Research Article

Characterization and Molecular Identification of *Vibrio cholerae* from Sea Beach Using 16S rRNA Sequencing

Kalpita Bhatta[1,*], Priyadarshani Samal[2], Jyoti Ranjan Rout[3] Santi Lata Sahoo[2]

[1]Department of Botany, School of Applied Sciences, Centurion University of Technology and Management, Odisha, INDIA.
[2]Department of Botany, Biochemistry and Molecular Biology Laboratory, Utkal University, Vani Vihar, Bhubaneswar, Odisha, INDIA.
[3]School of Biological Sciences, AIPH University, Bhubaneswar, Odisha, INDIA.

Submission Date: 19-06-2023; Revision Date: 13-07-2023; Accepted Date: 12-09-2023.

ABSTRACT

Background: Developed and developing countries were confronted with public health concerns due to the devastating contamination of deadly microbes. *Vibrio* is one among them which is associated with contaminated water resources and food materials. The disease cholera, a gastroenteric, has recorded a history of seven pandemics globally, but in much of Asia, Africa, and Latin America. The present investigation elucidates with characterization and molecular identification of *Vibrio* from contaminated water of the urban waste discharge point of the Puri Sea beach, Odisha, India as studies have shown that the Bay of Bengal has been the native for variants of *Vibrio cholerae*. **Materials and Methods:** The bacteria were isolated in nutrient agar and Thiosulfate Citrate Bile Salt (TCBS) agar medium with distinct cultural characteristics of *Vibrio*. 16S rRNA was identified DNA was isolated by centrifugation at 5000rpm for 10 min. Amplification of DNA was carried out and the 16 S rRNA was amplified. Then 16 S rRNA was subjected to molecular evolution analysis. **Results:** The bacteria were identified by both biochemical and molecular characterization and confirmed as *Vibrio cholerae* SPAB1, SPAB4, and SPAB5 (with NCBI Gene Bank Accession No: KT985959.1, KT985960.1, and KT985961.1, respectively). The bacteria were confirmed by 16S r RNA sequencing which shows a high degree of homology with the existing sequence and a comparison was carried out with amplified 16S rRNA gene sequence and NCBI sequence database. Molecular analysis of 16S rRNA generated an evolutionary and phylogenetic tree with a maximum identity of 95-99%. **Conclusion:** The three strains were new: KT985959.1, KT985960.1, and KT985961.1 are deposited in the NCBI database for future case studies. The strains were cardinal in designing the targeted drug.

Keywords: *Vibrio cholerae*, 16S rRNA, KT985959.1, KT985960.1, KT985961.1.

Correspondence:
Dr. Kalpita Bhatta,
Department of Botany, School of Applied Sciences, Centurion University of Technology and Management, Odisha, INDIA.

Email: kalpita.bhatta@cutm.ac.in

INTRODUCTION

Vibrio cholerae is a waterborne pathogen and the aetiological agent of cholera in humans. It belongs to the family Vibrionaceae, a facultatively anaerobic, non-spore-forming, curved rod Gram-negative ranging from 1.4-2.6 μm long and possesses fermentative metabolism. This single-sheathed polar flagellate bacterium is oxidase positive which reduces nitrates. These species are ubiquitous in marine environments and require 1-2% NaCl for optimal growth and metabolism.[1,2] Infectious diseases like diarrhoea and food-borne disorders are caused in humans due to some strains of *Vibrio cholerae*.[3-7] The Seventh pandemic and the new pandemic of cholera spread by O1 EI Tor biotype and non-O1 serogroup (O139 Bengal).[8,9] The epidemic with morbidity and mortality is an explosion of *Vibrio cholerae* in the affected areas which had an impact on the public as well as community life of people. Both pathogenic and non-pathogenic strains of *Vibrio cholerae* species are

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2023.12.54

present depending upon the virulence which may vary according to the gene content.[10] Different attempts have been made over several years for the isolation, identification, and characterization of *V. cholerae*.

