Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: December 31, 2022

Citation: Akinsinde K. A., Sack D. A., et al., 2022. Genotypes of *Vibrio cholerae* isolates from Nigeria show a founder-flush pattern, Medical Research Archives, [online] 10(12). https://doi.org/10.18103/mra.v10i12.3411

DOI: https://doi.org/10.18103/mra.v10i12.3411

ISSN: 2375-1924

RESEARCH ARTICLE

Genotypes of *Vibrio cholerae* isolates from Nigeria show a founder-flush pattern

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ABSTRACT

Introduction: We analyzed five variable tandem repeats in order to establish genotypes for toxigenic *Vibrio cholerae* O1 patient isolates from Nigeria collected by the Nigerian Institute of Medical Research between 2016 to 2021.

Findings: Forty distinct variable tandem-repeat genotypes were observed among the 144 Isolates. There were two large clonal complexes of related genotypes that differed by change at a single locus. The locus with the most alleles was VCA0283. Each genotype is represented by the number of repeats at each of the five loci, in order. The first clonal complex was observed from 2016 to 2019, but not afterwards. A single genotype of the first clonal complex was observed in 2016, 5 genotypes in 2017, 6 in 2018 including 2 previously observed in 2017 and 1 in 2019. The second clonal complex was observed only in 2021. Three genotypes - 8,4,6,18,y where y = 33, 34 or 35 – were observed in isolates collected at multiple locations. These genotypes were observed in 8, 7 and 7 of the 11 outbreaks, respectively. All three genotypes were present in Gombe, Plateau and Jigawa. Only one outbreak did not have any of these three genotypes. 15 of 18 other genotypes were unique to a location. In Gombe, Katsina, Kebbi, Lagos, Niger, Plateau and Taraba, an isolate with a founder genotype (8,4,6,18,y) was collected prior to isolates with genotypes unique to that site.

Discussion: The genotypes of *V. cholerae* isolates collected from the outbreaks of cholera follow a *founder-flush* pattern. One or more genotype(s) [the founders] give(s) rise to multiple other genotypes as the outbreak expands and spreads [the flush] over time and space in Nigeria. Our data and analyses are consistent with the founder-flush phenomena.

Keywords: cholera, MLVA, founder-flush, Nigeria, surveillance, Africa, *Vibrio cholera*

Introduction

The founder-flush phenomenon describes a pattern of genetic evolution during an outbreak, in which a founder genotype(s) occur(s) at the beginning of a population expansion and during the subsequent increase in population size of the pathogen, novel genotypes related to the founder genotypes occur and become established in the population. The initial description of this phenomenon was in butterflies[1] and subsequently has been applied to *Vibrio cholerae* outbreaks in India[2] and Mozambique[3]. This concept is similar to the understanding of the development of genetic variants for SARS-CoV-2[4].

Cholera outbreaks are common in Nigeria. Table 1 shows the number of cholera cases and cholera deaths reported to the World Health Organization for the ten-year period from 2012 to 2021. As noted, these numbers have varied from year to year but the years 2018 and 2021 were years with an unusually large number of cases and deaths.

Genotypes are used to create diagrams of genetic relatedness. One genotyping method relies on variable tandem repeats at specific loci to define the genotype for each isolate. The genotype is the number of repeated units at specific loci in order. The degree of genetic relatedness determined by analyses of variable tandem repeat loci can correlate closely with the relatedness found by whole genome sequencing[5] and may avoid distortion of the genetic relatedness pattern that is potentially introduced by recombination[6, 7].

Table 1. Number of cholera cases and cholera deaths reported to the World Health Organization from Nigeria between 2012 and 2021.

