Chapter 6: Laboratory Best Practices

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Preface

The laboratory has a unique role in healthcare outbreak response as a source of key information to help initiate and guide investigations. While previous chapters have introduced and developed some basic concepts regarding the role of laboratory partners, here we present more detailed explanations, examples, and considerations, with an emphasis on best practices.

6.0 Introduction

The role of the laboratory in healthcare-associated infection (HAI) outbreak response is critical, beginning with organism identification and routine antimicrobial susceptibilities. Moreover, with advanced technologies, communications, and networks, a laboratory may be able to provide information regarding novel resistance patterns and mechanisms, identify clusters of related illness, and generate data to be used by public health and healthcare partners to detect and respond to outbreaks.

Public health laboratories (PHLs) are required to notify public health authorities upon the identification of reportable diseases. PHLs are also well positioned for early recognition of sentinel cases (involving unusual pathogens or resistance patterns) or clusters. Additionally, they are encouraged to promptly alert epidemiology partners when in receipt of a request from a healthcare facility or provider to perform typing of multiple isolates for an apparent cluster or outbreak.

Beyond outbreak detection, many aspects of outbreak response benefit from active collaboration and coordination between the PHL and other public health and healthcare partners. Examples include clarifying requirements and streamlining procedures for reporting of potential outbreaks and the retention/submission of specimens and/or isolates by commercial, private, and academic laboratories, as well as in-state and out-of-state (incorporating these into guidance or administrative codes). PHLs also may serve a key function in support of outbreak response activities by developing and maintaining inventory of the specialized testing and characterization services available in-house or in other laboratories to and providing guidance to partners regarding how to access these services.

This chapter begins with an overview of laboratory types and their roles and a description of laboratory functions that support outbreak response, the importance of reliable and clearly communicated data will be discussed. For laboratory data to be meaningful and useful, it must be accurate, timely, of high quality, and presented in a clear and concise manner. Specific to laboratorians, safety practices when working with antimicrobial resistant (AR) pathogens and the validation of AR and HAI test methods will also be addressed.

6.1 Types of Laboratories and Roles

6.1.1 Public Health Laboratories

Each state in the US has at least one state public health laboratory, and often other governmental laboratories in large cities or counties, that, as part of their mission, are dedicated to promoting and protecting the health of citizens. There is diversity in discipline and range of capability in the types of laboratory services offered at each facility. As the national public health laboratory, the CDC offers a wide scope of testing, guidance, research and development services.

In 2016, the Centers for Disease Control and Prevention (CDC) established the Antimicrobial Resistance Laboratory Network (AR Lab Network) which serves to detect and characterize AR pathogens and communicate findings and resources to prevent infection. The seven AR Lab Network Regional Laboratories offer access to a wide variety of specialized testing including colonization testing, identification of resistance mechanisms, specialized susceptibility testing using reference methods and next generation sequencing (NGS). Some of these testing services may also be available at state or local public health laboratories, reference laboratories, or large clinical laboratories, but the regional laboratories assure a centralized mechanism to access this testing for all facilities.

The national, non-profit professional organization dedicated to strengthening these laboratory systems is the Association of Public Health Laboratories (APHL). As a representative of national state and local governmental health laboratories, APHL is positioned to capitalize on the available diversity in PHLs, foster communication, provide expert-derived guidance, and work with federal agencies to develop and execute national health initiatives, such as those related to HAIs and AR. Related toolkits, guidance documents, training opportunities, and various other resources are available at www.aphl.org.

6.1.2 Clinical Laboratories

Clinical laboratories, often based in hospitals, provide a wide range of laboratory procedures which aid clinicians in their diagnosis, treatment, and management of patients. Commercial laboratories, some of them quite large and national in scope, provide similar functions. Clinical laboratories serve an integral role in the detection and characterization of a wide array of HAIs and AR pathogens. However, more complex analyses of pathogens such as *Mycobacterium tuberculosis* complex (MTBC) and *Candida* spp. may require transfer to a commercial or reference laboratory or state PHL. Antimicrobial susceptibility testing services in clinical laboratories may include growth and molecular-based analyses of some of the more common Gram-positive and Gram-negative bacteria. Clinical laboratories should be knowledgeable of and consider applicable surveillance and reportable disease regulations or guidance when deciding to proceed with AR testing.

