arbovirus is yet known to cause birth defects, although several are neurotropic, and none has before been found, as Zika virus has been, to be sexually transmitted.[353] Virus isolates from Latin America are the Asian lineage, which had not been previously observed to cause birth defects, although a retrospective review of records from French Polynesia suggests an association between Zika virus infection and microcephaly was overlooked earlier.[354] If Zika virus is widely endemic in Africa and Asia, as recent investigations in Indonesia indicate,[355] it is possible many females reaching puberty there are naturally immune. Much attention is being devoted to improving serologic tests capable of reliably differentiating a history of Zika infection from dengue[356] and to developing a vaccine.[357]

Yellow Fever

Since 1937 hundreds of millions of people at risk from yellow fever virus have been immunized with a highly efficacious live-attenuated vaccine (17D and its derivatives). Nevertheless, yellow fever, the eponymous *Flavivirus,* continues to cause large deadly epidemics in South America and Africa. The virus is transmitted in both a sylvatic cycle among monkeys by forest dwelling *Aedes* mosquitoes and in an urban cycle among humans, predominately by *A. aegypti.* The cycles are linked when urban or semiurban dwellers are infected while working in the forest. In late 2015, a major urban epidemic began near Lusaka, Angola, later spreading to western regions of the Democratic Republic of the Congo (DRC).[358] Nearly 7500 suspected cases were reported, with fatality rates among 962 laboratory-confirmed cases of 14% to 21%. At least 11 cases were carried to China by migratory workers. Although China, like all of Asia, is yellow fever free, *A. aegypti* are common; fortunately there was no secondary transmission. A major epidemic of sylvatic yellow fever began in Brazil in late 2016,[359] affecting southeastern regions where vaccination rates were low. Up to June 2018, there had been more than 2000 confirmed cases with a fatality rate of approximately 30%. Urban transmission did not happen, possibly because of emergency mass distribution of more than 40 million vaccine doses. Together with about 30 million doses sent to West Africa by WHO, vaccine stockpiles became so depleted that efficacious fractional administration with 20% of the standard dose was successfully implemented in both Brazil and the DRC.[360]

Chikungunya

Unlike dengue, Zika, and yellow fever viruses, chikungunya is in the family Togaviridae, but like them it is a single-stranded, positive-sense RNA virus transmitted by *Aedes.* First described from Tanzania in 1953, a pandemic began in coastal northeastern Kenya in 2004 and by 2013 had spread through the Indian Ocean to South Asia, Southeast Asia, the Pacific, and the Caribbean.[361] By 2015 it had spread through much of Latin America. The disease is notable for the high proportion of infections developing symptoms, in particular painful arthralgia of the small joints; fatalities are rare. RNA viruses have high potential for mutation, and sometime early in the pandemic a single nucleotide substitution occurred that replaced an alanine with valine at position 226 in the viral envelope protein, E1.[362] This seemingly insignificant change increased the ability of one chikungunya lineage to infect *A. albopictus,* which extends farther into temperate zones than does *A. aegypti*. Sporadic outbreaks of chikungunya in Italy[344] and France[345] since 2007 were driven by *A. albopictus* transmission.

Plasmodium vivax, Plasmodium knowlesi, and Zoonotic Malaria

The primate malarias are transmitted by a wide range of *Anopheles* mosquito species. Although predominantly diseases of the tropics today, *Plasmodium* was endemic in North America and Europe well into the 20th century. Major public health initiatives in Africa and Asia have substantially reduced disease from *Plasmodium falciparum,* but these advances are countered by rising drug resistance, increased awareness of pernicious *P. vivax* disease, and the emergence of zoonotic malaria.[363]

There is not yet an efficacious, licensed malaria vaccine. Therapy for *P. falciparum* infections, and increasingly for *P. vivax,* has depended on artemisinin-based combination therapies. The recent rise of *P. falciparum* resistance in Southeast Asia to artemisinins and some partner compounds threatens elimination campaigns and complicates treatment.[364] For reasons that are poorly understood, an area of southwestern Cambodia has generated most examples of *P. falciparum* resistance, beginning with chloroquine in 1968 and followed by mefloquine, tafenoquine, fansidar, and, in 2016, artemisinin.[365] Rising resistance of the *Anopheles* vectors to insecticides, including the pyrethroids used in treated bed nets, also hamper control[366]; while there have been advances in engineering mosquitoes incapable of transmitting malaria, designing means to drive modified genes into wild populations remains a challenge.[343]

P. vivax is the most cosmopolitan human malaria and was long considered relatively benign compared to *P. falciparum* malaria. There is mounting evidence that it does, in fact, cause serious disease and death.[367,368] Complications of *P. vivax* infection, which might elicit greater inflammatory response than does *P. falciparum,* seem especially to afflict children with comorbidities, common in malaria-endemic regions.[369] The extent of *P. vivax* endemicity also continues to be reappraised. It had been thought that the invasion of reticulocytes by *P. vivax* merozoites was exclusively mediated by the Duffy antigen/chemokine receptor (DARC), which is absent in most sub-Saharan populations.[370,371] Since the discovery that *P. vivax* was being naturally transmitted to DARC-negative people in Kenya,[372] there have been mounting reports of *P. vivax* both in Africa and in the African diaspora.[373,374] The existence of alternate erythrocyte invasion pathways could affect development of *P. vivax* blood stage vaccine candidates designed to block the *P. vivax* merozoite's access to DARC.[375]

Until recently it was dogmatic that naturally acquired human malaria was caused by four species: *P. falciparum, P. vivax, Plasmodium malariae,* and *Plasmodium ovale.* It is now known that in Southeast Asia humans are not only being infected with the macaque malaria, *P. knowlesi,* but that in some places it is the predominant cause of clinical malaria.[376] It is not yet clear whether human cases all originate in monkeys or if human-to-human transmission is also occurring.[377] The importance of *P. knowlesi* was long overlooked because in the microscopic examination of thick, dehemoglobinized blood films, *P. knowlesi* is easily mistaken as *P. malariae* or *P. vivax.* Whereas *P. knowlesi* is a distinct simian malaria that has jumped to humans, in Brazil it appears that *Plasmodium simium,* which infects howler and capuchin monkeys and is increasingly reported infecting humans, was originally human *P. vivax.*[378] Similarly, *Plasmodium brasilianum,* a parasite of New World monkeys that has been found infecting people in Venezuela, has a genome nearly identical to *P. malariae.*[379] The increasing use of sophisticated genomic investigative methods in epidemiology are likely to further qualify our knowledge about human malaria. If zoonotic transmission of malaria is more common than presumed, it could complicate efforts to eliminate the disease in some places.

Tick-borne Pathogens

The epidemic potential of tick-borne pathogens is constrained by biology: unlike mosquito-borne pathogens, humans are always dead-end hosts and tick dispersion is more limited than for mosquitoes. But the persistence of tick-borne pathogens in the environment and the increasing rate of emergence make them major public health concerns. Lyme disease (*Borrelia burgdorferi*) is the most common vector-borne disease in the United States.[380] Recent analyses have estimated the incidence of Lyme borreliosis to be greater than 300,000 cases per year in the United States, 8- to 10-fold higher than reported cases.[381,382] Despite underreporting, it is also clear that the geographic range of the vector, the black-legged tick (*Ixodes scapularis*), has been steadily expanding from its core in New England westward to the Great Lakes, northward to southern Canada, and southward to the Mid-Atlantic states.[383] The black-legged tick is also the vector of two recently described relapsing fever agents—*Borrelia miyamotoi* and *Borrelia mayonii*—related to the Lyme spirochete. *B. miyamotoi* was first described in Japan in 1995[384] and in the United States in 2013[385] but is now known to occur in Europe as well,[386] suggesting it is not a recent introduction. *B. mayonii,* first reported in 2016 from a group of Lyme disease–negative patients, is characterized by an unusually high spirochete density in the blood.[387] Bourbon virus, the first human thogotovirus discovered in the Western Hemisphere,

was isolated from a postmortem specimen in Kansas in 2014.[388] It was initially suspected to be Heartland virus, a tick-borne phlebovirus virus discovered in Missouri in 2009.[389] Both viruses have been identified in *Amblyomma americanum,* the lone star tick, common in wooded and scrub areas in much of the eastern and midwestern regions of the United States, where it can also transmit species of *Ehrlichia.*

The range of Rocky Mountain spotted fever (*Rickettsia rickettsiae*) extends through much of North America, where the most common vectors are *Dermacentor variabilis,* the American dog tick, and *D. andersoni,* the Rocky Mountain wood tick. Recently, however, especially pernicious foci have been found on Native American lands in Arizona, where *Rhipicephalus sanguineus,* which feeds on both humans and dogs, is the vector. The close association between people and their dogs is the route of transmission, and a program to collar dogs with insecticide bands significantly reduced incidence.[390] There is increasing evidence that a significant proportion of reported *R. rickettsiae* cases are in fact *Rickettsia parkerii,* which presents with nearly identical symptoms and responds equally well to doxycycline, but has a much lower mortality when untreated.[391]

EBOLA AND MARBURG HEMORRHAGIC FEVERS

Ebola and Marburg viruses cause rare, severe, and often-fatal diseases in humans and nonhuman primates (monkeys, gorillas, and chimpanzees). Symptoms include fever, headache, joint and muscle aches, sore throat, and weakness, followed by diarrhea, vomiting, and stomach pain. Some patients also exhibit a rash, red eyes, hiccups, and internal and external bleeding. There is no licensed vaccine or standard treatment for Ebola or Marburg, and supportive therapy with fluid and electrolytes replacement, maintaining oxygen status and blood pressure, and providing treatment for any complicating infections remain the primary treatment.[392,393] Hemorrhagic fever outbreaks typically result from a single or small number of spillover events from the virus reservoir with subsequent chains of human-to-human transmission.[394] Prior to the 2014–16 outbreak of Ebola in West Africa, it was thought that transmission in health care settings could be prevented by adherence to basic infection control practices.[395] Experience gained during the outbreak response, including experience with "super-spreading" situations such as burial ceremonies, led to the adoption of more stringent control measures regarding use of personal protective equipment and disposal of potentially infectious items.[396]

The causative agents of Ebola and Marburg hemorrhagic fevers are filoviruses, belonging to the Filoviridae. These viruses are associated with fruit bats, which may be their natural animal reservoirs.[397–400] Marburg virus was first detected in 1967, when 31 cases (7 fatal) occurred in Germany and Yugoslavia among laboratory workers handling tissues from African green monkeys.[401] Eight years later, in 1975, a traveler returning from Rhodesia (now Zimbabwe) died in a hospital in Johannesburg, South Africa; his traveling companion and a nurse subsequently became ill, although both survived.[402] During the 1980s, two cases of Marburg hemorrhagic fever were reported in visitors to Kitum Cave in Mount Elgon National Park, Kenya.[403,404]

Ebola virus was first detected in 1976 as the cause of outbreaks with high fatality rates in Zaire (now the DRC)[405] and the Sudan.[406] The Ebola species associated with the 1976 outbreaks are Ebola virus and Sudan virus (formerly Ebola-Zaire and Ebola-Sudan). Other Ebola species that cause human disease include Tai Forest virus (isolated in 1994 when a scientist became ill after conducting an autopsy on a chimpanzee from the Tai Forest in Cote D'Ivoire)[407] and Bundibugyo virus (isolated in 2007 during an outbreak in Uganda).[408] A case of Sudan virus infection was reported in the United Kingdom in 1976 in a laboratory worker infected via the accidental stick of a contaminated needle,[409] and a case of Ebola virus infection was reported in South Africa in 1996 in a physician who had traveled to Johannesburg after treating Ebola virus–infected patients in Gabon (the site of three Ebola outbreaks during the 1990s). The physician survived, but a nurse who took care of him became infected and died.[410] A fifth species of Ebola was identified in Reston, Virginia, as the cause of severe illness and death in Philippine monkeys imported by research facilities in the United States in 1989 and 1990[411,412] and Italy in 1992.[413] In 2008, Reston virus was detected in pigs on two farms in the Philippines.[414] Six workers from the pig farm and from a slaughterhouse developed antibodies but did not become ill.

Since 2000, outbreaks of Ebola have recurred multiple times in central and East Africa, in the DRC, in Sudan, and in Uganda,[414a] and Marburg outbreaks have occurred in the DRC, Angola, and Uganda.[414b] The first Ebola outbreak in West Africa—and the largest outbreak of Ebola to date—began in Guinea and spread to neighboring Sierra Leone and Liberia, caused by the Ebola virus.[415,416] By the time it was controlled in the spring of 2016, it had caused more than 28,000 cases and 11,000 deaths in these three countries.[417] It was the first time Ebola transmission occurred in major urban settings. Secondary outbreaks occurred in Nigeria, Senegal, Mali, and the United States, and infected health care workers from Spain, Italy, the United Kingdom, and the United States were evacuated to their home countries (see also "Factors That Favor Disease Spread" previously). All of the secondary outbreaks were contained, and the epidemic virus did not spread outside of the affected countries in West Africa. The case-fatality proportion for patients managed in biocontainment units in Europe and the United States was 18.5% (versus 40%–70% in West Africa), highlighting the importance of critical care and advanced organ support.[418] The index case of the secondary outbreak in the United States was a hospitalized traveler from Liberia whose illness was initially misdiagnosed[44]; lessons learned from that experience led to improved local awareness and better understanding of infection control needs in hospitals.[392,393,396]

In addition to devastating health effects, Liberia, Sierra Leone, and Guinea experienced significant losses in gross domestic product.[419] An unprecedented international response was mounted to help control the outbreak, and trials were set up to evaluate promising therapeutics and vaccines, which advanced our toolbox to deal with Ebola. Helpful data on safety and immunogenicity of one of two vaccines was generated, but did not yield definitive efficacy results because the outbreak was waning.[420–423] With 17,000 Ebola survivors from the 2014–16 outbreak, long-term complications of joint and vision problems were observed.[424] It was also learned that the virus can persist in some body fluids, including semen, indicating potential risk of sexual transmission.[425–427]

Public health capacity building in West Africa to address viral hemorrhagic fevers, both during and after the Ebola outbreak, has included a focus on emergency response, laboratory capacity, disease surveillance, and workforce development.[428–431] Sustained efforts are needed to improve regional disease surveillance and to develop and test additional tools for prevention, early diagnosis, and treatment; clinical investigation during outbreaks is critical to evaluate vaccines and therapeutics and optimize clinical care and outcomes. Ecologic investigations of Ebola and Marburg viruses are also needed to better understand the natural reservoirs of these viruses and how they emerge and spread.

In May 2018, an Ebola vaccine developed during the outbreak in West Africa was deployed under a research protocol to help control an outbreak in Central Africa—in the DRC—that by May 2019 had infected 1600 people and claimed 1069 lives.[431a] Efforts to control that outbreak were hampered by violence and civil strife that limited responders' ability to provide treatment and immunization to affected communities.

CONCLUSION: CONTROLLING THE THREATS

Major infectious threats such as the AIDS pandemic, the 2003 SARS outbreak, and (more recently) the West Africa Ebola outbreak and the Zika virus epidemic in the Americas shock the world and spur renewed efforts to improve capacity to detect and control emerging threats. Efforts directed at emerging diseases must work in concert with initiatives to reduce high-burden infections that account for the majority of disease and disability caused by microbial agents. Each emergent event teaches particular lessons: HIV's emergence was long and insidious; Ebola's was explosive and logistically complex; Zika's dangers were unappreciated too long. In all cases, however, the most critical advantage would have been early detection of the initial cases of disease.

During the 1990s, the global devastation caused by AIDS, which spread undetected for many years, led nations around the world to address emerging diseases as a major diplomatic issue. In summit

TABLE 14.5 Global Health Initiatives to Address Emerging Infectious Diseases

The emergence and international spread of HIV/AIDS in the 1980s and 1990s led to the establishment of global health initiatives that address infectious diseases of international concern. They include:

1990s

- The Joint United Nations Program on HIV/AIDS (UNAIDS; http://www.unaids.org/)
- RBM Partnership to End Malaria (https://endmalaria.org/)
- Stop TB Partnership (http://www.stoptb.org/)
- International Partnership Against AIDS in Africa (IPAA; https://www.un.org/ga/aids/ungassfactsheets/html/fsipaa_en.htm)

2000s

- United Nations Development Programme (UNDP) Millennium Development Goals (2000–2015), including Goal 6: Combat HIV/AIDS, malaria, and other diseases (https://www.un.org/millenniumgoals/)
- Global Alliance for Vaccines and Immunization (Gavi; https://www.gavi.org/)
- Global Health Program, Bill and Melinda Gates Foundation (https://www.gatesfoundation.org/)
- Global Fund to Fight AIDS, Tuberculosis and Malaria (https://www.theglobalfund.org/en)
- International Health Regulations (IHR 2005; https://www.who.int/topics/international_health_regulations/en/)
- United States President's Emergency Plan for AIDS Relief (PEPFAR; https://www.pepfar.gov/)
- U.S. President's Malaria Initiative (PMI; https://www.pmi.gov/)
- One Health Initiative[a] (https://www.onehealthinitiative.com/)
- Emerging Pandemic Threats Program, USAID (https://www.usaid.gov/news-information/fact-sheets/emerging-pandemic-threats-program)

2010s

- Global Health Security Agenda (GHSA) partnership (https://www.ghsagenda.org/)
- UNDP Sustainable Development Goals (2016–2030), including Goal 3: Health (https://www.who.int/topics/sustainable-development-goals/targets/en/)
- Coalition for Epidemic Preparedness Innovations (CEPI; https://cepi.net/)
- U.S. National Strategy for Combating Antibiotic-Resistant Bacteria (CARB; https://www.cdc.gov/drugresistance/us-activities/national-strategy.html) issued in 2014, followed in 2015 by:
  - Publication of a US National Action Plan for Combating Antibiotic-Resistant Bacteria (https://www.cdc.gov/drugresistance/us-activities/national-action-plan.html)
  - Establishment of a Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB; https://www.cdc.gov/drugresistance/us-activities/paccarb.html)
- World Health Assembly endorsed a Global Action Plan on Antimicrobial Resistance in 2015 (https://www.who.int/antimicrobial-resistance/global-action-plan/en/)
- Meeting of global leaders at the United Nations General Assembly in New York in September 2016 to commit to fighting antimicrobial resistance together (https://www.who.int/antimicrobial-resistance/events/UNGA-meeting-amr-sept2016/en/)

[a]The One Health Initiative began as the One Health Initiative Team (2006–2015), which established a One Health Initiative website in 2008 (http://www.onehealthinitiative.com/news.php?query=History+of+the+One+Health+Initiative+team+(April+2006+through+September+2015)+and+the+One+Health+Initiative+website+since+October+1,+2008).

meetings in 1998 and 2000, the leaders of the Group of Eight industrialized nations pledged support for improving global surveillance for emerging threats, as well as for reducing deaths from "diseases of poverty" and vaccine-preventable diseases.[432] Global health initiatives such as the Bill and Melinda Gates Foundation, the Global Alliance for Vaccines and Immunization (now called Gavi, the Vaccine Alliance), and the Global Fund to Fight AIDS, Tuberculosis and Malaria were created to advance these goals (Table 14.5).

In the aftermath of the 2003 SARS outbreak, which spread overnight from Hong Kong to Canada via airplane travelers, the global public health community finalized the 2005 International Health Regulations (IHR 2005) as an international treaty that requires identification, reporting, and control of emerging threats.[432a] IHR 2005 requires member nations to report all Public Health Emergencies of International Concern (PHEICs) to the WHO, including not only outbreaks of infectious diseases but all biological, chemical, or radionuclear threats and natural disasters. IHR 2005 also requires prompt reporting of a single case of smallpox; poliomyelitis due to wild-type poliovirus; SARS; and human influenza caused by a new subtype. Although its primary goal is to improve national capacities to control threats at their source, IHR 2005 also encourages participation in international systems such as the WHO Global Outbreak Alert and Response Network (GOARN), which provides technical assistance during outbreak investigations, and the GISRS,[191] which tracks influenza viruses with pandemic potential (see "Influenza A and Pandemics" earlier). In 2014, government and nongovernment donors established the Global Health Security Agenda (GHSA) partnership, which provides guidance and training to help countries meet IHR requirements (https://www.ghsagenda.org/).

Containing and stopping the Ebola hemorrhagic fever epidemic in West Africa, which resembled historic outbreaks of pneumonic plague in its rapid person-to-person spread and high fatality rate, was especially challenging in a globalized world. Stopping transmission required close and complicated coordination among affected countries and their neighbors, donors, and international nongovernmental organizations. In the United States, lessons learned about coordination and continuity of outbreak operations informed the creation of the CDC Global Rapid Response Team (GRRT), which can be rapidly mobilized for extended missions and works in close partnership with GOARN.[433] A commitment was also made to help build public health capacity in high-risk countries, including the affected countries in West Africa.[434]

The outbreak also showed the importance of having plans and funds in place to develop and validate vaccines and identify the best available treatments while an outbreak is in progress.[420,435] The experience in West Africa led to calls for a global vaccine development fund[436] and for the creation of public-private research partnerships like the Coalition for Epidemic Preparedness Innovations (CEPI; Table 14.5), which aims "to stop future epidemics by developing new vaccines for a safer world" (https://cepi.net/). Efforts are also underway to develop universal influenza vaccines—vaccines that protect against all or most influenza viruses—as well as vaccines with enhanced immune protection and faster production in case of a pandemic.[437]

Other lessons include the need for greater innovation and investment to address vector-borne diseases such as Zika[380] (i.e., through improved vector control methods and enhanced expertise in entomology) and zoonotic diseases such as SARS and Ebola (i.e., through One Health approaches to monitoring infectious diseases in humans and animals).[438] Like the MERS epidemic, the SARS and Ebola epidemics were initiated by zoonotic pathogens that had gained capacity for human-to-human transmission, as had happened with HIV. Rapid detection of epizootic outbreaks in animals can forestall or mitigate human spillover, as has been demonstrated for influenza[439] and plague.[440] However, for obscure and emerging pathogens the surest warnings come from sensitive human surveillance, which is often poor in the places most receptive to emergence.[441]

In the future, technical advances in bioinformatics and next-generation sequencing will improve pathogen discovery[442] and eventually allow for earlier and more definitive identification of the causative agents of outbreaks, whether due to emerging pathogens, drug-resistant pathogens, or acts of bioterrorism. Public health laboratories in the United States are transitioning to rapid molecular methods with technical assistance from the CDC's Advanced Molecular Detection initiative.[442] As part of this transition, PulseNet and PulseNet International laboratories are using WGS to speed detection of outbreaks of foodborne diseases by linking cases of enteric disease.[443,444] Eventually diagnostic laboratories will likely further transition to metagenomic methods in which genetic material is recovered directly from clinical samples, without prior isolation (as needed for WGS), and analyzed by computer algorithms that rapidly identify pathogen sequences.

Further advances in data science that facilitate data-sharing and visualization of complex data will enable earlier recognition of emerging problems and better coordination of response efforts, especially in remote areas. Early warning systems such as the Program for Monitoring Emerging Diseases (ProMED-mail; https://www.promedmail.org/),[445] the Public Health Agency of Canada's Global Public Health Intelligence Network (GPHIN)[446], and HealthMap (https://www.healthmap.org/en/) are collecting, categorizing, and disseminating real-time outbreak

information from formal and informal sources. Internet and social media networks are increasingly being used for rapid exchange of health information and for broadening public health partnerships among nontraditional partners such as law enforcement, the media, and members of the public at local, national, and global levels.

Public health research in areas such as metagenomics[340,447,448] and mathematical modeling of infectious disease dynamics[449] are also moving us toward faster detection and control of emerging threats. Other promising areas include studies that improve understanding of host-pathogen interactions (e.g., microbiome research[450]) and develop tools for treatment and prevention (e.g., new antibiotics, environmentally safe pesticides, and strategies that use genetic manipulation to alter the population dynamics of disease-carrying mosquitoes[451]).

Combining modern innovations in disease surveillance and control with proven measures to reduce the burden of infectious diseases and advance global health equity can have a major impact on global health.

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Key References

The complete reference list is available online at Expert Consult.

1. Lederberg J. Pathways of discovery: infectious history. *Science*. 2000;288:287–293.
2. Morens DM, Fauci AS. Emerging infectious diseases: threats to human health and global stability. *PLoS Pathog*. 2013;9:e1003467.
3. Global Burden of Disease (GBD 2015) LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections (LRI) in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*. 2017;17:1133–1161.
4. Global Burden of Disease (GBD 2015) Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*. 2017;17:909–948.
11. Arwady MA, Bawo L, Hunter JC, et al. Evolution of Ebola virus disease from exotic infection to global health priority, Liberia, mid-2014. *Emerg Infect Dis*. 2015;21:578–584.
15. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature*. 2007;447:279–283.
16. Stärk KD, Morgan D. Emerging zoonoses: tackling the challenges. *Epidemiol Infect*. 2015 Jul;143:2015–2017.
22. Gargano LM, Hughes JM. Microbial origins of chronic diseases. *Annu Rev Public Health*. 2014;35:65–82.
28. Global Burden of Disease Liver Cancer Collaboration (GBD 2015). The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015. *JAMA Oncol*. 2017.
37. Conrad C, Bradley HM, Broz D, et al; Centers for Disease Control and Prevention (CDC). Community outbreak of HIV infection linked to injection drug use of oxymorphone–Indiana, 2015. *MMWR Morb Mortal Wkly Rep*. 2015;64:443–444.
38. Zibbell JE, Asher AK, Patel RC, et al. Increases in acute hepatitis C virus infection related to a growing opioid epidemic and associated injection drug use, United States, 2004 to 2014. *Am J Public Health*. 2018;108:175–181.
40. Rhoads WJ, Garner E, Ji P, et al. Distribution system operational deficiencies coincide with reported Legionnaires' disease clusters in Flint, Michigan. *Environ Sci Technol*. 2017;51:11986–11995.
48. Marston HD, Dixon DM, Knisely JM, et al. Antimicrobial resistance. *JAMA*. 2016;316:1193–1204.
54. World Bank. *Drug-Resistant Infections: A Threat to Our Economic Future*. Washington, DC: World Bank: March 2017. http://documents.worldbank.org/curated/en/323311493396993758/pdf/final-report.pdf.
55. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2013. http://www.cdc.gov/drugresistance/threat-report-2013/index.html.
69. Bolan GA, Sparling PF, Wasserheit JN. The emerging threat of untreatable gonococcal infection. *N Engl J Med*. 2012;66:485–487.
72. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis*. 2017;215(suppl_1):S28–S36.
74. Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother*. 2008;45:1151–1161.
75. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis*. 2010;10:597–602.
82. The Lancet Infectious Diseases. Tuberculosis reaches new milestones, good and bad. *Lancet Infect Dis*. 2015;15:1361.
98. Berendonk TU, Manaia CM, Merlin C, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol*. 2015;13:310–317.
122. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003;348:1967–1976.
123. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA*. 2005;102:12891–12896.
124. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med*. 2009;360:2605–2615.
125. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med*. 2012;367:1814–1820.
180. Widdowson MA, Bresee JS, Jernigan DB. The global threat of animal influenza viruses of zoonotic concern: then and now. *JID*. 2017;216(suppl 4):S493–S498.
207. Yang ZF, Mok CK, Peiris JS, et al. Human infection with a novel avian influenza A(H5N6) virus. *N Engl J Med*. 2015;373:487–489.
233. Uyeki TM, Katz JM, Jernigan DB. Novel influenza A viruses and pandemic threats. *Lancet*. 2017;389:2172–2174.
235. Zhou L, Chen E, Bao C, et al. Clusters of human infection and human-to-human transmission of avian influenza A(H7N9) virus, 2013-2017. *Emerg Infect Dis*. 2018;24.
271. Török TJ, Tauxe RV, Wise RP, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA*. 1997;278:389–395.
273. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States – major pathogens. *Emerg Infect Dis*. 2011;17:7–15.
274. Scallan E, Griffin PM, Angulo FJ, et al. Foodborne illness acquired in the United States - unspecified agents. *Emerg Infect Dis*. 2011;17:16–22.
311. He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. *Nat Genet*. 2013;45:109–113.
329. Editorial. The inexorable progress of norovirus. *Lancet Infect Dis*. 2013;13:97.
348. Sharp TM, Tomashek KM, Read JS, et al. A new look at an old disease: recent insights into the global epidemiology of dengue. *Curr Epidemiol Rep*. 2017;4:11–21.
352. Metsky HC, Matranga CB, Wohl S, et al. Zika virus evolution and spread in the Americas. *Nature*. 2017;546:411–415.
361. Zeller H, Van Bortel W, Sudre B. Chikungunya: its history in Africa and Asia and its spread to new regions in 2013-2014. *J Infect Dis*. 2016;214:S436–S440.
363. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. *Lancet*. 2018;391:1608–1621.
383. Mead PS. Epidemiology of Lyme disease. *Infect Dis Clin North Am*. 2015;29:187–210.
397. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature*. 2005;438:575–576.
400. Swanepoel R, Smit SB, Rollin PE, et al. Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis*. 2007;13:1847–1851.
418. Uyeki TM, Mehta AK, Davey RT Jr, et al; Working group of the U.S.–European clinical network on clinical management of Ebola virus disease patients in the U.S. and Europe. Clinical management of Ebola virus disease in the United States and Europe. *N Engl J Med*. 2016;374:636–646.
419. World Bank. 2014. The economic impact of the 2014 Ebola epidemic: short and medium term estimates for West Africa (English). Washington, DC: World Bank Group. http://documents.worldbank.org/curated/en/524521468141287875/The-economic-impact-of-the-2014-Ebola-epidemic-short-and-medium-term-estimates-for-West-Africa.
420. Samai M, Seward JF, Goldstein ST, et al; STRIVE Study Team. The Sierra Leone trial to introduce a vaccine against Ebola: an evaluation of rVSVΔG-ZEBOV-GP vaccine tolerability and safety during the West Africa Ebola outbreak. *J Infect Dis*. 2018;217(suppl_1):S6–S15.
438. Gebreyes WA, Dupouy-Camet J, Newport MJ, et al. The global one health paradigm: challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. *PLoS Negl Trop Dis*. 2014;8:e3257.
441. Rosenberg R. Detecting the emergence of novel, zoonotic viruses pathogenic to humans. *Cell Mol Life Sci*. 2015;72:1115–1125.
442. Gwinn M, MacCannell DR, Khabbaz RF. Integrating advanced molecular technologies into public health. *J Clin Microbiol*. 2017;55:703–714.
443. Ribot EM, Hise KB. Future challenges for tracking foodborne diseases: PulseNet, a 20-year-old US surveillance system for foodborne diseases, is expanding both globally and technologically. *EMBO Rep*. 2016 Nov;17:1499–1505.
448. Miller RR, Montoya V, Gardy JL, et al. Metagenomics for pathogen detection in public health. *Genome Med*. 2013;5:81.
450. Liang D, Leung RK, Guan W, et al. Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut Pathog*. 2018;10:3.

References

1. Lederberg J. Pathways of discovery: infectious history. *Science*. 2000;288:287–293.
2. Morens DM, Fauci AS. Emerging infectious diseases: threats to human health and global stability. *PLoS Pathog*. 2013;9:e1003467.
3. Global Burden of Disease (GBD 2015) LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections (LRI) in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*. 2017;17:1133–1161.
4. Global Burden of Disease (GBD 2015) Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*. 2017;17:909–948.
5. Gavi, the Vaccine Alliance. Facts and figures, vaccine support; 2017. https://www.gavi.org/about/mission/facts-and-figures/.
6. World Health Organization HIV/AIDS fact sheet; 2017. https://www.who.int/mediacentre/factsheets/fs360/en/.
7. UNAIDS. Global HIV & AIDS statistics—2018 fact sheet; 2019. http://www.unaids.org/en/resources/fact-sheet.
8. Global Burden of Disease (GBD 2015) HIV Collaborators. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. *Lancet HIV*. 2016;3:e361–e387.
9. Murray CJL, Ortblad KF, Guinovart C, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384:1005–1070.
10. World Health Organization. Fact sheet: world malaria report 2015; 2016. https://www.who.int/malaria/media/world-malaria-report-2016/en/.
11. Arwady MA, Bawo L, Hunter JC, et al. Evolution of Ebola virus disease from exotic infection to global health priority, Liberia, mid-2014. *Emerg Infect Dis*. 2015;21:578–584.
12. Heymann DL, Chen L, Takemi K, et al. Global health security: the wider lessons from the west African Ebola virus disease epidemic. *Lancet*. 2015;385:1884–1901.
13. Bausch DG. West Africa 2013 Ebola: from virus outbreak to humanitarian crisis. *Curr Top Microbiol Immunol*. 2017;411:63–92.
14. Coltart CE, Lindsey B, Ghinai I, et al. The Ebola outbreak, 2013-2016: old lessons for new epidemics. *Philos Trans R Soc Lond B Biol Sci*. 2017;32.
15. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature*. 2007 May 17;447:279–283.
16. Stärk KD, Morgan D. Emerging zoonoses: tackling the challenges. *Epidemiol Infect*. 2015 Jul;143:2015–2017.
17. McGrath EL, Rossi SL, Gao J, et al. Differential responses of human fetal brain neural stem cells to Zika Virus Infection. *Stem Cell Reports*. 2017;8:715–727.
18. Bartlett AW, McMullan B, Rawlinson WD, et al. Hearing and neurodevelopmental outcomes for children with asymptomatic congenital cytomegalovirus infection: a systematic review. *Rev Med Virol*. 2017.
19. Koneru A, Nelson N, Hariri S, et al. Increased Hepatitis C Virus (HCV) detection in women of childbearing age and potential risk for vertical transmission - United States and Kentucky, 2011-2014. *MMWR Morb Mortal Wkly Rep*. 2016;65:705–710.
20. Watts T, Stockman L, Martin J, et al. Increased risk for mother-to-infant transmission of hepatitis C virus among medicaid recipients - Wisconsin, 2011-2015. *MMWR Morb Mortal Wkly Rep*. 2017;66:1136–1139.
21. Bowen V, Su J, Torrone E, et al. Increase in incidence of congenital syphilis - United States, 2012-2014. *MMWR Morb Mortal Wkly Rep*. 2015;64:1241–1245.
22. Gargano LM, Hughes JM. Microbial origins of chronic diseases. *Annu Rev Public Health*. 2014;35:65–82.
23. Bagheri N, Shirzad H, Elahi S, et al. Downregulated regulatory T cell function is associated with increased peptic ulcer in Helicobacter pylori-infection. *Microb Pathog*. 2017;110:165–175.
24. Israeli E, Agmon-Levin N, Blank M, et al. Guillain-Barré syndrome–a classical autoimmune disease triggered by infection or vaccination. *Clin Rev Allergy Immunol*. 2012;42:121–130.
25. Ebert T, Kotler M. Prenatal exposure to influenza and the risk of subsequent development of schizophrenia. *Isr Med Assoc J*. 2005;7:35–38.
26. Brunham RC, Gottlieb SL, Paavonen J. Pelvic inflammatory disease. *N Engl J Med*. 2015;372:2039–2048.
27. Mackey TK, Liang BA, Cuomo R, et al. Emerging and reemerging neglected tropical diseases: a review of key characteristics, risk factors, and the policy and innovation environment. *Clin Microbiol Rev*. 2014;27:949–979.
28. Global Burden of Disease Liver Cancer Collaboration (GBD 2015). The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015. *JAMA Oncol*. 2017.

28a. National Cancer Institute. Cancer Moonshot Blue Ribbon Panel Report 2016. https://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative/blue-ribbon-panel/blue-ribbon-panel-report-2016.pdf.

29. Morgan NG, Richardson SJ. Enteroviruses as causative agents in type 1 diabetes: loose ends or lost cause? *Trends Endocrinol Metab*. 2014;25:611–619.
30. Jartti T, Gern JE. Role of viral infections in the development and exacerbation of asthma in children. *J Allergy Clin Immunol*. 2017;140:895–906.
31. Ponterio E, Gnessi L. Adenovirus 36 and obesity: an overview. *Viruses*. 2015;7:3719–3740.
32. Ivanova MV, Kolkova NI, Morgunova EY, et al. Role of Chlamydia in Multiple Sclerosis. *Bull Exp Biol Med*. 2015;159:646–648.
33. Dahal U, Sharma D, Dahal K. An Unsettled Debate About the Potential Role of Infection in Pathogenesis of Atherosclerosis. *J Clin Med Res*. 2017;9:547–554.
34. Leishman SJ, Ford PJ, West MJ, et al. Heat shock proteins: a double-edged sword linking periodontal and cardiovascular diseases. *Future Cardiol*. 2017.
35. Thompson JR. Is irritable bowel syndrome an infectious disease? *World J Gastroenterol*. 2016;22:1331–1334.
36. Mancuso R, Baglio F, Agostini S, et al. Relationship between herpes simplex virus-1-specific antibody titers and cortical brain damage in Alzheimer's disease and amnestic mild cognitive impairment. *Front Aging Neurosci*. 2014;6:285.
37. Conrad C, Bradley HM, Broz D, et al; Centers for Disease Control and Prevention (CDC). Community outbreak of HIV infection linked to injection drug use of oxymorphone–Indiana, 2015. *MMWR Morb Mortal Wkly Rep*. 2015;64:443–444.
38. Zibbell JE, Asher AK, Patel RC, et al. Increases in acute hepatitis C virus infection related to a growing opioid epidemic and associated injection drug use, United States, 2004 to 2014. *Am J Public Health*. 2018 Feb;108:175–181.
39. Saulnier DD, Brolin Ribacke K, von Schreeb J. No calm after the storm: a systematic review of human health following flood and storm disasters. *Prehosp Disaster Med*. 2017;32:568–579.
40. Rhoads WJ, Garner E, Ji P, et al. Distribution system operational deficiencies coincide with reported Legionnaires' disease clusters in Flint, Michigan. *Environ Sci Technol*. 2017 Oct 17;51:11986–11995.

40a. Centers for Disease Control and Prevention. 40 Years of Ebola virus disease around the world; 2019. https://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html.

41. Otu A, Ameh S, Osifo-Dawodu E, et al. An account of the Ebola virus disease outbreak in Nigeria: implications and lessons learnt. *BMC Public Health*. 2017;18:3.
42. Diarra B, Safronetz D, Sarro YD, et al. Laboratory response to 2014 Ebola virus outbreak in Mali. *J Infect Dis*. 2016;214(suppl 3):S164–S168.
43. Ka D, Fall G, Diallo VC, et al. Ebola virus imported from Guinea to Senegal, 2014. *Emerg Infect Dis*. 2017;23:1026–1028.
44. Chevalier MS, Chung W, Smith J, et al. Ebola virus disease cluster in the United States–Dallas County, Texas, 2014. *MMWR Morb Mortal Wkly Rep*. 2014;63:1087–1088.
45. Mora-Rillo M, Arsuaga M, Ramírez-Olivencia G, et al; La Paz-Carlos III University Hospital Isolation Unit. Acute respiratory distress syndrome after convalescent plasma use: treatment of a patient with Ebola virus disease contracted in Madrid, Spain. *Lancet Respir Med*. 2015;3:554–562.
46. Breakwell L, Gerber AR, Greiner AL, et al. Early Identification and Prevention of the Spread of Ebola in High-Risk African Countries. *MMWR Suppl*. 2016;65:21–27.
47. Cohen NJ, Brown CM, Alvarado-Ramy F, et al. Travel and Border Health Measures to Prevent the International Spread of Ebola. *MMWR Suppl*. 2016;65:57–67.
48. Marston HD, Dixon DM, Knisely JM, et al. Antimicrobial resistance. *JAMA*. 2016;316:1193–1204.
49. Shaw K, Singleton S. Antimicrobial resistance will kill millions–we must act now. *Nurs Times*. 2016;112:11.
50. Chioro A, Coll-Seck AM, Høie B, et al. Antimicrobial resistance: a priority for global health action. *Bull World Health Organ*. 2015;93:439.
51. Mendelson M, Matsoso MP. A global call for action to combat antimicrobial resistance: can we get it right this time? *S Afr Med J*. 2014;104:478–479.
52. Nolte O, et al. Antimicrobial resistance in the 21st century: a multifaceted challenge. *Protein Pept Lett*. 2014;21:330–335.
53. Fukuda K. Antimicrobial drug resistance threat: our duty towards future generations. *East Mediterr Health J*. 2013 May;19:399.
54. World Bank. *Drug-Resistant Infections: A Threat to Our Economic Future*. Washington, DC: World Bank: March 2017. http://documents.worldbank.org/curated/en/323311493396993758/pdf/final-report.pdf.
55. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2013. http://www.cdc.gov/drugresistance/threat-report-2013/index.html.
56. Molton JS, Tambyah PA, Ang BS, et al. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. *Clin Infect Dis*. 2013;56:1310–1318.
57. Rolain JM, Canton R, Cornaglia G. Emergence of antibiotic resistance: need for a new paradigm. *Clin Microbiol Infect*. 2012;18:615–616.
58. Bhullar K, Waglechner N, Pawlowski A, et al. Antibiotic resistance is prevalent in an isolated cave microbiome. *PLoS ONE*. 2012;7:e34953.
59. Levy SB. Microbial resistance to antibiotics: an evolving and persistent problem. *Lancet*. 1982;2:83–88.
60. Barber M, Rozwadowski-Dowzenko M. Infection by penicillin-resistant staphylococci. *Lancet*. 1948;2:641–644.
61. Barber M. Methicillin-resistant staphylococci. *J Clin Pathol*. 1961;14:385–393.
62. van Belkum A, Verbrugh H. 40 years of methicillin resistant *Staphylococcus aureus*. *BMJ*. 2001;323:644–645.
63. Hiramatsu K, Hanaki H, Ino T, et al. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*. 1997;40:135–136.
64. Smith TL, Pearson ML, Wilcox KR, et al. Emergence of vancomycin resistance in *Staphylococcus aureus*. *N Engl J Med*. 1999;340:493–501.
65. Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin—United States, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:565–567.
66. Fridkin SK. Vancomycin-intermediate and -resistant *Staphylococcus aureus*: what the infectious disease specialist needs to know. *Clin Infect Dis*. 2001;32:108–115.
67. Ortwine JK, Bhavan K. Morbidity, mortality, and management of methicillin-resistant *S. aureus* bacteremia in the USA: update on antibacterial choices and understanding. *Hosp Pract*. 2018;1–9.
68. Holubar M, Meng L, Deresinski S. Bacteremia due to methicillin-resistant *Staphylococcus aureus*: new therapeutic approaches. *Infect Dis Clin North Am*. 2016 Jun;30:491–507.
69. Bolan GA, Sparling PF, Wasserheit JN. The emerging threat of untreatable gonococcal infection. *N Engl J Med*. 2012;66:485–487.
70. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases treatment guidelines, 2010: oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep*. 2012;61:590–594.
71. Unemo M, Del Rio C, Shafer WM. Antimicrobial resistance expressed by Neisseria gonorrhoeae: a major global public health problem in the 21st century. *Microbiol Spectr*. 2016;4.
72. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis*. 2017;215(suppl_1):S28–S36.
73. Centers for Disease Control and Prevention (CDC). Vital signs: carbapenem-resistant Enterobacteriaceae. *MMWR Morb Mortal Wkly Rep*. 2013;62:165–170.
74. Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother*. 2008;45:1151–1161.
75. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis*. 2010;10:597–602.
76. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16:161–168.
77. Xavier BB, Lammens C, Ruhal R, et al. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. *Euro Surveill*. 2016;21.
78. Yin W, Li H, Shen Y, et al. Novel plasmid-mediated colistin resistance gene mcr-3 in Escherichia coli. *MBio*. 2017 Jun 27;8:pii: e00543-17.

