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Widespread epidemic cholera caused by a restricted subset of *Vibrio cholerae* clones

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Abstract

Since 1817, seven cholera pandemics have plagued humankind. As the causative agent, *Vibrio cholerae*, is autochthonous in the aquatic ecosystem and some studies have revealed links between outbreaks and fluctuations in climatic and aquatic conditions, it has been widely assumed that cholera epidemics are triggered by environmental factors that promote the growth of local bacterial reservoirs. However, mounting epidemiological findings and genome sequence analysis of clinical isolates have indicated that epidemics are largely unassociated with most of the *V. cholerae* strains in aquatic ecosystems. Instead, only a specific subset of *V. cholerae* El Tor 'types' appears to be responsible for current epidemics. A recent report examining the evolution of a variety of *V. cholerae* strains indicates that the current pandemic is monophyletic and originated from a single ancestral clone that has spread globally in successive waves. In this review, we examine the clonal nature of the disease, with the example of the recent history of cholera in the Americas. Epidemiological data and genome sequence-based analysis of *V. cholerae* isolates demonstrate that the cholera epidemics of the 1990s in South America were triggered by the importation of a pathogenic *V. cholerae* strain that gradually spread throughout the region until local outbreaks ceased in 2001. Latin America remained almost unaffected by the disease until a new toxigenic *V. cholerae* clone was imported into Haiti in 2010. Overall, cholera appears to be largely caused by a subset of specific *V. cholerae* clones rather than by the vast diversity of *V. cholerae* strains in the environment.

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Cholera is an acute diarrhoeal infection caused by the bacterium *Vibrio cholerae*, which provokes disease exclusively in humans. Since 1817, seven cholera pandemics have plagued humankind. Although the infection is often mild or asymptomatic, some patients present with profuse watery diarrhoea and vomiting [1]. If left untreated, severe cases show a massive loss of bodily fluids that can quickly result in dehydration, hypovolaemic shock, and death [2]. The clinical symptoms of the disease are directly associated with the major virulence factors cholera toxin (CTX) [1] and toxin co-regulated pilus, the latter being essential for bacterial colonization of the intestine [3]. Upon colonization of the small intestine, the bacterium produces and releases CTX, a protein complex composed of one A subunit and five B subunits [4]. Once CTX binds to epithelial cells, a portion of the A subunit is internalized [1,5] and subsequently induces constitutive cAMP production [6,7]. Increased cAMP production causes excessive secretion of water and electrolytes into the lumen of the small intestines and, in acute cases, eventually provokes the severe diarrhoea recognized as cholera [1,6,7].

A diverse spectrum of *V. cholerae* strains flourish in the aquatic environment. The bacterium is found in brackish and estuarine waters, either as planktonic bacilli or associated with a wide range of flora and fauna [8]. Elevated water temper-

atures, copepod blooms and rainfall have been suggested to correlate with elevated concentrations of *V. cholerae* in the environment [9–12]. Conversely, *V. cholerae* levels are controlled in the ecosystem by bacteriophages and predation by bacterivorous protozoa [13–15]. A study in Bangladesh has shown that increased concentrations of certain bacteriophages targeting toxigenic *V. cholerae* coincided with a decreased concentration of the corresponding *V. cholerae* serogroup in environmental water samples [16]. As this phenomenon was also associated with a reduced number of locally reported cholera cases, it has been suggested that bacteriophages may influence the course of cholera outbreaks [17] and the emergence of new *V. cholerae* clones.

