The Lake Chad Basin, an Isolated and Persistent Reservoir of Vibrio cholerae O1: A Genomic Insight into the Outbreak in Cameroon, 2010

Kaas, Rolf Sommer; Ngandjio, Antoinette; Nzouankeu, Ariane; Siriphap, Achiraya; Fonkoua, Marie-Christine; Aarestrup, Frank Møller; Hendriksen, Rene S.

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The prevalence of reported cholera was relatively low around the Lake Chad basin until 1991. Since then, cholera outbreaks have been reported every couple of years. The objective of this study was to investigate the 2010/2011 *Vibrio cholerae* outbreak in Cameroon to gain insight into the genomic make-up of the *V. cholerae* strains responsible for the outbreak. Twenty-four strains were isolated and whole genome sequenced. Known virulence genes, resistance genes and integrating conjugative element (ICE) elements were identified and annotated. A global phylogeny (378 genomes) was inferred using a single nucleotide polymorphism (SNP) analysis. The Cameroon outbreak was found to be clonal and clustered distant from the other African strains. In addition, a subset of the strains contained a deletion that was found in the ICE element causing less resistance. These results suggest that *V. cholerae* is endemic in the Lake Chad basin and different from other African strains.

**Introduction**

Cholera is a serious and potential life-threatening waterborne communicable disease caused by *Vibrio cholerae* [1,2]. The disease is associated with poor water quality and inadequate sanitation, [3] and is transmitted by the fecal-oral route [4,5]. Cholera outbreaks are commonly reported and often related to collateral damage of natural disasters or flooding [6–9]. However, asymptomatic healthy carriers have also been observed, mainly infected with the El Tor biotype [10].

*V. cholerae* produces the hallmark of cholera; the enterotoxin: CTXφ and is classified into approximately 200 serogroups of which O1 and O139 are mostly associated with clinical cases.
and have the potential to cause endemic cholera [11–14]. The serogroup O1 can be further subdivided into two biotypes; Classical and El Tor, but recently, variants of those biotypes have been reported [15,16]. In addition, each of the biotypes also display two distinct serotypes; Inaba and Ogawa [13].

Since 1817, when cholera spread from the Indian sub-continent, seven pandemics have been observed. In 1961, the seventh pandemic began in Southeast Asia caused by O1 El Tor [14,17–20]. Whole genome sequence (WGS) analysis has identified eight distinct phyletic lineages; the lineages have been named L1-L8. Lineages L1 and L3-L6 represent the former pandemics and L2 represents the current, seventh, El Tor pandemic. Lineages L7 and L8 are formed by unique isolates [19].

In 2013, a total of 129,064 cholera cases were reported to the World Health Organization, of which, 43% came from Africa. However, due to underreporting and insufficient surveillance data, the true global burden is estimated to be significantly higher, from 1.4 to 4.3 million cases, and 28,000 to 142,000 deaths per year (http://www.who.int/gho/epidemic_diseases/cholera/cases_text/en/).

In this study, it was of interest to determine the genetic relatedness of contemporary clinical O1 El Tor V. cholerae isolates originating from the 2010 outbreak in Cameroon by a phylogenetic analysis based on WGS. The study also includes an analysis of the occurrence of antimicrobial resistance genes and the mechanisms hereof. In addition, we want to elucidate if Lake Chad basin is an isolated and persistent reservoir of cholera by temporally comparing the surrounding countries’ outbreak data and spatially available genomic data in a global context.

Methods

Country outbreak data

The number of annual cholera cases per country was obtained from WHO (http://apps.who.int/globalatlas/dataQuery/). The database was queried for the total number of cases between 1970 and 2012, for each of the four countries surrounding Lake Chad (Cameroon, Chad, Niger, and Nigeria). The case numbers were imported into Excel and a stacked area plot was created (accumulated values).

Samples and bacterial isolates

Twenty-four O1 El Tor V. cholerae isolates were obtained from stool samples collected from patients diagnosed with cholera between 2010 and 2011 in Cameroon.