Year	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Number of cases	597	6,600	35,996	5,290	768	12,174	45,047	2,486	596	111,062
Number of deaths	18	229	755	186	32	288	836	43	47	3,604

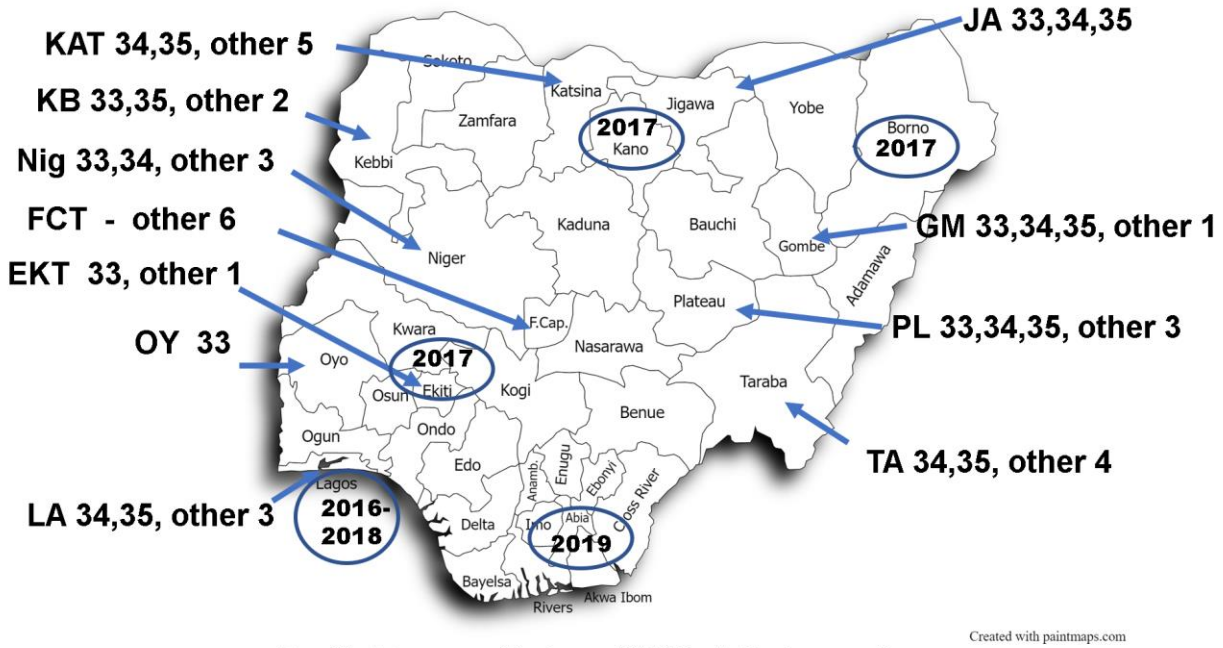
Variable tandem repeat analyses have been used to describe transmission events and the genetic relatedness of outbreaks during multiple outbreaks in Africa. In Kenya, multiple genetic lineages were circulating simultaneously and revealed that specific lineages were found at specific geographic locations[8]. In Tanzania, the analyses revealed that i) two distinct genetic lineages of *V. cholerae* caused independent outbreaks in a single location in different months of the same year; and ii) one genetic lineage caused outbreaks at geographically distinct locations in the same year. The results were confirmed by whole genome sequence analyses[9]. In Zambia, the analyses revealed that sequential outbreaks in Lusaka were due to three separate invasions of novel genetic lineages rather than from *V. cholerae* surviving from the previous outbreak[7].

In this study, we used variable tandem-repeat analyses to explore the genetic relatedness of isolates collected during 2016 through 2021 in Nigeria.

Methods.

The Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria collects *V. cholerae* serotype O1 isolates during cholera outbreaks wherever these occur in Nigeria in their role as a reference laboratory. Some of these isolates were made available for additional genetic studies. Strains were available from one state in 2016 (Lagos), from three states in 2017 (Kano, Borno and Lagos), from one state in 2018 (Lagos), from one state in 2019 (Abia), and from eleven states in 2021 when cholera was widespread in Nigeria (Figure 1).

Figure 1. **Map of Nigeria with outbreak locations.** For the years 2016-2019, the state where collections were made occurs within the oval along with the date, while in 2021, each location is identified by an arrow and an abbreviation (see Table 2). The numbers 33, 34 and 35 identify the founder genotypes at that location and number of other genotypes is also listed.



