Cholera was first detected in Papua New Guinea in July 2009, caused by *Vibrio cholerae* O1 El Tor serotype Ogawa. By late 2011, 15,500 cases had been reported throughout lowland Papua New Guinea with a case fatality rate of 3.2%. The epidemic has since slowed, with only sporadic cases reported in Western Province and the Autonomous Region of Bougainville (ARB). Accurate and timely diagnosis is a critical element of the public health response to cholera, yet in low-income countries where the burden of cholera is the greatest, diagnostic services are often limited. Here we report on the diagnostic challenges and the logistical factors that impacted on diagnosis during the first reported outbreak of cholera in Papua New Guinea.

The Port Moresby General Hospital (PMGH) laboratory is the only laboratory in Papua New Guinea that routinely conducts bacterial culture for diagnostic purposes. When cholera spread from the remote outbreak epicentre in rural Morobe Province to the provincial capital (Lae), bacterial culture was re-established at the provincial hospital in Lae (culture had not been conducted for many years due to limited funding and declining infrastructure). The disease spread to six other lowland provinces of Papua New Guinea and ARB where it was not feasible to re-establish culture facilities in a time frame that could have assisted with cholera diagnosis. Instead, specimens were sent by plane to the PMGH laboratory. Rapid diagnostic tests (RDTs) were not recommended by the National Department of Health; however, some Provincial Health Offices used RDTs locally during the outbreak.

Swabs were prepared from stool samples of patients older than five years of age with acute watery diarrhoea (AWD) (with no documented recent exposure to antibiotics) and placed in Cary-Blair transport medium. Culture was conducted following standard bacterial methods. In brief, enrichment was conducted using alkaline peptone water (6–12 hours at 37°C) then plated onto TCBS agar (37°C for 24 hours). Direct inoculation of samples onto TCBS agar was also conducted. Confirmation was done by biochemical profiling (API 20E, bioMerieux, Marcy-l’Étoile France) and serology to determine biotype and serotype. In total, 678 samples were analysed at PMGH from 17 of Papua New Guinea’s 20 provinces, with 331 (49%) being culture positive. Data are not available regarding the number of samples tested in Lae and bacterial culture has not been sustained at that site.

It is accepted that “prompt and accurate diagnosis of *Vibrio cholerae* is a key step in cholera outbreak surveillance that can greatly influence rapid intervention and prevention to minimize disease spread and mortality.” However, tracking the spread of cholera throughout Papua New Guinea and confirming cases in cholera-naïve regions was a long process. The remote location of some outbreak sites, the lack of roads linking with Port Moresby and the inability to conduct culture at nearby hospital laboratories delayed the confirmation time of cases. It often took three to four days to collect samples from the outbreak area and deliver them to PMGH. At least two days were required for confirmation of a culture-
positive result, resulting in delays of up to one week from the time when cholera was first suspected in a previously unaffected area of the country to confirmation. Patients with suspected cholera were treated empirically, following standardized rehydration algorithms, so delayed diagnosis did not impact on treatment. However, the time to diagnosis may have delayed public health responses aimed at reducing the spread of cholera within an outbreak area.

The World Health Organization (WHO) recommends laboratory confirmation (by culture) for the first 10–20 cases of suspected cholera. WHO also recommends that a few samples be taken during an outbreak to monitor antimicrobial sensitivity and about 20 stool samples tested to confirm the end of the outbreak (all should be culture negative). In Papua New Guinea, samples were collected and cultured sporadically during an outbreak in a new district, and no outbreaks were confirmed to have ended through culture. This opportunity was largely missed, as the added demands created by the cholera outbreak in Papua New Guinea stretched laboratory capacity to the limit. Confirming the end of the outbreaks would have enabled provincial governments to close cholera treatment centres in a timely manner, thus saving money and resources.

Although culture remains the mainstay of laboratory diagnosis for cholera, it may fail to detect many true cases. In a recent study of cholera in Bangladesh, 131/135 (97%) stool samples were deemed to be *V. cholerae* positive using a combination of culture, RDTs, direct fluorescent antibody detection, polymerase chain reaction or detection of lytic phage using a plaque assay; however, only 86 (64%) of positive samples were culture positive. The inability of culture to detect all cases of cholera may be a contributing factor to the <50% isolation rate of *V. cholerae* in Papua New Guinea. Moreover, it is difficult to ascertain the impact of storage and transportation of samples on the viability of *V. cholerae* during the outbreak. Improved laboratory capacity in major regional centres would ensure Papua New Guinea is better prepared to manage future epidemics while also aiding diagnosis of high-burden endemic infectious diseases.

The 49% culture-positive rate in samples sent to PMGH is comparable to rates of detection previously reported. The remaining 51% of AWD cases fulfilled the case-definition for cholera (in the context of a cholera outbreak) but did not have a definitive culture result. As the burden of other enteric infections is high in Papua New Guinea, it is possible that people with different diarrhoeal illnesses presented to health care facilities out of fear of having cholera. In the future, full etiological studies using culture and molecular techniques should be considered on a subset of samples to better understand the spectrum of pathogens associated with outbreaks of AWD in Papua New Guinea.

Improved diagnostic tools are required for the diagnosis of *V. cholerae* in low-income countries. At least two different RDTs were used during the Papua New Guinea outbreak (*Cholera Ag O1*, Standard Diagnostics Inc. Kyonggi province, Republic of Korea and SMART II, New Horizons Diagnostic, Corp., Columbia, Maryland, USA), but their use was neither widespread nor systematic. While RDTs are generally considered easy to use, during the early stages of the outbreak, junior clinical and laboratory staff (who were not trained to perform RDTs) falsely interpreted the first 20 test kits as negative and did not collect stool samples for culture. Although a rapid clinical outbreak response was initiated early and appropriately, the misinterpretation of cholera RDTs may have delayed its laboratory confirmation and highlights the need for adequate training when using RDTs before their introduction into the country.

Although RDTs may be useful, the role of bacterial culture should not be overlooked in low-income countries. Culture remains the gold standard for diagnosis of many bacterial infections. The isolation and preservation of clinical isolates can enable important public health data to be obtained, e.g. surveillance of antimicrobial drug resistance in bacteria. In a country of approximately 7 million people, with a high burden of infectious diseases and lack of transport infrastructure, one laboratory equipped to conduct bacterial culture is insufficient. The core function of the PMGH laboratory is routine diagnosis; the need to respond to the cholera epidemic was a strain on the capacity of the laboratory. Outbreak response and ongoing surveillance might be better suited to the central public health laboratory in Papua New Guinea. Large regional hospitals in Papua New Guinea should be equipped with culture facilities.

Increased capacity in bacterial culture is unlikely to occur in Papua New Guinea in the foreseeable
future; thus complementary tests should be considered to aid diagnosis. The currently available cholera RDTs have not gained widespread acceptance, but despite their shortcomings, they may have a role to play in cholera diagnosis. RDTs should not be considered as a replacement for culture but may be a useful adjunct to diagnosis by culture.\textsuperscript{3,11} Timely and accurate diagnosis leads to better patient outcomes, better public health responses and better epidemiological data; all of these were suboptimal in the Papua New Guinea cholera outbreak. Adequate planning and investment in resources at the national level would ensure Papua New Guinea and other countries in the Western Pacific Region are better situated to respond to future cholera outbreaks.

Conflicts of interest

None declared.

Funding

None.

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