Research Article

Molecular Characterization of *Vibrio cholerae* Outbreak Isolates from Western India

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Vibrio cholerae **isolates obtained from fecal samples referred in an outbreak of acute watery diarrhea in Rahude village in Nashik district of Maharashtra, India were characterized. The five culture-confirmed** ***Vibrio cholerae*** **isolates were subjected to molecular and phylogenetic analysis. The PCR and sequencing results of** ***ctxB*****,** ***rstA*****,** ***rstB*** **and** ***tcpA*** **genes confirmed that the Nashik** ***V.cholerae*** **strain belonged to subtype O1 Haitian-type strain of** ***ctxB*****7 genotype. Moreover, the phylogenetic analysis of** ***tcpA*** **gene suggested that the Nashik strain is closely related to the newly discovered polymyxin B sensitive strain. This supports the recent finding on the prevalence of polymyxin B sensitive strain in India. The mutation in** ***tcpA*** **protein was mapped to the** $\alpha\beta$**-loop region, which plays a role in pili formation as well as in interaction of pili to the local environment.**

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Introduction

Acute diarrheal disease is a major public health concern in India and across the world [1][2]. *Vibrio cholerae* is the etiological agent of cholera, a devastating watery diarrheal disease. The occurrence of cholera is divided into seven pandemics since 1817 [3][4][5]. In contrast to the previous pandemics which were caused by the classical biotype, the 7th pandemic was caused by the less virulent El Tor biotype [6]. However in the recent past, El Tor strains are becoming more virulent by acquiring genetic determinants of the classical strains [7].

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The three genetic determinants that account for the virulence of *V. cholerae* strains include the bacteriophage CTXΦ encoded core region and two *Vibrio* pathogenicity islands VPI1 and VPI2 [8]. The core region of CTXΦ phage contains *ctxA* and *ctxB* genes present in the O1/O139 strains and transcribes the potent A-B type enterotoxin *ctxA* and *ctxB* proteins. The ADP-ribosylation activity of ctxA is responsible for the secretory diarrhea [9]. Another toxin gene *rtxA* (repeat in toxin) is present in VPI2, which is also a marker for nonO1/nonO139 pathogenic strains of *V. cholerae* [10]. Other than the core region, the RS2 region contains *rstA*, *rstB* and *rstR* genes that are involved in replication, integration and regulation of site-specific recombination of CTXΦ [11]. The VPI1 contains *tcpA* gene, which encodes colonization factors - toxin co-regulated pili (TCP) that mediate attachment of bacteria to the intestinal epithelium [12][13].

Non-random genetic changes occur in the virulent genes of *V. cholerae*. These changes are systematically acquired in the *Vibrio* genome with a direct impact on the virulence of the bacteria. In 1990, an atypical El Tor variant with classical cholera toxin gene started circulating in South Asia. With passage of time, atypical El Tor strain acquired new single nucleotide polymorphisms (SNPs). Based on these new SNPs, strains are divided into three waves and the third wave containing strains like ctxB-3b strain and Haitian strain (ctxB-6) are known to cause severe cholera [7]. Thus, the sequence analysis of *V. cholerae* virulence genes and comprehensive evaluation of genetic mutations are critical for understanding the phylodynamics of *V. cholerae* [14]. The present study reports the molecular characterization of culture-confirmed *V. cholerae* isolates obtained from an outbreak of cholera in July 2018. The outbreak occurred in a remote tribal population in Rahude village of Nashik district Maharashtra, a state located in western India[5][15]. In addition, phylogenetic analysis of the virulence genes (*ctxB, rstA*, *rstB*, *tcpA* and *rtxA*) was also performed.

Materials and Methods

Bacterial Strains

V. cholerae

Five isolates of *V. cholerae* obtained from fecal samples referred from an outbreak of acute watery diarrhea at Rahude village, Nashik district, Maharashtra, in July 2018 were used in the study. The Institutional Ethics Committee was informed about the outbreak and approval taken for conducting post outbreak. The isolates were confirmed as *Vibrio cholerae* O1 Ogawa biotype El Tor using standard culture methods and

slide agglutination with specific *Vibrio cholerae* polyvalent O1 antisera, followed by Ogawa and Inaba serotype specific antisera (Denka Seiken, Japan) as well as phenotypic tests for biotype differentiation (hemolysis, Chick RBC agglutination and Voges-Proskauer reaction) [16]. The isolates plated on non inhibitory media (Trypticase Soy Agar-TSA) were further suspended in phosphate buffered saline (PBS) and used for extraction of genomic DNA as described below.