The isolated colonies were subcultured in broth at the NIMR and the broth cultures were placed on filter paper and allowed to dry. The dried filter paper samples were then sent to Johns Hopkins University in Baltimore where the extracted DNA from the filter paper samples were confirmed to be toxigenic *V cholerae* by PCR[10]. The DNA from the filter paper samples were then assayed for MLVA as described[11, 12]. Briefly, five loci (VC0147, VC0436-7 (intergenic), VC1650, VCA0171 and VCA0283) were assayed by PCR amplification. The size of the amplified product was measured using LIZ600 internal lane standards on ABI automatic sequencers. The size was converted to numbers of repeat units

to determine the allele size and the five alleles were the genotype[11, 12].

Results.

V. cholerae isolates were collected from 2016 to 2019 and 2021. Six from Lagos in 2016, 38 in 2017 from Lagos, Kano and Borno, 13 in 2018 from Lagos, one in 2019 from Abia, and 86 from 11 states in 2021 (Figure 1). Among the 144 isolates, 40 genotypes were identified. There were two large clonal complexes of related genotypes that differed by changes at a single locus (Figures 2 & 3). Each genotype is represented by the allele size at each of the five loci, in order. Genotypes that differ at a single locus are connected by line. The locus with the most alleles was VCA0283. In contrast, VC1650 was not variable.

The two genotypes found in equal frequency in Lagos in 2016 were related to genotypes in subsequent years. One genotype founded the first clonal complex, it was related by single allelic changes to two genotypes found in 2017 and to three additional genotypes by one additional change. Two of the 2017 genotypes were found in 2018, as were four more genotypes. Notably, the second genotype in 2016 was also observed in 2018 and was related to another genotype in 2019 by a single allelic change. No isolates were assayed by MLVA in 2020.

In 2021, the year with the largest number of cases, 27 distinct MLVA genotypes were observed among the 86 isolates. The observed genotypes are displayed in Figure 3. Seven of the genotypes differ solely at the VCA0283 and are shown around an oblong oval indicating that each individual genotypes could be derived from any of the other genotypes on the oval.

Figure 2. **Genotypes of isolates collected in 2016 to 2019 forming the first clonal complex.** The letters indicate the location (see Table 2). The question mark indicates an alternative explanation of how the 2019 genotype was derived.

