Asian Journal of Epidemiology

ISSN 1992-1462

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Asian Journal of Epidemiology 2 (3): 66-71, 2009
ISSN 1992-1462
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Transmission of *Vibrio cholera* O1 Serotype Inaba in a Rural Area of Qazvin, Iran Associated with Drinking Water

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Abstract: In this study, we characterized twelve *Vibrio cholerae* isolates from stool specimens of suspected diarrheal patients and one isolate from water sample in a rural area in Qazvin, Iran by Pulsed Field Gel Electrophoresis (PFGE) in order to identify the source of infection. All isolates were determined as *V. cholerae* O1 serotype Inaba by serotyping and were resistant to co-trimoxazole, nalidixic acid and furazolidone and susceptible to ampicillin, ciprofloxacin, tetracycline, erythromycin, cefexime and doxycycline and showed intermediate susceptibility to chloramphenicole. The MIC of the isolates to erythromycin was 1 μg mL^{-1}. The PFGE pattern of all 13 isolates were identical showing transmission of the same strain from contaminated water to all patients. The main difference between the epidemiology of cholera in our country during past years was variation between serotypes from Ogawa to Inaba. From present results it can be concluded that all strains of *V. cholera* O1 have similar pattern of PFGE and the source of contamination may be same for all infected patients.

Key words: *Vibrio cholerae*, Inaba, PFGE

INTRODUCTION

Cholera is a gastroenteritis infectious caused by enterotoxin-producing *Vibrio cholerae* (Faruque and Nair, 2008). Transmission of cholera vibrios to humans occurs through eating or drinking contaminated food or water. Aquatic environments can serve as good reservoirs of the bacteria (Islam *et al*., 1993). Cholera is predominantly a waterborne disease especially in countries with inadequate sanitation. Water maybe contaminated with fecal matter (Faruque *et al*., 1998; Reidl and Klose, 2002). A lack of clean drinking water caused some outbreak in different part of the world. In 1999 there was a transmission of *V. cholerae* associated with drinking water in rural western Kenya (Shapiro *et al*., 1999). An outbreak of *V. cholerae* was occurred in Ebeye Island, Republic of the Marshall Islands and India associated with use of an adequately chlorinated water source in 2000 and 2003, respectively (Beatty *et al*., 2004; Taneja *et al*., 2003) and recently an outbreak of cholera was occurred in Iraq in 2007 because of lacking appropriate sanitation (UN reports, 2007).

In Iran, an epidemic cholera spread throughout 1959 (Hamedy *et al*., 2004). Inadequate sanitation facilities was likely responsible for this spread of epidemic cholera. There was a rapid spread of disease in Iran and Iraq in 1965-1966 (Moureau, 1970). Although, *V. cholera* strains were isolated from different provinces in Iran in 1998 to 2001 (Pourshafie *et al*., 2000,

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2002), during 2001 to 2004 the range of cholera has remained of 90 to 120 cases annually that all were from rural areas of south eastern Iran (Izadi *et al*., 2006). However, in 2005 an outbreak of *V. cholera* serotype Inaba was occurred again in different provinces in Iran (Pourshafie *et al*., 2007).

Rapid identification for early detection of relationship between isolates in an outbreak using molecular typing methods may prevent regional and even national spread of outbreak-related strains. So, in this study *V. cholerae* that isolated from 12 patients taking apart in a wedding party in a rural area in Qazvin (a province near Tehran in Iran with the cold weather) were characterized by molecular technique PFGE.

