

Vibrio Cholerae: Endemic to the Philippines or reintroduced?



Background

Vibrio cholerae causes cholera, a disease which infects the small intestine and can cause life threatening diarrhea and vomiting.

The Philippines has many cases of cholera each year, yet it is unknown whether the infections are persistently endemic or reintroduced from other countries [6].

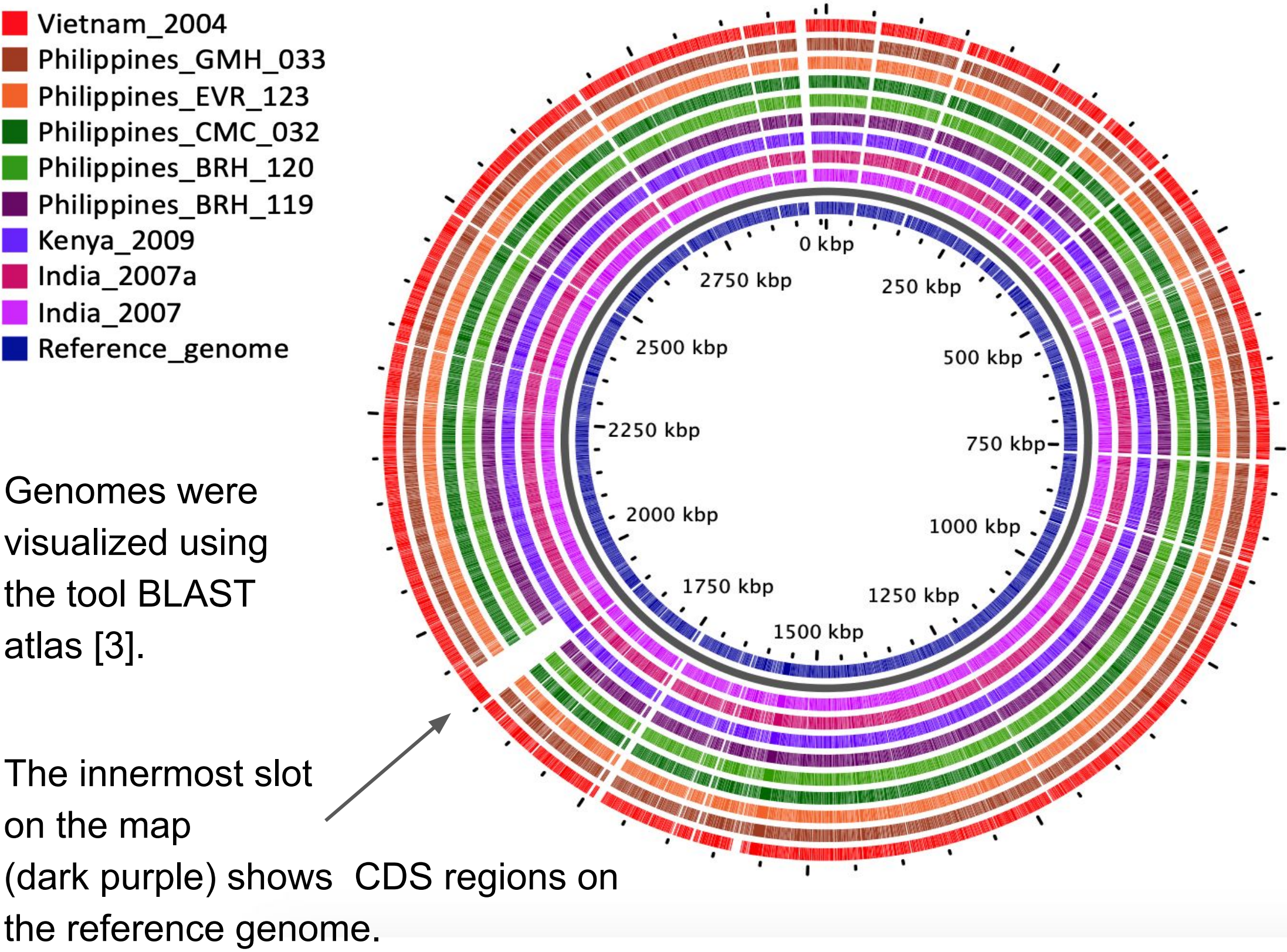
In this study, WGS was combined with bioinformatics tools to investigate the epidemiology of *Vibrio cholerae* in the Philippines and on other cholera affected countries. Our results could ultimately be applied in control measures and management of the disease.