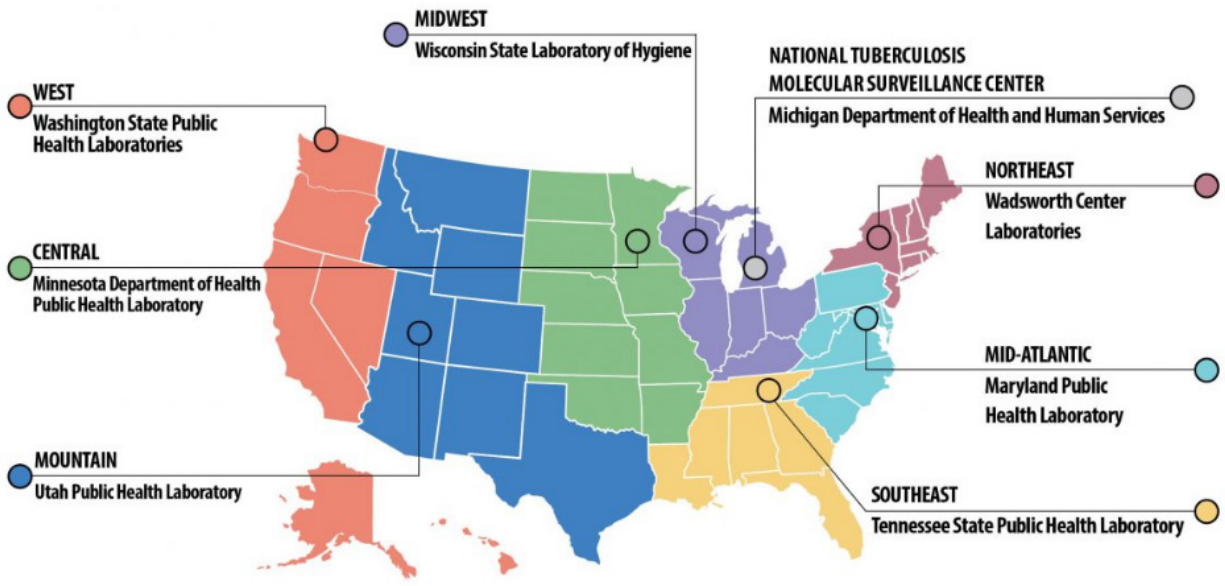
6.1.3 Reference Laboratories

Reference laboratories may also offer extensive and specialized testing to support surveillance activities. These facilities may be independent laboratories or associated with public health agencies or educational or research institutions. The same considerations described in the previous section regarding jurisdictional reporting requirements apply.

In addition to its national reference laboratory function, CDC established and supports the AR Lab Network, as first described in section 6.1.1, greatly expanding public health capacity to detect and respond to AR cases and outbreaks. The Network consists of laboratories in 50 states, four cities, and Puerto Rico, including seven regional laboratories and the National Tuberculosis Molecular Surveillance Center (Figure 1). The Network allows the public health community to rapidly detect emerging AR threats in healthcare, food, and the community; rapidly respond at a state and local level to contain any transmission; and increase understanding of emerging AR threats.[1]

The AR Lab Network assists each local jurisdiction with AR surveillance, but the Network as a whole functions as a surveillance entity with the capacity to provide information on national trends and detect outbreaks. The Network's regional laboratories provide additional testing when state/local laboratories do not have the capability or capacity; at the time of this writing this includes advanced testing for *Acinetobacter*, *Aspergillus fumigatus*, *Candida auris*, CRE, colistin resistance among extended-spectrum beta-lactamase (ESBL)-producing organisms, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, and *Streptococcus pneumoniae*. Regional laboratories which detect organisms and mechanisms of resistance of public health significance routinely alert public health partners to trigger investigations and other actions to prevent transmission.

Figure 1. Antimicrobial Resistance Laboratory Network Map of Regional Laboratories



6.2 Laboratory Functions in Support of Healthcare Outbreak Response

6.2.1 Surveillance

Surveillance involves collecting and analyzing health-related data to evaluate the quality of healthcare provided, identifying opportunities for improvement, and monitoring progress following intervention. Laboratories are an integral component to the surveillance process as they generate, analyze, and submit data to surveillance programs and may be the first to identify an unusual occurrence or frequency in results. In these capacities, laboratories serve as the first level of action in the surveillance process, and therefore, should be cognizant of how, when and to whom data can be shared to be most impactful.

Hospitals and clinical laboratories monitor and report certain drug-resistant organisms and HAIs to meet a variety of different regulatory requirements. The Centers for Medicare and Medicaid Services mandates the reporting of certain HAIs through the National Healthcare Safety Network (NHSN).[2] States and counties may require that hospitals report certain pathogens, diagnoses, and/or multidrug-resistant organisms. In addition, CDC provides guidance for the initial response to a novel or targeted MDRO or resistance mechanism. Such a response may involve a combination of prospective and retrospective laboratory surveillance, depending on the resistance pattern of interest. More information on surveillance, including reportable and notifiable diseases, is provided in Chapter 2.

6.2.2 HAI and AR Detection and Confirmation

As described in Chapter 5, Section 5.1.2, Verify the Diagnosis, early detection of the causative agent is critical to appropriate treatment and the prevention of additional cases. Numerous assays are available to the laboratory to support the identification and confirmation of HAI and AR cases and subsequently assist with the diagnostic aspects of these case definitions where needed. Tests that use physical characteristics of a microorganism are called phenotypic or growth-based (e.g., culture), whereas tests that use genetic properties are called genotypic or molecular-based (e.g., PCR or sequencing). When applying these tests to the detection and confirmation of new and emerging AR, each test type has advantages and limitations (Table 6.1).

6.2.2.1 Phenotypic Testing

The emergence of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has greatly improved the ability of laboratories to identify organisms rapidly and efficiently to the species level. This has contributed to the reporting of less familiar nomenclature such as unique species previously characterized as part of a group of organisms or a complex (e.g., identification of *Enterobacter asburiae* which would have been reported as *Enterobacter cloacae* complex using traditional biochemical tests). Similarly, the enhanced characterization of bacterial and fungal species through molecular techniques such as DNA sequencing has prompted reclassification or renaming of some species. The laboratory can be helpful in assisting the infection prevention (IP) team and epidemiologists in navigating these changes, particularly when including former microbial names in case-findings (e.g., the 2017 reclassification of *Enterobacter aerogenes* to *Klebsiella aerogenes*).[3] For laboratories in which organism identification is achieved via more traditional methods such as biochemical tests (e.g., API20E) or older automated instruments with less up to date software, it is important to remember there may be discrepancies when the organism identification is confirmed using newer technologies or more up to date software.

