Impacts of Culture-Independent Diagnostic Practices on Public Health Surveillance for Bacterial Enteric Pathogens

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For decades, culture has been the mainstay of diagnostic testing for bacterial enteric pathogens. This paradigm is changing as clinical laboratories adopt culture-independent methods, such as antigen-based tests and nucleic acid–based assays. Public health surveillance for enteric infections addresses 4 interrelated but distinct objectives: case investigation for localized disease control; assessment of disease burden and trends to prioritize and assess impact of population-based control measures; outbreak detection; and microbiologic characterization to improve understanding of pathogens, their virulence mechanisms, and epidemiology. We summarize the challenges and opportunities that culture-independent tests present and suggest strategies, such as validation studies and development of culture-independent tests compatible with subtyping, that could be adopted to ensure that surveillance remains robust. Many of these approaches will require time and resources to implement, but they will be necessary to maintain a strong surveillance system. Public health practitioners must clearly explain the value of surveillance, especially how outbreak detection benefits the public, and collaborate with all stakeholders to develop solutions.
tests to detect genes of multiple enteric pathogens simultaneously are being developed, although they are not yet FDA approved [6–8]. Culture-independent tests are usually faster than culture and, in some cases, can provide more types of information than were previously available, such as detection of Shiga toxin. However, they do not yield an isolate that can be forwarded to PHLs, and specimens collected for culture-independent testing may, in some cases, be incompatible with culture. In addition, performance characteristics of culture-independent tests are variable and different from those of culture.

We summarize the challenges and opportunities these new tests present for disease surveillance and discuss the impact on each of the 4 surveillance objectives. In addition, we suggest strategies that public health agencies, clinical laboratories, and industry could adopt to ensure that surveillance for bacterial enteric infections remains robust.

**DIAGNOSTIC TESTING FOR PATIENTS AND POPULATIONS**

Clinicians, clinical laboratorians, and public health practitioners value diagnostic tests that are accurate, rapid, and inexpensive and that allow for subtyping and other testing as needed. Clinicians primarily address the needs of individual patients, whereas public health practitioners focus on the health of populations and thus prioritize attributes of available tests differently.

For clinicians and clinical laboratorians seeking to diagnose a patient’s illness, the ideal test is fast and accurate. Antimicrobial susceptibility testing can be important, but initial treatment decisions are generally made before results are available. Subtyping or virulence testing results are seldom used for patient care. However, as more is learned about virulence factors, particularly for STEC, virulence testing may play a greater role in clinical management [9].

Like clinicians, public health practitioners value tests that are fast and accurate, but public health practitioners generally place greater emphasis on accuracy (ie, specificity and sensitivity) than speed. Even during outbreaks, when the relative importance of speed increases, accuracy remains essential to ensure outbreak-associated cases are unambiguously identified. Because surveillance is ongoing, public health practitioners value stability in the use and accuracy of diagnostic testing to minimize any artificial impact of changes in testing practices on trends in reported infections and to allow them to detect aberrations (ie, outbreaks) effectively.

In addition, public health practitioners often need more detailed information regarding isolates than do clinicians. For example, various methods of subtyping, such as serotyping and virulence factor characterization, allow for outbreak detection and tracking trends in infections with specific strains but may not be directly relevant to clinical care. Although clinicians and clinical diagnostic laboratories ultimately determine the type of testing that is performed in clinical settings, all stakeholders need to understand the public health impact of available methods.

**CHANGING LABORATORY PRACTICES**

Culture-independent tests for bacterial enteric pathogens have been adopted rapidly by clinical laboratories that face pressure to provide rapid and reliable results while minimizing costs. These methods include nucleic acid amplification tests such as PCR and antigen-based methods such as enzyme immunoassays and lateral flow assays [3–8]. Other methods may be in development. Culture often requires multiple days, whereas some culture-independent methods yield results in ≤1 day. Although culture-independent test materials may be more expensive, they often yield cost savings by reducing the need for highly trained microbiologists.