Diarrheagenic E.coli

Three to six lactose fermenting colonies and upto three non lactose fermenting colonies from MacConkey (MAC) agar plates were selected for testing by conventional procedures [16]. Identification of *E.coli* isolates was confirmed by VITEK® 2 COMPACT automated microbial identification system (bioMérieux, Inc. Hazelwood, USA) and VITEK 2 GN cards (bioMérieux, Marcy-l'Ètoile, France).These isolates were replated on TSA from which three isolated colonies were resuspended in PBS for further extraction of genomic DNA.

Polymerase chain reaction (PCR) assay and Phylogenetic analysis

The genomic DNA was extracted from the bacterial isolates using QIAamp DNA kit (Qiagen, USA) and singleplex PCRs were performed for specified targets using AccuPrime *Taq* DNA Polymerase System (Thermo Fisher Scientific). To confirm the identity of the PCR products, both the strands of amplified products were sequenced. using Big-Dye terminator cycle sequencing kit (Applied Biosystems) followed by sequencing in an automated nucleotide sequencer (Bioanalyzer 3100; Applied Bio systems).

The phylogenetic analysis of the *V. cholerae* isolates from Nashik outbreak was performed for *ctxB*, *rstA, rstB* and *tcpA* genes. The sequencing results were confirmed by alignment search using BLAST [17]. The BLAST hits mentioned in literature were selected and their sequences were retrieved for further analysis from public database - NCBI [18]. The *ctxB* and *rstA* genes sequence were aligned with the corresponding genes sequences of atypical El Tor strains [7]. The phylogenetic analysis for *tcpA* gene was performed by comparing with *tcpA* gene sequences from toxicogenic and non-toxicogenic isolates as reported by Kumar et al. (Kumar et al. 2011). In addition, the *tcpA* sequences from classical strains (O395), El Tor strain (MJ 1236), ZJ59 strain and WB-0050 polymyxin B sensitive strain were also included [19][20].

The multiple sequence alignment was performed using MUltiple Sequence Comparison by Log-Expectation (MUSCLE) tool available in Molecular Evolutionary Genetic Analysis program version 7

(MEGA) and gap regions removed. The phylogenetic tree of trimmed alignment was prepared using MEGA program Neighbor-joining method with 2000 bootstraps [21][22].

Mapping SNP on tcpA protein structure

Sequence alignment was performed for tcpA protein sequences of Nashik strain and El Tor strain. The coordinates of single chain (chain A) of $tcpA^{cla}$ from classical strain (PDB ID1OQV) and $tcpA^{El}$ from El Tor stain (PDB ID 3HRV) were obtained from the PDB database and structure-based sequence alignment was performed using $tcpA^{El}$ as template [23][24]. The mutated amino acid was mapped on $tcpA^{El}$ using UCSF Chimera software [25][26][27][28]. In addition, αβ-loop region and D-region were mapped on $tcpA^{El}$ structure. The residues that are different in $tcpA^{El}$ and $tcpA^{cla}$ were also mapped [29]. The solvent accessibility of $tcpA^{El}$ was determined by ASAVIEW [30].

Results and Discussion

Progressive changes in the genomic sequence of *V. cholerae,* in the form of SNPs, intra-strand and inter-strand chromosomal recombination are known, which lead to novel and diverse strains. The virulence regions like core region, RS2 region and *Vibrio* pathogenicity islands VPI1 and VPI2 gain such non-random genetic variations [31]. This study has reconfirmed *V. cholerae* as the main etiological agent of acute diarrhea in Rahude village in Nashik. The Nashik strain was found positive for virulence genes of all three virulence regions (*ctxA*, *rtxA*, *ctxB*, *rstA, rstB* and *tcpA*) suggesting the presence and intactness of both the core region and *Vibrio* pathogenicity islands. Presence of *ctxA* and *ctxB* confirmed the presence of main CT antigens. The sequencing comparison of virulence genes among different isolates implicated complete identity suggesting the prevalence of only a single strain of *Vibrio* in Rahude village.

