

List of ten best papers highlighting the important discoveries/contributions

- 1. 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. *Frontiers in Microbiology*. 2021;12.**

The origin, spread and molecular epidemiology of altered El Tor *Vibrio cholerae* O1 strains isolated from cholera outbreaks/surveillance studies between 1995 and 2019 from different district of Odisha were analyzed. The stock cultures of *V. cholerae* O1 strains from 1995 to 2019 were analyzed through molecular analysis using different PCR assays and pulse field gel electrophoresis (PFGE) analysis. The spread map (month, year and place) was constructed to locate the dissemination of altered El Tor variants of *V. cholerae* O1 in this region. A total of 13 cholera outbreaks were caused by *V. cholerae* O1 Ogawa biotype El Tor carrying *ctxB1* and *ctxB7* genotypes. **The *ctxB1* alleles of *V. cholerae* O1 mostly confined to the coastal areas, whereas the *ctxB7* genotypes, though originating in the coastal region of Odisha in 1999 first time reported from the globe, concentrated more in the tribal areas.** The positive correlation between virulence-associated genes (VAGs) was found through Pearson's correlation model, indicative of a stronger association between the VAGs. **The clonal relationship through PFGE between *ctxB1* and *ctxB7* genotypes of *V. cholerae* O1 strains exhibited 80% similarity indicating single- or multi-clonal evolution. It is evident from this study that the spread of multidrug-resistant altered El Tor *V. cholerae* O1 was dominant over the prototype El Tor strains in this region. The origin of altered El Tor variants of *V. cholerae* O1 occurred in the East Coast of Odisha established that the origin of cholera happened in the Gangetic belts of Bay of Bengal where all new variants of *V. cholerae* O1 might have originated from the Asian countries.**

- 2. Pal BB*, Nayak AK, Nayak SR. Emergence and spread of different *ctxB* alleles of *Vibrio cholerae* O1 in Odisha, India. *International Journal of Infectious Diseases*. 2021;105:730-2.**

This study reported variants of the *ctxB* allele of *Vibrio cholerae* O1 isolated between 1995 and 2019 in Odisha, **India. *ctxB1* genotypes dominated from 1995 to 2016. The Haitian**

variant and El Tor *ctxB3* genotypes of *V. cholerae* O1 emerged in 1999, and were most common in 2018-2019 and 2005-2011, respectively. The *ctxB7* genotype of the Haitian variant of *V. cholerae* O1 was quiescent from 2000 to 2006, but further spread was noted from 2007 to 2019.

3. Pal BB*, Nayak SR, Biswal B, Das BK. Environmental reservoirs of *Vibrio cholerae* serogroups in the flowing freshwater environs from the tribal areas of Odisha, Eastern India. *Environmental Microbiology Reports*. 2021;13(2):119-125.

The environmental reservoirs of different serogroups of *Vibrio cholerae* causing cholera in the flowing freshwater bodies of the tribal areas of Odisha are not known. So this study was conducted from June 2017 to March 2020 to find out the environmental reservoirs of *V. cholerae* serogroups in the water and plankton samples collected from the river, nala, stream and chua from Rayagada district. Similarly, rectal swabs were collected from diarrhoea patients and correlation was established among the *V. cholerae* strains isolated from diarrhoea patients and environmental *V. cholerae* isolates through routine culture, different multiplex PCR assays and pulse field gel electrophoresis (PFGE) analysis using standard techniques. **The multiplex PCR assays on biotypes and different toxic genes exhibited similar correlation between the clinical and water isolates, which was further strengthened by PFGE analysis. The planktonic DNA was positive for *ctxA* gene which established that the environmental water bodies were the reservoirs for virulence genes of *V. cholerae* serogroups. The detection of environmental reservoirs of *V. cholerae* serogroups in temporarily stagnant condition of water; partially encircled by stones, and near the bank of the river, nala and stream were the reservoirs which is a rare report from Odisha, India and Globe.**

4. Pal BB*, Mohanty A, Biswal B, Nayak SR. New variant of *Vibrio cholerae* O139 in Odisha, India. *Journal of clinical microbiology*. 2019;57(5):e01877-18.

Three interesting findings emerged from this investigation, namely (i) **the reemergence of *V. cholerae* O139 in Odisha after a hiatus of 10 years;** (ii) **the reemerged O139 strains carried a novel *ctxB* genotype, and this is the first report of such strains of O139 causing outbreaks of cholera;** and (iii) **a new variant of *V. cholerae* O139 has again emerged from the Bay of Bengal region.** By sequencing and comparing hundreds of bacterial genomes, recent

studies have shown that all of the explosive epidemics of cholera in Africa and the Americas in the past half-century arose after the arrival of new strains that had evolved in Asia. **Although the O139 serotype of *V. cholerae* has not spread to the African or American continents, the likelihood of this new variant spreading to other parts of the world should not be underestimated which is a new variant reported for the first time from Globe**

5. Pal BB*, Khuntia HK, Nayak SR, Mohanty A, Biswal B. *Vibrio cholerae* O1 Ogawa Strains Carrying the *ctxB7* Allele Caused a Large Cholera Outbreak during 2014 in the Tribal Areas of Odisha, India. Japanese journal of infectious diseases. 2017;70(5):549-553.