Antimicrobial susceptibility testing

The 24 O1 El Tor V. cholerae isolates were tested for antimicrobial susceptibility to ampicillin (AMP), azithromycin (AZI), cefotaxime (FOT), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MERO), nalidixic acid (NAL), sulfamethoxazole (SMX), ceftazidime (TAZ), tetracycline (TET), tigecycline (TGC), and trimethoprim (TMP). The testing was performed by broth microdilution to determine minimum inhibitory concentration (MIC) with a commercially prepared panel of dehydrated antimicrobials (Sensititre; TREK Diagnostic Systems Ltd., East Grinstead, England). The MIC results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints [21], except for tigecycline, for which the clinical breakpoint was used according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org). The Escherichia coli ATCC 25922 was used as reference strain for quality control, according to CLSI guidelines [21].
Whole genome sequencing

Genomic DNA was extracted from the 24 O1 El Tor *V. cholerae* isolates using an Invitrogen Easy-DNA™ Kit (Invitrogen, Carlsbad, CA, USA), and DNA concentrations were determined using the Qubit dsDNA BR assay kit (Invitrogen). The genomic DNA was prepared for Illumina using the Illumina (Illumina, Inc., San Diego, CA) NexteraXT® Guide 150319425031942 following the protocol revision C (http://support.illumina.com/downloads/nextera_xt_sample_preparation_guide_15031942.html). A sample of the pooled NexteraXT libraries was loaded onto an Illumina MiSeq reagent cartridge, using MiSeq Reagent Kit v2 and 500 cycles with a Standard Flow Cell. The libraries were sequenced using an Illumina MiSeq platform and MiSeq Control Software 2.3.0.3.

All 24 isolates were paired-end sequenced and ranged in insert size from 371 to 498 with an average of 427. The read coverage of the sequences was between 122X and 232X with an average of 187X.

Raw sequence data has been submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under study accession no.: PRJEB13614 (http://www.ebi.ac.uk/ena/data/view/PRJEB13614). A complete list of genomic sequence data is available in the S1 Table.

The raw data was trimmed and cleaned for adapters using AdapterRemoval v. 1.1 (https://github.com/slindgreen/AdapterRemoval) before any analysis was performed.

VelvetK (http://bioinformatics.net.au/software.velvetk.shtml) was applied to each set of cleaned and trimmed data to estimate the k parameter for the following Velvet assembly, the k that provided a k-mer coverage closest to 20X.

VelvetOptimiser v. 2.2.5 (http://bioinformatics.net.au/software.velvetoptimiser.shtml) was used to test the range of the parameter k for each isolate and choose the optimal assembly. The range of k was set to the previously estimated k value +20 and -20 with a maximum of k = 99 and a minimum of k = 15. Velvet v. 1.2.07 [22] was used by VelvetOptimiser to do the actual *de novo* assemblies.

Identification in silico of *V. cholerae*, antimicrobial resistance genes, SXT element, and the class 1 integron

The assembled sequences were analyzed to identify the species-specific gene (*ompW*), serogroup-specific genes (*rfbV*-O1, *wbfZ*-O139), the biotypes-specific genes (*ctxB*, *rstR*, *tcpA*), the MLST sequence type (ST) for *V. cholerae*, and the acquired antimicrobial resistance genes using the web-server MyDbFinder 1.0 with a selected threshold equal to 95% identity (https://cge.cbs.dtu.dk/services/MyDbFinder/) and the bioinformatic tool MLST (version 1.7) and ResFinder (version 2.1, 80% threshold for %ID/ 60% minimum length) available from Center for Genomic Epidemiology (CGE) [23,24].

The SXT element, the class 1 integron, and the presence of mutations in the DNA gyrase (*gyrA* gene) and in the DNA topoisomerase IV (*parC* gene) were determined using MyDbFinder. The nucleotide sequence of the integrase gene of the SXT element (*intSXT* gene, AF099172) and the class 1 integron (*intI* gene, EU436855), and *gyrA*, and *parC* genes in the quinolone-resistant *V. cholerae* strains (GQ502315, KJ596550, and GQ502316) in GenBank were used as references.