Consequently, molecular characterization of *ctxB*, *rstA, rstB* and *tcpA* was performed [32]. The SNPs in ctxB gene sequence serves as the basis of differentiating classical and El Tor biotypes and CTB epityping [33]. The presence of SNP A58 (change in amino acid residue 20) on *ctxB* suggested that the Nashik 2018 strain belongs to O1 *ctxB*7 genotype. Results of the phylogenetic analysis of Nashik strain *ctxB* gene sequences are atypical El Tor strains diverged from El Tor strains N16961 and India 1991. The phylogenetic tree of *ctxB* genes showed that the atypical El Tor strains are distributed into two clusters. The first cluster consisted of wave 1 and wave 2 strains. The third wave which includes ctxB-3b, Haitian strain (ctxB-6) and newly reported polymyxin B sensitive strain is included in second cluster (Figure 1) [14][34]. The Nashik strain was also found to cluster in the third wave. The SNPs mapping of *ctxB* suggested similarity between

Nashik strain, Haitian-type strain of (also categorized as CTX-6b) and CTX-3b type. Both the Haitian strain and CTX-3b are highly virulent strains are responsible for severe cholera outbreaks. The CTX-3b emerged in 1996 in India and is gradually replacing the CTX-3 strain in Indian subcontinent [35]. The difference in the Haitian and CTX-3b was determined by sequencing *rstB* gene. The absences of trinucleotide 'GTA' at position 74-76 nt in *rstB* implicated that Nashik strain is a Haitian type strain [7]. This result is in congruence with the finding that the *ctxB7* Haitian strain is presently in circulation in India [36].

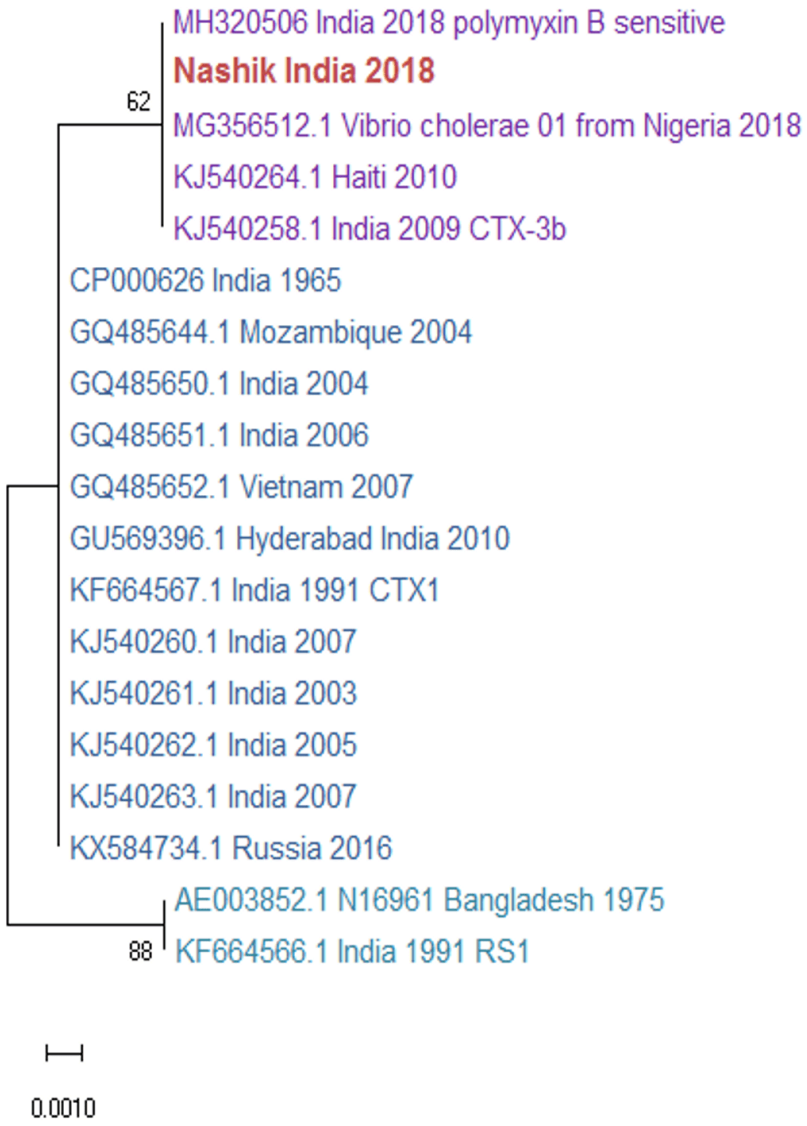

Figure 1. The phylogenetic tree of the *ctxB* gene constructed using neighbor-joining method as implemented in MEGA. Bootstrap values are the percentage of 2000 replications. First and second wave are in blue color and third wave is in violet color. Nashik strain is shown in red color

The molecular analysis of partial sequence of *rstA* (from 435 to 1083 nucleotides) gene revealed presence of C927, T933 and T942. The *rstA* phylogenetic analysis grouped all atypical El Tor strains, except V212-1, V212-2, CTX-2 Mozambique 2004 strain and CTX-3b India 2006 strain. The Nashik strain also clustered in the atypical El Tor strains cluster (Figure 2). Thus, the analysis of *ctxB* and *rstA* reinforces that the Nashik 2018 strain is part of the third wave of atypical El tor strains clustering with Haitian strain, CTX-3b strain

and polymyxin B sensitive strain [7][20]. The PCR analysis of the *rstB* gene detected the presence of trinucleotide GTA at nucleotide position 74-76 [34], indicating that Nashik strain is a Haitian type strain.