The large outbreak of cholera reported during July to September 2014 in the Narla block of Kalahandi district, India, was investigated to determine the causative organism. Rectal swabs collected from patients with diarrhea and environmental water samples were cultured following standard techniques. The causative organism was identified as *Vibrio cholerae* O1 Ogawa biotype El Tor, and analysis by double mismatch mutation assay PCR confirmed that all strains were the *ctxB7* variant of Haitian *V. cholerae* O1. **This *ctxB7* variant of *V. cholerae* O1 was first originated from Odisha after the super cyclone of 1999.** The environmental water samples were negative for *V. cholerae*. The *V. cholerae* O1 strains were sensitive to tetracycline, ciprofloxacin, norfloxacin, ofloxacin, doxycycline, and azithromycin, but were resistant to erythromycin, gentamicin, chloramphenicol, furazolidone, neomycin, cotrimoxazole, nalidixic acid, and ampicillin. In the 2014 cholera outbreak, the early reporting of the pathogen enabled the government authorities to implement adequate control measures in time to curtail the spread of the disease. **This was the second large cholera outbreak due to Haitian variants of *V. cholerae* O1 after the 2010 Haiti cholera outbreak reported from Odisha, India, and other locations globally.** Active surveillance is required to track the spread of this strain in the Odisha region.

6. Pal BB*, Khuntia HK, Samal SK, Kar SK, Pattnaik B. Epidemics of severe cholera caused by El Tor *Vibrio cholerae* O1 Ogawa possessing the *ctxB* gene of the classical biotype in Orissa, India. International Journal of Infectious Disease. 2010;14:384-389.

This study represents an investigation of epidemic of cholera that occurred in Kashipur and Dasmantpur blocks of Orissa, reported during July–September 2007. Sixty-two rectal swabs and

28 water samples collected from diarrhea patients at different hospitals and villages were bacteriologically analyzed for the identification, antibiogram, and detection of toxic genes of *Vibrio cholerae*. The cholera outbreaks were caused by *V. cholerae* O1 Ogawa biotype El Tor in both Kashipur and Dasmantpur blocks. All the *V. cholerae* isolates from the clinical and environmental samples were sensitive to tetracycline, gentamicin, azithromycin, and chloramphenicol, but were resistant to ampicillin, ciprofloxacin, norfloxacin, co-trimoxazole, nalidixic acid, neomycin, and furazolidone, except the water isolates, which were sensitive to ciprofloxacin and norfloxacin. **The multiplex PCR assay revealed that all the clinical and environmental *V. cholerae* isolates were positive for the *ctxA* and *tcpA* genes, showing biotype El Tor. Interestingly, 88% of the clinical and environmental isolates of *V. cholerae* were El Tor biotype with mutation at the *ctxB* gene of the classical strain, as confirmed by mismatch amplification of mutation (MAMA)-PCR assay. This is the first report of the El Tor variant of *V. cholerae* O1 Ogawa having the *ctxB* gene of the classical strain with altered antibiogram causing epidemics of cholera in Orissa, India and globe.**

7. Kar SK, Pal BB*, Khuntia HK, Samal SK, Khuntia CP, Achary KG. Emergence and spread of Tetracycline resistant *V. cholerae* O1 El Tor variant during 2010 cholera epidemic in the tribal areas of Orissa, India. *International Journal of Infectious Disease*. 2015;33:e45-e49.

The epidemics of cholera were reported in the Kashipur, K. singhpur, B cuttack blocks of Rayagada district and Mohana block of Gajapati district of Odisha during 2010. This study was carried out to isolate the bacterial pathogen, its drug sensitivity pattern and to describe the spread of the disease in those areas. A total of 68 rectal swabs collected from patients with severe diarrhea, admitted to different health centers and diarrhea affected villages were bacteriologically analyzed. Similarly 22 water samples collected from different villages from nala, chua, etc were tested for the presence of *V. cholerae*. Out of 68 rectal swabs tested 35 (51.5%) were *V. cholerae* O1 Ogawa and 14 (20.6%) were *E coli*; which might be commensals. All water samples were negative for *V. cholerae*. The *V. cholerae* strains were sensitive to gentamicin, norfloxacin, ciprofloxacin, azithromycin and ofloxacin; but were resistant to ampicillin, tetracycline, nalidixic acid, furazolidone, streptomycin, erythromycin, co-trimoxazole, neomycin and chloramphenicol. **All *V. cholerae* strains were 100% resistant to tetracycline and they were El Tor variants**

harboring *ctxB* gene of classical strain. The present study indicated the emergence and spread of tetracycline resistant *V. cholerae* O1 El Tor variant in the tribal areas which needs close monitoring.