Phylogenetic structure of *V. cholerae* using single nucleotide polymorphisms

In order to put the genomic data from Cameroon in a global context, raw read data and assembled genomes from 352 *V. cholerae* strains were obtained from the European Nucleotide
Archive (ENA) and GenBank, respectively. Only lineage 2 genomes belonging to the seventh El Tor pandemic were included in this investigation and only datasets that contained sufficient meta-data (collection date and location), however, there were a few exceptions (older strains missing location information).

Single Nucleotide Polymorphisms (SNPs) from the 24 O1 El Tor \textit{V. cholerae} genomes from Cameroon as well as the 352 global \textit{V. cholerae} strains were determined using the pipeline CSI Phylogeny 1.1 [25] available from the CGE website (https://cge.cbs.dtu.dk/services/all.php). Full genomic information is shown in S1 Table. Specific SNP information for the Cameroon strains is presented in S2 Table.

Briefly, the raw reads obtained from each isolate were mapped to the published completed reference strain N16961 (Acc. No.: NC_002505.1) using BWA version 0.7.2 [26]. SNPs were called using the ’mpileup’ module in SAMTools version 0.1.18 [27]. Subsequently, SNPs were selected when meeting the following criteria: (i) a minimum distance of 10 bps between each SNP, (ii) a minimum of 10% of the average depth and at least 10X, (iii) a mapping quality greater than 30, (iv) a SNP quality greater than 25, (v) the position of the SNP was significantly covered in all the analysed isolates, (vi) in cases with contradicting calls, the called base must obtain a Z-score of at least 1.96 (corresponding to a p-value of 0.05), and (vii) all indels were excluded. The qualified SNPs from each genome were concatenated to a single pseudo-alignment.

For assembled genomes, Nucmer was used to align the contigs to a reference and call SNPs. The “show-snps” (with options “-CIlrT”) application was used to retrieve the SNPs. Both of these applications are part of the software package MUMmer v. 3.23 [28].

Maximum likelihood trees were created using FastTree [29]. FastTree was compiled with the accuracy alterations suggested by Aaron Darling (http://darlinglab.org/blog/2015/03/23/not-so-fast-fasttree.html). The non-lineage 2 strain M66-2 (Acc. No.: CP001233.1) was used to root the final tree.

Results

Epidemiological country data

The emergence of the seventh cholera pandemic started around the Lake Chad basin in 1971 and led to more than 22,931, 9,265, and 8,230 cases in Nigeria, Niger, and Chad, respectively. In contrast, the 1971 outbreak affected Cameroon far less with only 2,167 cases http://www.who.int/cholera/countries/en/ (Fig 1). Between 1971 and 1991, only a few sporadic cases and minor outbreaks were reported from the Lake Chad basin, except for a single, larger isolated outbreak in Niger in 1984. A major outbreak among the four countries was observed in 1991; it was the largest outbreak ever recorded in Nigeria. Unfortunately, any information related to the cause seems to be unpublished. This outbreak coincides with the first emergence of atypical El Tor variants in the Ganges Delta area and could potentially be related due to the genomic clustering between genomes from Cameroon and the sub-Indian continent (Fig 2) [30]. Ever since, cholera has become endemic in the Lake Chad basin following a pattern of cross-border transmission among the four countries with large coinciding outbreaks reported from the area in the years 1996, 1998/1999, and 2004/2005/2006. In 2009, the latest coinciding outbreak in the region started and intensified in 2010/2011, affecting 41,787 people in Nigeria. In Cameroon and Chad, the outbreak likewise progressed with a total of 17,121 and 4,410 cases reported, respectively. In Niger, Chad, and Cameroon, the outbreak was the worst since 1971. The outbreak peaked in Nigeria (44,456 cases) in 2010, in Chad (17,267 cases) and Cameroon (22,433 cases) in 2011, and Niger (5,284 cases) in 2012 (Fig 1).
Antimicrobial resistance, antimicrobial resistance genes, the class 1 integron and the SXT element

