Diagnosis of *Vibrio cholerae* O1 Infection in Africa

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Isolation of *Vibrio cholerae* O1 is necessary for cholera outbreak confirmation. Rapid diagnostic testing of fecal specimens, based on lipopolysaccharide detection of *V. cholerae* O1 or O139, may assist in early outbreak detection and surveillance. Cary-Blair transport medium is recommended for specimen transport. Filter paper, although used in epidemics, needs evaluation against rectal swab specimens. Fecal specimens are subcultured onto selective and nonselective media, including 5% blood agar and TCBS agar, for detection of *V. cholerae* O1 or O139. Suspicious, oxidase-positive isolates are serotyped in monovalent antisera. Antimicrobial-susceptibility testing is performed to detect resistance. Molecular characterization supports phenotypic identification and outbreak investigations. The presence of genes encoding cholera toxin, lipopolysaccharide, and El Tor biotype traits can be confirmed. Standardized pulsed-field gel electrophoresis analysis facilitates strain comparison. Quality management ensures reliability of results through validation and verification of functional laboratory equipment; quality control of testing procedures, laboratory reagents, and consumables; and participation in proficiency-testing schemes.

**Keywords.** cholera; Africa; *Vibrio cholerae* O1; rapid diagnostic tests; diagnostics; antimicrobial susceptibility testing; molecular diagnostics; quality management.

Cholera remains an important cause of diarrhea in developing countries. Cholera in many African countries is endemic, defined as detection of cholera cases in at least 3 of the preceding 5 years, and Africa has reported the highest burden of disease globally during 2000–2008 [1], which has been principally drive by outbreaks. Notable outbreaks included those reported from Mozambique [2], Zimbabwe [3], and Namibia, where the disease had never previously been observed [4]. Many of these outbreaks are associated with high mortality rates [1]. Mortality may be grossly underreported, as has been shown by active-case-finding studies in Kenya [5]. Diagnostic delays may result in higher case numbers and case-fatality rates [6], resulting in an enormous economic burden to the continent [7].

Challenges remain regarding diagnosis, as resources are extremely limited, but isolation of *Vibrio cholerae* O1 is necessary for cholera outbreak confirmation. Ongoing interventions by the World Health Organization (WHO) and other bodies to optimize infectious diseases surveillance and diagnostic methods have included cholera [8] but have failed to have the necessary impact. Rapid and appropriate diagnostic methods and personnel development, using standardized methods, remain important considerations in combating disease. This review serves to highlight African diagnostic studies; to promote the use of standard diagnostic methods that are based on outcomes of various WHO workshops held on the continent; and to provide information about newer technologies that might become affordable as African countries develop resources.

**DIAGNOSIS OF CHOLERA AND IDENTIFICATION OF V. CHOLERAE O1**

**Rapid Methods**

Many challenges to early detection of cholera outbreaks remain. Although management of patients with acute
watery diarrhea is similar regardless of etiology, public health implications, epidemic spread, and management of cholera outbreaks are distinct, given that *V. cholerae* is highly virulent and has tremendous epidemic potential.

Isolation and identification of *V. cholerae* serogroup O1 or O139 by culture of a stool specimen remains the gold standard for laboratory diagnosis. Although rapid diagnostic tests are not a substitute for stool culture, owing to their lack of sensitivity, as reported in many studies [9], they can be used as an emergency diagnostic tool for individual patients in African outbreak situations where stool culture is not readily available. Possible scenarios include outbreaks that occur among remote or displaced persons or when the usual system for laboratory referral and testing is interrupted because of a humanitarian crisis or natural disaster. Rapid diagnostic tests could be used in point-of-care facilities or in field conditions by trained personnel to provide early outbreak detection in conditions where microbiological confirmation may delay intervention or for epidemiological surveillance to inform public health authorities. In all instances, efforts to confirm diagnosis by stool culture should be continued, and case management should be performed on the basis of clinical information even before laboratory confirmation is available.