The agent for causing cholera is one of the best adaptive bacteria found as it is capable of withstanding a wide variety of environmental stress. *Vibrio cholerae* generally reside in aquatic environments. It has 200 or more species in number depending upon the serogroup classified by O-polysaccharide specificity. Isolates in the sero O1 group consist of classical, El Tor, and serotypes Ogawa and Inaba. The O139 has been specified for causing the epidemic and pandemic of cholera. Several studies also emphasize that the single clonal expression of *Vibrio cholerae* O1 El Tor ancestor is assisted by horizontal gene transfer, which involves toxins and antibiotic-resistant genes mainly responsible for the pandemic and epidemics.[11-13] On the other hand, the occurrence of *Vibrio cholerae* is very common in urban water bodies due to poor wastewater management and/or poor sanitary conditions. Municipal wastewater contains a variety of pathogenic microorganisms including *Vibrio cholerae* that induces a high risk for the environment and public health.[14] So, the current study is primarily aimed at checking the possible occurrences of pathogenic bacteria from the urban discharge water of Puri, India which is directly exposed to seawater. This bacterium can be identified by taking into account morphological, biochemical, and molecular properties. The morphological and biochemical characterization is generally laborious and takes more time for identification.[15] However, the isolation and identification of bacterial isolates were done through biochemical and morphological methods which will pave the way for the isolation of new variants which is very tedious. Based on findings by Pal *et al.*, altered *Vibrio cholerae* O1 was born on the east coast of the Bay of Bengal and gradually spread to other areas, suggesting that the Gangetic belts of the Bay of Bengal were the origin of cholera.[16] The estuarine habitat of the Bay of Bengal and the temperature of 35°C give more impetus for the growth of *Vibrio*.[17,18] Better survival capacity in the environment leads to the production of a significant amount of Cholera Toxin (CT).

Over the years, advanced molecular techniques in microbiological systems have given more systematic and confirmative detection of microbial species. The PCR-based method is the latest widely conventional method of detection and identification of the 16S rRNA gene. A complete genetic DNA relatedness provides absolute resolution in bacterial categorization. In general, the basic taxonomic information is incorporated into the complete nucleotide sequence of the genome.[19] The 16S rRNA gene sequencing has developed into one of the essential pillars in polyphasic bacterial categorization and novel detection of bacteria.[20] The 16S rRNA is the most preserved of these three rRNAs, it has been future as an "evolutionary clock" that has shown the way to the renewal of the tree of life. It is becoming increasingly popular to compare the 16S rRNA gene sequence of bacteria.[21] 16Sr RNA gene sequences are very useful informatics to provide identification of species and genus for strains that are associated with human transmittable diseases.[22] The 16 s rRNA operons contain at least one promoter region. Clinical microbiology also uses 16S rRNA to identify pathogens at a species level. The bacterium *Vibrio cholerae* has two chromosomes[23] and the two chromosomes are believed to confer an evolutionary advantage to *Vibrio*. As they allow for replication in a quicker way than if they were to have one large genome, thus shorting the generation time of the bacteria.[24] The National Centre for Biotechnology Information (NCBI) provides more than 10,000 clinical and environmental isolates with sequence which are available online (www.ncbi.nlm.nih.gov) and in other websites which provides comparison algorithms. Moreover, with the help of bioinformatics tools, the phylogenetic tree is constructed by comparing it with other bacteria.[25]

The virulence of *Vibrio cholerae* depends upon the genes rtx A, rtx C and ctx B respectively.[18] This investigation will give further insight into variants of *V. cholrea* and their toxicity which can be explored to develop novel drugs in the near future.

MATERIALS AND METHODS

Isolation of bacteria

The sewage water samples were collected from the sea beach area of Puri, Odisha, India. Serial dilutions of each sewage sample (10 mL each) were carried out up to 10^{-5}. A spread plate technique was used to isolate *Vibrio cholerae* from 0.1 mL of a diluted sample using TCBS agar medium and nystatin and nalidixic acid as antifungal and antimicrobial agents. The number of colonies in each set of plates was scored and recorded as CFU/mL after a 48-hour incubation at 35°C.

Morphological and Biochemical characterization

The purified isolates were grown for 4 days at 30°C on TCBS agar plate and their cultural characteristics were observed. Some basic features like the nature of colony morphology and the color of the culture were initially observed. For biochemical analyses, the IMVIC test,

sugar utilization test, catalase test, and coagulate test were done as per the standard procedure.