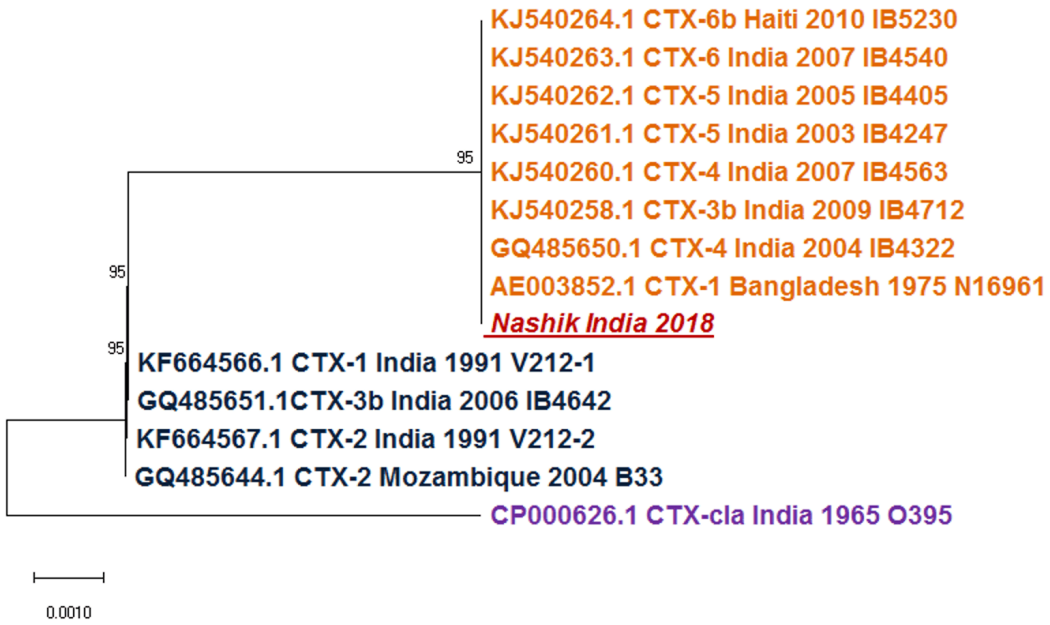

Figure 2. The phylogenetic tree of the *rstA* constructed by using neighbor-joining method as implemented in MEGA. Bootstrap values are the percentage of 2000 replications. Nashik strain is colored red, italicized and underlined

The phylogenetic tree of the *tcpA* gene revealed the distribution of *Vibrio* strains into 10 phylogenetically divided clusters as reported by Kumar et al. [37]. The Nashik strain *tcpA* gene was part of cluster number 9, along with MJ-1236 strain (El Tor strain). However, Nashik strain *tcpA gene* showed more sequence identity to *tcpA* gene from polymyxin B sensitive strain, thus originating a new subcluster (Figure 3). The polymyxin B sensitive strain in India is documented from 2016 with circulations of both polymyxin B resistant and sensitive type strains recently reported from Maharashtra and Gujarat [20]. The polymyxin B sensitivity is one of the consistent phenotypic traits of the classical strain, which is now being acquired by the El tor biotype. Thus, isolation of polymyxin resistant strain from Rahude village, a tribal area on the border of Maharashtra and Gujarat is of significance.

