

# We Cannot Do It Alone

## The Intersection of Public Health, Public Policy, and Clinical Microbiology



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### KEYWORDS

- Public health microbiology • Epidemiology • FoodNet
- Antimicrobial Resistant Laboratory Network • FDA-CDC Biobank
- Laboratory-developed test • New antibiotics • Multidrug resistance

### KEY POINTS

- National resources such as the FDA-CDC AR Isolate Bank can support clinical laboratories at a local level as they confront multidrug-resistant pathogens and should be supported, strengthened, and expanded.
- Distributed networks such as the Antimicrobial Resistance Laboratory Network offer specialized diagnostics to address specific needs such as unconventional antimicrobial susceptibility testing not yet available at a local level.
- Public resources should be made available to help laboratories develop and standardize tests to address pressing infectious disease diagnostic needs that are not commercially compelling for assay development.
- Continuously updated local, regional, and national antibiograms should be available to guide therapeutic decisions with granularity and guide public health interventions.
- Policies and regulations should balance reliability of laboratory testing with fostering rapid entrance of infectious diagnostics into the market.

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## INTRODUCTION

The intersection of public health with clinical microbiology has been apparent since John Snow established the connection of cholera with the Broad Street pump. As we have been challenged by communicable disease crises from the human immunodeficiency virus (HIV) epidemic to the rise of carbapenem-resistant Enterobacteriaceae (CRE), our society has amassed new tools to diagnose and treat these infections. Nevertheless, with evolving resistance and emerging infections, the urgent need to fight such threats in a coordinated fashion at a local and societal level continues. The authors therefore review microbiological public health resources and strategies, and reflect on policies needed to combat microbial threats of the future.

## NATIONAL RESOURCES AVAILABLE AT LOCAL LEVEL

*Bringing new drugs on board:* new antibiotics offer potentially life-saving options for multidrug-resistant infections. However, they are only useful clinically if the microbiology laboratory can provide timely antimicrobial susceptibility testing (AST) results. Historically there has been a time lag in the availability of susceptibility testing methods for new antibiotics. As a result, isolates must be sent to a reference laboratory delaying AST results for up to a week or more. However, for an AST result to be meaningful for patient management, it usually must be available in a few days at most.

In the recent past, the time delay between Food and Drug Administration (FDA) approval of new antimicrobials and the availability of corresponding AST methods has been a significant hindrance to the utilization of new drugs for clinical care. Cef-taroline, for example, did not have an FDA-cleared AST method until 7 months after the initial approval in 2010 and automated systems took another 2.5 to 3.5 years to gain clearance. The FDA recognized this problematic discordance and hence made efforts to coordinate release of antimicrobials and commercial AST methods.<sup>1</sup> However, it can still take years before novel antimicrobials become incorporated into commercial panels. Fortunately, diffusion-based methods may offer an interim solution.

Nevertheless, before implementation of any AST method for a new drug, clinical laboratories must still verify its performance per Clinical Laboratory Improvement Amendments (CLIA) of 1988 requirement. CLIA stipulations are nonspecific and for FDA-approved assays only indicate the need to verify accuracy and precision to an unstated degree. In the absence of explicit guidance, use of accepted standards in the field are a reasonable and commonly used substitute, codified in documents such as Cumitech 31A.<sup>2</sup>

Verification could entail comparing the new AST method with a reference standard such as broth microdilution (BMD), but this gold-standard method requires significant assay expertise, technologist effort, and ready availability of antimicrobial powder. Most hospital laboratories consequently opt to verify new AST methods using a set of strains already characterized by a reference method such as BMD (or a nonreference, FDA-cleared method that has been previously verified in a CLIA-accredited laboratory) and that has an appropriate representation of susceptible and resistant isolates.