MATERIALS AND METHODS

Bacterial Sources

Twelve *V. cholerae* isolates from stool specimens of suspected diarrheal patients taking apart in a wedding party in a rural area in Qazvin, a province near Tehran, Iran who had drank the same contaminated water with *V. cholerae* and one isolates from water sample recovered in October in 2008. Patients included 10 females and two males with age ranging from 4 to 75 years old. Stool specimens and water sample were plated directly on blood agar, MacConkey agar and Thiosulphate Citrate Bile Salt (TCBS) sucrose agar. The samples were also inoculated in alkaline peptone water and incubated overnight at 37°C. Subculture was made from alkaline peptone water on TCBS after 6 h. The isolates of *V. cholerae* identified morphologically and biochemically using standard recommended procedures (Winn *et al*., 2006). All suspected isolates serotyped using *Vibrio cholera* O1 polyvalent and monospecific Ogawa, Inaba and O139 antisera (BD, Becton Dickinson, NJ, USA).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing for the isolates was performed on Muller-Hinton agar by disk diffusion method as recommended by CLSI (former NCCLS) (Clinical and Laboratory Standards Institute, 2006). The antimicrobial drugs included ampicillin (10 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), tetracycline (30 μg), Erythromycin (15 μg), Nalidixic acid (30 μg), Furazolidone (100 μg) and chloramphenicole (30 μg), Cefexime (5 μg) and Doxycycline (30 μg) (Mast Diagnostic Group UK). The E-test MIC (AB Biodisk Solna, Sweden) method used for detection of Minimum Inhibitory Concentration (MIC) for erythromycin.

PFGE Analysis

Intact chromosomal DNAs of all 13 isolates were typed with PFGE. A one-day pulsenet protocol was used for typing of studied isolates (www.cdc.gov/pulsenet, Cooper *et al*., 2006). Strains was grown on BHI agar and adjusted to about 10^{10} CFU mL^{-1} in cell suspension buffer (100 mM Tris, 100 mM EDTA, pH 8.0) and were mixed with an equal volume of 2% low-melting point agarose and proteinase K (0.5 mg mL^{-1}) and allowed plugs to solidify. DNA embedded in plugs were incubated at 55°C with proteinase K in cell lysis buffer (50 mM Tris, 50 mM EDTA [pH 8.0], 1% sarcosine and 0.5 mg of proteinase K mL^{-1}) for 1 h. Then, plugs were washed four times by TE buffer (10 mM Ttis-HCl, 1 mM EDTA pH: 8.0) at 37°C by gentle shaking. Each washing step was performed 15-30 min. One-third of plugs were digested with 40 U *Not*I for 4 h. *Xba*I digested DNA of *Salmonella choleraesuis* serotype Branderup H9812 (www.cdc.gov/pulsenet) were used as size markers. Restriction fragments were separated by electrophoresis through 1% PFGE agarose in 0.5X

TBE buffer (10X TBE: 0.89 M Tris borate and 0.02 M EDTA (pH 8.3); Sigma Chemical Co., St. Louis, MO) at 14°C in a CHEF DR-II apparatus (Bio-Rad). The run time was 19 h, with a voltage of 200 V with ramped pulse time in the first block of 2 to 10 sec, with a run time of 13 h and the second block of 20 to a 25 sec, with a run time of 6 h.

RESULTS AND DISCUSSION

All isolates from patients and water diagnosed as *V. cholerae* using standard biochemical tests. All the isolates were determined as O1 serotype Inaba by serotyping. Antibiotic susceptibility testing revealed that all studied isolates were resistant to co-trimoxazole, nalidixic acid and furazolidone and were susceptible to ampicillin, ciprofloxacin, tetracycline, erythromycin, cefexime and doxycycline. All isolates showed intermediate susceptibility to chloramphenicole. Minimum Inhibitory Concentration (MIC) of isolates to erythromycin was 1 μg mL^{-1} by E-test.

The PFGE results of restriction pattern of all isolates (12 isolates from patients and one from water) were identical showing transmission of the same strain from contaminated water to all patients (Fig. 1).

