

Peer Review

Review of: "Molecular Characterization of *Vibrio cholerae* Outbreak Isolates from Western India"

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Comments

Very nice work, accept after a major revision

This work will add significant value in deciphering the molecular qualities of *Vibrio cholerae* outbreak isolates isolated from Western India. The calibration of the Nashik strain as an O1 Haitian-type ctxB7 genotype belonging to the third wave of atypical El Tor strains further emphasizes the genetic evolution and epidemiological importance of these new variants. The addition of phylogenetic analysis of major virulence genes (ctxB, rstA, rstB, and tcpA) to the results obtained strengthens the results and illustrates the genetic diversity and evolutionary strategies of the pathogen. Of special note was the finding of the $\alpha\beta$ -loop region mutation N64S in tcpA, whose activity requires further investigation, but which may be relevant to the structure of pili and host interaction. Moreover, the emergence of atypical EPEC was unanticipated in samples that were negative for *Vibrio* and brought to surface concerns about a putative multi-pathogen nature of diarrheal outbreaks, suggesting enhanced multi-nucleic acid surveillance methods for a holistic bosom pathogenography. In general, this is a well-balanced study covering important angles in cholera epidemiology, but more study of the functional consequences of tcpA mutations, antimicrobial resistance profiling, and clinical correlations would provide a better impact.

☒ Descriptions of the epidemiological context, such as case numbers, demographics, and clinical outcomes, are needed to document public health consequences.

- ✘ Provide further detail about the PCR conditions, the sequencing platform, and quality controls to enhance reproducibility.
- ✘ Represent graphs, such as the value of bootstrap and the distance of evolution, on figures for more powerful verification of phylogeny.
- ✘ Discuss how the N64S mutation in tcpA may affect pili function and virulence: 1) structural modeling or in-silico analysis to predict the impact of the N64S mutation on the structure of pili.
- ✘ Polymyxin B sensitivity is indicated; a full antimicrobial susceptibility profile for these isolates should be given to assess clinical treatment implications.
- ✘ The Nashik strain is evolutionarily contextualised beyond India, particularly with regard to Haitian ctxB7 global strains. What is the consequence of having only five isolates on the findings? Correlate the ctxB7 genetic findings with disease manifestation and other outcomes, if possible.
- ✘ What is the clinical importance of atypical EPEC in samples that were Vibrio negative in the course of a cholera outbreak? Explain the biology better. Atypical EPEC and Vibrio-negative EPEC were detected during the cholera outbreak. Explain the conclusions that can be drawn from the data and the rationale behind the findings. Discuss and enhance figure legends. Add explanatory legends, descriptions, and commentary to the phylogenetic trees and diagrams.

Declarations

Potential competing interests: No potential competing interests to declare.