Practically, for new antibiotics, where to find such characterized strain sets is unclear. Availability of appropriate strains sets is also needed for “off-label” verification of existing methods when breakpoints are adjusted to reflect evolving best practice consensus (eg, annual Clinical and Laboratory Standards Institute updates). The often-recommended fall back for the latter is to compare with the disk diffusion method using correspondingly updated zone sizes.<sup>3</sup> The rationale is that the disk diffusion method for common drugs was instituted before CLIA 1988 and therefore is

exempt from its own verification requirements,<sup>4</sup> a somewhat problematic strategy, as the disks were originally cleared based on categorical performance around former, but not updated breakpoints, and accordingly important essential agreement metrics cannot be assessed.

Obviously for new drugs, appropriate, well-characterized strain sets must be possessed by pharmaceutical manufacturers or affiliates, as data from these strains are required to establish the susceptibility breakpoints for the drug. Under current regulations, however, pharmaceutical companies are prohibited from proactively either providing or sourcing characterized strains sets for clinical laboratories. Oddly, clinical laboratories can independently inquire on a need-to-know basis, freeing pharmaceutical companies to reveal some potential options. Such obstructive policies should be remedied by governing bodies, as the ability for clinical laboratories to verify, and thereby enable clinicians to use novel antimicrobials, is just as important as their commercial availability.

*The FDA-Centers for Disease Control and Prevention (CDC) Antimicrobial Resistance Isolate Bank:* fortunately, the FDA-CDC Antimicrobial Resistance (AR) Isolate Bank now provides a way to circumvent this conundrum. Launched in July 2015 as a tool to combat antimicrobial resistance, this highly valuable public health resource provides a curated repository of genotypically and phenotypically characterized bacterial isolates with clinically important resistance mechanisms and reference minimum inhibitory concentrations (MICs) to novel and standard antimicrobials.<sup>5,6</sup>

The FDA-CDC AR Isolate Bank is a paradigm of a public health resource that supports clinical laboratories at a local level to provide potentially life-saving, rapid, and up-to-date AST reporting. For example, the AR Isolate Bank includes an *Enterobacteriaceae* carbapenem breakpoint panel designed to assist with verification and implementation of new Clinical and Laboratory Standards Institute (CLSI) carbapenem breakpoints, given emergence of novel resistance mechanisms. The gram-negative carbapenemase detection panel supports verification of tests for carbapenemase production such as the modified carbapenem inactivation method (mCIM) and EDTA-mCIM (eCIM), which can distinguish serine  $\beta$ -lactamases from metallo- $\beta$ -lactamases.<sup>7</sup> Importantly, these strain sets include an assortment of well-characterized multidrug-resistance mechanisms, such as a range of serine and metallo-carbapenemases, which would be difficult for clinical laboratories to collect comprehensively from their own patients or purchase, and thereby allow clinical laboratories to gain experience with detection of critical resistance elements in their own laboratories.

Extending this idea further, imagine strain sets distributed widely to clinical laboratories for which curated modal MIC data for each new antibiotic would be released coincident with FDA approval. Analogously, as CLSI updates breakpoints, including changes such as new susceptible dose-dependent (SDD) categories to address emerging resistance patterns, there would ideally be concomitant AR Isolate Bank deployment of strain sets with modal MICs within and bordering the relevant MIC ranges to aid laboratories in verifying and promptly adopting these revisions. Particularly in the superbug era, accurate AST reporting of SDD categories formerly classified as “intermediate” can be crucial in providing appropriate salvage therapeutic options for multidrug resistant infections.<sup>8</sup>

In summary, the recently created FDA-CDC AR Isolate Bank provides welcome support for clinical microbiology laboratories as well as a resource for researchers, diagnostics, and pharmaceutical companies. This resource should be supported and strengthened, and ongoing “free availability” should be maintained with release/ updating of panels to coincide with new drug approvals to counterbalance

disincentives for clinical laboratories and companies to invest in capacity for rarely used antimicrobials and testing.

