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The emergence of non-epidemic *Vibrio cholerae* O1 ST75 in South Africa

Cholera is a potentially fatal acute diarrhoeal disease resulting in large volumes of watery stool, causing rapid dehydration that can progress to hypovolaemic shock and metabolic acidosis. Every year, an estimated 3-5 million people worldwide contract cholera, with ~100 000 deaths. Cholera is caused by toxin-producing *Vibrio cholerae* serogroups O1 or O139. *V. cholerae* is a highly motile, comma-shaped Gram-negative bacterium with a single polar flagellum. It has more than 200 serogroups, based on the O-antigen of lipopolysaccharide. The serogroup O1 is classified into two biotypes (biological variants), termed classical and El Tor; each biotype is subdivided into two serotypes, Ogawa and Inaba.

Over the last 200 years, cholera has spread globally beyond Asia seven times, resulting in seven cholera pandemics. The first six pandemics (which began in 1817, 1829, 1852, 1863, 1881, and 1889 respectively) were caused by the classical biotype of *V. cholerae* serogroup O1. The seventh cholera pandemic (which is still ongoing) began in 1961, appearing first in Indonesia and subsequently spreading to South Asia, Africa, South America and the Caribbean islands. It is caused by *V. cholerae* serogroup O1 of the El Tor biotype, and named 7PET; it has been identified through whole-genome sequencing as *V. cholerae* sequence type 69 (ST69). The seventh pandemic continues to be a major public health threat for 175 countries in Asia, Africa and the Americas. In late 1992, *V. cholerae* serogroup O139 (Bengal) caused large outbreaks of cholera in India and Bangladesh; but never spread out of Asia and is now seldom isolated. Recently, there has been increased recognition of the role that non-01 and non-O139 serogroups may be playing in diarrhoeal disease.

The first laboratory-confirmed cholera case in South Africa was reported in 1974. South Africa is not considered endemic for cholera; outbreaks are typically associated with importation events, particularly from neighbouring countries. Large outbreaks have previously occurred in three provinces (Mpumalanga, Limpopo, and KwaZulu-Natal) caused by importation events from neighbouring countries, particularly Zimbabwe and Mozambique. The last cholera outbreak in South Africa was initiated by an importation of cases from a large outbreak in Zimbabwe during 2008. From November 2008 to April 2009, >12000 cases and 65 deaths were reported nationally, primarily from Mpumalanga and Limpopo provinces. Since the 2008-2009 outbreak, very few cases have been identified. From 2010 through 2014, five cases of cholera were reported (most proven to be imported), and from 2015 through 2017 no cases were identified.

Cholera is a category 1 notifiable medical condition in South Africa. All *V. cholerae* isolates (human and environmental) identified at private sector and NHLS laboratories in South Africa, are submitted to the Centre for Enteric Diseases, National Institute for Communicable Diseases (NICD), for further investigation. The case definition for confirmed cholera is the isolation of *V. cholerae* O1 or O139 from a person with diarrhoea.

From February 2018 through January 2020, the NICD received a total of 102 *V. cholerae* isolates for testing, of which nine were identified as *V. cholerae* O1. The isolates were phenotypically and genotypically characterised, including whole-genome sequencing (WGS), comparative genomics and phylogenetic analysis.

Of the nine *V. cholerae* O1 isolates tested, two isolates were identified as ST69 (7PET lineage) and seven as ST75. The ST69 isolates were recovered from two patients with cholera in a family cluster in October 2018. The index case-patient had travelled to Zimbabwe, where a cholera outbreak was ongoing, within the incubation period (7 days) before onset of symptoms. These ST69 isolates were confirmed as belonging to the highly resistant outbreak strain identified during the 2018 cholera outbreak in Zimbabwe. The seven ST75 isolates originated from KwaZulu-Natal and Limpopo provinces. Five isolates were recovered from patients with cholera, and two isolates were recovered from environmental samples collected during two of the case investigations (on-site sewage in Limpopo Province, and river water in KwaZulu-Natal Province, respectively). Case-patients were adults 37-57 years of age. The three cases in KwaZulu-Natal Province were located ~200-600 km apart, the first occurring in February 2018 and the last in January 2020. The 2 cases in Limpopo Province were located in the same district ~70 km apart and both occurred in November 2018. The cases in Limpopo Province were ≥900 km from the cases in KwaZulu-Natal Province. Epidemiological investigations included interviewing case-patients using a standardised case investigation form, visiting their places of residence to inspect water and sanitation services and interview other household members, collection of stool samples from household members and collection of environmental samples when indicated. There was no evidence of importation from another country, no epidemiological links between cases, no secondary transmission and no evidence of increased diarrhoea cases in local clinics and hospitals in the respective districts. Phenotypic characterisation of the O1 ST75 strains confirmed toxigenic *V. cholerae* 01 serotypes Ogawa and Inaba. All seven O1 ST75 isolates showed susceptibility to all antimicrobials tested, in contrast to African 7PET isolates which are reported to have become increasingly antimicrobial-resistant over time.