The MIC determination and the corresponding antimicrobial resistance genes and amino acid substitutions of the 24 V. cholerae isolates revealed two different profiles with 19 isolates resistant to and harboring the following substitutions/genes: ciprofloxacin—two amino acid substitutions in gyrA (Ser83Ile) and parC (Ser85Leu); sulphonamides (sul2); streptomycin (strA/strB); florfenicol (floR); chloramphenicol (catB9); and trimethoprim (dfrA1); and five isolates only resistant to the latter two antimicrobials and corresponding genes (Fig 3). Of the five less-resistant isolates, four isolates (#278_1A, #278_8A, #281_8A, #355_1A) originated from Douala situated close to the sea, and one isolate (#285_11A) originated from Foumban, closest to Lake Chad (Fig 4). Interestingly, all isolates carried the integrating conjugative element (ICE) variant corresponding to ICEVchInd5 [31] with the notable difference that the five less-resistant isolates revealed a gap from approximately bp. position 8600 to 19400 in the ICE fragment excluding the four resistance genes; sul2, strA, strB, and floR (Fig 5). The ICE variant was found using a new bioinformatic tool under development for typing of V. cholerae determined “VcTypeFinder”, which will be freely available online from the CGE website in 2016. The ICE variant result was confirmed with BLAST.

Serogroup, serotype, biotype, MLST, population structure of V. cholerae based on SNPs and genomic elements

All 24 V. cholerae strains belonged to the O1 serogroup, the atypical El Tor biotype, the MLST type ST-69 and harbored the ctxB7 toxin (found in silico).

Overall, 61 different SNPs were found among the Cameroon strains with the genome #342_10A from Douala being substantially different from the others (Fig 3). The closest
neighboring genome to #342_10A differed with 25 SNPs (#322_3E, Yaounde, 2010). Interestingly, the 25 SNPs were all singletons and only found in the genome #342_10A (S2 Table). The 25 SNPs, in general, do not seem to cluster, and from the reference genome annotation they do not seem to be located in mobile elements (S3 Table). The mutS gene was inspected to assess the presence of hypermutators but seemed functional. However, if the 25 SNPs were excluded from the analysis, #342_10A would be identical to #322_3E. In comparison, the greatest pairwise distance found between any of the other genomes were 17 SNPs. Thus, the outbreak isolates were all quite closely related and the phylogeny suggests the outbreak to be clonal. The less resistant sub-clones seemed to disappear in the beginning of the outbreak. However, it was not possible from the phylogenetic analysis to conclude whether the sub-clone was the original outbreak clone or a derivation of the more resistant sub-clone.

To establish a global perspective, the genomes from the Cameroon strains were compared with 352 *V. cholerae* genomes available from the European Nucleotide Archive (ENA) and GenBank, including African genomes originating from Kenya, Tanzania, Zambia, Malawi, Zimbabwe, Mozambique, South Africa, Djibouti, and Benin (Fig 2). The genomes from

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**Fig 2. The inferred phylogeny based on SNPs for the Cameroon isolates.** Each isolate is presented with a strain name followed by the city of origin and year of isolation. The vertical lines divide the isolates into two groups, representing the two different resistance profiles. The resistance genes that differ are marked in brown.

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Cameroon and the one from Benin branched out from a clade consisting of genomes from the Sub-Indian continent and Nepal, whereas other genomes originating from Africa formed their own unique clade or were scattered among genomes of a global origin (Fig 2). Thus, confirming that the clone responsible for the Cameroon outbreak in 2010/2011 was an isolated clade in comparison with other African strains presented in the phylogenetic tree.

