalanced—or skewed—T-helper lymphocyte response, resulting in a lower cellular memory.

Another interesting question is whether the vaccine itself exerts a mutational selective pressure on the circulating strains (Poland, 2012). Research in Australia has isolated newly emerging B. pertussis clones. These clones do not carry antigens included in the acellular pertussis vaccines.
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**

Karen B. Byers

Dana-Farber Cancer Institute, Boston, Massachusetts

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 biocontainment 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 (Faberov, 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 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