*Dare we ask?* We also might consider, if new AST methods were appropriately vetted by the FDA, the encore verification performance by clinical laboratories, whether limited or extensive, seems superfluous. It is estimated to take approximately 2 days of technologist and director time to validate a new E-test or disk method with 30 to 40 strains—that is, a discouraging barrier for bringing new AST tests on board. Importantly, laboratories also perform a mini-verification every time they perform a test by running quality control (QC) testing with confirmation that results are within specified limits (individualized QC plan, exceptions aside). Presumably QC requirements are deemed appropriately discriminatory for evaluation of ongoing assay performance, so why the initial extra verification step? Verification should be an issue for initial vetting by the manufacturer with appropriately large, representative strain sets, and test product deficits should not fall under the purview of postmarketing discovery by laboratories with greatly differing capabilities. If this seemingly redundant and purposefully vague verification requirement were lifted, the broad array of AST testing for new drugs could be implemented within days! Another option, although potentially burdensome and perhaps unnecessary, would be to task a set of high complexity clinical laboratories on a volunteer basis or possibly with some financial recompense to perform an independent assessment to verify manufacturer's claims that could be relied on by the field.

*Antimicrobial Resistance Laboratory Network (ARLN):* with emerging multidrug resistance, clinical laboratories are more frequently encountering pathogens for which there are no active agents based on routine or even reference laboratory-based AST. Although novel antimicrobials in clinical trials may be available on a compassionate-use basis, existing agents used in combination regimens are worthy of consideration as well. For example, aztreonam, a monobactam, remains active against metallo-carbapenemases such as the New Delhi metallo- $\beta$ -lactamase 1, and ceftazidime-avibactam provides activity against AmpC and extended-spectrum- $\beta$ -lactamases (ESBLs), which are enzymes that inactivate aztreonam. Accordingly, a regimen that inhibits AmpC and ESBL degradation of aztreonam, which then can function in the presence of potent metallo-carbapenemases should be active against “superbugs” carrying these dangerous resistance elements.<sup>9</sup> However, the question remains how a clinical laboratory would determine whether combinatorial salvage regimens are active against a given isolate.

The CDC has recently set up the ARLN to offer such testing. Established in 2016, the ARLN is composed of 7 regional laboratories and the National Tuberculosis Molecular Surveillance Center where clinical laboratories around the United States can send resistant isolates for additional testing. Their laboratory network has adopted inkjet printing technology for this AST testing, originally described by Smith and Kirby and Brennan-Krohn and Kirby, that allows highly accurate and precise at-will set-up and testing of any desired antimicrobial alone or in combination with reference broth microdilution equivalent AST results.<sup>10–14</sup> The ALRN currently offers, for example, the combination AST of aztreonam + ceftazidime-avibactam. Furthermore, it has the capacity to characterize isolates via whole genome sequencing and other molecular testing. Most importantly, the ARLN provides a distributed laboratory network that brings new AST and surveillance capabilities closer to the point of patient care. Alternatively, in the future, equivalent technology and antimicrobial reagents could and should be deployed at referral hospitals where superbugs are more prevalent.

*Central data and analyte repositories to support laboratory-developed test (LDT) design and validation:* there has been little industry interest in commercializing and

seeking FDA approval for molecular diagnostics for clinically important yet less common infectious diseases. LDTs fill this unmet need. LDTs are in vitro diagnostic tests developed and verified for local use. FDA-cleared methods that have been modified in any way by a clinical microbiology laboratory are also considered LDTs.<sup>2</sup>

Prominent examples of LDTs would include viral load testing for BK virus, Epstein-Barr virus, and cytomegalovirus (CMV) in the transplant setting. Although there are FDA-cleared assays for CMV viral load testing in blood, testing in other specimen types such as bronchoalveolar lavage, urine, and saliva provide added value for certain populations. Application of revised breakpoints to existing commercial AST methods are also considered a modification and therefore an LDT. Commercial manufacturers often take years to seek clearance for such updates, as the FDA does not have the authority to require companies to submit data within a certain timeframe. Accordingly, during this interval, clinical laboratories must verify accuracy and precision across revised breakpoints. Without the capacity or expertise to implement LDTs, laboratories presumably must continue to use outdated breakpoints, which could miss resistant strains and undermine patient care. As one example of the magnitude of this issue, 28% of laboratories in California had not yet lowered carbapenem breakpoints within 5 years of CLSI introducing revised, evidenced-based cut-offs in 2010.<sup>15</sup> Alternatively, LDT testing, whether for molecular diagnosis of target pathogens or AST determinations with revised breakpoints, may be performed at reference laboratories, which have extensive menus of LDTs but with suboptimal turnaround time delays.

