An Outbreak of Foodborne Gastroenteritis Caused by Dual Pathogens, *Salmonella enterica* serovar Weltevreden and *Vibrio fluvialis* in Kolkata, India

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Abstract

Salmonella enterica serovar Weltevreden and *Vibrio fluvialis* have been identified as etiological agents of a foodborne gastroenteritis outbreak associated with the consumption of mutton ghogni during an Iftar party on July 30th, 2012. This report describes a first foodborne outbreak of gastroenteritis caused by both these pathogens in North Dumdum, West Bengal, India. About 400 people had diarrhea and 248 people were admitted to the Infectious Diseases Hospital, Kolkata. Out of 44 stool samples collected and tested, 14 were positive for *Salmonella enterica* serovar Weltevreden, and 13 for *V. fluvialis*. In the pulsed-field gel electrophoresis profiles, *Salmonella enterica* serovar Weltevreden was identified as a single clone but diverged from others Weltevreden strains isolated during the previous years. However, the *V. fluvialis* strains were found to be closely related clones but different from previous years.

Introduction

Foodborne infections caused by *Salmonella* spp. and *Vibrio* spp. are common and often result in gastroenteritis. In India and other countries, gastroenteritis is an important human infections and gets attention of public health authorizes during outbreaks (Kumar SG. et al., 2012; Scallan E, *et al*., 2005). Foodborne outbreaks are mainly caused due to consumption of meat, egg, dairy, and plant products that may be contaminated at the different stages of production, processing and at the time of delivery. *Salmonella enterica* is one of the most common causes of human gastroenteritis. Salmonellosis is estimated to affect three billion people and to cause 200,000 deaths every year (Herikstad H. *et al*., 2002). More than 2,500 serovars of *S. enterica* have been identified; most have been described as the cause of human infections, but only a limited number of serovars are of public health importance (Galanis E. *et al*., 2006). *S. enterica* serovar Weltevreden (*S.* Weltevreden) has been reported as a frequent and increasingly common cause of human infection in the restricted areas of Southeast Asia including India (Bangtrakulnonth A. *et al*., 2004; Kim S. 2010; Antony B. *et al*.2009; Aggarwal P. *et al*., 1985) and Europe (D'Ortenzio E. *et al*., 2008). *Salmonella enterica* serovar Weltevreden is a leading cause of outbreaks of foodborne gastroenteritis but the involvement of *Vibrio fluvialis* are uncommon. Outbreaks comprising more than one pathogen are very rare. *V. fluvialis* is distributed worldwide in tropical and temperate marine coastal and estuarine waters. Diarrhoeal illness is the most common form of disease caused by *V. fluvialis* and usually occurs 4–96 h after ingestion of raw or improperly cooked seafood. Clinical manifestations include diarrhea, potentially severe abdominal cramps, nausea, and fever. Although infrequent, severe diarrhoea and dysentery-like illness with blood and mucus in the stool have also been described (Chowdhury G. *et al*., 2012). Outbreak of gastroenteritis caused by *V. fluvialis* also reported in previous study (Takedi RJ. *et al*., 1990;

Tokoro M. *et al*., 1984; Chowdhury G. *et al*., 2011). Interestingly, the clinical importance and the emerging trend in the prevalence of *V. fluvialis* have been reported in Kolkata, India (Chowdhury G. *et al*., 2012). We describe here the clinical, epidemiological, and bacteriologic features of an outbreak of gastroenteritis in the Iftar ceremony during the Ramzan month caused by *Salmonella enterica* serovar Weltevreden and *Vibrio fluvialis* in which the consumption of mutton ghogni was implicated as the cause for transmission.

Materials and Methods

Background of the Outbreak: In July 30th, 2012 about 500 peoples attended an Iftar ceremony during the Ramzan month at Bankra, Birati, North Dumdum, West Bengal, India. A total of 400 people developed typical symptoms of food-poisoning including watery diarrhea with abdominal pain, vomiting and fever after consumption of mutton ghogni (lentil) dish prepared by a caterer. Around 300 were referred to the Infectious Diseases Hospital (IDH), Kolkata and 248 people were admitted and rests were treated at home. Forty four stool samples were collected from the patients and sent to the laboratory for microbiological analysis. The sample collection was from the active surveillance of diarrheal infection at the IDH, in which every fifth patient will be enrolled followed by screening of more than 25 enteric pathogens from the stool samples.