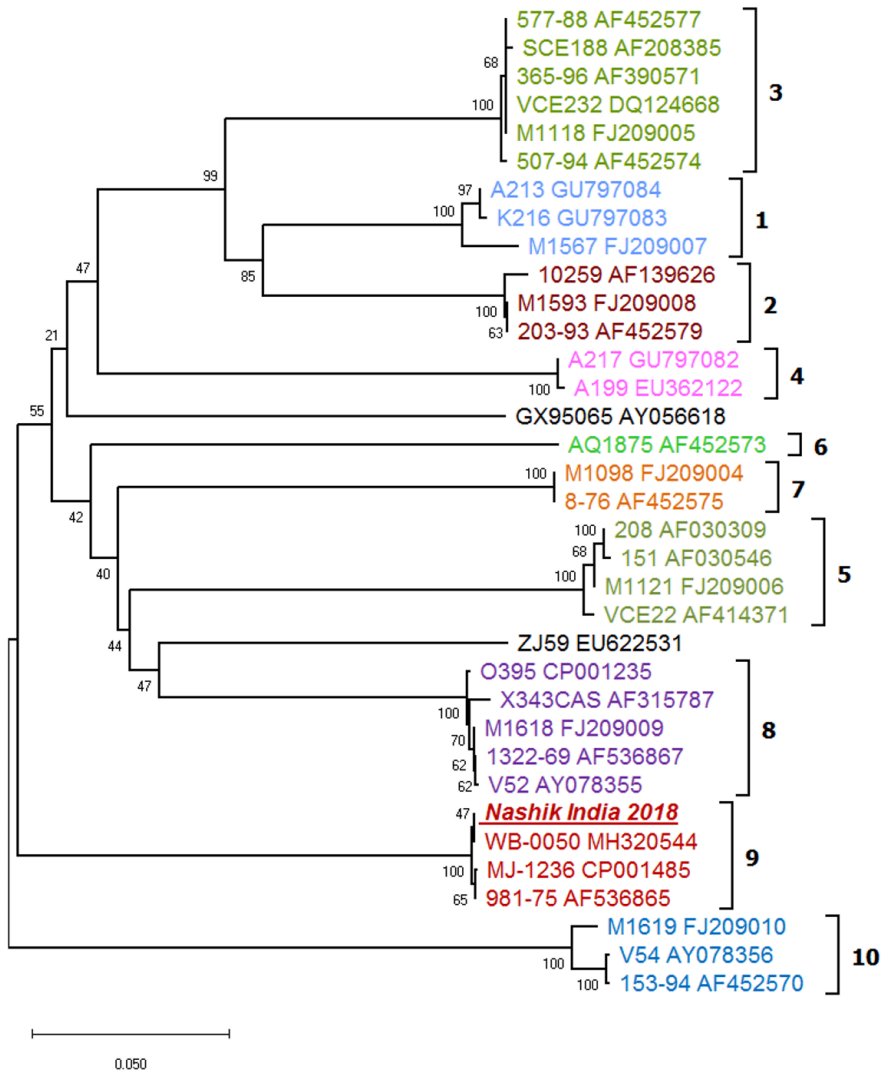

Figure 3. The phylogenetic tree of the *tcpA* gene constructed using neighbor-joining method as implemented in MEGA. Bootstrap values are the percentage of 2000 replications. The phylogenetic tree is categorized into 10 clusters. Nashik strain is in red font, italicized and underlined

The presence of SNP A266G in the *tcpA* of Nashik and polymycin B sensitive strain leads to mutation at the 64th position of the tcpA protein from Asparagine to Serine (N64S). The N64S mutation is mapped to the αβ-loop region, which play role in pili formation as well as interaction with the local environment [38][39][40][41]. The tcpA belongs to class of type IV pilin proteins and the αβ-loop region shows high structure as well as sequence variability among type IV pilin proteins from different species and also among different biotypes of *V. cholerae*. The functional relevance of N64S mutation is still unknown [36][42]. However this

has been reported that N64S mutation does not affect the virulence of *V. cholerae* [43]. The change from Threonine to Asparagine and then to Serine occurred at the 64th position in classical, El Tor and polymyxin-sensitive strain, respectively, indicates regain of hydroxyl (-OH) group. Notably tcpA not only play a role in adherence but also is critical in CTXΦ phage invasion to *Vibrio* bacterium [44]. The amino acid at 64th position has high solvent accessible (as determined by ASAVIEW, data not shown) area that might have functional role of N64 residue [30].

In addition, two *E.coli* isolates from fecal samples negative for *V.cholerae* were found to be positive for EPEC virulence *eae* (intimin) gene, a marker for pathogenic *E. coli* EPEC strain. However these isolates were negative for another EPEC gene *bfpA* (bundle forming pili) gene. Presence of (*eae*+, *bfpA*-) strains is suggestive of an atypical EPEC strain [45]. The molecular detection of atypical EPEC in two *Vibrio*-negative isolates (sample no. 52 and 78) indicates more than one microbial etiology in the diarrheal outbreak. The targets *bfpA* and *eae* genes are crucial for the three-stage model of infection of EPEC [46]. The non-intimate attachment is formed by the *bfpA* in first stage, whereas in third stage intimin (coded by *eae* gene) protein leads to intimate attachment [46]. Deletion of *eae* gene reduces the pathogenicity of EPEC, whereas no significant effects happen in absence of deletion of *bfpA* gene [47]. The positivity of atypical-EPEC (*eae*+, *bfpA*-) during cholera outbreak is intriguing, as *bfpA* and *tcpA* both belong to same class of type IVb pilins [46].