Phenotypic antimicrobial susceptibility testing (AST) describes conventional methods that establish resistance or susceptibility by measuring growth (or lack thereof) of an organism in the presence of a drug. Phenotypic testing methods require pure culture of growth of that organism, and for the organism to be identified to interpret the results. There are several manual and automated tests available. Among them are disk diffusion, agar dilution, broth microdilution, broth dilution, and gradient strip diffusion. The Kirby-Bauer disk diffusion test results in a zone of inhibition around a disk containing antibiotics of a known concentration. The size of the zone correlates to susceptibility or resistance of that organism to that drug. The zone size is inversely proportional to the Minimum Inhibitory Concentration (MIC), but zone size alone is meaningless and should not be reported to clinical providers.[4] The MIC is the minimum concentration of antibiotic necessary to inhibit growth. It can be determined by broth microdilution and Etest. It can also be referred to as the Minimum Bacterostatic Concentration as growth is inhibited but the organism is not killed. In contrast to the MIC, the Minimum Bactericidal Concentration (MBC) is the minimum concentration necessary to kill the organism. Both MIC and zone sizes can be interpreted to resistant, susceptible, or susceptible dose-dependent results based on defined breakpoints in the Clinical and Laboratory Standards Institute (CLSI) M100.

Regardless of the test used, laboratories should use the current interpretive breakpoints published by organizations developing standards, such as CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST). These sources will have the most up-to-date recommendations for breakpoints and detection strategies. Often, Food and Drug Administration (FDA) cleared products may not reflect current breakpoints, therefore, validation studies may be necessary. Validation studies are also warranted when using laboratory-developed tests (LDTs) or other methods that have not been approved by the FDA, such as those labeled for research use only (RUO) which are not intended for use in patient diagnostics. Laboratories should be aware of the new College of American Pathologists (CAP) requirement[5] that all breakpoints are to be identified and recorded, and any that were updated prior to 2021 must be current by January 1, 2024. APHL and the AR Laboratory working group have developed a toolkit to assist laboratories in this transition.

6.2.2.2 Genotypic Testing

Molecular methods may be used to predict antibiotic resistance in vivo through the detection of specific genetic targets or mutations. Identification of a gene target or mutation may be useful in predicting antibiotic resistance in vivo. The primary benefit to molecular ASTs is direct testing on clinical or environmental specimens without the need for culture. When this is the case, genotypic ASTs are more rapid than phenotypic methods. However, these systems lack the ability to distinguish between viable and non-viable organisms and genetic indicators of resistance do not always confer resistance phenotypically.

The intended use of the assay, whether for screening or identification, must be considered as this will dictate how results are reported and data are interpreted. Screening tests typically exhibit high sensitivity and low to moderate specificity since they are designed to quickly assess a specimen for the presence or absence of the target. Such tests allow for a presumptive result and should be reflexed to culture to isolate and identify the organism. Alternatively, identification tests usually possess characteristics of high sensitivity and specificity, and therefore are more accurate. Depending on the assay, additional testing may be necessary before reporting a confirmed result. Discerning a presumptive from a confirmatory result is critical when reporting data to epidemiologists and other partners. However, in many cases preventive action will still be taken to reduce the risk of transmission based on a presumptive or preliminary result. Confirmed and final results should be reported as soon as they are available.

6.2.2.3 Next Generation Sequencing

Over the past two years, advancements in next generation sequencing (NGS) technology allow the use of NGS not only for identification purposes but also for the detection of drug-resistant markers. NGS can play an important role in outbreak investigations in healthcare-associated infections including those involving multi-drug resistant organisms.

Currently, this technology may be cost-prohibitive due to the high upfront cost of equipment, and the need for highly specialized bioinformaticians. However, it is expected that in the near future, equipment will be affordable, and trained personnel will be more widely available.

NGS is relevant and useful to Antimicrobial Resistance Surveillance in two distinct ways. The first is in detection of novel resistance genes that may not be detected in current molecular (PCR) assays. This is illustrated in a recent example of a patient with a *Pseudomonas aeruginosa* infection. The organism was non-susceptible to most antibiotics tested, was positive for carbapenemase by the modified carbapenemase inactivation method (mCIM) but was negative for all the PCR targets for which it was tested. Next generation sequencing analysis detected the bla_{sim-1} gene, which is the first time this target was detected in the US.[6]

The second use for WGS among antimicrobial resistance surveillance is to determine the relatedness between strains. This is particularly relevant to assess transmission within or between facilities. Strains that are highly related to one another are more likely to share a common source.