Ideal characteristics of a field-friendly diagnostic test include rapidity; low cost; ease of use; need for limited training, equipment, or supplies; reliable procurement from the manufacturer; long shelf life (≥1 year); and storage without refrigeration. An extensive review of the literature identified 24 rapid diagnostic tests developed for cholera since 1990 [10]. Many recent tests developed are based on the detection of the lipopolysaccharide of *V. cholerae* O1 and O139 by monoclonal antibodies, using the vertical-flow immunochromatography principle. These commercial membrane-based rapid diagnostic tests have been evaluated and tested under laboratory and field conditions with variable sensitivity and specificity, depending on the reference standard (Supplementary Table 1).

Although the rapid diagnostic tests have limited usefulness for the diagnosis or management of the individual patient because their sensitivity (93%–98%) and specificity (67%–96%) are less than optimal [9], in the setting of an outbreak of acute watery diarrhea, in which epidemiological and clinical evidence suggest that ≥10 persons have the same illness, the pretest prevalence of cholera among these persons will either be close to 100% or close to 0%. As the disease prevalence in the test population approaches either extreme, the overall sensitivity and specificity of the test for the diagnosis of an outbreak of cholera improve. If the cause of the outbreak is cholera, most (an estimated 80%) of the rapid test results from the 10 individual patients will be positive; if the outbreak due to another cause, the majority (an estimated 60%) of the rapid test results will be negative. The difference (≥8 positive tests vs ≤4 positive tests) should be sufficient to provide a clear indication of whether the outbreak of acute watery diarrhea is due to cholera [17].

For optimal results, rapid diagnostic tests should be performed and interpreted in accordance with manufacturer’s instructions. Fecal specimens for testing should be collected in the acute stage of illness, when pathogens are typically present in the stool in highest numbers, and before antibiotic therapy is administered. Stool specimens should be collected from a minimum of 10 persons who are suspected of being part of an outbreak and who meet the clinical criteria for cholera illness. Stool specimens should be refrigerated if possible and processed within 2 hours of collection. Although some rapid diagnostic tests have been evaluated for use with rectal swab specimens [18], manufacturer guidance for acceptable specimens should be considered. Appropriate personal protective equipment and universal safety precautions should be used when handling and testing clinical specimens. Patients’ test results should be recorded in a worksheet or notebook as a permanent record. If a test needs to be repeated because of an invalid result, record the first result (invalid), resolve the problem, and record the repeated result. Assay results should be interpreted in conjunction with available clinical information.

### Stool Transport

Regardless of the rapid test results, isolation of the bacterium is necessary for a definitive diagnosis and to have an isolate available for molecular subtyping and antimicrobial-susceptibility testing. First, in the isolation of *V. cholerae* O1 or O139, an adequate and viable patient specimen needs to be transported to the laboratory. Several transport media are available that preserve the viability of microbes including *V. cholerae* and help maintain the ratio of the microbes in the specimens during its transportation over several hours to days before the bacteriological analysis.

The semisolid consistency of Cary-Blair transport medium for *V. cholerae*, the only one recommended by the WHO [19, 20], and its high pH (8.4), makes it a medium of choice for transport and preservation of *V. cholerae*. It is inexpensive, at around $1 per bottle, and has a long shelf life when stored at 25°C. Alkaline peptone water (APW) can be used for the same purpose only when Cary-Blair medium is unavailable and should be cultured within 6 hours of collection [19]. Stuart [22] and Amies [23] transport media seem inferior to Cary-Blair medium for transport of *V. cholerae*. Stuart and Amies transport media are nonnutritious, buffered with phosphate, and provide a reduced environment because of their formulation with sodium thioglycolate, suppressing other fecal flora [21]. Organisms in the sample material are protected from drying by moisture in the transport medium (BBL CultureSwab transport medium). Sodium thioglycolate is a reducing agent that prevents oxidation and bacterial death during transport. Agar also prevents oxygenation, as well as spillage and dehydration (PML Microbiologials). Rectal swab specimens or nonchlorinated stool material that cannot be immediately cultured or must be transported should be placed in Cary-Blair medium. The efficiency of this medium in maintaining the viability of *V. cholerae*
for up to 4 weeks has been verified. In the absence of available suitable transport medium, strips of blotting paper may be soaked in rice-water stool and inserted into air-tight plastic bags. Bile peptone transport medium is an alternate nutritive, alkaline, bile-salt, tellurite-peptone, selective holding medium used as a transport medium to maintain the viability of *V. cholerae* [23] in stool specimens during delayed transmission to the laboratory. Bile peptone medium is used for the safe collection, transport, and preservation of *V. cholerae* for a prolonged period (approximately 1 week), whereas plain alkaline-peptone-water is preferred for performing a rapid diagnostic test [24, 25].

