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Note: Characterization of *Vibrio cholerae* O139 Bengal isolated from water in Malaysia

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R. SON, G. RUSUL, L. SAMUEL, YUHERMAN, S. SENTHIL, A. RASIP, E.H. NASRELDIN AND M. NISHIBUCHI. 1998. Four *Vibrio cholerae* O139 Bengal strains isolated from surface water were characterized by antibiotic resistance, plasmid profile, presence of cholera toxin gene and random amplification of polymorphic DNA (RAPD) analysis. All four strains exhibit multiple resistance towards the antibiotics tested with a multiple antibiotic resistance index of 0·5–0·66, and harboured a 2·0 MDa non-conjugative plasmid. The *Vibrio cholerae* O139 Bengal were positive for the cholera toxin gene. Antibiotyping and random amplification of polymorphic DNA analysis with four primers proved to be useful in discriminating the isolates. RAPD proved to be more sensitive. These results reveal that there is significant genetic diversity among the *Vibrio cholerae* O139 Bengal strains studied.

INTRODUCTION

Vibrio cholerae belonging to serogroup O1 was considered to be the only causative agent of epidemic cholera. However, it was found recently that a highly epidemic form of a cholera-like disease on the Indian subcontinent in 1992 was strongly associated with a strain of *V. cholerae* non-O1, designated *V. cholerae* O139 Bengal (Albert *et al.* 1993; Shimada *et al.* 1993). As this clone shows striking similarity in biochemical and physiological characteristics to *V. cholerae* O1 biotype El Tor, the conventional methods of laboratory diagnosis of *V. cholerae* O1 infection are applicable to *V. cholerae* O139 (Ansaruzzaman *et al.* 1995). Recent studies of *V. cholerae* O139 Bengal from environmental surface water indicate that environmental water may be an important reservoir for infectious *V. cholerae* O139 (Faruque *et al.* 1997). To improve the determination of the health risk associated with exposure to *V. cholerae* O139 in the environment, epidemiological tracking of strains is required. This goal would be better achieved if molecular technologies were available which would allow rapid and sensitive differentiation between *V. cholerae* O139 Bengal strains. In the work reported here, *V. cholerae* O139 Bengal strains isolated from surface water over a 3-month period were characterized by antibiotic resistance, plasmid profiling, random amplified polymorphic DNA (RAPD) analysis and detection of the cholera toxin (CT) gene by the DNA hybridization test.

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MATERIALS AND METHODS

Bacterial strains

Four *V. cholerae* O139 Bengal strains originating from four of 60 samples from a location which received sewage drainage from a hospital within Peninsular Malaysia were investigated. The samples were collected in pre-sterilized bottles and brought to the laboratory in an ice container and processed within 2 h of collection. Water samples were diluted and plated onto thiosulphate-citrate-bile salts-sucrose agar (TCBS: Oxoid) for selective isolation of *V. cholerae*. After overnight incubation at 37 °C, identification of isolates was performed as described by Sakazaki and Shimada (1986). Bacteria that matched the biochemical profile of *Vibrio cholerae* were later serotyped at the Institute for Medical Research, Kuala Lumpur, Malaysia.