78a. Centers for Disease Control and Prevention. Tracking the *mcr* gene; 2017. https://www.cdc.gov/drugresistance/biggest-threats/tracking/mcr.html.
79. Tsay S, Welsh RM, Adams EH, et al. Notes from the field: ongoing transmission of Candida auris in health care facilities - United States, June 2016-May 2017. *MMWR Morb Mortal Wkly Rep*. 2017;66:514–515.
80. Shah NS, Wright A, Gill-Han B, et al. Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis*. 2007;13:380–387.
81. Keal JL, Davies PD. Tuberculosis: a forgotten plague? *J R Soc Med*. 2011;104:182–184.
82. The Lancet Infectious Diseases. Tuberculosis reaches new milestones, good and bad. *Lancet Infect Dis*. 2015; 15:1361.
83. Dean AS, Cox H, Zignol M. Epidemiology of drug-resistant tuberculosis. *Adv Exp Med Biol*. 2017;1019: 209–220.
83a. World Health Organization. Global tuberculosis report 2018; 2018. https://www.who.int/tb/publications/global_report/en/.
83b. Centers for Disease Control and Prevention. The urgent threat of TB drug resistance; 2018. https://www.cdc.gov/globalhivtb/images/DGHT_MDR-TB_Factsheet.pdf.
83c. World Health Organization. The End TB Strategy; 2019. https://www.who.int/tb/strategy/en/.
83d. United States Agency for International Development. United States Government global tuberculosis strategy, 2015-2019; 2019. https://www.usaid.gov/sites/default/files/documents/1864/Reach-Cure-Prevent-2015-2019-TBStrategy.pdf.
83e. World Health Organization. Global action plan on antimicrobial resistance; 2015. https://www.who.int/antimicrobial-resistance/global-action-plan/en/.
83f. World Health Organization. United Nations high-level meeting on antimicrobial resistance; 2016. https://www.who.int/antimicrobial-resistance/events/UNGA-meeting-amr-sept2016/en/.
83g. Centers for Disease Control and Prevention. National action plan for combating antibiotic-resistant bacteria; 2015. https://www.cdc.gov/drugresistance/pdf/national_action_plan_for_combating_antibiotic-resistant_bacteria.pdf.
83h. Centers for Disease Control and Prevention. CDC & FDA antibiotic resistance (AR) isolate bank; 2018. https://www.cdc.gov/drugresistance/resistance-bank/index.html.
83i. Centers for Disease Control and Prevention. Lab capacity: Antibiotic Resistance Laboratory Network (AR Lab Network); 2018. https://www.cdc.gov/drugresistance/solutions-initiative/ar-lab-networks.html.
83j. Centers for Disease Control and Prevention. Data summary of HAIs in the US: assessing progress 2001-2016; 2018. https://www.cdc.gov/hai/data/archive/data-summary-assessing-progress.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fhai%2Fsurveillance%2Fdata-reports%2Fdata-summary-assessing-progress.html.
83k. Centers for Disease Control and Prevention. Making health care safer; 2017. https://www.cdc.gov/vitalsigns/stop-spread/index.html.
84. Delit TH, Owens RC, McGowan JE Jr, et al. Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clin Infect Dis*. 2007;44: 159–177.
85. Bartlet JG. A call to arms: the imperative for antimicrobial stewardship. *Clin Infect Dis*. 2011;53(suppl 1):S4–S7.
86. Davey P, Marwick CA, Scott CL, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev*. 2017;(2): CD003543.
87. Kaki R, Elligsen M, Walker S, et al. Impact of antimicrobial stewardship in critical care: a systematic review. *J Antimicrob Chemother*. 2011;66:1223–1230.
88. Beardsley JR, Williamson JC, Johnson JW, et al. Show me the money: long-term financial impact of an antimicrobial stewardship program. *Infect Control Hosp Epidemiol*. 2012;33:398–400.
89. Standiford HC, Chan S, Tripoli M, et al. Antimicrobial stewardship at a large tertiary care academic medical center: cost analysis before, during, and after a 7-year program. *Infect Control Hosp Epidemiol*. 2012;33:338–345.
90. Fridkin S, Baggs J, Fagan R, et al; Centers for Disease Control and Prevention (CDC). Vital signs: improving antibiotic use among hospitalized patients. *MMWR Morb Mortal Wkly Rep*. 2014;63:194–200.
90a. Centers for Disease Control and Prevention. Antibiotic prescribing and use; 2018. https://www.cdc.gov/antibiotic-use/.
91. World Health Organization. *The Evolving Threat of Antimicrobial Resistance: Options for Action*. Geneva: World Health Organization; 2012.
92. Tang KL, Caffrey NP, Nóbrega DB, et al. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet Planet Health*. 2017;1:e316–e327.
93. Hoelzer K, Wong N, Thomas J, et al. Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence? *BMC Vet Res*. 2017;13:211.
94. Rinsky J, Nadimpalli M, Wing S, et al. Livestock-associated methicillin and multidrug resistant *Staphylococcus aureus* is present among industrial workers, not antibiotic-free livestock operation workers in North Carolina. *PLoS ONE*. 2013;8:e67641.
95. Bergeron CR, Prussing C, Boerlin P, et al. Chicken as reservoir for extraintestinal pathogenic Escherichia coli in humans. *Emerg Infect Dis*. 2012;18:415–421.
96. Cogliani C, Goossens H, Greko C. Restricting antimicrobial use in food animals: lessons from Europe. *Microbe*. 2011;6:274–279.
97. US Food and Drug Administration, Animal and Veterinary. Judicious Use of Antimicrobials. http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/default.htm.
98. Berendonk TU, Manaia CM, Merlin C, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol*. 2015;13:310–317.
99. Waseem H, Williams MR, Stedtfeld RD, et al. Antimicrobial Resistance in the Environment. *Water Environ Res*. 2017;89:921–941.
100. Topp E, Larsson DGJ, Miller DN, et al. Antimicrobial resistance and the environment: assessment of advances, gaps and recommendations for agriculture, aquaculture and pharmaceutical manufacturing. *FEMS Microbiol Ecol*. 2018;94.
101. Spellberg B. The future of antibiotics. *Crit Care*. 2014;18:228.
102. Infectious Diseases Society of America. The 10 × '20 initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. *Clin Infect Dis*. 2010;50:1081–1083.
103. Outterson K, Powers JH, Daniel GW, et al. Repairing the broken market for antibiotic innovation. *Health Aff (Millwood)*. 2015;34:277–285.
104. Luepke KH, Mohr JF 3rd. The antibiotic pipeline: reviving research and development and speeding drugs to market. *Expert Rev Anti Infect Ther*. 2017;15:425–433.
105. US Food and Drug Administration Safety and Innovation Act (FDASIA). http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FDASIA/default.htm.
105a. CARB-X. Progress against superbugs: annual report 2017-2018; 2018. https://carb-x.org/wp-content/uploads/2018/09/CARB-X_AR-2017-18.pdf.
106. Spellberg B, Bartlett J, Wunderink R, et al. Novel approaches are needed to develop tomorrow's antibacterial therapies. *Am J Respir Crit Care Med*. 2015;191:135–140.
107. Roca I, Akova M, Baquero F, et al. The global threat of antimicrobial resistance: science for intervention. *New Microbes New Infect*. 2015;6:22–29.
108. Penchovsky R, Traykovska M. Designing drugs that overcome antibacterial resistance: where do we stand and what should we do? *Expert Opin Drug Discov*. 2015;10:631–650.
109. Harris SR, Cartwright EJ, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis*. 2013;13:130–136.
110. Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. *Sci Transl Med*. 2012;4:148ra116.
111. Köser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N Engl J Med*. 2012;366: 2267–2275.
112. Pecora ND, Li N, Allard M, et al. Genomically informed surveillance for carbapenem-resistant Enterobacteriaceae in a health care system. *MBio*. 2015;6:e01030.
113. Kanamori H, Parobek CM, Juliano JJ, et al. A prolonged outbreak of KPC-3-producing Enterobacter cloacae and Klebsiella pneumoniae driven by multiple mechanisms of resistance transmission at a large academic burn center. *Antimicrob Agents Chemother*. 2017;61.
114. Kyaw MH, Lynfield R, Schaffner W, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant Streptococcus pneumoniae. *N Engl J Med*. 2006;354:1455–1463.
115. Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis*. 2010;201:32–41.
116. Griffin MR, Zhu Y, Moore M, et al. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. *N Engl J Med*. 2013;369:155–163.
117. Weil-Olivier C, van der Linden M, de Schutter I, et al. Prevention of pneumococcal diseases in the post-seven valent vaccine era: a European perspective. *BMC Infect Dis*. 2012;12:207.
118. Neves FPG, Cardoso NT, Snyder RE, et al. Pneumococcal carriage among children after four years of routine 10-valent pneumococcal conjugate vaccine use in Brazil: the emergence of multidrug resistant serotype 6C. *Vaccine*. 2017;35:2794–2800.
119. Steens A, Bergsaker MA, Aaberge IS, et al. Prompt effect of replacing the 7-valent pneumococcal conjugate vaccine with the 13-valent vaccine on the epidemiology of invasive pneumococcal disease in Norway. *Vaccine*. 2013;31:6232–6238.
120. Tin Tin Htar M, Christopoulou D, Schmitt HJ. Pneumococcal serotype evolution in Western Europe. *BMC Infect Dis*. 2015;15:419.
121. Van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001;7:719–724.
122. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003;348:1967–1976.
123. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA*. 2005;102: 12891–12896.
124. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med*. 2009;360:2605–2615.
125. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med*. 2012;367:1814–1820.
126. Jartti T, Jartti L, Ruuskanen O, et al. New respiratory viral infections. *Curr Opin Pulm Med*. 2012;18:271–278.
127. Gao R, Cao B, Hu Y, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med*. 2013;368:1888–1897.
128. Uyeki TM. Human infection with highly pathogenic avian influenza A (H5N1) virus: review of clinical issues. *Clin Infect Dis*. 2009;49:279–290.
129. Jassal M, Bishai WR. Extensively drug-resistant tuberculosis. *Lancet Infect Dis*. 2009;9:19–30.
130. Global Burden of Disease 2015 (GBD 2015) Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388:1459–1544.
131. Murphy SL, Xu J, Kochanek KD, et al. Deaths: final data for 2015. *Natl Vital Stat Rep*. 2017;66:1–75.
132. Schildgen V, van den Hoogen B, Fouchier R, et al. Human metapneumovirus: lessons learned over the first decade. *Clin Microbiol Rev*. 2011;24:734–754.
133. Hermos CR, Vargas SO, McAdam AJ. Human metapneumovirus. *Clin Lab Med*. 2010;30:131–148.
134. Sastre P, Ruiz T, Schildgen O, et al. Seroprevalence of human respiratory syncytial virus and human metapneumovirus in healthy population analyzed by recombinant fusion protein-based enzyme linked immunosorbent assay. *Virol J*. 2012;9:130.
135. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. *J Infect Dis*. 2010;201:1890–1898.
136. Hahn A, Wang W, Jaggi P, et al. Human metapneumovirus infections are associated with severe morbidity in hospitalized children of all ages. *Epidemiol Infect*. 2013;7:1–11.
137. Edwards KM, Zhu Y, Griffin MR, et al. Burden of human metapneumovirus infection in young children. *N Engl J Med*. 2013;368:633–643.
138. Papenburg J, Hamelin ME, Ouhoummane N, et al. Comparison of risk factors for human metapneumovirus and respiratory syncytial virus disease severity in young children. *J Infect Dis*. 2012;206:178–189.
139. Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis*. 2012;206:56–62.
140. Haas LE, Thijsen SF, van Elden L, et al. Human metapneumovirus in adults. *Viruses*. 2013;8:87–110.

141. Liao RS, Appelgate DM, Pelz RK. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility for the elderly in Oregon. *J Clin Virol*. 2012;53:171–173.
142. Midgley CM, Baber JK, Biggs HM, et al. Severe human metapneumovirus infections—North Dakota, 2016. *MMWR*. 2017;66:486–488.
143. Shahda S, Carlos WG, Kiel PJ, et al. The human metapneumovirus: a case series and review of the literature. *Transpl Infect Dis*. 2011;13:324–328.
144. Feuillet F, Lina B, Rosa-Calatrava M, et al. Ten years of human metapneumovirus research. *J Clin Virol*. 2012;53:97–105.
145. Wevers BA, van der Hoek L. Recently discovered human coronaviruses. *Clin Lab Med*. 2009;29:715–724.
146. Enserink M. War stories. *Science*. 2013;339:1264–1268.
147. Abdul-Rasool S, Fielding BC. Understanding human coronavirus HCoV-NL63. *Open Virol J*. 2010;4:76–84.
148. Woo PC, Lau SK, Yip CC, et al. More and more coronaviruses: human coronavirus HKU1. *Viruses*. 2009;1:57–71.
149. Balboni A, Battilani M, Prosperi S. The SARS-like coronaviruses: the role of bats and evolutionary relationships with SARS coronavirus. *New Microbiol*. 2012;35:1–16.
150. Anderson LJ, Tong S. Update on SARS research and other possibly zoonotic coronaviruses. *Int J Antimicrob Agents*. 2010;36:S21–S25.
151. Hui DSC, Chan PKS. Severe acute respiratory syndrome and coronavirus. *Infect Dis Clin North Am*. 2010;24:619–638.
152. Cleri DJ, Ricketti AJ, Vernaleo JR. Severe acute respiratory syndrome (SARS). *Infect Dis Clin North Am*. 2010;24:175–202.
153. World Health Organization. Summary of Probable SARS Cases with Onset of Illness from 1 November 2002 to 31 July 2003. http://www.who.int/csr/sars/country/table2004_04_21/en/index.html.
154. European Centre for Disease Prevention and Control. Updated Rapid Risk Assessment: Severe Respiratory Disease Associated with Middle East Respiratory Syndrome Coronavirus (MERS-CoV). http://www.ecdc.europa.eu/en/publications/Publications/mers-cov-risk-assessment-6-november-2013.pdf.
155. World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV) – The Kingdom of Saudi Arabia. Disease Outbreak News: Update 24 April 2019. https://www.who.int/csr/don/24-April-2019-mers-saudi-arabia/en/.
156. World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV). WHO MERS-CoV Global Summary and Assessment of Risk. 21 July 2017. WHO/MERS/RA.
157. Arabi YM, Al-Omari A, Mandourah Y, et al; Saudi Critical Care Trial Group. Critically ill patients with the Middle East respiratory syndrome: a multicenter retrospective Cohort Study. *Crit Care Med*. 2017 Oct;45:1683–1695.
158. Arabi YM, Balkhy HH, Hayden FG, et al. Middle East respiratory syndrome. *N Engl J Med*. 2017;376:584–594.
159. Ng DL, Al Hosani F, Keating MK, et al. Clinicopathologic, immunohistochemical, and ultrastructural findings of a fatal case of Middle East respiratory syndrome coronavirus infection in the United Arab Emirates, April 2014. *Am J Pathol*. 2016;186:652–658.
160. Centers for Disease Control and Prevention. First confirmed cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in the United States, updated information on the epidemiology of MERS-CoV infection, and guidance for the public, clinicians, and public health authorities—May 2014. *MMWR Morb Mortal Wkly Rep*. 2014;63:431–436.
161. Korea Centers for Disease Control and Prevention. Middle East Respiratory syndrome coronavirus outbreak in the Republic of Korea, 2015. *Osong Public Health Res Perspect*. 2015;6:269–278.
162. Uyeki TM, Erlandson KJ, Korch G, et al. Development of medical countermeasures to Middle East respiratory syndrome coronavirus. *Emerg Infect Dis*. 2016;22.
163. van Boheemen S, de Graaf M, Lauber C, et al. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *MBio*. 2012;3:e00473-12.
164. Perlman S, Zhao J. Human coronavirus EMC is not the same as severe acute respiratory syndrome coronavirus. *MBio*. 2013;4:e00002-13.
165. Annan A, Baldwin HJ, Corman VM, et al. Human betacoronavirus 2c EMC/2012–related viruses in bats, Ghana and Europe. *Emerg Infect Dis*. 2013;19:456–459.
166. Memish ZA, Mishra N, Olival KJ, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. *Emerg Infect Dis*. 2013;19:1819–1823.
167. Memish ZA, Cotten M, Meyer B, et al. Human infection with MERS coronavirus after exposure to infected camels, Saudi Arabia, 2013. *Emerg Infect Dis*. 2014;20:1012–1015.
168. Azhar EI, El-Kafrawy SA, Farraj SA, et al. Evidence for camel-to-human transmission of MERS coronavirus. *N Engl J Med*. 2014;370:2499–2505.
169. Müller MA, Corman VM, Jores J, et al. MERS coronavirus neutralizing antibodies in camels, Eastern Africa, 1983-1997. *Emerg Infect Dis*. 2014;20:2093–2095.
169a. Conzade R, Grant R, Malik MR, et al. Reported direct and indirect contact with dromedary camels among Laboratory-Confirmed MERS-CoV cases. *Viruses*. 2018;10:E425.
170. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature*. 2013;495:251–254.
171. Müller MA, Raj VS, Muth D, et al. Human coronavirus EMC does not require the SARS-coronavirus receptor and maintains broad replicative capability in mammalian cell lines. *MBio*. 2012;3:e00515–12.
172. Reusken CB, Haagmans BL, Muller MA, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infect Dis*. 2013;13:859–866.
173. Guido M, Tumolo MR, Verri T, et al. Human bocavirus: current knowledge and future challenges. *World J Gastroenterol*. 2016;22:8684–8697.
174. Jartti T, Hedman K, Jartti L, et al. Human bocavirus—the first 5 years. *Rev Med Virol*. 2012;22:46–64.
175. Lüsebrink J, Wittleben F, Schildgen V, et al. Human bocavirus—insights into a newly identified respiratory virus. *Viruses*. 2009;1:3–12.
176. Milder E, Arnold JC. Human metapneumovirus and human bocavirus in children. *Pediatr Res*. 2009;65:78R–83R.
177. Visher E, Whitefield SE, McCrone JT, et al. The mutational robustness of influenza A virus. *PLoS Pathog*. 2016;12:e1005856.
178. Lauring AS, Andino R. Quasispecies theory and the behavior of RNA viruses. *PLoS Pathog*. 2010 Jul 22;6:e1001005.
179. Stevens J, Blixt O, Glaser L, et al. Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. *J Mol Biol*. 2006;355:1143–1155.
180. Widdowson MA, Bresee JS, Jernigan DB. The global threat of animal influenza viruses of zoonotic concern: then and now. *JID*. 2017 Sep 15;216(suppl 4):S493–S498.
181. Yoon SW, Webby RJ, Webster RG. Evolution and ecology of influenza A viruses. *Curr Top Microbiol Immunol*. 2014;385:359–375.
182. Olsen B, Munster VJ, Wallensten A, et al. Global patterns of influenza A virus in wild birds. *Science*. 2006 Apr 21;312:384–388, Review.
183. Lee DH, Bertran K, Kwon JH, et al. Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. *J Vet Sci*. 2017 Aug 31;18(S1):269–280.
184. World Organisation for Animal Health (OIE). Terrestrial Animal Code: Ch 10.4. Infection with Avian Influenza Viruses. http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_avian_influenza_viruses.htm. Accessed April 26, 2018.
185. Ma W, Lager KM, Vincent AL, et al. The role of swine in the generation of novel influenza viruses. *Zoonoses Public Health*. 2009;56:326–337. Aug.
186. Centers for Disease Control and Prevention. Past Pandemics. https://www.cdc.gov/flu/pandemic-resources/basics/past-pandemics.html. Accessed April 3, 2018.
187. Johnson NP, Mueller J. Updating the accounts: global mortality of the 1918-1920 "Spanish" Influenza Pandemic. *Bull Hist Med*. 2002;76:105–111.
188. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science*. 2009;325:197–201.
189. Nelson MI, Vincent AL. Reverse zoonosis of influenza to swine: new perspectives on the human-animal interface. *Trends Microbiol*. 2015;23:142–153.
190. Vincent A, Awada L, Brown I, et al. Review of influenza A virus in swine worldwide: a call for increased surveillance and research. *Zoonoses Public Health*. 2014;61:4–17.
191. World Health Organization. Global Influenza Surveillance and Response System (GISRS). http://www.who.int/influenza/gisrs_laboratory/en/. Accessed April 3, 2018.
192. World Health Organization. International Health Regulations (2005) Second Edition. p.43. http://www.who.int/ihr/9789241596664/en/. Accessed April 3, 2018.
193. Freidl GS, Meijer A, de Bruin E, et al. Influenza at the animal-human interface: a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1). *Euro Surveill*. 2014;19:pii: 20793.
194. World Health Organization. Avian and other zoonotic influenza. http://www.who.int/influenza/human_animal_interface/en/. Accessed April 3, 2018.
195. Subbarao K, Klimov A, Katz J, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science*. 1998;279:393–396.
196. Chan PK. Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. *Clin Infect Dis*. 2002;34(suppl 2):S58–S64.
197. Bridges CB, Lim W, Hu-Primmer J, et al. Risk of influenza A (H5N1) infection among poultry workers, Hong Kong, 1997-1998. *J Infect Dis*. 2002;185:1005–1010.
198. Katz JM, Lim W, Bridges CB, et al. Antibody response in individuals infected with avian influenza A (H5N1) viruses and detection of anti-H5 antibody among household and social contacts. *J Infect Dis*. 1999;180:1763–1770.
199. Buxton Bridges C, Katz JM, Seto WH, et al. Risk of influenza A (H5N1) infection among health care workers exposed to patients with influenza A (H5N1), Hong Kong. *J Infect Dis*. 2000;181:344–348.
200. Peiris JS, Yu WC, Leung CW, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet*. 2004;363:617–619.
201. Lai S, Qin Y, Cowling BJ, et al. Global epidemiology of avian influenza A H5N1 virus infection in humans, 1997-2015: a systematic review of individual case data. *Lancet Infect Dis*. 2016;16:e108–e118.
202. Mounts AW, Kwong H, Izurieta HS, et al. Case-control study of risk factors for avian influenza A (H5N1) disease, Hong Kong, 1997. *J Infect Dis*. 1999;180:505–508.
203. Zhou L, Liao Q, Dong L, et al. Risk factors for human illness with avian influenza A (H5N1) virus infection in China. *J Infect Dis*. 2009;199:1726–1734.
204. Wang H, Feng Z, Shu Y, et al. Probable limited person-to-person transmission of highly pathogenic avian influenza A (H5N1) virus in China. *Lancet*. 2008;371:1427–1434.
205. Kandun IN, Wibisono H, Sedyaningsih ER, et al. Three Indonesian clusters of H5N1 virus infection in 2005. *N Engl J Med*. 2006;355:2186–2194.
206. World Health Organization. Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO, 2003-2018. 2 March 2018. http://www.who.int/influenza/human_animal_interface/2018_03_02_tableH5N1.pdf?ua=1.
207. Yang ZF, Mok CK, Peiris JS, et al. Human infection with a novel avian influenza A(H5N6) virus. *N Engl J Med*. 2015;373:487–489.
208. Pan M, Gao R, Lv Q, et al. Human infection with a novel, highly pathogenic avian influenza A (H5N6) virus: virological and clinical findings. *J Infect*. 2016;72:52–59.
209. Jiang H, Wu P, Uyeki TM, et al. Preliminary epidemiologic assessment of human infections with highly pathogenic avian influenza A(H5N6) virus, China. *Clin Infect Dis*. 2017;65:383–388.
210. World Health Organization. Influenza at the human-animal interface. Summary and assessment, 22 September to 1 November 2018. https://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_01_11_2018.pdf?ua=1].
211. Herfst S, Mok CKP, van den Brand JMA, et al. Human clade 2.3.4.4 A/H5N6 influenza virus lacks mammalian adaptation markers and does not transmit via the airborne route between ferrets. *mSphere*. 2018;3:pii: e00405-17.
212. Tsunekuni R, Yaguchi Y, Kashima Y, et al. Spatial transmission of H5N6 highly pathogenic avian influenza viruses among wild birds in Ibaraki Prefecture, Japan, 2016-2017. *Arch Virol*. 2018;163:1195–1207.
213. Beerens N, Koch G, Heutink R, et al. Novel Highly Pathogenic Avian Influenza A(H5N6) virus in the Netherlands, December 2017. *Emerg Infect Dis*. 2018;24.
214. Mellor KC, Meyer A, Elkholly DA, et al. Comparative epidemiology of highly pathogenic avian influenza virus H5N1 and H5N6 in Vietnamese live bird markets: spatiotemporal patterns of distribution and risk factors. *Front Vet Sci*. 2018;5:51.
215. Zhang J, Lao G, Zhang R, et al. Genetic diversity and dissemination pathways of highly pathogenic H5N6 avian influenza viruses from birds in Southwestern China along the East Asian-Australian migration flyway. *J Infect*. 2018;76:418–422.
216. Lu S, Zhao Z, Zhang J, et al. Genetics, pathogenicity and transmissibility of novel reassortant H5N6 highly pathogenic avian influenza viruses first isolated from migratory birds in western China. *Emerg Microbes Infect*. 2018;7:6.
217. de Vries E, Guo H, Dai M, et al. Rapid emergence of highly pathogenic avian influenza subtypes from a

subtype H5N1 hemagglutinin variant. *Emerg Infect Dis*. 2015;21:842–846.

218. Guo H, de Vries E, McBride R, et al. Highly pathogenic influenza A(H5Nx) viruses with altered H5 receptor-binding specificity. *Emerg Infect Dis*. 2017;23:220–231.
219. Lee DH, Bertran K, Kwon JH, et al. Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3.4.4. *J Vet Sci*. 2017;18(S1): 269–280.
220. Li Q, Zhou L, Zhou M, et al. Epidemiology of human infections with avian influenza A(H7N9) virus in China. *N Engl J Med*. 2014;370:520–532.
221. Liu B, Havers F, Chen E, et al. Risk factors for influenza A(H7N9) disease–China, 2013. *Clin Infect Dis*. 2014;59: 787–794.
222. Gao HN, Lu HZ, Cao B, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. *N Engl J Med*. 2013;368:2277–2285.
223. Hu Y, Lu S, Song Z, et al. Association between adverse clinical outcome in human disease caused by novel influenza A H7N9 virus and sustained viral shedding and emergence of antiviral resistance. *Lancet*. 2013;381:2273–2279.
224. Marjuki H, Mishin VP, Chesnokov AP, et al. Characterization of drug-resistant influenza A(H7N9) variants isolated from an oseltamivir-treated patient in Taiwan. *J Infect Dis*. 2015;211:249–257.
225. Xu C, Havers F, Wang L, et al. Monitoring avian influenza A(H7N9) virus through national influenza-like illness surveillance, China. *Emerg Infect Dis*. 2013;19:1289–1292.
226. Chen Z, Liu H, Lu J, et al. Asymptomatic, mild, and severe influenza A(H7N9) virus infection in humans, Guangzhou, China. *Emerg Infect Dis*. 2014;20:1535–1540.
227. Yu H, Cowling BJ, Feng L, et al. Human infection with avian influenza A H7N9 virus: an assessment of clinical severity. *Lancet*. 2013;382:138–145.
228. Yu H, Wu JT, Cowling BJ, et al. Effect of closure of live poultry markets on poultry-to-person transmission of avian influenza A H7N9 virus: an ecological study. *Lancet*. 2014;383:541–548.
229. Virlogeux V, Feng L, Tsang TK, et al. Evaluation of animal-to-human and human-to-human transmission of influenza A (H7N9) virus in China, 2013-15. *Sci Rep*. 2018;8:552.
230. Zhou L, Ren R, Ou J, et al. Risk factors for influenza A(H7N9) disease in China, a matched case control study, October 2014 to April 2015. *Open Forum Infect Dis*. 2016;3:ofw182.
231. Wang X, Jiang H, Wu P, et al. Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland China, 2013-17: an epidemiological study of laboratory-confirmed case series. *Lancet Infect Dis*. 2017;17:822–832.
232. Kile JC, Ren R, Liu L, et al. Comparison of the first three waves of avian influenza A(H7N9) virus circulation in the mainland of the People's Republic of China. *MMWR Morb Mortal Wkly Rep*. 2017;66:928–932.
233. Uyeki TM, Katz JM, Jernigan DB. Novel influenza A viruses and pandemic threats. *Lancet*. 2017;389: 2172–2174.
234. Zhou L, Tan Y, Kang M, et al. Preliminary epidemiology of human infections with highly pathogenic avian influenza A(H7N9) virus, China, 2017. *Emerg Infect Dis*. 2017;23:1355–1359.
235. Zhou L, Chen E, Bao C, et al. Clusters of human infection and human-to-human transmission of avian influenza A(H7N9) virus, 2013-2017. *Emerg Infect Dis*. 2018;24.
236. William T, Thevarajah B, Lee SF, et al. Avian influenza (H7N9) virus infection in Chinese tourist in Malaysia, 2014. *Emerg Infect Dis*. 2015;21:142–145.
237. Chang SY, Lin PH, Tsai JC, et al. The first case of H7N9 influenza in Taiwan. *Lancet*. 2013;381:1621.
238. Skowronski DM, Chambers C, Gustafson R, et al. Avian Influenza A(H7N9) virus infection in 2 travelers returning from China to Canada, January 2015. *Emerg Infect Dis*. 2016;22:71–74.
239. World Health Organization. Influenza at the human-animal interface. Summary and assessment, 26 January to 2 March 2018. http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_02_03_2018.pdf?ua=1.
240. Fouchier RA, Schneeberger PM, Rozendaal FW, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci USA*. 2004;101:1356–1361.
241. Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet*. 2004;363:587–593.
242. Lopez-Martinez I, Balish A, Barrera-Badillo G, et al. Highly pathogenic avian influenza A(H7N3) virus in poultry workers, Mexico, 2012. *Emerg Infect Dis*. 2013;19:1531–1534.
243. Avian influenza A/(H7N2) outbreak in the United Kingdom. Editorial team, Collective, Weekly releases (1997–2007), 12, 3206 (2007), https://doi.org/10.2807/esw.12.22.03206-en.
244. Tweed SA, Skowronski DM, David ST, et al. Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis*. 2004;10:2196–2199.
245. Nguyen-Van-Tam JS, Nair P, Acheson P, et al; Incident Response Team. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. *Euro Surveill*. 2006;11:E060504.2.
246. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. *Lancet*. 1996;348:901–902.
247. Arzey GG, Kirkland PD, Arzey KE, et al. Influenza virus A (H10N7) in chickens and poultry abattoir workers, Australia. *Emerg Infect Dis*. 2012;18:814–816.
248. Wei SH, Yang JR, Wu HS, et al. Human infection with avian influenza A H6N1 virus: an epidemiological analysis. *Lancet Respir Med*. 2013;1:771–778.
249. Terebuh P, Adija A, Edwards L, et al. Human infection with avian influenza A(H7N2) virus-Virginia, 2002. *Influenza Other Respir Viruses*. 2018;12:529–532.
250. Uyeki TM, Chong YH, Katz JM, et al. Lack of evidence for human-to-human transmission of avian influenza A (H9N2) viruses in Hong Kong, China 1999. *Emerg Infect Dis*. 2002;8:154–159.
251. Cheng VC, Chan JF, Wen X, et al. Infection of immunocompromised patients by avian H9N2 influenza A virus. *J Infect*. 2011;62:394–399.
252. World Health Organization. Influenza at the human-animal interface. Summary and assessment as of 23 June 2015. http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_23_June_2015.pdf?ua=1.
253. Ostrowsky B, Huang A, Terry W, et al. Low pathogenic avian influenza A (H7N2) virus infection in immunocompromised adult, New York, USA, 2003. *Emerg Infect Dis*. 2012;18:1128–1131.
254. World Health Organization. Human infection with avian influenza A(H7N4) virus – China. Disease outbreak news 22 February 2018. http://www.who.int/csr/don/22-february-2018-ah7n4-china/en/.
255. World Health Organization. Influenza at the human-animal interface. Summary and assessment, 21 January to 25 February 2016. http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_25_02_2016.pdf?ua=1.
256. Chen H, Yuan H, Gao R, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. *Lancet*. 2014;383:714–721.
257. World Health Organization. Influenza at the human-animal interface. Summary and assessment, 20 July to 3 October 2016. http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_10_03_2016.pdf?ua=1.
258. Lee CT, Slavinski S, Schiff C, et al; Influenza A(H7N2) Response Team. Outbreak of influenza A(H7N2) among cats in an animal shelter with cat-to-human transmission-New York City, 2016. *Clin Infect Dis*. 2017;65:1927–1929.
259. Webster RG, Geraci J, Petursson G, et al. Conjunctivitis in human beings caused by influenza A virus of seals. *N Engl J Med*. 1981;304:911.
260. Freidl GS, Meijer A, de Bruin E, et al; FLURISK Consortium. Influenza at the animal-human interface: a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1). *Euro Surveill*. 2014;19:pii: 20793.
261. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. *Clin Infect Dis*. 2007;44:1084–1088.
262. Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. *N Engl J Med*. 2009;360:2616–2625.
263. Jhung MA, Epperson S, Biggerstaff M, et al. Outbreak of variant influenza A(H3N2) virus in the United States. *Clin Infect Dis*. 2013;57:1703–1712.
264. Rovida F, Piralla A, Marzani FC, et al. Swine influenza A (H1N1) virus (SIV) infection requiring extracorporeal life support in an immunocompetent adult patient with indirect exposure to pigs, Italy, October 2016. *Euro Surveill*. 2017;22.
265. Fraaij PL, Wildschut ED, Houmes RJ, et al. Severe acute respiratory infection caused by swine influenza virus in a child necessitating extracorporeal membrane oxygenation (ECMO), the Netherlands, October 2016. *Euro Surveill*. 2016;21:pii: 30416.
266. Greenbaum A, Quinn C, Bailer J, et al. Investigation of an outbreak of variant influenza A(H3N2) virus infection associated with an agricultural Fair-Ohio, August 2012. *J Infect Dis*. 2015;212:1592–1599.
267. Centers for Disease Control and Prevention. Summary of Influenza Risk Assessment Tool (IRAT) Results. https://www.cdc.gov/flu/pandemic-resources/monitoring/irat-virus-summaries.htm. Accessed April 23, 2018.
268. World Health Organization. Tool for Influenza Pandemic Risk Assessment (TIPRA). http://www.who.int/influenza/areas_of_work/human_animal_interface/tipra/en/. Accessed April 23, 2018.
269. Brooks N, Regmi A, Jerardo AUS. *Food Import Patterns, 1998–2007. Outlook No. FAU-125*. Washington, DC: U.S Department of Agriculture, Economic Research Service; 2009.
270. McLaughlin JB, DePaola A, Bopp CA, et al. Outbreak of Vibrio parahaemolyticus gastroenteritis associated with Alaskan oysters. *N Engl J Med*. 2005;353:1463–1470.
271. Török TJ, Tauxe RV, Wise RP, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA*. 1997;278:389–395.
272. Global Burden of Disease (GBD 2015) Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388:1545–1602.
273. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States – major pathogens. *Emerg Infect Dis*. 2011;17:7–15.
274. Scallan E, Griffin PM, Angulo FJ, et al. Foodborne illness acquired in the United States - unspecified agents. *Emerg Infect Dis*. 2011;17:16–22.
275. Messner M, Shaw S, Regli S, et al. An approach for developing a nation estmate of waterborne disease due to drinking water and a national estimate model aplication. *J Water Health*. 2006;4:201–240.
276. Sheth AN, Hoekstra M, Patel N, et al. A national outbreak of Salmonella serotype Tennessee infections from contaminated peanut butter: a new food vehicle for salmonellosis in the United States. *Clin Infect Dis*. 2011;53:356–362.
277. Crowe SJ, Bottichio L, Shade LN, et al. Shiga toxin-producing E. coli infections associated with flour. *N Engl J Med*. 2017;377:2036–2043.
278. Langer AJ, Ayers T, Grass J, et al. Nonpasteurized dairy products, disease outbreaks, and state laws-United States, 1993–2006. *Emerg Infect Dis*. 2012;18:385–391.
279. Sharapov UM, Wendel AM, Davis JP, et al. Multistate outbreak of Escherichia coli O157:H7 infections associated with consumption of fresh spinach: United States, 2006. *J Food Protection*. 2016;79:2024–2030.
280. Jay MT, Cooley M, Carychao D, et al. Escherichia coli O157:H7 in feral swine near spinach fields and cattle, Central California Coast. *Emerg Infect Dis*. 2007;13:1908–1911.
281. Herwaldt BL, Ackers ML, Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *N Engl J Med*. 1997;336: 1548–1556.
282. Soave R. Cyclospora: an overview. *Clin Infect Dis*. 1996;23:429–437.
283. Hlavsa MC, Roberts VA, Kahler AM, et al. Outbreaks of illnesses associated with recreational water- United States, 2011-2012. *MMWR*. 2015;64:668–672.
284. Hale CR, Scall E, Cronquist AB, et al. Estimates of enteric illness attributable to contact with animals and their environments in the United States. *Clin Infect Dis*. 2012;54(suppl 5):S472–S479.
285. Basler C, Nguyen TA, Anderson TC, et al. Outbreaks of human Salmonella infections associated with live poultry, United States, 1990-2014. *Emerg Infect Dis*. 2016;22:1705–1711.
286. Morris JG Jr. Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. *Clin Infect Dis*. 2003;37:272–280.
287. Begue RE, Castellares G, Hayashi KE, et al. Diarrheal disease in Peru, after the introduction of cholera. *Am J Trop Med Hyg*. 1994;51:585–589.
288. Vugia DJ, Rodriguez M, Vargas R, et al. Epidemic cholera in Trujillo, Peru, 1992: utility of a clinical case definition and shift in Vibrio cholerae O1 serotype. *Am J Trop Med Hyg*. 1994;50:566–569.
289. Walton DA, Ivers LC. Responding to cholera in post-earthquake Haiti. *N Engl J Med*. 2011;364:3–5.
290. Dowell SF, Tappero JW, Frieden TR. Public health in Haiti – challenges and progress. *N Engl J Med*. 2011;364:300–301.
291. Chin C-S, Sorenson J, Harris JB, et al. The origin of the Haitian cholera outbreak strain. *N Engl J Med*. 2011;364:33–42.

292. Piarroux R, Barrais R, Faucher B, et al. Understanding the cholera epidemic, Haiti. *Em Inf Dis*. 2011;17:1161–1167.
293. Dowell SF, Braden CR. Implications of the introduction of cholera to Haiti. *Emerg Infect Dis*. 2011;17:1299–1300.
294. Kupferschmidt. Second bacterium theory stirs Haiti's cholera controversy. *Science*. 2012;336:1493.
295. Cholera 2016. *Wkly Epidemiol Rec*. 2017;92:521–530.
296. Rohde H, Qin J, Cui Y, et al. Open-source genomic analysis of Shiga-toxin-producing E. coli O104:H4. *N Engl J Med*. 2011;365:718–724.
297. Blaser MJ. Deconstructing a lethal foodborne epidemic. *N Engl J Med*. 2011;365:1835–1836.
298. Bielaszewska M, Mellmann A, Zhang W, et al. Characterization of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis*. 2011;11:671–676.
299. Rasko DA, Webster DR, Sahl JW, et al. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med*. 2011;365:709–717.
300. DuPont HL. The growing threat of foodborne bacterial enteropathogens of animal origin. *Clin Infect Dis*. 2007;45:1353–1361.
301. Glynn MK, Bopp C, Dewitt W, et al. Emergence of multidrug-resistant Salmonella enterica serotype Typhimurium DT104 infections in the United States. *N Engl J Med*. 1998;338:1333–1338.
302. Helms M, Ethelberg S, Mølbak K, the DT104 Study Group. International Salmonella Typhimurium DT104 infections, 1992-2001. *Emerg Inf Dis*. 2005;11:859–867.
303. Varma JK, Mølbak K, Barrett TJ, et al. Antimicrobial-resistant nontyphoidal Salmonella is associated with excess bloodstream infections and hospitalizations. *J Infect Dis*. 2005;191:554–561.
304. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant Campylobacter jejuni infections in Minnesota, 1992-1998. *N Engl J Med*. 1999;340:1525–1532.
305. Shiferaw B, Solghan S, Palmer A, et al. Antimicrobial susceptibility patterns of Shigella isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000-2010. *Clin Infect Dis*. 2012;54(suppl 5): S458–S463.
306. Reporter R, Pulido M, Bowen A, et al. Outbreak of infections caused by Shigella sonnei with decreased susceptibility to azithromycin – Los Angeles, California, 2012. *Morbid Mortal Wkly Rep*. 2013;62:171.
307. Feasey NA, Dougan G, Kingsley RA, et al. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet*. 2012;379:2489–2499.
308. Okor CK, Kinglsey RA, Connor TR, et al. Intracontinental spread of human invasive Salmonella pathovariants in sub-Saharan Africa. *Nat Genet*. 2012;44:1215–1221.
309. McDonald LC, Owings M, Jernigan DB. *Clostridium difficile* infection in patients discharged from US short-stay hospitals, 1996-2003. *Emerg Inf Dis*. 2006;12:409–415.
310. Senior K. Concern over *Clostridium difficile* in the USA. *Lancet Infect Dis*. 2008;8:352.
311. He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. *Nat Genet*. 2013;45:109–113.
312. Khanna S, Baddour LM, Huskins WC, et al. The epidemiology of Clostridium difficile infection in children: a population-based study. *Clin Infect Dis*. 2013;56:1401–1406.
313. Lessa FC, Mu Y, Bamberg W, et al. Burden of *Clostridium difficile* in the United States. *N Engl J Med*. 2015;372:825–834.
314. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353:2433–2441.
315. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high mobidity and mortality. *N Engl J Med*. 2005;353:2442–2449.
316. Hubert B, Loo VG, Bourgault AM, et al. A portrait of the geographic dissemination of the *Clostridium difficile* North American pulsed-field type 1 strain and the epidemiology of C.difficile-associated disease in Quebec. *Clin Infect Dis*. 2007;44:238–244.
317. McDonald LC, Lessa F, Sievert D, et al. Vital signs: preventing *Clostridium difficile* infections. *Morbid Mortal Wkly Rep*. 2012;61:149–152.
318. Goorhuis A, Bakker D, Corver J, et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin Infect Dis*. 2008;47:1162–1170.
319. Rodriguez-Palacios A, Staempfli HR, Duffield T, et al. *Clostridium difficile* in retail ground meat, Canada. *Emerg Inf Dis*. 2007;13:485–487.
320. Bakken JS, Borody T, Brand LJ, et al. Treating *Clostridium difficile* infection with fecal microbiota transplantation. *Clin Gastroenterol Hepatol*. 2011;9:1044–1049.
321. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med*. 2013;368:407–415.
322. Barash JR, Arnon SS. A novel strain of *Clostridium botulinum* that produces type B and type H botulinum toxins. *J Infect Dis*. 2013;209:183–191.
323. Centers for Disease Control and Prevention, Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review of the List of Select Agents and Toxins and Enhanced Biosafety Requirements. Final rule. *Fed Regist*. 2017;82:6278–6294.
324. Keim P. A novel botulinum neurotoxin and how it tested our scientific institutions. *J Infect Dis*. 2016;213:332–334.
325. Maslanka SE, Luquez C, Dykes JK, et al. A novel botulinum neurotoxin, previously reported as serotype H, has a hybrid-like structure with regions of similarity to structures of serotypes A and F and is neutralized with serotype A antitoxin. *J Infect Dis*. 2016;213:379–385.
326. Widdowson MA, Sulka A, Bulens SN, et al. Norovirus and foodborne disease, United States, 1991-2000. *Emerg Inf Dis*. 2005;11:95–102.
327. Blanton LH, Adams SM, Beard RS, et al. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000-2004. *J Infect Dis*. 2006;193:413–421.
328. Dolin R. Noroviruses — challenges to control. *N Engl J Med*. 2007;357:1072–1073.
329. Editorial. The inexorable progress of norovirus. *Lancet Infect Dis*. 2013;13:97.
330. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:725–730.
331. Barclay L, Wikswo M, Gregoricus N, et al. Emergence of new norovirus strain GII.4 Sydney – United States, 2012. *Morbid Mortal Wkly Rep*. 2013;62:55.
332. Gould LH, Mungai EA, Johnson SD, et al. Surveillance for foodborne disease outbreaks – United States, 2009-2010. *Morbid Mortal Wkly Rep*. 2013;62:41–47.
333. Payne DC, Vinje J, Szilagyi PG, et al. Norovirus and medically attended gastroenteritis in U.S. children. *N Engl J Med*. 2013;368:1121–1130.
334. van Beek J, Ambert-Balay K, Botteldoorn N, et al. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. *Euro Surveill*. 2013;18:8–9.
335. Desai R, Hembree CD, Handel A, et al. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks; a systematic literature review. *Clin Infect Dis*. 2012;55:189–193.
336. Debbink K, Lindesmith LC, Donaldson EF, et al. Emergence of new pandemic GII.4 Sydney norovirus strain correlates with escape from herd immunity. *J Infect Dis*. 2013;208:1877–1887.
337. Ramanan P, Bryson AL, Binnicker MJ, et al. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev*. 2018;31:e00024-17.
338. Jackson BR, Tarr C, Strain E, et al. Implementation of nationwide real-time whole genome sequencing to enhance listeriosis outbreak detection and investigation. *Clin Infect Dis*. 2016;63:380–386.
339. Cronquist AB, Mody RK, Atkinson R, et al. Impacts of culture-independent diagnostic practices on publi health surveillance for bacterial enteric pathogens. *Clin Infect Dis*. 2012;54(suppl 5):S432–S439.
340. Besser J, Carleton HA, Gerner-Smidt P, et al. Next-generation sequencing technologies and their application to the study and control of bacterial infections. *Clin Microbiol Infect*. 2017;pii: S1198-743X(17)30575-X.
341. Löwy I. Leaking containers: success and failure in controlling the mosquito Aedes aegypti in Brazil. *Am J Public Health*. 2017;107:517–524.
342. Moyes CL, Vontas J, Martins AJ, et al. Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. *PLoS Negl Trop Dis*. 2017;11:e0005625.
343. Burt A. Heritable strategies for controlling insect vectors of disease. *Philos Trans R Soc Lond B Biol Sci*. 2014;369:20130432.
344. Cella E, Riva E, Salemi M, et al. The new Chikungunya virus outbreak in Italy possibly originated from a single introduction from Asia. *Pathog Glob Health*. 2017;28:1–3.
345. Delisle E, Rousseau C, Broche B, et al. Chikungunya outbreak in montpellier, France, September to October 2014. *Euro Surveill*. 2015;20. pii: 21108.
346. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature*. 2013;496: 504–507.
347. Vasilakis N, Cardosa J, Hanley KA, et al. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat Rev Microbiol*. 2011;9:532–541.
348. Sharp TM, Tomashek KM, Read JS, et al. A new look at an old disease: recent insights into the global epidemiology of dengue. *Curr Epidemiol Rep*. 2017;4:11–21.
349. World Health Organization. Handbook for clinical management of dengue. 2012. http://www.who.int/tdr/publications/handbook_dengue/en/.
350. Wichmann O, Vannice K, Asturias EJ, et al. Live-attenuated tetravalent dengue vaccines: the needs and challenges of post-licensure evaluation of vaccine safety and effectiveness. *Vaccine*. 2017;35:5535–5542.
351. Tsai WY, Lin HE, Wang WK. Complexity of human antibody response to Dengue virus: implication for vaccine development. *Front Microbiol*. 2017;8:1372.
352. Metsky HC, Matranga CB, Wohl S, et al. Zika virus evolution and spread in the Americas. *Nature*. 2017;546:411–415.
353. Mead PS, Duggal NK, Hook SA, et al. Zika Virus shedding in semen of symptomatic infected men. *N Engl J Med*. 2018;378:1377–1385.
354. Musso D, Bossin H, Mallet HP, et al. Zika virus in French Polynesia 2013-14: anatomy of a completed outbreak. *Lancet Infect Dis*. 2018;18:e172–e182.
355. Sasmono T, Dhenni R, Yohan B, et al. Endemicity of Zika virus in Indonesia. *Emerg Infect Dis*. 2018;24.
356. Landry ML, St George K. Laboratory diagnosis of Zika virus infection. *Arch Pathol Lab Med*. 2017;141:60–67.
357. McArthur MA. Zika virus: recent advances towards the development of vaccines and therapeutics. *Viruses*. 2017;9:E143.
358. Ahmed QA, Memish ZA. Yellow fever from Angola and Congo: a storm gathers. *Trop Doct*. 2017;47:92–96.
359. World Health Organization. Yellow Fever Situation Reports. http://www.who.int/csr/don/archive/disease/yellow_fever/en/.
360. Ahuka-Mundeke S, Casey RM, Harris JB, et al. Immunogenicity of fractional-dose vaccine during a yellow fever outbreak - Preliminary report. *N Engl J Med*. 2018;[Epub ahead of print].
361. Zeller H, Van Bortel W, Sudre B. Chikungunya: its history in Africa and Asia and its spread to new regions in 2013-2014. *J Infect Dis*. 2016;214:S436–S440.
362. Tsetsarkin KA, Vanlandingham DL, McGee CE, et al. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog*. 2007;3:e201.
363. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. *Lancet*. 2018;391:1608–1621.
364. Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. *Nat Med*. 2017;23:917–928.
365. Woodrow CJ, White NJ. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. *FEMS Microbiol Rev*. 2017;41:34–48.
366. Rabinovich RN, Drakeley C, Djimde AA, et al. malERA: an updated research agenda for malaria elimination and eradication. *PLoS Med*. 2017;14:e1002456.
367. Val F, Machado K, Barbosa L, et al. Respiratory complications of Plasmodium vivax malaria: systematic review and meta-analysis. *Am J Trop Med Hyg*. 2017;97:733–743.
368. Quispe AM, Pozo E, Guerrero E, et al. Plasmodium vivax hospitalizations in a monoendemic malaria region: severe vivax malaria? *Am J Trop Med Hyg*. 2014;91:11–17.
369. Anstey NM, Douglas NM, Poespoprodjo JR, et al. Plasmodium vivax: clinical spectrum, risk factors and pathogenesis. *Adv Parasitol*. 2012;80:151–201.
370. Miller LH, Mason SJ, Clyde DF, et al. The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy. *N Engl J Med*. 1976;295: 302–304.
371. Rosenberg R. Plasmodium vivax in Africa: hidden in plain sight? *Trends Parasitol*. 2007;23:193–196.
372. Ryan JR, Stoute JA, Amon J, et al. Evidence for transmission of Plasmodium vivax among a duffy antigen negative population in Western Kenya. *Am J Trop Med Hyg*. 2006;75:575–581.
373. Gunalan K, Niangaly A, Thera MA, et al. Plasmodium vivax infections of Duffy-negative erythrocytes: historically undetected or a recent adaptation? *Trends Parasitol*. 2018;34:420–429.
374. Carvalho TA, Queiroz MG, Cardoso GL, et al. Plasmodium vivax infection in Anajás, State of Pará: no differential resistance profile among Duffy-negative and Duffy-positive individuals. *Malar J*. 2012;11:430.
375. Mueller I, Shakri AR, Chitnis CE. Development of vaccines for Plasmodium vivax malaria. *Vaccine*. 2015;33:7489–7495.

