Indian J Med Res 141, February 2015, pp 239-241

Correspondence

Salmonella enterica serovar Weltevreden ST1500 associated foodborne outbreak in Pune, India

Sir,

Foodborne gastroenteritis outbreaks due to non-typhoidal *Salmonella* represent important public health problem globally[1]. In the last two decades, *Salmonella enterica* serovar Weltevreden *(S*. Weltevreden) has emerged as a dominant foodborne pathogen globally especially in South-East Asian countries, being increasingly isolated from vegetables, meat and seafoods[1-6]. *S*. Weltevreden associated foodborne outbreaks of gastroenteritis have been reported from Scandinavian countries, Réunion Island (France) and India in the past[7-10]. Although, the complete genome sequence of *S*. Weltevreden outbreak strain 2007-60-3289-1 isolated in Scandinavia was available in EMBL nucleotide sequence database[7], the implicating agent of *S*. Weltevreden associated outbreaks in India have not been characterized earlier.

Here we report occurrence of *S*. Weltevreden associated foodborne gastroenteritis outbreak among student staying in a hostel at Pune, Maharashtra, India, and characterization of the isolates by antimicrobial susceptibility pattern, plasmid profiling and typing, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

On January 19, 2010, 150 students aged between 20-30 yr had consumed food comprising milk, rice, sprouted pulses and curd from their hostel canteen for lunch in Pune, Maharashtra. Almost all of them developed acute watery diarrhoea within 12 h of having food, associated with fever, abdominal cramps, nausea and vomiting. Most of them were immediately admitted to the local private hospitals (Aditya Birla Group Hospital) for treatment. Stool samples (n=25) were collected from the admitted patients, processed by standard microbiological method and the isolated organism (n=14) were sent to B. J. Medical College, Pune, for identification. The patients were discharged following treatment with either ciprofloxacin metronidazole combination or ceftriaxone alone. Food samples were not available to identify the source of infection due to delay (>12 h) in carrying out the investigation. A total of nine isolates, provisionally identified as *Salmonella* spp. were sent to National Institute of Cholera and Enteric Diseases (NICED), Kolkata, for confirmation of identification and characterization.

At NICED, the *Salmonella* isolates were identified by standard biochemical tests, followed by serotyping using *Salmonella* specific poly- and monovalent O and H antisera (Denka Seiken Pvt. Ltd, Tokyo, Japan) and nomenclature was done following Kauffmann White Scheme[11]. The isolates were tested for their antimicrobial susceptibility using 17 antibiotic discs (BD BBL™, Marryland, USA); ampicillin, chloramphenicol, tetracycline, co-trimoxazole, nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, gentamicin, amikacin, streptomycin, cefotaxime, ceftazidime, ceftriaxone, aztreonam, azithromycin and erythromycin, by disc diffusion method and results were interpreted following CLSI (Clinical and Laboratory Standards Institute) guidelines[12]. *Escherichia coli* ATCC 25922 was used as control. Plasmid DNA was extracted by Kado and Liu method[13], electrophoresed on 0.8 per cent agarose gel and stained with ethidium bromide to determine the plasmid profiles. *E. coli* V517 was used as plasmid molecular weight marker. The plasmid incompatibility type was determined by PCR using published primers followed by sequencing[14].

DNA fingerprinting was performed by PFGE in CHEF-DRIII system (BioRad Laboratories, Hercules, CA, USA), following standard PulseNet one-day protocol (*http://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf*) and using *Salmonella* Braenderup H9812 as control. The PFGE