Stool transport on filter paper has been developed and evaluated by the nongovernmental medical organization Médecins Sans Frontières and Institut Pasteur [26]. A blotting paper disk (commercially available nonimpregnated paper disks, 6.35 mm in diameter, and unsterilized; Bio-Rad, Marnes-la-Coquette, France) soaked with liquid stool was placed in a screw-cap microtube, and a few drops (2–3, or 200-µL) of normal saline solution (0.9% NaCl) is added to prevent drying [27, 28]. Blotting paper should be available in most diagnostic laboratories, is inexpensive, and, because of lighter loads and smaller containers, may provide an inexpensive method of specimen transport [28]. Filter paper may be as effective as use of Cary-Blair medium for specimen transport for culture in field conditions [26, 29], but it needs to be evaluated using rectal swab specimens. Specimens collected in this way may remain viable for up to 5 weeks.

Regardless of the transport medium, stool samples should be collected in an environment free of detergent or chlorine to ensure preservation of *V. cholerae* in transport medium and, later, its growth in appropriate culture medium.

**Phenotypic Characterization**

Given the challenges regarding rapid methods and the frequent need for additional information on isolates, including antimicrobial susceptibility, epidemiological information, and extended strain characterization, culture of *V. cholerae* O1 or O139 in outbreaks remains critical. Although the majority of cases would still be clinically defined and rapid diagnosis in the field would be supported through the use of the rapid diagnostic tests described above, the role of the traditional microbiology laboratories and reference laboratory within a country remains core to outbreak management. This may have a positive impact on morbidity, mortality, and even case numbers, as earlier interventions may disrupt disease transmission.

Conversely, laboratory errors in the phenotypic identification of *V. cholerae* are rarely reported but may result in inappropriate consumption of laboratory resources and personnel [30]. Accurate identification is therefore mandatory, and a simple algorithm may be best in resource-limited settings (Supplementary Figure 1). *V. cholerae* will grow on a variety of commonly used agar media, and selective medium is recommended for the initial isolation, which should be available to all clinical and reference laboratories in Africa [19]. Many laboratories may manufacture these in-house, and if quality control procedures (see “Quality Management,” below) are followed, these media are appropriate for this purpose. Thiosulfate-citrate-bile-salts-sucrose (TCBS) agar is the selective agar of choice for culture [31], but the prepared medium has a short shelf-life of 1 week. This may artificially increase the actual cost of production above $70–$75 per 500 g of dry powder, if expired medium must be discarded.

APW is recommended as an enrichment broth (see “Stool Transport,” above). *Vibrio* species grow rapidly in APW, within 6–8 hours, and will be greater in numbers than non-*Vibrio* organisms [19]. Both media should be used for patients’ specimens, individuals with suspected asymptomatic infections, and environmental specimens and when high numbers of competing organisms are likely to be present in the specimen [19]. Clinical specimens should be processed as soon as possible after arrival in the laboratory. For isolation of *V. cholerae*, the specimen should be inoculated onto 5% blood agar, TCBS, and APW and incubated at 35°C–37°C for 18–24 hours. After incubation, colonies suspicious for *V. cholerae* will appear yellow and shiny with a diameter of 2–4 mm on TCBS agar. The yellow color is due to fermentation of sucrose (other sucrose-fermenting *Vibrio* species include *Vibrio fluvialis* and *Vibrio alginolyticus*).