376. Lubis IND, Wijaya H, Lubis M, et al. Contribution of Plasmodium knowlesi to multispecies human malaria infections in North Sumatera, Indonesia. *J Infect Dis*. 2017;215:1148–1155.
377. Millar SB, Cox-Singh J. Human infections with Plasmodium knowlesi–zoonotic malaria. *Clin Microbiol Infect*. 2015;21:640–648.
378. Brasil P, Zalis MG, de Pina-Costa A, et al. Outbreak of human malaria caused by Plasmodium simium in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. *Lancet Glob Health*. 2017;5:e1038–e1046.
379. Lalremruata A, Magris M, Vivas-Martínez S, et al. Natural infection of Plasmodium brasilianum in humans: man and monkey share quartan malaria parasites in the Venezuelan Amazon. *EBioMedicine*. 2015;2:1186–1192.
380. Rosenberg R, Lindsey NP, Fischer M, et al. Vital signs: trends in reported vectorborne disease cases - United States and territories, 2004-2016. *MMWR Morb Mortal Wkly Rep*. 2018;67:496–501.
381. Nelson CA, Saha S, Kugeler KJ, et al. Incidence of clinician-diagnosed lyme disease, United States, 2005-2010. *Emerg Infect Dis*. 2015;21:1625–1631.
382. Hinckley AF, Connally NP, Meek JI, et al. Lyme disease testing by large commercial laboratories in the United States. *Clin Infect Dis*. 2014;59:676–681.
383. Mead PS. Epidemiology of Lyme disease. *Infect Dis Clin North Am*. 2015;29:187–210.
384. Fukunaga M, Takahashi Y, Tsuruta Y, et al. Genetic and phenotypic analysis of Borrelia miyamotoi sp. nov., isolated from the ixodid tick Ixodes persulcatus, the vector for Lyme disease in Japan. *Int J Syst Bacteriol*. 1995;45:804–810.
385. Gugliotta JL, Goethert HK, Berardi VP, et al. Meningoencephalitis from Borrelia miyamotoi in an Immunocompromised Patient. *N Engl J Med*. 2013;368:240–245.
386. Krause PJ, Fish D, Narasimhan S, et al. Borrelia miyamotoi infection in nature and in humans. *Clin Microbiol Infect*. 2015;21:631–639.
387. Pritt BS, Mead PS, Johnson DKH, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *Lancet Infect Dis*. 2016;16:556–564.
388. Kosoy OI, Lambert AJ, Hawkinson DJ, et al. Novel thogotovirus associated with febrile illness and death, United States, 2014. *Emerg Infect Dis*. 2015;21:760–764.
389. McMullan LK, Folk SM, Kelly AJ, et al. A new phlebovirus associated with severe febrile illness in Missouri. *N Engl J Med*. 2012;367:834–841.
390. Drexler N, Miller M, Gerding J, et al. Community-based control of the brown dog tick in a region with high rates of Rocky Mountain spotted fever, 2012-2013. *PLoS ONE*. 2014;9:e112368.
391. Herrick KL, Pena SA, Yaglom HD, et al. Rickettsia parkeri Rickettsiosis, Arizona, USA. *Emerg Infect Dis*. 2016;22:780–785.
392. Lamontagne F, Fowler RA, Adhikari NK, et al. *Lancet*. 2018;391:700–708, Evidence-based guidelines for supportive care of patients with Ebola virus disease.
393. Leligdowicz A, Fischer WA 2nd, Uyeki TM, et al. Ebola virus disease and critical illness. *Crit Care*. 2016;20:217.
394. MacNeil A, Farnon EC, Morgan OW, et al. Filovirus outbreak detection and surveillance: lessons from Bundibugyo. *J Infect Dis*. 2011;204:S761–S767.
395. World Health Organization. Interim Infection Control Recommendations for Care of Patients with Suspected or Confirmed Filovirus (Ebola, Marburg) Haemorrhagic Fever. March 2008. http://www.who.int/csr/bioriskreduction/filovirus_infection_control/en/index.html.
396. Hageman JC, Hazim C, Wilson K, et al. Infection prevention and control for Ebola in health care settings - West Africa and United States. *MMWR Suppl*. 2016;65:50–56.
397. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature*. 2005;438:575–576.
398. Leroy EM, Epelboin A, Mondonge V, et al. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne Zoonotic Dis*. 2009;9:723–728.
399. Towner JS, Pourrut X, Albariño CG, et al. Marburg virus infection detected in a common African bat. *PLoS ONE*. 2007;2:e764.
400. Swanepoel R, Smit SB, Rollin PE, et al. Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis*. 2007;13:1847–1851.
401. Luby JP, Sanders CV. Green monkey disease ("Marburg virus" disease): a new zoonosis. *Ann Intern Med*. 1969;71:657–660.
402. Conrad JL, Isaacson M, Smith EB, et al. Epidemiologic investigation of Marburg virus disease, Southern Africa, 1975. *Am J Trop Med Hyg*. 1978;27:1210–1215.
403. Johnson ED, Johnson BK, Silverstein D, et al. Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. *Arch Virol Suppl*. 1996;11:101–114.
404. Smith DH, Johnson BK, Isaacson M, et al. Marburg-virus disease in Kenya. *Lancet*. 1982;1:816–820.
405. World Health Organization. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ*. 1978;56:271–293.
406. World Health Organization. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull World Health Organ*. 1978;56:247–270.
407. Le Guenno B, Formenty P, Wyers M, et al. Isolation and partial characterisation of a new strain of Ebola virus. *Lancet*. 1995;345:1271–1274.
408. Towner JS, Sealy TK, Khristova ML, et al. Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog*. 2008;4:e1000212.
409. Edmond RT, Evans B, Bowen ET, et al. A case of Ebola virus infection. *BMJ*. 1977;2:541–544.
410. Sidley P. Fears over Ebola spread as nurse dies. *BMJ*. 1996;313:1351.
411. Rollin PE, Williams J, Bressler D, et al. Isolated cases of Ebola (subtype Reston) virus among quarantined non-human primates recently imported from the Philippines to the United States. *J Infect Dis*. 1999;179(suppl 1):S108–S114.
412. Centers for Disease Control. Update: filovirus infection in animal handlers. *MMWR Morb Mortal Wkly Rep*. 1990;39:221.
413. World Health Organization. Viral hemorrhagic fever in imported monkeys. *Wkly Epidemiol Rec*. 1992;67:142.
414. Miranda ME, Ksiazek TG, Retuya TJ, et al. Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. *J Infect Dis*. 1999;179(suppl 1):S115–S119.
414a. Centers for Disease Control and Prevention. History of Ebola virus disease; 2018. https://www.cdc.gov/vhf/ebola/history/summaries.html.
414b. Centers for Disease Control and Prevention. Outbreaks chronology: Marburg hemorrhagic fever; 2014. https://www.cdc.gov/vhf/marburg/outbreaks/chronology.html.
415. Dixon MG, Schafer IJ, Centers for Disease Control and Prevention. Ebola viral disease outbreak—West Africa, 2014. *MMWR Morb Mortal Wkly Rep*. 2014;63:548–551.
416. Baize S, Pannetier D, Oestereich L, et al. Emergence of Zaire Ebola virus disease in Guinea. *N Engl J Med*. 2014;371:1418–1425.
417. Centers for Disease Control and Prevention. 2014 Ebola Outbreak in West Africa—Case Counts. http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html.
418. Uyeki TM, Mehta AK, Davey RT Jr, et al; Working Group of the U.S.–European Clinical Network on Clinical Management of Ebola Virus Disease Patients in the U.S. and Europe. Clinical Management of Ebola Virus Disease in the United States and Europe. *N Engl J Med*. 2016 Feb 18;374:636–646.
419. World Bank. 2014. The economic impact of the 2014 Ebola epidemic: short and medium term estimates for West Africa (English). Washington, DC: World Bank Group. http://documents.worldbank.org/curated/en/524521468141287875/The-economic-impact-of-the-2014-Ebola-epidemic-short-and-medium-term-estimates-for-West-Africa.
420. Samai M, Seward JF, Goldstein ST, et al; STRIVE Study Team. The Sierra Leone trial to introduce a vaccine against Ebola: an evaluation of rVSVΔG-ZEBOV-GP vaccine tolerability and safety during the West Africa Ebola outbreak. *J Infect Dis*. 2018;217(suppl_1):S6–S15.
421. Davey RT Jr, Nordwall J, Proschan MA, Prevail II study team. Trial of ZMapp for Ebola virus infection. *N Engl J Med*. 2017 Feb 16;376:700–701.
422. Fitzpatrick G, Decroo T, Draguez B, et al. Operational Research during the Ebola Emergency. *Emerg Infect Dis*. 2017;23:1057–1062.
423. National Academies of Sciences, Engineering, and Medicine, Health and Medicine Division, Board on Health Sciences Policy, Board on Global Health, Committee on Clinical Trials During the 2014-2015 Ebola Outbreak, Busta ER, Mancher M, et al, eds. *Integrating Clinical Research into Epidemic Response: The Ebola Experience*. Washington (DC): National Academies Press (US); 2017.
424. Scott JT, Sesay FR, Massaquoi TA, et al. Post-Ebola Syndrome, Sierra Leone. *Emerg Infect Dis*. 2016;22:641–646.
425. Fischer RJ, Judson S, Miazgowicz K, et al. Ebola virus persistence in semen ex vivo. *Emerg Infect Dis*. 2016;22:289–291.
426. Thorson A, Formenty P, Lofthouse C, et al. Systematic review of the literature on viral persistence and sexual transmission from recovered Ebola survivors: evidence and recommendations. *BMJ Open*. 2016;6:e008859.
427. Chughtai AA, Barnes M, Macintyre CR. Persistence of Ebola virus in various body fluids during convalescence: evidence and implications for disease transmission and control. *Epidemiol Infect*. 2016;144:1652–1660.
428. Marston BJ, Dokubo EK, van Steelandt A, et al. Ebola response impact on public health programs, West Africa, 2014-2017. *Emerg Infect Dis*. 2017;23.
429. André AM, Lopez A, Perkins S, et al. Frontline field epidemiology training programs as a strategy to improve disease surveillance and response. *Emerg Infect Dis*. 2017;23.
430. Raftery P, Condell O, Wasunna C, et al. Establishing Ebola Virus Disease (EVD) diagnostics using GeneXpert technology at a mobile laboratory in Liberia: impact on outbreak response, case management and laboratory systems strengthening. *PLoS Negl Trop Dis*. 2018;12:e0006135.
431. Van den Bergh R, Chaillet P, Sow MS, et al. Feasibility of Xpert Ebola assay in médecins sans frontières Ebola program, Guinea. *Emerg Infect Dis*. 2016;22:210–216.
431a. World Health Organization. Ebola virus disease - Democratic Republic of the Congo. Disease outbreak news: Update 9 May 2019. https://www.who.int/csr/don/09-may-2019-ebola-drc/en/.
432. Watts J. Targets now set by G8 countries to reduce "diseases of poverty. *Lancet*. 2000;356:408.
432a. World Health Organization. Strengthening health security by implementing the International Health Regulations (2005); 2019. http://www.who.int/ihr/publications/9789241580496/en/.
433. Stehling-Ariza T, Lefevre A, Calles D, et al. Establishment of CDC global rapid response team to ensure global health security. *Emerg Infect Dis*. 2017;23.
434. Angulo FJ, Cassell CH, Tappero JW, et al. Progress and opportunities for strengthening global health security. *Emerg Infect Dis*. 2017;23.
435. Graham BS, Sullivan NJ. Emerging viral diseases from a vaccinology perspective: preparing for the next pandemic. *Nat Immunol*. 2018;19:20–28.
436. Plotkin SA, Mahmoud AA, Farrar J. Establishing a global vaccine-development fund. *N Engl J Med*. 2015;373:297–300.
437. Sautto GA, Kirchenbaum GA, Ross TM. Towards a universal influenza vaccine: different approaches for one goal. *Virol J*. 2018;15:17.
438. Gebreyes WA, Dupouy-Camet J, Newport MJ, et al. The global One Health paradigm: challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. *PLoS Negl Trop Dis*. 2014;8:e3257.
439. Ramey AM, DeLiberto TJ, Berhane Y, et al. Lessons learned from research and surveillance directed at highly pathogenic influenza A viruses in wild birds inhabiting North America. *Virology*. 2018;518:55–63.
440. Boegler KA, Atiku LA, Enscore RE, et al. Rat fall surveillance coupled with vector control and community education as a plague prevention strategy in the West Nile Region, Uganda. *Am J Trop Med Hyg*. 2018;98:238–247.
441. Rosenberg R. Detecting the emergence of novel, zoonotic viruses pathogenic to humans. *Cell Mol Life Sci*. 2015;72:1115–1125.
442. Gwinn M, MacCannell DR, Khabbaz RF. Integrating advanced molecular technologies into public health. *J Clin Microbiol*. 2017;55:703–714.
443. Ribot EM, Hise KB. Future challenges for tracking foodborne diseases: PulseNet, a 20-year-old US surveillance system for foodborne diseases, is expanding both globally and technologically. *EMBO Rep*. 2016;17:1499–1505.
444. Deng X, den Bakker HC, Hendriksen RS. Genomic epidemiology: whole-genome-sequencing-powered surveillance and outbreak investigation of foodborne bacterial pathogens. *Annu Rev Food Sci Technol*. 2016;7:353–374.
445. Madoff LC. ProMED-mail: an early warning system for emerging diseases. *Clin Infect Dis*. 2004;39:227–232.
446. Mykhalovskiy E, Weir L. The Global Public Health Intelligence Network and early warning outbreak detection: a Canadian contribution to global public health. *Can J Public Health*. 2006;97:42–44.
447. Forbes JD, Knox NC, Ronholm J, et al. Metagenomics: the next culture-independent game changer. *Front Microbiol*. 2017;8:1069.
448. Miller RR, Montoya V, Gardy JL, et al. Metagenomics for pathogen detection in public health. *Genome Med*. 2013;5:81.
449. Siettos CI. Editorial: mathematical modeling of infectious disease dynamics. *Virulence*. 2016;7:119–120.
450. Liang D, Leung RK, Guan W, et al. Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut Pathog*. 2018;10:3.
451. Adli M. The CRISP R tool kit for genome editing and beyond. *Nat Commun*. 2018;9:1911.

15 Bioterrorism: An Overview

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Exposures to biological agents, such as microbes and toxins, are usually unintentional. Notably, these agents can be used as weapons to cause intentional harm to humans, animals, or plants as well as to contaminate the environment. Unlike nuclear, radiologic, chemical, and explosive threat agents, the diseases and conditions caused by biological agents as well as their treatment could be encountered during the practice of medicine. Similarly, the surveillance of and response to these agents is within the purview of many public health systems. This feature of encountering these biological agents in practice highlights the importance of understanding which of the biological agents are associated with the greatest health consequences (Table 15.1) both for the individual and for the public, when to suspect their presence, how to diagnose them, and what epidemiologic clues may suggest that the case(s) identified may have been caused intentionally.[1]

The anthrax letter attacks that occurred in the United Sates in 2001 highlighted the need for improvements in diagnosis, detection, treatment, preparedness, and response by clinicians, health care systems, and public health professionals.[2] Subsequently, guidelines for the medical management of anthrax and other high-consequence biological agents, in deliberate use settings, have continued to improve, reflecting our increased understanding of these pathogens (Table 15.2).

DEFINING BIOLOGICAL WARFARE, BIOTERRORISM, AND BIOCRIMES

Some confusion exists over how to categorize incidents associated with the intentional use of biological agents (pathogens and toxins) to cause harm. Although there is no universally accepted definition of these three terms, the underlying motivation and objectives for intentional use of these biological agents has been suggested as a way to distinguish biological warfare, bioterrorism, and biocrimes. Descriptions of each may help guide clinicians and public health professionals in determining when interactions with law enforcement personnel may be anticipated.

Bioterrorism is commonly viewed as an action by a non–state actor to achieve a political, ideological, or religious goal.[3] Implied in the word *bioterrorism* is the desire to terrorize as much or more than causing casualties. A biocrime, on the other hand, occurs when a biological agent is used by a person or group against an individual or small group, often for revenge or extortion, in the absence of an underlying ideology; the perpetrator(s) are often believed to have underlying psychopathology. Biological warfare occurs when a state actor uses a biological agent as part of its armamentarium in waging war. Importantly, biological agents have been used intentionally in all three settings—bioterrorism, biocrime, and biological warfare.

HISTORY OF BIOLOGICAL WEAPONS DEVELOPMENT AND USE IN WARFARE AND AGAINST INSURGENCIES

Proven biological weapons use by countries (or state actors) to wage warfare against other states as well as internally against insurgents has been documented since the 18th century and has been purported to have occurred prior to the "germ theory." However, there is no reliable evidence that previously alleged use of biological agents in war occurred in the ancient world or in medieval times, including use of *Yersinia pestis,* although plague outbreaks were identified during periods of conflict.[4] The scale of programs and manner of use of biological weapons varied over time. Increasing sophistication of state-run programs was noted beginning in World War I (WWI) through termination of offensive programs by most countries in the 20th century.

Pre–World War I Biological Weapons Development and Use

The first well-documented use of biological weapons occurred in 1763 when the British used contaminated blankets and clothing in an attempt to infect Delaware tribe Native Americans with smallpox[5]; there is no evidence of a successful outcome.[6] A similar attempt to use fomites to cause disease deliberately was documented during the American Civil War when a Confederate physician attempted to spread yellow fever (before its vector-borne transmission was known) to populations living in Union cities through the introduction of contaminated clothing and bedding.[7] It was not until the latter half of the 19th century that high-quality microscopes of good optical quality, underpinning the new science of bacteriology, and the work of Louis Pasteur and Robert Koch made modern biological warfare possible.

Development and Use of Biological Weapons During World War I

During WWI, Germany had the first documented state-run biological weapons program (Table 15.3). It began in 1915, with documented use of anthrax against animals being shipped for use by Allied forces. France had the second documented biological weapons program (see Table 15.3).[8] In 1925, following WWI, the Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare (also known as the Geneva Protocol),[9] was drawn up and signed under the auspices of the League of Nations and entered into force as a treaty in 1928. Although the Geneva Protocol, which had been officially agreed to by 137 State Parties as of March 15, 2013,[10] banned the use of biological weapons, it did not proscribe the research, production, or possession of biological weapons, and many of the State Parties to the treaty reserved the right to retaliate in kind should they or their allies be attacked. In addition, no provision was made for verification, and compliance was voluntary. A number of countries agreeing to the Geneva Protocol began or continued biological weapons programs after signing the treaty, including Canada, France, Germany, Italy, Japan, Poland, the United Kingdom, and the Soviet Union (see Table 15.3). The United States did not ratify the Geneva Protocol until 1975, after it had ended its biological weapons program.[8]

Development and Use of Biological Weapons During World War II

Seven countries had biological weapons programs during World War II (WWII): Canada, France, Hungary, Italy, Japan, the Soviet Union, the United Kingdom, and the United States (see Table 15.3). Among these seven countries, only Japan waged biological warfare during WWII. Its biological weapons program—organized at its Unit 731 and based in Harbin, China—used multiple biological agents and employed more than 3000 scientists plus smaller units at a number of other sites in China, which were under Japanese occupation. More than 10,000

[a]The views expressed in this chapter do not necessarily represent the views of the United States government.

TABLE 15.1 Bioterrorism Agents/Diseases Posing a Risk to National Security, by Priority Category

Category Designation	A	B	C
Priority/Consequence	1	2	3
Definition	• Easily disseminated *or* easily spread person-to-person AND • High case fatality rate and potential serious public health impact AND • May cause social disruption or panic AND • Requires deliberate and special action for public health preparedness	• Moderately easy to disseminate AND • Low case fatality rate but moderate morbidity rate AND • Requires enhanced disease surveillance and specific enhancements to the CDC's diagnostic capacity	• Could be engineered for widespread dissemination due to: • Availability • Ease of production and dissemination AND • High case fatality rate and morbidity rate potential
Disease Agent	Anthrax (*Bacillus anthracis*) Botulism (*Clostridium botulinum* toxin) Plague (*Yersinia pestis*) Smallpox (*Variola major*) Tularemia (*Francisella tularensis*) Viral hemorrhagic fevers: Filoviruses (Ebola virus, Marburg virus) Arenaviruses (Lassa virus, Machupo virus)	Brucellosis (*Brucella* species) Unknown pathogenesis in humans (*Clostridium perfringens* types B or D epsilon toxin[a]) Foodborne safety threats: *Salmonella* species *Escherichia coli* O157:H7 *Shigella* species Glanders (*Burkholderia mallei*) Melioidosis (*Burkholderia pseudomallei*) Psittacosis (*Chlamydia psittaci*) Q fever (*Coxiella burnetii*) Ricin intoxication/poisoning[b] (*Ricinus communis* toxin [from castor beans]) SEB intoxication/poisoning[c] (*Staphylococcus aureus* enterotoxin B) Typhus fever (*Rickettsia prowazekii)* Viral encephalitis (Alphaviruses [e.g., EEE, VEE, WEE]) Water safety threats: *Vibrio cholera* *Cryptosporidium parvum*	Engineered pathogens or emerging pathogens that could be engineered and could cause various diseases/syndromes: Nipah virus Hantavirus species

[a]Produces fatal enterotoxemia in ruminant animals but no data to support human disease. Concern for humans is theoretical based on animal data, particularly if exposed to aerosolized toxin (see reference 43).
[b]Ricin intoxication-related manifestations are dependent upon the route of exposure: inhalation vs. injection vs. ingestion vs. skin vs. eye (see reference 42).
[c]SEB intoxication-related manifestations are dependent upon dose and route of exposure, which have an impact on disease expression (see reference 44).
CDC, Centers for Disease Control and Prevention; *EEE,* eastern equine encephalitis; *SEB,* staphylococcal enterotoxin B; *VEE,* Venezuelan equine encephalitis; *WEE,* western equine encephalitis.
Modified from Centers for Disease Control and Prevention. Emergency preparedness and response: bioterrorism agents/diseases; 2017. https://emergency.cdc.gov/agent/agentlist-category.asp. Accessed November 15, 2017.

prisoners died as a result of experimental infections or execution after experimentation.[11] Significant adverse impact among the civilian populations of at least 11 cities in China was recorded following Japanese attacks using various delivery mechanisms to deliver *Bacillus anthracis, Vibrio cholera, Shigella* spp., *Salmonella* spp., and *Y. pestis.*[11] Additionally, during the war, Polish resistance fighters used biological agents as weapons for assassination.[4]

Biological Weapons in the Post–World War II Period Through the End of the Cold War

Revelations of the successful use of biological weapons by the Japanese during WWII led several countries to expand their programs and others to create them; 11 countries had a biological weapons program at some time following WWII (see Table 15.3). In the post-WWII period, three new programs in Israel,[12] Rhodesia,[13] and South Africa[14] used biological agents on a small scale, with limited, if any, proven consequences. Use by the governments of Rhodesia and South Africa were internally directed against insurgents seeking regime change.[13,14]

In the post-WWII period, the only large biological weapons programs were found in the Soviet Union and the United States; the former is estimated to have been 10 times the size of the latter and much larger than both the Iraqi (1000 times the size) and South African (10,000 times the size) programs.[15] The US program was terminated in 1969; it is unknown if Russia has a current biological weapons program.

Offensive Biological Weapons Programs in the United States

Although the biological weapons program operated by the Soviet Union was the largest in the world, the US offensive biological weapons program and toxin weapons program, before termination by President Richard Nixon in 1969[16] and 1970,[17] respectively, were the most scientifically sophisticated. Human, animal, and plant pathogens were studied; human disease–causing pathogens assessed were *B. anthracis,* botulinum toxins, *Francisella tularensis, Brucella suis, Coxiella burnetii,* staphylococcal enterotoxin B, and Venezuelan equine encephalitis. Technical advances were made that permitted large-scale fermentation and storage of biological warfare agents as well as optimal aerosol dissemination techniques, although no new discoveries regarding the physics of aerosol particles were made by the US program.[18] Rather, the focus was on identifying optimal battlefield dissemination techniques to create extensive clouds of high concentration using small amounts of a biological agent.[18]

In an effort to learn more about how to protect against these agents under "field conditions," the US Department of Defense conducted a series of classified biological warfare and vulnerability tests from 1962 through 1973.[19] Both land-based and ship-based tests were conducted under the name Project 112/SHAD.[19] Humans, plants, and animals were all potentially exposed to biological agents, chemical agents, biological stimulants, decontaminants, or tracers; approximately 6000 service members were possibly exposed, but no long-term exposure health effects were identified.[19,20]

TABLE 15.2 Management Guidelines and Recommendations for the Management of Disease Caused by High-Consequence Bioterrorism Threat Agents

CONDITION	MANAGEMENT GUIDELINE OR RECOMMENDATION	YEAR PUBLISHED	ON-LINE LINK
Anthrax[b,c,d,e]	Clinical Framework and Medical Countermeasure Use During an Anthrax Mass-Casualty Incident	2015	https://www.cdc.gov/mmwr/pdf/rr/rr6404.pdf
Botulism	Botulism: Information for Health Professionals	2017	https://www.cdc.gov/botulism/health-professional.html
Ebola virus disease	Ebola Virus Disease (EVD) Information for Clinicians in US Healthcare Settings	2016	https://www.cdc.gov/vhf/ebola/healthcare-us/preparing/clinicians.html
Glanders (and melioidosis)	Workshop on Treatment of and Postexposure Prophylaxis for *Burkolderia pseudomallei* and *B. mallei* Infection, 2010	2012	https://wwwnc.cdc.gov/eid/article/18/12/12-0638_article
Plague	Plague: Resources for Clinicians	2015	https://www.cdc.gov/plague/healthcare/clinicians.html
Smallpox[f]	Clinical Guidance for Smallpox Vaccine Use in a Postevent Vaccination Program	2015	https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6402a1.htm?s_cid=rr6402a1_w

[a]Guidelines and recommendations of the Centers for Disease Control and Prevention. Health care provider bioterrorism training modules and video webcasts can be found at https://emergency.cdc.gov/bioterrorism/training.asp.
[b]Mass casualty: Bower WA, Hendricks K, Pillai S, et al. Clinical framework and medical countermeasure use during an anthrax mass-casualty incident. *MMWR Recomm Rep.* 2015;64(No. RR-4):1–22.
[c]Anthrax prevention and treatment in adults: Hendricks KA, Wright ME, Shadomy SV, et al. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. *Emerg Infect Dis.* 2014;20:e130687.
[d]Pediatric anthrax guideline: Bradley JS, Peacock G, Krug SE, et al. Pediatric anthrax clinical management. *Pediatrics.* 2014;133:e1411–e1436.
[e]Considerations in pregnant women: Meaney-Delman D, Zotti ME, Creanga AA, et al. Special considerations for prophylaxis for and treatment of anthrax in pregnant and postpartum women. *Emerg Infect Dis.* 2014;20:e130611.
[f]Smallpox vaccine use guideline: Petersen BW, Damon IK, Pertowski CA, et al. Clinical guidance for smallpox vaccine use in a postevent vaccination program. *MMWR Recomm Rep.* 2015;64(No. RR-2):1–26.

TABLE 15.3 Known Biological Weapons Programs, by Region, Country, and Functioning Program Dates

REGION	COUNTRY	FUNCTIONING PROGRAM DATES
Africa (sub-Saharan)	Rhodesia[a]	1977
	South Africa	1981–1993
The Americas	Canada	1940–1958
	United States of America	1941–1971
Asia	Japan	1934–1945
Europe	France	1915–1916 1922–1926 1934–1940 1947–1966 or 1967
	Germany	1915–1918
	Italy	1934–1940 or 1943
	Poland	Unknown[b]
	Soviet Union[c]	1918–1991[c]
	United Kingdom	1940–1957
Middle East[d]	Egypt	1960s–?[e]
	Iraq	1974–1978 1981–1991
	Israel	1948–?[e]
	Syria	1970s–?[e]

[a]Name changed to Zimbabwe with independence.
[b]Polish scientists fleeing German takeover in World War II admitted to destroying program elements.
[c]Does not apply to current-day Russia.
[d]Includes Iran, Asia Minor, Mesopotamia, the Levant, the Arabian Peninsula, and Egypt (15 countries and Palestine).
[e]Probable continuing program; other probable biological weapons programs exist in China, Iran, and North Korea.
Data from Carus WS. A Short History of Biological Warfare: From Pre-History to the 21st Century. *Center for the Study of Weapons of Mass Destruction, Occasional Paper, No. 12. Washington, DC: National Defense University Press; 2017.*

LIMITING USE OF BIOLOGICAL WEAPONS: THE BIOLOGICAL WEAPONS CONVENTION

Over time there was increasing international concern that the Geneva Protocol did not ban the research, production, or possession of biological weapons and lacked the means to verify adherence by signatory countries. In 1969, draft proposals for a new protocol were submitted to the Committee on Disarmament of the United Nations (UN).[21] Then in 1972, the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons and on their Destruction—commonly referred to as the Biological Weapons Convention (BWC)—was opened for signature.[22] State Parties to the BWC—which entered into force in 1975—are obligated to not develop, produce, stockpile, or otherwise acquire or retain microbial or other biological agents or toxins of types and in quantities that have no justification for prophylactic, protective, or other peaceful purposes. They may not use any means of delivery of such agents or toxins for hostile purposes and must take necessary measures to prohibit or prevent such activities in their territories. Further, State Parties should destroy or divert to peaceful purposes all agents, toxins, weapons, equipment, and means of delivery and not transfer to any recipient or in any way assist, encourage, or induce to manufacture or otherwise acquire biological agents, toxins, weapons, equipment, or means of delivery. A total of 196 State Parties may become members of the BWC. As of September 2017, 179 State Parties have acceded to or ratified the treaty.[23] The BWC does not explicitly address non–state actors, such as terrorists or criminals.

Monitoring and Compliance Under the Biological Weapons Convention

There is no formal verification protocol to monitor compliance. Accordingly, verifying adherence to and compliance with the BWC by State Parties has proven challenging.

Civilian Research in the Soviet Union

The Soviet Union became a State Party to the BWC in 1975. Little is known of the country's biological weapons efforts from the end of WWII to the early 1970s. In 1973, a large, science-based research and development program to address offensive issues related to biological agents,

called Biopreparat, was created in the civilian sector, complementary to a less sophisticated program in the Ministry of Defense.[24,25] In the late 1980s and early 1990s serious concerns arose regarding the biological weapons capability of the Soviet Union,[21] heightened by information provided by defectors and revelations by program scientists after the fall of the Soviet Union describing a biological weapons program that was far more extensive and sophisticated than anyone had imagined at the end of the Cold War.[26,27] Biopreparat was a system of 18 nominally civilian research laboratories employing approximately 30,000 staff prior to the fall of the Soviet Union; research was undertaken on biological agents, often at the request of the Ministry of Defense.[28] One of the larger and more sophisticated of these laboratory centers, the State Research Center of Virology and Biotechnology (VECTOR), continues to function in a role stated to be similar to that of the US Centers for Disease Control and Prevention (CDC).

Military Activities in the Soviet Union

Another facility of concern was the Soviet Union's principal production center for smallpox virus located near Moscow, at Sergiev Posad. It was reportedly able to produce upwards of 20 tons of smallpox virus annually, primarily for delivery via intercontinental ballistic missile as a strategic weapon.[27] Currently, the site houses the Russian Federation's Ministry of Defense Microbiology Scientific Research Institute, a laboratory research complex known to maintain a national collection of dangerous pathogens, including Ebola, Marburg, and Lassa viruses.[29] In 1992, Russian officials confirmed the existence of a biological weapons program it had inherited from the Soviet Union and committed to dismantling it.[30] The dissolution of the Soviet Union in 1991 and the halting of the inherited Soviet offensive biological weapons program by Russian President Yeltsin in 1992 resulted in profound reductions in Biopreparat funding and personnel, raising concerns that former biological weapons scientists may sell their expertise to states or groups seeking such knowledge.[31] In 2000 it was estimated that approximately 15,000 Biopreparat scientists remained employed within the system, which could pose a proliferation risk.[32]

Iraq's Biological Weapons Program

Another example that illustrates the challenges of verifying compliance to the BWC is the case of Iraq, which signed the BWC in 1972. After the first Gulf War (Operation Desert Storm) in 1991, UN Security Council Resolution 687 established, among other things, the UN Special Commission (UNSCOM) to carry out on-site inspections of Iraq's biological and chemical weapons and missile capabilities and to oversee their destruction or removal, or procedures to render them harmless.[33] In April 1991, under its initial declaration (as required under UN Resolution 687), Iraq declared that it did not have a biological weapons program and accepted Resolution 687; in August 1991 it declared to the first Biological Weapons Inspection Team that it had conducted only "biological research activities for defensive military purposes."[34] It was not until July 1995, after 4 years of UNSCOM investigations and "in the light of irrefutable evidence," that Iraq admitted for the first time that it had an offensive biological weapons program.[34] Notably, Iraq initially denied creating weapons for use, but a defector with responsibility for Iraq's program, General Hussein Kamel, revealed to UNSCOM the full extent of the Iraqi program upon arrival in Jordan in August 1995. Subsequently, Iraq admitted to "a far more extensive biological warfare programme" than previously admitted, including weaponization.[34]

HISTORY OF BIOLOGICAL WEAPONS USE TO COMMIT ACTS OF BIOTERRORISM

Non–state actors, including individuals and groups (e.g., terrorist groups, criminal networks), are not explicitly covered under the BWC. They present a unique, complex, and growing challenge with respect to the development and use of biological weapons. During the 20th century, confirmed or threatened use of biological weapons against humans was more common by criminals than by terrorists. However, use by both types of these non–state actors increased over this 100-year period.[3] Additionally, in the first years of the 21st century, one confirmed use of a biological agent, with human health outcomes, in an act of bioterrorism occurred in the United States.[2] Multiple subsequent deliberate hoaxes using nonpathogens followed this latter bioterrorism incident, and all have been classified as crimes.[35]

During the 20th century there were 15 confirmed incidents during which a biological weapon was used by non–state actors with resulting adverse human health effects.[3] Six events (40%) occurred in the final 2 decades of the century, one of which was due to bioterrorism, compared with an average of one incident in each of the preceding 8 decades. The biological agents were delivered in food in nine (60%) of the confirmed intentional incidents, five (33%) by injection, and one by an unknown delivery mechanism; all but one of the perpetrators was trained or practicing in a health-related field (Table 15.4). There was only one event associated with the use of a highest consequence pathogen, referred to as a CDC Category A agent (see Table 15.1); nine involved use of a CDC Category B agent, and five were not on the CDC "ABC" list of bioterrorism agents.

20th-Century Bioterrorism Against Humans: The Rajneeshee Cult

The only confirmed terrorist use of a biological agent against humans in the 20th century occurred in 1984.[37] In 1990, a second alleged case was perpetrated by a Japanese cult, the Aum Shinrikyo, but without documentary evidence of use.[3] In the 1984 incident, members of a religious cult, the Rajneeshees, in an effort to influence a local county election, aimed to incapacitate opposition voters by deliberately contaminating salad bars located along a stretch of an Oregon interstate highway with *Salmonella typhimurium* (now *Salmonella enterica* subsp. *enterica* serovar Typhimurium), a CDC Category B agent (see Table 15.1 and Table 15.3). The pathogen was legally obtained by the cult's health care system and prepared by a cult member, who was a health care provider who also had laboratory skills. Although there were no deaths among the 751 persons who became ill, 45 were hospitalized.[37] Importantly, the public health investigation of this incident in 1984 initially failed to determine how the salad bars became contaminated. It was not until 1 year after the outbreak that dissension among the perpetrators led law enforcement officials to discover that the contamination was deliberate with the ultimate goal of disrupting the local election.[3] This episode illustrates the difficulty in differentiating an ordinary foodborne outbreak from a small-scale biological weapons attack conducted by non–state actors.

21st-Century Bioterrorism Against Humans: The Anthrax Letters

Since the commencement of the 21st century, there has only been one successfully executed bioterrorism incident. Notably, some experts classify it as a crime rather than an act of terrorism due to assessed motivation by the alleged perpetrator. In late September and October 2001, multiple letters containing *B. anthracis* spores were sent through the US mail, targeting members of Congress and the media, resulting in 22 cases of anthrax—11 inhalational and 11 cutaneous.[2] Five of the victims died. A key concept is the health care provider as a first responder. Physicians provided the initial evaluation that led to diagnosis and needed treatment. Public health professionals responded to the situation with the outbreak investigative skills needed. When a deliberate etiology of the outbreak was suspected, law enforcement expertise was required to identify the perpetrator(s) and to stop further attacks, if possible.

Over the subsequent 8 years, law enforcement carried out the so-called Amerithrax Investigation.[38] This was a complex, expensive, and lengthy investigation conducted mainly by the Department of Justice's Federal Bureau of Investigation (FBI) and the US Postal Inspection Service. Although a number of persons initially were investigated as possible perpetrators, there were two major persons of interest, both with work experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID).[38] In 2002, the first major suspect, a physician, was subjected to an intensive and wide-ranging investigation but was not indicted when it was determined that there was no documented evidence and insufficient circumstantial evidence to support arrest.[38] The investigation subsequently focused on an anthrax vaccine expert who had worked at USAMRIID since late 1980—a civilian with a doctorate in microbiology—as a person of interest; however, in 2008

TABLE 15.4 Incidents of Confirmed Deliberate Use of Biological Agents to Commit a Crime or as an Act of Bioterrorism, With Resulting Human Morbidity and Mortality, 1900-2017

YEAR	LOCATION	TYPE[a]	MOTIVE	BIOLOGICAL AGENT	MODE OF DELIVERY	CDC CATEGORY[b]	PERPETRATOR[c]
20th Century[d]							
1910	Russia	C	Financial gain	Diphtheria toxin	Injection	NA	Physician
1912	France	C	Financial gain	*Salmonella typhi*	Food	B	Pharmacist
1913	Germany	C	Financial gain	*S. typhi*	Food	B	Not an HCP
1916	USA	C	Financial gain	Several including *S. typhi*	Unclear	B	Dentist
1933	India	C	Financial gain	*Yersinia pestis*	Injection	A	Physician
1936	Japan	C	Revenge	*S. typhi*	Food	B	Physician
1939	Japan	C	Revenge	*S. typhi*; *S. paratyphi*	Food	B	Physician
1964	Japan	C	Revenge	*S. typhi*; *Shigella* spp.	Food	B	Physician
1970	Canada	C	Revenge	*Ascaris suum*	Food	NA	Parasitologist[e]
1984	USA	B	Political	*S. typhimurium*	Food	B	Nurse[f]
1992	USA	C	Revenge	HIV	Injection	NA	Phlebotomist
1993	Netherlands	C	Revenge	HIV	Injection	NA	Unknown
1994	USA	C	Revenge	HIV	Injection	NA	Physician
1995	USA	C	Revenge	Ricin	Food	B	Physician
1996	USA	C	Unknown	*Shigella* spp.	Food	B	Laboratory technician
21st Century[g]							
2001	USA	B[h] vs. C[h]	Political vs. – Revenge – Career preservation	*Bacillus anthracis*	Aerosol (primary) Cutaneous (secondary)	A	Research microbiologist and anthrax vaccine expert

[a]Type of deliberate use: B, bioterrorism; C, crime.
[b]CDC Category of Bioterrorism Biological Agents, from highest consequence (Category A) to lowest consequence (Category C) if used in an act of bioterrorism (see Table 15.1).
[c]Perpetrator: Healthcare-related training or experience.
[d]Twentieth century data from Carus W. *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents since 1900 (Revised).* Washington, DC: Center for Counterproliferation Research, National Defense University; 2001. http://www.fas.org/irp/threat/cbw/carus.pdf. Accessed July 18, 2017.
[e]Graduate student in parasitology.
[f]Family nurse practitioner with additional laboratory skills.
[g]Twenty-first century incident data from United States Department of Justice. Amerithrax investigative summary: released pursuant to the Freedom of Information Act. Washington, DC: US Department of Justice; 2010. http://www.justice.gov/archive/amerithranx/docs/amx-investigative-summary.pdf. Accessed September 15, 2017; and Expert Behavioral Analysis Panel. The Amerithrax case: report of the Expert Behavioral Analysis Panel. Vienna, VA: Research Strategies Network; 2011. http://web.archive.org/web/20110708081506/http://www.dcd.uscourts.gov/dcd/sites/dcd/files/unsealedDoc031011.pdf. Accessed September 20, 2017.
[h]Post facto analysis of unsealed mental health provider notes and documents suggest motives that are more akin to criminal activity. However, statements made to colleagues suggested political or ideological motivation. (See reference 36.)
HCP, Health care professional; *HIV,* human immunodeficiency virus.

he committed suicide before he could be arrested, indicted, and tried.[38] Following this alleged perpetrator's death, the FBI requested a review by the National Research Council of the investigative forensic methods; the panel concluded that there was insufficient scientific evidence to definitively conclude that the anthrax spores came from this microbiologist's laboratory.[39] However, circumstantial evidence collected during the Amerithrax Investigation and the findings of a court-ordered comprehensive postmortem behavioral analysis expert panel[36] led the FBI to conclude that the late microbiologist acted alone and planned and executed the 2001 anthrax attack.[40] The case was then closed and no additional suspects were sought.

