

## **Identification of clinical isolates as *Vibrio navarrensis* by molecular and phenotypic approaches.**

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*Vibrio navarrensis* is an aquatic bacterium that has been isolated from sewage and it shares phenotypic similarities with the human pathogen *V. vulnificus*. Little is known about the prevalence of *V. navarrensis* in the environment or if it is associated with human illness. In 2008, the *Vibrio* Reference Lab at the CDC received four isolates that were phenotypically similar to each other, yet could not be classified as one of the 12 known pathogenic species using biochemical tests or *rpoB* sequencing. The isolates were similar to *V. vulnificus* biotype 1, but differed from the typical profile as follows: isolates were negative for lysine and salicin, and positive for acetate utilization and urea hydrolysis. Phylogenetic analysis of *rpoA* gene sequences from the isolates suggested that they were *V. navarrensis*. We used multilocus sequence analysis (MLSA) of seven housekeeping genes to further investigate the evolutionary relationships of these and other isolates in the CDC reference collection. We included 12 unidentified *Vibrio* strains that were isolated from 1980-2001 that exhibited similar biochemical profiles to the recent *V. navarrensis* strains. Sources for the clinical isolates included blood, wounds, stool, and ear infections. Biochemical characterization of a total of 20 strains, including two type strains, separated the strains into two groups based on differences in 7 biochemical tests. One group was similar to the classical *V. navarrensis* biotype and one group of four isolates did not resemble the classical type or *pommerensis* biotype. Phylogenetic analysis revealed a similar pattern: although all 20 strains examined resembled *V. navarrensis*, the same four isolates were clustered separately in the gene tree and their divergence from other *V. navarrensis* suggests they represent a unique biotype, or a separate *Vibrio* species. In addition, the classical *V. navarrensis* strains examined in this study possessed a DNA region that was amplifiable using *V. vulnificus* primers to detect the cytotoxin-hemolysin, *vvhA*. Interestingly, the more distantly related group of four strains did not possess this region. *vvhA* is frequently used as a marker to identify *V. vulnificus* strains, and studies that rely solely on positive results for this assay to identify *V. vulnificus* may inadvertently include *V. navarrensis* strains. Further molecular characterization is underway to determine the extent of the diversity of the *V. navarrensis* and related clinical strains.