Table 6.1 Phenotypic and Genotypic Tests

Test Type	Method, output	Examples	Advantage	Limitation
Phenotypic	Zone of inhibition, millimeters	Kirby Bauer Disk Diffusion	• Simple to perform • Applicable to several antibiotics • Applicable for diverse organisms (e.g., *Haemophilus influenzae*, *H. parainfluenzae*, *Neisseria gonorrhoeae*, *N. meningitidis*)[7] • Standardized method • 16 to 24 hours for result • Cost-effective • Results correlate to known resistance/ susceptibility based on defined breakpoints for known resistance	• Detection is limited to the growth rate of the organism • Requires a pure culture of actively growing organism • Visual/manual data interpretation requires expertise and competency • Unable to detect novel resistance mechanisms
			• Simple to perform • Applicable to several antibiotics	• Detection is limited to the growth rate of the organism

Phenotypic	MIC, reported concentration, ug/mL	Automated: Vitek, Microscan, Sensititre, Phoenix Manual: Etest or MTS strips	• Applicable for diverse organisms (e.g., *Haemophilus influenzae*, *H. parainfluenzae*, *Neisseria gonorrhoeae*, *N. meningitidis*)[7] • Standardized method • 16 to 24 hours for results • Cost-effective	• Requires a pure culture of actively growing organism • Visual/manual data interpretation requires expertise and competency • Unable to detect novel resistance mechanisms • High volume of reagents • Requires multiple dilutions • Expertise
Genotypic	PCR	Lab-developed tests, CDC developed tests, Commercial Platforms: Streck, Cepheid	• Inability to distinguish viable and non-viable organism • Gene target associated with resistance must be known • Presence of target does not always confer phenotypic resistance • High technical skill required • High instrument and consumable cost • Increased potential for cross-contamination • Can be culture-independent • Rapid • Sensitive • Specific • Detection of multiple targets simultaneously • High throughput	• Presence of resistance genes or mechanisms does not always confer phenotypic resistance • If performed without prior culture, no isolate for further investigation • Requires specialized sequencing techniques and bioinformatic processing • Expensive • Inability to distinguish viable and non-viable organism • Gene target associated with resistance must be known • Presence of target does not always confer phenotypic resistance
Genotypic	Sequencing	Targeted Sequencing, Next Generation Sequencing,	• Novel resistance mechanisms can be detected	• Detection is limited to processing time of sequencing and analysis which can be time-consuming

		Long Read, Short Read	• Identify genetic relatedness among isolates • Single nucleotide polymorphism (SNP)/mutations that confer new resistance or altered resistance patterns may be detected	• Inability to distinguish viable and non-viable organism • Presence of target does not always confer phenotypic resistance

6.2.2.4 Terminology

Laboratories should remain current with accepted definitions for various multi-drug resistant organisms (MDRO) either resistant to a primary drug or to one or more drugs from different drug classes. Some practical MDROs described by CDC are listed in Table 6.2.[8]

Table 6.2 Common MDROs

Organism	Drug Resistance
Staphylococcus aureus	Methicillin-resistant *S. aureus* (MRSA) Vancomycin-intermediate *S. aureus* (VISA) Vancomycin-resistant *S. aureus* (VRSA)
Enterococci	Vancomycin-resistant Enterococci (VRE)
E. coli *Klebsiella*	Extended spectrum cephalosporin-resistant
Proteus mirabilis	Extended spectrum cephalosporin-resistant *ampC* phenotype
Enterobacterales	Carbapenem-resistant Enterobacterales (CRE)
Pseudomonas aeruginosa	Carbapenem-resistant *P. aeruginosa* (CRPA)
Acinetobacter	Carbapenem-resistant *Acinetobacter* (CRAB)

No single list of MDROs is comprehensive, but standard terminology applies[9]:

- Susceptible (S) – growth inhibited by drug treatment
- Intermediate (I) – growth inhibited with higher than S drug dose
- Resistant (R) – growth is not inhibited by treatment with at least 1 drug
- multi-drug resistant (MDR) – acquired resistance; "not susceptible" to at least 1 drug in ≥ 3 drug classes
- extensively-drug resistant (XDR) – acquired resistance; "not susceptible" to almost all classes but is sensitive to at least one drug class
- pan-drug resistant (PDR) – acquired resistance to all drugs available

6.2.2.5 Saving Specimens and Isolates

During an outbreak investigation, all relevant organism isolates should be retained by either the clinical lab, public health lab, or the reference laboratory to ensure availability for strain typing. In the event culture-independent diagnostic tests (CIDTs) are used and an isolate is unavailable, laboratories should send CIDT-positive samples to the PHL within 24 hours of the positive result. Clinical laboratories should coordinate with PHL staff prior to shipping. For circumstances outside of outbreak management, laboratories should work with the IP team to develop a routine laboratory policy for saving isolates. The policy should define which isolates are retained and for how long. The policy should also address the retention of original specimens, their derivatives and any specimens with uncommon results.[10] This is valuable to the laboratory so that specimens can be retained for repeat or additional testing when needed, further investigation for public health purposes, quality control purposes and new test validation. Extended storage (for up to 10 years) is ideal for specimens and isolates exhibiting unusual, emerging, and novel resistance mechanisms. An inventory system of retained specimens and isolates should be in place for the biosafety and biosecurity of the laboratory. The laboratory must consider the needs of the patient, the storage capacity for the laboratory and future test development.

6.2.2.6 Characterization Testing

Considerations and best practices for establishing a case definition and managing case findings are discussed in Chapter 5, Section 5.1.6. This section provides information regarding laboratory testing that may be used to support an outbreak investigation through characterization and relatedness testing. Outbreak response may require laboratory support beyond the workflows associated with typical clinical specimens. Each clinical laboratory needs to be able to rapidly identify AR pathogens for subsequent referral to a PHL or reference laboratory for full characterization. Timely communication and collaboration between laboratories are critical. Outbreak investigation and response may include surveillance activities such as point prevalence surveys and admission screening which can require substantial laboratory resources. It can involve processing a large number of samples using methods not routinely performed in that laboratory. It may require environmental testing and healthcare personnel testing if personnel or an environmental reservoir is potentially implicated in the outbreak during the investigation. During an investigation it may be appropriate to perform molecular analyses such as PCR

and NGS in order to identify mechanisms of resistance and determine genetic relatedness between clinical isolates and/or environmental sources.