Molecular characterization

DNA extraction from *Vibrio cholerae*

For genomic DNA isolation, 1.5 mL of overnight grown bacterial culture was taken and centrifuged at 5,000 rpm for 10 min. The residue obtained was resuspended in 200µL of lysis buffer, and 66 µL of 5M sodium chloride to remove proteins along with cell debris. Again, the suspension mixture was centrifuged and the remaining residue was suspended with isoamyl alcohol: chloroform (1:1, v/v). The mixture was gently inverted several times to obtain a white liquid substance. A further step included centrifuging at 14,000 rotations per minute for 10 min, followed by interacting the collected supernatant with ice-cooled ethanol. The solution was gently mixed 5 to 6 times and again spun to get the pellet to which cold 70% ethanol was added mixed thoroughly and spun to obtain the DNA pellet and it was dissolved in 100µL TE buffer and stored at -20°C for further analysis. The quantity of DNA was checked by taking the optical density at 260 nm and comparing it with standard herring sperm DNA.[26]

PCR amplification and DNA Sequencing

Amplification of DNA sequence was carried out in Eurofins Genomics India Pvt. Ltd., Lab, Bengaluru, India. Electrophoresis analysis has shown a single band of high-molecular weight DNA. The 16S rRNA gene was amplified by taking 27F (*5′-AGAGTTTGATCCTGGCTCAG-3′*) and 1492R (*5′-GGTTACCTTGTTACGACTT-3′*) universal primers with ABI 3730xl Genetic Analyzer (Applied BioSystems, USA).

Phylogenetic analysis

The 16S rRNA gene sequenced from isolated *Vibrio cholerae* was subjected to molecular evolutionary analysis. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Pennsylvania State University, PA, USA; https://www.megasoftware.net/). The sequences were submitted to NCBI database with Genebank accession number KT985959.1 (SPAB1), KT985960.1 (SPAB4) and KT985961.1 (SPAB5). Phylogeny construction was achieved through a maximum likelihood tree and the detailed analysis of the nucleotides was carried out using the Tamura-Nei substitution model based on a 1,000-replication bootstrap method.[27]

RESULTS

Morphological and biochemical identification

For the morphological study, only the color characteristic of bacterial isolates was observed on the TCBS medium and found that all selected isolates (SPAB1, SPAB4 and SPAB5) showed a yellowish in color on the petri dish culture (Figure 1).

IMVIC tests were performed for biochemical analysis and the findings are represented in Table 1.

The isolated bacterial species showed positive results for different biochemical tests like catalase, coagulase, indole and methyl red which aided the additional steps for identification of *Vibrio* spp. (Table 1 and Figure 2).

Yellow colonies on TCBS agar medium primary indicates the presence of *Vibrio cholerae* which is a typical culture characteristic of *Vibrio* spp. In TCBS

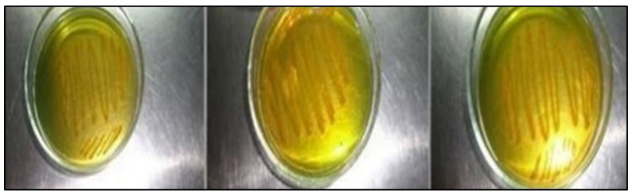

Figure 1: Thiosulfate citrate bile salt medium containing plate showing the characteristic *Vibrio* spp. Colonies (yellow) during enumeration procedures.

Table 1: Biochemical characteristics of *V. cholerae* SPAB1, SPAB4 and SPAB5.

Test	Observation
Catalase	+
Coagulase	+
Indole	+
Methyl red	+
Voges-Proskauer	Variable
Citrate utilization	+
Oxidase	+
Gelatin hydrolysis	+
Nitrate reduction	+
Galactose	+
sucrose	+
Mannitol	+
Lactose	Variable
Glucose	+
Mannose	+
Maltose	+
Tartrate	+
Trehalose	+
Sorbitol	-
Myo-inositol	-

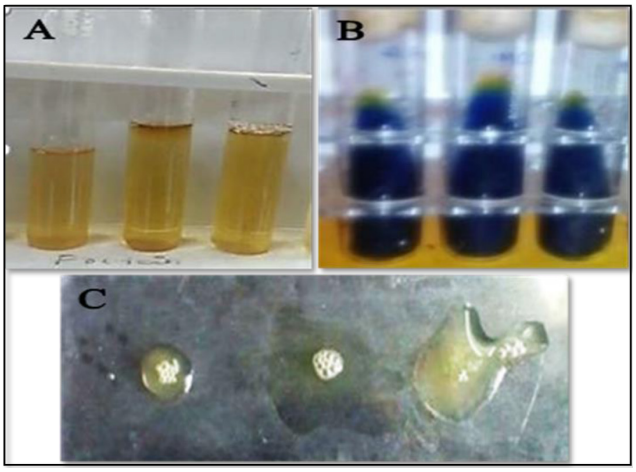

Figure 2: Image showing the various biochemical tests. Voges-Proskauer test (A), Citrate utilization test (B) and Coagulase test (C).