Materials

To answer our research question, 9 local and global strains of *Vibrio cholera* were characterized to determine phenotypic and genotypic traits, and to investigate their genetic relatedness. All 9 strains were confirmed as *Vibrio cholerae* by KmerFinder [2].

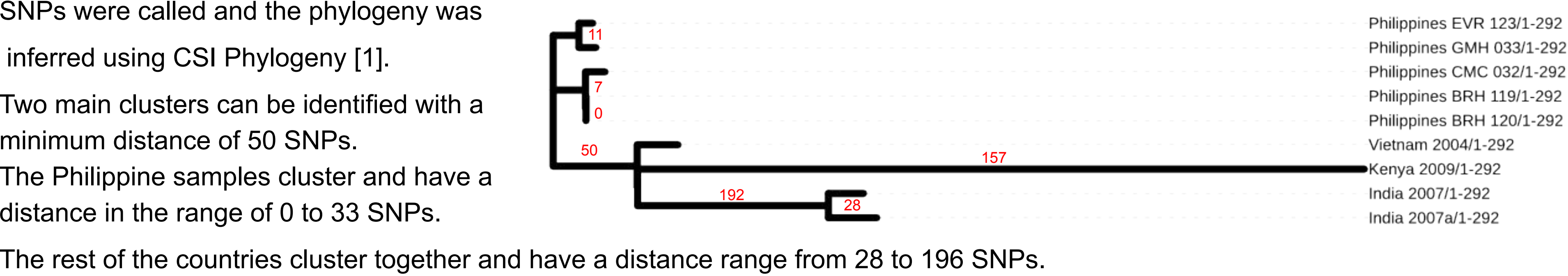
| Strain ID | Country | Year | KmerFinder |
|---------------------|-------------|------|------------------------|
| India_2007 | India | 2007 | <i>Vibrio cholerae</i> |
| India_2007a | India | 2007 | <i>Vibrio cholerae</i> |
| Kenya_2009 | Kenya | 2009 | <i>Vibrio cholerae</i> |
| Philippines_BRH_119 | Philippines | 2013 | <i>Vibrio cholerae</i> |
| Philippines_BRH_120 | Philippines | 2013 | <i>Vibrio cholerae</i> |
| Philippines_CMC_032 | Philippines | 2013 | <i>Vibrio cholerae</i> |
| Philippines_EVR_123 | Philippines | 2013 | <i>Vibrio cholerae</i> |
| Philippines_GMH_033 | Philippines | 2013 | <i>Vibrio cholerae</i> |
| Vietnam_2004 | Vietnam | 2004 | <i>Vibrio cholerae</i> |

Genome comparison



All empty regions on the query slots indicate areas where there were no BLAST hits were found between the reference and the query genomes. Only sequences present in the reference can be shown. Since the reference lacks resistance genes (ResFinder result [4]) and misses other relevant genes found in this study (MyDbFinder result [5]), these are not shown.

Phylogenetic Tree



| Country |
|-------------|
| Philippines |
| India |
| Vietnam |
| Kenya |

| Biotype |
|----------------|
| Classical |
| El Tor Variant |
| El Tor |

| Resistance genes |
|------------------|
| Aminoglycoside |
| Fluroquinolone |
| MLS |
| Phenicol |
| Sulphonamide |
| Tetracycline |
| Trimethoprim |

The Philippines have the same biotype “Classical” as Vietnam, and the same resistance gene profile as Kenya with only the catB9 gene which causes Phenicol resistance [5].

Conclusion

In silico analysis showed: All 9 strains shared a common genetic background, being sequence type 69, serogroup O1. However, investigation of the genes revealed different biotypes and a SNP analysis showed that Philippines isolates clustered together.