**Discussion**

Not a lot of scientific typing data on cholera are available from the investigated region or in general from Africa. However, the outbreak around the Lake Chad basin in 2010 was

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**Fig 3.** Location of *Vibrio cholerae* cases in Cameroon and around Lake Chad basin. Red dots in Cameroon indicate the location of the cities from where the isolates originate.

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**Discussion**

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**Fig 4.** A graphical representation of the ICE fragment ICEVchInd5. The arrows represent the genes. The numbers indicate bp. position on the ICE fragment, with position 1 starting at the first bp of the fragment. The brown arrows represent the genes that were missing from the less-resistant sub-clone.

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Fig 5. The Cameroon outbreak in a global perspective and phylogeny. Fig A. Map of Africa presenting the origin of the sequenced *V. cholerae* from the African continent. Green indicates Cameroon and Benin and brown indicates the rest of Africa. Fig B. Global phylogeny inferred from SNPs of 376 *V. cholerae* lineage 2 isolates. The origin of the majority of isolates in each cluster is indicated by the vertical lines to the right of the figure. The tree is rooted on the non-7th pandemic strain M66-2. Ten branches have been shortened to fit the figure; this is indicated with a small gap flanked by vertical lines near the end of the branch.

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previously believed to be caused by a multi-drug resistant atypical El Tor O1 \textit{V. cholerae} strain similar to the Indian Orissa variant [32–35], which was confirmed in this study.

To date, only a few strains from the 2010 outbreak have been characterized by pulsed field gel electrophoresis (PFGE). The strains that have been characterized, generally show similar PFGE patterns and relationships to the ones from the 1971 outbreak [36]. In addition, only a single strain from Cameroon has been WGS in order to investigate possible links to the outbreak in Haiti in 2010 [37]. The true link between the reservoirs and countries requires a more advanced and appropriate genetic analysis such as a SNP analysis using WGS [38], as applied in this study to determine the clonality of the strains originating from the 2010/2011 outbreak in Cameroon. The topology of the global phylogenetic analysis performed in this study was in agreement with previous global studies of \textit{V. cholerae} [19, 39]. This study showed that the clone responsible for the Cameroon outbreak in 2010/2011 was a single introduction and formed an isolated clade in comparison with other African strains presented in the global phylogenetic tree. This analysis combined with the epidemiological data from WHO support the hypothesis that \textit{V. cholerae} is endemic to this area of Africa, and the Lake Chad basin is an isolated reservoir for cholera. To verify this hypothesis, isolates including also potentially stored historical strains from the other countries around Lake Chad should be obtained and sequenced. Unfortunately, the stability and laboratory capacity of these countries continues to be fragile, hampering sampling efforts.

There seems to be several major drivers responsible for the transmission of cholera and for it being endemic in the Lake Chad basin. Typically, the spread of cholera is caused by rain and flooding in endemic areas [32]. However, this seems not to be the case around the Lake Chad basin because several outbreaks have occurred in the dry seasons. However, several studies have reported outbreaks in areas of severe morbidity and mortality caused by multiple factors such as poor sanitation, contaminated potable water, fecal-contaminated wells (due to run-off), travel, and trade [40–42]. This could potentially explain why minor clonal differences were observed in the genomes from Cameroon such as the loss of gene cassettes from the ICE fragment and how strains from different cities were related.

The antimicrobial susceptibility data and corresponding antimicrobial resistance indicated the presence of the SXT integrase harbored in the ICE. Previous studies from countries around the Lake Chad basin and of the same time period reported similar \textit{V. cholerae} isolates resistant to trimethoprim (\textit{dfr}A1), sulfonamides (\textit{sul}2), nalidixic acid, and reduced susceptibility to ciprofloxacin caused by amino acid substitutions in the DNA gyrase, \textit{gyr}A (Ser83Ile) and the DNA topoisomerase IV, \textit{par}C (Ser85Leu) [34,36] which correspond well to the data of this study. In addition, resistance was also reported to chloramphenicol (\textit{flo}R), ampicillin, and \textit{str}A and \textit{str}B in other studies [36,43]. These findings support the local phylogeny as well as the perception that cholera in the Lake Chad basin has been persistent and an isolated reservoir for decades.