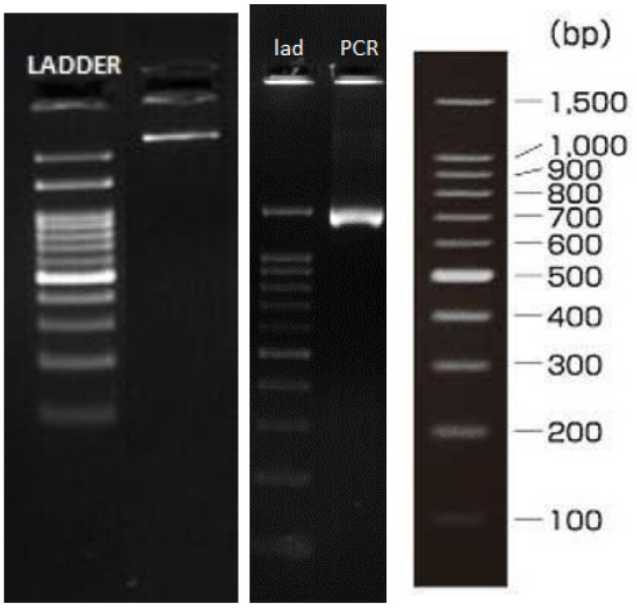

Figure 3: Agarose gel electrophoresis for PCR product of ctxB gene in *Vibrio cholerae* (460 bp).

agar medium *Vibrio cholerae* produces yellow colonies due to sucrose fermentation which is visible against a dark green background.[19] Bacteria produce acid through the fermentation of glucose can be identified by methyl red indicator test that confirms the red coloration under an acidic medium. In Voges-Proskauer (VP) test, microorganisms can produce the neutral end product acetyl methyl carbinol from pyruvic acid. A deep red color indicates the presence of VP positive organism.[28] Moreover, the existence of citrate permease enzyme in *Vibrio cholerae* allows for obtaining energy from citrate when no other carbon source is present. The citrate is converted into oxaloacetic acid which is

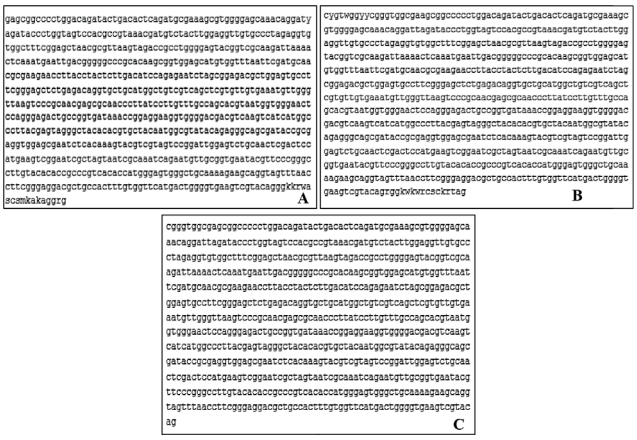

Figure 4: PCR product sequencing of 16S r RNA gene of isolated strains SPAB1 (A), SPAB4 (B) and SPAB5 (C).

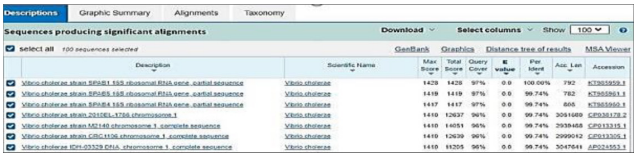

Figure 5: NCBI blasting of isolate sequence 16S rRNA with world sequences of 16S rRNA.

further converted to pyruvic acid and carbon dioxide. Carbon dioxide reacts with sodium and water, forming sodium carbonate, which transforms bromothymol blue indicator into Prussian blue which inferred positive results.[29]

Molecular identification

16S r RNA was used for molecular-level characterization using the NCBI database (Figures 3-5) by the BLAST search program (https://www.ncbi.nlm.nih.gov).

The strains have 99% maximum similarity and were given accession no. KT985959.1, KT985960.1 and KT985961.1

These were used to construct a phylogenetic dendrogram to find out the homology between the isolated strains (Figure 5), so that their evolutionary homology can be determined (https://www.ncbi.nlm.nih.gov).[30]

The homology search made using BLAST showed 99-100% maximum identity with that of *V. cholerae* (Figure 4) with NCBI gene bank accession no. KT985959.1, KT985960.1 and KT985961.1.