The Foodborne Diseases Active Surveillance Network (FoodNet) conducts active surveillance for 9 foodborne pathogens with >650 clinical laboratories in 10 states [1]. From 2003 to 2007, the proportion of laboratories using Shiga toxin testing, a culture-independent method, increased from 6% to 11% [3]; a 2010 survey suggests that this percentage has continued to increase (Centers for Disease Control and Prevention [CDC], unpublished data). Similarly, from 2009 to 2010, the percentage of clinical laboratories in FoodNet using culture-independent tests for *Campylobacter* increased from 2% to 7%, a rapid increase that appears to be continuing (CDC, unpublished data).

**IMPACT ON PUBLIC HEALTH SURVEILLANCE OBJECTIVES**

The shift toward culture-independent testing has different implications for each surveillance objective (Tables 1 and 2). These impacts will vary by pathogen.

**Case Investigation for Disease Control**

The fundamental goal of notifiable case surveillance is to identify infected persons in order to perform case investigation and disease control. Because culture-independent testing has the potential for ease of use and lower cost, its adoption may increase the number of tests performed and, hence, the number of cases ascertained. Some tests might also detect pathogens that are not detectable using culture. For example, routine identification of non-O157 STEC was not practical until Shiga toxin testing became commercially available. As laboratories have adopted Shiga toxin testing, there has been a concomitant increase in the number of non-O157 STEC cases reported [3, 10].
Performance characteristics of many culture-independent tests appear to be different from culture. For some, sensitivity or specificity or both appear to be lower than culture. Although reduced test performance may be justified in exchange for speed, it can pose problems for clinicians and public health practitioners alike. Culture-independent methods with low specificity may produce a number of false-positive results, especially when used in low-prevalence populations. A recent study reported a specificity of 94.2% and a positive predictive value (PPV) of 92.6% for a new antigen-based Campylobacter assay. However, 42% of the samples included were positive by culture, substantially higher than would probably be seen in practice [11]. Translating these results to a real-world setting where the prevalence of Campylobacter might be closer to 3%–5% of diarrheal stools [12, 13], the expected PPV would be only 47%. Preliminary results from a multisite, real-world validation study of Campylobacter culture and culture-independent methods were similar [14]. In contrast, 2 enzyme immunoassays for Shiga toxin detection have been shown to have PPVs of 93% and 81% based on a reference standard of culture for STEC O157 in studies with populations with realistic prevalence of infection [15, 16]. Less is known about several newer PCR tests, but their characteristics will probably be different from antigen-based tests and culture. Commercially available PCR tests have not yet received FDA approval. It is important to remember that the accuracy of any test, including culture, can vary due to the skill level of laboratory staff, calibration of equipment, and other factors.

False-positive results are problematic for patients, who may receive unnecessary therapy and increased medical costs, and for public health practitioners, who may waste resources investigating individual cases that are not true infections. False-positive results could lead to unnecessary follow-up tests and exclusion from work or childcare, raising questions about the evidence needed for a person to safely return to work or childcare after being excluded because of diagnosis of enteric infection. Little or no guidance is available to help in choosing tests or resolving discrepancies between culture and culture-independent results. To prevent the spread of infection, health departments need tests that are sensitive enough to detect all or most persons with infections that might pose a risk to others while avoiding the burden imposed by using tests that might detect nonviable organisms or be falsely positive for other reasons.

Timely real-world validation studies are needed to address these challenges (Tables 3 and 4). These studies should include collection of demographic and clinical information from patients and should be performed in settings in which the prevalence of infection reflects that of the population for which the test is used. Whenever possible, validation studies should be conducted using all patient specimens submitted for testing without respect to results at the diagnostic laboratory. Based on their findings, best practice documents can be developed for clinical laboratories and PHLs, similar to those published for STEC [17], Chlamydia trachomatis, and Neisseria gonorrhoeae [18]. Networks such as FoodNet can monitor adherence to guidelines. The results of validation and other studies can be used to develop new public health testing algorithms for safe return to work or childcare that maintain public safety but do not place undue burdens on patients.