Processing of stools

Stool specimens were collected using sterile catheters and examined within 2 hrs in the laboratory for common enteric pathogens [Nair et al., 2010, Panchalingam et al., 2012]. Briefly, stools were cultured on MacConkey, Xylose-lysine deoxycholate (XLD) and Hektoen enteric (HE) agars were used in the isolation of *Escherichia coli*, *Shigella* spp and *Salmonella* spp,

respectively. Ryan medium and thiosulfate citrate bile-salts sucrose (TCBS) agar were using in the isolation of *Aeromonas* spp. and *Vibrio* spp., respectively. In addition to the direct plating, fecal samples were inoculated into selenite-F and alkaline peptone water for enrichment of *Salmonella* spp and *Vibrio* spp, and subcultured on XLD/HE and TCBS agar plates, respectively. All the agar plates and enrichment broths were incubated at 37°C with appropriate incubation periods. In addition to the enteric bacteria, protozoans were detected by commercial kits (TechLab, Radford, VA, USA) and the enteric viruses by RT-PCR (Panchalingam et al., 2012).

Serotyping of salmonellae

Serotyping of *Salmonella* was done using a commercially available antisera test kit, following manufacturer's instructions (S&A Reagents Lab, Bangkok, Thailand).

PCR assays

Three typical lactose fermenting colonies confirmed as *Escherichia coli* by biochemical testing were tested in the multiplex PCR assay for the detection of three pathogroups of diarrheagenic *E. coli* (ETEC, EPEC and EAEC, Panchalingam et al., 2012). PCR was also performed to detect the *V. fluvialis* spp. by using the primers described previously (Chowdhury et al., 2011). Template DNA was prepared with the growth of the test strains in Luria broth (Difco) supplemented with 3% NaCl. The bacterial cells were washed and resuspended the cell pellets in sterile distilled water. The cells were lysed and the DNA was denatured after boiling the cell suspension for 10 mins. Supernatant of this cell lysates were used as templates in the PCR assays. The PCR amplicons were electrophoresed in a 1% agarose gel stained in ethidium bromide and visualized under UV light using a gel documentation system (Geldoc 2000, BioRad, Hercules, CA).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using disk diffusion method with commercially available discs such as ampicillin (10 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), furazolidone (100µg), norfloxacin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg) and erythromycin (15 µg) (Becton Dickinson, Sparks Glencoe, MD, USA) in accordance with the criteria recommended by Clinical and Laboratory Standards Institute (CLSI, 2011). Since there is no interpretive criterion for *V. fluvialis* in the CLSI guidelines, breakpoint for Enterobacteriaceae was adopted. *E. coli* ATCC 25922 was used as a quality control strain.

Pulsed-field gel electrophoresis **(PFGE)**

PFGE was performed according to the PulseNet protocol (Ribot EM, 2006, Kam KM., 2003). DNA digestion with restriction endonuclease *Xba*I for *Salmonella* and *Not*I for *V. fluvialis* (50 U/plug; Fermentas, Germany) was performed at 37°C overnight. The digested DNA of *Salmonella* Braenderup strain H9812 with *Xba*I was used as a molecular weight marker. The restriction fragments were resolved on a CHEF Mapper system (Bio-Rad) in 0.5X TBE. Gels were stained in ethidium bromide (25 min), destained in distilled water for 45 min and photographed under UV light. The PFGE patterns were analyzed using the BioNumerics version 4.0 software (Applied Maths, Sint Martens Latem, Belgium) after normalization of the TIFF images with *Salmonella enterica* serotype Braenderup size standard. Clustering was performed using the unweighted pair group method (UPGMA) and the Dice correlation coefficient with a position tolerance of 1.5%.

Results

Of the 500 people attended Iftar ceremony, 400 developed typical clinical symptoms of diarrhea including watery stool, moderate dehydration, abdominal pain, vomiting and some with mild fever. Due to the severity diarrhea and dehydration, 248 patients were admitted in the IDH for treatment and fluid rectification. Thirty seven patients (84%) had vomiting and 34 (77%) and 24 (55%) had abdominal pain and tenesmus respectively. About 66% (29 patients) had the fever. The age of the patients varied from 7 to 55 years and adult male (31 male and 13 female) were mostly infected than children. All the 400 patients eat the mutton ghogni which was cooked in an open ground under a tree by a caterer. After the consumption of food, diarrheal symptoms appeared between 8 and 10 hrs in all most all the cases. Most of the patients were recovered within 48 hrs, but two patients aged 7yrs and 12yrs were died due to other clinical complications. All the admitted cases were treated with IVF or ORF depends on the severity and oral ciprofloxacin and metronidazole was given in divided doses.