Furthermore, the phylogenetic dendrogram was constructed based on the sequences of bacteria isolates to determine the genetic relatedness between them. To know the evolutionary history

all the closed homologs of identified *V. cholerae* were used to draw the phylogenetic dendrogram (Figure 6).

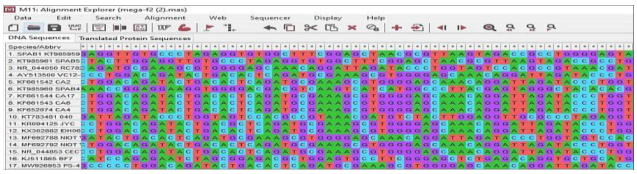

Figure 6: Homology analysis of different strains by NHEJ by using Mega 6.0. Results show nucleotide alignment similarity (*) with different V. cholerae isolates.

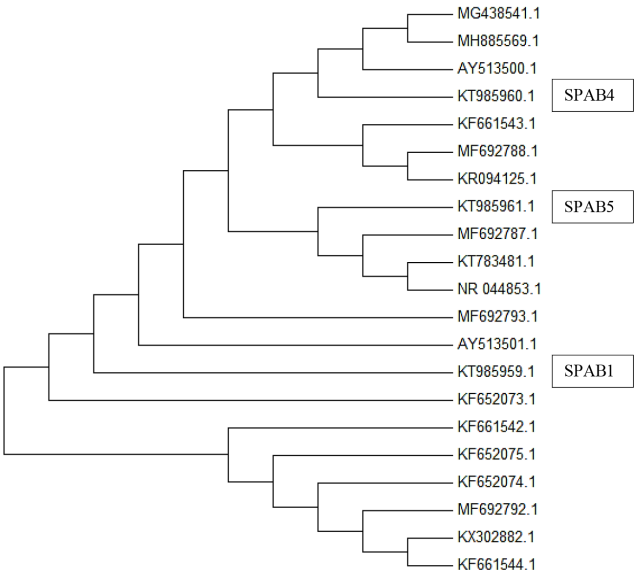

Figure 7: Phylogenetic tree representing close homology of *Vibrio cholerae* KT985959.1 (SPAB1), KT985960.1 (SPAB4) and KT985961.1 (SPAB5).

The dendrogram showing the relation between *Vibrio cholerae* and their close homologs is shown in Figure 7.

The customary method of identifying bacteria based on phenotypic characteristics is generally not accepted. The phenotypic characterisation of bacteria which includes morphological, physiological and physiological parameters to be studied is very time-consuming and tedious. Which is one of the major limitations of the process. The method needs some improvement over the time. For a phylogeny tree closely related taxa and the type strain of appropriate genera should be taken into consideration for comparing phenotypic traits.[31] Accurate identification based on genetic markers is well acknowledged. Therefore, proper identification based on genetic methods comparing bacterial 16S rRNA sequences has emerged as the preferred genetic technique. It is the most suitable methodology for the identification and characterization of bacteria which is applied all over the world. The 16S rRNA sequencing further strengthens the finding and the validity of the results as it has been already proven by published papers.[12,13]