It is the fact that *V. cholerae* continues to be an important cause of cholera outbreaks in different part of the world. Although, this bacterium is usually isolated from warm and humid climates, but in this research it was also isolated from Qazvin that is located in the north west of Tehran with the cold weather. The main difference between the epidemiology of 1997-2004 and recent years was variation between serotypes. In 1997 to 2004 the prevalent serotype was Ogawa (Moureau, 1970; Pourshafie *et al*., 2000, 2002; Izadi *et al*., 2006) while in 2005 and this study Inaba serotype was the prevalent serotype (Pourshafie *et al*., 2007). It suggested two possibilities for this variation; first is seroconversion due to mutation in the wbeT region because *V. cholerae* O1 strains are known to interconvert between the Ogawa and Inaba forms (Colwell *et al*., 1995) and the second is transmission of this organism from other part of the world especially our neighbor countries such as Pakistan, Turkey, India and Iraq by travelers to our country so that they had *V. cholerae* serotype Inaba outbreak in recent years in their countries (Jabeen *et al*., 2008; De Schrijvera *et al*., 2007; Rajeshwari *et al*., 2008; WHO, 2007). More molecular analysis is needed to find out which hypothesis is the reason of this seroconversion.

Fortunately, *V. cholerae* isolates in this study were susceptible to tetracycline and erythromycin which are two choice antibiotics for cholera treatment.

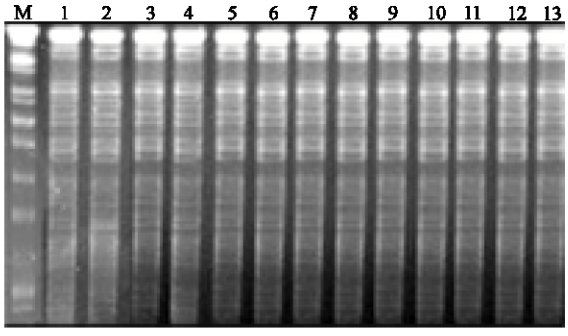

Fig. 1: PFGE pattern of *V. cholerae* isolates digested with *Not*I. M: *S. branderup*, lanes 1-12: isolates recovered from patients and lane 13: isolate recovered from water

In accordance with a 4 year old patient in this study and previous finding it appears that WHO recommendations to age range in case definition of cholera in endemic areas needs reconsideration.

Different phenotypic and genotypic techniques have been developed for the characterization and subtyping of *V. cholerae* (Faruque *et al*., 1998; Singh *et al*., 2001). While serotyping is internationally standardized, this method has rather poor discrimination power with epidemic strains belonging to serogroups, O1 and O139 and more recently with a new serogroup O141, which may also possess epidemic potential (Crump *et al*., 2003). Molecular methods like Pulsed-Field Gel Electrophoresis (PFGE) have been successfully used to study the relationship among *V. cholerae* strains from outbreaks around the world (Cooper *et al*., 2006). Dissemination of a major *V. cholerae* clone was seen in different provinces in Iran in 2005 by PFGE (Pourshafie *et al*., 2007).

Interestingly, according to our PFGE results genomic patterns of these strains have close relationship with a major clone detected in previous study in Iran (Pourshafie *et al*., 2007). It reveals that these strains were possibly derived from a clonal lineage that was already circulating in our country as causing small outbreaks.

It seems that the role of contaminated food and poor hygienic practices are the most important sources of cholera transmission. As in 2003 a 50 days long outbreak in Sistan-Baluchestan province in Iran was occurred in result of contaminated food (Khazaei *et al*., 2005).

However, the result of testing water sample confirmed that among the risk factors for cholera (such as contaminated water and food, poor hygienic practice like hand washing, drinking beverage from street vendors eating remaining food from previous meals without reheating and delay between cooking and eating), drinking water contaminated with cholera is the route of transmission in this report.

Only appropriate sanitation and safe drinking water could greatly reduce cholera transmission within a community, as safe municipal reservoirs and treatment facilities defeated cholera before the discovery of antibiotics and Oral Rehydration Solution (ORS) (Quick *et al*., 1996; Mintz *et al*., 1995). In the regions especially without appropriate water pipe system, community education and increasing level of culture among people, use of chlorination water could be very effective to prevention of water born disease.

In conclusion we suggested use of standard methods of typing such as PFGE with high discriminatory power to rapid identification of the source of infections particularly those which are simply spread among people such as cholera.

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