The decline in WGS cost coupled with the growing availability of user-friendly free bioinformatic tools, such as the tools used in this study (CSI Phylogeny, MLST, ResFinder [23–25], and VcTypeFinder in development) enables researchers to do fast characterizations and globally link cholera outbreaks, enhancing the ability to control and understand \textit{V. cholerae} in the future.

In conclusion, the Cameroon outbreak was found to be clonal with a single introduction. The genomes of the isolates were in general found to be very similar. However, in the ICE, which was found to be of the ICEVchInd5 variant, a deletion was found in a small subset of the isolates; the deletion caused these isolates to be less resistant. The results presented in this study along with the case reports obtained from WHO supports the hypothesis that \textit{V. cholerae} is endemic to the Lake Chad basin. It further suggests that it is clonal and different from other
African *V. cholerae*, this however needs to be further investigated by inclusion of more and historical strains.

**Supporting Information**

S1 Table. Supplementary_table1-Sequence_info. (XLSX)

S2 Table. Supplementary_table2-Cameroon_SNPs. (XLSX)

S3 Table. Supplementary_table3-Cameroon_SNP_annotations. (XLSX)

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**Author Contributions**

Conceived and designed the experiments: RSK RSH. Performed the experiments: A Ngandjio A Nzouankeu AS RSK MCF. Analyzed the data: AS RSK RSH FMA. Contributed reagents/materials/analysis tools: A Ngandjio A Nzouankeu AS RSK MCF. Wrote the paper: A Ngandjio A Nzouankeu AS RSK MCF RSH FMA.

