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Occurrence, significance & molecular epidemiology of cholera outbreaks in West Bengal

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***Background & objectives*: Diarrhoeal disease outbreaks are causes of major public health emergencies in India. We carried out investigation of two cholera outbreaks, for identification, antimicrobial susceptibility testing, phage typing and molecular characterization of isolated *Vibrio cholerae* O1, and to suggest prevention and control measures.**

***Methods*: A total of 22 rectal swabs and 20 stool samples were collected from the two outbreak sites. The *V. cholerae* isolates were serotyped and antimicrobial susceptibility determined. Pulsed- field gel electrophoresis (PFGE) was performed to identify the clonality of the *V. cholerae* strains which elucidated better understanding of the epidemiology of the cholera outbreaks.**

***Results*: Both the outbreaks were caused by *V. cholerae* O1 (one was caused by serotype Ogawa and the other by serotype Inaba). Clinically the cases presented with profuse watery diarrhoea and dehydration. All the tested *V. cholerae* isolates were sensitive to tetracycline, gentamycin and azithromycin but resistance for ampicillin, co-trimoxazole, nalidixic acid, and furazolidone. PFGE pattern of the isolates from the two outbreaks revealed that they were clonal in origin. Stoppage of the source of water contamination and chlorination of drinking water resulted in terminating the two outbreaks.**

***Interpretation & conclusion*: The two diarrhoeal outbreaks were caused by *V. cholerae* O1 (Inaba/Ogawa). Such outbreaks are frequently seen in cholera endemic areas in many parts of the world. Vaccination is an attractive disease (cholera) prevention strategy although long-term measures like improvement of sanitation and personal hygiene, and provision of safe water supply are important, but require time and are expensive.**

Key words Cholera - Inaba - molecular epidemiology - ogawa - outbreak

Globally 1.3 billion cases of acute diarrhoea occur in children below 5 yr annually of which more than 3 million die and 80 per cent of these deaths are in children below 2 yr of age[1]. Acute diarrhoea can be caused by a number of bacterial, viral or parasitic agents. The most important bacterial agents causing outbreaks of acute diarrhoea are *V. cholerae* O1 and O139. Diarrhoeal disease outbreaks are causes of major public health emergencies in India. West Bengal located in the Gangetic delta has been hailed as the "homeland of cholera", with frequent localized outbreaks being reported[2,3]. Lack of availability of clean water and poor environmental sanitation and personal hygiene are significant contributing factors for the spread of diarrhoeagenic organisms resulting in outbreaks particularly of cholera[4,5].

With the development of molecular tools, identification and molecular characterization of organisms have become easier and faster for prompt diagnosis and better understanding of the epidemiology (molecular) of the disease. Between November 2004 and April 2005, two diarrhoeal disease outbreaks in widely separated areas (approximately 120 km apart) caused by *V. cholerae* O1 serotypes Ogawa and Inaba occurred in Murshidabad and North 24 Parganas districts of West Bengal, India. Here, we describe the detailed account of the investigation of two cholera outbreaks.

Material & Methods

Demography of the outbreak sites *Outbreak* 1: The first outbreak occurred in Beldanga Block of Murshidabad, West Bengal. Increased number of acute diarrhoea cases started reporting to the Block Health Center of Beldanga Block 1 of Murshidabad district towards the end of October 2004. This health centre is often referred to as "diarrhoea hospital". A team from National Institute of Cholera and Enteric Diseases (NICED), Kolkata investigated the reported outbreak at the end of November 2004.

Outbreak 2: The second outbreak occurred in Garulia town, which is located by the side of river Hooghly in North 24 Parganas, West Bengal. The total population of the area is around 80,000, living mostly in congested environment. An investigating team from NICED observed that out of 21 wards, seven (1-5,7,8) adjacent wards were affected. The population at risk was approximately 7000.

Collection of samples from the outbreak regions : A total of 14 rectal swab samples in Cary-Blair's transport media and 6 stool samples were collected from hospitalized patients of the first outbreak, whereas 8 rectal swabs and 14 stool samples were collected from new cases visiting Garulia emergency clinic and hospitalized patients respectively. The stool samples were transported in icebox for performing PAGE (polyacrylamide gel electrophoresis) to screen for any viral aetiology. Eight water samples from each of the outbreak sites were collected from different sources (which included sample from the leakage point in ward no.2 of Garulia municipality, water from different pumping stations and stored water from the households) in sterile containers to detect any faecal contamination.