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**Table 1. Advantages and Challenges of Culture-Independent Testing From the Clinical (Patient-Level) Perspective**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Challenges</th>
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<tbody>
<tr>
<td>Rapid diagnosis</td>
<td>False-positive findings resulting in unnecessary treatment or incorrect diagnosis</td>
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<tr>
<td>Potential for lower cost</td>
<td>Loss of antimicrobial susceptibility testing</td>
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<tr>
<td>Potential for easier specimen collection (dry swab specimen)</td>
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<tr>
<td>Detection of wider range of pathogens (eg, non-O157 STEC)</td>
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<tr>
<td>Better sensitivity for some pathogens</td>
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Abbreviation: STEC, Shiga toxin–producing Escherichia coli.

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**Table 2. Advantages and Challenges of Culture-Independent Testing From the Public Health (Population-Level) Perspective**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Challenges</th>
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<tbody>
<tr>
<td>Rapid detection of cases</td>
<td>Resources wasted on unnecessary case follow-up</td>
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<tr>
<td>Potential increased testing leading to increased case ascertainment</td>
<td>Incorrect and or unstable estimates of actual number of illnesses</td>
</tr>
<tr>
<td>Detection of wider range of pathogens (eg, non-O157 STEC)</td>
<td>Disruption of trend monitoring</td>
</tr>
<tr>
<td>Better sensitivity for some pathogens</td>
<td>Loss of subtyping for outbreak detection</td>
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<tr>
<td></td>
<td>Possible investigation of pseudo-outbreaks</td>
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<td></td>
<td>Decreased ability to monitor trends in subtypes, such as Salmonella serovar Enteritidis</td>
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<tr>
<td></td>
<td>Loss of antimicrobial susceptibility testing</td>
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<td></td>
<td>Increased public health costs for surveillance</td>
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<table>
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<tr>
<th>Table 3. Summary of Proposals for Public Health: Next 1–2 Years</th>
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<tr>
<td><strong>Short-term Proposals</strong></td>
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<tr>
<td>Timely, real-world validation studies in collaboration with key stakeholders of new testing methods to define performance characteristics</td>
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<tr>
<td>Develop best practice documents and clinical guidelines for laboratories to follow and disseminate widely</td>
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<tr>
<td>Routine surveys of clinical laboratories to monitor uptake of new test methods</td>
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<tr>
<td>Adopt multilevel case definitions to capture cases tested only with culture-independent methods; develop criteria that are needed before national case definitions are changed based on culture-independent methods; consider initial implementation in geographically limited systems, such as FoodNet</td>
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<tr>
<td>Immediately begin collecting data on cases not meeting confirmed case definitions and modify data systems to capture more detailed laboratory data for cases</td>
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<tr>
<td>Conduct provider or health system surveys to determine populations for which new testing is being used</td>
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<tr>
<td>Recommend reflexive culture of positive specimens at clinical laboratories</td>
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<tr>
<td>Request forwarding of clinical material to state public health laboratories for culture, prioritizing STEC and Salmonella, as resources allow</td>
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<tr>
<td>Prepare for shifts in clinical laboratory practice by assessing the need to change specimen submission requirements (or add them); determine specifics about shipping, media, etc, to be ready for submission of clinical material to public health laboratories</td>
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**Assessment of Disease Burden and Trends**

Public health practitioners use notifiable disease reports to estimate total illnesses and monitor trends, thus informing decisions about resource allocation and determining the effectiveness of public health interventions. Culture-independent testing poses at least 5 challenges to accurately monitoring disease burden and trends: (1) artifactual increases or decreases in reports due to variations in test performance or the use of new tests for different clinical indications, (2) shifts in demographic characteristics of patients with detected infections, (3) the need to reassess multipliers used to estimate total illnesses, (4) loss of strain differentiation, (5) and the need to modify surveillance case definitions.

Changes to any surveillance system can pose challenges in interpreting data. Therefore, changes in laboratory testing practices can be problematic. Even a test with far-from-perfect performance characteristics can yield reliable data for monitoring trends if used in a stable manner. For example, Campylobacter culture is not 100% sensitive [8] but has been used for decades. Surveillance systems that track culture-confirmed infections provide reliable trend information. As laboratories adopt culture-independent methods with specificity and sensitivity that differ from culture, surveillance data will need to be interpreted with caution and in conjunction with data about laboratory uptake of new tests.