Conclusion

The study highlights the molecular and phylogenetic characterization of *V. cholerae* strains isolated during the Nashik outbreak 2018. The information obtained shows that it is an altered El Tor O1 *ctxB*7 genotype Haitian-type strain, with genotypic similarity to polymyxin B sensitive strain. The mutation present in tcpA protein of Nashik strain and polymyxin B sensitive strain was mapped on the αβ-loop region, suggesting a probable effect on pili formation or the interaction of pili with local environment. In addition, atypical EPEC lacking *bfpA* gene was also detected. Taken together, the finding supported the molecular characterization of genetic makeup of *Vibrio* strain with its virulence along with providing evidence for more than microbial etiology. In conclusion, the study reinforces the need for continuous molecular surveillance of diarrheal outbreaks.

Statements and Declarations

Conflicts of interest

The authors declare no conflicts of interest.

Ethics

This study was approved by ICMR-NIV ethics committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Availability

The sequence data analyzed in this study are available in public databases NCBI (NCBI IDs -MK873008 (ctxB), MK873009 (rstA), MK873010 (rstB) and MK873011(tcpA)). Additional data may be available from the corresponding author upon reasonable request.

Author Contributions

Anuj Kumar performed the molecular analyses and drafted the manuscript. Rajlakshmi Viswanathan contributed to sample collection, bacterial culture, and manuscript revision. All authors read and approved the final manuscript.

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References

1. ^*Lakshminarayanan S, Jayalakshmy R (2015). "Diarrheal Diseases Among Children in India: Current Scenar io and Future Perspectives." J Nat Sci Biol Med.*
2. ^*Fischer Walker CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. (2013). "Global Burden of Childh ood Pneumonia and Diarrhoea." Lancet.*
3. ^*Barua D (1992). "History of Cholera." In: Cholera.*
4. ^*Gopalkrishna V, Joshi M, Viswanathan R, Malu G, Ganorkar N, Chavan N, et al. (2019). "Cholera Outbreak in Aurangabad, Maharashtra, Western India." Indian J Med Res. **150**(6):640. http://www.ijmr.org.in/text.asp?201*

9/150/6/640/277417.

5. a, b *Kumar A, Barve U, Gopalkrishna V, Tandale BV, Katendra S, Joshi MS, et al. (2022). "Outbreak of Cholera i n a Remote Village in Western India." Indian J Med Res.* ***156****(3):442–8. http://www.ncbi.nlm.nih.gov/pubmed/ 36751742*.
6. ^ *Hu D, Liu B, Feng L, Ding P, Guo X, Wang M, et al. (2016). "Origins of the Current Seventh Cholera Pandemi c." Proc Natl Acad Sci U S A.*
7. a, b, c, d, e *Kim EJ, Lee D, Moon SH, Lee CH, Kim SJ, Lee JH, et al. (2014). "Molecular Insights Into the Evolutiona ry Pathway of Vibrio cholerae O1 Atypical El Tor Variants." PLoS Pathog.*
8. ^ *Murphy T, Bisgard K, Sanden G (2000). "Chapter 2 — Diagnosis and Laboratory Methods." Methods. (Jun e):1–16.*
9. ^ *Bharati K, Ganguly NK (2011). "Cholera Toxin: A Paradigm of a Multifunctional Protein." Indian J Med Res.* ***133****:179–87.*
10. ^ *Chow KH, Ng TK, Yuen KY, Yam WC (2001). "Detection of RTX Toxin Gene in Vibrio cholerae by PCR." J Clin Microbiol.* ***39****(7):2594–7.*
11. ^ *Boyd EF, Waldor MK (1999). "Alternative Mechanism of Cholera Toxin Acquisition by Vibrio cholerae: Gener alized Transduction of CTXΦ by Bacteriophage CP-T1." Infect Immun.* ***67****(11):5898–905.*
12. ^ *Tay CY, Reeves PR, Lan R (2008). "Importation of the Major Pilin TcpA Gene and Frequent Recombination Drive the Divergence of the Vibrio Pathogenicity Island in Vibrio cholerae." FEMS Microbiol Lett.*
13. ^ *Nielsen AT, Dolganov NA, Rasmussen T, Otto G, Miller MC, Felt SA, et al. (2010). "A Bistable Switch and Anat omical Site Control Vibrio cholerae Virulence Gene Expression in the Intestine." PLoS Pathog.* ***6****(9):e1001102.*
14. a, b *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.*
15. ^ *Viswanathan R, Kumar A (2019). "Frontier Warriors—Combating Cholera in Rural India." Am J Trop Med H yg.* ***100****(5):1071–2. http://www.ajtmh.org/content/journals/10.4269/ajtmh.19-0027.*
16. a, b *Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (2003). Manual of Clinical Microbiology. Ameri can Society for Microbiology, Washington, DC.*
17. ^ *Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). "Basic Local Alignment Search Tool." J Mol Biol.* ***215****(3):403–10.*
18. ^ *Agarwala R, Barrett T, Beck J, Benson DA, Bollin C, Bolton E, et al. (2017). "Database Resources of the Natio nal Center for Biotechnology Information." Nucleic Acids Res.*