Limiting Development and Use of Biological Weapons by Terrorists

In response to increasing concerns regarding the risk that non–state actors might acquire and use biological, chemical, and nuclear weapons and the fact that the BWC does not explicitly address non–state actors, UN Security Council Resolution 1540 was appended to Chapter VII of the UN Charter by unanimous vote on April 28, 2004.[41] Chapter VII sets out the UN Security Council's powers to maintain peace. Resolution 1540 adds the requirement that all member states develop laws with regulatory enforcement measures aimed at preventing the creation, proliferation, delivery, and spread of chemical, biological, and nuclear weapons by non–state actors. The desired intended outcome is to reduce the threat of non–state actors gaining access to and disseminating these weapons. The objectives of this Resolution were reiterated and the mandate was extended under UN Security Council Resolutions 1673, 1810, and 1977, with the Resolution 1540 Committee mandate extended to the year 2021 to ensure full implementation of the original resolution through capacity building and technical assistance.[41]

CLASSIFICATIONS OF BIOLOGICAL AGENTS OF CONCERN

Three systems are used for identifying biological agents that would have potential for highly adverse outcomes for humans, animals, or plants. The first, commonly used by clinicians and public health professionals, is the CDC Category A, B, and C agents (the CDC "ABC" list; see Table 15.1), which helps to prepare for, diagnose/detect, and treat/respond to national security threats posed by biological agents. The

second system, a derivative of the first, is the National Institute of Allergy and Infectious Diseases (NIAID) Category A, B, and C Priority Pathogens.[42] In addition to the CDC "ABC" list, the NIAID list adds pathogens of public health interest that are not necessarily viewed as agents of or diseases likely to be caused by an act of bioterrorism. The list was created as an intramural and extramural program management tool in keeping with the NIAID biodefense and emerging infection missions. The third list of biological agents, the Select Agents and Toxins List, are those that are monitored by the mandated Federal Select Agent Program. The scrutinized agents may be possessed or transferred by nonclinical locations, such as research laboratories, and have the potential to pose a severe threat to the public, animal, or plant health if released. This is a regulatory program with established regulations; program personnel inspect locations in possession of these agents, set compliance standards, and conduct investigations when noncompliance is suspected. The CDC "ABC" list is not the same as the Select Agents list,[43] although many agents are on both lists. The US Department of Health and Human Services (HHS) contribution to the list was formulated based on the following criteria for each agent or toxin: (1) its effect on human health following exposure; (2) its degree of communicability and the route of transmission; (3) the availability and effectiveness of medical countermeasures (MCMs) to treat or prevent it; and (4) other criteria such as needs of vulnerable populations, including children.[44] The Tier 1 select agents on the list are very similar to the CDC Category A agents because these are considered to have the highest potential adverse associated consequences following exposure.

Formulation of the CDC List of Category A, B, and C Agents/Diseases of Bioterrorism

The CDC "ABC" list is the classification system most commonly used by healthcare providers and public health professionals to identify potential bioterrorism threats (see Table 15.1). It reflects the 1999 initial assessment undertaken by a multidisciplinary group of experts and the Working Group on Civilian Biodefense consensus panel.[45] There were two critical inputs to this process. One was a threat assessment to evaluate the likelihood that a particular intentional bioterrorism event will occur given estimated capabilities and intentions of the potential perpetrator(s).[46] This process is fraught with uncertainties.[47] Therefore the second element, a risk assessment, was added to complement the threat assessment. The risk assessment is used to estimate the probability of adverse public health impacts from each biological agent under specified exposure conditions, to identify the production capabilities needed, and to examine person-to-person transmissibility, the estimated public level of concern/panic should there be a bioterrorism event involving each pathogen, and if special preparation is needed, and at what level, in advance of a bioterrorism event to minimize casualties.[48] These assessments are revisited on a regular basis.

Category A agents are those with the highest priority, specifically defined as those that (1) are most likely to have the highest consequence to individuals and populations due to their ease of dissemination or ease of person-to-person transmission, (2) may result in high mortality rates and have the potential for a major public health impact, (3) may cause associated public panic, and (4) require special actions to attain public health preparedness.[45] These highest adverse consequence conditions, the Category A diseases (see Table 15.1), include anthrax, botulism, plague, smallpox, tularemia, and viral hemorrhagic fevers, which are rarely, if ever, seen by clinicians and public health professionals.

The Category B agents are considered to have lower associated adverse health outcomes. Some of the agents, particularly food safety threats (see Table 15.1), are commonly seen by health care providers in routine practice and by public health practitioners undertaking commonly occurring foodborne outbreak investigations. The Category C agents (see Table 15.1) capture the concern of intentional engineering of existing pathogens to render them more virulent and then disseminating them to cause deliberate harm. This category also captures the possibility that a newly emerging infection or condition could be used in nefarious ways.

The CDC and NIAID Biodefense and Priority Pathogen Lists in Perspective

The impact of those endemic and epidemic emerging infectious diseases not found on the CDC "ABC" list but found on the NIAID list is orders of magnitude greater than what was noted with the deliberate use of biological weapons. A proposed impact measure helps to put the outcomes of biological weapons use in perspective. Using this impact typing system, deliberately caused bioterrorism incidents and biocrimes have fallen into the lowest impact tier, Individual level (type A), affecting less than 1000 persons.[49] Interestingly, since the end of WWII the use of biological weapons by state actors also has been in this lowest impact tier.[12-14] This is in contrast to past planned usage by state-sponsored offensive biological weapons programs targeting outcomes at the Town level (type B), affecting between 1000 to up to 1 million persons, or at the Country level (type C), affecting 1 million to under 1 billion.[49] At the Global level (type D), there is a potential for 1 billion or more deaths—unlikely to be found outside of science fiction novels.

Dual Use Research of Concern, Gain-of-Function Research, and Synthetic Biology

A subtext to the three categorization schema discussed previously is the concept of research conducted on one of the listed pathogens, ostensibly for peaceful purposes, which may be directed at future nefarious use with broad adverse consequences to health and safety—so-called dual use research of concern (DURC).[50] Relatedly, research that inadvertently or deliberately seeks to enhance virulence of existing less pathogenic organisms—gain-of-function (GOF) research—is a concern.[51] This latter concern has engendered extensive debate on the impact of curtailing such research when used to gain insights that may be critical for the development of improved MCMs. Following a pause in US government funding for GOF research involving influenza, severe acute respiratory syndrome, and Middle East respiratory syndrome viruses, which was initiated in October 2014, the ban was lifted on December 19, 2017 after a multidisciplinary review was completed and a policy guidance issued.[52]

In addition to DURC and GOF research is the concern regarding the use of synthetic biology to create modified or new biological agents to deliberately cause harm. The definition of synthetic biology is often debated but generally is thought to encompass either the redesign of existing natural biological systems or the design and creation of new biological systems or parts for useful purposes.[53] This burgeoning and relatively new field has coupled advances in computing power with our ability to quickly synthesize complete genomes and edit genes through the use of new and improved techniques, such as clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9).[54] Although considered an academic field of study, the tools of synthetic biology can be moved from academia into other sectors to develop needed useful products. The power of synthetic biology is exemplified in the creation and then production of synthetic artemisinin for treatment of *Plasmodium falciparum* following shortage of the naturally grown *Artemisia annua* plant in Asia.[55,56] However, this same suite of powerful tools could be misused by large and small enterprises with requisite knowledge, skills, and resources to create a biological weapon without first acquiring a pathogen or toxin.[57]

ADDRESSING THE HIGHEST PRIORITY BIOLOGICAL AGENTS

Using the results of the mandated biannual Biological Terrorism Risk Assessment[58] conducted by the US Department of Homeland Security, coupled with evaluations undertaken by the US government's interagency Public Health Emergency Medical Countermeasures Enterprise (PHEMCE), potential national security threats needing a high-priority for MCM development are identified.[59] The focus of MCM development efforts are to mitigate the health outcomes through the use of vaccines, other biologic products, drugs, diagnostic tests, and personal protective equipment. All CDC Category A biological agents are targets for MCM development by PHEMCE, as well as multidrug-resistant *B. anthracis*, found on the Select Agent list, and *Rickettsia prowazekii*, *Burkholderia mallei*, and *B. pseudomallei*, found on the CDC Category B list (see

Table 15.1). Pandemic influenza (see Chapter 165) is also found on this list of PHEMCE high-priority agents but is not discussed here.

Approval and Use of Medical Countermeasures for Priority Biological Agents

The infrequent occurrence of most of the priority agents means that there may not be MCMs that are approved for use by the US Food and Drug Administration (FDA). Despite being targets for product development, many of these MCMs are unapproved (i.e., in development and under Investigational New Drug [IND] status). The terrorist attacks in 2001, particularly the anthrax letter attacks, highlighted the need for regulatory mechanisms to authorize the use of unapproved MCMs with the potential for preventing or treating biological agents, if needed, in large populations during emergencies. Separately, the Animal Rule (also known as the Animal Efficacy Rule) was established as a regulatory pathway for the development of MCMs whose efficacy could not be readily studied in human clinical trials.[60,61] Not surprisingly, the first use of these two statutory remedies was for products to prevent or treat anthrax.[62] Similar approaches for streamlining the development and use of investigational products in an emergency exist in other countries.

Emergency Use Authorization

During a public health emergency potentially involving a large number of persons—including during an outbreak of disease due to a biological agent, such as one following a bioterrorist attack—needed MCMs may not have completed their development pathway to approval by the FDA. In such a public health emergency, if the diagnosis and treatment of large numbers of people requires use of a large number of investigational MCMs, applying the regulatory requirements for use under IND status could lead to delayed care with possible heightened morbidity and mortality. In anticipation of such conditions, a regulatory mechanism was created that could be applied not only for bioterrorism but also for other serious or life-threatening diseases or conditions resulting from chemical, radiologic, or nuclear threats.

The Project Biosheld Act of 2004 (Public Law [P.L.] 108-276)[61] added provisions to the Food, Drug, and Cosmetic Act to create statutory remedies, with subsequent amendments to the law made by the Pandemic and All-Hazards Preparedness Reauthorization Act (PAHPRA) of 2013 (P.L. 113-5)[63] and then in 2016 by the 21st Century Cures Act (P.L. 114-255).[64] An emergency use authorization (EUA) allows use of certain unapproved medical products or certain unapproved use of approved medical products, in a time-limited manner, when certain conditions are met (Fig. 15.1), including that there is no other readily available and existing regulatory mechanism. The authority to issue an EUA for a product is delegated by the Secretary of HHS to the Commissioner of the FDA. There are five statutory requirements, all of which must be met for an EUA to be issued, but even if met, the Commissioner of the FDA may opt not to use the authority (see Fig. 15.1). First, the Secretary of HHS must declare that there is a emergency or threat that would permit EUA use. Second, the threat in the Secretary's declaration must be capable of causing a serious or life-threatening disease or condition. Third, there must be evidence of product effectiveness for its intended use. Fourth, a risk-benefit analysis favors likely benefit over risk. Fifth, there are no existing adequate alternatives among available FDA-approved products.

The steps in the process are exemplified by the first EUA issued in 2005 for the use of anthrax vaccine adsorbed (AVA), a vaccine licensed, at the time, for prevention of cutaneous anthrax but not approved for prevention of inhalational anthrax. The first step was the issuance, on December 10, 2004, of the required determination by the Deputy Secretary of Defense that there was a significant potential for a military emergency involving a heightened risk to US military forces of an attack with anthrax.[65] The Secretary of HHS then issued, on January 14, 2005, the required declaration that circumstances existed to warrant an EUA; the EUA was also issued on that day and was terminated on February 1, 2006.[66]

The Animal Rule Use for Approval of Drugs, Vaccines, and Biologics

In the wake of the anthrax letter attacks, the 107th Congress passed the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (P.L. 107-188) instructing the Secretary of HHS to complete the final rulemaking, started in 1999, to permit the use of animals for approval of MCMs, under rule-specified conditions.[67,68] This act allowed the use of animals to approve an MCM that is targeted for use to prevent or ameliorate serious or life-threatening conditions caused by exposure to substances, including biological agents, when human efficacy studies are not ethical or field trials are not feasible. The final rule, known as the Animal Efficacy Rule or the Animal Rule, was added to the Code

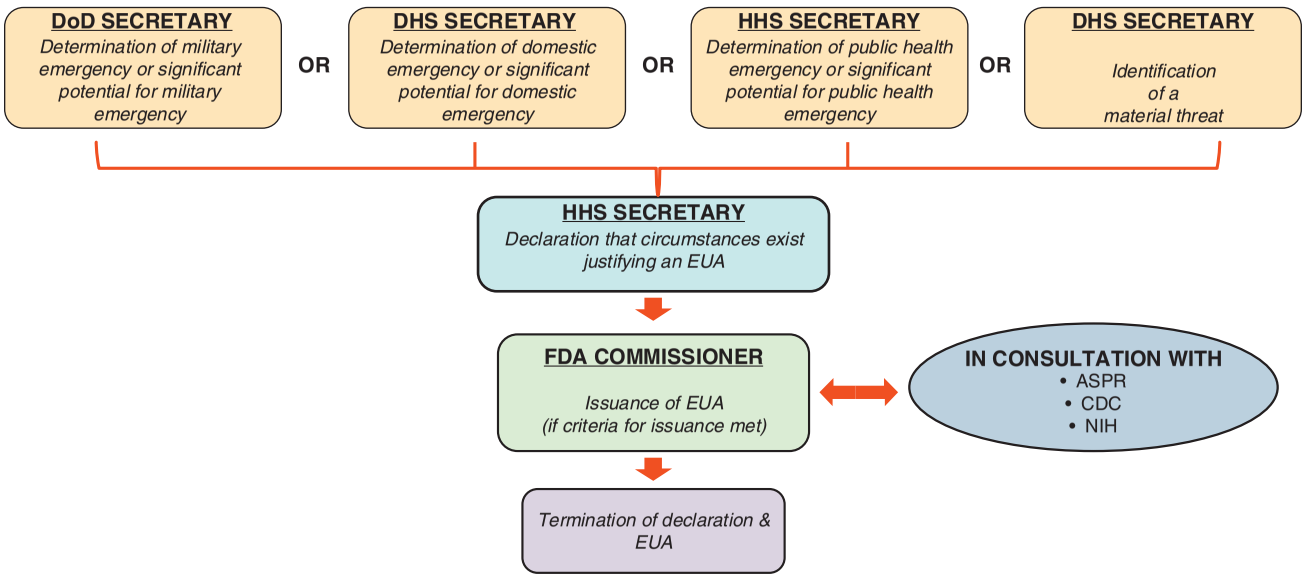

FIG. 15.1 Summary of the process for an Emergency Use Authorization *(EUA)* issuance. *ASPR,* Office of the Assistant Secretary for Preparedness and Response; *CDC,* Centers for Disease Control and Prevention; *DHS,* US Department of Homeland Security; *DoD,* US Department of Defense; *FDA,* US Food and Drug Administration; *HHS,* US Health and Human Services; *NIH,* National Institutes of Health. *(Modified from US Food and Drug Administration. Summary of process for EUA issuance; 2018.* https://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm411445.htm.*)*

TABLE 15.5 Criteria for Approval of Medical Countermeasures Under the Animal Rule and Approved High-Consequence Biological Agents, 2002–2018

Effective Evidence Criteria for Use of the Animal Rule[a]

CRITERION	DESCRIPTION
1	Reasonably well-understood pathophysiological mechanism of toxicity or the biological agent and its prevention or substantial reduction by the MCM submitted for licensure or approval.
AND	
2	Effect of the MCM is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized model for predicting the response in humans.
AND	
3	Animal study end point is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.
AND	
4	Data or information on the kinetics and pharmacokinetics of the product or other relevant data or information, in animals AND humans, allows selection of an effective dose in humans.

Approved High-Consequence Biological Agent MCM[b]

CLASS OF MCM	MCM	PATHOGEN TARGETED	SPECIFIC INDICATIONS	DATE OF APPROVAL
Antibiotics				
	Levofloxacin	*Yersina pestis*	ID and SD Rx and PEP[c]	April 22, 2012
	Ciprofloxacin	*Yersinia pestis*	ID and SD Rx and PEP[c]	February 2, 2015
	Moxifloxacin	*Yersinia pestis*	ID and SD Rx and PEP[c]	May 8, 2015
Nonvaccine Biologics				
	Raxibacumab (Abthrax®)	*Bacillus anthracis*	ID Rx and PEP + Abx	December 12, 2012
	Botulinum antitoxin, heptavalent (A, B, C, D, E, F, G), equine	*Clostridium botulinum* toxin, types A–G	Symptomatic botulism following documented or suspected exposure to types A–G	March 22, 2013
	Anthrax immune globulin intravenous, human (Anthrasil®)	*Bacillus anthracis*	ID Rx + Abx	March 25, 2015
	Obiltoxaximab (Anthim®)	*Bacillus anthracis*	ID Rx[d] + Abx PEP[e]	March 21, 2016
Vaccines				
	Anthrax vaccine adsorbed	*Bacillus anthracis*	PEP + Abx	November 23, 2015
Antivirals				
	Tecovirimat	Variola virus (smallpox virus)	Treatment[f]	June 13, 2018

[a]See Code of Federal Regulations: 21 CFR 314.610a for drugs and 21 CFR 601.9 for vaccines and other biologic products.
[b]Approved for use in adult and pediatric patients except where indicated.
[c]Moxifloxacin approved only for use in adults.
[d]Does not cross the blood-brain barrier and does not prevent or treat meningitis.
[e]Only when alternative therapies are not available or not appropriate for use.
[f]Oral treatment only; children weighing 13 kg or more and adults.
Abx, Antibacterial drugs; *ID,* inhalational disease; *MCMs*, medical countermeasures; *PEP,* prevention after exposure; *Rx,* treatment; *SD,* septicemic disease.
Modified from US Food and Drug Administration. CDER drug and biologic animal rule approvals as of July 17, 2018. https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/NDAandBLAApprovalReports/UCM578137.pdf. Accessed July 25, 2018.

of Federal Regulations on May 31, 2002 and became effective on July 1, 2002,[69] with multiple subsequent revisions, the latest in April 2017.[70] The initial published draft guidance for use of the Animal Rule was updated and published in final form by the FDA on October 28, 2015.[71] To date, four new MCMs and four new uses of previously approved MCMs have been approved under the Animal Rule to prevent or treat high-priority biological agents—the first in 2012 (Table 15.5).

CLINICAL ASPECTS OF HIGH-CONSEQUENCE BIOTERRORISM PATHOGENS

Variola (Smallpox) Virus

Smallpox, a disease caused by variola virus (see Chapter 132), was declared eradicated by the World Health Organization (WHO) in 1980.[72] Concerns have been raised about the risk of a bioterrorism attack using this virus because of the possibility that all remaining stocks of smallpox virus were not destroyed following declared eradication. The VECTOR laboratory in Novosibirsk Region in the Russian Federation and the CDC in Atlanta, Georgia in the United States are the only two declared repositories designated by WHO to maintain stocks of the smallpox virus. Both institutions continue to do research on smallpox, albeit under the close scrutiny of the WHO Advisory Committee on Variola Virus Research.[73] It is difficult to ascertain whether other, undisclosed, repositories exist. The 2014 discovery of vials containing smallpox on the research campus of the National Institutes of Health highlights this reality,[74] along with the potential use of synthetic biologic techniques to recreate this pathogen.[75] Although the risk is unknown, and is thought to be very low, a release of smallpox today could result in a public health catastrophe.

It is estimated that a single virus particle in contact with the mucosa could initiate infection.[76] The virus is relatively stable as an aerosol and can be stored in a lyophilized form for many years. If an aerosol were deliberately released, the initial cases would likely present with a hemorrhagic pulmonary form of smallpox due to the high exposure dose. This hemorrhagic form of disease was a rare presentation during the days of endemic smallpox. Typical smallpox lesions are deep-seated vesicles or pustules in the same stage of development. In the past, endemic smallpox had an associated mortality rate of 30% of those infected.

A clinical guideline for the use of smallpox vaccine in the postevent setting has been published by the CDC (see Table 15.2). The Strategic

National Stockpile holds a large stockpile of licensed live, attenuated smallpox vaccine (ACAM2000®) and a smaller stockpile of a second investigational live, attenuated virus vaccine. A third vaccine, nonreplicating modified vaccinia Ankara, which was never evaluated in the field when smallpox was an endemic disease, is available as an investigational MCM for use in certain immunocompromised persons. Most countries have little or no vaccine, and worldwide production capacity is minimal.

Additionally, there is an antiviral treatment, tecovirimat (ST-246), now approved for use in the treatment of smallpox (see Table 15.5).[77]

Bacillus anthracis

A CDC Category A agent, *Bacillus anthracis* (see Chapter 207) usually causes anthrax in nonhuman warm-blooded animals, primarily ruminant herbivores, and is enzootic in most parts of the world. In the United States it is enzootic most commonly in Texas, North Dakota, and Nevada. Humans in contact with infected animals are at risk of developing anthrax, primarily as a cutaneous infection. In the United States up to 2 cases of human anthrax, usually cutaneous, are reported annually, commonly among persons in contact with infected, unvaccinated livestock.[78] Occasional cases of inhalational or gastrointestinal diseases have been reported among persons handling untreated, spore-contaminated animal hides or drums made from these hides.[78] It is not transmitted person-to-person. Additionally, *Bacillus cereus* Biovar *anthracis,* which has caused animal anthrax in sub-Saharan Africa, is classified as a Tier 1 select agent.[79,80]

As previously noted, *B. anthracis* was one of the principal biological weapons in the arsenal of former state-run biological weapons programs, including in the former Soviet Union and in Iraq (see Table 15.3). Non–state actors have also pursued anthrax for weapons purposes. For example, the Japanese Aum Shinrikyo cult's attempt to develop an anthrax weapon went undetected for 5 years until the cult's sarin gas attack in the Tokyo subway system in 1995 attracted the attention of authorities.[3] In the years prior to the US military engagement in Afghanistan in 2001, the terrorist group Al-Qaeda pursued establishing a biological weapons capability that included *B. anthracis*.[81]

The organism's ubiquitous nature and long-lived spore form highlight its availability and, when coupled with the minimal technical skills and permissive growth requirements needed to grow this organism, underscore why it may be explored for use by terrorists. Additionally, methods to induce multidrug resistance in the organism have been published in the peer-reviewed scientific literature.[82,83] Whether such strains maintain virulence is unknown. If used intentionally, an aerosol delivery route of exposure, as seen with the anthrax letter attacks, would likely be the most efficient and the most deadly.[84]

There are multiple FDA-approved MCMs to prevent and treat infection, including AVA for use prior to exposure; AVA is commonly given to large animal veterinarians, for example, who care for ruminants. AVA is also recommended in combination with antibiotics for postexposure treatment for persons who are without symptoms of disease.[85] There are now three licensed biologic treatments in the United States. The first is anthrax immune globulin for intravenous infusion (Anthrasil®), approved in 2015 (see Table 15.5). It is manufactured from the plasma of persons vaccinated against anthrax who have mounted documented antibody that neutralizes anthrax toxins produced by the organism.[86,87] The product is indicated for treatment of persons with inhalational anthrax and is used in combination with antibacterial drugs. The second product is raxibacumab (Abthrax®), a monoclonal antibody that binds domain IV of the anthrax protective antigen,[86,88] which was approved in 2012 (see Table 15.5). The third product is obiltoxaximab (Anthim®), also a monoclonal antibody and approved in 2016. All are given intravenously to treat inhalational anthrax in combination with antibacterial drugs. These MCMs were tested in humans for safety but efficacy was demonstrated in animals using the Animal Rule,[71] which permits licensure of a product based upon animal efficacy studies when a human clinical trial would be unethical. The CDC has produced a guideline for the clinical management and use of MCMs during an anthrax mass casualty incident (see Table 15.2).

Clostridium botulinum Toxin

Botulism is a neurologic intoxication caused by one of the seven botulinum toxins (designated type A to type G) produced by *Clostridium botulinum* (see Chapter 245), a CDC Category A bioterrorism agent. Like *B. anthracis,* the microorganism is ubiquitous in soil in its spore form, which can remain dormant for 30 years or more.[89] The toxins, produced by the vegetative form of the organism, are the most potent poison known and are associated with high case fatality if untreated. In the United States approximately 200 cases of botulism are reported annually.[78] Most cases in the United States occur following ingestion of food containing the preformed toxin. Other forms include wound botulism or intestinal botulism, in which the spore undergoes in situ germination to produce toxin that is then systemically absorbed.[89] Notably, very dilute amounts of type A and type B toxin have therapeutic and cosmetic purposes.[90] Importantly, botulism following inhalation of aerosol purified toxin has occurred in the research laboratory setting following minute exposure doses.[91]

Botulinum toxins were one of the principal weapons in the arsenal of the former Soviet Union and are known to have been produced as a weapon by Iraq.[92] One terrorist group, the Aum Shinrikyo, tried unsuccessfully to produce and disseminate botulinum toxin.[93] *Clostridium botulinum* static and fermenter-based growth requirements for toxin production are well known and have been perfected over the years, particularly for the production of therapeutic and cosmetic products, making the production technology readily available.[94] Deliberate aerosol dissemination would create an initial diagnostic dilemma because standard serologic and body fluid detection methods often give confusing results.[95] Furthermore, deliberate use of preformed botulism toxin by contamination of food or by aerosol release could cause a large number of casualties that could cripple the health care delivery system if the number of casualties exceeds the intensive care and ventilator support capacity available for treatment.[96]

Yersinia pestis

Plague is caused by *Yersinia pestis* (see Chapter 229), a CDC Category A bioterrorism agent, and is both endemic in the United States[78] and periodically epidemic in various parts of the world. It is usually a zoonotic infection of humans.[97] Untreated bubonic plague can lead to septicemia that is associated with pneumonia and subsequent aerosol transmission to others manifesting as pneumonic plague.[98] Although uncommon, infected dogs can transmit to humans via the respiratory route.[99] The person-to-person transmissibility of pneumonic plague made *Y. pestis* a candidate for development and use in state-sponsored biological weapons programs.[98] Primary pneumonic plague would result from an aerosol exposure and lead to a rapidly progressive and lethal infection absent immediate medical intervention; this form of plague is transmissible to others.

There is no longer an FDA-licensed vaccine for the prevention of plague. An inactivated *Y. pestis* vaccine, plague vaccine USP, was licensed in the United States on October 5, 1994, but production discontinued in 1999.[100] A recombinant F1-V fusion protein plague vaccine completed a phase IIa clinical trial in healthy adults in 2012.[101] It received Orphan Drug designation for the prevention of plague (*Y. pestis*) by the FDA on November 30, 2016.[102] The vaccine is intended for administration for persons at high risk for exposure to aerosolized *Y. pestis;* it is anticipated that future licensure could follow if efficacy is demonstrated in clinical studies or through the Animal Rule. There is no known stockpile of this vaccine.

There are now three FDA-approved bactericidal antibiotics indicated for use to treat inhalational plague or those persons exposed to the pathogen but not yet ill—levofloxacin, ciprofloxacin, and moxifloxacin (see Table 15.5). Streptomycin, not widely available in the United States, has long been an approved parenteral treatment for plague. Gentamicin, although not FDA approved for the treatment of plague, has been used off-label to treat plague, usually in combination with other drugs.[103,104] Like a large aerosol release of botulinum toxin, a large *Y. pestis* release could overwhelm critical care resources needed for immediate management of pneumonic disease, although for a shorter period than that anticipated for botulism.

Francisella tularensis

Tularemia is caused by the bacterium *Francisella tularensis* (see Chapter 227), a CDC Category A bioterrorism agent (see Table 15.1) found in many northern hemisphere countries, including the United States (all states except Hawaii).[78] There are six clinical forms of this zoonotic

infection depending upon exposure route.[105] Unintentional routes of infection include direct contact with animals, ingestion of contaminated food or water, arthropod bites, and inhalation of infected aerosols. It is a hardy organism capable of surviving for weeks in the environment.[106] Between 2005 and 2015 there were between 100 and 300 reported cases of tularemia annually, usually in the south central United States, the Pacific Northwest, and parts of Massachusetts.[78]

Francisella tularensis was developed into an aerosol biological weapon by several countries in the past, including the United States while it was operating its offensive biological weapons program. There are several reasons for selection of this pathogen. First, inhalation of as few as 10 organisms can cause disease—and may lead to serious illness and death.[105] Second, given its widespread occurrence in nature, it is theoretically easy to obtain. Third, it can be grown on common nonselective media that are not difficult to obtain. If used as a bioweapon in the future, it is likely to be introduced by the aerosol route. Importantly, it is not transmitted person-to-person, even the respiratory form of infection.

A live tularemia vaccine strain brought to the United States from the Soviet Union in 1942 was used to develop a vaccine in the United States, designated as a live vaccine strain (LVS).[107] The vaccine has been used as an IND product for prevention of tularemia in laboratory workers, primarily at Department of Defense laboratories. Existing and new lots of the vaccine were tested in a phase II, multicenter, double-blind, randomized comparison safety and immunogenicity trial in 228 subjects ending in 2012; no differences between the vaccines were identified.[108] No further development plan has been identified.

Existing approved antibacterial agents have been recommended for the treatment of inhalational tularemia.[109] However, none of these products has an on-label indication for this specific pathogen or form of disease.

Rickettsia prowazekii

Louse-borne or epidemic typhus is caused by *Rickettsia prowazekii* (see Chapter 189), a bacterium carried and transmitted by body lice. It is a CDC Category B agent (see Table 15.1) and a Tier 1 select agent.[43,44] Unintentionally occurring disease is typically associated with war, famine, and other poor causes of unhygienic conditions associated with human lice infestation. Transmission is from person to person via lice. Additionally, in societally disrupted settings, the environment can be contaminated with dried louse feces carrying the microorganism, leading to aerosolized bacteria in louse fecal dust.[110] The median infectious dose is fewer than 10 organisms.[111]

In 1940, the Japanese Unit 731 biological weapons program conducted tests over a 5-month period, spreading 70 kg of *R. prowazekii* by an unclear dissemination method and precipitating a typhus epidemic in Ning Bo, a Chinese seaside resort town.[112] This pathogen was also reported by a defector to have been one of the first biological weapons developed during the 1930s in the Soviet Union.[26]

The concern is the intentional delivery of this biological agent by the aerosol route at a dose much higher than that seen under natural conditions, which could alter the natural history, expression, and fatality associated with the disease. There is no licensed *R. prowazekii* vaccine in the United States. Treatment with doxycycline is usually effective.[111]

Burkholderia mallei

Glanders is caused by infection with the bacterium *Burkholderia mallei* (see Chapter 221). It is CDC Category B agent (see Table 15.1) and a Tier 1 select agent. *Burkholderia mallei* causes an infection primarily of solipeds, found in lower resource settings. The last case in the United States was in 1942 in a horse.[113] Transmission from solipeds to humans is uncommon, even in the setting of close contact animals, likely because the usual exposure settings are associated with dosages too low to cause disease.[114] Notably, when it did occur in the preantibiotic era, over 90% of human cases were fatal. Usual modes of acquisition involve infectious animal material coming in contact with nonintact skin or mucosal surfaces. Unintentional disease is rarely fatal with prompt and appropriate antibiotic treatment. Rare cases have occurred following laboratory accidents, most often by the aerosol route, when a higher infecting dose is delivered.[114]

Importantly, *B. mallei* has been used as a biological weapon. It was the first biological weapon used by the Germans during WWI by infecting livestock and horses with the agent before they could be shipped to the Allies for use by cavalry members.[115] During WWII, this pathogen was part of the Japanese Unit 731 biological weapons program, which included infecting prisoners with *B. mallei* and performing autopsies on 21 fatal cases.[112] The concern today is that *B. mallei* could be deliberately released as a high-dose aerosol, causing a large number of rapidly evolving pulmonary and septicemic cases, with many initial deaths due to delays in diagnosis and treatment. Prolonged therapy to prevent relapse is needed. There is no preventive licensed vaccine available to prevent disease.[116]

Burkholderia pseudomallei

Melioidosis is caused by *Burkholderia pseudomallei* (see Chapter 221),[116] a CDC Category B agent (see Table 15.1) and a Tier 1 select agent.[43,44] Unlike its zoonotic cousin *B. mallei*, which causes glanders, *B. pseudomallei* is a saprophytic bacterium found in soil and water in tropical and semitropical areas of the world, particularly Southeast Asia and northern Australia. In endemic areas, the routes of transmission are primarily by inoculation of abraded areas of nonintact skin, percutaneous, by ingestion, and by inhalation in persons with direct soil contact. Inhalational transmission was supported epidemiologically when significantly different rates of acute pulmonary melioidosis were noted among US military helicopter winch operators in Vietnam compared with other troops.[117] Person-to-person transmission rarely occurs, even in severe pulmonary disease.

The disease is associated with a high mortality due to the speed with which septicemia develops, particularly in immunocompromised hosts—especially those with comorbidities in whom metabolic acidosis is present—and the inherent resistance of the bacteria to several classes of antibiotics.[118] Prolonged courses of antibiotics are required to treat melioidosis, with the risk of recurrence following treatment.[116] As with glanders, prolonged antimicrobial therapy is required to treat it and to prevent its relapse.

There is no evidence that *B. pseudomallei* has been included in any past known state-sponsored biological weapons program. However, the organism can survive for months to years in endemic areas, particularly in waterlogged, heavy clay soils following monsoons, typhoons, and hurricanes.[117] The organism persists in biofilms, in drinking water with generally used chlorine levels, and within amoebic cysts.[117]

Viral Hemorrhagic Fever Viruses

Several important viral hemorrhagic fever viruses are classified as CDC Category A agents (see Table 15.1). These include the Filoviridae Ebola virus and Marburg virus (see Chapter 164) and the Arenaviridae Lassa virus and Machupo virus (see Chapter 167). These viruses usually have high associated morbidity and mortality.[119] None of these pathogens is known to have been used in the past as a biological warfare agent (see Table 15.3) or to commit an act of terrorism or a biocrime (see Table 15.4). However, concerns exist that the Russian Federation may have developed and stockpiled one or more of these agents as biological weapons.[29-31,32]

The large West African Ebola virus disease outbreak, extending from late 2013 (initially undetected) through early 2015,[120] served to hasten development of MCMs for this pathogen and to update management guidelines (see Table 15.2). Health care workers, including burial attendants, had the highest case fatality rate.[121] There are now investigational MCMs for use following exposure, including investigational vaccines, one of which has been shown to be efficacious in a postexposure setting when used within 6 days of exposure; specific monoclonal antibody combinations; and small-molecule antiviral agents.[122] Aerosolization of Ebola virus or Marburg virus with subsequent inhalation of virus, a concern should viral hemorrhagic fever virus be used deliberately, has not been proven to be a mode of transmission.

BIOTERRORISM INCIDENTS

Clinical Provider Preparedness and Response

The early detection of a bioterrorism attack is critical to the most efficient and effective response by health care and public health systems, directed at minimizing morbidity and mortality. Health care providers are on the detection front line.[123] It is anticipated that primary care providers,

emergency medicine physicians, and infectious disease specialists will continue to be key to the earliest identification of a suspect event through their application of epidemiologic principles while evaluating the acute illness presentations of their patients.[1]

Many of the CDC Category A high-consequence pathogens are endemic in parts of the United States but at a low background level. Therefore most providers have little experience with these conditions, even when they are naturally occurring. Preevent basic knowledge of the pathogens and their usual presentations is important in helping to trigger a clinical index of suspicion that a bioterrorism even may be occurring (Table 15.6). Health care providers can complete bioterrorism training modules and video webcasts to enhance knowledge in this area as well as reviewing biological agent—specific clinical management guidances (see Table 15.2).

A suspicion that a presenting infection or intoxication may be deliberately caused is often triggered by being alert for patterns of illness and diagnostic clues. For example, if a healthy young person presents with a relatively acute onset of shortness of breath and a widened mediastinum on a chest radiograph and reports recently attending a summertime event at a local arena with three friends who also are experiencing a similar but less severe illness, this should alert the clinician to the presence of possible deliberately caused inhalational anthrax. This brief patient-presented history provides both diagnostic clues and suggests a clustering of illness. Other alerting features include the occurrence of a relatively severe, acute illness in a previously healthy person, and a report of illness in others who attended the same event, suggesting a possible point source.

In addition to diagnostic testing and treatment initiation for this and other possible conditions, contacting local public health authorities immediately along with the health care system leadership, if employed in this setting, is important. Sharing this information as quickly as possible will ensure that the next steps in detection, investigation, response, and public outreach are undertaken in a timely manner. The frontline clinician is generally not expected to inform law enforcement. This generally falls to public health authorities, especially should the diagnosis be confirmed.

Supplementing Health Care Provider Resources

In the event of a large bioterrorism event, there may be insufficient providers to care for patients. When supplementation of clinical response efforts is needed, the National Disaster Medical System (NDMS) and its Disaster Medical Assistance Teams (DMATs)[124] and the Medical Reserve Corps (MRC)[125] can be activated.

The NDMS, a system coordinated by the HHS, acts to temporarily supplement state and local medical care needs after a disaster of any kind. The NDMS can provide personnel, supplies, and equipment at the site or at definitive care sites in unaffected areas, and can provide patient care movement. DMATs are local units activated for 2-week deployments with sufficient supplies and equipment to be self-sustaining for at least 72 hours before resupply is necessary.[124] In the event of a national disaster, DMATs may be moved from their local area, at which time they are made federal employees with medical credentials recognized in all states and protected under the Federal Tort Claims Act[126] against any malpractice claim. Predisaster employment is protected under the Uniformed Services Employment and Reemployment Rights Act.

The MRC was created in 2002 as community-based and locally organized groups of health care volunteers who donate their time and expertise to prepare for and respond to existing emergency medical and public health resources when needed. There are more than 300 units in the United States. Units have been active, for example, in providing services after hurricanes. The Office of the Surgeon General acts as a clearinghouse for information and best practices in establishing and maintaining MRC units. Liability protection for individual MRC practitioners is determined by each state.

Supplementing MCMs With the Strategic National Stockpile

In fiscal year 1999, the CDC received $51 million for the development of an emergency stockpile of MCMs, then called the National Pharmaceutical Stockpile, primarily antibiotics for anthrax and a vaccine for smallpox. Now named the Strategic National Stockpile, this is an important part of the response armamentarium after a bioterrorism incident.[127] The Strategic National Stockpile contains antibiotics, antitoxins, vaccines, life-support medications, and medical supplies that can be used to supplement state and local resources during a large-scale public health emergency. Within 12 hours of a request, a Push-Package containing an initial supplemental cache of MCMs and supplies can be at the targeted destination. These packages have been prepositioned in strategically located secure warehouses to facilitate prompt delivery. If additional support is necessary, a vendor-managed inventory is called on to deliver ongoing needed MCMs and supplies. Distribution is coordinated in concert with the receiving jurisdiction.

Health Care Facility Preparedness and Response

Persons with severe illness following deliberate exposure to a biological agent may likely present first to a hospital emergency room or be directly admitted to a hospital by their primary care provider. Preparedness for addressing public health emergencies, with resulting stress on the care systems, is critically important for a successful response.[128]

Through its Hospital Preparedness Program, the HHS aims to assist in the preparation of the health care delivery system through the development of health care coalitions.[129] Specific performance measures in four capability areas for hospital readiness to respond to a public health emergency, including a bioterrorism attack, have been created. These targets include creating a sustainable health care coalition among health care facilities, emergency medical services, emergency management organizations, and public health agencies for planning, training, exercising, and managing resources; coordinating health care and medical response approaches and teams; ensuring continuity of services even in the setting of a disrupted health care delivery system; and determining how to maintain surge response in the setting of a public health emergency. Although all hospitals do not need to have the capacity to completely manage one or more patients needing care in a highly contained area due to infection with a highly communicable high-consequence pathogen, each facility does need to know how to properly respond to and safely transfer such a patient.

Responding to Ebola virus provides an example of a tiered approach to manage future patients with possible or confirmed Ebola virus disease.[130] The majority of hospitals in the United States are designated as Frontline Health Care Facilities. These first-tier facilities function to quickly identify and isolate a patient with possible Ebola virus infection, notify infection control and state and local health officials, and have enough personal protective equipment for at least 12-24 hours of patient care. The second tier consists of 55 designated Ebola Assessment Hospitals that can isolate the patient, perform immediate laboratory studies, and coordinate Ebola testing with the state or the CDC,[130] and are able to care for the patient for up to 5 days with enough personal protective

TABLE 15.6 Recognizing a Possible Illness Caused by Exposure to a Biological Weapon

- Have a high index of suspicion.
- Be alert for patterns of illness and diagnostic clues.
- Think like an epidemiologist—consider the patient within a population context. **Ask yourself several questions:**
  - Does the patient have an unusually high fever? Unique features of a respiratory tract or gastrointestinal infection?
  - Does the patient have symptoms or signs or a confirmed condition not commonly seen in your location of practice, and the patient has not traveled to an endemic location?
  - Is the illness appearing in an unusual age group or as part of a cluster of cases?
  - Is there a simultaneous human outbreak of the condition with an epizootic in local animals?
  - Is there an unusual temporal or geographic clustering suggesting a point source exposure?

If you think what you are seeing is unusual, call your Health Department immediately.

equipment on hand. The final tier consists of Ebola Treatment Centers, which can care for the patient throughout the duration of illness. There is now a regional Ebola treatment network in the United States consisting of 10 facilities with so-called biocontainment units that have the capacity to receive in transfer and admit or directly admit patients with known or suspect Ebola virus or other high-consequence pathogen infections.[130]

Clinical laboratories within health facilities are considered as Sentinel Laboratories in the Laboratory Response Network (LRN) for bioterrorism.[131] There are approximately 25,000 private and commercial laboratories in the United States at this level. Most are hospital based or part of clinical institutions. The key role of these laboratories is early detection of biological threat agents, application of "rule-out" algorithms, and referral of needed samples to the next level of the LRN, one of about 155 reference laboratories.[132]

Hospital epidemiology and infection control (HEIC) plays a critical role in the overall management of a potentially transmissible high-consequence pathogen in the health care setting. Within the hospital setting HEIC functions to manage any facility outbreak related to the identified pathogen; ensure that standards are in place and followed for the protection of health care workers, volunteers, and patients; and control anxiety/concerns of families, employees, and unaffected persons within the health system.[133]

Public Health Preparedness and Response

Communication

A major challenge to public health officials is the institution of necessary measures to avoid panic in the face of an epidemic of a traditionally feared disease.[134,135] Reviews of past epidemics indicate that the most essential factor is effective leadership and competent, frequent, and open communication with the public, the press, professionals, and others concerned in dealing with the epidemic. This is an area that is too often neglected. The 2001 anthrax outbreak illustrated the problems resulting from inadequate lines of communication.[136] Health departments at all levels were overwhelmed by requests for information from the public, from health professionals, and especially from the media. None had experienced an epidemic threat such as this previously and none was prepared.[136] Frequent, authoritative, up-to-date reports through the media to the public proved absolutely vital, but it took time before a pattern for these became established.[136] The need for communication between and among professionals was clear, and this is now being addressed in part by the national Health Alert Network, which is financed by federal preparedness funds.[137]

Another important lesson learned from the anthrax letter attack response was the need for command centers to coordinate and direct operations and to facilitate the flow of information; these took time to become established and to begin to function well. Sophisticated centers are now in place in the office of the Secretary of HHS, in the CDC, and in many states and cities; they are now staffed on a 24-hour-per-day, 7-day-per-week schedule. The creation of such emergency operations centers during any public health crisis worldwide is now a part of the Global Health Security Agenda to meet the requirements of the WHO International Health Regulations of 2005.[138-140] Information and educational materials have been prepared with respect to Category A diseases and are available throughout the health system.[141] At federal, state, and local levels, exercises are being conducted to test response systems to determine how well they are actually functioning.[142]

A second factor in muting the likelihood of panic is to do everything possible to keep the normal day-to-day activities of citizens and the city as minimally disrupted as possible. Public officials at all levels have often been prone to want to invoke quarantine measures, whether to close airports or other parts of the transportation network or to forbid entry into or departure from cities or other large areas. This was the situation in all countries that reported cases of severe acute respiratory syndrome in 2003.[143] Experience has shown that quarantine measures are seldom effective and, in fact, often lead to more serious problems as many people seek to flee an area or deny the presence of possible cases in family or friends, thus precluding appropriate containment measures.[144]

Epidemiology

The epidemiologic challenges associated with responding to a bioterrorism attack are uniquely different from those associated with responding to an explosion or to the release of a chemical agent. The effects of the latter two events are readily apparent, allowing early approximations to be made as to the geographic extent of the problem and the number and nature of casualties to be expected. Needed response efforts can thus be gauged and initiated immediately. When a microbe or toxin is used, however, the incubation period of the biological agents means there is an inherent delay from the time a covert attack is launched until the realization that an attack has occurred—most likely by the identification of a cluster of sick patients or a single person with a condition associated with suspect clinical clues (see Table 15.6). The varying incubation periods of the disease inevitably mean a delay in gauging the magnitude and scope of the attack and deploying appropriate response efforts on the basis of the epidemiology of the ensuing outbreak. Simultaneous attacks with an aerosolized biological agent in several locations, for example, could generate a large, complex geographic distribution of cases, thereby delaying the timely development of an epidemic curve used as a tool to assist in characterizing the event. In addition, exposure to a large inoculum of aerosolized biological agents could result in atypical disease presentations and clinical courses (e.g., shorter incubation periods, compressed and severe disease course), thus creating both clinical and epidemiologic conundrums.