There is also evidence that the demographics of reported cases with culture confirmation and those without culture confirmation can differ. FoodNet has collected information on persons with positive culture-independent tests since June 2009. During June 2009–December 2010, FoodNet received reports of 10,350 persons with culture-confirmed infection and 573 with only a positive culture-independent test. The median age for persons with culture-confirmed infection was 37 years and for persons with only a positive culture-independent test was 49 years (P < .0001). Among persons with culture-confirmed infection, 45% were female, compared with 52% of persons with only a positive culture-independent test (P < .0001) (CDC, unpublished data). These demographic differences might be explained at least in part if the new tests are being used for different clinical indications or if there is variability in the populations served by laboratories that adopt culture-independent testing. However, the recent multisite validation study found even more pronounced demographic differences and was conducted in a setting where all stools were tested with the same series of tests, suggesting a third, as yet unexplained, mechanism that affects performance of the culture-independent tests (Collette Fitzgerald, CDC, personal communication). Whatever the cause or causes, if case definitions are expanded to include the results of culture-independent testing, these demographic differences will result in variations in age- and sex-specific trends reported for Campylobacter cases and could lead to challenges in accurately interpreting surveillance data.

 notified disease surveillance captures a fraction of all diarrheal illnesses in the population. For a case to be reported to surveillance, a lengthy chain of events must occur (Figure 1). FoodNet has conducted population, physician, and laboratory surveys to estimate the proportion of cases missed at each level of this surveillance pyramid, providing critical data for estimates of the number of foodborne illnesses in

<table>
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<th>Table 4. Summary of Proposals for Public Health: Shaping the Future</th>
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<tr>
<td><strong>Long-term Proposals</strong></td>
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<tr>
<td>Establish sentinel site surveillance sites to ensure isolate submission for characterization of specific attributes</td>
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<tr>
<td>Partner with industry, clinicians, and academia to develop subtyping methods that will function independent of pathogen isolation and advocate for funding for development of these techniques</td>
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<tr>
<td>Advocate for adequate public health funding for surveillance activities</td>
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<tr>
<td>Epidemiology: enhanced data collection and analysis</td>
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<tr>
<td>Public health laboratories: culture for Salmonella, STEC, and other pathogens on material received from clinical laboratories</td>
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the United States [1, 19, 20]. Because culture-independent tests could alter the parameters at several levels, surveys will be needed to update future burden estimates.

Characterization below the species level is essential to surveillance and disease control measures for several enteric pathogens. For example, Salmonella has >2500 serotypes, many of which have particular ecologic niches and routes of transmission. One of the most frequently isolated serotypes, Salmonella enterica serovar Enteritidis (SE), is commonly associated with eggs and chicken; assessing the impact of control measures to improve the safety of eggs or chicken requires specifically tracking SE. Because current culture-independent tests do not yield an isolate that can be used for further characterization [7], widespread adoption of these tests for Salmonella could erode the ability to monitor trends in SE and other Salmonella infections. Like Salmonella, STEC has considerable diversity. Shiga toxin assays have made it possible to routinely identify non-O157 STEC infections. However, Shiga toxin assays in themselves do not differentiate between O157 and non-O157 strains; such differentiation usually depends on characterization at PHLs. As more laboratories adopt Shiga toxin testing, PHLs may need additional resources to manage higher volumes of specimens requiring strain characterization in order to further define the epidemiology of these important pathogens.

Currently, ill persons with positive results only from culture-independent tests will not be captured in national public health surveillance. The standard definitions of confirmed and probable cases approved by the Council of State and Territorial Epidemiologists (CSTE) include infections that are culture-confirmed or epidemiologically linked to culture-confirmed cases [21]. Increasing uptake of culture-independent tests will mean that case definitions will need to be reconsidered.

