

**Population dynamics of *Vibrio cholerae* in the Vaal Barrage**

by

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Cholera continues to be an important cause of morbidity and mortality in many areas of Asia, Africa, and Latin America. The diarrheal disease is caused by the gram-negative bacterium *Vibrio cholerae*, which is a natural inhabitant of the aquatic environment. These environmental *V. cholerae* strains are suggested to be a reservoir for future epidemics and can be converted to enterotoxin producing strains through the addition of genetic elements by the filamentous lysogenic phage CTX $\phi$ . A better understanding of the population dynamics in these environmental reservoirs could give insight into the potential risk of future epidemics and aid in the management of current cholera epidemics.

A polymerase chain reaction technique was evaluated for use as a detection and identification approach, and compared to traditional biochemical identification. The PCR primers targeted a 304bp region of the gene coding for the *V. cholerae* outer membrane protein. The PCR approach used was found to be a highly reliable and rapid technique for

the identification of *V. cholerae* isolates, and proved to be more accurate than the biochemical approaches used.

Environmental *V. cholerae* strains were screened for the presence of two virulence factors, the toxin co-regulated pili and the cholera toxin genetic element, by a developed multiplex PCR. A hundred strains were screened and none were found to harbor these virulence factors. These strains thus do not possess the genetic potential for enterotoxin production, and are unlikely to undergo toxigenic conversion due to the fact that they lack the receptor for CTX $\phi$ .

Amplified fragment length polymorphism DNA fingerprinting was used to assess the genetic diversity of the studied *V. cholerae* population. A high level of genetic diversity was observed within the Vaal Barrage population, with single sampling sites hosting diverse genotypes and highly related strains only found within short time frames and localized regions. The high level of genetic diversity observed in this study suggests that environmental *V. cholerae* populations are genetically highly flexible and capable of extended survival. These populations may persist and act as pre-cursors for future epidemic strains, or at the very least serve as models for studying the survival and persistence of enterotoxigenic strains.

