EMERGENCE OF MULTI-DRUG RESISTANT STRAINS OF EL TOR BIOTYPE OF VIBRIO CHOLERAE O1 IN BANGLADESH AND REVERSION OF THEIR SUSCEPTIBILITY TO TETRACYCLINE AFTER TWO-YEARS

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During October 2004 through December 2005, we identified 953 (565 Ogawa and 388 Inaba) and 344 (197 Ogawa and 147 Inaba) strains of El Tor biotype V. cholerae O1 from cholera patients admitted to the Dhaka (urban area) and Matlab (rural area) hospitals of ICDDR,B respectively. These strains were tested for their susceptibility to tetracycline, erythromycin, trimethoprim-sulfamethoxazole, and furazolidone using the disc-diffusion technique on Mueller-Hinton agar (Difco, Detroit, Michigan, USA) with commercial disks (Oxoid, UK) and appropriate control strains. The overall resistances to tetracycline, erythromycin, trimethoprim-sulfamethoxazole, and furazolidone were 525 (55%), 420 (44%), 944 (99%) and 948 (100%) respectively for the Dhaka strains, and 186 (54%), 165 (48%), 332 (97%) and 343 (100%) respectively for the Matlab strains. Before October 2004, most V. cholerae O1 strains were resistant to trimethoprim-sulfamethoxazole and furazolidone but they were uniformly susceptible to tetracycline, erythromycin, and ciprofloxacin. The first multi-drug-resistant (furazolidone, trimethoprim-sulfamethoxazole, tetracycline, and erythromycin) strains of V. cholerae O1 were observed in October 2004 at Matlab Hospital among the Ogawa serotype, and by February 2005 all (100%) of strains of this serotype became resistant. In Dhaka, the multi-drug-resistant strains appeared a month later, in November 2004, among both Ogawa (13%) and Inaba (5%) strains of V. cholerae, and by February 2005, all of the Ogawa strains exhibited the multi-drug-resistant phenotype. By March 2005, nearly all of the El Tor Ogawa isolates (5/6 i.e. 83% at the Matlab Hospital and 43/45 i.e. 96% at the Dhaka Hospital) were multi-drug-resistant. The findings suggest circulation of two or more different clones of V. cholerae O1 at different time points, and we are in the process of understanding the clonality by subjecting representative strains to molecular typing analysis. Earlier studies have observed that resistance to tetracycline can serve as a proxy indicator for resistance to doxycycline. We determined antimicrobial susceptibility using both disc-diffusion technique and E-test methods. In the absence of a reference zone size for V. cholerae resistance to erythromycin and azithromycin, we used the cut-off zone size used for other gram-negative pathogens to determine the susceptibility of our V. cholerae strains (zone of inhibition ≥23 mm for erythromycin and ≥18 mm for azithromycin). Seventeen (49%) V. cholerae strains were resistant to both erythromycin and azithromycin, and the remaining 51% of strains were susceptible to both erythromycin and azithromycin. The strains resistant to erythromycin had minimum inhibition concentrations (MIC) of 8-32 µg/mL, while the MICs of the susceptible strains ranged from 0.25 to 1.0 µg/mL. Similarly, the MICs of the strains resistant to azithromycin ranged from 0.75-3 µg/mL, and the MICs of the susceptible strains ranged from 0.047-0.125 µg/mL. These findings suggest that resistance to erythromycin could be a marker for resistance to azithromycin. We have, for the first time, encountered this unique, multi-drug-resistant pattern that includes most of the drugs known to be effective in the treatment of cholera, including erythromycin. Throughout the period of this investigation, all isolates from Matlab and Dhaka remained susceptible to ciprofloxacin, as determined by disc-diffusion method. We determined MICs for ciprofloxacin using E-test (AB-BIODISK, Sweden) with the zone size interpretive criteria for susceptibility corresponding to 0.06 µg/mL. We noted a consistent increase in the median MIC of V. cholerae O1 strains isolated at the Dhaka Hospital over the years: from 0.003 mg/mL in 1994 to 0.023 mg/mL in 2001 to 0.38-0.5 mg/mL in 2005 (this increase in MIC has been associated with a corresponding decrease in the efficacy of ciprofloxacin for treatment of cholera. In August 2006, we observed re-emergence of the Inaba serotype and a sharp reduction in the isolation rate of the Ogawa serotype. In Bangladesh, such changes in serotypes, and the appearance of short-lasting, multi-drug resistance, have been noted earlier. Commensurate with this, the proportion of V. cholerae strains resistant to tetracycline decreased substantially, from 75% in January 2006 to 10% in August 2006. However, resistance to tetracycline persisted for nearly two years before subsiding, a finding that we have not observed earlier. The current situation clearly demonstrates the need to monitor MICs of drugs known to be effective in the treatment of severe cholera in areas where the disease is endemic and to assess their clinical efficacy in the treatment of cholera. There is also a need to identify effective alternative antimicrobials for the management of cholera caused by such strains. Through this communication, we would also like to alert our neighbouring countries and the region of the circulation of this new multi-drug-resistant strain of V. cholerae O1.