Non–sucrose-fermenting organisms, *Vibrio parahaemolyticus*, *Vibrio parahaemolyticus*, *Vibrio mimicus*, and most *Vibrio vulnificus* appear as blue to green colonies on TCBS agar [32]. Phenotypic characterization of *Vibrio* is included in Supplementary Table 2 [33]. A study evaluating 6 new systems available commercially for identification of *Vibrio* species showed that Crystal E/NF correctly identified 90% of the strains [34]. For accurate identification of *V. cholerae*, Crystal E/NF (96.7%) was the best, followed by Microscan Neg ID2 (86.6%) and Vitek ID-GNB (73.3%). API 20E identified 50% of the strains [34]. Such systems may have a role in selected reference laboratories and research centers in Africa.

Serological confirmation must follow phenotypic identification (including automated systems) for reporting *V. cholerae*. *V. cholerae* O1 is confirmed by isolate agglutination in polyvalent antisera raised against the O1 antigen [35]. Antiseria cost $85–$90 for the polyvalent O1 and monovalent Ogawa and Inaba, but it keeps well. If correctly refrigerated, it may be used beyond the expiration dates on the bottle, provided that quality control has been done with known strains of *V. cholerae* O1 and O139. It may not unnecessary to purchase culture-type collection strains, which are expensive, if the laboratory has a well-characterized clinical or external quality assessment strain of *V. cholerae* O1 isolate available. Slide agglutination testing is done using 24-hour cultures from non-selective (blood) agar. Growth from TCBS may result in false-negative reactions. A drop of sterile normal saline is placed onto a clean disposable glass slide and a suspension of the culture made in the saline. If agglutination is observed in the suspension, it is termed “non–typeable.” Nontypeable isolates can be suspended in normal
saline and boiled for 15 minutes or, alternatively, subcultured and incubated for 6 hours at 35°C–37°C. If the isolate remains nontypeable in saline after repeating the serotyping procedure, the isolate should be biochemically reconfirmed as *V. cholerae*.

If no agglutination is observed in the saline suspension, equal volumes of polyvalent O1 antisera must be added. To conserve antisera, particularly in resource-limited settings, volumes as small as 10 µL can be used, and 10 µL of saline can be used for the suspension; equal volumes of antisera and growth suspension are mixed [19]. Agglutination of *V. cholerae* O1 occurs 0.5–1 minutes after tilting the slide back and forth [19]. If agglutination is seen with the polyvalent O1 antisera, 2 additional suspensions in saline are made, and monovalent Inaba and Ogawa antisera are added simultaneously to each (Supplementary Figure 1). The stronger and more rapid agglutination reaction is reported. If agglutination is observed equally in both the Inaba suspension and the Ogawa suspension, the result is referred to as “possible serotype Hikojima,” an unstable state that rarely occurs. Hikojima strains should be submitted to a reference laboratory for further examination [19].

A suspected *V. cholerae* isolate that agglutinates in O139 antisera (and has negative results after testing in *V. cholerae* O1 antisera) should be sent to a reference laboratory for confirmation. Additional testing is required for confirmation [32]. Once the organism is identified and even prior to the antimicrobial-susceptibility patterns being established, results should be transmitted as soon possible to relevant public health officials.

Because of the increasing prevalence of antimicrobial resistance, monitoring of the susceptibility of *V. cholerae* O1 and O139 to antimicrobials has become important. Antimicrobial therapy reduces the duration of illness, the volume of stool, and shedding of *Vibrio* species in the feces [32]. The cost of the illness in terms of patient management, loss of patient wages, and salaries of healthcare workers, can be substantial [34] and must be weighed against the negative impact of extensive development of multidrug resistant *V. cholerae* and other enteric pathogens [36].