Microbiological analysis of the 44 samples collected from the hospitalized patients revealed that 6 (13.6%) samples were positive for *Salmonella enterica* serovar Weltevreden, 5 (11.4%) for *V. fluvialis* and for both these pathogens (mixed infection) in 8 samples (18.2%). In addition, 2 samples each were positive for *V. cholerae* non-O1, non-O139 and EPEC and one for LT-ETEC. In rest of the 20 samples, we could not identify any enteric pathogens. There is no correction between age group of the patient and prevalence of any pathogen.

The results of antimicrobial susceptibility testing of *Salmonella* Weltevreden showed that most of the strains susceptible to all the commonly used antibiotics except for erythromycin. In case

of *V. fluvialis*, most of the strains were resistance to co-trimoxazole and erythromycin. Three strains resistance to 8 or 9 antimicrobials (Table 1). The *V. fluvialis* strains did not harbor any virulence genes or the encoding regions of virulence associated genes of other vibiros including *ctxA, tcpA, rtxA,* TTSS*, mshA, stn, tdh* and *trh*. In the PFGE analysis of all the S. Weltevreden, exhibited identical *Xba*I profile and hence belongs to the single clone (Fig. 1). However, they varied from profiles of strains isolated during 2010 and 2011 in Kolkata. Of the 11 *V. fluvialis* strains, 8 were found to be clonally related with overall similarity of 93% in the Cluster A (Fig. 2). One *V. fluvialis* strain isolated during 2008 from a diarrheal patient had close lineage with strain IDH453 (96%), IDH4535 and IDH 4536 (92%) in the Cluster B (Fig. 2).

Discussion

Foodborne infections are still a major challenge in public health, especially while controlling the outbreaks. Surveillance of foodborne illness requires proper planning in the detection of the pathogens. This strategy will help in timely reporting of positive cases and trends in the prevalence of different pathogens to the public health authorities. The protocol used in this study helped in the identification many pathogens in this outbreak, of which *S.* Welteverdan and *V. fluvialis* are predominantly involved in the outbreak of gastroenteritis. Detection of dual pathogens during outbreak situations is very rare. As an emerging trend concomitant infections have been reported in several outbreaks (Chakraborty et al., 2001; Nyachuba, 2010). In developing countries, polymicrobial infections seems common in diarrheal patients (Bhavnani et al., 2012, Nair et al. 2010, Chen et al., 2009, Nimri et al., 2004). To our knowledge, this is the first foodborne gastroenteritis outbreak involving both *S.* Welteverdan and *V. fluvialis* in India.

The duration of present outbreak of gastroenteritis was relatively short; affecting almost 80% people who attended ceremony and 60% of them were hospitalized. Based on the questioner, probable source of the infection is mutton ghogni that was consumed by almost all the patients than other food items. We could not obtain the served foods to identify the cause of this outbreak. Foods prepared in an open place under the tree. Catering settings are also considered one of the sources in of foodborne outbreaks related with salmonellosis (OzFoodNet Working Group, 2005). *S.* Weltevreden is gaining global importance as a significant pathogen causing non-typhoidal salmonellosis. In India and other countries, *S.* Weltevreden constituted less than 4% of the total number of cases of human salmonellosis during 1970s (Sood LR. *et al*., 1984, Bangtrakulnonth A. *et al*., 2004). However, this is one the most frequently isolated serotype from humans in Thailand and Malaysia during 1983-2002 (Bangtrakulnonth A. *et al*., 2004, Yasin RM. *et al*., 1996). In India also *S.*Weltevreden is one of the top ten *Salmonella* serotypes and its importance of has been investigated in several findings (Patil BA. *et al*., 2006; Ghadage DP. *et al*., 2002; Antony B. *et al*., 2009).

It is known that *V. fluvialis* gastroenteritis has been associated with consumption of improperly cooked food (Takedi RJ. *et a*l., 1990; Tokoro M. *et al*., 1984). Diarrheal outbreaks caused by this pathogen have been reported in US and other countries (Seidler et al., 1980, Huq et al. 1980, Thekdi et al., 1982). Recently, the prevalence status of *V. fluvialis* has been reported in Kolkata (Chowdhuary et al., 2012). As observed in the current finding, our previous report also showed that majority of the *V. fluvialis* strains were multidrug resistance (Chowdhuary et al., 2012). Interestingly, the antimicrobial resistance not seen in *S.*Weltevreden strains as most the strains were susceptible for many drugs.

PFGE is a high discriminatory molecular subtyping and is useful in identifying the clonality iof many bacterial pathogens (Swaminathan B. *et al*., 2001). In the PFGE, clonality of *S*. Weltevreden was identical but differed with a strains isolated in this region during 2010 and 2011. Though the *V. fluvialis* strains shared more than 90% PFGE profile similarity, they were found to be in close lineage with a strains isolated in Kolkata during 2008.