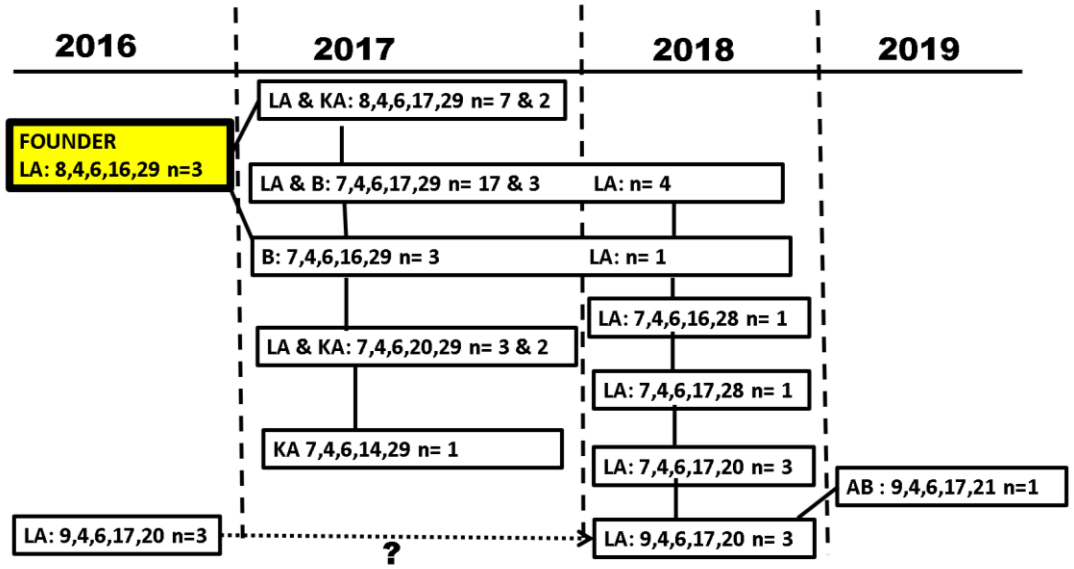
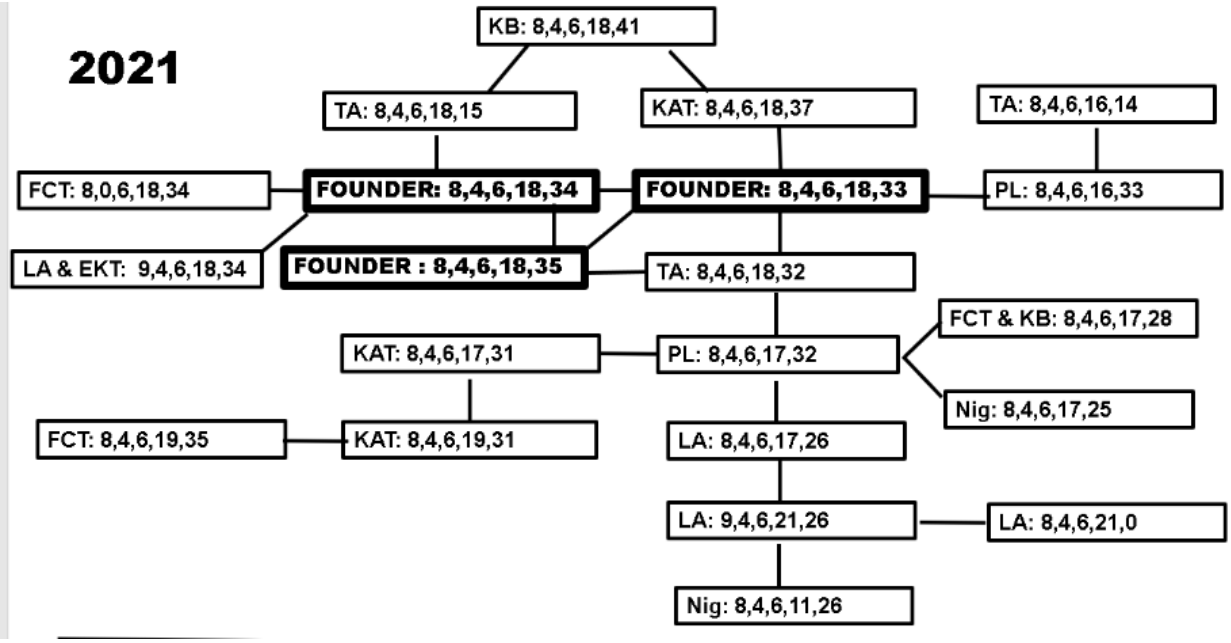


Figure 3. **Genotypes identified for isolates in 2021 composing the second clonal complex.** The letters indicate the location (see Table 2).



Worth noting is that three genotypes - 8,4,6,18,y where y = 33, 34 or 35 – were observed in isolates collected at multiple locations. These genotypes were observed in eight, seven and seven of the eleven outbreaks, respectively (Table 2). All three genotypes were present in Gombe, Plateau and Jigawa. Only one outbreak (Federal City) did not have any of these three genotypes among the isolates that were collected. Of the other genotypes, 15 of 18 (83%) were unique to a location.

Also, worth noting is that in Gombe, Katsina, Kebbi, Lagos, Niger, Plateau and Taraba, an isolate with a common genotype (8,4,6,18,y) was collected prior to isolates with genotypes unique to that site. In Jigawa and Oyo, only common genotypes (8,4,6,18,y) were observed, while in the Federal City, only isolates with derived genotypes were observed. Thus, the common genotypes were observed prior to the other genotypes in 7 of 8 (87%) of the provinces in which a test could be made.

Finally, there were seven 'singleton' genotypes that had alleles that differed from all other genotypes at 2 or more loci. These are considered unrelated to the other genotypes.