There is ongoing debate about the appropriate level of regulation required for LDTs and whether routine laboratory quality assurance activities under CLIA 1988 are sufficient. Given the rapid growth of LDTs in personalized medicine, the American Society for Clinical Pathology recommended that “the regulatory infrastructure adopted must be sufficiently meticulous to safeguard the public without being so burdensome that it impedes emerging technology.”<sup>16</sup> As a comparator, in Europe most diagnostic tests are considered low-risk and exempt from premarket evaluation. Therefore, clinical quality of LDTs is managed through professionally driven quality assessment infrastructure.<sup>16</sup> The authors agree with this latter approach.

By analogy to the FDA-CDC AR Isolate Bank, the authors envision a public health resource to assist in LDT development that would have the added benefit of greater standardization of assays between institutions. Currently, microbiology laboratories independently construct and validate LDTs for similar sets of pathogens, given comparable clinical needs and the lack of commercial testing options. A free centralized publicly available database of pooled procedural and validation information would provide a much more comprehensive understanding of assay design and performance and allow laboratories to benefit from collective experience instead of each reinventing the wheel on its own. Best practice procedures including reagent and assay performance characteristics could then be described in consensus guidelines, which would ultimately increase the quality of overall diagnostic testing.

An expansion of interinstitutional comparable LDTs would also significantly bolster surveillance programs, as smaller facilities that otherwise may not have had the technical expertise to adopt LDTs may now be able to contribute to the nationwide diagnostic capacity to understand important microbiological concerns such as spread of viral subtypes, sexually transmitted infections, or antimicrobial resistance. To expand this idea further, the authors also propose a repository of free publicly available critical analytes that would allow standardization of LDT assays across facilities (eg, viral load standards) and ensure robust detection, for example, of critical viral subtypes in the face of genetic drift and emerging variants.

## IT IS TIME TO ADOPT A DIFFERENT MODEL FOR DIAGNOSTIC TEST APPROVAL IN AREAS OF UNMET MEDICAL NEED

An alternative and bolder strategy would be to lower the regulatory burden for approval of infectious disease diagnostics in areas of unmet need. Our proposal would be to lower the approval threshold for areas of focused need that would not normally be appealing for commercial development under current regulations. Specifically, companies would still have to establish robust analytical performance for their methodology, however, without the need for extensive and costly clinical trials to establish clinical performance/utility. This would spur innovation, development, and implementation of laboratory tests in areas such as detection of rare emerging diseases (MERS, Ebola, carbapenemase detection and discrimination, blood parasites, seasonal influenza subtyping for therapeutic discrimination, tick-borne bloodstream infection, and *Candida auris* to forestall hospital outbreaks). Transplant and immunocompromised host infectious disease testing could also be extended to the range of sample types of importance (eg, bronchoalveolar lavage fluid and other respiratory specimens for molecular detection of *Pneumocystis jiroveci* pneumonia (PJP) and toxoplasma among others). The European diagnostics market, for example, offers excellent diagnostic support for clinical care without the extra layer of regulatory burden.

Freed of the need to determine clinical validity, companies could confirm analytical performance in multiple sample types, thereby in turn freeing clinical laboratories from replicative efforts to develop LDTs when existing testing platforms would suffice. Those companies that could offer testing on the multitude of sample types of interest would have a competitive advantage, and competition would then spur a comprehensive testing menu to the benefit of the patients.

Furthermore, the demand for expensive reference laboratory testing would be decreased and more timely local diagnosis would reduce inefficiencies in the health care system, avoid unnecessary expense-associated delayed diagnosis, and contribute positively to patient well-being. The authors therefore encourage a rethinking of current regulatory framework in the United States. For areas of unmet need, we should put decision-making capability about clinical utility into the hands of medical specialists (laboratory medicine/clinical microbiology/infectious diseases) who can evaluate the most up-to-date medical and scientific literature in concert with evaluation of analytical performance capabilities, published in product inserts and vetted by the FDA, and make appropriate decisions about assays and platforms.