DISCUSSION

The abundance and community composition of *Vibiro* has been well documented previously in estuaries and coast. However, it has been little studied in the Bay of Bengal ecosystem which upholds a key position for its origin. Parida *et al*., (2010) first studied the bacteria in the sea beach of Puri and well documented their results by findings of two-gram negative bacteria *Aeromonas schubertii* and *Serratia plyumuthia*.[32] Therefore, the present study is the first of its kind to give due importance to *Vibiro* as Asian countries have always been the origin of this bacteria.[16] The present investigation aimed to study the spatial heterogeneity of *Vibiro* species collected from marine environments. Three analytical methods such as culturing, bacterial universal 16S rRNA and *Vibrio* specific 16S RNA molecular techniques were used to find out the different species of *Vibrio*. Which is followed by researchers all over the world.[12,13] Our results suggest three new species of *Vibrio* such as KT985959.1 (SPAB1), KT985960.1 (SPAB4), and KT985961.1 (SPAB5). Which were abundant in presence at the discharge point of sewage. The paper gives a comprehensive view of the study that the molecular method is more appropriate and relevant to study while analysing low-biomass seawater samples rather than biochemical methods. The molecular technique is a better perspective for targeted *Vibiro* identification than the culture procedure. *Vibrio* diversity is generally assessed through high-throughput sequencing of primers targeting bacterial or *Vibrio* specific 16S rRNA genes. The sequence of the 16S rRNA gene has been widely used as an evolutionary clock to estimate relations among bacteria (progeny). In recent years, it has also become a useful tool for identifying unknown bacteria to genus or species levels. It has been common practice to review the sequence of 16S rRNA to review the bacterial phylogeny and taxonomy since it has several advantages over most other genetic markers. Patel J.B. (2001) emphasized the use of 16S rRNA as the sequence that has not changed over time and the sequence 1500 bp is large enough to contribute toward genetic analysis.[33] In the past using *Vibrio* specific 16S rRNA gene primers, for phylogeny tree construction has been improved largely.[34] In a *Vibiro* species, the alignment of sequence between the different strains is very low. In the present investigation, we have discarded the similar sequences present below 95% to any *Vibrio* 16S rRNA sequence in NCBI database. This study evaluated the use of 16S rRNA amplicons in combination with culturing techniques to survey the *Vibrio* community and its abundance at Puri Sea Beach located in an estuarine environment. However,

for a better understanding the *Vibrio* abundance and its presence in the Bay of Bengal we have to incorporate a wide range of environmental factors that regulate *Vibiro* It will be easier to understand and monitor its occurrence in the coastal environment, in particular in tropical and subtropical regions.

CONCLUSION

Identification of new strains of bacteria with the help of molecular techniques is now gaining importance where the usage of 16S rRNA sequence method is vividly used. In this study, three strains (like *V. cholerae* SPAB1, *V. cholerae* SPAB4 and *V. cholerae* SPAB5) are identified through morphological, biochemical, and molecular analysis. These three new strains are new to record and submitted to NCBI database. *Vibrio cholerae* is a natural inhabitant of aquatic ecosystems globally. Strains of the bacteria cause the epidemic diarrheal disease known as cholera. This study deals with the characterization of the phenotypic and taxonomic status of new strains of cholera found in the urban sewage of the Puri Sea beach area. Documentation of new strains will give a new impetus to the ongoing research activity on cholera and will play a pivotal role in public epidemiology. The isolates and their pathogenicity can prove to be a major cornerstone for developing medicine for drug-resistant strains. Cholera is a well-known communicable disease and it is a major concern for Asian countries. Its fatality can be measured by the fact that various serotypes of *V. cholera* have reached to pandemic level seven times. The result found in the current study may be a benchmark in exploring more serotypes of *Vibrio* and ways to undermine the effects of its toxin cholera. Which will be useful in controlling and curing the disease.

ACKNOWLEDGEMENT

All the authors thank the Department of Botany, Utkal University for providing the laboratory facility to carry out the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NCBI: National Center for Biotechnology Information; **NHEJ:** Non-homologous end joining; **IMVIC:** Indole methyl red Voges-proskauer citrate; **PCR:** Polymerase Chain Reactions.

SUMMARY

Cholera is a severe disease which has been present since time immemorial. It is indigenous to the estuarine environment of the Bay of Bengal. The temperature and low salinity favour its growth and multiplication. Many strains of the bacteria *Vibiro chlorea* have been developed over these years as a result of an assortment of genes. The present work findings of new bacterial strains of the *Vibiro chlorea* at Puri Sea Beach will manifest a novel way of drug designed to control the disease. It is concluded that three strains, KT985959.1 (SPAB1), KT985960.1 (SPAB4) and KT985961.1 (SPAB5) were new to the record and thus open up avenues for targeted drug delivery and discovery of new medications for the control of epidemic.