A modified Kirby-Bauer antimicrobial-susceptibility test (standardized by Clinical and Laboratory Standards Institute [CLSI] guidelines) provides data that can reliably predict the in vivo effectiveness of the antibiotic, although currently none are published by the European Committee on Antimicrobial Susceptibility Testing (available at: http://www.eucast.org). Antimicrobial disks are inexpensive, costing $8 for 100 disks, but must be stored in a refrigerated environment to ensure that they retain their potency. Antimicrobial disks also need to undergo quality control (see “Quality Management,” below), and users should be aware that, because the antimicrobials in different disks may decay at different rates, regular quality control of all the disks is necessary. Antimicrobial susceptibilities should be determined for the first 30–50 isolates identified by the laboratory at the beginning of an outbreak or epidemic to provide information for treatment policy [32]. If the outbreak continues for a long time (ie, >1 month), changes in drug resistance should be monitored, particularly for drugs being used for treatment of severe cases. WHO-recommended antimicrobials for testing against *V. cholerae* are listed in Table 1. Zone size interpretative criteria for *V. cholerae* O1 and O139 have been established by the CLSI: guidelines for amoxicillin, chloramphenicol, sulfonamides, tetracycline, cotrimoxazole, and the fluoroquinolones are available [37]. Isolates tested against these drugs by disk diffusion correlate well with the minimum inhibitory concentrations determined by broth microdilution [37]. Microdilution rather than disk diffusion is recommended for doxycycline. Results from tetracycline disk diffusion can be used to predict susceptibility to doxycycline [37]. Resistance to azithromycin may be predicted using the Etest [38], which has been used previously for testing minimum inhibitory concentrations of erythromycin against *V. cholerae* [39]. Etests are expensive, costing around $300 per 100 strips; use can be limited to only those antibiotics for which no disk-susceptibility values have been established.

Mueller-Hinton agar medium is, in general, the antimicrobial-susceptibility medium validated by the CLSI for disk diffusion during antimicrobial-susceptibility testing [37], and it should also be available to diagnostic microbiology and reference laboratories in Africa. Agar plates are incubated at 35°C–37°C for 16–20 hours. Zones of growth should be compared to the zone size interpretive values, according to the CLSI guidelines [37, 40].

**Molecular Characterization**

Further characterization of *V. cholerae* strains at the molecular level is available in limited numbers of African laboratories. Laboratory capacity for molecular characterization of toxigenic
**Table 1. Antimicrobials for Testing Against Vibrio cholerae**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Disk Potency, µg</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Zone Diameter Limits, mm E. coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol*</td>
<td>30</td>
<td>≥18</td>
<td>13–17</td>
<td>≤12</td>
<td>21–27</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
<td>≥21</td>
<td>16–20</td>
<td>≤15</td>
<td>30–40</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>100</td>
<td>≥18</td>
<td>. . .</td>
<td>&lt;18</td>
<td>22–26</td>
</tr>
<tr>
<td>Tetracyclineb</td>
<td>30</td>
<td>≥15</td>
<td>12–14</td>
<td>≤11</td>
<td>18–25</td>
</tr>
<tr>
<td>ATCC 25922</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are from [37]. For azithromycin, there are currently no zone diameters; isolates would test as susceptible at a minimum inhibitory concentration of <2 µg/mL.

* Use interpretive standards with caution as the disk diffusion test may misclassify many organisms (higher minor error rate).

b The results from tetracycline disk susceptibility testing are used to predict susceptibility to doxycycline.

**V. cholerae** (serogroups O1 and O139) has become an essential requirement to support phenotypic identification of the pathogen and to assist in epidemiological and outbreak investigations internationally [41, 42]. Molecular capacity should include diagnostic and subtyping capabilities and will invariably have a domino effect on related and downstream activities, leading to enhanced turnaround times in obtaining results, more rapid and efficient epidemiological investigations, rapid implementation of intervention strategies, and, consequently, decreased morbidity and mortality in populations affected by cholera.