*Setting the standard:* strong national and international standards for quality assurance, method performance, and interpretative criteria should be strengthened and maintained. The authors acknowledge the contribution of both national and international organizations such as CLSI, EUCAST (European Committee on Antimicrobial Susceptibility Testing), USCAST (United States Committee on Antimicrobial Susceptibility Testing), SIS (Swedish Standards Institute), CEN (European Committee for Standardization), and ISO (International Organization of Standardization) that establish such standards. Many are volunteer-driven, membership- and/or government-supported not-for-profit entities. The authors also applaud coordination between organizations such as the FDA and CLSI. They encourage their continued, proactive review of breakpoints based on the most current understanding of pharmacokinetics and pharmacodynamics, which may suggest revisiting of values established during original drug approval.

## STRENGTHENING PUBLIC HEALTH LABORATORY SURVEILLANCE

National surveillance programs represent a key intersection between public health and microbiology laboratories. One of the oldest examples is the Foodborne Diseases

Active Surveillance Network (FoodNet), established in 1995 as a collaboration between 10 state health departments, that monitors for significant infectious enteric pathogens.<sup>17</sup> FoodNet determines the burden and trends in foodborne illness in order to appropriately design prevention and intervention programs.

Several other CDC surveillance systems for tracking food and waterborne diseases include Foodborne Disease Outbreak Surveillance System (FDOSS), National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS), and Waterborne Disease and Outbreak Surveillance (WBDOS) among others.<sup>18</sup> Although certain programs function more closely with Infection Control and Epidemiology departments to gather relevant patient clinical data, all of these systems require interaction with the microbiology laboratory for appropriate identification and isolate collection.

Some of the programs such as PulseNet provide bacterial DNA fingerprinting (previously pulsed-field gel electrophoresis now transitioning to whole genome sequencing) of foodborne illnesses. This data revolutionized epidemic investigations, because outbreaks could be identified and intervened on in hours to days instead of weeks in the previous era when epidemiologists had to wait for new patients to meet appropriate case criteria in order to identify clinical patterns that suggest a novel outbreak<sup>19</sup>.

The need for shared surveillance and diagnostic data repositories has been recognized among international collaborations as well. TBnet is one illustration of a partnership of European pulmonologists, epidemiologists, and infectious disease specialists organized on the premise of shared research goals, with a particular interest in immunodiagnostic tools. They accordingly have developed their own TB Biobank in addition to a data repository using common collection methods to simplify cross-study comparison.<sup>20</sup>

Similarly, the Program for Monitoring Emerging Diseases (ProMED-mail) is an entity founded in 1994 and maintained by the International Society of Infectious Diseases. Conceived as a free Internet listserv tool for rapid detection and report of emerging infectious or toxin-mediated diseases, ProMED-mail expanded from only 40 subscribers at its inception to greater than 83,000 in more than 150 countries. Subscribers receive e-mail reports filtered and moderated by a specialist panel on outbreaks and disease emergence. ProMED-mail voiced the earliest public account of severe acute respiratory syndrome and warned the medical community throughout the world of this outbreak.<sup>21,22</sup>

In this era of globalization with common threats and pathogens facing individual hospitals, states, and nations, it makes intuitive sense that these efforts to collect and share data should be fostered and strengthened.