19. ^Takemura T, Murase K, Maruyama F, Tran TL, Ota A, Nakagawa I, et al. (2017). "Genetic Diversity of Enviro nmental Vibrio cholerae O1 Strains Isolated in Northern Vietnam." Infect Genet Evol. **54**:146–51.

20. a, b, cSamanta P, Saha RN, Chowdhury G, Naha A, Sarkar S, Dutta S, et al. (2018). "Dissemination of Newly E merged Polymyxin B Sensitive Vibrio cholerae O1 Containing Haitian-Like Genetic Traits in Different Parts o f India." J Med Microbiol.

21. ^Edgar RC (2004). "MUSCLE: Multiple Sequence Alignment With High Accuracy and High Throughput." Nuc leic Acids Res. **32**(5):1792–7.

22. ^Kumar S, Stecher G, Tamura K (2016). "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bi gger Datasets." Mol Biol Evol.

23. ^Craig L, Taylor RK, Pique ME, Adair BD, Arvai AS, Singh M, et al. (2003). "Type IV Pilin Structure and Assem bly: X-Ray and EM Analyses of Vibrio cholerae Toxin-Coregulated Pilus and Pseudomonas aeruginosa PAK Pilin." Mol Cell. **11**(5):1139–50.

24. ^Lim MS, Ng D, Zong Z, Arvai AS, Taylor RK, Tainer JA, et al. (2010). "Vibrio cholerae El Tor TcpA Crystal Stru cture and Mechanism for Pilus-Mediated Microcolony Formation." Mol Microbiol. **77**(3):755–70.

25. ^Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. (2004). "UCSF Chimera - A Visualization System for Exploratory Research and Analysis." J Comput Chem.

26. ^Dobhal S, Chauhan K, Kumar S, Shikha S, Jogi M, Kumar D, et al. (2024). "In Silico Identification of MHC Dis played Tumor Associated Peptides in Ovarian Cancer for Multi-Epitope Vaccine Construct." Endocr Metab I mmune Disord Drug Targets. http://www.ncbi.nlm.nih.gov/pubmed/38275062.

27. ^Kaur R, Kumar P, Kumar A (2023). "Insights on the Nuclear Shuttling of H2A-H2B Histone Chaperones." Nu cleosides Nucleotides Nucleic Acids. 1–13. http://www.ncbi.nlm.nih.gov/pubmed/38133493.

28. ^Garg K, Kumar A, Kizhakkethil V, Kumar P, Singh S (2023). "Overlap in Oncogenic and Pro-Inflammatory P athways Associated With Areca Nut and Nicotine Exposure." Cancer Pathog Ther. https://linkinghub.elsevier. com/retrieve/pii/S2949713223000538.

29. ^Lim MS, Ng D, Zong Z, Arvai AS, Taylor RK, Tainer JA, et al. (2010). "Vibrio cholerae El Tor TcpA Crystal Stru cture and Mechanism for Pilus-Mediated Microcolony Formation." Mol Microbiol. **77**(3):755–70.

30. a, bAhmad S, Gromiha MM, Fawareh H, Sarai A (2004). "ASAView: Database and Tool for Solvent Accessibilit y Representation in Proteins." BMC Bioinformatics.

31. ^Chakraborty S, Mukhopadhyay AK, Bhadra RK, Ghosh AN, Mitra R, Shimada T, et al. (2000). "Virulence Ge nes in Environmental Strains of Vibrio cholerae." Appl Environ Microbiol.