Molecular methods provide data that support and augment phenotypic test results; costs of these newer techniques are decreasing. African laboratories need to weigh these costs, as part of a mechanism for rapid diagnosis and outbreak, against the cost of cholera to a country [7]. Techniques for molecular diagnosis of toxigenic *V. cholerae* infection are well described in the literature, so the question is which methods to choose, considering the African laboratory setting where resources are limited. From the viewpoint and experiences of a surveillance laboratory in South Africa, the primary molecular diagnostic assay that should be available is one with the ability to detect nucleic acid sequences encoding the 2 major virulence determinants associated with toxigenic *V. cholerae*: cholera toxin and toxin-coregulated pilus (encoded by the tcpA gene) [44]. This multiplex PCR offers the added advantage of determining the *V. cholerae* O1 biotype by exploiting the sequence difference between classic biotype and El Tor biotype tcpA genes to distinguishing the two [44] (Figure 1). Conventional PCR is very affordable, with reagents and consumables for a single multiplex PCR costing approximately $4 (the cost estimate is based on use in the South African setting).

Real-time PCRs offer an alternative PCR platform to target the above described virulence determinants [46–48]. However, real-time PCR costs more (although it is still fairly affordable), with reagents and consumables for a single duplex real-time PCR costing approximately $9 (the cost estimate is based on use in the South African setting). TaqMan detection chemistry offers simple and uncomplicated real-time technology. An example of TaqMan real-time technology is described by Koskela et al [49], who simultaneously targeted the cholera toxin gene, the tcpA gene, and a regulatory gene (*toxR*) commonly found in all *V. cholerae* species. This PCR assay determines whether the organism is *V. cholerae* and whether it has outbreak potential (based on the presence of cholera toxin; Supplementary Figure 3).

Laboratories in Africa should consider having a secondary molecular diagnostic assay in place to detect DNA sequences specific for O1- and O139-antigen biosynthesis, to assist with confirmation of diagnosis of *V. cholerae* of serogroups O1 or O139. Various PCR methods have been described for this purpose [48, 50, 51]. Hoshino et al described an appropriate and uncomplicated example of the method, using conventional PCR for concurrent detection of *wbe* gene sequences specific for O1- and O139-antigen biosynthesis associated with *V. cholerae* serogroups O1 and O139, respectively [51].

Bacterial subtyping (ie, genotyping) provides a measure of the genetic relatedness of strains and is vital for accurate epidemiological investigations of bacterial infections. Techniques for subtyping toxigenic *V. cholerae* are well described in the literature [52–58]. Subtyping methods must provide data suitable for laboratory-based surveillance and outbreak detection and investigation; ideally, standardized and internationally validated
methods must be followed, to allow for interlaboratory comparison of subtyping data and global comparison of strains. Currently, 1 subtyping method possibly meets these requirements: pulsed-field gel electrophoresis (PFGE) performed according to methods standardized by PulseNet International (Figure 2). African laboratories should use PFGE analysis as their primary method to subtype toxigenic *V. cholerae* and strictly follow PulseNet protocols and methods [59]. Reagents and consumables for PFGE analysis (with single-enzyme digestion) of a single bacterial strain cost approximately $10 (the cost estimate is based on use in the South African setting).

PulseNet Africa (available at: http://www.pulsenetinternational.org/networks/Pages/africa.aspx) was established in August 2010 and is coordinated by the Centre for Enteric Diseases, National Institute for Communicable Diseases, South Africa. PulseNet Africa currently has 11 member countries, which are actively working on building and strengthening capacity for PFGE analysis of enteric pathogens in Africa and the establishment of country and regional databases of PFGE subtyping data. The PulseNet Africa network is operational and ready; submission of PFGE subtyping data for surveillance purposes and outbreak investigations is invited, and data can be compared and investigated against data in PulseNet International databases, including the PulseNet Global *V. cholerae* database hosted by the National Institute of Cholera and Enteric Diseases, India. The network encourages countries to assist one another, should any country have insufficient capacity to evaluate enteric isolates. In the past, such assistance has improved our understanding of the molecular epidemiology of cholera outbreaks in African countries, as well as in international outbreaks in which African countries may be implicated as the source [4, 41, 42].