Table 2. **Founder and non-founder genotypes at each location in 2021.**

	Number of isolates with allele at VCA0283 (aka y) = 33, 34, 35			
State	y=33	y=34	y=35	Non-founder genotypes
Ekiti	0	2	0	1
Federal City	0	0	0	6
Gombe	1	2	3	1
Jigawa	2	3	2	0
Katsina	0	1	3	5
Kebbi	3	0	2	2
Lagos	0	3	1	3
Niger	2	2	0	3
Oyo	3	0	0	0
Plateau	2	3	1	3
Taraba	0	2	1	4

Discussion.

Our data are consistent with the founder-flush phenomenon in which a founder genotype occurs at the beginning of a population expansion and during the expansion (aka the outbreak), novel genotypes related to the founder genotypes occur.

In 2016 to 2019, the founder-flush phenomenon was observed over four years, chiefly in Lagos, with some spread to other provinces. One genotype in 2016 was related to all five of the genotypes occurring in 2017 and all six occurring in 2018. Thus, the genotype from 2016 was considered the founder and the genotypes in 2017 and 2018 to be the result of the flush. In 2021, the *V. cholerae* isolates collected from the outbreaks of cholera in Nigeria were genetic diverse with 21 MLVA genotypes among 86 isolates. The three most frequently observed genotypes were 8,4,6,18,y where y =33, 34 or 35. These genotypes were observed in multiple outbreaks (Table 2). These three genotypes were considered the founders (Figure 3). Only one outbreak did not have any of these three genotypes among the isolates that were collected. Eighty-three percent of the other genotypes were unique to one location. Fifteen of the other 18 genotypes that were related to 8,4,6,18,y by allelic changes at a single locus are considered to have arisen at their location. The three genotypes that were

found in more than one location could have arisen in both locations or could have spread from one location to the other.

Worth noting is that the genotype: 8,4,6,21,0 (0 because it did not amplify), occurred six times and every time it was found in an isolate from Lagos. That all six isolates were independent and were from the same location, serves as an internal replication that confirms the result. Thus, we interpret 8,4,6,18,y to be the founder genotypes and the rest of the related genotypes to result from the population flush occurring during the outbreak(s).

A previous study using 'whole' genome sequencing using strains from West Africa found that most of the isolates were from the 12th Transmission event from South Asia to Africa[13]. The genomic sequences that were analyzed did not identify subgroups. The tandem repeats were not included in the analysis; however, an application that determines the repeat lengths from sequence data exists[14].

Additionally, there were seven unrelated genotypes that may be related one of the clonal complexes, although we did not detect an intermediate genotype or be from another genetic lineage. Other surveys have observed isolates that were unrelated to the local epidemic isolates.

Our sampling is very limited at any one location (range=3 to 15) and genotypes may have been missed at any given location. One potential example is the genotype (9,4,6,17,20) that was seen in Lagos in 2016 and 2018, but not 2017. It could be that isolates with that genotype existed in 2017 but were not collected; or it could be that isolates with that genotype were indeed extinct in 2017, and then a novel lineage with the same genotype arose in 2018. The first explanation is far simpler than the second. The first possibility is represented by the dotted line and an accompanying question mark in Figure 2. Similarly, to our potential missing genotypes, it is also possible that the limited reporting may have missed other minor outbreaks in other states. Despite these limitations, it is clear the founder-flush phenomenon describes the underlying genetics of *Vibrio cholerae* outbreaks in Nigeria during the period between 2016 and 2021.

Conclusion.

Variable tandem repeat analyses of *Vibrio cholerae* are consistent with the founder-flush phenomenon. There were two separate events, one occurring during the period 2016 to 2019 inclusive and the other over the entire country in 2021. Thus, our analyses are consistent with the analyses of isolates from outbreaks in India and Mozambique suggesting the founder-flush phenomenon is wide-spread.

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Conflict of Interest Statement:
There are no conflicts of interest to declare.

Acknowledgements:
None.

Funding Statement
This work was supported by a grant from the National Institute of Allergy and Infectious Disease [NIAID] (5R01AI123422).

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