Bacteriological processing of the stool specimens and water samples : The rectal swabs and stool samples were processed for the entire gamut of established diarrhoeagenic pathogens following standard procedures using enrichment and selective media[6]. Serotyping was done by slide agglutination test using polyvalent O1 and monospecific Inaba and Ogawa antisera prepared at NICED. PAGE was run with the six stool samples to detect concomitant presence of rotavirus. Eight samples of water were analyzed for total coliform bacteria and faecal *Escherichia coli* by membrane filtration method using Lauryl sulphate broth or an equivalent medium which is inhibitory to many non-coliform bacteria and on which coliforms grow as yellow coloured colonies due to presence of lactose and phenol red in it[7]. The water samples were also processed for diarrheaogenic pathogens. Phage typing of the *V. cholerae* isolates was carried out using Basu and Mukherjee's[8] and new phage typing scheme[9].

Antimicrobial susceptibility and pulsed-field gel electrophoresis (PFGE) : The antimicrobial susceptibility test was done using standard methods[10]. The ATCC strains of *E. coli* 25922 and *Staphylococcus aureus* 25923 were used as quality control strains. PFGE was done according to previously established methods[11].

Results

Outbreak 1: From the beginning of the outbreak till the date of investigation, 554 cases had been admitted and the number was increasing (Fig. 1). All age groups were affected including 53.6 per cent of cases in those below 15 yr of age. Both the sexes were equally affected. Majority (90%) of the cases had history of acute watery diarrhoea at the time of admission.

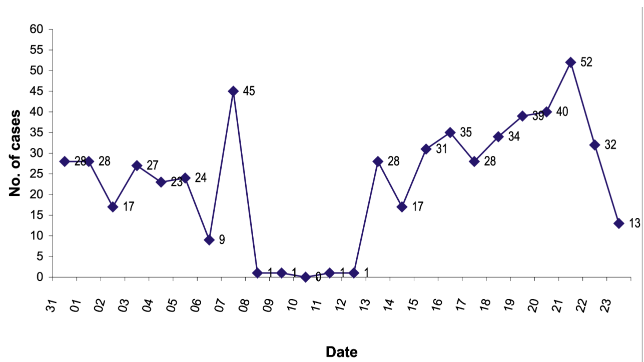

Fig. 1. Daily incidence of admitted cases of acute diarrhoea at Health Center, Beldanga-1, Murshidabad in November 2004.

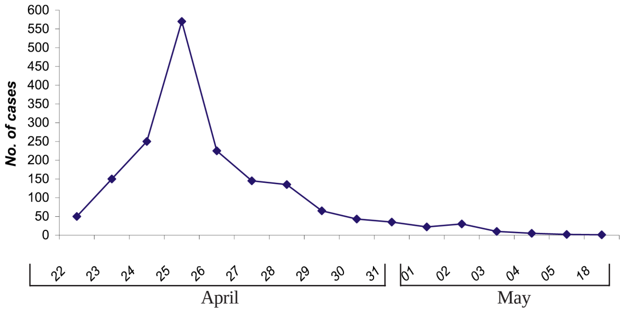

Fig. 2. Daily cases of diarrhoea during the outbreak at Garulia, North 24 Parganas in 2005.

It was noted that there was no pipeline water supply in the study area and people were dependent on tube wells for drinking water supply. Most of the tube wells were fitted with pipes of length 80-100 ft. The areas within 15m of the tube wells, which needed to be kept free from pollution with liquid and solid waste were not well maintained in most places.

Of the 20 cases examined, 19 (95%) had rice watery stool, eighteen (90%) had vomiting and most of them had dehydration (some dehydration 14, severe dehydration 4)[12]. None gave history of any movement outside the locality or taking food outside their houses during the last 7 days. They did not use soap to wash their hands before taking meal and after defaecation. Only 5 (25%) had used sanitary latrines for defecation and 11 (55%) had secondary cases in their vicinity.

The aetiological agent was confirmed to be *V. cholerae* O1 ElTor Ogawa (isolation rate 50%). Water samples were found positive (50%) for faecal *E.coli* (by demonstrating that faecal *E.coli* can form acid and gas from lactose at 44^0C and indole from tryptophan at 44^0C) due to contamination of sub-soil water from open area defaecation and indiscriminate disposal of garbage. *V. cholerae* could not be isolated from any water sample possibly due to chlorination of water done by the health authority immediately prior to the visit of the investigating team.