*Information exchange:* real-time publicly available data to track infectious diseases is essential to control and prevent efforts and ever more relevant as demonstrated by ProMED-mail's internet-based success. FluNet is a model prototype that should be extrapolated to other emerging infectious threats. Established in 1997, FluNet is a global web-based data collection and reporting tool for influenza and logs viruses by subtype with records updated weekly.<sup>23</sup> SENTRY and ATLAS provide worldwide tracking of AST data for currently available antimicrobials.<sup>24,25</sup>

Expanded surveillance programs that, for example, track CRE by genotype should be public health goals achievable with current bioinformatic platforms. As one example of potential impact, the Israel National Center for Infection Control initiated an effort in 2008 within long-term care facilities where they collected a real-time database of all CRE carriers and events leading to acquisition. The program facilitated supervised information exchange and encompassed approximately 25,000 beds over 300 institutions enabling early detection of carriers and implementation of



population-specific contact precautions.<sup>26</sup> These efforts achieved more than a 10-fold reduction of CRE point prevalence in their acute hospital network and 50% reduction in all facilities. There is no doubt that such efforts will become increasingly important as new resistance emerges.

Annually updated hospital-based antibiograms are insufficient to guide empirical therapy with emerging antimicrobial resistance. Automated, deidentified input from hospital and laboratory information systems that provide regional to national metadata to track and forecast patterns of antibiotic resistance is a reasonable goal for our public health infrastructure. Daily updated facility; regional, national, and international (for travelers) species; and clone-specific antibiograms should be available to guide empirical therapeutic choice. Integration with whole-genome sequencing will facilitate clone tracking, illuminate resistance evolution, and inform local and public health countermeasures. As sources of new epidemics, infections, and/or resistance may be identified, there may be local opposition to participation. However, with balanced levels of access by health care providers and the public, the overarching public good of this early detection and control infrastructure should outweigh economic disincentives.

## SUMMARY

Microbiological data are necessary to inform public health goals and strategies, and conversely public health goals help guide the diagnostic strategies pursued in laboratories. In an era of rising global infectious disease threats, the public health laboratory infrastructure requires maintenance and strengthening to forestall harm to individual patients and populations. A pressing public health and societal need is the framework and infrastructure to streamline adoption of new antimicrobials and diagnostics. We analogously need streamlined, real-time output from the microbiology laboratories with centralized data aggregation to detect spread of resistant organisms and direct appropriate local and public health countermeasures. Here, the authors review some of the major existing resources that have supported our public health efforts and also identify programs and policies that could be of significant benefit. Governments, standards organizations, researchers, industry, and clinical microbiology laboratories should continue to collaborate to better address unmet public health goals and individual needs of infected patients.

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## REFERENCES

1. Humphries RM, Kircher S, Ferrell A, et al. The continued value of disk diffusion for assessing antimicrobial susceptibility in clinical laboratories: report from the clinical and laboratory standards Institute methods development and standardization working group. *J Clin Microbiol* 2018;56(8) [pii:e00437-18].