REFERENCES

1. Thompson FL, Klose KE, AVIB Group. *Vibrio* 2005: The first international conference on the biology of vibrios. J Bacteriol. 2006;188(13):4592-6. doi: 10.1128/JB.00141-06, PMID 16788166.
2. Frans I, Michiels CW, Bossier P, Willems KA, Lievens B, Rediers H. *Vibrio anguillarum* as a fish pathogen. Virulence factors, diagnosis and prevention. J Fish Dis. 2011;34(9):643-61. doi: 10.1111/j.1365-2761.2011.01279.x, PMID 21838709.
3. Lee YL, Hung PP, Tsai CA, Lin YH, Liu CE, Shi ZY. Clinical characteristics of non-O1/ non-O139 *Vibrio cholerae* isolates and polymerase chain reaction analysis of their virulence factors. J Microbiol Immunol Infect. 2007;40(6):474-80. PMID 18087626.
4. Chandrasekhar MR, Krishna BV, Patil AB. Changing characteristics of *Vibrio cholerae* emergence of multidrug resistance and non- O1, non-O139 serogroups. Southeast Asian J Trop Med Public Health. 2008;39(6):1092-7. PMID 19062701.
5. Thomas A, Straif-Bourgeois S, Sokol TM, Ratard RC. *Vibrio* infections in Louisiana:twenty-five years of surveillance 1080-2005. J La State Med Soc. 2007;159(4):205-11. PMID 17987958.
6. Dalsgaard A, Forslund A, Bodhidatta L, Serichantalergs O, Pitarangsi C, Pang L, *et al*. A high proportion of *Vibrio cholerae* strains isolated from children with diarrhoea in Bangkok, Thailand is multiple antibiotic resistant and belong to heterogenous non O1, nonO 139-serotypes. Epidemiol Infect. 1999;122(2):217-26. doi: 10.1017/s0950268899002137, PMID 10355785.
7. Altekruse SF, Bishop RD, Baldy LM, Thompson SG, Wilson SA, Ray BJ, *et al*. *Vibrio gastroenteritis* in the US Gulf of Mexico region: the role of raw oysters. Epidemiol Infect. 2000;124(3):489-95. doi: 10.1017/s0950268899003714, PMID 10982073.
8. Sack DA, Sack RB, Nair GB, Siddique AK. Cholera. Lancet. 2004;363(9404):223-33. doi: 10.1016/s0140-6736(03)15328-7, PMID 14738797.
9. Faruque SM, Mekalanos JJ. Pathogenicity islands and phages in *Vibrio cholerae* evolution. Trends Microbiol. 2003;11(11):505-10. doi: 10.1016/j.tim.2003.09.003, PMID 14607067.
10. Faruque SM, Albert MJ, Mekalanos JJ. Epidemiology genetics and ecology of toxigenic *Vibrio cholerae*. Microbiol Mol Biol Rev. 1998;62(4):1301-14. doi: 10.1128/MMBR.62.4.1301-1314.1998, PMID 9841673.
11. Abioye OE, Osunla AC, Okoh AI. Molecular detection and distribution of six medically important *Vibrio* spp. In selected freshwater and brackish water resources in Eastern Cape Province, South Africa. Front Microbiol. 2021;12:617703. doi: 10.3389/fmicb.2021.617703, PMID 34149632.
12. Al-Sa'ady AT, Baqer KA, Al-Salim ZKS. Molecular detection and phylogenetic analysis of *Vibrio cholerae* genotypes in Hillah, Iraq. New Microbes New Infect. 2020;37:100739. doi: 10.1016/j.nmni.2020.100739, PMID 32874595.

