Letter to Editor

# The First Appearance of Classical-like Phenotype *Vibrio cholerae* in Nepal

Dear Editor,

*Vibrio cholerae* is the etiology agent of cholera and is mainly spread through contaminated drinking water. *V. cholerae* O1 and O139 serogroups are known to cause cholera epidemics worldwide. Until the end of the sixth pandemic (1899-1923), all cholera cases were considered to be caused by the *V. cholerae* O1 of the classical biotype, before the characterization of the O1 El Tor biotype in 1905. The El Tor biotype was implicated in major epidemics approximately 60-years later (1961) with the emergence of the seventh pandemic,[1] although the classical biotype was reportedly identified in Bangladesh in 1988-1989.[2]

From July to August 2012, a total of 503 patients with acute watery diarrhea were hospitalized at Sukraraj Tropical and Infectious Disease Hospital, Kathmandu. Stool specimens were inoculated on thiosulfate citrate bile salt sucrose agar (TCBS) (Hi-media, Mumbai, India) and incubated at 37°C for 24 h. Subcultures were further done on MacConkey Agar (MA) and incubated overnight at 37°C. The colonies were analyzed and biochemical tests were performed. Of these, 21 (4.1%) were culture positive for *V. cholerae*. Isolates were further serotyped using specific antisera (Denka Seikan, Tokyo, Japan). All the isolates were identified as O1 Ogawa serotype. Biotyping was performed using Polymyxin B (50 U) susceptibility test, Chicken red cell agglutination (CCA), and Voges-Proskauer reaction test (VP test) according to standard recommended procedures.[3,4] All isolates were negative for VP [Figure 1a] and CCA tests [Figure 1b] but sensitive to Polymyxin B (50 U) [Figure 1c]. Repeat analysis of these assays confirmed that the isolates carried phenotypic traits specific for the classical biotype. Until recently, *V. cholerae* showing phenotypic traits of the classical biotype was not reported in Nepal. This study, therefore, highlights the first appearance of *V. cholerae* of the classical biotype in patients with acute diarrhea in Nepal.

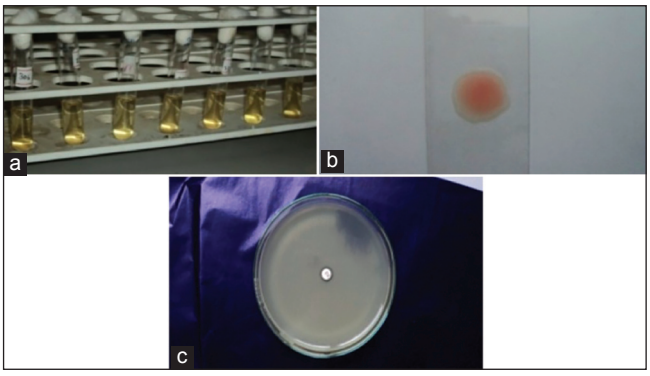


**Figure 1:** (a-c) Laboratory tests showing phenotypic results of the isolates

Previous studies have shown that *V. cholerae* strains detected in Nepal were restricted to El Tor phenotype.[5,6] In a recent study, Nepalese *V. cholerae* strains were found to be of the El Tor phenotype but carrying the classical cholera toxin.[6] Contrary to expectations, isolates in the present study demonstrated phenotypic characteristics of the classical biotype. A strain designated as "Matlab type II" belonging to the Ogawa serotype having characteristics of the classical biotype was identified in a hospitalized patient with acute diarrhea during a study period of 1991 to 1994 in Bangladesh.[7] Interestingly, this strain was found to have the *tcpA* gene of the classical biotype, whereas the *rstR* gene was of the El Tor type. The *V. cholerae* strains isolated in this study may possess similar phenotypes of both the classical and El Tor strains. Genetic exchange between co-occurring classical and El Tor biotypes could be one of the major factors leading to hybrid or variant *V. cholerae* strains. *V. cholerae* classical strains have never been reported in Nepal. Thus, the Nepalese isolates with classical phenotypic properties in this study, therefore, may not be indigenous strains but were presumably introduced from a distant geographic location. At present, it is not known whether Nepalese strains solely consist of classical genome or acquired El Tor genes, as this study investigated phenotypic traits only; it is not known whether the Nepalese strains consist of the classical genotype or whether they have acquired El Tor genes. Genetic testing, therefore, may reveal whether these strains are of the classical biotype or if they carry El Tor-specific alleles. The frequent exchange of genes between classical and El Tor biotypes may promote the emergence of a "classical variant", a potential new variant of *V. cholerae*, though such designation has not yet been proposed or included in the redefined biotype scheme.[8] Nevertheless, it is possible to hypothesize the emergence of "classical variant" *V. cholerae*, as genetic exchange between strains carrying traits of both biotypes might occur in the environment, albeit slowly, particularly in areas where cholera is endemic.

During the study period, none of the samples were shown to have El Tor biotypic properties, contrary to previous studies in Nepal. The El Tor strain was not

included as a negative control during analysis is another limitation of this study. However, the likelihood of contamination during laboratory analysis was low, as isolates carrying classical phenotypes of *V. cholerae* have never been reported in Nepal, and reexamination of these strains was performed to confirm the results.

Past studies have shown that classical strains are likely to cause more severe disease compared with El Tor strains,[9] although the role of hybrid type of *V. cholerae* in pathogenesis is not yet well studied. Although Kathmandu has witnessed both rapid population growth and urbanization over the past decade, progress toward the provision of safe water supplies and improved sanitation facilities remain slow. It can thus be postulated that this newly emerging *V. cholerae* having classical-like phenotype may lead to severe forms of cholera because there may be little or no immune protection against neither classical nor genetic hybrid strains of *V. cholerae* in the human population. This, therefore, underscores the immediate need for increased vigilance in monitoring and preparedness for the future outbreaks.

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