Phenotypic and Genetic Characterization of *Vibrio Cholerae* O1 Strains

Isolated From Various Regions of Kenya between 2007 and 2010

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ABSTRACT

Cholera remains an important public health concern in developing countries that lack basic infrastructure especially access to safe drinking water and proper sanitation. In this laboratory based study, 76 \textit{Vibrio cholerae} O1 isolates that represents 2007-2010 cholera epidemics in Kenya were phenotypically and genetically characterized. Fifty-six \textit{Vibrio cholerae} O1 isolates were used to represent 2009 cholera outbreaks, whereas 20 \textit{Vibrio cholerae} O1 isolates represented outbreaks from 2007, 2008 and 2010 in Kenya. The inclusion of \textit{Vibrio cholerae} O1 strains from 2007, 2008 and 2010 was to allow for comparison with those from 2009. These were characterized by serotyping, biotyping, antimicrobial susceptibility testing, toxin and pathogenicity gene detection, ribotyping and Pulse Field Gel Electrophoresis (PFGE). \textit{V. cholerae} O1 Inaba was the dominant serotype (88.2\%) whereas Ogawa was isolated in 11.8\%. All the strains were resistant to polymyxin B. The multiplex and simplex PCR assay showed that all the \textit{V. cholerae} isolates were positive for \textit{ctxA}, \textit{tcpA} (El Tor) and \textit{rtxC} genes, showing Kenyan isolates belonged to biotype El Tor. The MAMA-PCR assays for the differentiation of cholera toxin B subunit of classical and El Tor biotypes of \textit{V. cholerae} O1 indicated that the 76 strains were \textit{V. cholerae} O1 biotype El Tor variant that harbors the classical \textit{ctxB} gene. Based on CLSI guidelines of defining susceptibility, 100\% of the strains were susceptible to tetracycline, doxycycline, ofloxacin, azithromycin, norfloxacin and ceftriaxone. Susceptibility to ciprofloxacin was 97.7\%. Intermediate susceptibility to ciprofloxacin, nalidixic acid, ampicillin and chloramphenicol was observed in 2.3\%, 59.1\%, 11.4\%, and 93.2\% of the strains respectively. The strains were 100\% resistant to furazolidone, streptomycin and sulfamethoxazole-trimethoprim, while 88.6\% were resistant to nalidixic
acid, 40.9% resistant to ampicillin and 6.8% resistant chloramphenicol. The *V. cholerae* O1 Isolates from the different regions of Kenya had genetically similar pattern on ribotyping of *Bgl* I digested chromosomal DNA and Pulse Field Gel Electrophoresis (PFGE) patterns of *Not* I-digested chromosomal DNA. All the tested strains belonged to ribotype RIII. The strains had similar antimicrobial susceptibility patterns, toxin and pathogenicity genes, ribotype pattern and PFGE pattern. These findings suggest that the *V. cholerae* O1 strains that caused cholera outbreaks between 2007 and May 2010 had a clonal origin regardless of year and place of isolation and that the outbreaks may have been linked epidemiologically.

Tetracycline and doxycycline currently used in treatment of cholera cases in Kenya were found to be effective and thus should continue being used. A coordinated multidisciplinary approach and working with communities to encourage behavioral change to diminish the risks of infection may be the most efficient way to prevent and contain cholera outbreaks, as increase in number of cholera cases reported in Kenya in 2009 may not have been as a result of change in the *V.cholerae* O1 strains in circulation. Further sequence based typing of *V. cholera* O1 strains may be useful in confirming epidemiological linkage of the cholera outbreaks in Kenya.