On the basis of experience gained during the anthrax letter attacks, it is to be expected that there will be widespread apprehension, fear, and concern about the possibility of further cases—from the spread of a contagious agent and/or from sequential attacks. Many people may live in fear that they or their families will be the next victims. Experience shows that this inevitably complicates event investigation and response efforts.[145-148] For example, in the 2001 anthrax attack it was not until more than 2 weeks after the initial anthrax-laden letters were sent through the US Postal Service that the index case was diagnosed and reported.[2,136] By that time nine cases of anthrax had actually occurred (two inhalational and seven cutaneous). As health and law enforcement authorities subsequently worked to determine what had happened and to implement appropriate response measures, additional anthrax-laden letters were sent (3 weeks after the initial letters), resulting in an additional 13 cases of anthrax (9 inhalational and 4 cutaneous). There was widespread heightened concern among various segments of not only the US population but citizens in other areas of the world as well, who were experiencing a large number of hoaxes with envelopes containing powders—all of which required investigation by public health and law enforcement officials.[136]

Laboratory Preparedness and Response

A network of National, Reference, and Sentinel Laboratories defines the Laboratory Response Network, which was established by the HHS at the CDC in 1999.[132] The LRN includes a component that specifically addresses biological terrorism in collaboration with the Association of Public Health Laboratories and the FBI. It defines a tiered system of laboratories for the identification and verification of biological agents. The Sentinel Laboratories, previously described, are part of the health care system and function in the Biosafety Level 1 (BLS-1) or BLS-2 environment. The American Society of Microbiology works closely with the CDC and Sentinel Laboratories to provide needed protocols and training for laboratorians. The Sentinel Laboratories refer questionable samples to the second tier of approximately 150 Reference Laboratories, which form the backbone of the LRN's public health laboratory arm and function as high as BSL-3 for further identification and investigation. This tier includes state and local public health, military, veterinary, agriculture, food, and water testing laboratories. Additionally, certain countries, such as Australia, Canada, the United Kingdom, Mexico, and South Korea, have their own Reference Laboratories and are part of the LRN. If needed, final confirmation of a threat agent is done at one of the National Laboratories capable of functioning at BSL-4 that is also part of the LRN. The CDC and USAMRIID, for example, have BSL-4 capacity.

Surveillance

In the United States, governments (federal, state, local, tribal, and territorial), health care systems, and private sector companies, such as those

involved in the preparation and sale of foodstuffs, have taken steps to improve surveillance to detect unusual outbreaks and cases of infectious diseases as quickly as possible. There are federal, state, and local human, animal, and plant disease surveillance systems that identify specific pathogens. In recent years, improved surveillance systems at the international, national, and local levels—including an improved network of public health laboratories—have enabled the detection of outbreaks of novel infectious diseases with an exceedingly small number of cases.[132]

A multicomponent, interagency biosurveillance initiative was created and funded beginning in fiscal year 2004 to fill the gap in surveillance and to provide early warning of a potential terrorist attack or infectious disease outbreak. This effort was further enhanced in 2012 with the first-ever National Strategy for Biosurveillance.[149] Because there may be delays in final identification of pathogens, syndromic surveillance systems[150,151] have been utilized and integrated to provide for timely communication across multiple sectors reaching from the community to the national level.

The National Syndromic Surveillance Program (NSSP), coordinated by the CDC, serves as a community-based and community-driven effort for data collection.[152] The effort utilizes clinical data collected from hospitals, pharmacies, emergency departments, and urgent care facilities and contextual data collected from schools, poison control centers, environmental data, and large events. Collected data are placed on BioSense, a cloud-based platform, and then public health agencies at all levels, associations, and other partners can utilize these data for improving situational awareness and monitoring specific conditions and patterns of illness that indicate a new or different event requiring rapid assessment and follow-up. Statistical tools are used to detect, monitor, and characterize unusual events for further investigation.

BioSense, which was mandated by Congress in 2002, has evolved to become an essential part of the NSSP.[153] It has undergone iterative improvements from its initial CDC-centric and bioterrorism-only focus from 2002 to 2009, to a more technology-focused and stakeholder-driven effort from 2010 to 2013. It is now serving multiple stakeholders at all levels and is capable of quickly identifying unusual events that may represent a new intentional or unintentional threat for further investigation.

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Key References

The complete reference list is available online at Expert Consult.

1. O'Toole T, Inglesby TV, Henderson DA. Why understanding biological weapons matters to medical and public health professionals. In: O'Toole T, Inglesby TV, Henderson DA, eds. *Bioterrorism: Guidelines for Medical and Public Health Management*. Chicago: American Medical Association Press; 2002:1–7.
2. Jernigan DB, Raghunathan PL, Bell BP, et al. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. *Emerg Infect Dis*. 2002;8:1019–1028.
3. Carus W. *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900 (revised)*. Washington, DC: Center for Counterproliferation Research, National Defense University; 2001. http://www.fas.org/irp/threat/cbw/carus.pdf. Accessed July 18, 2017.
4. Carus WS A Short History of Biological Warfare: From Pre-History to the 21st Century, Center for the Study of Weapons of Mass Destruction, Occasional Paper, No 12; 2017.
8. Carus WS. A century of biological weapons programs (1915-2015): reviewing the evidence. *Nonproliferation Rev*. 2017;24:129–153.
11. Guillemin J. *Hidden Atrocities: Japanese Germ Warfare and American Obstruction of Justice at the Tokyo Trial*. New York: Columbia University Press; 2017.
15. Leitenberg M. *The Problem of Biological Weapons*. Stockholm, Sweden: The Swedish National Defence College; 2006.
18. Rosebury T. *Experimental Air-Borne Infection*. Baltimore: Williams & Wilkins; 1947.
24. Domaradskij IV, Orent LW. *Biowarrior: Inside the Soviet/Russian Biological War Machine*. New York: Prometheus Books; 2003.
25. Domaradskij IV, Orent LW. Achievements of the Soviet biological weapons programme and implications for the future. *Rev - Off Int Epizoot*. 2006;25:153–161.
31. Tucker J, Vogle KM. Preventing the proliferation of chemical and biological weapon materials and know-how. *Nonproliferation Rev*. 2000;7:88–96.
36. Expert Behavioral Analysis Panel. *The Amerithrax Case: Report of the Expert Behavioral Analysis Panel*. Vienna, VA: Research Strategies Network; 2011. http://web.archive.org/web/20110708081506/http://www.dcd.uscourts.gov/dcd/sites/dcd/files/unsealedDoc031011.pdf. Accessed September 20, 2017.
37. Török TJ, Tauxe RV, Wise RP, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA*. 1997;278:389–395.
45. Rotz LD, Khan AS, Lillibridge SR, et al. Public health assessment of potential biological terrorism agents. *Emerg Infect Dis*. 2002;8:225–230.
49. Rozsa L. A proposal for the classification of biological weapons senu lato. *Theory Biosci*. 2014;133:129–134.
51. Sharples F, Husbands JO, Mazz A-M, et al, eds. *Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop*. Washington, DC: National Academies of Sciences; 2015.
57. Gronwall GK. *Synthetic Biology: Safety, Security, and Promise*. Baltimore, MD: Health Security Press; 2016.
72. Henderson DA. *Smallpox —the Death of a Disease: The Inside Story of Eradicating a Worldwide Killer*. Amherst, New York: Prometheus Books; 2000.
80. Klee SR, Brzuszkiewicz EB, Nattermann H, et al. The genome of a *Bacillus* isolate causing anthrax in chimpanzees combines chromosomal properties of *B. cereus* with *B. anthracis* virulence plasmids. *PLoS ONE*. 2010;5e10986.
84. Inglesby TV, Henderson DA, Bartlett JG, et al. Anthrax as a biological weapon: medical and public health management. *JAMA*. 1999;281:1735–1745.
85. Bower WA, Hendricks K, Pillai S, et al. Clinical framework and medical countermeasure use during an anthrax mass-casualty incident. *MMWR Recomm Rep*. 2015;64(RR–4).
91. Holzer E. Botulism caused by inhalation. *Med Klin*. 1962;41:1735–1740.
92. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA*. 2001;285:1059–1069.
93. Danzig R, Sageman M, Leighton T, et al. *Aum Shinrikyo: Insights Into How Terrorists Develop Biological and Chemical Weapons*. 2nd ed. Washington DC: Center for a New American Security; 2012.
95. Bossi P, Tegnell A, Baka A, et al. Bichat guidelines for clinical management of botulism and bioterrorism-related botulism. *Euro Surveill*. 2004;9:E9–E10.
96. Ajao A, Nystrom SV, Koonin LM, et al. Assessing the capacity of the healthcare system to use additional ventilators during a large-scale public health emergency (PHE). *Disaster Med Public Health Prep*. 2015;9:634–641.
98. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. *JAMA*. 2000;283:2281–2291.
100. Williamson ED, Oyston PC. Plague vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*. 6th ed. Beijing, China: Elsevier Saunders; 2013.
103. Boulanger LL, Ettestad P, Fogarty JD, et al. Gentamicin and tetracyclines for the treatment of human plague: review of 75 cases in New Mexico, 1985-1999. *Clin Infect Dis*. 2004;35:663–669.
104. Mwengee W, Butler T, Mgema S, et al. Treatment of plague with gentamicin or doxycycline in a randomized clinical trial in Tanzania. *Clin Infect Dis*. 2006;42:614–621.
105. Sjökstedt A. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann N Y Acad Sci*. 2007;1105:1–29.
109. Bossi P, Tegnell A, Baka A, et al. Bichat guidelines for the clinical management of tularaemia and bioterrorism-related tularaemia. *Euro Surveill*. 2004;9:27–28.
110. Bechah Y, Capo D, Mege J-L, et al. Epidemic typhus. *Lancet Infect Dis*. 2008;8:417–426.
111. Azad AF. Pathogenic rickettsia as bioterrorism agents. *Clin Infect Dis*. 2007;45:S52–S55.
112. Harris S. Japanese biological warfare research on humans: a case study of microbiology and ethics. *Ann NY Acad Sci*. 1992;666:21–52.
116. Lipsitz RG, Garges S, Aurigemma R, et al. Workshop on treatment of and postexposure prophylaxis for *Burkholderia pseudomallei* and *B. mallei* infection, 2010. *Emerg Infect Dis*. 2012;18:e2.
117. Inglis TJJ, Sagripanti J-L. Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. *Appl Environ Microbiol*. 2006;72:6865–6875.
119. Borio L, Inglesby TV, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA*. 2002;287:2391–2405.
121. Mulligan MJ, Siebert PN. Era of global ebola: risk of exposure in health-care workers. *Lancet Infect Dis*. 2015;15:1248–1249.
122. Fischer WA 2nd, Vetter P, Bausch DG, et al. Ebola virus disease: an update on post-expousre prophylaxis. *Lancet Infect Dis*. 2018;18:e183–e192.
128. Masci JR, Bass E. *Bioterrorism: A Guide for Hospital Preparedness*. Boca Raton FL: CRC Press; 2005.
130. Kratochvil CJ, Evans L, Ribner BS, et al. The national ebola training and education center: preparing the United States for ebola and other special pathogens. *Health Secur*. 2017;15:4–10.
133. Petrosillo N. Bioterrorism agents and hospital infection control: no time to drop the guard. *J Bioterr Biodef*. 2012;3:e103.
135. Gould R, Connell ND. The public health effects of biological weapons. In: Levy BS, Sidel VW, eds. *War and Public Health*. New York: Oxford Press; 1997:98–116.
136. Gursky E, Inglesby TV, O'Toole T. Anthrax 2001: observations on the medical and public health response. *Biosecur Bioterror*. 2003;1:97–110.
144. Barbera J, Macintyre A, Gostin L, et al. Large-scale quarantine following biological terrorism in the United States. *JAMA*. 2002;286:2711–2717.
150. Henning KJ. Overview of syndromic surveillance: what is syndromic surveillance. *MMWR Morb Mortal Wkly Rep*. 2004;53:5–11.
151. Pavlin JA, Mostahari F, Kortepeter MG, et al. Innovative methods for rapid detection of disease outbreaks and bioterrorism: results of an interagency workshop on health indicator surveillance. *Am J Public Health*. 2003;93:1230–1235.

References

1. O'Toole T, Inglesby TV, Henderson DA. Why understanding biological weapons matters to medical and public health professionals. In: O'Toole T, Inglesby TV, Henderson DA, eds. *Bioterrorism: Guidelines for Medical and Public Health Management*. Chicago: American Medical Association Press; 2002:1–7.
2. Jernigan DB, Raghunathan PL, Bell BP, et al. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. *Emerg Infect Dis*. 2002;8:1019–1028.
3. Carus W. *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents Since 1900 (revised)*. Washington, DC: Center for Counterproliferation Research, National Defense University; 2001. http://www.fas.org/irp/threat/cbw/carus.pdf. Accessed July 18, 2017.
4. Carus WS A Short History of Biological Warfare: From Pre-History to the 21st Century, Center for the Study of Weapons of Mass Destruction, Occasional Paper, No 12; 2017.
5. Ranlet P. The British, the Indians, and smallpox: what actually happened at Fort Pitt in 1763? *Pa Hist*. 2000;67:427–441.
6. Fenna EA. Biological warfare in eighteenth century North America: beyond Jeffery Amherst. *J Am Hist*. 2000;86:1552–1580.
7. Singer J. *The Confederate Dirty War: Arson, Bombings, Assassinations, and Plots for Chemical and Germ Attacks on the Union*. Jefferson, NC: McFarland and Co; 2005.
8. Carus WS. A century of biological weapons programs (1915-2015): reviewing the evidence. *Nonproliferation Rev*. 2017;24:129–153.
9. 1925 Geneva Protocol. http://www.un.org/disarmament/WMD/Bio/1925GenevaProtocol.shtml. Accessed March 15, 2013.
10. Disarmament Treaties Database: 1925 Geneva Protocol. http://disarmament.un.org/treaties/t/1925. Accessed March 15, 2013.
11. Guillemin J. *Hidden Atrocities: Japanese Germ Warfare and American Obstruction of Justice at the Tokyo Trial*. New York: Columbia University Press; 2017.
12. Cohen A. Israel and chemical/biological weapons: history, deterrence, and arms control. *Nonprolif Review*. 2001;Fall/Winter:27–53.
13. Cross G. *Dirty War: Rhodesia and Chemical Biological Warfare, 1975-1980*. West Midlands, England: Helion & Co Ltd; 2017.
14. Gould C, Folb P. *Project Coast: Apartheid's Chemical and Biological Warfare Programme*. Geneva, Switzerland: United National Institute for Disarmament Research; 2002. http://www.unidir.org/files/publications/pdfs/project-coast-apartheid-s-chemical-and-biological-warfare-programme-296.pdf. Accessed August 15, 2017.
15. Leitenberg M. *The Problem of Biological Weapons*. Stockholm, Sweden: The Swedish National Defence College; 2006.
16. National Security Decision Memorandum 35—United States Policy on Chemical Warfare Program and Bacteriological/Biological Research Program; 1969. http://history.state.gov/historicaldocuments/frus1969-76ve02/d165. Accessed March 15, 2013.
17. National Security Decision Memorandum 44—United States Policy on Toxins; 1970. http://history.state.gov/historicaldocuments/frus1969-76ve02/d190. Accessed March 15, 2013.
18. Rosebury T. *Experimental Air-Borne Infection*. Baltimore: Williams & Wilkins; 1947.
19. National Academies of Sciences, Engineering, and Medicine. *Assessing Health Outcomes Among Veterans of Project SHAD (Shipboard Hazard and Defense)*. Washington, DC: The National Academies Press; 2016.
20. Project 112/SHAD Fact Sheets. https://health.mil/Military-Health-Topics/Health-Readiness/Environmental-Exposures/Project-112-SHAD/Fact-Sheets. Accessed September 1, 2017.
21. Martin J, Christopher GW, Eitzen EM. History of biological weapons: from poisoned darts to intentional epidemics. In: Dembek Z, ed. *Medical Aspects of Biological Warfare*. Washington, DC: Office of the Surgeon General, US Army Medical Department Center and School, Borden Institute; 2007:1–20.
22. The Biological and Toxin Weapons Convention (BTWC) website. https://www.unog.ch/80256EE600585943/(httpPages)/04FBBDD6315AC720C1257180004B1B2F?OpenDocument. Accessed September 17, 2017.
23. Membership of the Biological Weapons Convention. http://www.unog.ch/80256EE600585943/%28httpPages%29/7BE6CBBEA0477B52C12571860035FD5C?OpenDocument. Accessed September 17, 2017.
24. Domaradskij IV, Orent LW. *Biowarrior: Inside the Soviet/Russian Biological War Machine*. New York: Prometheus Books; 2003.
25. Domaradskij IV, Orent LW. Achievements of the Soviet biological weapons programme and implications for the future. *Rev - Off Int Epizoot*. 2006;25:153–161.
26. Alibek K. *Biohazard*. New York: Random House; 1999.
27. Tucker J. Biological weapons in the former Soviet Union: an interview with Kenneth Alibek. *Nonproliferation Rev*. 1999;6:1–10.
28. Davis CJ. Nuclear blindness: an overview of the biological weapons program of the former Soviet Union and Iraq. *Emerg Infect Dis*. 1999;5:509–512.
29. Guillemin J. *Biological Weapons: From the Invention of State-Sponsored Programs to Contemporary Bioterrorism*. New York: Columbia University Press; 2005.
30. *Adherence to and Compliance With Arms Control, Nonproliferation, and Disarmament Agreements and Commitments*. Washington, DC: US Department of State; 2012.
31. Tucker J, Vogle KM. Preventing the proliferation of chemical and biological weapon materials and know-how. *Nonproliferation Rev*. 2000;7:88–96.
32. *Biological Weapons—Effort to Reduce Former Soviet Threat Offers Benefits, Poses New Risks*. Washington, DC: United States General Accounting Office; 2000. GAO/NSIAD-00-138.
33. United Nations Security Council Resolution 687; 1991. http://www.un.org/Depts/unmovic/documents/687.pdf. Accessed March 15, 2013.
34. UNSCOM: chronology of main events. http://www.un.org/Depts/unscom/Chronology/chronologyframe.htm. Accessed March 18, 2013.
35. Leask A, Delpech V, McAnulty J. Anthrax and other suspect powders: initial responses to an outbreak of hoaxes and scares. *NSW Public Health Bull*. 2003;14:218–221.
36. Expert Behavioral Analysis Panel. *The Amerithrax Case: Report of the Expert Behavioral Analysis Panel*. Vienna, VA: Research Strategies Network; 2011. http://web.archive.org/web/20110708081506/http://www.dcd.uscourts.gov/dcd/sites/dcd/files/unsealedDoc031011.pdf. Accessed September 20, 2017.
37. Török TJ, Tauxe RV, Wise RP, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA*. 1997;278:389–395.
38. United States Department of Justice. *Amerithrax Investigative Summary: Released Pursuant to the Freedom of Information Act*. Washington, DC: 2010. http://www.justice.gov/archive/amerithraxnx/docs/amx-investigative-summary.pdf. Accessed September 15, 2017.
39. National Research Council. *Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters*. Washington, DC: National Research Council, National Academy of Sciences; 2011.
40. US Department of Justice. Justice Department and FBI announce formal conclusion of investigation into 2001 anthrax attacks; 2010. http://www.justice.gov/opa/pr/2010/February/10-nsd-166.html. Accessed March 18, 2013.
41. United Nations Security Council Resolution 1540. http://www.un.org/en/ga/search/view_doc.asp?symbol=S/RES/1540%282004%29. Accessed August 20, 2017.
42. National Institutes of Health, National Institute of Allergy and Infectious Diseases, Biodefense & Emerging Infectious Diseases Priority Pathogens website. https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens. Accessed September 1, 2017.
43. Department of Health and Human Services, Centers for Disease Control and Prevention and US Department of Agriculture, Federal Select Agent Program. Select Agents and Toxins List. https://www.selectagents.gov/selectagentandtoxinslist.html Accessed October 10, 2017.
44. Department of Health and Human Services. Possession, use, and transfer of select agents and toxins: biennial review of the list of select agents and toxins and enhanced biosafety requirements. Final rule. *Fed Regist*. 2017;82:6278–6294.
45. Rotz LD, Khan AS, Lillibridge SR, et al. Public health assessment of potential biological terrorism agents. *Emerg Infect Dis*. 2002;8:225–230.
46. *Department of Homeland Security Risk Lexicon*. Washington, DC: US Department of Homeland Security; 2010.
47. *The Commission on the Intelligence Capabilities of the United States Regarding Weapons of Mass Destruction—Report to the President of the United States*. Washington, DC: Commission on the Intelligence Capabilities of the United States Regarding Weapons of Mass Destruction; 2005.
48. National Research Council. Improving the Department of Homeland Security's biological threat risk assessment and adding risk management. In: *Department of Homeland Security Bioterrorism Risk Assessment: A Call for Change*. Washington, DC: The National Academies Press; 2008. https://www.nap.edu/read/12206/chapter/9. Accessed September 4, 2017.
49. Rozsa L. A proposal for the classification of biological weapons senu lato. *Theory Biosci*. 2014;133:129–134.
50. Drew TW, Mueller-Doblies UU. Dual use issues in research—a subject of increasing concern? *Vaccine*. 2017;35:5990–5994.
51. Sharples F, Husbands JO, Mazz A-M, et al, eds. *Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop*. Washington, DC: National Academies of Sciences; 2015.
52. US Department of Health and Human Services. *Framework for Guiding Funding Decisions About Proposed Research Involving Enhanced Potential Pandemic Pathogens*. Washington, DC: 2017. https://www.phe.gov/s3/dualuse/Documents/p3co.pdf. Accessed December 19, 2017.
53. Synthetic biology. https://www.nature.com/subjects/synthetic-biology. Accessed December 27, 2017.
54. Lander ES. The heroes of CRISPR. *Cell*. 2016;164:18–28.
55. Ro DK, Paradise EM, Ouellet M, et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature*. 2006;440:940–943.
56. Paddon CJ, Westfall PJ, Pitera DJ, et al. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature*. 2013;496:528–532.
57. Gronwall GK. *Synthetic Biology: Safety, Security, and Promise*. Baltimore, MD: Health Security Press; 2016.
58. Homeland Security Presidential Directive-10. Biodefense for the 21st Century, April 28, 2004. The White House. https://fas.org/irp/offdocs/nspd/hspd-10.html. Accessed September 7, 2017.
59. Department of Health and Human Services 2017-2018 Public Health Emergency Medical Countermeasures Enterprise Strategy and Implementation Plan; December 2017. https://www.phe.gov/Preparedness/mcm/phemce/Documents/2017-phemce-sip.pdf Accessed December 15, 2017.
60. Food and Drug Administration. *Emergency Use Authorization of Medical Products and Related Authorities: Guidance for Industry and Other Stakeholders*. Silver Spring, MD: Office of Counterterrorism and Emerging Threats, Office of the Chief Scientist, Office of the Commissioner, Food and Drug Administration; 2017. https://www.fda.gov/downloads/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/UCM493627.pdf. Accessed May 2, 2017.
61. Public Law 108-256 108th Congress. Project BioShield Act of 2004. https://www.gpo.gov/fdsys/pkg/PLAW-108publ276/pdf/PLAW-108publ276.pdf. Accessed July 22, 2004.
62. Beasley DWC, Brasel TL, Comer JE. First vaccine approval under the FDA animal rule. *NPJ Vaccines*. 2016;1:16013.
63. Public Law 113-5 113th Congress. Pandemic and All-Hazards Preparedness Act of 2013. https://www.gpo.gov/fdsys/pkg/PLAW-113publ5/pdf/PLAW-113publ5.pdf Accessed March 15, 2013.
64. Public Law 114-255 114th Congress. 21st Century Cures Act of 2016. https://www.gpo.gov/fdsys/pkg/PLAW-114publ255/pdf/PLAW-114publ255.pdf. Accessed December 15, 2016.
65. Thompson TG A notice by the Health and Human Services Department. Determination and Declaration Regarding Emergency Use of Anthrax Vaccine Adsorbed for Prevention of Inhalation Anthrax. Federal Register Doc. 05-2027, Filed 1-31-20; 11:39 a.m. and published 2 Feb 2005. https://www.federalregister.gov/documents/2005/02/02/05-2027/determination-and-declaration-regarding-emergency-use-of-anthrax-vaccine-adsorbed-for-prevention-of. Accessed March 1, 2005.
66. US Food and Drug Adminiistration. Emergency Use Authorization—Archived Information. https://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm264224.htm. Accessed October 17, 2017.
67. Federal Register. 64FR53960. Proposed rule: new drug and biological drug products; Evidence needed to demonstrate efficacy of new drugs for use against lethal or permanently disabling toxic substances when efficacy studies in humans ethically cannot be conducted. *Fed Regist*. 1999;64:5. https://www.gpo.gov/fdsys/granule/FR-1999-10-05/99-25377/content-detail.html. Accessed October 27, 2013.
68. Public Law 107-188 107th Congress. Public Health Security and Bioterrorism Preparedness and Response Act of 2002. https://www.gpo.gov/fdsys/pkg/PLAW-107publ188/pdf/PLAW-107publ188.pdf. Accessed June 30, 2002.
69. Department of Health and Human Services, Food and Drug Administration. Final Rule—new drug and

biological drug products; Evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible, 21 CFR parts 314 and 601. *Fed Regist*. 2002;62:105. https://www.fda.gov/OHRMS/DOCKETS/98fr/053102a.htm. Accessed September 11, 2016.

70. Code of Federal Regulations, 21CFR314, Revised as of 1 April 2017. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=314&showFR=1&subpartNode=21:5.0.1.1.4.9. Accessed October 31, 2017.
71. Center for Biologics Evaluation and Research, Food and Drug Administration. Product Development Under the Animal Rule Guidance for Industry, 28 October 2015. https://www.fda.gov/downloads/drugs/guidances/ucm399217.pdf. Accessed October 10, 2017.
72. Henderson DA. *Smallpox—the Death of a Disease: The Inside Story of Eradicating a Worldwide Killer*. Amherst, New York: Prometheus Books; 2000.
73. WHO. *Advisory Committee on Variola Virus Research: Report of the Eighteenth Meeting*. Geneva, Switzerland: World Health Organization; 2017. http://www.who.int/csr/resources/publications/smallpox/18-ACVVR-Final.pdf. Accessed July 7, 2017. Licence: CC BY-NC-SA 3.0 IGO.
74. Director of Laboratory Science and Safety, Food and Drug Administration. *Report to the Commissioner: FDA Review of the 2014 Discovery of Vials Labeled "Variola" and Other Vials Discovered in an FDA-Occupied Building on the NIH Campus*. Silver Spring, MD: Food and Drug Administration; 2016.
75. WHO. *The Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox—a Report to the Director-General of WHO*. Geneva, Switzerland: World Health Organization; 2015. http://apps.who.int/iris/bitstream/10665/198357/1/WHO_HSE_PED_2015.1_eng.pdf?ua=1. Accessed December 26, 2017.
76. Nicas M, Hubbard AE, Jones PM, et al. The infectious dose of variola (smallpox) virus. *Appl Biosafety*. 2004;9:118–127.
77. Grosenbach DW, Honeychurch K, Rose EA, et al. Oral tecovirimat for the treatment of smallpox. *N Engl J Med*. 2018;379:44–53.
78. Centers for Disease Control and Prevention. Summary of Notifiable Diseases website. https://www.cdc.gov/mmwr/mmwr_nd/index.html. Accessed September 4, 2017.
79. Antonation KS, Grützmacher K, Dupke S, et al. *Bacillus cereus* biovar *anthracis* causing anthrax in sub-saharan Africa—chromosomal monophyly and broad genetic distribution. *PLoS Negl Trop Dis*. 2016;10:e0004923.
80. Klee SR, Brzuszkiewicz EB, Nattermann H, et al. The genome of a *Bacillus* isolate causing anthrax in chimpanzees combines chromosomal properties of *B. cereus* with *B. anthracis* virulence plasmids. *PLoS ONE*. 2010;5:e10986.
81. Mowatt-Larssen R. Al Qaeda's pursuit of weapons of mass destruction. Foreign Policy 25 Jan 2010. http://foreignpolicy.com/2010/01/25/al-qaedas-pursuit-of-weapons-of-mass-destruction/. Accessed September 8, 2017.
82. Grohs P, Podglajen I, Gutmann L. Activities of different fluoroquinolones against *Bacillus anthracis* mutants selected in vitro and harboring topoisomerase mutations. *Antimicrob Agents Chemother*. 2004;48:3024–3027.
83. Athamna A, Athamna M, Abu-Rashed N, et al. Selection of *Bacillus anthracis* isolates resistant to antibiotics. *J Antimicrob Chemother*. 2004;54:424–428.
84. Inglesby TV, Henderson DA, Bartlett JG, et al. Anthrax as a biological weapon: medical and public health management. *JAMA*. 1999;281:1735–1745.
85. Bower WA, Hendricks K, Pillai S, et al. Clinical framework and medical countermeasure use during an anthrax mass-casualty incident. *MMWR Recomm Rep*. 2015;64.
86. Huang E, Pillai SK, Bower WA, et al. Antitoxin treatment of inhalation anthrax: a systematic review. *Health Secur*. 2015;13:365–377.
87. BioThrax and anthrasil for anthrax. *Med Lett Drugs Ther*. 2016;58:62–63.
88. Raxibacumab for anthrax. *Med Lett Drugs Ther*. 2013;55:27–28.
89. Peck MW. Biology and genomic analysis of *Clostridium botulinum*. *Adv Microb Physiol*. 2009;55:183–265.
90. Katona P. Botulinum toxin: therapeutic agent to cosmetic advancement to lethal biothreat. *Anaerobe*. 2010;18:240–243.
91. Holzer E. Botulism caused by inhalation. *Med Klin*. 1962;41:1735–1740.
92. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA*. 2001;285:1059–1069.
93. Danzig R, Sageman M, Leighton T, et al. *Aum Shinrikyo: Insights Into How Terrorists Develop Biological and Chemical Weapons*. 2nd ed. Washington DC: Center for a New American Security; 2012.
94. Siegel LS, Metzger JF. Toxin production by *Clostridium botulinum* type a under variouis fermentation conditions. *Appl Environ Microbiol*. 1979;38:606–611.
95. Bossi P, Tegnell A, Baka A, et al. Bichat guidelines for clinical management of botulism and bioterrorism-related botulism. *Euro Surveill*. 2004;9:E9–E10.
96. Ajao A, Nystrom SV, Koonin LM, et al. Assessing the capacity of the healthcare system to use additional ventilators during a large-scale public health emergency (PHE). *Disaster Med Public Health Prep*. 2015;9:634–641.
97. Dennis DT, Gage KL, Gratz N, et al Plague Manual: Epidemiology, Distribution, Surveillance and Control. Document WHO/CDS/CRS/EDC/99.2. World Health Organziation. http://www.who.int/csr/resources/publications/plague/WHO_CDS_CSR_EDC_99_2_EN/en/. Accessed September 21, 2017.
98. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. *JAMA*. 2000;283:2281–2291.
99. Runfola JK, Hourse J, Miller L, et al. Outbreak of human pneumonic plague with dog-to-human and possible human-to-human transmission—Colorado, June-July 2014. *Morb Mortal Wkly Rprt*. 2015;64:429–434.
100. Williamson ED, Oyston PC. Plague vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*, 6th ed. Beijing, China: Elsevier Saunders; 2013.
101. Sun W. Plague vaccines: status and future. In: Yang R, Anisnov D, eds. *Yersinia pestis: Retrospective and Perspective*. Dordrecht, Netherlands: Springer Nature; 2016.
102. Eslava-Kim L Plague vaccine granted orphan drug designation. *Infect Dis Advisor*, 20 Mar 2017. http://www.infectiousdiseaseadvisor.com/prevention/plague-vaccine-orphan-drug-designation/article/645129/. Accessed November 17, 2017.
103. Boulanger LL, Ettestad P, Fogarty JD, et al. Gentamicin and tetracyclines for the treatment of human plague: review of 75 cases in New Mexico, 1985-1999. *Clin Infect Dis*. 2004;35:663–669.
104. Mwengee W, Butler T, Mgema S, et al. Treatment of plague with gentamicin or doxycycline in a randomized clinical trial in Tanzania. *Clin Infect Dis*. 2006;42:614–621.
105. Sjökstedt A. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *Ann N Y Acad Sci*. 2007;1105:1–29.
106. Berrada ZL, Telford SRIII. Survival of *Francisella tularensis* type A in brackish-water. *Arch Microbiol*. 2011;193:223–226.
107. Pittman PR, Plotkin SA. Biodefense and special pathogen vaccines. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*. 6th ed. Beijing, China: Elsevier Saunders; 2013.
108. ClinicalTrials.gov. Continued safety and immunogenicity study of a live *Francisella tularensis* vaccine. Identifier NCT00787826. Last posted update 13 Oct 2017. https://clinicaltrials.gov/ct2/show/NCT00787826. Accessed December 24, 2017.
109. Bossi P, Tegnell A, Baka A, et al. Bichat guidelines for the clinical management of tularaemia and bioterrorism-related tularaemia. *Euro Surveill*. 2004;9:27–28.
110. Bechah Y, Capo D, Mege J-L, et al. Epidemic typhus. *Lancet Infect Dis*. 2008;8:417–426.
111. Azad AF. Pathogenic rickettsia as bioterrorism agents. *Clin Infect Dis*. 2007;45:S52–S55.
112. Harris S. Japanese biological warfare research on humans: a case study of microbiology and ethics. *Ann NY Acad Sci*. 1992;666:21–52.
113. Sellon DC, Long MT. *Equine Infectious Diseases*. St. Louis, MO: Saunders Elsevier; 2007.
114. Van Zandt KE, Greer MT, Galhaus HC. Ganders: an overview of infection in humans. *Orphanet J Rare Dis*. 2013;8:131.
115. Wheelis M. First shots fired in biological warfare. *Nature*. 1998;395:213.
116. Lipsitz RG, Garges S, Aurigemma R, et al. Workshop on treatment of and postexposure prophylaxis for *Burkholderia pseudomallei* and *B. mallei* infection, 2010. *Emerg Infect Dis*. 2012;18:e2.
117. Inglis TJJ, Sagripanti J-L. Environmental factors that affect the survival and persistence of *Burkholderia pseudomallei*. *Appl Environ Microbiol*. 2006;72:6865–6875.
118. Healey GD, Elvin SJ, Morton M, et al. Humoral and cell-mediated adaptive immune responses are required for protection against *Burkholderia pseudomallei* challenge and bacterial clearance postinfection. *Infect Immun*. 2005;73:5945–5951.
119. Borio L, Inglesby TV, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA*. 2002;287:2391–2405.
120. Chan M. Ebola virus disease in West Africa—no early end to the outbreak. *N Engl J Med*. 2014;317:1183–1185.
121. Mulligan MJ, Siebert PN. Era of global ebola: risk of exposure in health-care workers. *Lancet Infect Dis*. 2015;15:1248–1249.
122. Fischer WA 2nd, Vetter P, Bausch DG, et al. Ebola virus disease: an update on post-expousre prophylaxis. *Lancet Infect Dis*. 2018;18:e183–e192.
123. Catlett C, Perl T, Jenckes MW, et al. *Training of Clinicians for Public Health Events Relevant to Bioterrorism Preparedness. Evidence Report/Technology Assessment, Number 51*. AHRQ Pub. No. 02-E011. Rockville, MD: Agency for Healthcare Research and Quality.; 2002.
124. Toner E, Waldhorn R, Franco C. A national disaster medical system for the 21st century. *Biosecur Bioterror*. 2007;5:192–193.
125. About the Medical Reserve Corps. https://mrc.hhs.gov/pageViewFldr/About. Accessed December 31, 2017.
126. About the Federal Tort Claims Act (FTCA). 28 US, Part VI, Code Chap 171—Tort Claims Procedure. https://www.law.cornell.edu/uscode/text/28/part-VI/chapter-171. Accessed September 18, 2017.
127. Centers for Disease Control and Prevention. Strategic National Stockpile. https://www.cdc.gov/phpr/stockpile/index.htm. Accessed September 25, 2017.
128. Masci JR, Bass E. *Bioterrorism: A Guide for Hospital Preparedness*. Boca Raton FL: CRC Press; 2005.
129. Department of Health and Human Services, Office of the Assistant Secretry for Preparedness and Response. 2007-2022 Hospital Program Performance Measures Implementation Guidance. https://www.phe.gov/Preparedness/planning/hpp/reports/Documents/hpp-pmi-guidance-2017.pdf. Accessed September 1, 2017.
130. Kratochvil CJ, Evans L, Ribner BS, et al. The national ebola training and education center: preparing the United States for ebola and other special pathogens. *Health Secur*. 2017;15:4–10.
131. Brown CS, Mepham S, Shorten RJ. Ebola virus disease: an update on epidemiology, symptoms, laboratory findings, diagnostic issues, and infection prevention and controls for laboratory professionals. *Clin Lab Med*. 2017;37:269–284.
132. Centers for Disease Control and Prevention. Laboratory Response Network (LRN); last updated September 30, 2014. https://www.cdc.gov/vhf/ebola/healthcare-us/preparing/clinicians.html. Accessed September 17, 2017.
133. Petrosillo N. Bioterrorism agents and hospital infection control: no time to drop the guard. *J Bioterr Biodef*. 2012;3:e103.
134. Kosal ME. A new role for public health in bioterrorism deterrence. *Front Public Health*. 2014;2:278.
135. Gould R, Connell ND. The public health effects of biological weapons. In: Levy BS, Sidel VW, eds. *War and Public Health*. New York: Oxford Press; 1997:98–116.
136. Gursky E, Inglesby TV, O'Toole T. Anthrax 2001: observations on the medical and public health response. *Biosecur Bioterror*. 2003;1:97–110.
137. Centers for Disease Control and Prevention. Resources for Emergency Health Personnel. Health Alert Network (HAN); last updated December 27, 2017. https://emergency.cdc.gov/han/index.asp Accessed December 29, 2017.
138. World Health Organization. *International Health Regulations (2005)*. 2nd ed. Geneva, Switzerland: World Health Organization; 2008.
139. Global Health Security Agenda. https://www.ghsagenda.org/. Accessed December 26, 2017.
140. World Health Organization. *Joint External Evaluation Tool. International Health Regulations (2005) Monitoring and Evaluation Framework*. Geneva, Switzerland: World Health Organization; 2016.
141. Center for Health Security. Fact sheets. http://www.centerforhealthsecurity.org/resources/fact-sheets. Accessed September 9, 2017.
142. Hupert N, Cuomo J, Callahan MA, et al. *Community-Based Mass Prophylaxis: A Planning Guide for Public Health Preparedness. Prepared by Weill Medical College of Cornell University, Department of Public Health Under Contract No. 290-02-0013-3*. AHRQ Pub No. 04-0044. Rockville, MD: Agency for Healthcare Research and Quality.; 2004. https://archive.ahrq.gov/downloads/pub/biotertools/cbmprophyl.pdf. Accessed November 15, 2012.
143. Institute of Medicine. Knobler S, Mahmoud A, Lemon S, et al, eds. Forum on Microbial Threats. Board on Global Health. Learning From SARS: Preparing for the Next Outbreak—Workshop Summary. Washington, DC: The National Academies; 2004.
144. Barbera J, Macintyre A, Gostin L, et al. Large-scale quarantine following biological terrorism in the United States. *JAMA*. 2002;286:2711–2717.
145. Austin PC, Mamdani MM, Chan BT, et al. Anxiety-related visits to Ontario physicians following September 11, 2001. *Can J Psychiatry*. 2003;48:416–419.

146. Allegra PC, Cochrane D, Dunn E, et al. Emergency department visits for concern regarding anthrax—New Jersey, 2001. *MMWR Morb Mortal Wkly Rep*. 2005;54:163–167.
147. Begley S, Isikoff M. War on terror. Anxious about anthrax. *Newsweek*. 2001;138:28–35.
148. van Furth AM, Zaaijer HL. Meningococcal disease in the Netherlands: media hype, but not an epidemic. *Ned Tijdschr Geneeskd*. 2001;145:1716–1718.
149. The White House. National Strategy for BioSurveillance; July 2012. https://obamawhitehouse.archives.gov/sites/default/files/National_Strategy_for_Biosurveillance_July_2012.pdf. Accessed August 8, 2012.
150. Henning KJ. Overview of syndromic surveillance: what is syndromic surveillance. *MMWR Morb Mortal Wkly Rep*. 2004;53:5–11.
151. Pavlin JA, Mostahari F, Kortepeter MG, et al. Innovative methods for rapid detection of disease outbreaks and bioterrorism: results of an interagency workshop on health indicator surveillance. *Am J Public Health*. 2003;93:1230–1235.
152. Centers for Disease Control and Prevention. National Syndromic Surveillance Program. https://www.cdc.gov/nssp/biosense/index.html. Accessed November 15, 2017.
153. Gould DW, Walker D, Yoon PW. The evolution of BioSense: lessons learned and future directions. *Public Health Rep*. 2017;132:7S–11S.

D Clinical Microbiology

16 The Clinician and the Microbiology Laboratory: Test Ordering, Specimen Collection, and Result Interpretation

Robin Patel

SHORT VIEW SUMMARY

BACKGROUND
- Optimal care for infected patients requires collaboration between clinicians and the clinical microbiology laboratory.
- Challenges may include distancing of diagnostic laboratories from health care providers, outsourcing of tests, financial and time constraints on the laboratory and clinicians, and ongoing rapid changes in diagnostic microbiology.
- This chapter provides guidelines to the clinician for making best use of the microbiology laboratory.

CLINICIAN'S RESPONSIBILITY TO THE MICROBIOLOGY LABORATORY
- Alert laboratory to specimens that may pose a hazard to laboratory personnel.
- Obtain appropriate specimens and order appropriate tests.
- Prioritize tests if specimen amounts are limited.
- Advise laboratory about selection of antimicrobial agents for susceptibility testing, as appropriate.
- Maintain open communication for:
  - Specimens needing specialized testing
  - Need to add new tests to or remove obsolete old tests from test menu

MICROBIOLOGY LABORATORY RESPONSIBILITIES TO CLINICIANS
- Maintain a system that provides an appropriate test menu, easily interpretable results, and rapid clinical alerts regarding new significant test results.
- Provide information about performance, clinical utility, turnaround time, and cost of tests.
- Maintain open communication about policy, test menu, and reporting changes.
- Collaborate with infection prevention and control and antimicrobial stewardship programs
  - Provide periodic data on local antimicrobial susceptibility results.
  - Maintain data needed by hospital epidemiology and antimicrobial stewardship groups.

Infectious diseases clinicians are highly reliant on diagnostic microbiology testing and should work in concert with clinical microbiologists to maximize the value and impact of clinical microbiology testing. This includes working with laboratory staff to ensure that the ideal menu of testing is offered, that testing is appropriately ordered (i.e., neither overused nor underused), and that important results are correctly and expeditiously acted on. These activities ensure quality patient care, guide appropriate interventions, and help control cost and appropriate use of antimicrobial agents. In some cases, laboratory testing may be performed off-site or in reference laboratories. Off-site testing potentially increases the time between specimen collection and laboratory processing, possibly compromising specimen integrity if not carefully managed, and may result in delays in the availability of critical test results. Conversely, point-of-care infectious diseases diagnostics are increasingly available,[1] substantially decreasing test turnaround time. These changes make it increasingly important for infectious diseases clinicians and clinical microbiologists to work together. A number of expectations regarding the responsibilities of the infectious diseases clinician should be recognized, as delineated in Table 16.1.

The infectious diseases clinician should be knowledgeable about the test menu and ought to work with the laboratory director to ensure that the test menu is up-to-date and that test turnaround time is sufficient to satisfy patient care needs. Selection of tests on the menu and availability of infrequently offered tests should be determined after discussions with key members of the medical staff and should be revised as patient care needs change. New commercial tests become available on a regular basis; infectious diseases clinicians should be involved in decision making regarding the merits or lack thereof in bringing in individual new tests. Infrequent, expensive, or sophisticated testing may need to be performed in a reference laboratory; this can be associated with significant cost. The infectious diseases clinician should work with diagnosticians to ensure that the send-out menu and method for vetting send-out tests is appropriate. The laboratory should provide the medical staff with a menu of the offered tests, indications and performance characteristics for each test, and turnaround times for test results. Information about appropriate collection of specimens including timing and technique of specimen collection and appropriate transportation of the specimens to the laboratory should be specified. This information should be readily available in the hospital computerized information system and ideally integrated with electronic ordering of tests. Poorly collected specimens may yield recovery of colonizing or commensal organisms and may be rejected by the laboratory.