Occasionally, PFGE subtyping data need to be corroborated and supported by alternative subtyping methods. These situations usually arise when investigations call for information about the evolutionary dynamics and genetic lineage of particular strains. Applicable subtyping methods could involve PCR-based profiling methods [60] but, more appropriately, would involve DNA sequencing of selected genes and comparison of sequence data to publicly available databases; housekeeping genes and virulence genes (*ctxAB*, *rstR*, and *tcpA*) are commonly selected for such analysis [61, 62]. Investigations may culminate with the ultimate method for analysis and comparison of strains: whole-genome-sequence analysis [63, 64]. A recent study investigated 2010–2011 *V. cholerae* O1 strains associated with the ongoing outbreak of cholera in Haiti [41, 42] and is an elegant example of the use of PFGE analysis of strains as a primary subtyping method, followed by supporting analysis using DNA sequencing methods. Because of the sophistication of these techniques, these studies are often completed at international reference laboratories. The primary goal for an African laboratory in such circumstances involves its inclusion in a network of laboratories, whether locally or abroad, that can assist in such cases. PFGE analysis of Haiti outbreak strains, for instance, showed that 1 PFGE pattern predominated; this primary pattern was shared by strains from India, Nepal, Pakistan, Oman, Afghanistan, Cameroon, and South Africa. Further analysis involving DNA sequencing of virulence genes and whole-genome-sequence analysis determined that the Haiti outbreak strain was a hybrid El Tor strain, carrying a classic cholera toxin type. The outbreak strain was genetically most closely related to strains from India and Cameroon; this work was completed by US and European laboratories [41, 42, 65]. Molecular characterization thus also benefits from quality management, to ensure that its performance meets internationally acceptable standards.

**Quality Management**

International standards [66] and guidelines [67, 68] highlight the importance of quality management in laboratory testing. The strict adherence to quality requirements produces high-quality, reliable results that are delivered in a timely fashion to

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Figure 2. Example of pulsed-field gel electrophoresis fingerprint patterns for *Vibrio cholerae* O1 strains from Africa, incorporating analysis with digestion of genomic DNA by NotI. Lanes 2–5, *V. cholerae* O1 strains; lanes 1 and 6, PulseNet reference standard (*Salmonella* Braenderup strain H9812 digested with XbaI).
health workers responsible for patient management [30]. Quality assurance procedures can be addressed by means of the following systematic 4-point plan [69].

Quality Control Plan
A quality control plan must be established to determine when, how, what, and which internal quality controls are performed for the examination procedures; to determine the acceptance and rejection criteria for internal quality control; and to determine the criteria for reviewing documented internal quality control results [66]. For internal quality control of V. cholerae O1 testing, African laboratories should retain a well-documented and known strain of V. cholerae O1. Ideally, American Type Culture Collection (available at: http://www.atcc.org) or National Collection of Type Cultures (available at: http://www.hpacultures.org.uk) strains can be used, but these may prove expensive, and a known external quality assessment strain or clinical strain may be used as an alternative (see "Phenotypic Characterization," above). For internal quality control of antimicrobial susceptibility tests, these strains are defined by the methods followed; for instance, CLSI uses American Type Culture Collection strains [37]. These may not be replaced by alternative clinical or external quality assessment strains. Documentation and review of the internal quality control results and the corrective actions taken, when results fall outside the acceptance criteria, is evidence that quality assurance objectives are being fulfilled [66]. Documenting records of laboratory consumables and reagents (eg, the date received, the date used, the lot used, and the expiration date), laboratory equipment (eg, the date in use, maintenance and servicing details, and description of malfunctions), internal quality control (to establish trends), and results (eg, the test personnel and the raw findings of analyses) are crucial to assist in developing audit trails for the traceability of a specimen and in helping the laboratory produce reliable results. Documentation of the examination procedure in the form of a standard operating procedure ensures that laboratory personnel perform procedures correctly and that consistent results are produced [67, 68].