Outbreak 2: The first case was reported from ward No.4 of Garulia town where a two year old boy suffered from several bouts of rice watery stools on April 22, 2005. Cases were then reported from different wards of the area with the total number of cases mounting to 1590 till 18.05.2005, giving an attack rate of 22.7 per cent (Fig. 2). They were mostly treated in Garulia emergency clinic with oral rehydration salt solution (ORS). Those with severe dehydration requiring iv fluids and antibiotic(s) were admitted to nearby hospitals. Total number of hospital admissions was 634, of which 616 were discharged after treatment and 15 were referred to tertiary health care centres. Four diarrhoeal deaths were reported throughout the outbreak.

All 28 cases (100%) investigated by the team, complained of watery diarrhoea with an average of 15-20 motions daily. Eleven cases (40%) complained of vomiting. Twelve had some dehydration while 14 had severe dehydration. Cases admitted in Infectious Diseases Hospital (IDH), Kolkata with severe dehydration were treated with iv fluids and inj. tetracycline and/or metrogyl or fluoroquinolones and/ or metrogyl and inj. ampicillin and inj. amikacin and discharged after complete recovery.

Garulia municipality supplies treated drinking water from its Treatment Plant twice a day to major portion of the region and the rest have tube well supply. Most of the pipelines were not maintained well and chances of contamination were high. The survey revealed the existence of a leakage point of connecting pipelines supplying potable water in ward no.2. Most houses have sanitary privies, though few katcha privies were seen. Drainage system is mostly open, inadequate and nonfunctioning in most parts of the area. Source of infection was detected as gross sewage contamination of drinking water. Outbreak was controlled eventually with effective chlorination of piped water supply in households as well as supply of drinking water temporarily through mobile tankers till effective chlorination was achieved and sinking of 5 deep tube wells in the affected area.

All the stool samples taken from Garulia emergency clinic were positive for *V. cholerae* O1 Inaba. Of the 14 samples collected from IDH, 12 were positive for *V. cholerae* O1 Inaba. Of the 8 water samples, 5 were positive for *V. cholerae* O1 Inaba and faecal *E. coli*.

All the isolated *V. cholerae* strains from both the outbreaks were sensitive to tetracycline, gentamicin, azithromycin and ciprofloxacin (but with reduced susceptibility to ciprofloxacin for the *V. cholerae* isolated from the Garulia outbreak) but resistant to ampicillin, co-trimoxazole, nalidixic acid, and furazolidone. In Kolkata, increased incidence of reduced susceptibility to fluoroquinolones amongst *V. cholerae* isolates was reported during 1998[13].

In both the outbreaks, the PFGE pattern of all the *V. cholerae* O1 Inaba and Ogawa strains tested had similar pattern (type H) irrespective of their source of isolation (Fig. 3). *V. cholerae* isolated from both the outbreaks