If it is determined through NGS that two or more organisms are genetically related, it is likely they have a common source. This could be evidence of patient-to-patient transmission or a common reservoir of infection. Species identification and susceptibility results may provide evidence for (or against) an epidemiological link. However, because many organisms have predictable resistance patterns, susceptibility patterns are not discriminatory enough, and additional tests are required. Thus, genotypic or DNA-based typing methods have replaced phenotypic typing methods, which discriminate poorly among isolates. Given the dramatic reduction in cost and time needed to sequence a bacterial or viral genome, NGS has now become the gold standard for molecular typing of healthcare-associated pathogens and has largely replaced older genotypic methods such as pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). If the laboratory cannot perform strain typing when it has been deemed necessary, isolates can be sent to the PHL for testing.

6.2.3 Reporting to Epidemiology and Other Partners

Detection of clusters and possible outbreaks can originate from a variety of sources as described in Chapter 4. As epidemiology staff gather information, they rely on laboratory results to provide meaningful details relevant to a possible event. Thus, the laboratory plays a key role in outbreak detection through the generation of testing results and compilation into reports. Laboratory testing should be performed accurately and in a timely manner, with reports available upon completion. Laboratory results are crucial in identifying true cases associated with an event. Data must be reported in a clear and concise manner so that it may be evaluated without interpretation biases, as is possible when technical details are provided without proper context or guidance.

Reports such as antibiograms, which include antimicrobial resistance surveillance data for a defined population, may be shared with epidemiologists, infection control practitioners, clinicians, and other stakeholders. Within a facility, antibiograms may be developed for specific area such as an intensive care unit or infectious disease unit. Clinical laboratories should provide periodic summaries of selected microbiology results, such as antibiograms specifically for HAI pathogens or trends in selected AR pathogen incidence over time. Hospital laboratory personnel should call the IP program personnel directly to report some results to ensure that timely control measures are implemented (e.g., transmission-based isolation precautions, prophylaxis of contacts, etc.). The list of results that require such urgent test reporting may vary based upon federal, state, or local regulations in addition to any required or requested by the facility, but examples of organisms requiring immediate notification include:

- *Neisseria meningitidis* from a sterile site
- *Legionella*
- *Mycobacterium tuberculosis* (or positive AFB-smears from respiratory samples)
- Potential agents of bioterrorism* (e.g., *Bacillus anthracis*, *Yersinia pestis*)

    - *If a potential agent of bioterrorism cannot be ruled out in the laboratory, it is important to reduce access to the primary specimen or cultured isolate, and contact the state or local PHL immediately
- AR pathogens (e.g., carbapenem-resistant *Enterobacterales*, vancomycin-resistant *Staphylococcus aureus*, *Candida auris*)

Epidemiologists and infection preventionists might be able to use these reports to support an investigation regarding the source and spread of disease within a facility. They might also collaborate with other partners to support the development of guidelines to prevent future outbreaks and reduce the incidence of antimicrobial resistance. It is important to establish and maintain good working relationships with partners in epidemiology and HAI Programs, hospital infectious diseases and infection control departments, and microbiology laboratory directors. One way to do that is to establish a committee that meets 2-3 times a year. More information regarding communication among partners can be found in Chapter 5, Section 5.1.3.3, Communication Among Partners.

Additionally, the reporting procedures in place must allow for timely transmission of laboratory results to infection prevention personnel and relevant state and local reporting systems. Because different facilities often use highly variable methods for storing and tracking data, it is essential to allow for reliable data exchange so that relevant information is not lost during transmission. It is also beneficial to allow various options for reporting to be available. These options can include secure transmission via legacy systems such as fax and telephone as well as electronic submission such as secure email and electronic laboratory reporting (ELR).

In addition to the above reporting, hospital laboratory staff should meet regularly with IP personnel to ensure that communication channels are direct and effective, and to discuss areas of mutual concern, such as the status of all ongoing cluster or outbreak investigations. Together they can also determine whether supplementary testing such as organism typing, or environmental cultures will be necessary. It may prove beneficial to bring in state and local public health partners as well.

Ensuring that the above reporting mechanisms are in place may be challenging if a hospital has outsourced laboratory services (e.g., to a commercial laboratory or to a central laboratory within a large healthcare system) but remains necessary to provide optimal HAI and AR outbreak detection and response.

6.2.4 Detection of HAI Outbreaks by the Laboratory

Chapter 4 established that detection of an HAI outbreak can occur at any level, but next we will explore how the laboratory can support its detection. Essentially, laboratories provide support through characterization testing that may be used to guide response and monitor developments. The use of PHLs and the AR Lab Network regional laboratories can provide the necessary structured framework for improved communication, coordination, and tracking during an HAI outbreak.

Characterization of isolates may be performed to assist with identifying the source of an outbreak and to link clinical cases and/or environmental sources, but the resulting data from such analyses may be

complex and require interpretation. Next generation sequencing is commonly used to investigate isolates at the genetic level and yields large amounts of data that need subsequent analyses with sophisticated software programs. Multiple sequences can then be further examined to determine genetic relatedness which is depicted using a phylogenetic tree. When data from multiple patients or sources are compiled and reported in such a manner, a description should be included to clearly indicate which are and are not likely to be genetically related. These data, along with other epidemiological findings, may be used to define the scope of the outbreak, source attribution, risk factors, or to otherwise link cases based on common features. For this to be successful, communication among partners in a timely manner is essential.