2. Clark RB, Lewinski ML, Loeffelholz MJ, et al. Verification and validation of procedures in the clinical microbiology laboratory. In: Sharp SE, editor. *Cumitech*, vol. 31A. Washington, DC: American Society of Microbiology; 2009.
3. Infectious Diseases Society of America. A minimal validation study for application of revised CLSI beta-lactam breakpoints to interpret lower range MICs Generated by a commercial susceptibility Device. ISDA Practice Guidelines. 2018. Available at: <https://www.idsociety.org/globalassets/idsa/topics-of-interest/antimicrobial-resistance/appendix-a-brief-validation-protocol-final.pdf>. Accessed April 23, 2019.
4. Heil EL, Johnson JK. Impact of CLSI breakpoint changes on microbiology laboratories and antimicrobial stewardship programs. *J Clin Microbiol* 2016;54(4): 840–4.
5. CDC & FDA antibiotic resistance (AR) Isolate Bank. US Centers for disease control and prevention. Available at: <https://www.cdc.gov/drugresistance/resistance-bank/index.html>. Accessed April 5, 2019.
6. Lutgring JD, Machado MJ, Benahmed FH, et al. FDA-CDC antimicrobial resistance Isolate Bank: a publicly available resource to support research, development, and regulatory requirements. *J Clin Microbiol* 2018;56(2) [pii:e01415-17].
7. CLSI. Performance standards for antimicrobial susceptibility testing— 28th Edition. CLSI Supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2018. p. 108–18.
8. Smith KP, Brennan-Krohn T, Weir S, et al. Improved accuracy of cefepime susceptibility testing for extended-spectrum-beta-lactamase-producing Enterobacteriaceae with an on-demand digital dispensing method. *J Clin Microbiol* 2017; 55(2):470–8.
9. Marshall S, Hujer AM, Rojas LJ, et al. Can ceftazidime-avibactam and aztreonam overcome beta-lactam resistance conferred by metallo-beta-lactamases in enterobacteriaceae? *Antimicrob Agents Chemother* 2017;61(4) [pii:e02243-16].
10. Brennan-Krohn T, Kirby JE. Antimicrobial synergy testing by the inkjet printer-assisted automated checkerboard array and the manual time-kill method. *J Vis Exp* 2019;146:e58636.
11. Brennan-Krohn T, Truelson KA, Smith KP, et al. Screening for synergistic activity of antimicrobial combinations against carbapenem-resistant Enterobacteriaceae using inkjet printer-based technology. *J Antimicrob Chemother* 2017;72(10): 2775–81.
12. Smith KP, Kirby JE. How inkjet printing technology can defeat multidrug-resistant pathogens. *Future Microbiol* 2016;11:1375–7.
13. Smith KP, Kirby JE. Verification of an automated, digital dispensing platform for at-will broth microdilution-based antimicrobial susceptibility testing. *J Clin Microbiol* 2016;54(9):2288–93.
14. Brennan-Krohn T, Pironti A, Kirby JE. Synergistic activity of colistin-containing combinations against colistin-resistant enterobacteriaceae. *Antimicrob Agents Chemother* 2018;62(10) [pii:e00873-18].
15. Humphries RM, Hindler JA, Epton E, et al. carbapenem-resistant Enterobacteriaceae detection practices in California: what are we missing? *Clin Infect Dis* 2018;66(7):1061–7.
16. Regulation of laboratory developed tests (LDTs) (Policy Number 10-02). American Society for Clinical Pathology. 2016. Available at: <https://www.ascp.org/content/docs/default-source/policy-statements/ascp-pdf/pp-regulation-of-ltds.pdf?sfvrsn=0>. Accessed April 5, 2019.

17. Foodborne diseases active surveillance network (FoodNet). Atlanta, GA: US Centers for Disease Control and Prevention; 2018. Available at: <https://www.cdc.gov/foodnet/index.html>. Accessed April 5, 2019.
18. Surveillance and data systems. Atlanta, GA: US Centers for Disease Control and Prevention; 2018. Available at: <https://www.cdc.gov/ncezid/dfwed/keyprograms/surveillance.html>. Accessed April 5, 2019.
19. What is PulseNet? Association of public health laboratories. 2016. Available at: <http://www.aphlblog.org/what-is-pulsenet/>. Accessed April 5, 2019.
20. TBnet. 2019. Available at: <http://www.tb-net.org/index.php/home>. Accessed April 5, 2019.
21. Carrion M, Madoff LC. ProMED-mail: 22 years of digital surveillance of emerging infectious diseases. *Int Health* 2017;9(3):177–83.
22. Madoff LC. ProMED-mail: an early warning system for emerging diseases. *Clin Infect Dis* 2004;39(2):227–32.
23. Influenza: FluNet. Geneva (Switzerland): World Health Organization; 2019. Available at: [https://www.who.int/influenza/gisrs\\_laboratory/flunet/en/](https://www.who.int/influenza/gisrs_laboratory/flunet/en/). Accessed April 5, 2019.
24. Fuhrmeister AS, Jones RN. The importance of antimicrobial resistance monitoring Worldwide and the Origins of SENTRY antimicrobial surveillance program. *Open Forum Infect Dis* 2019;6(Suppl 1):S1–4.
25. ATLAS: Antimicrobial testing Leadership and surveillance. Available at: <https://atlas-surveillance.com/>. Accessed April 25, 2019.
26. Ben-David D, Masarwa S, Fallach N, et al. Success of a national intervention in controlling carbapenem-resistant Enterobacteriaceae in Israel's long-term care facilities. *Clin Infect Dis* 2019;68(6):964–71.