32. ^ Kotetishvili M, Stine OC, Chen Y, Kreger A, Sulakvelidze A, Sozhamannan S, et al. (2003). "Multilocus Sequence Typing Has Better Discriminatory Ability for Typing Vibrio cholerae Than Does Pulsed-Field Gel Electrophoresis and Provides a Measure of Phylogenetic Relatedness." J Clin Microbiol.

33. ^ Safa A, Nair GB, Kong RYC (2010). "Evolution of New Variants of Vibrio cholerae O1." Trends Microbiol. **18** (1):46–54.

34. a, b Kim EJ, Lee D, Moon SH, Lee CH, Kim DW (2014). "CTX Prophages in Vibrio cholerae O1 Strains." J Microbiol Biotechnol. **24**(6):725–31.

35. ^ Naha A, Pazhani GP, Ganguly M, Ghosh S, Ramamurthy T, Nandy RK, et al. (2012). "Development and Evaluation of a PCR Assay for Tracking the Emergence and Dissemination of Haitian Variant ctxB in Vibrio cholerae O1 Strains Isolated From Kolkata, India." J Clin Microbiol.

36. a, b Satchell KJF, Jones CJ, Wong J, Queen J, Agarwal S, Yildiz FH (2016). "Phenotypic Analysis Reveals That the 2010 Haiti Cholera Epidemic Is Linked to a Hypervirulent Strain." Infect Immun. **84**(9):2473–81.

37. ^ Kumar P, Thulaseedharan A, Chowdhury G, Ramamurthy T, Thomas S (2011). "Characterization of Novel Alleles of Toxin Co-Regulated Pilus A Gene (tcpA) From Environmental Isolates of Vibrio cholerae." Curr Microbiol. **62**(3):758–63.

38. ^ Craig L, Volkmann N, Arvai AS, Pique ME, Yeager M, Egelman EHH, et al. (2006). "Type IV Pilus Structure by Cryo-Electron Microscopy and Crystallography: Implications for Pilus Assembly and Functions." Mol Cell.

39. ^ Malik S, Kumar P, Yadav CP, Kumar D, Kumar A (2025). "Histone Chaperones as Potential Epidrug Targets Against Cancer." Nucleosides Nucleotides Nucleic Acids. 1–15.

40. ^ Singh PK, Jain P, Pandey V, Saxena S, Tripathi S, Kumar A, et al. (2021). "Smokeless Tobacco Quitting During COVID-19: A Mixed-Methods Pilot Study Among Participants Screened for a Cessation Trial in India." Clin Epidemiol Glob Health. **12**:100902. http://www.ncbi.nlm.nih.gov/pubmed/34786519.

41. ^ Deoshatwar A, Salve D, Gopalkrishna V, Kumar A, Barve U, Joshi M, et al. (2021). "Evidence-Based Health Behavior Interventions for Cholera: Lessons From an Outbreak Investigation in India." Am J Trop Med Hyg. **106** (1):229–32. http://www.ncbi.nlm.nih.gov/pubmed/34695790.

42. ^ Son MS, Megli CJ, Kovacikova G, Qadri F, Taylor RK (2011). "Characterization of Vibrio cholerae O1 El Tor Biotype Variant Clinical Isolates From Bangladesh and Haiti, Including a Molecular Genetic Analysis of Virulence Genes." J Clin Microbiol. **49**(11):3739–49.

43. ^ Ghosh P, Naha A, Basak S, Ghosh S, Ramamurthy T, Koley H, et al. (2014). "Haitian Variant tcpA in Vibrio cholerae O1 El Tor Strains in Kolkata, India." J Clin Microbiol.

44. ^Kim EJ, Yu HJ, Lee JH, Kim JO, Han SH, Yun CH, et al. (2017). "Replication of Vibrio cholerae Classical CTX Phage." Proc Natl Acad Sci U S A. **114**(9):2343–8.

45. ^Bouzari S, Aslani MM, Oloomi M, Jafari A, Dashti A (2011). "Comparison of Multiplex PCR With Serogrouping and PCR-RFLP of fliC Gene for the Detection of Enteropathogenic Escherichia coli (EPEC)." Braz J Infect Dis.

46. a, b, cDonnenberg MS, Kaper JB (1992). "Enteropathogenic Escherichia coli." Infect Immun. **60**(10):3953–61.

47. ^Donnenberg MS, Tacket CO, James SP, Losonsky G, Nataro JP, Wasserman SS, et al. (1993). "Role of the eaeA Gene in Experimental Enteropathogenic Escherichia coli Infection." J Clin Invest. **92**(3):1412–7.

Declarations

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