

Peer Review

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

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This manuscript titled "*Molecular Characterization of Vibrio cholerae Outbreak Isolates from Western India*" presents a molecular and phylogenetic analysis of *V. cholerae* strains isolated during a cholera outbreak in Rahude village, Nashik district, Maharashtra. The authors provide evidence that the isolates belong to the Haitian-type ctxB7 genotype, with additional findings suggesting polymyxin B sensitivity. The study also reports the detection of atypical EPEC strains from a subset of samples.

Major Comments:

Methodology:

The PCR and sequencing methodology lacks critical detail necessary for reproducibility. The authors should include the primer sequences, expected amplicon sizes, and PCR cycling conditions in either the main text or as a supplementary table.

Phenotypic Confirmation of Polymyxin B Sensitivity:

The manuscript refers to polymyxin B sensitivity based on genetic similarity to known sensitive strains. However, no antimicrobial susceptibility testing was performed. The authors should clarify that this inference is based solely on sequence comparison, or ideally, provide MIC or disc diffusion data if available.

Missing Figures:

Figures 1 to 3 referenced in the Results section are not included in the submitted document. These are critical to the interpretation of the phylogenetic analysis. The authors must include these figures with appropriate annotations (bootstrap values, strain names, accession numbers).

E. coli Findings:

The detection of *eae*⁺/*bfpA*⁻ atypical EPEC strains in the same outbreak setting is of interest. This finding should be discussed further in terms of its epidemiological significance and whether such co-infections are common in similar outbreaks.

Terminology and Strain Classification:

The use of terms such as “Haitian-type,” “CTX-3b,” and “ctxB7” appears inconsistently throughout the manuscript. The authors should standardize terminology and clearly explain how these classifications relate to one another, especially in the context of the three cholera pandemic waves.

Minor Comments:

Several long and complex sentences may benefit from restructuring for improved clarity.

References should be checked for consistency and completeness, particularly in-text formatting and citation of online sources.

Accession numbers for submitted gene sequences (*ctxB*, *rstA*, *rstB*, *tcpA*) should also be mentioned in the Results section for ease of access.

Conclusion:

The manuscript presents relevant findings on the molecular epidemiology of *V. cholerae* in India. With appropriate revisions to methodology transparency, inclusion of figures, and clarification of phenotypic inference, the study will be suitable for publication.

Additional Discussion Needed on Atypical EPEC Strains

The section on atypical *E. coli* strains (*eae*⁺/*bfpA*⁻) is currently underdeveloped. The authors should consider including the following points in the discussion:

a. Epidemiological Relevance:

Explain whether detection of atypical EPEC in cholera outbreaks is rare or increasingly reported in mixed infections.

Cite prevalence data or previous outbreak reports, if available, from India or similar regions.

b. Pathogenic Potential:

Elaborate on the clinical significance of *eae*⁺/*bfpA*⁻ strains:

These strains lack bundle-forming pili (bfpA) and may use alternative mechanisms for adherence.

Atypical EPEC is often associated with **prolonged or persistent diarrhea**, particularly in children.

c. Implications for Outbreak Investigation:

Discuss whether these EPEC strains may have contributed to the outbreak severity, co-infection dynamics, or complicated the clinical diagnosis and response.

Emphasize that the detection of more than one diarrheagenic pathogen in outbreak settings suggests the need for **broad-spectrum diagnostic panels** or **multiplex PCR** approaches.

d. Zoonotic or Environmental Reservoirs:

Atypical EPEC can have animal or environmental reservoirs. The authors could hypothesize whether contaminated water sources (as in cholera) may harbor EPEC strains as well.

Suggested Add-on Sentence (for Discussion Section): The presence of *eae*-positive, *bfpA*-negative atypical EPEC strains in *Vibrio*-negative fecal samples highlights the possibility of mixed or co-existing enteric pathogens during cholera outbreaks. Given that atypical EPEC is increasingly recognized in both sporadic and outbreak-associated pediatric diarrhea, this finding warrants further investigation into its contribution to outbreak severity, particularly in resource-limited or endemic settings.”

Declarations

Potential competing interests: No potential competing interests to declare.