Inventory Management
Adequate supplies of reagents and consumables is needed for reliable testing services. Laboratory reagents and consumables must be inspected on delivery for damage, correctness of items ordered, and expiration date. Reagents and consumables must be stored at the correct temperatures (as per manufacturer’s instructions). Reagents must be verified before use in the examination procedure. Reagents used in examination procedures must be identified and their lot number recorded (on worksheets or automated equipment) for traceability of the reagents [67].

Laboratory Equipment
Operational laboratory equipment is crucial to producing quality results. All equipment must be routinely maintained (daily, weekly, and monthly) and serviced (as per manufacturer’s instructions). Measuring equipment usually requires annual calibration. Equipment should be validated before it is installed for routine use in the laboratory. Laboratory equipment must be verified before use in the examination procedure, and this must be documented. Each piece of equipment requires a file that documents the history of the life of the equipment. The equipment file will contain the following information: serial number, date placed in use, dates serviced, validation report, equipment maintenance records, servicing records, and a history of equipment malfunction and corrective actions [67].

Proficiency Testing Schemes
Participation in proficiency testing schemes assists in providing external evidence of the laboratory’s technical competency. Documentation of receipt of the proficiency testing samples, testing of the samples and these results, will be used to review the overall outcomes of the scheme. Corrective actions may need to be taken, if these outcomes are not within the acceptance limits. Currently in Africa, there are very few proficiency testing schemes for V. cholerae, but a major program run from the NICD in South Africa, on behalf of the WHO Regional Office for Africa has promoted improvements in diagnostic testing capabilities and reporting of outbreaks [8]. An alternative, which may supplement existing external quality assessment plans in resource-limited African countries, involves implementing an isolate exchange program with other clinical, public health, and reference laboratories working with similar pathogens. The rationale is that participating laboratories exchange unknown isolates as “clinical” isolates or specimens. Such partnerships are most easily developed between laboratories working in close proximity to each other and in the same country, to decrease transport costs and avoid the necessity of obtaining permits to transport materials across borders and of material transfer agreements. Laboratories may alternate in sending and receiving unknown isolates, which would include V. cholerae O1. The receiving laboratory processes the isolate or specimen as it would normally, testing the criteria listed above. The receiving laboratory can document progress in dealing with such specimens (A. Sooka, personal communication). Challenges may remain, however, because of frequent shortages of selective medium and of antisera, affecting overall performance [8].

A common fallacy is that quality management is expensive, because of the need for additional laboratory consumables and resources, including the time required to perform these activities. The reality is that accurate, reproducible, and reliable results save time because of the need to repeat tests due to laboratory errors, facilitate outbreak management because of improved turnaround times, and decrease the overall costs of outbreak management and response in the event of a suspected outbreak, resulting in public health savings within the country.
DISCUSSION

Ongoing challenges remain in Africa in the laboratory diagnosis of *V. cholerae*, although certain countries in Africa are recognized emerging economies and may thus be developing infrastructure and resources for both phenotypic and genotypic identification of outbreaks of *V. cholerae* O1 infection. The minimum requirement for rapid diagnosis of cholera outbreaks necessitates reliable specimen transport and phenotypic identification, which may be supported by the use of rapid diagnostic tests and molecular characterization. Despite attempts at interventions, most African countries struggle to retain expertise, access laboratory equipment and consumables, and maintain full capabilities to diagnose this disease. This is compounded by major natural disasters and internal strife [70–73], situations in which cholera is frequently seen on the continent and that distinguish African outbreaks from those seen in highly cholera-endemic areas, such as the Indian subcontinent. Ironically, it is often in association with these situations, that diagnostic challenges become magnified, as resources are stretched even further [71, 72]. Political will and support from senior decision makers in health departments within the country is mandatory to promote diagnostic and reference laboratory functions, ensuring the health of the African population. Ultimately, cholera is an international disease in Africa, requiring international solutions, including cross-border cooperation during epidemics, intercountry assistance with outbreak investigation and control, and the willingness to openly share data with the international community.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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