Several approaches may help to address these challenges in monitoring disease burden and trends. Public health departments could collect information on the testing method for each reported positive result to allow interpretation of the result in the context of knowledge of test performance. State health departments could regularly survey clinical laboratories regarding testing practices and could also consider monitoring clinician testing practices. Here, too, validation studies will be crucial to understanding changes in frequency of reports of positive results and possible changes in the demographics of reported cases. FoodNet has implemented several of these practices, collecting data on positive culture-independent tests results for STEC since 2008 and Campylobacter since 2009. FoodNet has also initiated routine surveillance of clinical microbiology laboratories’ testing practices and is currently modifying its surveillance methods to capture all positive test results for the pathogens under surveillance, whether by culture or culture-independent testing, along with information on the testing methods.

**Outbreak Detection**

Subtyping is critical for detecting outbreaks. Currently, culture-derived isolates are necessary for subtyping methods such as antimicrobial resistance testing, serotyping, and pulsed-field gel electrophoresis (PFGE). The PFGE patterns from Salmonella, STEC, Listeria, and other enteric pathogens are routinely uploaded to PulseNet, the national molecular subtyping network for these pathogens. State health departments and the CDC continuously monitor for clusters of PFGE matches to detect outbreaks that might otherwise go undetected. Since its establishment in 1996, PulseNet has revolutionized outbreak detection [22]. Outbreak investigations initiated as a result of PulseNet have resulted in discoveries of novel food vehicles (eg, Salmonella in peanut products [23] and frozen microwavable pot pies [24], STEC in raw cookie dough [25], and Listeria on cantaloupe [26]). It has also reinforced concerns about pathogens emerging in food items (eg, multidrug-resistant Salmonella in ground beef and turkey [27, 28]) and highlighted the role of other routes of transmission (eg, Salmonella associated with pet frogs [29]). The results of these investigations have led to industry-wide changes in food processing and monitoring, improved regulatory focus, public education campaigns, and other control activities. Effective outbreak detection, particularly of widely dispersed outbreaks caused by commercially distributed foods, depends...
on subtyping isolates from a large percentage of cases. As clinical laboratories move away from tests that yield isolates, it will be critical to maintain a system in which subtyping can be performed on a high percentage of positive specimens, particularly *Salmonella*, STEC, and *Listeria*.

Several options to maintain capacity for outbreak detection during this period of changing diagnostic practices can be considered. First, clinical laboratories could perform reflexive culture of specimens that have a positive result with a culture-independent test to yield isolates for submission to PHLs for subtyping. Second, clinical laboratories could be requested or required to submit clinical materials that have tested positive with a culture-independent test to their PHL for culture. To ensure receipt of these clinical materials, state health departments may need to modify their specimen submission requirements and resolve logistical issues in advance. Public health laboratories would also need to address significant workload issues if they were to begin receiving and culturing specimens that have tested positive with culture-independent tests for relatively common pathogens such as *Salmonella*. Third, culture-independent subtyping methods could be developed for the clinical laboratory or PHL. This may be necessary if specimens that are sometimes incompatible with culture, such as dry fecal swab specimens, are used for culture-independent tests. Development of these methods would require a concerted research effort and retooling of the national and international subtype-based surveillance infrastructure. It would be catastrophic for outbreak surveillance if clinical laboratories were to abandon culture before new subtyping technologies and informatics systems were developed or other systems implemented for specimen collection.

The relatively low specificity of many culture-independent tests presents a different challenge to outbreak detection and investigation. Already we are aware of several situations in which apparent falsely positive culture-independent test results led to inappropriate public health responses during outbreaks. Two outbreaks of norovirus infection, 1 in a childcare center and 1 in a university, were first reported as outbreaks of STEC infection, based on presumably false-positive Shiga toxin results at a clinical laboratory [30, 31]. More recently, “outbreaks” of *Campylobacter* infection have been reported in 2 Colorado skilled nursing facilities and 1 childcare center (Colorado Department of Public Health and Environment, personal communication). In each instance, 1 case was reported to have a positive culture-independent test for *Campylobacter* in the setting of additional diarrheal illnesses at the facility. The initial *Campylobacter*-positive stools were not available for culture at the PHL. Further testing of ill persons at these facilities yielded norovirus. While the clinical characteristics of these outbreaks might ultimately have been inconsistent with campylobacteriosis, initial control measures are often implemented based on limited, preliminary information. Although resource-intensive, public health practitioners should work to verify reported pathogens through testing at PHLs when investigating possible outbreaks in which persons have been diagnosed exclusively with culture-independent methods, particularly in high-risk settings.