8. Khuntia HK, Pal BB and Chhotray GP. Quadriplex PCR assay simultaneous for detection of biotype, serotype, toxigenic potential and central regulating factor of *V. cholerae*. *Journal of clinical Microbiology*. 2008;46:2399-2401.

A quadruplex PCR was developed for the simultaneous detection of genes specific for *Vibrio cholerae* O1 and/or O139 serogroup (*wbe* and/or *wbf*), cholera toxin A subunit (*ctxA*), toxin-coregulated pilus (*tcpA*), and central regulating protein ToxR (*toxR*) in a single tube reaction. This is a simple, rapid, and accurate approach for the detection of toxigenic *V. cholerae* O1 and/or O139 and can prevent the rapid spread of the disease by early detection.

9. Chhotray GP, Pal BB, Khuntia HK, Chowdhury NR, Chakraborty S, Yamasaki S, Ramamurthy T, Takeda Y, Bhattacharya SK, Nair GB. Incidence and molecular analysis of *Vibrio cholerae* associated with cholera outbreak subsequent to the super cyclone in Orissa, India. *Epidemiol Infect*. 2002;128:131-138.

An epidemiological study was carried out to find out the etiological agent for diarrheal disorders in the cyclone and flood affected areas of Orissa, India. Rectal swabs collected from 107 hospitalized diarrhea patients were bacteriologically analyzed to isolate and identify the various enteropathogens. Detection of toxic genes among *E. coli* and *V. cholerae* was carried out by polymerase chain reaction (PCR) assay. Of the 107 rectal swabs analyzed, 72.3% were positive for *V. cholerae* O1 Ogawa, 7.2% for *V. cholerae* O139, 1.2% for *E. coli* (EAggEC) and 1.2% for *Shigella flexneri* type 6. Using multiplex PCR assay it was found that all *V. cholerae* isolates were *ctxA* positive and El Tor biotype. Strains of *V. cholerae* O1 were observed to be resistant to nalidixic acid, furazolidone, streptomycin, co-trimoxazole and ampicillin. Except for nalidixic acid, the resistance pattern for O139 was identical to that of O1 strains. Representative strains of *V. cholerae* were further characterized by randomly amplified polymorphic DNA (RAPD) analysis and ribotyping. Both O1 and O139 *V. cholerae* strains exhibited the R3 pattern of ribotype and belonged to a similar pattern of RAPD compared with that of Calcutta strains. Early bacteriological and epidemiological investigations have revealed the

dominance of *V. cholerae* O1 among the hospitalized patients in cyclone affected areas of Orissa. **Drinking water scarcity and poor sanitation; environmental water, open well, tube wells were positive for *V. cholerae* O1. Early reporting and identifying the source of infection could enable the Govt. to implement control measures in time procuring bleaching powder through air as railway lines were disrupted. So the large cholera epidemic was controlled and millions of lives were saved.**

10. Pal BB*, Mohanty A, Biswal B, Nayak SR, Das BK, Lenka PP. Haitian variant *Vibrio cholerae* O1 Ogawa caused cholera outbreaks in Odisha. *Indian Journal of Medical Microbiology*. 2021;39(4):513-7. Best Paper award on bacteriology by MICROCON, 2021.

Diarrheal disorders particularly cholera cause a significant threat resulting in high morbidity and mortality in the coastal and tribal areas of Odisha. Two sequential diarrheal outbreaks reported in 2016 from Balasore and Rayagada districts of Odisha were investigated to find out the causative organisms, antibiogram profile and molecular analysis of the isolated pathogens. Bacteriological analysis and antibiogram profiles of the pathogens were carried out as per the standard procedure followed. The double mismatch amplification mutation (DMAMA) PCR for *ctxB* gene, sequencing and pulse-field gel electrophoresis (PFGE) were carried out on *Vibrio cholerae* O1 strains. The rectal swabs and water samples from these districts were positive for *V. cholerae* O1 Ogawa biotype El Tor. The *V. cholerae* O1 strains isolated from Balasore district were multidrug resistant to many antibiotics which differed from the isolates of Rayagada district. **The DMAMA PCR assay on all clinical and water isolates from these areas and some strains from other districts exhibited *ctxB7* allele of *V. cholerae* O1 which correlates with the sequencing results having different pulsotypes. The Haitian variant of *V. cholerae* O1 strains which were compared with the *V. cholerae* O1 strains of 1999 and 2000 exhibited different pulsotypes. This study reports cholera outbreaks due to multidrug resistant *ctxB7* allele of *V. cholerae* O1 from both coastal (Balasore) and tribal (Rayagada) areas of Odisha.**