In summary, we could identify the causative pathogens associated in this gastroenteritis using our existing surveillance system. However, proper planning should be in place for the identification of source of contamination and have the capability for performing case-control studies in the future outbreaks/epidemics.

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Table 1. Antimicrobial resistance profile of *S.* Weltivreden *and V. fluvialis* strains

Sample ID	Resistance profile	
	***S.* Weltiveredan**	***V. fluvialis***
IDH 4513	E	
IDH 4516	E	
IDH 4517	E	
NT 5513	E	
NT 5520	E	
NT 5526	E	
IDH 4528	E	SXT, E
IDH 4529	E	SXT, E
IDH 4533	E	AM, S, SXT, E
IDH 4539	E	SXT, E
IDH 4540	E	SXT, E
IDH 4541	E	SXT, E
NT 5521	E	E
NT 5524	E	SXT, E
IDH 4532		AM, S, SXT, E
IDH 4535		NA, CIP, NOR, OFX, AM,S, SXT, E, C
IDH 4536		NA, CIP, NOR, OFX, AM,S, SXT, E, C
IDH 4537		SXT, E, N
IDH 4538		NA, CIP, OFX, AM,S, SXT, E, C

Fig. 1. *Xba*I digested chromosomal DNA banding patterns of *S.* Weltivreden strains.

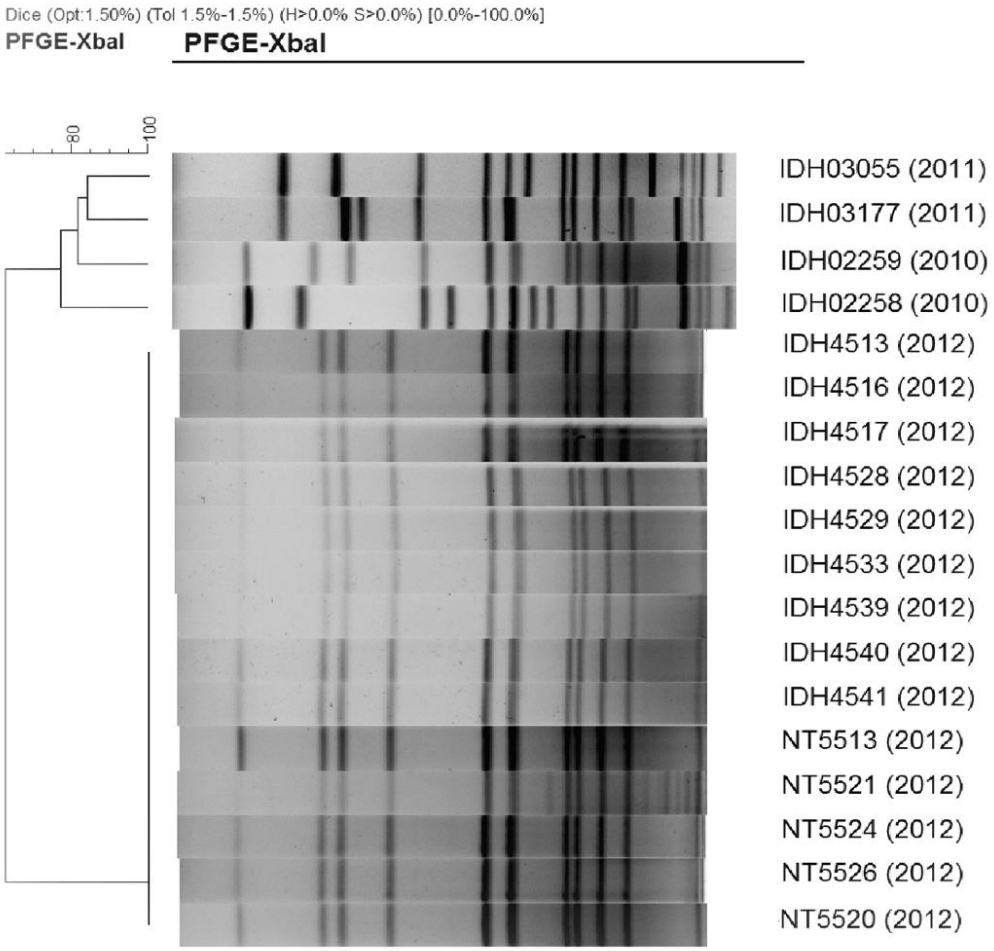

Fig. 2. *Not*I digested chromosomal DNA banding patterns of *V. fluvialis* strains.