Cholera has been extensively portrayed as a prototypical waterborne disease. Some studies, primarily conducted in the Bay of Bengal, have revealed links between cholera outbreaks and fluctuations in climatic and aquatic conditions [11,12,16]. These observations have led many experts in the field to conclude that cholera epidemics are triggered by exposure to local reservoirs of *V. cholerae* [18], driven directly by environmental factors that promote bacterial growth in water bodies [19]. However, the mechanisms of cholera outbreaks around the Bay of Bengal are poorly understood, perhaps because of the lack of large-scale spatial and temporal studies conducted in the area. In fact, in contrast to sub-Saharan African countries, Bangladesh and India are reluctant to report suspected cholera cases. Furthermore, there is little evidence that blooms of toxigenic strains occur in aquatic environments prior to epidemics; therefore, toxigenic *V. cholerae* present in water sources may be derived from patients with diarrhoea. Seasonal cholera epidemics in the Bay of Bengal may also be associated with other factors such as rainfall and drought, which can promote the contamination of drinking water with patient-derived toxigenic *V. cholerae*. Recent studies examining the evolution of a variety of *V. cholerae* strains responsible for the current pandemic have provided novel insights into cholera epidemiology. In the current article, we show that mounting epidemiological findings and genome sequence analysis of clinical isolates indicate that cholera epidemics are largely unassociated with most of the *V. cholerae* strains found in aquatic ecosystems. Indeed, the current evidence demonstrates that only a specific subset of *V. cholerae* 'types', which have become increasingly specialized in interhuman transmission, have triggered epidemics in the current cholera pandemic. Moreover, the current pandemic can be largely attributed to *V. cholerae* strains derived from a single ancestral clone that has spread globally in successive waves.

Surveys of *V. cholerae* strains derived from clinical and environmental samples have shown the species to be highly diverse, comprising >200 serogroups [1,20]. However, only serogroups O1 and O139, a derivative of serogroup O1 that was first identified in 1992, cause cholera epidemics [20–23]. Although serogroup O1 is widespread throughout affected regions, serogroup O139 is almost exclusively restricted to Asia [21].

Pandemic *V. cholerae* O1 strains can be divided into two distinct biotypes: 'classical' and 'El Tor'. The classical biotype was responsible for at least the sixth cholera pandemic and reputedly more [1,24]. Indeed, a recent study has applied targeted high-throughput sequencing to reconstruct the genome of a *V. cholerae* isolate recovered from the intestine of a victim of the 1849 Philadelphia cholera outbreak (i.e. the second pandemic). According to these results, this O1 isolate showed 95–97% genetic similarity with the classical *V. cholerae* O395 genome [25]. The El Tor biotype has been described as the causative agent of the seventh pandemic, which emerged in 1961 in the Sulawesi Archipelago [1,26]. Recent high-definition genomic evidence (see below) and isolated historical records indicate the Bay of Bengal as a major hub linking the spread of cholera around the globe during the 19th and early 20th centuries. To understand the underlying phylogeny of the lineage responsible for the current pandemic, Mutreja *et al.* have performed a genome-wide high-resolution marker analysis by defining single-nucleotide polymorphisms (SNPs) in the core genome of 123 seventh pandemic strains, using a pre-seventh pandemic strain (M66) as an outgroup to root the constructed phylogenetic tree. Genomic elements including SNPs in probable genomic islands were excluded, as these regions are mobile and therefore show different phylogenies from that of their bacterial host. As recombination can also hamper the ability to obtain an accurate phylogeny, these SNPs were also excluded from the final phylogenetic analysis. This work on the core genome sequence of *V. cholerae* O1 El Tor unequivocally demonstrated that the current pandemic is monophyletic and originated from a single clone that is very distinct from the classical strains of the previous pandemic. Phylogenetic analysis of the seventh pandemic strains also shows that *V. cholerae* has continually evolved in South Asia, and spread around the globe in at least three independent but overlapping waves. The three waves share a common ancestor dating back to the 1950s [22].

The evolution of toxigenic *V. cholerae* has been characterized by the acquisition, loss and rearrangement of specific mobile genetic elements that play a major role in causing cholera epidemics [22]. Strains responsible for the sixth pandemic harboured certain mobile elements specific to the classical biotype: the *tcpA* gene, the CTX core genes (i.e. *ctxA* and *ctxB*), and RS elements (*rstR*, *rstA*, and *rstB*), which play a role in the replication and chromosomal integration of CTXΦ, the active lysogenic bacteriophage that carries the CTX genes