### Microbiologic Characterization of Reported Infections

Public health practitioners monitor microbiologic characteristics of reported infections to improve understanding of pathogens and their epidemiology. Examples include tracking the susceptibility of *Salmonella* and *Campylobacter* to antimicrobial agents and assessing virulence factors in STEC. These types of testing are generally performed at PHLs, the CDC, or both on isolates submitted by clinical laboratories. For example, the National Antimicrobial Resistance Monitoring System performs antimicrobial susceptibility testing on a systematic sample of *Salmonella* isolates submitted to PHLs.

Monitoring antibiotic susceptibility is important at the population level because infection with pathogens resistant to key antimicrobial agents has been associated with more severe illness [32, 33]. Key public health regulatory decisions have been based on surveillance data on antimicrobial resistance. For example, an increase in ciprofloxacin-resistant *Campylobacter* resulted in a major policy change banning the use of fluoroquinolones in poultry production in the United States [34]. Recently, the Food Safety and Inspection Service began requesting that companies recall ground beef and ground turkey implicated in multidrug-resistant *Salmonella* outbreaks [35, 36]. Antimicrobial susceptibility data can also provide helpful subtyping information for outbreak investigations and for attributing illness to specific food commodities. For example, outbreaks of pan-susceptible *Salmonella* serovar Newport infections linked to foods are often associated with produce, whereas multidrug-resistant *Salmonella* serovar Newport outbreaks are more likely to be associated with meat and dairy products, foods derived from animals in which antibiotics are commonly used [27, 37].

Similarly, virulence factor testing performed by PHLs is used as a subtyping method and for understanding the clinical and public health impact of STEC to better target public health prevention measures. Enhanced understanding of STEC virulence factors might lead to improved guidance for responding to non-O157 STEC outbreaks in childcare centers and other settings. Virulence characterization, currently isolate-based, was essential for case assessment and public health action during the large 2011 sprouts-associated outbreak of enteropathogenic *Shiga* toxin–producing *E. coli* O104:H4 infections centered in Germany [38].

Microbiologic characteristics of isolates such as subtypes and susceptibility profiles are also used in combination with other
data to attribute illness to specific sources [39]. Should isolates become less available, it will be more difficult to use surveillance data as a platform for attribution analyses, such as case-control studies of sporadic infections and comparisons of isolates from patients, foods, and animals.

Culture-independent methods that produce no isolate and possibly no specimen for forwarding pose a substantial but not insurmountable challenge to public health goals related to monitoring specific characteristics of enteric pathogens. For this purpose, testing all specimens is often unnecessary. If adequate statistical power can be attained, one solution would be to establish sentinel sites to obtain isolates for special surveillance projects. Routine testing for gonorrhea has been culture-independent for many years. The Gonorrhea Isolate Surveillance Project monitors trends in antibiotic susceptibility through sentinel sites that collect specimens for culture from a systematic sample of patients [40]. Alternately, as for subtyping for outbreak detection, culture-independent methods could be developed that include testing for known resistance determinants. However, some isolate-based susceptibility monitoring will remain necessary for the foreseeable future because resistance mechanisms continually evolve in ways that cannot be predicted by specific genetic targets.

CONCLUSIONS

The landscape of surveillance for enteric bacterial pathogens is changing rapidly. Culture-independent testing provides advantages for diagnostic laboratories and clinicians treating patients with diarrheal illness that will very likely lead to continued uptake of these methods. However, without timely and effective action, such testing will significantly hamper public health efforts to prevent and control these infections.

We have described several possible approaches to address these challenges (Tables 3 and 4). Many of these approaches will require time and resources to implement, but they will be necessary to maintain a strong surveillance system that can identify cases, monitor trends, detect outbreaks, and characterize pathogens. Such a system benefits everyone. To meet the challenges of culture-independent testing, public health practitioners need to clearly explain the value of surveillance for enteric pathogens, especially how outbreak detection benefits the public, and collaborate with all stakeholders—patient groups, clinicians, regulatory agencies, and industry—to develop solutions.

Notes

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