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Molecular evaluation of *Vibrio cholerae* O1 strains in India to decipher the mechanism of higher virulence and antimicrobial resistance

Abstract

Vibrio cholerae, the causative agent of the diarrheal disease cholera can frequently modify their genotypic and phenotypic attributes to adapt well in the environment and cause severe outbreaks in the community. The present study aimed to characterize the changing genetic features of recently circulating *V. cholerae* O1 strains in India with special emphasis on alterations in the resistance profile towards third generation cephalosporin antibiotics.

In a study period of 4 years (2018-2021), a total of 474 *V. cholerae* O1 strains isolated from different cholera endemic states which were further classified into two groups, West Bengal (n=116) and other regions of India (n=358) (Maharashtra, Gujarat, Delhi, Tamil Nadu, and Karnataka). All the isolates from other regions of India belonged to El Tor biotype and carried the Haitian alleles of virulence-associated genes, *ctxB, tcpA,* and *rtxA* with polymyxin B-sensitive phenotype. Moreover, a deletion in the VSP II region, a remarkable trait of Yemen cholera outbreak strains has been checked by a newly developed PCR which demonstrated the presence of this deletion (VSP-IIC) within all the isolates from other regions of India. However, only 6% (7/116) of the West Bengal strains exhibited classical *ctxB* with El Tor *rtxA* along with polymyxin B-resistant, non-hemolytic trait and harboured VSP-II rather than VSP-IIC type. These strains were found to contain a new variant of *hlyA* with a 6 bp deletion in addition to the 11 bp deletion which is a characteristic feature of non-hemolytic classical *hlyA*. We have also evaluated various roles of polymyxin B-sensitive allele ($carR^S$) in comparison to the resistant allele ($carR^R$) in terms of pathogenicity and observed that polymyxin B sensitivity has been associated with higher IL-8 production in INT 407 cell line which is linked to further higher motility and adherence compared to the resistant strains. The mRNA expression study of flagellar genes *flhDC* is significantly higher in the case of polymyxin B-sensitive Haitian variant strains. However, the contribution of $carR^S$ allele in suckling mice colonization and fluid accumulation in rabbit illeal loop model has given similar results to the $carR^R$ allele.

All of the studied strains followed the antibiogram pattern similar to that exhibited by multidrug resistant El Tor vibrios. Interestingly six of the isolates from Gujarat (2019) revealed resistance against carbapenem and hence were further characterized. The whole genome sequencing of these isolates indicated the presence of a 142kb mega-sized plasmid belonging to IncA/C1 which harbours genes such as bla_{NDM-1}, bla_{CMY-6}, bla_{DHA-7}; *aph(3')-VI, aph(6)-Id, aac(6')-Ib10, aac(3)-IId, rmtC* conferring resistance against the antibiotics beta-lactam and aminoglycosides, respectively. The plasmid was conjugally transferred into *Escherichia coli*, *V. parahaemolyticus, Shigella flexneri* 4a, *Salmonella enterica*, and *V. cholerae* O1 Ogawa and depicted similar resistance patterns which may pose a high risk in the management of enteric diseases. The analysis by pulse field gel electrophoresis also showed that all of the six strains were clonally similar to each other.

This study indicated the prevalence of Haitian variant *V. cholerae* O1 strains linked to various hypervirulent features as well as the developing resistance against carbapenem antibiotics, which may aid in the spreading of this catastrophic disease. Therefore, continuous monitoring is important for better management and the control of antibiotic resistant *V. cholerae* O1 strains.

AKMukhopadhyay 10/10/2023

Sreeja Shaw
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Dr. Asish K. Mukhopadhyay
Scientist F (Sr. Deputy Director)
Division of Bacteriology
ICMR National Institute of Cholera and Enteric Diseases
P-33, C.I.T Road, Scheme XM
Kolkata-700010; India