13. Azwai SM, Alfallani EA, Abolghait SK, Garbaj AM, Naas HT, Moawad AA, *et al*. Isolation and molecular identification of *Vibrio* spp. By sequencing of 16S rDNA from seafood, meat and meat products in Libya. Open Vet J. 2016;6(1):36-43. doi: 10.4314/ovj.v6i1.6, PMID 27004169.
14. Eddabra R, Moussaoui W, Prévost G, Delalande F, Van Dorsselaer AV, Meunier O, *et al*. Occurrence of *Vibrio cholerae* non-O1 in three wastewater treatment plants in Agadir (Morocco). World J Microbiol Biotechnol. 2011;27(5):1099-108. doi: 10.1007/s11274-010-0556-7.
15. Harwood VJ, Gandhi JP, Wright AC. Methods for isolation and confirmation of *Vibrio vulnificus* from oysters and environmental sources: a review. J Microbiol Methods. 2004;59(3):301-16. doi: 10.1016/j.mimet.2004.08.001, PMID 15488274.
16. Pal BB, Behera DR, Nayak SR, Nayak AK. Origin and dissemination of altered El Tor *Vibrio cholerae* O1 causing cholera in Odisha, India: two and half decade's view. Front Microbiol. 2021;12:757986. doi: 10.3389/fmicb.2021.757986, PMID 34867883.
17. Alam M, Hasan NA, Sadique A, Bhuiyan NA, Ahmed KU, Nusrin S, *et al*. Seasonal cholera caused by *Vibrio cholerae* serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. Appl Environ Microbiol. 2006;72(6):4096-104. doi: 10.1128/AEM.00066-06, PMID 16751520.
18. Moore S, Thomson N, Mutreja A, Piarroux R. Widespread epidemic cholera caused by a restricted subset of *Vibrio cholerae* clones. Clin Microbiol Infect. 2014;20(5):373-9. doi: 10.1111/1469-0691.12610, PMID 24575898.
19. Goris J, Konstantinidis K. T, Klappenbach J.A. DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol. 2007;57:81-91. doi: 10.1099/ijs.0.64483-0.
20. Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use of 16S rRNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect. 2008;14(10):908-34. doi: 10.1111/j.1469-0691.2008.02070.x, PMID 18828852.
21. Clarridge JE III. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev. 2004;17(4):840-62. doi: 10.1128/CMR.17.4.840-862.2004, PMID 15489351.
22. Malik S, Beer M, Megharaj M, Naidu R. The use of molecular techniques to characterize the microbial communities in contaminated soil and water. Environ Int. 2008;34(2):265-76. doi: 10.1016/j.envint.2007.09.001, PMID 18083233.
23. Trucksis M, Michalski J, Deng YK, Kaper JB. The *Vibrio cholerae* genome contains two unique circular chromosomes. Proc Natl Acad Sci U S A. 1998;95(24):14464-9. doi: 10.1073/pnas.95.24.14464, PMID 9826723.
24. Thompson FL, Thompson CC, Vicente AC, Klose KE. Mol Microbiol *Vibrio* the third international conference on biology of Vibrios. 2010;77:1663-71.
25. Sambrook J, Fritsch E. R, Maniatis T. Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press Book Company; 1989.
26. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. Mega 6: Molecular evolutionary genetics. Mol Biol Evol. 2013;30(12):2725-9. doi: 10.1093/molbev/mst197, PMID 24132122.
27. Rohomania T, Saha ML, Hossain A, Rahman MS. Morphological and biochemical characterization of bacteria isolated from fresh and salted Hilsa, *Tenualosa Ilisha* (Hamilton, 1822). Bangladesh J Microbiol. 2015;32:7-13. doi: 10.3329/bjm.v32i0.28471.
28. Wang X, Liu Y, Sun J. Physiological and biochemical characterization of isolated bacteria from a coccolithophore *Chrysotila dentata* (Prymnesiophyceae) culture. Diversity. 2022;14(1):2. doi: 10.3390/d14010002.
29. Sacchi CT, Whitney AM, Mayer LW, Morey R, Steigerwalt A, Boras A, *et al*. Sequencing of 16S rRNAGene: A rapid tool for Identification of *Bacillus anthracis*. Emerg Infect Dis. 2002;8(10):1117-23. doi: 10.3201/eid0810.020391, PMID 12396926.
30. Xu W, Lin W, Wang Z, Gao Y, Luo Y, Grossart HP, *et al*. Disentangling the abundance and structure of *Vibrio* communities in a semi-enclosed Bay with mariculture (Dongshan Bay, Southern China). Comp Struct Biotechnol J. 2021;19:4381-93. doi: 10.1016/j.csbj.2021.07.040, PMID 34429854.
31. Li Q, Chen X, Jiang Y, Jiang C. Cultural, physiological, and biochemical identification of Actinobacteria. Actinobacteria-Basics Biotechnol Appl. 2016:87-111.
32. Parida P, Patit SS, Ray P. Negative bacterial isolates from coastal belt of Bay of Bengal at Shamuka Sea Beach puri, Orissa, India. Int J Appl Environ Sci. 2010;5(6):899-908.
33. Patel JB. 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Mol Diagn. 2001;6(4):313-21. doi: 10.1054/modi.2001.29158, PMID 11774196.
34. Yong L, Guanpin Y, Hualei W, Jixiang C, Xianming S, Guiwei Z, *et al*. Design of *Vibrio* 16S rRNA gene specific primers and their application in the analysis of seawater *Vibrio* community. J Ocean Univ China. 2006;5(2):157-64. doi: 10.1007/BF02919216.

Cite this article: Bhatta K, Samal P, Rout JR, Sahoo S. Characterization and Molecular Identification of *Vibrio cholerae* from Sea Beach Using 16S rRNA Sequencing. Asian J Biol Life Sci. 2023;12(2):408-14.