**References**

1. McLeod SM, Kimsey HH, Davis BM, Waldor MK (2005) CTXphi and *Vibrio* *cholerae*: exploring a newly recognized type of phage-host cell relationship. Mol Microbiol 57: 347–356. PMID: 15978069
2. Harris JB, LaRocque RC, Qadri F, Ryan ET, Calderwood SB (2012) Cholera. Lancet 379: 2466–2476. doi: 10.1016/S0140-6736(12)60436-X PMID: 22748592
3. Mengel MA, Delrieu I, Heyerdahl L, Gessner BD (2014) Cholera outbreaks in Africa. Curr Top Microbiol Immunol 379:117–44. doi: 10.1007/82_2014_369 PMID: 24827501
4. Sugimoto JD, Koepeke AA, Kenah EE, Halloran ME, Chowdhury F, Khan AI, et al. (2014) Household Transmission of *Vibrio* *cholerae* in Bangladesh. PLoS Negl Trop Dis 8: e3314. doi: 10.1371/journal.pntd.0003314 PMID: 25411971
5. Harris JB, LaRocque RC, Qadri F, Ryan ET, Calderwood SB (2012) Cholera. Lancet 379: 2466–2476. doi: 10.1016/S0140-6736(12)60436-X PMID: 22748592
6. de Magny GC, Thiaw W, Kumar V, Manga NM, Diop BM, Gueye L, et al. (2012) Cholera outbreak in Senegal in 2005: was climate a factor? PLoS One 7: e44577. doi: 10.1371/journal.pone.00044577 PMID: 22952995
7. Rebaudet S, Sudre B, Faucher B, Piarroux R (2013) Environmental determinants of cholera outbreaks in inland Africa: a systematic review of main transmission foci and propagation routes. J Infect Dis 208 Suppl 1:S46–54. doi: 10.1093/infdis/jit195 PMID: 24101645
8. Shah MA, Mutreja A, Thomson N, Baker S, Parkhill J, Dougan G, et al. (2014) Genomic epidemiology of *Vibrio* *cholerae* O1 associated with floods, Pakistan, 2010. Emerg Infect Dis 20: 13–20. doi: 10.3201/eid2001.130428 PMID: 24378019
9. Hendriksen RS, Price LB, Schupp JM, Gillece JD, Kaas RS, Engelthaler DM, et al. (2011) Population Genetics of *Vibrio* *cholerae* from Nepal in 2010: Evidence on the Origin of the Haitian Outbreak. MBio 2 (4). pii: e00157-11.
10. Nair GB, Qadri F, Holmgren J, Svennerholm AM, Safa A, Bhuiyan NA, et al. (2006) Cholera due to altered El Tor strains of *Vibrio* *cholerae* O1 in Bangladesh. J Clin Microbiol 44: 4211–4213. PMID: 16957040
11. Chatterjee SN, Chaudhuri K (2003) Lipopolysaccharides of *Vibrio* *cholerae*. I. Physical and chemical characterization. Biochim Biophys Acta 1639: 65–79. PMID: 14599113
12. Bhattacharya MK, Bhattacharya SK, Garg S, Saha PK, Dutta D, Nair GB, et al. (1993) Outbreak of *Vibrio* *cholerae* non-O1 in India and Bangladesh. Lancet 341: 1346–1347.
13. Kaper JB, Morris JG Jr., Levine MM (1995) Cholera. Clin Microbiol Rev 8: 48–86. PMID: 7704895
14. Moore S, Thomson N, Mutreja A, Piarroux R (2014) Widespread epidemic cholera caused by a restricted subset of Vibrio cholerae clones. Clin Microbiol Infect 20: 373–379. doi: 10.1111/1469-0691.12610 PMID: 24575898
15. Safa A, Nair GB, Kong RY (2010) Evolution of new variants of Vibrio cholerae O1. Trends Microbiol 18: 46–54. doi: 10.1016/j.tim.2009.10.003 PMID: 19942436
16. Nair GB, Faruque SM, Bhuiyan NA, Kamruzzaman M, Siddique AK, Sak D (2002) New variants of Vibrio cholerae O1 biotype El Tor with attributes of the classical biotype from hospitalized patients with acute diarrhea in Bangladesh. J Clin Microbiol 40: 3296–3299. PMID: 12202569
17. Cho YJ, Yi H, Lee JH, Kim DW, Chun J (2010) Genomic evolution of Vibrio cholerae. Curr Opin Microbiol 13: 464–465. doi: 10.1016/j.mib.2010.08.007 PMID: 20851041
18. Chun J, Grims CJ, Hasan NA, Lee JH, Choi SY, Haley BJ, et al. (2009) Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic Vibrio cholerae. Proc Natl Acad Sci U S A 106: 15442–15447. doi:10.1073/pnas.0907787106 PMID: 19720995
19. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH, Kariuki S, et al. (2011) Evidence for several waves of global transmission in the seventh cholera pandemic. Nature 477: 462–465. doi:10.1038/nature10392 PMID: 21866102
20. Clinical and Laboratory Standards Institute (2014) Performance Standards for Antimicrobial Susceptibility Testing. 24th Informational Supplement.
21. Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18: 821–829. doi:10.1101/gr.074492.107 PMID: 18349386
22. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. (2012) Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50: 1355–1361. doi:10.1128/JCM.06094-11 PMID: 22238442
23. Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agerso Y, Lund O, et al. (2012) Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. J Antimicrob Chemother.
24. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760. doi:10.1093/bioinformatics/btp324 PMID: 19451168
25. Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 5: e9490. doi: 10.1371/journal.pone.0009490 PMID: 20224823
26. Kim EJ, Lee CH, Nair GB, Kim DW (2015) Whole-genome sequence comparisons reveal the evolution of Vibrio cholerae O1. Trends Microbiol 23: 479–489. doi: 10.1016/j.tim.2015.03.010 PMID: 25913612
27. Ceccarelli D, Spagnolletti M, Bacci D, Nin-Poleg Y, Mendiatta DK, Kashi Y, et al. (2011) ICEVchInd5 is prevalent in epidemic Vibrio cholerae O1 El Tor strains isolated in India. Int J Med Microbiol 301: 318–324. doi: 10.1016/j.ijmm.2010.11.005 PMID: 21276749
28. Piarroux R, Faucher B (2012) Cholera epidemics in 2010: respective roles of environment, strain changes, and human-driven dissemination. Clin Microbiol Infect 18: 231–238. doi: 10.1111/j.1469-0691.2012.03763.x PMID: 22285640
29. Goel AK, Jiang SC (2010) Genetic determinants of virulence, antibiogram and altered biotype among the Vibrio cholerae O1 isolates from different cholera outbreaks in India. Infect Genet Evol 10: 815–819. doi: 10.1016/j.meegid.2009.06.022 PMID: 19580888
30. Quilici ML, Massenet D, Gake B, Bwaiki B, Olson DM (2010) Vibrio cholerae O1 variant with reduced susceptibility to ciprofloxacin, Western Africa. Emerg Infect Dis 16: 1804–1805. doi: 10.3201/eid1611.100568 PMID: 21029554
35. Cartwright EJ, Patel MK, Mbopi-Keou FX, Ayers T, Haenke B, Wagenaar BH, et al. (2013) Recurrent epidemic cholera with high mortality in Cameroon: persistent challenges 40 years into the seventh pandemic. Epidemiol Infect 141: 2083–2093. doi: 10.1017/S0950268812002932 PMID: 23290586

