Phenotypic and molecular characterization of Vibrio cholerae

isolated from cholera outbreaks in western Kenya

Robert Momanyi Maranga

A thesis submitted in partial fulfillment for the Degree of Master of Science in Biotechnology in the Jomo Kenyatta University of Agriculture and Technology

2010

ABSTRACT

Cholera disease is a major public health problem in Kenya and is caused by toxigenic Vibrio cholerae. V. cholerae are curved or straight Gram-negative bacilli. Epidemic cholera occurs in areas with water contamination and poor sanitation. This research investigated strains of Vibrio cholerae isolated from western Kenya during the 2007 to 2009 outbreaks. Stocked isolates were sub-cultured onto BTB agar and 24hr-colonies were used to perform specific biochemical tests on the isolates to determine their biotypes, serotypes and sub- serotypes. Presence of cholera toxin (ctx) and antibiotic resistance genes in the genome of the isolates was detected by amplification using PCR. Pulsed field gel electrophoresis (PFGE) was done to determine the clonal relatedness of the strains. The collected data were coded and analysed using statistical package for social sciences (SPSS) version 11 to determine chi square distributions for qualitative data. All the isolates were confirmed to be V. cholerae O1 El Tor biotype. Majority (97%) of them were Inaba serotype while 3% were Ogawa serotype and none was Hikojima serotype. PCR assays showed that 97% of the isolates were toxigenic and contained the ctx gene. The isolates showed varied antibiotic resistance patterns while the presence of resistant genes to commonly used antibiotics was not uniform but random in their genomes. Analyses of the PFGE bands to infer population relationships using the unweighted Neighbor-joining method viewed using the program DARwin software did not show any significant genetic diversity. Further investigation on the Western Kenya isolates using the *in-vivo* as well as *in-vitro* bioassays methods and comparison to other isolates from endemic regions in Kenya is required