The complexity of laboratory testing for infectious diseases has increased in recent years and is expected to continue to do so. As such, it is increasingly important to ideally display laboratory tests in the electronic medical record and implement and maintain clinical decision support systems vis-à-vis laboratory testing. Such clinical decision support systems may, for example, limit repeat ordering of some tests, limit the numbers of specimens that can be submitted for a specific test, or restrict test ordering to certain types of practitioners. Decisions about what is appropriate in this regard should be made by the clinical microbiologist and the infectious diseases clinician working together. Test utilization is becoming increasingly important and is the domain of both the clinical microbiologist and the infectious diseases clinician. For example, these two can work together to reduce ordering of urine cultures in asymptomatic patients or, conversely, to ensure that *Legionella* urinary antigen is ordered in patients with community-acquired pneumonia who need this type of testing. The computer system should

TABLE 16.1 Responsibilities of Infectious Diseases Clinician Vis-à-Vis Laboratory Testing

- Maintain knowledge of the laboratory test menu
- Assist the laboratory director with display of laboratory tests in the electronic medical record (ordering and resulting) and in implementing and maintaining clinical decision support systems vis-à-vis laboratory testing
- Assist in defining which antimicrobial agents should be routinely tested for individual bacteria, mycobacteria, and fungi as well as when routine additional susceptibility testing is needed and the nature thereof
- Alert the laboratory when an organism that could be hazardous to laboratory personnel is suspected
- Prioritize test requests when limited quantities of specimen will be or have been collected
- Establish open communication with the laboratory director when testing needs are not satisfied by the available test menu (e.g., specific testing is unavailable)
- Establish open communication with the laboratory director when special handling or testing of a specimen (e.g., testing for a particularly fastidious pathogenic organism) is required or questions about appropriate laboratory testing arise

TABLE 16.2 Organisms About Which the Laboratory Should Be Notified if Suspected

Avian influenza
Bacillus anthracis
Brucella spp.
Burkholderia mallei
Burkholderia pseudomallei
Clostridium botulinum
Coccidioides immitis/Coccidioides posadasii
Coxiella burnetii
Francisella tularensis
Hemorrhagic fever viruses (e.g., Ebola, Marburg, Chapare, Crimean-Congo, Guanarito, Hanta, Junin, Kyasanur Forest disease, Lassa fever, Lujo, Machupo, Omsk hemorrhagic fever, Sabia)
Measles
Middle East respiratory syndrome–associated coronavirus, severe acute respiratory syndrome–associated coronavirus
Nipah virus, Hendra virus
Smallpox
Yersinia pestis

document receipt of specimens in the laboratory, provide a record of testing in progress, and report preliminary and final results. Each institution should establish and communicate which results constitute critical results and therefore require immediate telephone notification. Although telephone communication provides the optimal and most reliable means to communicate information, it can be disruptive to the health care provider and the laboratory alike and so must be targeted and focused; other methods of communication such as text messaging, paging, and electronic messaging can be used to connect with health care providers in other contexts.[2]

The laboratory must ensure that its operation meets all current regulatory requirements including initial verification and ongoing validation of procedures that are used and training and competency of staff. Participation in proficiency testing, an effective program of quality control, and the use of established quality assurance benchmarks help ensure accuracy of the laboratory test results. Infectious diseases clinicians also play a role in maintaining quality of laboratory testing. If important results are observed that do not make clinical sense, especially if an important and unexpected false-positive result is suspected, the laboratory director should be notified. A system of short-term storage of select specimens should be established to facilitate additional testing if required; infectious diseases clinicians should work in concert with laboratories to select specimen types appropriate for this.

When unusual organisms that are challenging to isolate (e.g., *Corynebacterium diphtheriae*) are suspected, the laboratory should be notified. Even with non–organism-specific cultures, the laboratory should be notified if a specific organism is suspected; this facilitates optimized testing. Infectious diseases clinicians should understand testing from a specimen standpoint (i.e., what tests can be performed on specific specimen types and how these specimens should be collected), from a microorganism standpoint (i.e., what are the best tests to diagnose infection with a specific microorganism), and from a syndromic standpoint (i.e., what are the best tests for a patient with a specific syndrome [e.g., acute gastroenteritis, meningitis, community-acquired pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia]).

Infectious diseases clinicians should be prepared to prioritize test requests when only a limited quantity of specimen can be or has been collected. In this situation, discussions with the laboratory director can facilitate optimization of the testing process (e.g., need for routine stains, selection of media, testing conditions to optimize recovery of both bacteria and fungi using a limited number of media). There should be a professional discussion with the laboratory director when testing needs are not satisfied by the available test menu (e.g., specific testing is unavailable or an available test is not offered because it has not been validated for a specific specimen type). Communication with the laboratory director is also important when clinical testing needs are not satisfied by test turnaround time or when a laboratory policy causes patient care problems. With the explosion in rapid diagnostic testing, there is increasing need for clinical microbiologists to work together with infectious diseases clinicians to communicate results to the clinical practice in real time on a patient-by-patient basis to ensure that such results are appropriately acted on. Likewise, an anticipated explosion in deep sequencing-based microbial diagnostics will be best addressed by infectious diseases clinicians working closely with diagnosticians.

MICROORGANISMS HAZARDOUS TO LABORATORY WORKERS

Infectious diseases clinicians should alert the laboratory when an organism that could be hazardous to laboratory personnel is suspected (Table 16.2). This notification should cover all specimens from implicated patients that could pose a danger to laboratory staff. For example, isolation of *Brucella* species in culture can be hazardous to laboratory staff if cultures are not handled in a biological safety cabinet. This organism grows in routine blood culture bottles and on other culture media such that if brucellosis is suspected, all cultures from the patient in question should be carefully handled. Similarly, as *Coccidioides immitis* and *Coccidioides posadasii* grow readily on bacteriology media, bacteriology cultures from a patient with suspected coccidioidomycosis need to be specially handled. Infectious diseases clinicians should work with their institutions and laboratories to determine how high-consequence infectious diseases such as Ebola virus should be handled from the minute a patient with a suspected infection enters the system. This involves handling the patient and his or her specimens and includes how specimens should be collected, transported, and tested and whether any restrictions should be placed on laboratory testing to maximize patient outcome while protecting clinical and laboratory staff. Infectious diseases clinicians may be called on to help in managing laboratory exposures to hazardous agents and therefore should be familiar with appropriate postexposure management strategies.

TAXONOMY

A challenge that confronts the microbiologist and clinician alike is the continuous revision of the taxonomic nomenclature, particularly for bacteria and fungi. Much of the taxonomic reorganization now under way is driven by our increased ability to classify organisms using DNA sequencing. Moreover, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (see "Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry") in addition to gene and genome sequencing has enhanced the ability of laboratories to identify uncommon bacterial and fungal species on a routine basis. The advantage of more detailed identification is better understanding of the relationship between microorganisms and disease. The disadvantage is the practical challenge of remembering a rapidly expanding list of new names and the difficulty this presents for performing searches of the medical literature. In addition, if clinicians are not familiar with a new name and do not spend the time to research its significance, they may fail to recognize its clinical implications. An up-to-date list

of validly published names of bacteria is accessible at https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.html. The International Committee on Taxonomy of Viruses publishes a similar database for viruses (www.ictvonline.org). The *Journal of Clinical Microbiology* published taxonomy updates for parasites and mycobacteria in 2017.[3,4] A useful reference for fungal nomenclature is the MycoBank Database (http://www.mycobank.org/).

INFECTION PREVENTION AND CONTROL

The clinical microbiology laboratory plays an important role in infection prevention and control; infection prevention and control staff and the laboratory should establish a strong and collaborative relationship. Together, they should agree on what the clinical microbiology laboratory staff should notify infection prevention and control about and how that notification should occur. Given the rapid evolution of antimicrobial-resistant organisms and evolving diagnostic tests, there should be a regular review of these practices. Changes in laboratory testing, reporting, or informatics that may impact infection prevention and control should be appropriately handled. The type of isolation that should be used if patients are hospitalized (or ideally systems to automatically make this happen) based on detection of specific microorganisms and resistance factors should be defined. The clinical microbiologist and infection prevention and control specialist should work together to determine what, if any, additional or specialized testing is to be done for infection prevention and control and how billing of such testing should be handled. An example of specialized testing is screening for carbapenemase genes. A list should be put together of key clinical isolates that should be retained for potential future typing to determine relatedness and define potential nosocomial transmission. Clinical microbiology laboratory staff have the unique perspective of seeing all the microorganisms isolated from patients and knowing about all organisms detected in patients throughout the institution and therefore may be the first to recognize a small cluster of unique organisms. However, they are not necessarily systematically looking for this. Infection prevention and control staff also have a unique perspective that involves the ability to assess the likelihood that an infection in an individual patient is nosocomial and a systematic approach to looking for nosocomial transmission. Working in concert, clinical microbiology laboratory staff and infection prevention and control staff play an important role in guiding a potential outbreak investigation.

BACTERIAL, MYCOBACTERIAL, AND FUNGAL IDENTIFICATION

Bacteria, mycobacteria, and fungi were traditionally identified by assessing colony morphology, odor, and hemolytic patterns in addition to results of staining, agglutination tests, and manually generated biochemical tests. Today biochemical profiles are often determined by assessing reactions of individual organisms with substrates using commercial systems. Proteomic analysis using MALDI-TOF mass spectrometry (see following section) enables identification of bacteria and fungi in minutes and is used in many clinical microbiology laboratories. Nucleic acid amplification and, in many cases, sequencing technologies are also used for organism identification. Metabolomic analyses, which may enable identification during growth, are currently in their early stages.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

MALDI-TOF mass spectrometry is the state-of-the art identification system for bacteria, mycobacteria, and fungi.[5] MALDI stands for matrix that assists in desorption and ionization of highly abundant bacterial and fungal proteins through energy from a laser (Fig. 16.1). Material from a bacterial or fungal colony is placed on a spot on a MALDI-TOF mass spectrometry target plate (a reusable or disposable plate with test spots). Spots are overlain with matrix (or first mixed with a formic acid solution, which is dried, and then overlain with matrix) and dried, and the target plate is placed into a mass spectrometer. The matrix (e.g., α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid) isolates microbial molecules from one another, protecting them from fragmentation and enabling their desorption by laser energy; most of the laser energy is absorbed by the matrix, changing it to an ionized state. As a result of random collision in the gas phase, charge is transferred from matrix to microbial molecules; ionized microbial molecules are then accelerated through a positively charged electrostatic field into a time of flight (TOF) tube. Inside the tube, which is under vacuum, ions travel toward an ion detector, with small analytes traveling the fastest, followed by increasingly larger analytes; a mass spectrum is produced, representing the number of ions of a given mass impacting the detector over time. Highly abundant (mainly ribosomal) proteins produce the mass spectrum. Although they are not separately characterized, together they provide a profile unique to specific types of organisms, with peaks specific to species and genera. Computer software compares the produced mass spectrum with a databank of reference spectra, generating a list of the most closely related organisms with numeric rankings. Depending on how high the value (percent or score) of the topmost match is (and bearing in mind the next best matches), the organism is identified at the family, complex, group, genus, species, or subspecies level. The main commercial MALDI-TOF mass spectrometry systems for clinical microbiology testing are from Bruker Daltonics Inc. (Billerica, MA) and bioMérieux Inc. (Durham, NC).

ANTIBACTERIAL SUSCEPTIBILITY TESTING

The gold standard method for susceptibility testing is phenotypic susceptibility testing, which involves growing the organism with and without specific antimicrobial concentrations and comparing results. The traditional measurement of susceptibility is the minimal inhibitory concentration (MIC)—the lowest concentration of the antimicrobial agent that inhibits the growth of the organism. Methods used to determine MICs are standardized and comprise use of uniform organism amounts and growth conditions including media, incubation conditions, and incubation durations. To measure the MIC, the organism is grown in multiple concentrations of an antimicrobial agent spanning a clinically significant range. Dilutions can be prepared in wells of a microtiter well plate (or alternatively tested in test tubes or incorporated into agar plates) and, by convention, are doubled using a base of 1 μg/mL, for example, 0.5, 1, 2, 4, 8, 16, and so on. A standardized inoculum of the isolate is added, and after incubation for a defined time, wells are examined for turbidity produced by microbial growth. The first well in which visible growth is absent (e.g., indicated by clear broth) is the MIC of that organism. The MIC obtained is converted to susceptible, intermediate, susceptible dose dependent, or resistant categories by referring to an interpretative table from the US Food and Drug Administration (FDA), a standards development organization such as the Clinical and Laboratory Standards Institute, or the US or European Committees on Antimicrobial Susceptibility Testing. The 2016 US 21st Century Cures Act has created a system to accelerate the recognition of antimicrobial susceptibility test interpretive criteria or breakpoints. Antibacterial susceptibility test interpretive criteria can be found at https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm575163.htm. Antifungal susceptibility test interpretive criteria can be found at https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm575166.htm. Use of the term *susceptible* indicates that the MIC is at a concentration achievable in blood or other appropriate body fluid using typically suggested doses. The term *resistant,* the opposite of *susceptible,* denotes that the MIC is not exceeded by normally attainable levels. As in all biologic systems, the MIC of some organisms lies between susceptible and resistant levels. Borderline results may be referred to as intermediate or susceptible dose dependent due to technical variability or to indicate that the antimicrobial agent may still be used but at increased doses. Urine levels of some antimicrobial agents may be so high as to enable their use to treat urinary tract infections in the face of high MICs. Hence for some antimicrobial agents, different interpretations of *susceptible* may apply to urinary versus nonurinary isolates. Likewise, for central nervous system infections, there may be separate interpretations. Susceptibility testing may be automated using commercial systems in which organisms are incubated with multiple antimicrobial agents in specialized modules that are read automatically at regular intervals.

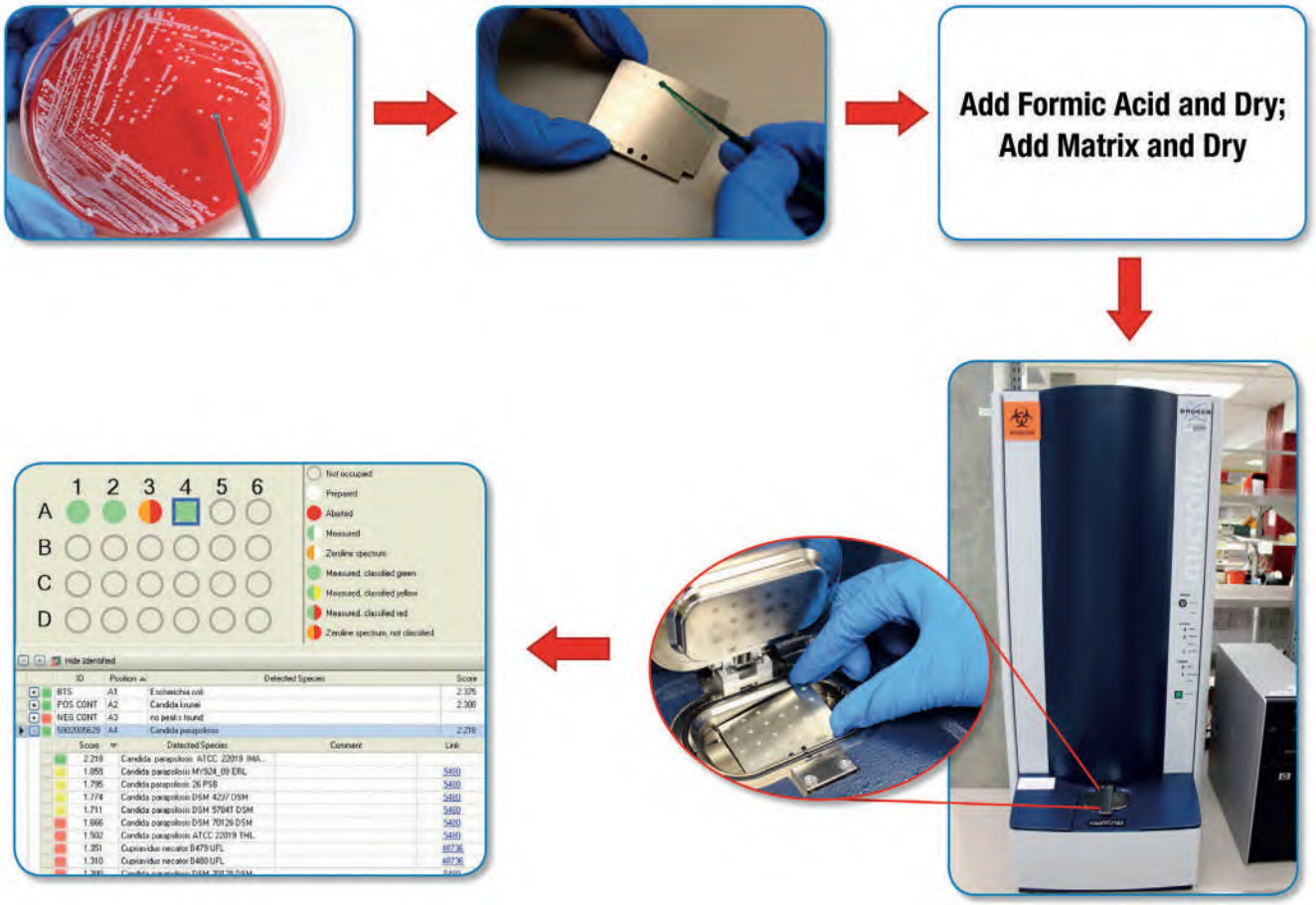

FIG. 16.1 Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. A colony is transferred from a culture plate to a MALDI-TOF mass spectrometry target plate. One or many isolates may be tested at a time, each on a different spot on the MALDI-TOF mass spectrometry target plate. In this example, cells are treated with formic acid on the target plate and allowed to dry. The spot is overlain with matrix and dried. The plate is placed in the ionization chamber of the mass spectrometer. A mass spectrum is generated and compared against a database of spectra by the software, resulting in identification of the microorganism. *(Modified from and used with permission of Mayo Foundation for Medical Education and Research. All rights reserved.)*

With disk diffusion testing, the inoculum is seeded onto the surface of an agar plate, to which disks containing defined amounts of antimicrobials are applied. While the plates are incubating, antimicrobial agents diffuse into the medium, producing a circular gradient around the disk. After incubation, the diameter of the zone of growth inhibition around the disks is used as an indirect measurement of the MIC of the organism. Such testing is influenced by the growth rate of the microorganism, how well the antimicrobic diffuses into the medium, and other technical factors. The diameter of the zone of inhibition obtained is converted to susceptible, intermediate, susceptible dose dependent or resistant categories by referring to an interpretative table. An additional diffusion procedure uses a strip that contains a gradient concentration of an antimicrobic, which produces elliptical zones of inhibition that can be directly correlated with MICs.

Standards development organizations may reset breakpoints over time. New breakpoints should be implemented in a timely manner. Changes in susceptibility testing should be promptly announced by the laboratory to clinicians to avoid interpretive errors. Delays in the adoption of new breakpoints by laboratories may occur; sometimes the manufacturers of automated platforms represent a barrier to implementation of the most current breakpoints. Clinicians should be aware of whether or not their laboratory is using updated breakpoints.

Molecular methods are increasingly applied to antimicrobial susceptibility testing. In some cases, resistance mechanisms for specific organism-antimicrobial agent combinations are so specific as to enable molecular susceptibility testing (e.g., *mecA/mecC* detection for diagnosis of methicillin resistance in *Staphylococcus aureus*). In other cases, molecular mechanisms of resistance are complex, precluding accurate molecular susceptibility testing (e.g., ceftriaxone resistance in *Klebsiella pneumoniae*).

ANTIMICROBIAL STEWARDSHIP

As with infection prevention and control, the microbiology laboratory plays an important role in antimicrobial stewardship; the laboratory director should play an active role in the institution's antimicrobial stewardship program and work collaboratively with antimicrobial pharmacists, if they are present, and infectious diseases clinicians involved in antimicrobial stewardship. In many countries, especially outside of the United States, clinical microbiologists undertake clinical roles outside the laboratory, especially if they are also trained in infectious diseases. Such dual training has certain advantages vis-à-vis antimicrobial stewardship, but either way, clinical microbiologists can play an important role in antimicrobial stewardship. Examples of activities that clinical microbiologists and stewardship groups can undertake in partnership include putting together antibiograms or cumulative antimicrobial susceptibility reports, providing enhanced culture and susceptibility reports, offering guidance in the preanalytic phase, making available rapid diagnostic tests, educating providers, and creating alert and surveillance systems.[2]

Laboratories must publish (preferably online or in an application software ["app"]) the antimicrobial susceptibility patterns for common

bacteria and fungi tested in their laboratory in the recent past (antibiograms or cumulative antimicrobial susceptibility reports) at least annually.[6] The Clinical and Laboratory Standards Institute publishes guidelines for the analysis and presentation of cumulative susceptibility tests.[6] Only diagnostic (i.e., not surveillance) isolates and only routinely tested agents should be included. For *Streptococcus pneumoniae,* data should be reported using both meningitis and nonmeningitis breakpoints, and for *S. aureus,* data should be reported for all isolates and methicillin-resistant *S. aureus*. The antibiogram can be used to guide empirical therapy while culture results are pending, inform and update local guidelines for empirical treatment of common infection syndromes, update procedural or surgical prophylaxis recommendations, inform antimicrobial formulary selection, survey local resistance patterns, identify targets for antimicrobial stewardship interventions and best practices, and provide a background for new drug susceptibility testing.[2] The antibiogram and communication of its results should be reviewed by infection prevention and control staff, pharmacy staff, and the antimicrobial stewardship team of the institution before release to identify and correct errors that might lead to antimicrobial misuse.[2] The antibiogram can be put together only if there are at least 30 isolates in the particular category being addressed. If the number is adequate and there are important differences, data may be stratified by service, unit, resistance mechanism, or specimen type. Combination therapy susceptibilities can be considered and may be useful to guide empirical double therapy when culture results are pending and resistance may be present (e.g., defining which two antimicrobial agents best cover *Pseudomonas aeruginosa*).[2] With the adoption of electronic medical records, antibiograms may be prepared without the participation of the microbiologist, in some cases by systems themselves. Although the information provided can be accurate, errors in interpretation and reporting may lead to the conveyance of misinformation to prescribers, so the clinical microbiologist should remain involved at some level.[2]

The laboratory director should work with infectious diseases clinicians, pharmacy staff, and the antimicrobial stewardship team at their institution to develop guidelines for performing and reporting antimicrobial susceptibility results for bacteria, mycobacteria, and fungi. The primary agents tested and reported should be determined based on the patient population served, local resistance patterns, the hospital formulary, and cost. It should also be determined when additional susceptibility testing should be routinely performed and reported (e.g., when certain resistance patterns are observed). There is a relationship between the antibiotics listed in the clinical report and antibiotic prescription. Cascade or selective reporting (e.g., not reporting carbapenem susceptibility when a microorganism is susceptible to drugs with a narrower spectrum) can be used to promote the judicious use of antimicrobials.[7]

Infectious diseases physicians should work with their laboratories to routinely add reporting comments when specific organisms or resistances are detected in particular specimen types, including advising specific treatment, interpreting results, and suggesting infectious diseases consultations. For example, for staphylococci for which rifampin has been tested and found to be susceptible, a comment such as "Rifampin should not be used as monotherapy" could be added. Automatically added messages are preferred, as their addition is less likely to be overlooked by laboratory technologists than messages added manually.

SPECIMEN SELECTION, COLLECTION, TRANSPORTATION, AND INITIAL PROCESSING

Selection of the appropriate specimens and testing for the detection of an organism responsible for a patient's disease is the ultimate objective. If a specific pathogen is suspected, selection of the appropriate specimen for the specific diagnostic test is relatively straightforward (Table 16.3); however, the differential diagnosis is typically not limited, and a variety of pathogens are usually considered as possibilities. This increases the types of specimens that must be collected and the number of tests ordered. Although this may not pose a problem if the quantity of specimen is sufficient (e.g., blood, urine, stool, sputum), this can present a challenge if specimen quantity is limited or a specimen is difficult to obtain (e.g., tissue biopsy). Thus the clinician must carefully consider potential pathogens and the diagnostic tests that should be ordered. The clinician also must ensure that specimens representative of the site of infection are collected in the appropriate container and transported to the laboratory in a timely fashion. In addition to delaying results, delays while specimens remain in the patient care areas may adversely affect diagnostic testing results.

There are several points to consider regarding specimen quality. Specimens must be accurately labeled. Inadequately labeled specimens as well as specimens of poor quality should be rejected. Commensal microbiota must be avoided when collecting specimens from body sites (e.g., respiratory tract, wounds) that have normal microbiota that can contaminate an inappropriately collected specimen and complicate interpretation. Swabs are appropriate for throat, nares, nasopharyngeal, and some ocular specimens but are not substitutes for tissues, aspirates, or fluids when these are collectable (e.g., at surgery) because swabs hold exceptionally small volumes of specimen. Flocked swabs are more effective than Dacron, rayon, and cotton swabs because their flocked nature allows for efficient release of contents for assessment. Clinicians should not request that the laboratory report "everything that grows" from specimens harboring commensal microbiota or large numbers of species, as this can provide irrelevant information and result in inaccurate diagnosis and inappropriate therapy and is time consuming and costly. Antimicrobial susceptibility testing should be done only on clinically significant isolates and not on all organisms recovered in culture.

Selection of appropriate specimens depends on multiple factors. If the diagnostic test is isolation of the organism in culture, the specimen must contain viable organisms; care should be taken to avoid organisms that may either suppress or overgrow the pathogen or confound the interpretation of the culture. Ideally, specimens for culture (and even for non–culture-based testing based on microbial detection) should be collected before administration of antimicrobial agents. An adequate volume of specimen should be collected to maximize recovery of the pathogen, and an adequate number of specimens should be collected if the organism is transiently present, present in low quantities, or unevenly distributed in clinical specimens. Microscopy is a rapid but typically insensitive method for detection of pathogens. If the quantity of specimen is limited, the value of microscopy must be balanced against the need for other diagnostic tests. In some situations, microbial stains are of such limited value (e.g., diagnosis of prosthetic joint infection) as to not be recommended, whereas in others, microscopy is the primary diagnostic test (e.g., examination of blood smears for malaria). Selection of appropriate specimens for detection of microbial antigens is determined by the suspected pathogen. For example, cerebrospinal fluid (CSF) is appropriate for diagnosis of cryptococcal meningitis, but both CSF and urine may be collected for diagnosis of pneumococcal meningitis. Likewise, the optimal specimens for diagnosis of *Legionella* pneumonia are respiratory secretions for nucleic acid amplification tests (NAATs) and culture and urine for antigen testing. Serologic tests are useful if the collection of blood is properly timed to coincide with the peak level of antibodies or to demonstrate a significant rise in antibodies but may be affected by antibodies elicited by vaccination, prior exposure to the same or similar organisms, or cross-reacting antibodies. Because the value of serologic testing varies for individual pathogens, a pathogen-specific and patient-specific approach is needed.

Transportation of specimens to the microbiology laboratory must be done in a timely fashion and particularly so for urgent requests. Efforts to minimize transit time (e.g., use of pneumatic tube systems) should be made. Generally transport times should be a maximum of 2 hours. Prolonged transportation times can result in loss of microbial viability, compromising sensitivity, or paradoxically, overgrowth of organisms, compromising specificity (e.g., urine cultures). Transit times should be monitored systematically as part of a diagnostic quality assurance program. If there are unanticipated delays, they should be indicated in the clinical report. It is also important to time collection and transportation of specimens to ensure they arrive in the laboratory before established cutoff times for performance of batched tests, especially if such batched tests are infrequently performed. If there are regular delays because of test batching, timing of specimen collection or test

Text continued on p. 202

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens

MICROORGANISM[a]	DETECTION METHOD					COMMENTS
	Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	
Adenovirus		±		√		NAAT preferred for active infection
Anaplasma phagocytophilum, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia muris	±			√	√	NAAT preferred for active infection
Aspergillus spp.	√	√	√	±		
Babesia spp.	√			√	√	NAAT or microscopy preferred for active infection
Bacillus anthracis	√	√		±		Notify laboratory for safety reasons and to ensure laboratory knows to look for this organism
Bartonella spp.	±			±	√	Culture insensitive
BK virus	±			√		NAAT preferred for active infection and monitoring response to therapy
Blastomyces spp.	√	√	√	±	√	
Bordetella pertussis		±		√	±	NAAT preferred for active infection
Borrelia burgdorferi sensu lato complex (*B. burgdorferi* [US], *B. garinii* [Europe, Asia], *B. afzelii* [Europe, Asia], *B. mayonii* [US])		±		±	√	
Borrelia miyamotoi				√	√	
Brucella spp.	±	√		±	√	Notify laboratory for safety reasons
Burkholderia pseudomallei	±	√			±	Notify laboratory for safety reasons
Candida spp.	√	√		√		
Chlamydia psittaci				±	√	
Chlamydia trachomatis				√		
Clostridium botulinum	±	√	√			Notify laboratory to ensure appropriate testing is performed
Clostridioides difficile (formerly *Clostridium difficile*)		±	√	√		See text
Clostridium tetani	±	√				Diagnosis primarily clinical; notify laboratory to ensure appropriate testing is performed
Coccidioides immitis/Coccidioides posadasii	√	√	√	±	√	Notify laboratory for safety reasons
Coronaviruses		±		√		
Corynebacterium diphtheriae		√				
Coxiella burnetii				±	√	Notify laboratory for safety reasons; serology test of choice; acute Q fever: fourfold increased titer to phase II antigen or IgM titer ≥50 and IgG titer ≥200; chronic Q fever: IgG titer ≥800 to phase I antigen
Cryptococcus neoformans/ Cryptococcus gattii	√	√	√	±		CSF cryptococcal antigen test preferred for cryptococcal meningitis; fungal stain and culture should also be ordered; NAATs not as sensitive as antigen tests for monitoring CSF on therapy
Cryptosporidium spp.	±		√	√		
Cyclospora cayetanensis	√			√		
Cytomegalovirus	±	±	±	√	√	Quantitative NAATs of plasma used for immunocompromised hosts; antibody detection used to assess immune status and for workup of infection in immunocompetent hosts
Dengue			√	√	√	Detection of dengue virus RNA, nonstructural protein 1 antigen, or anti–dengue virus IgM suggestive of acute infection with dengue virus
Dermatophytes (*Epidermophyton* spp., *Trichophyton* spp., *Microsporum* spp.)	√	√				Submit skin scrapings, hair follicles, nail scrapings, tissue biopsy specimen
Ebola				√		Notify laboratory for safety reasons; viral culture should not be performed on patients with suspected Ebola virus disease

Continued

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens[a]—cont'd

MICROORGANISM[a]	DETECTION METHOD					COMMENTS
	Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	
Entamoeba histolytica	√		√	√	√	Antibody detection useful only for invasive/disseminated disease (e.g., amebic liver abscess)
Enterobius vermicularis	√					Submit paddle or cellophane tape after sampling perianal area
Enterovirus/parechovirus		±		√	±	NAAT preferred for acute diagnosis
Epstein-Barr virus				√	√	Quantitative NAATs of blood used for immunocompromised hosts; antibody detection used to assess immune status and for workup of infection in immunocompetent hosts
Erysipelothrix rhusiopathiae	±	√				
Francisella tularensis		√			±	Notify laboratory for safety reasons and to ensure appropriate media are inoculated for culture
Free-living amebas (*Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri*)	√	√		√		
Giardia spp.	±		√	√		
Haemophilus ducreyi	±	√				Selective and differential medium needed for culture
Helicobacter pylori	√	±	√	±		See text; urea breath test also recommended
Hepatitis A, B, C, D, and E			√	√	√	See Chapter 117
Herpes simplex virus	±	±		√	±	NAAT preferred for diagnosis of acute infection; antibody detection useful to assess immune status
Histoplasma capsulatum	√	√	√	±	√	
Human herpesvirus 6				√	√	NAAT preferred for diagnosis of acute infection
Human immunodeficiency virus		±	√	√	√	See Chapter 120
Human metapneumovirus				√		
Human papillomavirus	√			√		
Influenza		±	√	√		NAAT preferred for diagnosis of acute infection; negative result by rapid antigen testing should be confirmed by NAAT or viral culture
Intestinal nematodes (*Ascaris lumbricoides, Strongyloides stercoralis, Trichuris trichiura,* hookworms)	√	[b]			[b]	
JC virus				√	±	NAAT preferred for diagnosis of acute infection
Kingella kingae	±	√		√		NAAT particularly useful in young children with osteoarticular infection
Klebsiella granulomatis	√			±		Microscopy (Giemsa stain, not Gram stain) test of choice; does not grow on conventional media
La Crosse virus					√	
Legionella spp.		√	√	√	±	Urine antigen tests sensitive (>80%) for *Legionella pneumophila* serogroup 1. *Legionella* culture and NAATs preferred (sensitive and specific, although NAATs are more sensitive than culture); antibody detection less sensitive, as antibodies may develop slowly and IgM may persist for ≥1 year
Leishmania spp.	√	√		√	√	Serology useful for visceral leishmaniasis; species identification (used to guide treatment of American cutaneous leishmaniasis) requires culture or NAAT or both
Leptospira spp.		±		±	√	Culture requires specialized techniques with organisms isolated in blood during first week of illness and urine thereafter; microscopy (darkfield) insensitive and requires experienced observer; serology most frequently performed test; microagglutination titer ≥200 or seroconversion diagnostic; serology is insensitive in early stages of disease, and seroconversion may be delayed
Listeria monocytogenes	√	√		±		
Lymphocytic choriomeningitis virus				±	√	

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens[a]—cont'd

MICROORGANISM[a]	DETECTION METHOD					COMMENTS
	Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	
Malassezia spp.	√	√				Notify laboratory when suspected; some species require addition of lipids to culture medium for growth
Measles				√	√	
Mucorales	√	√		±		Ask laboratory to avoid grinding tissue if suspected
Mumps				√	√	
Mycobacterium, nontuberculous (other than species below)	√	√				Microscopy and culture most commonly performed; molecular probes used to identify cultured isolates
Mycobacterium genavense	√	√				Notify laboratory when suspected; requires addition of mycobactin J to medium for ideal growth in culture
Mycobacterium haemophilum	√	√				Notify laboratory when suspected; requires addition of X-factor to medium and lower temperature (30°C) for ideal growth in culture
Mycobacterium marinum	√	√				Notify laboratory when suspected; requires lower temperature (30°C) for ideal growth in culture
Mycobacterium tuberculosis	√	√		√		See text; microscopy, culture, and NAATs most commonly performed; interferon-γ release assays and tuberculin skin tests provide evidence of infection (latent or active) but may be negative with active infection
Mycobacterium ulcerans	√	√		√		Notify laboratory when suspected; requires lower temperature (30°C) for ideal growth in culture
Mycoplasma pneumoniae				√	±	NAAT preferred; seroconversion may be delayed; cold agglutinin testing not recommended
Neisseria gonorrhoeae	±	±		√		See text; NAATs preferred; microscopy and culture less sensitive compared with NAATs; culture used if an isolate is needed for susceptibility testing
Neisseria meningitidis	√	√		±		
Nocardia spp.	√	√		±		Grow on mycobacterial and fungal cultures (and sometimes on bacterial media such as that used for *Legionella* culture)
Norovirus			√	√		
Parainfluenza		√		√		
Parvovirus B19	±			√	√	
Plasmodium spp.	√		±	±		Confirm antigen detection test results (positive and negative) by microscopy or NAAT when possible
Pneumocystis jirovecii	√		√	√		Does not grow in culture; β-D-glucan test may be positive
Powassan/deer tick virus				√	√	
Rabies virus	√		√	√	√	
Relapsing fever *Borrelia* spp. (*B. hermsii* [western US], *B. parkeri* [western US], *B. turicatae* [southwestern US], *B. mazzottii* [southern US], *B. recurrentis* [sub-Saharan Africa])	√	±		±	±	Darkfield microscopy or Wright, Giemsa, or Diff-Quik stains of peripheral thin or thick blood smears (or both) recommended; may be seen in direct wet preparation of blood in some cases
Respiratory syncytial virus		±	√	√		
Rhodococcus equi	√	√				
Rubella					√	Antibody detection useful to assess immune status
Sapovirus				√		
Scabies/lice	√					Macroscopic and microscopic visualization
Sporothrix schenckii complex	√	√			√	
St. Louis encephalitis virus					√	
Streptococcus pneumoniae	√	√	√	±		Microscopy and culture most commonly used tests; urine antigen tests can be helpful

Continued

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens[a]—cont'd

MICROORGANISM[a]	DETECTION METHOD: Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	COMMENTS
Streptococcus pyogenes (group A)	√	√	√	√	±	Antigen detection methods (backed up by culture, if negative) and NAATs used to test throat swabs; antibody detection used for diagnosis of poststreptococcal diseases
Taenia solium (neurocysticercosis)	±				√	
Tick-borne encephalitis virus					√	Detection of specific IgM in either blood or CSF
Toxocara spp.					√	Larvae rarely seen in histopathologic sections of biopsies of infected tissues (e.g., liver)
Toxoplasma gondii	±			±	√	
Treponema pallidum	±			±	√	See text
Trichinella spiralis	±				√	
Tropheryma whipplei	√			√		
Trypanosoma cruzi	√				√	
Trypanosomiasis, African (African sleeping sickness; *Trypanosoma brucei gambiense* [West African], *T. b. rhodesiense* [East African])	√				√	
Varicella-zoster virus	±	√		√	±	NAAT preferred for diagnosis of acute infection; antibody detection useful to assess immune status
Vibrio spp.		√		±		
West Nile virus				√	√	
Yersinia pestis	√	√	±	±		
Zika				√	√	

[a]Bacteria and fungi not specifically listed are most commonly detected by microscopy and culture. For esoteric organisms not listed, contact the local laboratory.
[b]Antibody detection and stool agar culture may be useful for *S. stercoralis*.
CSF, Cerebrospinal fluid; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *NAAT,* nucleic acid amplification test.

performance should be addressed. Table 16.4 summarizes normally accepted guidelines for specimen collection and transportation of common specimen types for microbiology testing. Not all situations are covered; for special instances, clinicians should contact their laboratory to discuss the best procedures for ensuring optimal handling of specimens.

SPECIFIC SPECIMEN GUIDELINES AND INITIAL LABORATORY PROCESSING

Blood Cultures

Blood cultured for bacteria and *Candida* species is one of the most essential tests performed in the clinical microbiology laboratory. Because relatively few bacteria and *Candida* species are present in the blood of patients with bacteremia or candidemia, a sufficient volume of blood should be cultured. Current blood culture systems include commercial purpose-specific blood culture bottles that are incubated on specifically configured blood culture instruments that are fully automated from the moment blood culture bottles are loaded onto the instruments until the bottles signal positive. These systems monitor blood culture bottles for microbial growth every 10 to 24 minutes. A variety of blood culture bottle types are available for these systems, including aerobic and anaerobic bottles, bottles configured for children, and fungal and mycobacterial bottles. Resin-supplemented bottles are designed to bind and remove antibiotics and therefore improve recovery of bacteria in patients who have been treated with antibiotics.[8] Blood cultures are routinely incubated for 5 days, although most are positive within 48 hours. Incubation beyond 5 days is seldom required, although it may be useful for recovering *Cutibacterium acnes* (e.g., in cases of *C. acnes* endocarditis). Bacteria that historically required special blood culture procedures such as HACEK bacteria (*Haemophilus* spp. *[H. parainfluenzae], Aggregatibacter* spp. *[A. actinomycetemcomitans, A. aphrophilus], Cardiobacterium* spp. *[C. hominis, Cardiobacterium valvarum]/Eikenella corrodens,* and *Kingella* spp.), *Abiotrophia* and *Granulicatella* species, and *Brucella* species are readily isolated using today's routine blood cultures. *Brucella* species will usually grow within a standard 5-day incubation period, although some experts recommend holding blood cultures for *Brucella* species for 7 days.[9] *Bartonella* species do not grow well in current routine blood culture systems; bartonellosis is best diagnosed serologically or using NAATs. Likewise, *Coxiella burnetii* is best diagnosed serologically or with a NAAT.

Specially designed fungal and mycobacterial blood culture bottles are available. A lysis-centrifugation system such as the ISOLATOR system (Abbott/Alere, Waltham, MA) with prolonged incubation can be used for recovery of dimorphic fungi, molds, mycobacteria, and *Nocardia* species (*Nocardia* species may also grow in routine blood culture bottles). Although lysis-centrifugation offers advantages for these organisms, it is labor intensive and has high rates of contamination; therefore it should not be used for routine blood cultures. *Candida* species typically grow in standard blood culture bottles.