Further phylogenetic analysis showed the South African O1 ST75 isolates to be very closely related to each other but split into two clusters based on province of origin, with Limpopo Province isolates differing from the KwaZulu-Natal isolates by 4-5 alleles on core-genome multilocus sequence typing (cg-MLST). On single nucleotide polymorphism (SNP) analysis, all isolates clustered into a previously defined L3b.1 clade (Figure 1). Further comparison of the South African O1 ST75 isolates with a larger global collection of O1 ST75 of closely related genomes showed the closest relationship with isolates collected from the Russian Federation in 2005 and 2011. The L3b.1 isolates from

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Taiwan and China were genetically more distant (Figure 1).

The first *V. cholerae* O1 ST75, the US Gulf Clone, was identified in the United States in 1973. Since then clinical and environmental isolates have been identified in multiple countries in several regions including South America, Europe, Asia and South East Asia. Cases due to *V. cholerae* O1 ST75 are reported to be primarily sporadic, although small clusters have been reported (usually household transmission) and the isolates are typically drug susceptible. This is the first report of *V. cholerae* O1 ST75 isolates from Africa.

Recent reports from Taiwan and Argentina provide further insight into the epidemiological characteristics of O1 ST75. Taiwan investigated the epidemiology of cholera during 2002-2018 and described the emergence of an ST75 clone in 2009. This clone has since become more prevalent than the O1 ST69 clone from a previous pandemic. Sixty-one of the sixty-three ST75 cases were sporadic; two were part of a family cluster. Closely related ST75 strains had been identified in China and two other South East Asia countries suggesting the ST75 clone may be spreading more widely in Asia. Argentinian scientists describe that during the cholera epidemic of 1992-1998, the epidemic clone (7PET lineage) co-existed alongside highly diverse non-7PET *V. cholerae* strains. Four of sixty-five non-7PET isolates sequenced were serogroup O1 and two were members of the Gulf Coast lineage of O1. The authors suggest that these non-7PET strains lack the propensity to cause epidemics and do not pose the same relative risk to public health as 7PET, and that this difference should be accounted for in epidemic preparedness responses.

The findings from South Africa align with those from Taiwan in that ST75 cholera cases now outnumber ST69 cholera cases. In addition, in keeping with observations in the literature, all South African O1 ST75 cases have been sporadic, with no secondary cases and no demonstrable epidemiological links. No associated outbreaks occurred, even when the strains were present in surface water sources used by multiple vulnerable communities with very poor WaSH (safe water, sanitation and hygiene). The O1 ST75 isolates were found across large spatial and geographical distances, suggesting local spread.

The emergence and dominance of non-epidemic (non-7PET) *V. cholerae* O1 ST75 in South Africa has major implications for the public health response to cholera cases. The level of public health response must be commensurate with the risk of outbreak, and WGS and cgMLST will need to be expedited to guide the response. Ongoing WGS of all clinical and environmental *V. cholerae* isolates is essential to describe the dynamics of O1 ST75 in South Africa and to identify emergence of other non-7PET strains.