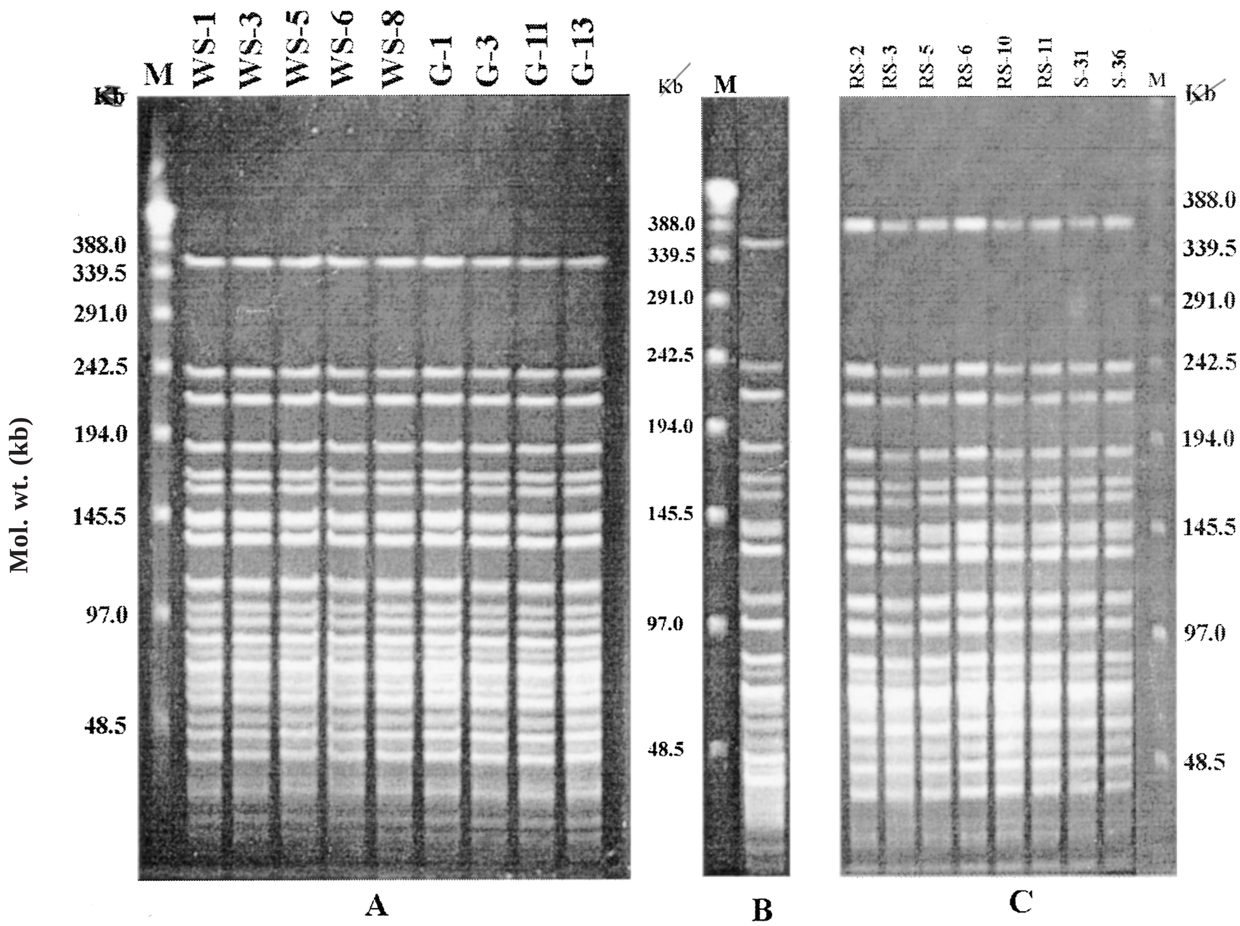

Fig. 3. PFGE of *V. cholerae* O1 after digestion with *NotI*. **(A)** isolates collected from Garulia water samples (Ws 1, 3, 5, 6, 8) and stool specimens (G-1, 3, 11, 13), **(B)** 'H' PFGE profile of *V. cholerae* O1 and **(C)** isolates collected from the stool specimens of Murshidabad cholera patients (Rs-2, 3, 5, 6, 10, 11, S-31, S-36). M, mol.wet marker (kb).

were included for phage typing study. It was observed that the isolates were clustered as phage type 4 of Basu and Mukherjee and type 27 of new phage typing scheme.

Discussion

Cholera being mainly a water-borne disease, contamination of water sources is the main cause of transmission of the disease. There was no regular monitoring of water sources, especially following rains and floods when chances of pollution are very high and there was no proper sanitation hygienic practices. Certainly these factors led to the rapid spread of these two outbreaks.

In both the outbreaks, the *V. cholerae* O1 isolates were resistant to some of the commonly used antibiotics like co-trimoxazole, nalidixic acid and furazolidone. Nalidixic acid resistance may be a forerunner of resistance to fluoroquinolones and occurrence of such resistance may create a therapeutic challenge in future, although presently the strains are sensitive to tetracycline, the drug of choice for the treatment of severe cholera as an adjunct to rehydration therapy. Moreover, rationalization of therapy of cholera cases is important in any cholera outbreak. The investigations also revealed the irrational use of antidiarrhoeals and antiamoebics.

In the PFGE, all the tested *V. cholerae* O1 Inaba isolates had identical pattern (type H) irrespective of their source of isolation. This strongly suggests that the source of contamination was drinking water supplied through the leaking pipeline. Prevalence of 'type H' PFGE pattern was reported among Ogawa serotypes after the emergence of the O139 serogroup[11]. As reported before[14], the recent Inaba strains would have emerged from the prevailing *V. cholerae* O1 Ogawa strains, which was indicated by the clonal nature of both the serotypes as determined by PFGE. Thus, the study reinforces the usefulness of molecular tools like PFGE and phage typing for elucidating the common source of origin and spread of disease outbreaks like cholera in both rural and urban settings.

Both the outbreaks were caused by *V. cholerae* O1 (one by serotype Ogawa and the other Inaba). Sur *et al*[15] reported a similar outbreak in a slum community in Kolkata recently. Manna *et al*[16] recommended vaccination against cholera as an attractive additional tool to combat the disease in endemic areas.

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