36. Marin MA, Thompson CC, Freitas FS, Fonseca EL, Aboderin AO, Zailani SB, et al. (2013) Cholera outbreaks in Nigeria are associated with multidrug resistant atypical El Tor and non-O1/non-O139 Vibrio cholerae. PLoS Negl Trop Dis 7: e2049. doi: 10.1371/journal.pntd.0002049 PMID: 23459673

37. Reimer AR, Van DG, Stroika S, Walker M, Kent H, Tarr C, Talkington D, Rowe L, Olsen-Rasmussen M, Frace M, Sammons S, Dahourou GA, Boncy J, Smith AM, Mabon P, Petkau A, Graham M, Gilmour MW, Gerner-Smidt P (2011) Comparative genomics of Vibrio cholerae from Haiti, Asia, and Africa. Emerg Infect Dis 17: 2113–2121. doi: 10.3201/eid1711.110794 PMID: 22099115

38. Orata FD, Keim PS, Boucher Y (2014) The 2010 cholera outbreak in Haiti: how science solved a controversy. PLoS Pathog 10: e1003967. doi: 10.1371/journal.ppat.1003967 PMID: 24699938

39. Didelot X, Pang B, Zhou Z, McCann A, Ni P, Li D, et al. (2015) The role of China in the global spread of the current cholera pandemic. PLoS Genet 11: e1005072. doi: 10.1371/journal.pgen.1005072 PMID: 25768799

40. Adagbada AO, Adesida SA, Nwaokorie FO, Niemogha MT, Coker AO (2012) Cholera epidemiology in Nigeria: an overview. Pan Afr Med J 12:59. Epub Jul 2. PMID: 22937199

41. Akoachere JF, Omam LA, Massalla TN (2013) Assessment of the relationship between bacteriological quality of dug-wells, hygiene behaviour and well characteristics in two cholera endemic localities in Douala, Cameroon. BMC Public Health 13:692. doi: 10.1186/1471-2458-13-692 PMID: 23895357

42. Akoachere JF, Mburntcha CK (2014) Water sources as reservoirs of Vibrio cholerae O1 and non-O1 strains in Bepanda, Douala (Cameroon): relationship between isolation and physico-chemical factors. BMC Infect Dis 14:421. doi: 10.1186/1471-2334-14-421 PMID: 25073409

43. Akoachere JF, Masalla TN, Njom HA (2013) Multi-drug resistant toxigenic Vibrio cholerae O1 is persistent in water sources in New Bell-Douala, Cameroon. BMC Infect Dis 13:366. doi: 10.1186/1471-2334-13-366 PMID: 23919373