HAI outbreaks are defined by an increase in the number of infections among patients or staff above the expected number of cases, and this can be determined through ongoing surveillance. Pathogen-specific surveillance can be used to monitor select pathogens through reporting by healthcare providers and laboratorians and should consider inclusion of information on patient exposure, risk, and underlying conditions. The full spectrum of specific pathogens under surveillance may be determined by infection prevention and control units within healthcare settings and may also be reportable beyond the facility and may include submission of a specimen from the laboratory serving the healthcare facility to the local or state public health laboratory. Further, notification to the CDC is required for nationally notifiable pathogens or for select reporting programs, and specimen submission to the CDC or AR Lab Network regional laboratory may be necessary as a requirement or if additional testing is requested. As cases are identified and reported, a response could occur at multiple levels, beginning first with the IP at the healthcare facility and next with public health epidemiologists working closest with the reporting laboratory. A first response effort would include collection of additional follow-up data to help identify how acquisition or transmission occurred. These data can be used to link cases based on relevant findings. Specific metadata for each isolate are invaluable for epidemiological study and could include basic specimen details (e.g., collection date, source, submitting facility, test results), patient information (e.g., age, gender, residence), and significant risk factors (e.g., co-morbidities, recent travel, unique exposures or behaviors).

Concurrent review of microbiology data remains the most common HAI and AR case-finding method used by hospital IP programs, and requires prompt, accurate, and reliable reporting of positive laboratory test results. This communication may occur in a number of ways, but most hospital IP programs now have electronic surveillance systems that interface directly with the laboratory information system (LIS) or electronic medical record (EMR). Such electronic surveillance systems allow IP teams to configure alerts and efficiently monitor test results in real time.

Detection may also occur at the local or state health department through regular systematic review of routine surveillance data, review of patient reports, or review of reports from alert healthcare personnel. When an outbreak is identified at the public health level, an outbreak number is often assigned which allows communications, laboratory findings, and reports to be connected. With adequate staff and expertise, the local health department would initiate and coordinate the responsibilities of the investigation to determine who will lead the response and what is needed from the participating laboratories. If local public health capabilities are insufficient, then state health

department would lead the response. Details such as laboratory testing methods and the facility at which testing will be performed, timeframe, and resources are agreed upon in order to effectively manage the investigation. Due to logistical challenges with analyzing large datasets, having electronic accessioning systems in place would allow seamless linkage of isolate test results to epidemiology data, while allowing for additional laboratory or epidemiology data to be added.

Whether an increase in cases is detected by healthcare personnel or the laboratory, public health officials and infection preventionists should be contacted to coordinate specimen submission and initiate the formal chain of reporting. Public health officials should also collaborate with healthcare personnel to assist the facility with coordination of effective control measures as well as additional specimen collection if further testing or confirmation is needed. If the laboratory providing testing is located offsite from the healthcare facility, then enhanced coordination may be needed with the facility as well as the health department due to greater logistical challenges associated with specimen collection, transport, testing, and data transmission between different systems.

To ensure swift detection of outbreaks, effective communication of test results between the laboratory and the infection prevention program is key. In particular, electronic systems that communicate laboratory results to the IP team in real time may help to identify outbreaks as they are happening. It is important to note, however, that concerns about a cluster or an outbreak are sometimes first raised by an astute laboratory technologist, nurse, or another member of the healthcare team. Outbreak detection should therefore be a multi-disciplinary effort that encourages all personnel to report concerning nosocomial infections to the IP program.

6.2.5 Environmental Testing

Environmental testing is an attractive addition to outbreak investigations because it can test hypotheses about transmission, identify pathogen reservoir(s) and later evaluate the efficacy of interventions. However, environmental testing is generally not encouraged except in circumstances where an environmental source has been implicated or the literature supports environmental testing and should be pursued only after consulting with an epidemiologist experienced in outbreak investigations. Many clinical microbiology laboratories do not have expertise in testing environmental samples, and most do not validate their existing tests for use on non-human specimens. When there is limited capacity in the laboratory to perform such testing, specimens should be referred to laboratories that specialize in environmental microbiology or to the jurisdictional public health laboratory. Some of these public health laboratories may have environmental, food safety or water quality testing laboratories that have methods, equipment and personnel that enhance the environmental testing capacity of their HAI or AR laboratories.

Diverse environmental samples may be analyzed to support outbreak investigations and may include swabs of inanimate objects in the outbreak setting (i.e., hospital furniture, water fixtures and equipment), water and cooling systems. Air samples may be of interest during invasive fungal infections. Outbreak responders may want to consult with laboratorians regarding the ecology of the target organism to help develop epidemiologic hypotheses and guide sample collection. Additionally, identifying the laboratory's capacity to not only sample, but also process the sampling devices is crucial

to developing an environmental sampling strategy. The selection of collection devices for environmental sampling depends on many factors, such as the target fomites' and the sampling devices' size, porosity, hydrophobicity and ease of downstream processing. Swabs come in a variety of materials such as foam, cotton and rayon and are ideal for sampling small surfaces and crevices. For larger surfaces, using paddle, sponge and wipe devices increases the likelihood of recovering microbes. Pre-moistening the selected sampling device with a sterile buffer that also neutralizes any residual disinfectants will also improve the chances of recovering the outbreak organism (Table 6.3). It is ideal for environmental samples to be transported refrigerated and processed within 24 hours of collection. Establishing and maintaining chain of custody (COC) is especially important for outbreak investigations that may implicate medical products or devices.