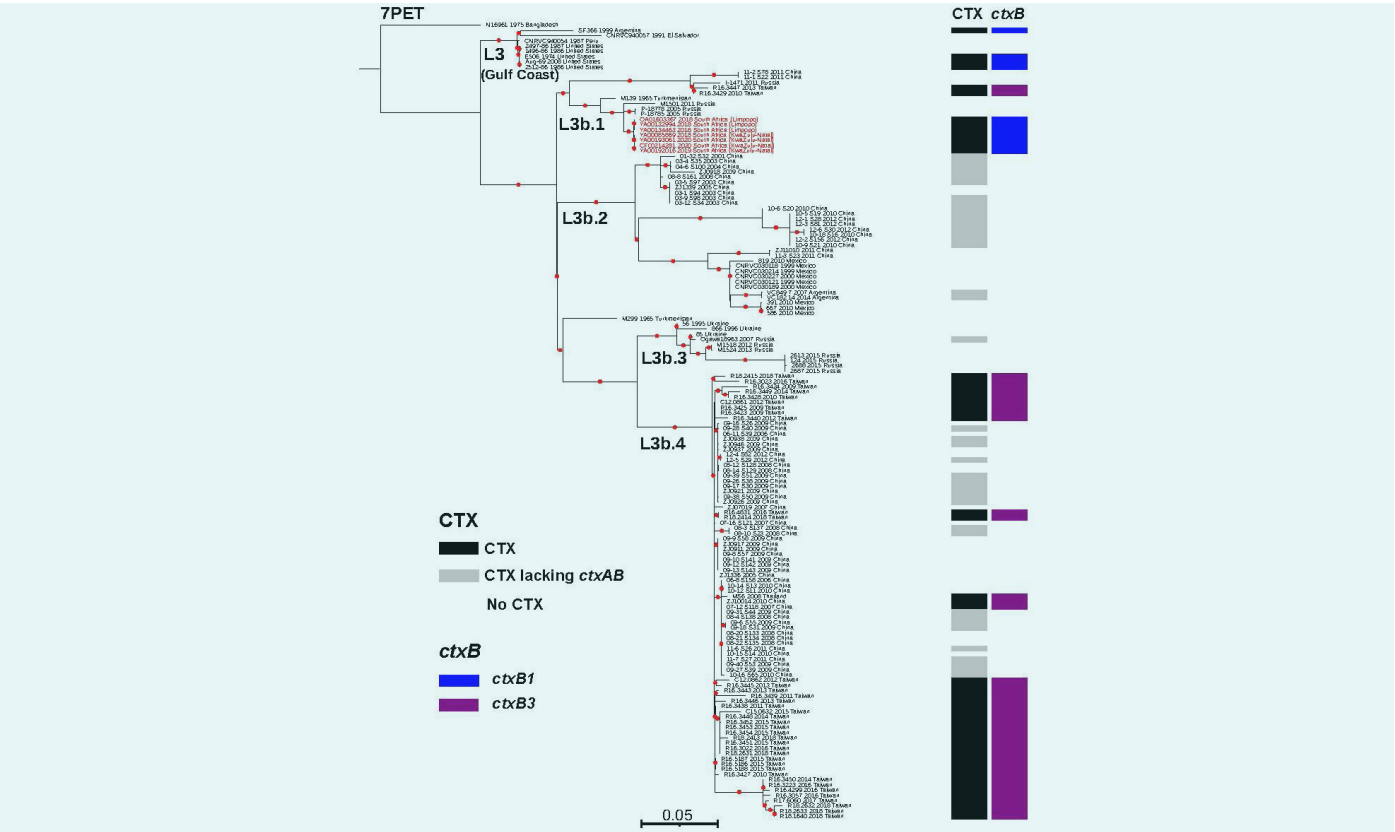

Figure 3. Phylogenomics of *V. cholerae* O1 ST75 isolates from South Africa, 2018-2020. Maximum likelihood phylogeny for 151 ST75 (or closely related STs) and one ST69 *V. cholerae* O1 genomic sequences. The seventh pandemic *V. cholerae* O1 El Tor (7PET) genome N16961 (ST69) was used as an outgroup. For each genome, its name, year (when known), and country of isolation (plus province of isolation for the South African isolates) are orderly shown at the tips of the tree. The genomes from South Africa are highlighted in red. The lineages, presence of the CTX prophage or its variant form, types of *ctxB* allele are also shown. The 7PET outgroup genome, N16961 contains CTX with an *ctxB3* allele (not represented in the figure). Bootstrap values greater than or equal to 95% are shown at the branch of the nodes as a red dot. The scale bar denotes substitutions per variable site (SNVs).

Source: Centre for Enteric Diseases, NICD-NHLS; junot@nicd.ac.za