From these samples it seems like the *Vibrio cholerae* pandemic in the Philippines was indeed endemic to the country. Nevertheless, because the Philippines samples were from same year and possibly from the same outbreak, even though this particular outbreak was not introduced from other countries other outbreaks may be.

To conclude with more confidence if cholera is persistently endemic or reintroduced to the Philippines, more sampling from different time points in the Philippines, and from more neighboring countries is needed.

Gene Investigation

- All strains were investigated by MyDbFinder [4].
- All isolates had the ctxA gene and were part of the 7th pandemic clone.
 - **mutL gene**
 - Only present in India 2007/2007a and Vietnamese strains
 - **floR gene**
 - Only present in Vietnamese strain
 - One of two genes for Phenicol resistance
 - catB9 is present in all strains
 - **T6SS**
 - Type 6 Secretion System
 - Only present in Philippines_BRH_119 strain

Most genes that were found here were also found in the ResFinder, and therefore give no new information.

All 9 strains had 4 pathogenicity islands (VPI-1, VPI-2, VSP-1, VSP-2). And no integrons or SXT elements were found.

Biotype and Serogroup Determination

| Serogroups | [4],[5] | | | MLST |
|---------------------|---|-------------------------------|---------------------------------|-------|
| | Vibrio cholera species-specific ompW | O1 serogroup-specific rfvB | O139 serogroup-specific wbfx | |
| India_2007 | x | x | o | ST 69 |
| India_2007a | x | x | o | ST 69 |
| Kenya_2009 | x | x | o | ST 69 |
| Philippines_BRH_119 | x | x | o | ST 69 |
| Philippines_BRH_120 | x | x | o | ST 69 |
| Philippines_CMC_032 | x | x | o | ST 69 |
| Philippines_EVR_123 | x | x | o | ST 69 |
| Philippines_GMH_033 | x | x | o | ST 69 |
| Vietnam_2004 | x | x | o | ST 69 |

ompW: *V. cholerae*
Serogroup: O1
MLST: ST69

| Biotype | ctxBet | ctxBcc | tcpAet | rstRet | rstRcc | tcpAcc |
|---------------------|--------|--------|--------|--------|--------|--------|
| India_2007 | o | x | x | x | o | o |
| India_2007a | o | x | x | x | o | o |
| Kenya_2009 | x | o | x | x | o | o |
| Philippines_BRH_119 | o | x | x | x | x | o |
| Philippines_BRH_120 | o | x | x | x | x | o |
| Philippines_CMC_032 | o | x | x | x | x | o |
| Philippines_EVR_123 | o | x | x | x | x | o |
| Philippines_GMH_033 | o | x | x | x | x | o |
| Vietnam_2004 | o | x | x | x | x | o |

- Biotypes were determined according to the harbored virulence genes:
- Indian strains were El Tor Variant (ctxBcc, tcpAet, rstRet).
 - Kenya strain was El Tor (ctxBet, tcpAet, rstRet).
 - Philippines and Vietnam strains were Classical (ctxBcc, rstRcc).

References:

[1]: Rolf S. Kaas, Pimlapas Leekitcharoenphon, Frank M. Aarestrup, Ole Lund. Solving the Problem of Comparing Whole Bacterial Genomes across Different Sequencing Platforms. PLoS ONE 2014; 9(8): e104984.

[2]: Hasman H, Saputra D, Sicheritz-Pontén T, Lund O, Svendsen CA, Frimodt-Møller N, Aarestrup. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. FM. J Clin Microbiol. 2014 Jan;52(1):139-46

[3]: BLAST atlas tool (<https://serve.r.gview.ca/>)

[4]: myDBFinder tool (<https://cge.cbs.dtu.dk/services/MyDbFinder/>)

[5]: Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. J Antimicrob Chemother. 2012 Nov;67(11):2640-4.

[6]: Richard A. Finkelstein. Cholera, *Vibrio cholerae* O1 and O139, and Other Pathogenic Vibrios