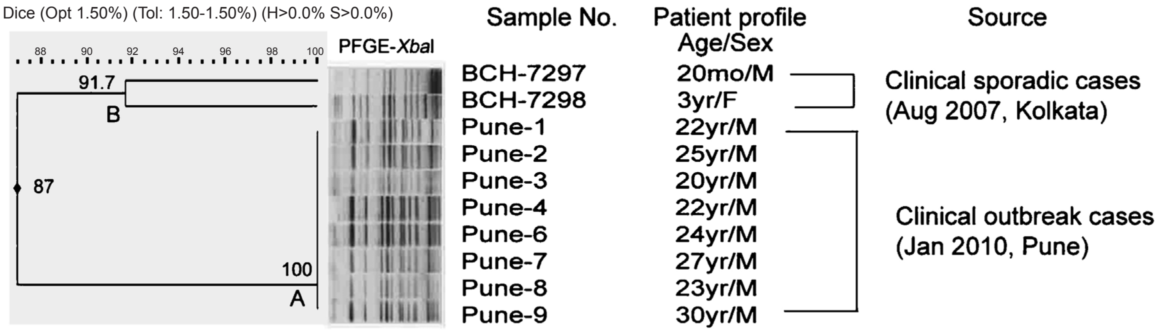

Fig. Pulsed field gel electrophoresis (PFGE) profiles of *Xba*I digest of DNA of *S.* Weltevreden Pune outbreak isolates, 2010 by cluster analysis and comparison with *S.* Weltevreden Kolkata sporadic isolates. Band comparison was performed using Dice coefficient with 1.50 per cent optimization (Opt) and 1.50 per cent position tolerance (Tol). H, minimum height; S, minimum surface; mo, months; M, male; F, female.

profiles were analyzed using FP Quest™ software version 4.5 (Bio-Rad, CA, USA). The extent of homology was determined by Dice coefficient, and clustering was based on unweighted pair group method with arithmetic mean (UPGMA)[15]. The isolates with ≥ 90 per cent similarity threshold were grouped under one cluster.

Phylogenetic relatedness of the isolates was studied by MLST. The amplification of the seven housekeeping genes followed by sequencing was done as previously described[16]. Sequences obtained were submitted to the MLST database (*http://mlst.ucc.ie/mlst/dbs/Senterica*) to assign sequence type (ST) of each isolate based on the set of allele types derived from all the seven loci.

Two local *S.* Weltevreden isolates from sporadic diarrhoea cases in Kolkata were also included in the study and characterized for comparison.

Out of nine outbreak isolates, eight were identified as *S.* Weltevreden. All the study isolates (Pune outbreak and Kolkata sporadic) were uniformly susceptible to the 17 antimicrobials tested. Pan susceptible *S.* Weltevreden isolates have earlier been reported from outbreaks in France (2007) and Mangalore (2008)[8,9]. However, isolation of multi-drug resistant *S.* Weltevreden (resistant to ampicillin, streptomycin, co-trimoxazole, erythromycin and tetracycline) has also been reported from environmental samples in different countries[2-6].

Single plasmid of 63kb size belonging to IncFIIS type was observed in all the study isolates. Earlier studies have documented presence of single or more plasmids ranging from <2 to 89.1 kb sizes[5]. The plasmids of type IncFIIS are considered as virulence plasmids in *Salmonella*[14], but its role in disease pathogenesis is yet to be established.

The Pune outbreak (A) and Kolkata sporadic (B) isolates formed distinct clusters in PFGE (Figure). All the outbreak isolates were found to be clonal (100%) and had 87 per cent similarity with the sporadic isolates. MLST database characterized all the study isolates as ST1500 [Clonal complex (CC) type 205] based on their sequence data. The allelic types of the seven genes were *aro*C: 130, *dna*N: 97, *hem*D: 25, *his*D: 125, *pur*E: 422, *suc*A: 9, and *thr*A: 101. The current data have been entered in the MLST database and are available at the website (*http://mlst.ucc.ie/mlst/dbs/Senterica/GetTableInfo_html*). *S.* Weltevreden human isolates from Sri Lanka, Australia, Indonesia, Zimbabwe, Philippines and Germany belonging to ST365 and that of an environmental isolate from UK belonging to ST1500 were available in the aforesaid MLST database. This report showed that the foodborne outbreak caused by Indian *S.* Weltevreden isolates belonged to ST1500 (CC205).

The use of molecular methods and antibiotic susceptibility profiles for characterization of *Salmonella* outbreak isolates in various geographical regions is useful for studying global epidemiology of the organism which might result in controlling further spread of the organism.

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Acknowledgment

The study was supported by the Indian Council of Medical Research (ICMR) intramural fund. ICMR-SRF to the first author (PJ) and technical assistance by NICED staff are acknowledged. Authors also thank the Curator of the MLST database (*http://mlst.ucc.ie*), which is currently supported by a grant from the Science Foundation of Ireland (05/FE1/B882) for enabling us to submit our data.

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