The most important factor that influences recovery of organisms in blood cultures is the volume of blood collected; typically the number of organisms in the blood of a septic patient is low (an average of less than 1 organism/mL).[10] The current recommendation for adult patients is that 20 to 30 mL of blood be collected and distributed into two to three blood culture bottles per draw and that two draws are routinely performed. Routine use of a blood culture from a single draw is not acceptable. To maximize yield, six bottles, collected across two to three sets, should be collected. Although the concept of spacing blood culture draws (i.e., to detect continuous bacteremia) has been commonly practiced, there is little evidence supporting the need for this; separation of blood culture draws over time should not be the standard for routine blood culture draws. For endocarditis diagnosis, three sets of blood cultures, with each set including one aerobic and one anaerobic bottle, may be collected; alternatively, two sets may be collected, with two aerobic bottles and one anaerobic bottle per set (i.e., a total of six blood culture bottles).[11] Repeat blood cultures are generally not useful in patients with recent negative blood cultures. For neonates and children,

TABLE 16.4 Collection and Transportation of Specimens for Microbiologic Testing

SPECIMEN	COLLECTION GUIDELINES	OTHER COMMENTS
Abscess		
	Aspirate pus or fluid in anaerobic transport vial; clean surface of closed abscess with 70% alcohol; collect specimens at margins of abscess	Aspirates in anaerobic transport tubes acceptable for aerobic and anaerobic bacterial, fungal, and mycobacterial cultures; separate vessel, without media, should be used for NAATs, if desired
Blood for Culture		
Routine aerobic and anaerobic bacteria and *Candida* spp.	Disinfect bottle tops with 70% isopropyl alcohol; disinfect phlebotomy site with alcohol, followed by tincture of iodine, chlorine pexoide, or chlorhexidine; allow disinfectants to dry Collect 20–30 mL in adults for each blood culture; divide blood into two or three blood culture bottles, consisting of one to two aerobic bottles and an anaerobic bottle; perform two blood culture draws routinely for adults	Blood culture bottles should be loaded onto blood culture instruments within 2 hours of collection Blood cultures should be collected before administering antibiotics; resins designed to inactivate antibiotics may be helpful for patients who have received antibiotics, but administration of antibiotics should be avoided, if possible, before blood culture collection
Mycobacteria, and fungi	Use special fungal/mycobacterial blood culture bottles	*Candida* spp. will grow in routine blood culture bottles
Blood for Microscopic Examination		
Plasmodium spp., *Anaplasma* spp., *Ehrlichia* spp., *Borrelia* spp. causing relapsing fever	Prepare thick and thin smears from fingerstick or EDTA blood	Transport rapidly; NAATs more sensitive than direct smears for *Anaplasma* and *Ehrlichia* spp.
Intravascular Catheter Tip		
	Remove aseptically, cut at least 2-inch segment from tip, place segment in sterile container	Transport rapidly (prevents drying out) Culture of Foley and drainage catheters not recommended
Ear		
Inner	For intact eardrum, clean ear canal with soap solution or 70% alcohol, then aspirate by syringe For ruptured eardrum, collect drainage with swab	If specimen collected by aspiration, inoculate media at time of collection or immediately transport syringe to laboratory
Outer	Remove crusted debris and firmly scrape or swab outer ear canal	
Eye		
Conjunctiva	Roll swab over conjunctiva; stains should be prepared and media inoculated at time of collection	Number of stains prepared and media inoculated limited because of small amount of material collected
Cornea	Use sterile spatula to scrape surface of cornea; stains should be prepared and media inoculated at time of collection	Number of stains prepared and media inoculated limited because of small amount of material collected; anesthetics may inhibit growth of some bacteria
Vitreous and anterior chamber fluids	Because limited amounts of fluid can be collected by aspiration, fluid should be transported immediately to laboratory in properly enclosed collection syringe, or stains should be prepared and media inoculated at time of collection	Number of stains prepared and media inoculated limited because of small amount of material collected
Stool		
	Collect in sterile container and deliver immediately to laboratory or collect and transport in Cary-Blair medium depending on local laboratory policies and tests ordered	Notify laboratory if specific pathogen is suspected (e.g., *Vibrio* spp., *Aeromonas* spp.).Testing for bacterial pathogens other than *Clostridioides difficile* (formerly *Clostridium difficile*) not recommended for patients hospitalized for more than 3 days
Body Fluids (Other Than Blood, Urine)		
Pleural, pericardial, peritoneal fluids	Collect sufficient volume for all tests ordered, including potential future "add-on" tests	If sufficient material can be collected, inoculate aerobic and anaerobic blood culture bottles for bacterial and candidal cultures; separate vessel needed for other tests (e.g., Gram stain, NAATs)
Synovial fluid	Collect sufficient volume for all tests ordered, including potential future "add-on" tests	If sufficient material can be collected, inoculate aerobic and anaerobic blood culture bottles for bacterial and candidal cultures; separate vessel needed for other tests (e.g., Gram stain, NAATs)
Cerebrospinal fluid	Collect sufficient volume for all tests ordered, including potential future "add-on" tests	Do not inoculate blood culture bottles
Genital Tract (Commonly Submitted Specimens)		
Cervical, urethral	Remove mucus and secretions from cervical os; using fresh swab, sample endocervical canal Collect material expressed from urethra on sterile swab or use thin urethral swab to sample urethra	Specimens collected for culture or stains should be inoculated onto media at collection or immediately transported to laboratory; do not allow swab to dry If specimen being submitted for a NAAT, use specific collection device designed for the system
Vaginal	Obtain secretions from vagina with swab	
Nails and Hair		
	Clip affected areas; transport to laboratory in dry, sterile container	Culture for yeasts and dermatophytes

Continued

TABLE 16.4 Collection and Transportation of Specimens for Microbiologic Testing—cont'd

SPECIMEN	COLLECTION GUIDELINES	OTHER COMMENTS
Respiratory, Upper		
Anterior nares	Insert swab 1–2 cm into anterior nares and rotate against nasal mucosa	Generally used to detect carriage of *Staphylococcus aureus*
Nasopharynx	Nasopharyngeal washings and swabs used for respiratory viruses, *Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*	
Paranasal sinuses	Ideally collect by aspiration (see text); if bacteria suspected, submit in anaerobic transport system	Aerobic and anaerobic bacterial cultures should be performed if bacterial etiology suspected; fungal stains and culture should be performed if fungal infection suspected
Throat or pharynx	Swab posterior pharynx, avoiding saliva, for detection of *Streptococcus pyogenes*	Notify laboratory if an organism other than *S. pyogenes* suspected and testing for it desired
Respiratory, Lower		
Bronchoalveolar lavage fluid	Large volume of fluid may be collected; transport in sterile container	
Bronchial brushing or biopsy	Transport specimen in sterile container	
Sputum, expectorated	Have patient rinse mouth with water to get rid of food particles or saliva; instruct patient to cough deeply and expectorate secretions from lower airways; collect and transport in sterile container	Presence of epithelial cells indicative of contamination with oral flora; contaminated specimen unacceptable for routine bacterial culture but may be processed for mycobacterial and fungal culture
Sputum, induced	Induced with sterile saline using nebulizer	
Endotracheal aspirate or tracheal secretions	Acceptable for same tests as expectorated sputum but not screened for acceptability	Contamination with oral bacteria common; specimen processed for bacteria despite presence of epithelial cells
Tissues and Biopsies		
	Keep specimen moist and transport rapidly to laboratory; transport in anaerobic transport vial if testing for anaerobic bacteria desired; do not use bacteriostatic saline or formalin; biopsies suitable specimens for aerobic and anaerobic bacterial, fungal, and mycobacterial cultures, depending on site of biopsy	Specify type of tissue and anatomic location from which it was collected
Urine		
Midstream	Instruct women to wash hands, hold labia apart, discard first portion of voided urine, and collect midstream portion in sterile container Instruct men to wash hands, retract foreskin, discard first portion of voided urine, and collect midstream portion in sterile container	Cleansing before voiding does not consistently improve specimen quality; if patient is unable to provide proper specimen, cleansing and supervised collection may be helpful Collect first-voided urine for urethral pathogens (e.g., *Chlamydia trachomatis, Neisseria gonorrhoeae*) Urine specimens should be cultured within 2 hours of collection if stored at room temperature or within 24 hours of collection if refrigerated; if refrigeration is not an option, addition of boric acid to prevent overgrowth of contaminating organisms may be considered *Actinotignum schaalii* is a uropathogen that typically grows in 48 hours (or under anaerobic conditions) with 5% carbon dioxide
Catheterized	If patient has an indwelling Foley catheter, it should ideally be removed and urine collected either by midstream voided or repeat catheterization Transport as for midstream specimen	
Suprapubic aspirate	Transport as for midstream specimen	Only urine specimen acceptable for anaerobic culture; expel air bubbles from syringe and inject into anaerobe culture vial

EDTA, Ethylenediaminetetraacetic acid; *NAAT,* nucleic acid amplification test.
Data from Miller JM, Binnicker MJ, Campbell S, et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis. *2018;67:e1–e94.*

proportionately smaller volumes of blood are collected; guidelines regarding recommended volumes of blood for culture in children have been published.[12]

Peripheral venipuncture is the preferred approach for obtaining blood for culture because intravascular catheter collection may be associated with a higher likelihood of contamination. Contaminants, typically originating from the skin, are common in blood cultures, typically in approximately 1.5%, but up to 3% is acceptable. Ideally suspected contaminants (e.g., single blood cultures positive for coagulase-negative *Staphylococcus* spp.) should be rapidly but minimally identified and reported with a comment indicating that they are likely contaminants, such as the following: "Possible blood culture contaminant (unless isolated from more than one blood culture draw or clinical case suggests pathogenicity). No antibiotic treatment is indicated for blood culture contaminants." To minimize the risk of contamination, scrupulous care should be taken in skin disinfection before venipuncture. Following scrubbing of the skin with isopropyl alcohol, the skin is prepared with tincture of iodine, chlorine peroxide, or chlorhexidine gluconate before collecting blood cultures.[13,14] Chlorhexidine gluconate is not recommended in infants younger than 2 months of age. The septum of the culture bottle or tube should be wiped with 70% isopropyl alcohol and allowed to air dry before puncture. Products that allow diversion and

discard of the first few milliliters of blood that are most likely to contain skin contaminants (e.g., as a result of containing a skin plug) can decrease contamination rates[15] but add cost to blood cultures.[16]

Blood can be collected with a needle and syringe or directly into the culture bottles with a collection system. If a needle and syringe are used, the needle need not be changed between collection and inoculation of the blood culture system because this poses an unnecessary risk of needlestick injury and does not reduce contamination rates.[17] In patients with central venous catheters in whom catheter-associated bacteremia is suspected, one blood culture should be collected via venipuncture and another through the catheter. If the catheter has multiple lumina, blood cultures should be collected through each of the lumina if trying to diagnose catheter-associated bacteremia. Blood cultures collected through a contaminated line lumen have a higher inoculum than cultures collected from a peripheral vein; therefore cultures drawn through the contaminated line lumen will be positive before those drawn peripherally. If both blood cultures grow the same organism and the blood culture drawn from the line lumen is positive more than 2 hours before the blood culture drawn by peripheral venipuncture, there is a high likelihood of catheter-associated bloodstream infection.[18]

A number of techniques can be used to rapidly test positive blood culture bottles to determine the organisms present and, in some cases, their susceptibility. Direct testing of positive blood culture bottles by MALDI-TOF mass spectrometry after short-term subculture plate incubation (i.e., 2–6 hours) of high-inoculum subcultures on solid media has been adopted by many laboratories as a cost-effective approach to rapid organism identification. (Methods for testing positive blood culture bottles directly using MALDI-TOF mass spectrometry have also been described.) In addition to assays that detect small numbers of organisms and resistance genes, there are multiplex assays approved by the FDA that simultaneously detect a panel of microorganisms as well as select resistance genes directly from positive blood culture bottles; examples include the FilmArray Blood Culture Identification panel (BioFire Diagnostics LLC, Salt Lake City, UT) and the Nanosphere Verigene Gram-Positive and Gram-Negative Blood Culture tests (Luminex, Northbrook, IL).[19] The Accelerate Pheno system (Accelerate Diagnostics, Inc., Tucson, AZ) is an automated system that uses gel electrofiltration and fluorescence in situ hybridization for identification of a limited number of bacteria and yeast directly from positive blood cultures. It is the first assay approved by the FDA to offer rapid phenotypic susceptibility testing directly from positive blood cultures. The system provides MIC values by analyzing bacterial growth in the presence of antibiotics using automated microscopy and time lapse imaging.[20] Given the costs of the multiplex molecular assays, numerous studies have assessed their economic and clinical effects. Although these studies generally demonstrate a decrease in time to optimization of antimicrobial therapy, there have been inconclusive results in terms of impact on mortality and lengths of stay. Institution-specific variables, such as distinct local resistance rates and patient populations, and the availability of antimicrobial stewardship programs probably influence the clinical impact of rapid molecular assays for testing positive blood culture bottles. These panels have the most impact when results are reported as quickly as possible and properly acted on by providers caring for patients. Antibiotic deescalation is ideally accomplished in the setting of delivery of results to an expert in antimicrobial stewardship (e.g., infectious diseases pharmacist, infectious diseases physician, doctoral-level clinical microbiologist) who can then deliver individualized and rapid direction to providers caring for patients.[21]

Intravenous Catheter Tips

The semiquantitative method of Maki et al.,[22] performed by rolling the tip across a blood-agar plate, is the most common method for culturing catheter tips. The detection of 15 or more colonies is indicative of a catheter infection. Adjustments of the Maki method have been described, as have approaches that use vortexing, sonication, flushing, or brushing of the catheter tip, but are not routinely performed.

Cerebrospinal Fluid

Three or four tubes of CSF are usually submitted for laboratory studies. The first has the most potential for contamination with skin flora and therefore should not be sent to the microbiology laboratory for direct smears, culture, or NAATs. The volume of CSF submitted should be sufficient for all tests ordered, as well as for those tests that may be added on at a later time; the total volume needed can be calculated by consulting the laboratory's test catalog. Mycobacterial and fungal cultures in particular ideally require larger volumes of CSF for ideal sensitivity. When the specimen volume is less than necessary for numerous test requests, prioritization of testing must be provided to the laboratory.

The most common causes of acute meningitis/encephalitis today are enteroviruses, herpes simplex virus (HSV), and bacteria (*S. pneumoniae* and *Neisseria meningitidis*). CSF NAATs for enterovirus and HSV types 1 and 2 are the tests of choice for central nervous system infection caused by these organisms. CSF NAATs for parechoviruses and varicella-zoster virus may be considered under relevant clinical conditions. If possible, specimens for bacterial culture should be obtained before antimicrobial therapy initiation, but antimicrobial therapy should not be delayed to collect CSF. CSF Gram stains should be performed on cytocentrifuged specimens, with positive results called to the patient care area immediately. Gram stain is generally positive in patients with bacterial meningitis (infection with *Listeria monocytogenes* is an exception); however, prior exposure to antibiotics can rapidly eliminate organisms in CSF or alter their Gram stain properties. Aside from the possible exception of *S. pneumoniae,* use of direct antigen tests for bacteria in CSF is not recommended. Antigen tests for *Cryptococcus neoformans* and *Cryptococcus gattii* are, however, sensitive and specific. Cultures for the classic causes of bacterial meningitis (e.g., *Streptococcus agalactiae, S. pneumoniae, N. meningitidis, L. monocytogenes*) are generally positive within 1 to 2 days; however, their recovery in culture can be decreased by prior exposure to antibiotics. The cryptococcal antigen test has replaced India ink staining for rapid diagnosis of cryptococcal meningitis. For central nervous system infection, this test is more sensitive when performed on CSF rather than serum.

Organisms that cause chronic meningitis include *Mycobacterium tuberculosis,* fungi, and spirochetes. Because the sensitivity of NAATs for *M. tuberculosis* in CSF may be poor, acid-fast bacillus (AFB) smear and mycobacterial cultures, performed on large volumes (≥5 mL) of CSF, should be requested in addition to NAATs if tuberculous meningitis is suspected.[23] For diagnosis of central nervous system infection caused by *Histoplasma capsulatum, C. immitis* and *C. posadasii, Blastomyces* spp., or *Sporothrix schenckii* complex, CSF fungal stains and culture should be supplemented with serologic testing (with concomitant serum testing), urine antigen testing (for *H. capsulatum, C. immitis* and *C. posadasii, Blastomyces* spp.), and, where available, NAATs performed on CSF. Serologic testing is the test of choice for *Treponema pallidum* (neurosyphilis) and *Borrelia burgdorferi* (neuroborreliosis).

Infection with a number of other central nervous system infection pathogens including bacteria (e.g., agents of ehrlichiosis and anaplasmosis, *Bartonella* spp., *C. burnetii, Leptospira* spp., *Mycoplasma pneumoniae, Nocardia* spp., *Rickettsia* spp., *Tropheryma whipplei*), viruses (e.g., adenovirus, cytomegalovirus, human herpesvirus 6, influenza virus, human immunodeficiency virus, lymphocytic choriomeningitis virus, measles virus, mumps virus, rabies virus, West Nile virus, Zika virus, California virus, Eastern equine encephalitis virus, St. Louis encephalitis virus, Western equine encephalitis virus, Powassan virus, and Jamestown Canyon virus, as well as other arboviruses), parasites (e.g., *Acanthamoeba* spp., *Balamuthia mandrillaris, Baylisascaris procyonis, Naegleria fowleri, Trypanosoma brucei, Toxoplasma gondii*), and prions can be assessed by testing CSF. Details regarding testing for each of these can be found in individual chapters of this book and the Infectious Diseases Society of America guidelines.[12] In shunt-associated or postneurosurgical infections or focal infections of the brain parenchyma, the differential diagnosis is broadened; in this case, CSF should be cultured for aerobic bacteria as well as anaerobic bacteria, and a broader list of fungi should be considered in the differential diagnosis.

A multiplex polymerase chain reaction panel for testing CSF (FilmArray Meningitis/Encephalitis panel; BioFire Diagnostics LLC) has been approved by the FDA. The panel includes 14 targets (*Escherichia coli* K1, *H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, S.*

pneumoniae, cytomegalovirus, enterovirus, HSV types 1 and 2, human herpesvirus 6, human parechovirus, varicella-zoster virus, and *C. neoformans* and *C. gattii*) and generates results in approximately 1 hour.[24] Sanger sequencing-based (e.g., 16S ribosomal RNA gene) and shotgun metagenomics approaches are also being applied. Studies are needed to define the impact on treatment decisions, cost-effectiveness, and, ultimately, patient outcomes of these types of tests.

Peritoneal, Pleural, and Pericardial Fluids

Peritoneal, pleural, and pericardial fluids may be cultured in aerobic and anaerobic blood culture bottles if the presence of multiple organisms is reasonably uncertain (e.g., spontaneous bacterial peritonitis). If inoculated into blood culture bottles at the bedside, additional fluid in a sterile container should be submitted for any desired tests other than bacterial culture (e.g., Gram stain). Culture in blood culture bottles is not ideal for polymicrobial specimens, as this strategy may not accurately assess the variety of organisms present because some organisms may be obscured by overgrowth of other more rapidly growing organisms. Negative cultures in the presence of other indicators of infection should prompt an assessment for fastidious or slow-growing organisms (e.g., *M. tuberculosis,* fungi, *Neisseria gonorrhoeae*). As with CSF, fungal and mycobacterial cultures of peritoneal, pleural, and pericardial fluids are best performed with large specimen volumes. The microbiology laboratory should be contacted if *N. gonorrhoeae* is under consideration because special culture media or NAAT, or both, may be appropriate. Swabs are inappropriate for testing peritoneal, pleural, and pericardial fluids.

Bone and Joint Specimens

Processing synovial fluid is similar to processing peritoneal, pleural, and pericardial fluids except that a smaller volume of fluid is generally available, and infection is most commonly caused by a single organism. As with peritoneal, pleural, and pericardial fluids, swabs are inappropriate for testing synovial fluid. Synovial fluid is ideally cultured in aerobic and anaerobic blood culture bottles. Additional fluid in a sterile container should be submitted for any desired tests other than bacterial culture (e.g., Gram stain). Gram stain is recommended for native joint septic arthritis. In young children with septic arthritis in whom *Kingella kingae* is being considered, NAAT is recommended because this organism does not always grow in culture. In patients with an arthroplasty at the site from which the synovial fluid is aspirated, some organisms otherwise considered contaminants may be pathogens (e.g., coagulase-negative staphylococci such as *Staphylococcus epidermidis*). *C. acnes,* which can cause prosthetic joint infection, may require up to 2 weeks for isolation.

For diagnosis of osteomyelitis, as much specimen as possible should be submitted for aerobic and anaerobic cultures and (except in the case of prosthetic joint infection) Gram stain; specimens may include pieces of intact bone and scrapings, shavings, or excised or aspirated necrotic material. Swabs and cultures of sinus tracts are generally not recommended because recovered organisms, aside from *S. aureus,* do not correlate with organisms found in deep cultures. In patients with diabetic foot osteomyelitis, specimens for aerobic and anaerobic culture of bone can be obtained by open débridement, needle puncture, or transcutaneous biopsy. Readers are referred to an Infectious Diseases Society of America guideline on the diagnosis of diabetic foot infections.[25]

For vertebral osteomyelitis, disk space infection, and spondylodiskitis, aerobic and anaerobic bacterial and candidal blood cultures are recommended; *Brucella* blood cultures and serologic tests should be obtained in patients in areas endemic for brucellosis, fungal blood cultures should be obtained in patients with relevant epidemiologic or host risk factors, and a purified protein derivative test or interferon-γ release assay should be obtained in patients at risk for tuberculosis. Patients suspected to have native vertebral osteomyelitis based on clinical, imaging, and laboratory studies with *S. aureus, Staphylococcus lugdunensis,* or *Brucella* bloodstream infection or, in an endemic setting with a positive *Brucella* serology, do not need further testing. For all others, imaging-guided aspiration or biopsy of a disk space or vertebral endplate with the specimens submitted for Gram stain and aerobic and anaerobic culture and, if adequate tissue can be obtained, histopathology, is recommended. If results are negative or inconclusive (e.g., *Corynebacterium* spp. is isolated), a second imaging-guided aspiration biopsy, percutaneous endoscopic diskectomy and drainage procedure, or open excisional biopsy should be considered to collect additional specimens for repeat and additional testing. Readers are referred to an Infectious Diseases Society of America guideline on the diagnosis of native vertebral osteomyelitis in adults.[26]

In patients with potential prosthetic joint infection, multiple tissue specimens should be submitted for aerobic and anaerobic cultures—four if using conventional plate and broth cultures and three if culturing tissues in aerobic and anaerobic blood culture bottles, a strategy that increases sensitivity.[27,28] Two or more intraoperative cultures or a combination of preoperative aspiration and intraoperative cultures that yield the same organism are considered definitive evidence of prosthetic joint infection. Single positive tissue or synovial fluid cultures, especially for organisms that may be contaminants (e.g., *S. epidermidis, C. acnes*), should not be considered definitive evidence of prosthetic joint infection. Tissue Gram stain is not recommended for prosthetic joint infection diagnosis. Isolation of *C. acnes* may require culture incubation times of 14 days. If the arthroplasty is resected, the implant components may be vortexed and sonicated and the resultant sonication fluid semiquantitatively cultured to sample biofilms on its surface.[29] As mycobacteria and fungi are rare in this setting, they should not be routinely sought.

Skin and Soft Tissue Specimens

Skin and soft tissue specimens sent to the laboratory include tissue biopsies, aspirates or swabs of abscesses, wound swabs, material obtained by surgical débridement, and drainage samples. The usefulness of processing these specimens by microscopy and culture is limited by site of the lesion. Lesions that communicate directly with the skin or mucosal surfaces are commonly contaminated with mixed populations of organisms. For meaningful test results, surgically obtained tissue samples or aspirates of closed abscesses, pus, or fluid are preferred. When anaerobic bacteria are expected, specimens should be placed into an anaerobic transport container and delivered promptly to the laboratory. Swabs, including swabs of superficial skin ulcers, from the skin surface of a sinus tract or from open abscesses commonly yield mixed flora and often do not reflect organisms of real infectious significance; they should be avoided.

Wound infections and abscesses are caused by many different organisms including aerobic and anaerobic bacteria, mycobacteria, and fungi. It is important for the clinician and the laboratory to recognize that certain organisms are often associated with particular types of wounds or abscesses. An infected animal bite may yield *Pasteurella multocida* or *Capnocytophaga* species; a posttraumatic hand infection may yield *S. aureus, Mycobacterium marinum,* or *S. schenckii* complex depending on the source of trauma; a postoperative wound infection may yield *P. aeruginosa* or *Acinetobacter baumannii,* among others. Fungal, mycobacterial, and anaerobic cultures must be specifically requested if clinically indicated. When *Francisella tularensis* or *Brucella* species are sought, the laboratory should be notified so that cultures can be set up appropriately and isolated organisms can be safely handled. Bartonellosis is best diagnosed by histopathology, serology, and, if needed, NAAT. Providing the laboratory with the type or location of the wound, abscess, or tissue is frequently useful because it may hasten recognition of specific pathogens known to be associated with a particular type of infection or anatomic site (e.g., cat bite, brain abscess).

If sufficient specimen is available, microscopy should be performed to obtain a preliminary indication of the infecting organism. Additionally, if antimicrobial therapy has been initiated, direct smear may be the single available guide to the cause because growth may be inhibited. Impression smears can be made by gentle pressing of a freshly cut surface of the tissue onto a slide, which can then be stained for bacteria, fungi, or mycobacteria. Direct examination of specimens by microscopy can provide preliminary information about the quality of the specimen (e.g., many epithelial cells in a wound specimen indicates skin contamination), guide empirical therapy, and direct culture strategies. For example, if organisms resembling *Nocardia* species or mycobacteria are observed in a Gram-stained specimen submitted for routine culture, additional processing to recover these organisms can be performed.

Organisms observed by microscopy should be correlated with culture results. Discrepancies may help identify an organism that would not otherwise be detected. For example, faintly staining gram-negative bacilli that do not grow on routine aerobic plates may suggest the possibility of an anaerobic organism, such as *Bacteroides* species.

Direct Gram smears of known quantities of specimen can be used to give an assessment of organism load. Quantitative wound, burn eschar, and tissue biopsy specimen cultures have been used to help predict the likelihood of wound sepsis, burn infection, or the success of skin grafting. They require weighing and careful preparation of the specimen for serial dilutions to determine colony counts. Because quantitative cultures are time consuming and labor intensive, not all laboratories perform these assays. The availability of quantitative wound cultures should be checked by consultation with the laboratory before such tests are ordered.

Respiratory Tract Specimens

Respiratory tract specimens can be divided into two major categories, upper and lower respiratory specimens. Common upper respiratory tract specimens include throat swabs and nasopharyngeal swabs or washings. Common lower respiratory tract specimens include expectorated and induced sputum; endotracheal aspirate (tracheal secretions); and bronchoalveolar lavage (BAL), brushing, and biopsy. Fine-needle lung aspirate and open lung biopsy specimens may also be collected.

Throat swabs submitted from patients with bacterial pharyngitis are typically processed for *Streptococcus pyogenes* only unless a specific request is submitted to look for other agents. Although other bacteria including *Streptococcus dysgalactiae, Arcanobacterium haemolyticum, Fusobacterium necrophorum*, *N. gonorrhoeae, C. diphtheriae,* and *M. pneumoniae* can cause pharyngitis, they are not routinely tested for and must be specifically requested if they are sought. *N. gonorrhoeae* and *M. pneumoniae* are best detected using NAATs. If *C. diphtheriae* is being considered, a specialized culture (which uses selective and differential media) should be specifically requested. If culture for *C. diphtheriae* is positive, toxin testing of the isolate is required to demonstrate production of diphtheria toxin. NAATs and direct antigen tests including point-of-care tests are used for rapid detection of *S. pyogenes* in throat swabs. Some direct antigen tests are not sensitive. The Infectious Diseases Society of America and the American Heart Association recommend performing cultures for children and adolescents with negative rapid antigen detection tests but do not recommend reflex cultures for adults with negative rapid antigen detection tests results, given the lower incidence of *S. pyogenes* pharyngitis and rheumatic fever in this population.[30,31] However, evidence suggests that culture of antigen-negative throat swabs may be indicated for adults as well as children and adolescents, as rapid antigen detection tests fail to detect *S. pyogenes* pharyngitis in a significant number of adults.[32] Antimicrobial susceptibility testing is not routinely done on *S. pyogenes,* as it is universally susceptible to penicillin, although it is not universally susceptible to macrolides and azalides. Although respiratory viruses are common causes of pharyngitis, it is usually of limited usefulness to define a specific etiology in patients with pharyngitis due to respiratory viruses because there is usually no pathogen-directed treatment required for these agents.

Detection of viruses in respiratory samples has changed with the introduction of FDA-approved multiplex NAATs that detect 17 or more respiratory viruses.[19] These panels have largely replaced culture-based and antigen-based tests for viral detection in respiratory samples. The preferred sample is a swab passed through each nostril all the way to the back of the nasopharynx and placed into viral transport medium. Once in the laboratory, test time is 1 to 8 hours, depending on platform. Some laboratories have validated testing on lower respiratory samples (e.g., BAL fluid). The specific organisms included vary and include adenoviruses, coronaviruses, human bocavirus, human metapneumovirus, influenza A virus (including subtypes H1, H3, and 2009 H1N1), influenza B virus, parainfluenza virus 1–4, and respiratory syncytial virus (including A and B); bacteria including *Chlamydia pneumoniae, M. pneumoniae, Bordetella pertussis, Bordetella parapertussis* and *Bordetella bronchiseptica,* and *Bordetella holmesii* are included in some panels. NAAT performed on a posterior nasopharyngeal swab or aspirate is the recommended test of choice for *B. pertussis* and *B. parapertussis* detection. Although *B. pertussis* can be cultured on specialized media, culture is less sensitive than NAATs.

Aspirates of the middle ear are occasionally submitted. In patients with otorrhea or myringotomy tubes, the ear canal can be cleaned and drainage collected with mini-tip swabs.

Aspirates of paranasal sinuses may be submitted for diagnosis of sinusitis; nasopharyngeal swabs or washings should not be submitted for this indication. Endoscopically collected swab cultures may recover bacterial pathogens but infrequently detect the causative fungi. In maxillary sinusitis, antral puncture with sinus aspiration and, in adults, swabs of material draining from the middle meatus collected under endoscopic guidance are suitable specimens. To establish a fungal etiology, an endoscopic sinus aspirate is suggested. Surgical procedures are required to sample sphenoid, frontal, or ethmoid sinuses.

Some laboratories screen high-risk patients for methicillin-resistant *S. aureus* or *S. aureus* carriage using anterior nares swabs. A variety of selective, differential media have been developed for this purpose, as have NAATs. (Additional sites that may be sampled for assessing *S. aureus* carriage include the axillae, groins, stool, and throat.)

Expectorated and induced sputa and tracheal aspirates (tracheal secretions) are the most common specimens submitted for diagnosis of lower respiratory tract infections. Although these are readily obtained, results can be difficult to assess because microorganisms colonizing the upper airways including those that can be pathogenic in the lower respiratory tract can contaminate the specimen. Although it is difficult to avoid contamination of induced sputa and tracheal aspirates, this can be reduced in expectorated sputa by instructing the patient to cough deeply and expectorate secretions from their lower chest directly into a clean container. A single coughed specimen is sufficient for bacterial culture. Laboratories screen expectorated specimens submitted for bacterial culture and reject them if they are contaminated with oral secretions, as identified by the presence of epithelial cells. Although not ideal, contaminated sputum specimens can be processed for *Legionella* species, *Nocardia* species, *Mycobacterium* species, and mold cultures because selective media are used to suppress growth of contaminants.

Noninvasive sampling of the respiratory tract is recommended for hospital-acquired and ventilator-associated pneumonia.[33] In nonventilated patients, appropriate specimens include expectorated sputum, induced sputum, or, in an uncooperative patient, nasotracheal aspirates; in ventilated patients, endotracheal aspirates are appropriate.[33] Cultures of endotracheal aspirates, although likely to contain the true pathogen, may yield more mixtures of species of bacteria, including colonizing bacteria, than specimens obtained bronchoscopically; this can lead to needless antibiotic therapy. Quantitative assessment of invasively obtained samples such as BAL and protected specimen brush specimens may be performed.[33] Bacterial quantities above a threshold (dependent on timing of antimicrobial therapy) are diagnostic of pneumonia, whereas those below that threshold are more consistent with colonization. Generally accepted thresholds are 10^6 CFU/mL for endotracheal aspirates, 10^4 CFU/mL for BAL fluid, and 10^3 CFU/mL for protected specimen brush samples. Quantitative cultures require a large amount of specialized laboratory work and are not endorsed by current guidelines.[12] Isolation of respiratory pathogens in biopsy specimens or fine-needle aspirates of the lungs is almost always significant.[33] Likewise, regardless of the collection method or number of organisms present, some pathogens should always be considered significant (e.g., *Legionella* spp., *Nocardia* spp., *M. tuberculosis, H. capsulatum, Blastomyces* species, *C. immitis* and *C. posadasii, C. neoformans* and *C. gattii*). Multiplex NAATs that detect multiple bacterial species and resistance genes as well as viruses have been approved by the FDA for testing sputum, tracheal aspirates, and BAL fluid. Infections with some respiratory pathogens (e.g., *S. pneumoniae, Legionella* spp.) may be diagnosed by detecting specific antigens in urine; these tests should be performed in addition to culture. Microscopy (e.g., Gram stain, acid-fast stain, calcofluor white stain) can provide a rapid diagnosis if positive but is insensitive, so alternative test methods should also be used. The use of a specific NAAT is becoming the approach of choice for some pathogens and an important supplementary test for others. NAATs are particularly important when a specific organism is considered in

the differential diagnosis (e.g., *M. tuberculosis, Pneumocystis jirovecii, M. pneumoniae, Legionella* spp.).

The microbiology of pulmonary infections in patients with cystic fibrosis is different from that in patients without cystic fibrosis. Specialized culture techniques for cystic fibrosis respiratory specimens should be used; these techniques help recover pathogens in the context of the presence of mucoid *P. aeruginosa* strains. Mycobacterial and fungal stains and cultures may also be appropriate.

Pulmonary infections in immunocompromised hosts have a broader differential diagnosis than infections in nonimmunocompromised hosts and may be associated with severe morbidity and mortality. As such, when rapid and noninvasive tests are not revealing, bronchoscopy with BAL with or without transbronchial biopsy is usually performed. Microbiology laboratories should collaborate with infectious diseases clinicians and pulmonologists to develop an algorithm for processing samples that comprises testing for all major categories of pathogens. At my institution, for example, an immunocompromised host BAL panel is offered that includes cell count and differential, cytology, viral NAATs (influenza, respiratory syncytial virus, adenovirus), galactomannan, Gram stain, bacterial culture, fungal stain and culture, *Nocardia* stain, mycobacterial stain and culture, viral culture, *Legionella* culture and NAAT, and *Pneumocystis* NAAT. Cytology or histopathology or both can also be helpful (e.g., to identify pulmonary hemorrhage), as can serum and BAL galactomannan and (1–3)-β-D-glucan tests. Transthoracic needle aspiration, computed tomography–guided biopsy, and open lung biopsy may be considered if less invasive diagnostic tests are unrevealing.

Urine

Urine, including voided midstream and catheterized urine, is the most common specimen processed in the clinical microbiology laboratory. Reliable results are substantially impacted by appropriateness of testing, as it is impossible to differentiate asymptomatic bacteriuria from urinary tract infection based on microbiology results alone. With a few exceptions (e.g., pregnant women and patients about to undergo urologic surgery), performance of urine cultures on asymptomatic patients is not recommended. Reliable results are affected by specimen quality and transportation to the laboratory. Culture contamination is an issue because the urethra is colonized with large numbers of bacteria (e.g., *Lactobacillus* spp., streptococci), introduction of which into the specimen during collection can compromise the specimen. Similarly, contamination of the specimen with vaginal or fecal bacteria can compromise results. The impact of contaminated urine cultures is amplified if urine is not promptly transported to the laboratory. Because urine is so easily contaminated, urine specimens for bacterial culture should be collected with attention to curbing contamination from perineal and superficial mucosal microbiota. Specific, detailed instructions should be provided to patients at the time of urine collection to minimize bacterial contamination. This includes discarding the first portion of the voided specimen, which could be contaminated with the urethral flora (i.e., collection of midstream urine). Although some studies suggest that skin cleansing in preparation for collection of midstream urine specimens may be of little benefit, many laboratories find that culture of specimens obtained without skin cleansing regularly contain mixed flora and accordingly yield high numbers of one or more potential pathogens, rendering interpretation of results challenging.[12] Urine collected in a sterile container and transported at room temperature should be plated for culture within 2 hours of collection or, if this is not possible, transferred to tubes with agents (e.g., boric acid) that inhibit bacterial overgrowth or refrigerated. An exception to the preference for collection of midstream urine is collection of urine for diagnosis of urethritis (e.g., *N. gonorrhoeae, Chlamydia trachomatis*), as a first-voided urine is preferred in this situation to sample the urethra. Suprapubic aspirates, primarily performed on infants or other patients in whom assessment of midstream urine is difficult or in whom an anaerobic infection is suspected, as well as specimens obtained by cystoscopy or by other invasive procedures should be clearly labeled as such, as all growth should be identified and reported.

Rapid screening techniques for urinary tract infection include direct Gram stain and a variety of commercially available products, such as dipsticks and fluorescence-based flow cytometry. A positive Gram stain is equivalent to greater than or equal to 10^5 CFU/mL. Because urine specimens from infected patients may have quantities of bacteria less than 10^5 CFU/mL, Gram staining is a relatively insensitive screening method for these specimens (i.e., patients can have negative Gram stains but positive cultures). The presence of mixed bacterial types or a moderate amount of squamous epithelial cells suggests contamination with genital flora. In-and-out catheterization of a correctly prepared patient usually offers a less contaminated specimen than a midstream specimen. Laboratories should routinely perform susceptibility testing on potential pathogens present in significant numbers.

Specimens from urinary catheters in place for more than a few hours often have colonizing flora due to biofilm formation on the catheter surface; accordingly, positive urine cultures collected from these catheters may not represent urinary tract infection, and culture from indwelling catheters is strongly discouraged. If urine cultures are needed in catheterized patients, the catheter should be removed and midstream urine collected, or a new catheter should be placed and urine collected from the sampling port of the newly inserted device. Cultures of Foley catheter tips are of no clinical value. Collection from urinary diversions such as ileal loops is also not suggested because of the tendency of these locations to be chronically colonized. Chronic nephrostomy collections and bagged urine collections are of dubious value for culture.

Yeasts, particularly *Candida* species, may be isolated from routine midstream urine cultures; their quantitation is not useful in assessing clinical significance. Recovery of *Candida* species even in high numbers is not infrequent from patients who do not actually have yeast urinary tract infection. Mycobacterial urine cultures (typically looking for *M. tuberculosis*) in addition to urine cultures for *C. neoformans* and *C. gattii, Blastomyces* species, *H. capsulatum,* and *C. immitis* and *C. posadasii* require large volumes of first-voided urine (typically at least 20 mL) and use of specialized media.

For acute bacterial prostatitis, a midstream urine culture with or without culture of expressed prostate secretions is recommended; for chronic bacterial prostatitis, a midstream urine culture with culture of expressed prostatic secretions is recommended.[12] The traditional Meares-Stamey four glass specimen involving collection of first void urine, midstream urine, expressed prostate secretions, and post–prostate massage urine is positive if there is a 10-fold higher bacterial count in the expressed prostate secretions than the midstream urine.[34] A two-specimen variant involving only the midstream and the expressed prostate secretions specimens is easier to use and is recommended. Clinicians should be aware that prostatic massage in a patient with acute bacterial prostatitis may precipitate bacteremia. For epididymitis testing, clinicians are referred to a recently published guideline.[12]

Detection of adenovirus in cases of cystitis is usually done by NAAT. BK virus nephropathy is best diagnosed by renal biopsy; however, plasma BK virus viral load testing may be used as a screening test for BK virus nephropathy, prompting renal biopsy if the viral load is >4 log copies/mL. Alternatively, urine testing for decoy cells or BK virus using a quantitative NAAT can be used as a screening test, and if positive, may be followed by BK viral load testing of plasma, which has a higher specificity.

Stool

Acute diarrhea can have a variety of etiologies including bacteria, viruses, and parasites in addition to noninfectious causes. Not all patients with acute diarrhea need testing, as most cases are self-limited and do not require specific treatment. The reader is referred to recent recommendations regarding who should be tested and with what tests.[19,35] Recommendations for minimizing unnecessary stool cultures include rejection of routine bacterial stool cultures in patients who have been hospitalized for 3 days or longer. Likewise, testing for *Clostridioides difficile* (formerly *Clostridium difficile*) is usually appropriate only on diarrheal stools, defined as specimens that are loose enough to take the shape of the container. Most laboratories have guidelines in place that limit repeat testing for enteric pathogens within specified time intervals (e.g., maximum weekly testing for *C. difficile*).[36,37] Repeat testing of patients previously positive for *C. difficile* as a "test of cure" is not appropriate.

Laboratories should have a routine in place to evaluate for common bacterial agents of acute gastroenteritis, which should include at a minimum *Campylobacter, Salmonella,* and *Shigella* species in addition to Shiga toxin–producing *E. coli* (typically detected using NAAT or antigen test). Laboratory test catalogs and reports should indicate which of the enteric pathogens would be detected with a routine stool culture because this may vary from institution to institution. Comprehensive workup of fecal specimens requires a variety of selective, differential culture media as well as microscopy, antigen tests, and NAATs. Enrichment broths to detect small numbers of organisms may or may not be used. Rectal swabs provide an insufficient quantity of specimen and are discouraged. Stool should be immediately sent to the laboratory or mixed in Cary-Blair transport medium after the specimen is collected. Laboratories should be notified if pathogens are suspected that are not included in routine stool cultures (e.g., *Vibrio cholerae*) because selective culture methods or the use of antigen tests or NAATs may be required.

Numerous methods may be employed for the diagnosis of *C. difficile*–associated diarrhea. Toxigenic culture and cytotoxin assay are sensitive but time consuming and labor intensive. Enzyme immunoassay and immunochromatographic methods for toxin detection have lower sensitivities but are faster; assays that detect both toxin A and toxin B are ideal. Glutamate dehydrogenase antigen assays are sensitive but have poor specificity. NAATs for *C. difficile* are as sensitive as toxigenic culture. To minimize turnaround time, mitigate costs, and improve accuracy of diagnosis of clinically significant *C. difficile*–associated diarrhea, some laboratories employ an algorithm that uses glutamate dehydrogenase as a rapid screening test followed, if results are positive, by toxin A and B detection or NAAT or both.

There are currently multiple FDA-approved multiplex NAATs that simultaneously detect five or more stool pathogens (and several more that detect smaller numbers of stool pathogens); examples include the Luminex xTAG Gastrointestinal Pathogen panel (Luminex GPP; Luminex), the BioFire FilmArray Gastrointestinal Panel (BioFire GI Panel; BioFire Diagnostics LLC), and the Verigene Enteric Pathogens Nucleic Acid Test (Verigene EP; Luminex). The Luminex GPP detects *Campylobacter* species, *C. difficile, E. coli* O157, enterotoxigenic *E. coli,* Shiga toxin–producing *E. coli, Salmonella* species, *Shigella* species, *V. cholerae,* adenovirus 40/41, norovirus, rotavirus A, *Cryptosporidium* species, *Entamoeba histolytica,* and *Giardia* species in approximately 5 hours. The BioFire GI Panel can identify 22 targets in approximately 1 hour, and targets include all of those on the Luminex GPP plus *Vibrio* species, *Yersinia enterocolitica,* enteropathogenic *E. coli,* enteroaggregative *E. coli, Plesiomonas shigelloides,* astrovirus, sapovirus, and *Cyclospora cayetanensis*; *Shigella* species and enteroinvasive *E. coli* are targeted instead of *Shigella* species. The Verigene EP targets *Campylobacter* group, *Salmonella* species, *Shigella* species, *Vibrio* group, *Y. enterocolitica,* Shiga toxin–producing *E. coli,* norovirus, and rotavirus and runs on the Nanosphere Verigene system, yielding results in <2 hours. These panels are FDA approved on the basis of accurate detection and identification of the pathogen. Determination of clinical significance of results is the responsibility of the clinician. In the absence of evidence of a new infection, follow-up testing should be limited; these panels should not be used as a test of cure.[38] They are expensive and should be used with a test utilization strategy in place.[19] Conventional methods are required to detect pathogens not covered by the panels (e.g., *Aeromonas* spp.) and provide antimicrobial susceptibility information when required. To provide isolates for susceptibility testing, clinical laboratories using multiplex panels may contemplate culturing for the organisms identified molecularly (i.e., reflexive culture), a strategy that can also be adopted when public health laboratories need isolates.[39,40] The ideal panel of organisms to be included has not been well defined.

Some institutions screen selected patients for fecal carriage of multidrug-resistant organisms such as vancomycin-resistant enterococci or carbapenemase-producing gram-negative bacilli. Selective media or NAATs targeting resistance genes of interest (e.g., *vanA, vanB,* bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48}$, bla_{VIM}, bla_{IMP}) can be used to test rectal swabs or stool for this purpose. Perianal swabs for *S. pyogenes* cultures are appropriate for diagnosis of perianal cellulitis, which most commonly occurs in children.

Vaginitis and Vaginosis Diagnostics

Vaginitis with discharge can be caused by *Candida* species, bacterial vaginosis, or *Trichomonas vaginalis*. Swabs of vaginal discharge are typically collected. Vaginal candidiasis is best diagnosed by a fungal smear showing many budding yeasts and pseudohyphae. Although candidal culture can be performed, growth of *Candida* species is not necessarily diagnostic of candidal infection because many asymptomatic women harbor *Candida* species.

For bacterial vaginosis, a graded Gram stain of vaginal discharge is recommended. The healthy vagina has a predominance of lactobacilli, whereas the flora of a patient with bacterial vaginosis is dominated by coccobacilli, reflecting an increase in growth of *Gardnerella vaginalis* and other anaerobes. Nugent criteria can be used to quantify or grade bacteria via Gram stain of vaginal samples. Nugent criteria evaluate *Lactobacillus* species, *Bacteroides* species and *Gardnerella* species, and *Mobiluncus* species by Gram stain, grading each on a scale of 1 to 4 (1+ is <1 cell per field, 2+ is 1–4 cells per field, 3+ is 5–30 cells per field, and 4+ is >30 cells per field). *Lactobacillus* species and *Bacteroides* species and *Gardnerella* species are then given scores between 0 and 4, with *Mobiluncus* species receiving scores of 0 and 2. Total scores are calculated and used as follows: 0 to 3, consistent with normal bacterial vaginal flora; 4 to 6, altered vaginal flora not consistent with bacterial vaginosis (this frequently represents a transitional stage); and 7–10, consistent with bacterial vaginosis. As an alternative to the Nugent criteria, Hay/Ison criteria can be used.[41] Vaginal discharge with a pH greater than 4.5, a fishy amine odor after addition of potassium hydroxide, and the presence of clue cells on saline wet mount are also used to support a diagnosis of bacterial vaginosis. *G. vaginalis* cultures are not recommended. Tests that assess the vaginal microbiome using NAATs have a similar performance to the graded Gram stain.

For *T. vaginalis* vaginitis, vaginal pH is also typically >4.5. Visualization of motile trichomonads allows a diagnosis but is not as sensitive as NAATs. Other approaches including culture, DNA hybridization probe detection, and rapid antigen testing are also not as sensitive as NAATs. Notably, NAATs for trichomoniasis can be performed on a number of specimen types including vaginal, endocervical, urethral, rectal, or pharyngeal swabs and urine or liquid-based cytology specimens.

Genital Lesions and Syphilis Diagnostics

Genital lesions are a challenge to diagnose. Patients with genital lesions should be assessed with a serologic test for syphilis as well as diagnostic tests for HSV and, where chancroid is prevalent, *Haemophilus ducreyi*.

Darkfield examination for motile spirochetes and NAAT are useful for diagnosing primary syphilis but are unavailable in the most laboratories. *Treponema pallidum* cannot be cultured in the routine laboratory. Accordingly, testing for syphilis is most commonly performed by serology. Traditional testing has consisted of initial screening with a nontreponemal test (e.g., rapid plasma reagin [RPR]), followed by testing of reactive specimens with a treponemal test (e.g., *T. pallidum* particle agglutination). Alternatively, a reverse testing sequence can be used beginning with a specific treponemal test such as an enzyme immunoassay or chemiluminescence immunoassay and then retesting reactive results with a nontreponemal test such as RPR to confirm the diagnosis. Screening with a treponemal test can identify persons previously positive, treated, or partially treated for syphilis as well as yield false-positive results in patients with low likelihood of infection. If the follow-up confirmation test (RPR) is negative, the laboratory should perform a different treponemal specific test (e.g., fluorescent treponemal antibody absorption).

For suspected cases of HSV genital lesions, NAATs are recommended, as viral culture or direct fluorescent antibody testing is less sensitive. A swab can be rubbed over the surface of the lesion, even a closed vesicle, for NAATs. Alternatively, a vesicle may be aspirated or unroofed and the liquid material swabbed. NAAT should test for HSV types 1 and 2. Consideration may also be given to additionally testing for varicella-zoster virus if the clinical picture is suggestive of this possibility.