Table 6.3 Tips for Collecting Environmental Samples[11]

Sample Type	Sampling device or mechanism
Small surface	• Use pre-moistened swab
Large surface	• Use pre-moistened paddle, sponge or wipe
Bulk water and ice	• Collect one liter
Drinking water	• Collect one liter • Add sodium thiosulfate to neutralize disinfectants
Fluid from medical device line	• Run device pumps prior to collection to suspend non-motile organisms
Medical device	• Consult with biomedical engineer for best collection strategy that does not adulterate the device • Neutralize cleansers and disinfectants that may be present

6.2.6 Healthcare Worker Testing

Healthcare workers (HCWs) are also occasionally screened during outbreaks, particularly those involving methicillin-resistant *Staphylococcus aureus* or *Streptococcus pyogenes*. For these two organisms, screening methods are well-established, but for many others (e.g., multiple-drug resistant Gram negative organisms), such methods are still being developed and will continue to evolve as more complex resistance phenotypes emerge. Results may be difficult to interpret as recovery of the outbreak from screening cultures obtained from the HCW does not establish the direction of transmission or definitively implicate a healthcare worker as the source of the outbreak. Also, culturing HCWs is a fraught procedure and may be perceived as hostile if mandated. HCW testing may fall under human

subjects testing which requires institutional review board (IRB) approval and has potential legal ramifications. HCWs should therefore be screened only after consulting with an epidemiologist experienced in outbreak investigation, ideally in groups with similar roles to focus interventions on practices as opposed to individuals. Additionally, healthcare providers should be engaged and consulted as appropriate when addressing health concerns or treatment needs of an individual healthcare worker that is being tested.

6.3 Safety, Quality Control, and Validation

Quality testing in a safe environment is a primary goal in any laboratory, but the processing of AR, novel, and emerging pathogens contribute complexities that can increase turnaround time for reporting. The impacts of self-infection or laboratory contamination with these organisms can compromise health or integrity of the testing space, respectively, so laboratorians may take extra precautions such as wearing additional personal protective equipment (PPE) and work in a laboratory with heightened safety infrastructure. Donning, doffing, and decontaminating PPE and working in an enhanced safety environment all increase the amount of time required to safely process a specimen. Similarly, working with AR, novel, and emerging pathogens requires the use of quality controls that may not be readily available to non-reference laboratories; therefore, additional time may be needed to acquire the proper control materials. Finally, laboratory testing of these organisms is rapidly evolving, and several tests have not received required FDA approval or have been developed at a laboratory (laboratory-developed test, LDT) which would require validation by the user prior to use and often requires considerable time.

6.4 Laboratory Data Management

Laboratory data can play a significant role in detecting an outbreak that involves healthcare-associated drug resistant pathogens. Laboratory information systems (LIS) are software systems used by most laboratories to process, manage and store data. The electronic centralization of data provides a mechanism for rapid analyses of large data sets and identification of trends. Some LIS can be configured to send alerts that remind laboratory personnel to save an isolate when it meets predefined criteria and generate reports to identify patients with specific test results. These reports can be used to help identify cases and isolates that should be saved for additional analysis such as sequencing. Some national networks and resources managed by CDC that may be of assistance include:

- National Healthcare Safety Network (NHSN): tracks HAIs
- Emerging Infections Programs (EIP): surveillance, prevention, and control of emerging infectious diseases
- Healthcare-associated infections – community interface: HAI threats, advanced tracking methods, and AR in the US

Other suggested best practices for using laboratory data include:

- Communicate routinely with State Epidemiology/Healthcare Associated Infection Program, Hospital Infectious Diseases and Infection Control Departments, and Microbiology Laboratory Directors and other key partners
- Compile and report significant and unusual drug resistant findings to individual health care facilities' infection control departments on regular (weekly/monthly) basis
- Generate an annual statewide antibiogram that can be shared with health care facilities
- Share characterization data (i.e., NGS) of highly resistant or rare isolates

6.4.1 Ensuring Chain of Custody

A chain of custody (COC) document should accompany all sample handling from receipt through disposition, or "cradle to grave," with the goal of preventing any opportunity for tampering. This section does not provide comprehensive guidance regarding COC. Rather, the intention is to provide an awareness of the utility of a COC in the context of AR and HAIs and general information for consideration.

The need for a COC may not be common practice for laboratorians primarily involved in clinical laboratory testing of AR and/or HAIs; however, there are situations in which it may be prudent or requested by a submitter. For example, if a pathogen with a novel resistance profile, one that has the potential to severely threaten the public's health, is identified, a laboratory may elect to implement an internal COC to prevent theft and misuse. HAI investigations in which law enforcement is involved due to negligence, intentional harm, or otherwise, may prompt the submission of a sample already under COC.

While each laboratory's resources and needs are unique, there are critical elements of COC documentation and procedures that are standard including; (1) submitter contact information, (2) description of the evidence, (3) signatures for transfer of custody and (4) documentation of final disposition of sample. To strengthen recordkeeping in support of the COC, laboratories may photograph the evidence, document and track aliquot transfers, document disposition, document communications, and compile all resulting records in a single "case file" for ease of retrieval. However, a laboratory decides to proceed, quality, not quantity, of documentation is paramount in COC and is critical to the legal defensibility of data generated.