Chancroid, caused by *Haemophilus ducreyi;* lymphogranuloma venereum caused by *C. trachomatis* serovars L1, L2, or L3; and granuloma inguinale (donovanosis) caused by *Klebsiella granulomatis* are uncommon causes of genital ulcers in the United States and are typically diagnosed

based on clinical presentation, identification of risk factors, and exclusion of the more common causes of genital lesions, syphilis and HSV. Chancroid may be identified by Gram stain and specialized culture. The laboratory must be notified if this organism is suspected. NAATs for *C. trachomatis* detect, but do not call out, lymphogranuloma venereum serovars; none are approved by the FDA for genital ulcer sites. *K. granulomatis* does not grow on conventional media. Diagnosis is made by observing the organism in tissue with the Giemsa stain.

Testing for human papillomavirus is beyond the scope of this chapter and is discussed in Chapter 143. The diagnosis of genital warts is normally through visual inspection; biopsy may be performed if there is any question about the diagnosis. High-risk human papillomavirus testing of genital warts is not recommended. Likewise, molluscum contagiosum is typically a clinical diagnosis; biopsy may be performed if there is any question about the diagnosis.

Urethritis and Cervicitis Diagnostics

For laboratory diagnosis of *C. trachomatis* and *N. gonorrhoeae,* NAATs are preferred because of their sensitivity and specificity and ability to be performed on urine. Urine or vaginal specimens (either collected by the provider or self-collected) in women and urine specimens in men are preferred specimen types. Other specimen types include endocervical, urethral, rectal, pharyngeal, and conjunctival swabs. Not all of these are approved by the FDA, so clinicians should check with their laboratories to determine whether these sources will be tested. Antibacterial resistance in *N. gonorrhoeae* is an emerging challenge that may necessitate culture for antimicrobial susceptibility testing. *N. gonorrhoeae* is labile; therefore, if culture is performed, the specimen should be sent promptly to the laboratory in appropriate transport media (not swabs) or plated at the point of care.

Mycoplasma genitalium causes urethritis in men and women and cervicitis and pelvic inflammatory disease in women. Antimicrobial resistance is a challenge. An NAAT is the test of choice for diagnosis of *M. genitalium*. *T. vaginalis* may cause urethritis or cervicitis; diagnostics are discussed in "Vaginitis and Vaginosis Diagnostics."

Helicobacter pylori Diagnostics

Noninvasive tests for *Helicobacter pylori* include stool antigen and urea breath tests.[42] Serology is not recommended. If endoscopy is performed, biopsy can be performed for histopathology or rapid urease testing or both in addition to specialized culture to recover an isolate for antimicrobial susceptibility testing, which is particularly germane to patients who fail to respond to therapy, given *H. pylori* antimicrobial resistance.[43]

Mycobacterium tuberculosis Diagnostics

Latent tuberculosis can be diagnosed using an interferon-γ release assay or a tuberculin skin test (TST). The former is recommended over the latter in individuals who have a history of bacillus Calmette-Guérin vaccination or are unlikely to return to have their TST read. Although both interferon-γ release assay and TST provide evidence for infection with *M. tuberculosis,* they cannot distinguish active from latent tuberculosis.

To diagnose active tuberculosis, AFB smear microscopy and mycobacterial cultures are recommended for patients suspected to have pulmonary tuberculosis. False-positive microscopy results (due to infections with nontuberculous mycobacteria) and false-negative results occur. Testing of three respiratory specimens each of 5 to 10 mL using AFB smear and mycobacterial culture is recommended. Concentrated respiratory specimens and fluorescence microscopy are preferred for AFB smears.

An NAAT is also recommended on the initial respiratory specimen from patients suspected to have pulmonary tuberculosis. In AFB smear–positive patients, a negative NAAT makes tuberculosis disease unlikely. In AFB smear–negative patients with an intermediate to high level of suspicion for disease, a positive NAAT is presumptive evidence of tuberculosis, but a negative result cannot be used to exclude pulmonary tuberculosis. It is recommended that rapid molecular drug susceptibility testing for rifampin with or without isoniazid be performed in individuals with risk factors for resistance. Collection of induced sputum rather than BAL fluid is recommended for adults with suspected pulmonary tuberculosis who are unable to expectorate sputum, with collection of BAL fluid recommended if collection of induced sputum proves unsuccessful. Postbronchoscopy sputum specimens can be collected for AFB smears and mycobacterial cultures. Testing for extrapulmonary disease is well outlined in the American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines on diagnosis of tuberculosis in adults and children.[44]

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Key References

The complete reference list is available online at Expert Consult.

1. Patel R, Karon BS. Advances afoot in microbiology. *J Clin Microbiol*. 2017;55:1984–1988.
2. Morency-Potvin P, Schwartz DN, Weinstein RA. Antimicrobial stewardship: how the microbiology laboratory can right the ship. *Clin Microbiol Rev*. 2017;30:381–407.
3. Simner PJ. Medical parasitology taxonomy update: January 2012 to December 2015. *J Clin Microbiol*. 2017;55:43–47.
4. Forbes BA. Mycobacterial taxonomy. *J Clin Microbiol*. 2017;55:380–383.
7. Barlam TF, Cosgrove SE, Abbo LM, et al. Executive summary: implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clin Infect Dis*. 2016;62:1197–1202.
11. Liesman RM, Pritt BS, Maleszewski JJ, et al. Laboratory diagnosis of infective endocarditis. *J Clin Microbiol*. 2017;55:2599–2608.
12. Miller J, Binnicker M, Campbell S, et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clin Infect Dis*. 2018;67:e1–e94.
19. Ramanan P, Bryson AL, Binnicker MJ, et al. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev*. 2018;31.
21. Banerjee R, Teng CB, Cunningham SA, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. *Clin Infect Dis*. 2015;61:1071–1080.
25. Lipsky BA, Berendt AR, Cornia PB, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis*. 2012;54:e132–e173.
26. Berbari EF, Kanj SS, Kowalski TJ, et al. 2015 Infectious Diseases Society of America (IDSA) clinical practice guidelines for the diagnosis and treatment of native vertebral osteomyelitis in adults. *Clin Infect Dis*. 2015;61:e26–e46.
31. Shulman ST, Bisno AL, Clegg HW, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2012;55:e86–e102.
35. Riddle MS, DuPont HL, Connor BA. ACG Clinical Guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. *Am J Gastroenterol*. 2016;111:602–622.
44. Lewinsohn DM, Leonard MK, LoBue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of tuberculosis in adults and children. *Clin Infect Dis*. 2017;64:111–115.

References

1. Patel R, Karon BS. Advances afoot in microbiology. *J Clin Microbiol*. 2017;55:1984–1988.
2. Morency-Potvin P, Schwartz DN, Weinstein RA. Antimicrobial stewardship: how the microbiology laboratory can right the ship. *Clin Microbiol Rev*. 2017;30:381–407.
3. Simner PJ. Medical parasitology taxonomy update: January 2012 to December 2015. *J Clin Microbiol*. 2017;55:43–47.
4. Forbes BA. Mycobacterial taxonomy. *J Clin Microbiol*. 2017;55:380–383.
5. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. *Clin Chem*. 2015;61:100–111.
6. Clinical and Laboratory Standards Institute. Analysis and presentation of cumulative antimicrobial susceptibility test data; approved guideline. CLSI document M39-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
7. Barlam TF, Cosgrove SE, Abbo LM, et al. Executive summary: implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clin Infect Dis*. 2016;62:1197–1202.
8. Zadroga R, Williams DN, Gottschall R, et al. Comparison of 2 blood culture media shows significant differences in bacterial recovery for patients on antimicrobial therapy. *Clin Infect Dis*. 2013;56:790–797.
9. Yagupsky P. Detection of *Brucellae* in blood cultures. *J Clin Microbiol*. 1999;37:3437–3442.
10. Bouza E, Sousa D, Rodriguez-Creixems M, et al. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? *J Clin Microbiol*. 2007;45:2765–2769.
11. Liesman RM, Pritt BS, Maleszewski JJ, et al. Laboratory diagnosis of infective endocarditis. *J Clin Microbiol*. 2017;55:2599–2608.
12. Miller J, Binnicker M, Campbell S, et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clin Infect Dis*. 2018;67:e1–e94.
13. Baron E, Weinstein M, Dunne W, et al. *Cumitech 1C, Blood Cultures IV*. Washington, DC: ASM Press; 2005.
14. Clinical Laboratory Standards Institute. Principles and procedures for blood cultures; Approved Guideline. CLSI document M47-A. Wayne, PA: Clinical and Laboratory Standards Institute.
15. Rupp ME, Cavalieri RJ, Marolf C, et al. Reduction in blood culture contamination through use of initial specimen diversion device. *Clin Infect Dis*. 2017;65:201–205.
16. McAdam AJ. Reducing contamination of blood cultures: consider costs and clinical benefits. *Clin Infect Dis*. 2017;65:206–207.
17. Leisure MK, Moore DM, Schwartzman JD, et al. Changing the needle when inoculating blood cultures. A no-benefit and high-risk procedure. *JAMA*. 1990;264:2111–2112.
18. Raad I, Hanna HA, Alakech B, et al. Differential time to positivity: a useful method for diagnosing catheter-related bloodstream infections. *Ann Intern Med*. 2004;140:18–25.
19. Ramanan P, Bryson AL, Binnicker MJ, et al. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev*. 2018;31.
20. Pancholi P, Carroll K, Buchan B, et al. Multicenter evaluation of the Accelerate PhenoTest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. *J Clin Microbiol*. 2018;56:e01329–17.
21. Banerjee R, Teng CB, Cunningham SA, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. *Clin Infect Dis*. 2015;61:1071–1080.
22. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med*. 1977;296:1305–1309.
23. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol Assess*. 2007;11:1–196.
24. Liesman R, Strasburg A, Heitman A, et al. Evaluation of a commercial multiplex molecular panel for the diagnosis of infectious meningitis and encephalitis. *J Clin Microbiol*. 2018;56:e01927–17.
25. Lipsky BA, Berendt AR, Cornia PB, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis*. 2012;54:e132–e173.
26. Berbari EF, Kanj SS, Kowalski TJ, et al. 2015 Infectious Diseases Society of America (IDSA) clinical practice guidelines for the diagnosis and treatment of native vertebral osteomyelitis in adults. *Clin Infect Dis*. 2015;61:e26–e46.
27. Peel TN, Spelman T, Dylla BL, et al. Optimal periprosthetic tissue specimen number for diagnosis of prosthetic joint infection. *J Clin Microbiol*. 2017;55:234–243.
28. Peel TN, Dylla BL, Hughes JG, et al. Improved diagnosis of prosthetic joint infection by culturing periprosthetic tissue specimens in blood culture bottles. *MBio*. 2016;7:e01776-15.
29. Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med*. 2007;357:654–663.
30. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute Streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. *Circulation*. 2009;119:1541–1551.
31. Shulman ST, Bisno AL, Clegg HW, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2012;55:e86–e102.
32. Dingle TC, Abbott AN, Fang FC. Reflexive culture in adolescents and adults with group A streptococcal pharyngitis. *Clin Infect Dis*. 2014;59:643–650.
33. Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis*. 2016;63:e61–e111.
34. Schaeffer AJ. Clinical practice. Chronic prostatitis and the chronic pelvic pain syndrome. *N Engl J Med*. 2006;355:1690–1698.
35. Riddle MS, DuPont HL, Connor BA. ACG Clinical Guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. *Am J Gastroenterol*. 2016;111:602–622.
36. Aichinger E, Schleck CD, Harmsen WS, et al. Nonutility of repeat laboratory testing for detection of *Clostridium difficile* by use of PCR or enzyme immunoassay. *J Clin Microbiol*. 2008;46:3795–3797.
37. Khanna S, Pardi DS, Rosenblatt JE, et al. An evaluation of repeat stool testing for *Clostridium difficile* infection by polymerase chain reaction. *J Clin Gastroenterol*. 2012;46:846–849.
38. Park S, Hitchcock MM, Gomez CA, et al. Is follow-up testing with the FilmArray gastrointestinal multiplex PCR panel necessary? *J Clin Microbiol*. 2017;55:1154–1161.
39. Fang FC, Patel R. 2017 Infectious Diseases Society of America Infectious Diarrhea Guidelines: a view from the clinical laboratory. *Clin Infect Dis*. 2017;65:1974–1976.
40. Gebrehiwot S, Rucinski S, Schwab J, et al. *"Reflexive Culture"—a Strategy for Laboratories Adopting Molecular Testing for Enteric Pathogens*. Boston: American Society for Microbiology General Meeting; 2014.
41. Ison CA, Hay PE. Validation of a simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics. *Sex Transm Infect*. 2002;78:413–415.
42. Chey WD, Leontiadis GI, Howden CW, et al. ACG Clinical Guideline: treatment of *Helicobacter pylori* infection. *Am J Gastroenterol*. 2017;112:212–239.
43. Chen D, Cunningham SA, Cole NC, et al. Phenotypic and molecular antimicrobial susceptibility of *Helicobacter pylori*. *Antimicrob Agents Chemother*. 2017;61.
44. Lewinsohn DM, Leonard MK, LoBue PA, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: diagnosis of tuberculosis in adults and children. *Clin Infect Dis*. 2017;64:111–115.

E Antiinfective Therapy

17 Principles of Antiinfective Therapy

Brad Spellberg

SHORT VIEW SUMMARY

History
- Antimicrobial agents revolutionized the practice of medicine, resulting in dramatic reductions in death from a variety of diseases. As a result, death from infections in the United States declined from approximately 280 per 100,000 population in 1936 (presulfa) to approximately 60 per 100,000 population by 1950 (postpenicillin), with virtually no further decline since then.

Societal Trust
- Antibiotic efficacy is lost with each use of the drugs due to antibiotic resistance.
- Antibiotic resistance is transmissible from person to person such that each individual's use of an antibiotic affects the ability of everyone else in society to have the drugs remain effective for their own use. Thus antibiotics are a shared societal trust.
- No one has a right to misuse these drugs because one person's misuse hurts everyone else in society. Infectious diseases practitioners have added responsibility for stewarding these drugs to maintain their effectiveness for society.

Principles of Antimicrobial Use
- Ten fundamental principles underpin the use of antibiotics to optimize cure rates while minimizing unnecessary/inappropriate use. Medicine is complex, and individual clinical scenarios may result in the need to deviate from such principles.
- Principle 1: Proper selection of empirical antibiotics starts with an accurate differential diagnosis that includes likely bacterial infection.
- Principle 2: Even in a patient with an obvious bacterial infection, treat only when therapy will alter the patient's clinical course.
- Principle 3: Empirically target the microbes that cause the diseases identified in the differential diagnosis.
- Principle 4: In critically ill patients there should be a lower threshold for the use of empirical therapy.
- Principle 5: Host factors affect the breadth of empirical therapy by altering the probabilities of the likely agents of infection.
- Principle 6: Pharmacokinetic-pharmacodynamic principles can influence optimal treatment.
- Principle 7: Clinicians should deescalate antibiotic therapy based on microbiology results and clinical and biomarker responses.
- Principle 8: If therapy is not working, consider source control and alternative diagnoses before blaming resistance and broadening therapy.
- Principle 9: Distinguish new infection from the failure of initial therapy.
- Principle 10: The duration of therapy should be evidence based, with a bias toward a short course whenever possible.

Controversial Principles:
- The distinction between bacteriostatic versus bactericidal is an in vitro construct. Clinical trial data do not support an intrinsic clinical efficacy difference between static and cidal antimicrobial agents.
- Combination therapy may be beneficial in specific clinical circumstances. However, its use is not supported by clinical data in many circumstances, and its use may result in increased toxicity, disruption of the host microbiome, and/or selection for antimicrobial resistance.

THE ANTIBIOTIC MIRACLE: WHAT WE ARE FIGHTING FOR

A Brief History of "Antibiotics"

From the earliest history of human civilization, we have sought "magic bullets" to cure diseases.[1] The ancient Greeks used natural substances, including aromatic extracts from woods, such as myrrh, as well as wine, honey, and flakes of copper and aluminum minerals to prevent or treat wound infections.[2] Ancient Chinese and Egyptian physicians used moldy tofu and bread, respectively, to treat skin infections.[3] These ancient treatments likely did have some, albeit limited, clinical benefit because modern science demonstrates that each of these substances possess antimicrobial effects.[1,2]

The crude nature of these ancient therapeutic approaches would not be advanced upon until the 20th century. Dr. Paul Ehrlich won the Nobel Prize in 1908 for his studies of the chemistry of antibody-antigen interactions. He reasoned that because chemistry governed the benefits of immune serum, it should be possible to recapitulate those benefits by creating or discovering small molecules that could similarly chemically interact with microbes.[4] He coined the term *chemotherapy* to describe the use of small molecules to treat diseases and principally infectious diseases.[4]

Ehrlich's efforts led to his discovery of the first antiinfective small molecule, arsphenamine (Salvarsan), a derivative of arsenic, which he found could successfully treat syphilis.[5] The drug became an enormous commercial success, which quickly faded when it was realized that the drug had potentially fatal side effects. Nevertheless, the sophisticated chemical library screen Ehrlich designed and conducted to discover arsphenamine became a blueprint for subsequent drug discovery that ultimately led to identification of many other antibiotics and other drugs as well.

The word *antibiosis*—meaning *against life*—was coined in 1889 by the French scientist Jean Paul Vuillemin.[1,6] He used the term to refer to the process of "one creature destroying the life of another to sustain its own."[6] Half a century later, in 1942, future Nobel laureate Dr. Selman Waksman built on this definition, coining the term *antibiotic* to mean "a chemical substance, of microbial origin, that…[inhibits] the growth or the metabolic activities of bacteria and other microorganisms."[6] Thus the term *antibiotic* technically refers to a natural substance produced by a living organism to inhibit the growth of another. However, in common parlance (and in the remainder of this chapter) the term is often used more liberally to encompass both natural and synthetic antimicrobial substances because at the clinical level the distinction is not important.

Alexander Fleming discovered penicillin-containing mold by-products in 1928. However, penicillin's first clinical use as a purified substance was not until 1940. Thus the first safe and effective, specific antibiotics (inclusive of synthetic drugs) to be clinically deployed were the synthetic sulfa agents discovered by Dr. Gerhard Domagk and his team in 1931.[1,7] Domagk's team developed sulfa drugs by chemical modification of industrial red dyes originally intended for clothing. The first sulfa drug, prontosil rubrum, had miraculous curative effects in mice with lethal bacterial infections.[7] By late 1932 news of the miracle had spread, and local physicians began to treat patients with sulfa drugs before the animal studies were even published.[7]

On May 17, 1933 a colleague of Domagk used a new sulfa drug to cure a 10-month-old boy with what had been considered an inevitably fatal bloodstream infection caused by *Staphylococcus aureus*.[7] This child was the first person in history ever to be cured of a lethal bacterial infection using a safe and effective antibiotic.

As powerful as sulfa drugs were at saving lives, they had limited or no efficacy against many types of infections. Purified penicillin, when it came along, was far more effective. The first patient ever treated with purified penicillin was a British police constable with severe staphylococcal facial cellulitis.[8] He improved dramatically but then died after the small supply of penicillin ran out. On March 14, 1942, Mrs. Ann Sheafe Miller became the first patient in the United States to have her life saved by penicillin.[9] She was dying from bacteremic, postpartum streptococcal sepsis that had failed to respond to sulfa drugs. Her treating physician procured penicillin, which resulted in such a dramatic eradication of the virtually invariably fatal infection that one of Mrs. Miller's consultants was overhead muttering to himself, "black magic!" while reviewing her chart.[9] Indeed, so powerful was penicillin at reducing death from infections that within 3 years of its widespread availability in the United States experts were already speculating on the end of bacterial infections as a significant clinical problem.[10]

The success of sulfa drugs and penicillin at curing disease inspired a generation of scientists, resulting in the discovery of the other major antibiotic classes in the 1940s and 1950s. All told, more than 150 antibiotics of various classes have been discovered and developed since 1935. And all have quickly suffered from the clinical emergence of resistant bacteria, which eroded the miraculous gains. It is the clinical use and protection of these antibacterial agents, as well as antifungal, antiviral, and antiparasitic agents, that concern us for the remainder of this chapter and the succeeding chapters on antiinfective therapy.

The Power of Antibiotics: What We Stand to Lose Due to Resistance

Antibiotics have unquestionably revolutionized the practice of medicine. According to a reliable eyewitness, the most common substance prescribed by physicians in Boston before sulfa drugs became available was bourbon; prescriptions were written in Latin script so it seemed impressive to patients and made them feel reassured.[11] Indeed, before sulfa drugs, placebos were used frequently in medicine because few truly effective therapies were available. The sudden availability of effective antibacterial therapy transformed the practice of medicine from one in which the primary focus was prognostication to one in which cure became expected.[11]

The massive declines in death enabled by antibiotics ran across all types of invasive bacterial infections (Table 17.1). For example, the mortality rate of cellulitis fell from an extraordinary 11% in the preantibiotic era to approximately 0.3% with the availability of penicillin.[12] Thus in the preantibiotic era, the mortality of cellulitis approximated that of myocardial infarction, and the reduction in death resulting from treating cellulitis with antibiotics (>10% absolute reduction in death) is far greater than that resulting from treating myocardial infarction with aspirin or thrombolytics (3% absolute reduction in death).[13]

At a population level the death rate from infectious diseases (ID) in the United States fell from approximately 280 per 100,000 in 1936 (the year before sulfonamides were available in the United States), to approximately 200 per 100,000 in 1945 (the year before penicillin became widely available), to approximately 60 per 100,000 by the early 1950s.[14] Despite the advent of modern intensive care unit (ICU) methodologies, and all other medical technologies over the past half century, there has been virtually no further decline in the death rate from infections in the United States since the penicillin era began,[14] underscoring how powerful the transformation of medical practice was.

Beyond saving the lives of infected patients, the enormous efficacy of antibacterial agents enabled conduct of complicated and deeply invasive surgery; aggressive chemotherapy for treatment of cancer; fundamental elements of critical care, such as central venous catheter placement and mechanical ventilation; care for premature neonates; and solid and liquid organ transplantation. None of these medical advances would be feasible without effective antibacterial agents to deal with the infections that result as a side effect of the advances themselves.

In short, the power of antibacterial therapy resulted in a revolution in the practice of medicine, transforming it from a primarily diagnostic-focused field to a therapeutic, interventional profession. As one eyewitness to the miracle of the emergence of antibiotic therapy wrote, antibiotics "place in the hands of a barefoot, nonliterate villager more real power to affect the outcome of a…critically ill [patient]…than could have been exerted by the most highly trained urban physician [from the preantibiotic era]."[15]

THE SOCIETAL TRUST OF ANTIBIOTICS MUST UNDERPIN PRINCIPLES OF USE

In many parts of the world antibiotics are available over the counter and without a prescription, which contributes to marked overuse and misuse of the drugs by patients who feel ill but do not have bacterial infections. Unfortunately, even in countries where antibiotics are available only by prescription, providers overuse and misuse the drugs for a variety of complex psychosocial reasons.[16,17]

Indeed, antibiotic overprescription is an example of the "tragedy of the commons."[16] The tragedy of the commons was originally described by Garrett Harding in 1968.[18] His example of this complex psychosocial phenomenon was the illegal overgrazing of public, shared lands in feudal Europe ("the commons") by livestock owned by private citizens. The tragedy of the commons results when individuals undertake an action that they perceive to be in their own self-interest but which causes harm to society at large. When such an action is undertaken rarely, the harm to society is not noticeable. When it happens tens of millions of times per year, as with inappropriate antibiotic prescriptions, the collective harm to society is catastrophic.[16]

TABLE 17.1 Antibiotic-Mediated Mortality Reductions for Specific Infections

DISEASE	PREANTIBIOTIC MORTALITY RATE	ANTIBIOTIC MORTALITY RATE	CHANGE IN MORTALITY
Community-acquired pneumonia	~35%	~10%	–25%
Nosocomial pneumonia	~60%	~30%	–30%
Bacterial endocarditis	~100%	~25%	–75%
Gram-negative bacteremia	~70%	~10%	–60%
Bacterial meningitis	>80%	<20%	–60%
Cellulitis	~11%	<0.5%	–10%

[a]By comparison, treatment of myocardial infarction with aspirin or streptokinase reduces death by 3%.
Data from references 12 and 139–153.

Given the power of antibiotics to save lives and the constant erosion of that power through use, one of the most important functions of the ID specialist (whether physician or pharmacist) is to serve as an expert in the use and protection of antimicrobial agents, fighting against the tragedy of the commons. Any licensed provider can prescribe an antibiotic; it takes an expert to know when not to prescribe and when to stop those already prescribed.

Genetic analysis indicates that antibiotic resistance mechanisms were developed by microbes as early as 2 to 2.5 billion years ago.[19] Microbes have thus been waging war among themselves with these weapons for 20 million times longer than humans have known of their existence.[17] We may presume that in 2 billion years of evolutionary warfare, microbes have invented poisons to disrupt every biochemical pathway that can be disrupted and, out of necessity, resistance mechanisms to protect all of those pathways.

The widespread existence in nature of antibiotic resistance mechanisms is underscored by a study in which microbes were cultured from the walls of a cave system that had never been explored by humans before and had been isolated from the surface of the planet for 4 million years.[20] Among the microbes cultured, resistance was encountered to every class of modern antibiotics. Resistance was encountered not just to natural substances (e.g., penicillin) but to synthetic drugs that did not exist on planet Earth until the 1960s–80s (e.g., fluoroquinolones, linezolid, daptomycin).

This remarkable finding underscores a critical—and perhaps poorly understood—point about antibiotic resistance. Resistance does not newly develop when we begin to use antibiotics clinically. Rather, there are already, widespread in nature, resistance mechanisms to drugs we have not yet invented. Our clinical use of antibiotics applies selective pressure that results in the increasing frequency of preexisting antibiotic-resistance mechanisms that were already in nature at low levels before our use of antibiotics.

Antibiotics Are a Societal Trust

As early as 1945 the man who discovered penicillin, Alexander Fleming, began warning the public that doctors and patients were abusing the miracle drug by using it to treat diseases not caused by bacteria and that resistance was being bred out as a result.[21] In his Nobel Prize lecture, he admonished that those who so abused penicillin would be "morally responsible" for the deaths of patients killed by resistant bacteria that could no longer be treated with it. This clarion call for antibiotic stewardship, more than 70 years ago, went unheeded.

As a result, resistance caught up to the antibiotic armamentarium by the late 1950s and early 1960s, sending industry back to their chemical libraries and natural substance screens. A roundtable of leading ID experts concluded as early as 1965 that there was a critical need for new antibiotics to "overcome the problems of resistance, to use against gram-negative bacillary infections, more active and less toxic ones for fungal infections, better ones for mycobacteria."[22] A half century later, after having been bailed out of the resistance problem by a flurry of new compounds discovered from the 1970s to 1980s, we are again having the same conversation with little institutional memory of what preceded us.

What history teaches us, therefore, is that in only 80 years of experience with compounds that microbes have kept successfully active for 2 billion years, we have already on several occasions nearly burned through the precious limited resource that is active antibiotic therapy. We will never win a war against microbes, which outnumber us by a factor of 10^{22} outweigh us by 100 million times, can replicate 500,000 times faster than we can, and have been doing this for 10,000 times longer than our species has existed.[23] Resistance will always be with us. It is the natural result of use of antibiotics. These facts place an extraordinary responsibility upon society, and upon ID experts as stewards in society, to protect antibiotics from abuse and misuse.

Antibiotics are a societal trust, different from all other classes of drugs (and indeed virtually any other technology).[24] Every individual's use of an antibiotic negatively affects the ability of everyone else in society to have the antibiotic available as an effective therapy. Thus the burden is on those trained in our specialty to help teach both our medical colleagues and the lay public how precious these drugs are and how they are to be properly deployed. We are the stewards of their incredible power to save lives and reduce morbidity.

FUNDAMENTAL PRINCIPLES OF ANTIBIOTIC THERAPY

Given the critical importance of antibiotics as a lifesaving medical intervention, fundamental principles should underpin their use. The practice of medicine is complex, and clinical scenarios can of course result in the need to deviate from individual principles in specific circumstances and with specific justification. Nevertheless, incorporation of fundamental principles of appropriate use of antibiotics into practice can help improve proper antibiotic use.

Ten fundamental, sequential principles guiding appropriate antimicrobial use are described here. These principles build on one another, from the initial decision regarding whether antibiotics are needed or not, to selection of which antibiotics to use empirically and then definitively, and ultimately to deciding for how long to prescribe them.

1. Proper Selection of Empirical Antibiotics Starts With an Accurate Differential Diagnosis That Includes Likely Bacterial Infection

Antibiotics are usually started empirically, meaning without knowing precisely which bacteria are infecting a patient, or even if the patient actually has a bacterial infection. Thus the first principle of appropriate use of antibiotics depends on establishing a rational and accurate differential diagnosis for the patient's current illness. Antibiotics should be given only if the differential diagnosis includes likely invasive bacterial infections. Although it may seem obvious that antibiotics should be given only to patients who have bacterial infections, the failure to adhere to this first principle is one of the most common causes of unnecessary antibiotic use.

Many viral infections and noninfectious diseases mimic the signs and symptoms of bacterial infections. Overestimating the probability of bacterial infections, or the harm that may come by not treating them (such as for upper respiratory tract infections), is a potent driver of inaccurate differential diagnoses, leading to inappropriate antibiotic prescriptions. Furthermore, providers frequently underestimate the harm of antimicrobial prescriptions—one in five patients given antibiotic prescriptions are harmed by them due to adverse events or superinfections by resistant pathogens or *Clostridioides difficile* (formerly *Clostridium difficile*).[25] This combination of fear from uncertainty of the diagnosis and lack of appreciation of how dangerous antibiotics can be is a particularly important driver of inappropriate prescriptions for upper respiratory tract illnesses in outpatient settings. Novel psychological approaches to combat the fear driving such prescriptions including audit and feedback and the gentle nudge of public commitment,[16,24,26,27] have been demonstrated in large clinical trials to markedly reduce inappropriate prescriptions in this setting.

Another common driver of inappropriate prescription results from mistaking positive cultures as evidence of infection. The presence of a positive culture in the absence of signs or symptoms of infection should not reflexively trigger antibiotic prescription. In most cases, absent signs or symptoms of infection, positive cultures represent colonization or contamination and should not trigger treatment.

Cultures from nonsterile sites are a particular problem. Surface swabs from skin or wounds, or cultures taken from respiratory (even via bronchoalveolar lavage), urinary, or gastrointestinal tracts all sample the microbiome. Growth of organisms from such cultures does not indicate infection in asymptomatic patients. Even if patients have symptoms, one cannot necessarily discern the microbial etiology of infection by growing organisms from nonsterile sites, which have polymicrobial microbiomes. There often is a tendency to apply therapy for every strain of bacteria grown, even though most of the organisms encountered are typically not pathogenic, and among those with pathogenic potential, it can be impossible to distinguish bystanders from etiologic pathogen(s).

Absent signs or symptoms of clinical disease, generally antibiotics should not be administered irrespective of culture results from skin, wound swabs, respiratory secretions, or urine.

2. Even in a Patient With a Bacterial Infection, Treat Only When Therapy Will Alter the Patient's Clinical Course

The administration of antibiotics should not be a reflexive response to infection, but rather should be incorporated into an overall, rational therapeutic plan for the patient. Patients who lack bacterial infections cannot have their clinical course improved by antibiotics (as discussed in Principle 1). But even patients who do have bacterial infections still may not warrant antibiotic therapy.

One classic example of this scenario is an osteomyelitis in chronically exposed bone. In this case, the driver of antibiotic efficacy for the osteomyelitis will be whether there is a feasible plan for long-term flapping of the wound so that the bone does not remain exposed.[28] It is not rational to attempt to cure the osteomyelitis with antibiotics absent such a plan. A brief course of antibiotics may be warranted if there is a superimposed acute cellulitis in the tissue around the osteomyelitis, but the course should not be prolonged to attempt cure of the underlying osteomyelitis.[28] All that will be achieved by administering prolonged antibiotics to such a patient is selection for antibiotic resistance and exposure to the risk of side effects from the antibiotics, without the possibility of cure of the bone infection. Once the wound is flapped, a curative course can be considered.

Other examples of irrational use of antimicrobial therapy in patients with confirmed infections include the use of antibiotics to treat infection in a terminally ill patient who will die shortly due to their underlying disease irrespective of the antibiotics, or administration of antiretroviral therapy to a patient with acquired immunodeficiency syndrome who is not yet ready to be adherent with the medications. The former scenario raises complex medical ethics questions. Should the wishes of a patient or their family be adhered to regarding administering antibiotics even at the cost of harming other people in society by driving antibiotic resistance? It is important to include consideration of the harm to society caused by the prescription in decision making, as it may outweigh the limited benefit of antimicrobial therapy in such cases. In the latter scenario, administering antiretroviral agents will cause resistance to develop, converting a treatable infection into a nontreatable one. Thus giving antimicrobial agents (inclusive of antivirals) to such patients will not be of benefit and can cause harm. Such use fails the core of the Hippocratic oath: *primum non nocere.*

3. Empirically Target Microbes That Cause the Diseases in the Differential Diagnosis

Once antiinfective therapy is determined to be necessary based on a rational differential diagnosis, and appropriate based on its likely ability to change the course of the patient's outcome, the proper agents must be selected. In general, preferred antimicrobial regimens are those previously established by clinical investigations to be safe and effective for the target disease(s). However, clinicians frequently encounter patients who would not have met enrollment criteria for comparative studies. Furthermore, multiple regimens may have been established to be safe and effective for the target disease(s). In either case the spectrum of activity is an important consideration when selecting which antimicrobial regimen to use.

The spectrum of activity of the empirical antiinfective agents prescribed should be only as broad as necessary to cover the likely microbial flora that cause the diseases in the differential diagnosis.[24] Use of overly broad therapies to treat pathogens unlikely to be encountered is inappropriate and will select for resistance while providing no benefit to the patient.

As a basic principle, nosocomial infections are often caused by more resistant pathogens, and hence require coverage for *Pseudomonas* or other nonfermenting gram-negative bacilli. Conversely, generally speaking community-onset infections are not caused by such bacteria, and empirical antibiotic coverage for community infections should not routinely include such coverage.

The issue of spectrum of activity raises another critical distinction between antibiotics and other drugs. For most drugs the diseases for which they were found to be safe and effective treatments in clinical trials define the relevant treatment population after approval. For antibiotics the issues are more complex.[17,24] Even if a drug with antipseudomonal activity is safe and effective for the disease in question, if the disease is typically caused by streptococci or other less resistant pathogens (e.g., community-onset skin infections or pneumonia), the first-line use of the agent with antipseudomonal coverage is inappropriate despite its safety and efficacy, and irrespective of US Food and Drug Administration indication.[24] Thus safety and efficacy are not sufficient, per se, to define appropriate use of an antibiotic. The spectrum of activity targeted to likely microbial etiology must be considered.

4. A Lower Threshold for Empirical Therapy Should Be Used in Critically Ill Patients

The margin for error is an important consideration in selecting empirical antimicrobial therapy. A patient who is hemodynamically stable and complains only of a fever and malaise may be able to wait without starting any empirical therapy while the workup proceeds. If therapy is to be started in such a patient due to a high pretest probability of bacterial infection, it can be focused on pathogens highly likely to be encountered.

In contrast, a hypotensive patient who is being treated with pressors in the ICU cannot wait. Multiple studies have found that delays of even as short as an hour in initiation of effective antibiotic therapy in such patients markedly increases the risk of progression to septic shock and death.[29–35] Thus the threshold for triggering initiation of empirical therapy should be lower in unstable patients.

The breadth of therapy in critically ill patients can also be wider, but therapy should still focus on the pathogens most likely to be encountered. For example, community-acquired pneumonia, intra-abdominal, skin, and urinary infections typically are not caused by *Pseudomonas* outside very specific circumstances (e.g., patient with cystic fibrosis or with history of bronchiectasis, chronic dialysis patient, or patient with indwelling catheters or recent surgery). Thus even in a critically ill patient with community-onset infections, antipseudomonal therapy generally should not be included outside such circumstances.

Hemodynamically compromised patients should have antibiotics infusing as soon as possible. It is important to emphasize that just ordering the antibiotic is not enough; its infusion should be begun immediately in such patients. Follow-on doses should be given on schedule, as a delay in the second dose has also been associated with higher mortality.[36]

When providers order multiple antibiotics to be administered to a critically ill patient, it may be useful to indicate on order sets or protocols the "anchor" antibiotic that is to be infused first. Delineating the anchor antibiotic streamlines the process so that the pharmacy and nursing unit are able rapidly to deliver the most time-sensitive therapy.

5. Host Factors Affect Breadth of Empirical Therapy by Altering Likely Microbial Causes of Infection

The primary impact that an immunocompromised state should have on selecting antimicrobial therapy is to broaden the differential diagnosis for pathogens likely to be encountered. Unusual bacterial etiologies, such as *Listeria* and *Nocardia*, viral and fungal etiologies, and even parasitic etiologies may need to be considered and therefore treated empirically in a patient who is immunocompromised. Generally speaking, viral and bacterial infections occur acutely in immunocompromised hosts (e.g., after hours to days), and fungal and parasitic infections occur subacutely or chronically (e.g., after weeks to months).

Different forms of immune compromise predispose to different types of pathogens. For example, neutropenic patients are at high risk for acute pyogenic bacterial pathogens and subsequently invasive mycoses. In contrast, patients with defects in T cells are at higher risk for chronic infections caused by atypical pathogens, such as *Pneumocystis* pneumonia, *Cryptococcus* meningitis, tuberculosis (TB), and so forth, as well as pneumococcal pneumonia. Patients with defects in humoral immunity (e.g., from congenital B-cell defects, acquired B-cell defects such as from lupus or treatment with rituximab, antibody-wasting diseases such as nephrotic syndrome) acquire infections caused by encapsulated

bacterial pathogens (e.g., pneumococci, *Neisseria meningitidis*, *Haemophilus influenzae*). A thorough understanding of immune mechanisms and how they defend against specific pathogens is important to enable proper prioritization of microbes likely to be encountered, and hence empirical antimicrobial therapy, in immunocompromised patients.

However, it is not true that immunocompromised patients are intrinsically more likely to be infected by antibiotic-resistant bacterial pathogens. The immune system does not dictate the nature of genetic resistance mechanisms present in bacteria. Rather, environmental and prior antimicrobial exposures do. There is no evidence that immunocompromised patients who acquire their infections in the community, and without having recently been exposed to health care environments (e.g., hemodialysis) or antimicrobial agents, are likely to be infected with more resistant pathogens than immunocompetent patients. Thus the breadth of coverage for highly antibiotic-resistant bacterial pathogens should be dictated by environmental and recent antimicrobial exposure rather than immune status.

For some types of lethal pyogenic infections, blunting the immune system is actually required to enhance efficacy of antimicrobial agents. Morbidity and mortality from many infections are driven as much by the host inflammatory response to the organism as to the bacterial density, and administration of antimicrobial therapy can result in sudden lysis of microbes, releasing highly inflammatory antigens into systemic circulation. The classically described example of this phenomenon is the Jarisch-Herxheimer reaction of syphilis.[37] Similarly, patients with high-grade bacteremia caused by gram-negative bacilli may suddenly decompensate shortly after antimicrobial therapy is begun, and organisms begin to lyse and release endotoxin into the bloodstream.[37] Other proven examples where a sudden lysis of pathogens exacerbates morbidity and mortality of infection, and administration of adjunctive corticosteroids on top of antimicrobial therapy improves morbidity and mortality, are patients with *Pneumocystis* pneumonia, pneumococcal meningitis, or tuberculous meningitis.[38–40]

The differential diagnosis of infection should drive the breadth of antimicrobial coverage. If the differential includes unusual pathogens or nonbacterial pathogens, which is more likely in an immunocompromised patient, then therapy should indeed be broader. However, if the differential diagnosis is clearly focused on acute pyogenic bacterial infections, antibacterial therapy generally does not need to be broader in spectrum in an immunocompromised host than in an immunocompetent host. Highly resistant pathogens generally do not need to be targeted with therapy in immunocompromised hosts who acquire their infections in the community and without other risk factors for developing antibiotic-resistant infections.

6. Pharmacokinetic-Pharmacodynamic Principles Can Influence Optimal Treatment

Having considered clinical trial data supporting specific antimicrobial use, the requisite spectrum of activity based on the differential diagnosis, its likely microbial etiologies, and the patient's clinical stability and risks for antibiotic-resistant pathogens, pharmacologic principles should be considered. Infections in the bloodstream may be treated preferably with antibiotics that achieve higher blood levels. Antibiotics with large volumes of distribution, which penetrate widely into tissues but have lower blood levels, may be less desirable for treatment of bacteremia. Such antibiotics include macrolides, tetracyclines, and rifamycins. Because many antibiotics penetrate into the central nervous system (CNS) poorly, higher than standard doses may be required to optimize achievable drug levels for such infections (e.g., ceftriaxone 2 g intravenous [IV] every 12 hours in lieu of 1 g IV once per day). In contrast, many drugs penetrate well into the kidney and urine. Among those with the highest urinary levels are aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole (TMP-SMX), and β-lactam antibiotics. Fluoroquinolones and TMP-SMX also have excellent prostatic penetration. Hence such antibiotics may be preferred for prostate infections.

For many years medical lore suggested that IV therapy was required to treat chronic bone infections. However, multiple antibiotics achieve levels in bone adequate to treat osteomyelitis when administered orally, including TMP-SMX, clindamycin, linezolid, rifampin, metronidazole, and so forth.[41] Validating the pharmacology data, multiple randomized controlled clinical trials have found that oral therapy with TMP-SMX (especially with rifampin), clindamycin, or fluoroquinolones are as effective as IV therapy for the treatment of osteomyelitis and are associated with fewer severe side effects due to the sparing of prolonged IV catheterization.[41–43]

In critically ill patients oral antimicrobial therapy may be less desirable for two reasons. First, there are concerns about the absorption of drugs from the intestines in critically ill patients, who may be experiencing diminished blood flow to the bowel. Second, all drugs will achieve peak levels faster when administered intravenously, which may be important in a critically ill patient.

Different antibiotics kill via different pharmacodynamic principles.[44–47] Some antibiotics, such as β-lactams, are time-dependent killers, and the time spent above the minimum inhibitory concentration (MIC) is a much better predictor of efficacy than how high the antibiotic concentration is above the MIC. For such drugs it is rational to prolong the infusion, which results in greater time spent above the MIC, while smoothing out the peak and troughs of the drug concentrations.[48–50] Indeed, clinical data validate that prolonged infusions of β-lactams may result in superior clinical outcomes,[51,52] and such strategies are increasingly used clinically. It is important to emphasize, however, that lower peak drug levels will be achieved with prolonged infusion. As such, if the organism being treated has a high MIC, prolonging the infusion may diminish efficacy by precluding achievement of antibiotic concentrations above the MIC. For example, at carbapenem MIC greater than 16 μg/mL, prolonging the infusion of the carbapenem will preclude achieving concentration of the drug above the MIC, which will result in diminished bacterial killing.[49] Hence intermittent dosing to achieve peak levels above the MIC may be preferred for isolates with higher MICs and particularly those in the intermediate-to-resistant range.

Some antibiotics have nonlinear dosing pharmacokinetics, and optimal dosing can result in superior drug levels. For example, increasing the dose of levofloxacin from 500 to 750 mg once per day (a 50% increase) enables a 100% increase in achieved serum concentration.[53] Similarly, because its biliary transport becomes saturated at a dose of approximately 450 mg, dosing rifampin at 600 mg once per day results in far superior 24-hour area-under-the-curve serum concentrations than dosing 300 mg twice per day.[54,55]

Finally, it is important to ensure that the antibiotic will retain antimicrobial activity at the site of infection. Although most antibiotics are active in most tissue environments, there are exceptions. For example, low oxygen tension and low pH environments, such as those found in dense abscesses, can inactivate aminoglycoside efficacy by blocking their uptake.[56] Hence aminoglycosides are not desirable agents to treat abscesses.[57] A unique situation was discovered with daptomycin during its pivotal clinical trial for the treatment of community-acquired pneumonia. Daptomycin was found to be inferior in efficacy to the comparator,[58] and a subsequent study discovered that pulmonary surfactant inactivates daptomycin.[59] Thus daptomycin should not be used to treat alveolar pneumonia. These are specific examples of tissue microenvironments altering antimicrobial effectiveness. In most cases, if adequate drug levels can be achieved at the site of infection, effective antimicrobial action will be achieved.

7. Deescalate Antibiotic Therapy Based on Microbiology Results and Clinical and Biomarker Responses

Having started empirical antimicrobial therapy in the absence of knowledge of an etiologic pathogen, the therapy should then be tailored based on several factors. The first is subsequent microbial identification or not. The Gram stain result or identification of the pathogen's species is often sufficient to enable "deescalation" of antibiotics, even without the results of susceptibility testing. For example, for patients growing gram-negative bacilli in blood culture bottles, empirical vancomycin can be stopped. Conversely, for a patient growing gram-positive cocci in the blood, empirical antibiotics targeting gram-negative bacilli can be stopped. Similarly, for patients with community-acquired pneumonia who grow *Streptococcus pneumoniae*, the atypical coverage can be stopped