6.5 Epidemiology-Laboratory Communication

The communication between laboratorians and epidemiologists during all stages of an outbreak is crucial for comprehensive and suitable public health action. Communication should begin as soon as possible to ensure proper specimen collection and accurate laboratory test results. Prior to specimen

collection, laboratorians can advise on relevant factors for consideration, including sample type to be collected, storage medium and conditions, time constraints to ensure sample viability, and testing turnaround time. Clinical samples from residents or patients, as well as environmental samples from the facility and equipment may be suitable, and appropriate collection and storage guidance is vital since incorrect temperatures and wrong conditions may negatively influence laboratory results. Some outbreak investigations may require testing in specialized laboratories. The public health laboratory will be able to facilitate specimen collection, testing, and reporting of results. The public health laboratory can serve as the single point of contact for all partners throughout the investigation. Coordination and communication are critical, especially when multiple facilities and laboratories are involved. Thus, effective communication with the testing laboratories at the outset is necessary to understand specific needs and to prepare for all potential challenges. Optimally, the channels of communication are established, and relationships fostered prior to an incident to facilitate an expedited response.

The laboratory's ability to respond to an outbreak can vary depending on available reagents and supplies, and even personnel. Once the scope of an outbreak has been determined, there may be the need for additional laboratory staff to be trained. Existing protocols may require modifications including additional validation or verification. This highlights the importance of early communication between the laboratory and epidemiology. Laboratorians and epidemiologists should coordinate specimen collection and shipping to the lab, and the expected timeline to the availability of results. For example, specimens collected on a Thursday and received on a Friday may require additional weekend staff for processing and testing. It may be better to collect specimens on a Wednesday so that results can be reported before the weekend. Thus, communication between epidemiology and the laboratory should occur through an open channel to ensure priorities are met without compromising testing quality and results.

6.5.2 Other Testing

There are occasions when it is necessary to investigate an outbreak or suspected outbreak of an organism other than one mentioned in this chapter. In these cases, it is crucial to maintain proper chain of custody of all samples and specimens, and to ensure proper quality control of all testing. Communication is vital to ensuring a timely and accurate response to every outbreak. These outbreaks can involve toxin testing such as for endotoxin using LAL and gel-clot, *Staphylococcus* exfoliative toxin, *Clostridioides difficile toxin*, product sterility testing such as with USP 71 products or USP 61 for non-sterile products, or histopathology samples.

6.6 Quality Control and Assurance

As with all laboratory testing, in addition to the appropriate regulatory certifications, quality control and assurance are vital to ensuring actionable and timely results. Commercial reagents and FDA approved kit-based tests need to be quality checked as described in their package inserts. Prior to beginning any new method, a proper validation or verification must be completed. These methods can vary by

jurisdiction, but general principles apply. There must be a written plan which includes the number of isolates or specimens tested, and acceptance criteria for sensitivity and specificity, accuracy and precision, and inter and intra-run variability. The plan and final report must be approved and signed by the laboratory director. All tests must include appropriate positive and negative controls as described in the package insert and relevant CLSI guidance, and in accordance with CLIA Standards. All tests must be performed as described in standard operating procedures. Results must be checked for accuracy prior to reporting. When performing PCR and sequencing involving amplified material, best practice is to conduct “wipe tests” of the environment to rule out contamination. Unusual results or resistance patterns, or results that are not reproducible, should be discussed with the laboratory director and quality manager before action is taken.

CORHA Keys to Success

Laboratory as a Key Team Member

- Perform clinical testing
  - Support and/or confirm diagnosis
  - Detect outbreak
- Perform environmental testing
  - Determine outbreak source
- Perform organism identification
- Perform AST
- Identify novel AR patterns
- Identification of clusters of illness and potential outbreaks
- Perform advanced testing as able and appropriate to determine relatedness of clinical cases
- Determine mode of transmission
- Collaborate with other laboratories (i.e., PHLs and reference laboratories), epidemiologists and hospital IP staff to ensure adequate capability and capacity to respond to HAIs and established, as well as emerging, AR pathogens
- Provide sample collection and shipping materials, including any required requisition forms and guidance for transport
- Transport specimens to reference, environmental, or other specialized laboratory testing, as necessary
- Communicate reportable HAIs and AR pathogens to appropriate authorities, including local epidemiology
- Participate in AR surveillance (local, state, federal) to support rapid identification of novel AR pathogens and earlier outbreak identification in order to prevent additional illness and spread of infection
- Provide interpretation of laboratory test results and technical consultation to epidemiologists, public health members, healthcare workers, hospital IP staff, and others:
  - To guide/focus investigations
  - To assist with the development of case definitions
  - To identify appropriate number and type of specimens for collection
- Host visiting epidemiologists and/or hospital IP staff during rounds
- Store samples, as able and requested, to support additional testing requests
- Maintain chain of command of samples, as necessary
- Employ electronic laboratory reporting for rapid communication of quality data
- Utilize LIS to mine data and assist epidemiologists and hospital IP staff identify trends
- Communicate routinely with other outbreak team members to understand the needs and roles of each

CORHA Keys to Success

Appropriate and Rapid Testing

1. Communication between partners is crucial and must begin early
2. Communicate about the expected number of specimens, collection date, transport, and expected turnaround time
3. Results and reports should be shared in real time
4. Sequencing can play a pivotal role in detection of novel resistance mechanisms and determination of relatedness between strains

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