

Reemergence of Cholera in Lakhimpur District of Assam, India

Jitendra Sharma*¹ and Sashi Gupta²

¹District Epidemiologist, Office of the Joint Director of Health Services, Lakhimpur, Assam, India

²Microbiologist, Dhubri Civil hospital, Dhubri, Assam, India

ABSTRACT

Objective: The study aims to find out the causative agent of severe gastroenteritis cases reported during an outbreak in Lakhimpur, Assam.

Methods: Stool specimens were collected from randomly selected 23 severe patients. Culture and sensitivity test was done for detection of *Vibrio cholerae*-like organisms. Water samples were also collected from their drinking water sources for H₂S and most probable number (MPN) test.

Results: In September, 2014, a total of 152 numbers of cases having symptoms of vomiting, watery diarrhoea, fever and abdominal pain were reported from Joyhing and Koilamari tea garden under Boginodi block public health centre (BPHC). Male to female ratio was 1:1.17. The initial case was detected on 4th September, thereafter slowly increasing and reached at peak level on 8th September. Culture showed growth of *Vibrio cholerae* O1, *El Tor*, *Ogawa* in 13 numbers of samples after 24 hours of aerobic incubation and was confirmed by biochemical test. All age groups were affected. Females were more vulnerable for *Vibrio cholerae* infection. The overall case fatality rate was 2.63% and fatality rate of cholera was 7.69%. Large numbers of the cholera positive cases (4 nos) were detected from Tarbine line. The sensitivity test showed that *Vibrio Cholerae* infected patients were resistance to common antimicrobials like Penicillin G, Bacitracin, Co-trimoxazole and Trimethoprim. Fecal-oral route of transmission was established as the major cause of the outbreak. All the collected water samples were also found to be contaminated.

Conclusion: The people from this area are mostly uneducated. They are having very poor knowledge on proper hygiene. So, special task should be needed to spread awareness among the people of this garden.

Keywords: Assam, Boginodi, H₂S, Joyhing TE, Most probable number, *Vibrio cholerae*.

Address for Correspondence

District
Epidemiologist, Office
of the Joint Director of
Health Services,
Lakhimpur, Assam,
India.

E-mail:
jitendra.du.biotech@gmail.com

INTRODUCTION

Cholera is an acute intestinal infection. It is caused by the ingestion of contaminated food or water. The causative agent is gram-negative, comma-shaped bacterium known as *Vibrio cholerae*. It has a short incubation period, from less than one day to five days. The bacteria produces an enterotoxin that causes copious, painless, watery diarrhoea that can quickly lead to severe dehydration. Vomiting is also occurs in most patients. Sometimes people may die also if treatment is not promptly given. Cholera is spreading in a community at a very faster rate. The major source of infection is water contaminated with the causative bacterium *Vibrio cholerae*. Beside this there are several other factors like contaminated foods, especially raw shellfish, may also transmit the cholera-causing bacteria. Any persons having complained of rice-watery stool are presumptively diagnosed as suspected Cholera. However the presence of *Vibrio cholerae*-like organisms is further confirmed by microscopic examination after culture and sensitivity test.

Annually about 3-5 million cases of Cholera occurs with more than 100,000 deaths around the world.¹ Cholera is a major problem in India. The people from North eastern region of India are highly vulnerable for getting *Vibrio cholerae* infection. Cases of acute diarrhoea with high rate of morbidity are commonly occur in rural areas of northeast India, throughout the year.² At any times it might becomes an epidemic form.² In 2010, an outbreak of cholera took place in Lakhimpur district of Assam affecting almost more than 100 people.³ Beside this, people from tea garden areas of Assam are mostly susceptible for *Vibrio cholerae* infection. It has been observed that during the month of August 2014, the numbers of gastroenteritis cases reported from Joyhing tea estate (TE) showed

gradually increasing trend as compared to previous months. It was assumed that there will be some cause behind the high incidence of gastroenteritis cases. Taking this data in mind, a study was conducted in Joyhing TE and adjacent Koilamari TE under koilamari division to find out the causative agents as well as to observe the risk factors associated with the high incidence of diarrheal diseases. By the early detection of causative agent, control measures could be taken in time to prevent the further transmission of disease.

MATERIALS AND METHODS

Lakhimpur district is situated in the Eastern corner of Assam lies between 26°48' and 27°53' northern latitude and 93°42' and 94°20' eastern longitude. This place is known as vulnerable for occurrence of many diseases.⁴⁻⁹ The study was carried out in Joyhing and Koilamari TE under Koilamari division which is nearly 20 Km apart from Lakhimpur town. The total population in Joyhing TE was 5287 and Koilamari TE was 4500 including the garden labours and stuffs. There are total 20 numbers of lines in the garden. This tea garden is included under the Seajuli mini public health centre (MPHC) of Boginodi area. The patients attended in Joyhing and koilamari TE hospital having complaint of vomiting, watery diarrhoea, fever, abdominal pain were presumptively diagnosed as gastroenteritis's cases and accordingly the patients were treated by the medical officers in that respective garden. Stool samples were collected from randomly selected 23 severe patients. The specimens were transported in carry blair media to the microbiology division, North Lakhimpur Civil Hospital for culture and sensitivity test. Initially stool inoculation was done on MacConkey's agar (Mac Agar) and

Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS Agar). The incubation period was overnight at 37°C followed by enrichment in Alkaline Peptone Water (APW). The growth of *bacilli* in Mac and TCBS agar media was observed by gram staining. Finally in *Vibrio Cholera* Antisera (Poly O1) test, a small portion of growth of bacteria in TCBS media was mixed with Poly O1 *Vibrio Cholera* antisera in a slide. By observing agglutination reaction, the presence of *Vibrio Cholera* O1 serotype in the collected specimen was confirmed.

Media/broth used for culture and sensitivity test

TCBS agar media (used to isolate *Vibrio* species), blood agar media, Mac agar media (designed to selectively grow Gram-negative bacteria), Müller-Hinton agar media (used for antibiotic susceptibility testing) and peptone broth (*Vibrio cholerae* and *Vibrio mimicus* can grow in peptone water mediums).

Biochemical test

Indole test, Oxidase test, Methylene Red test, String test, Urease test, *Vibrio Cholerae* polyvalent, *Vibrio cholerae* Ogawa and *Vibrio cholerae* Antisera, O139 Bengal tests were done. Based on the growth characterization nature in different agar media, microscopic examination and different biochemical results, the isolated bacteria was confirmed. Sensitivity results were obtained based on Kirby-Bauer disk diffusion susceptibility test method in Müller-Hinton agar media.¹⁰

RESULTS AND DISCUSSION

Epidemiological observation

Very poor sanitary living situation was observed in the affected areas (Figure 1). Almost, 90% people were having habit of open air defecation adjacent to a running river. It can be harmful for other unaffected

and non immune people. The people of Joyhing and koilamari tea gardens are using drinking water mostly from wells. Some people have using hand pumps. Although they were using the hand pumps, but there was a pipe connected with the wells. Many of drinking water sources were observed without brim or platform. People of these gardens were very much immune as they went immediately for the daily work after initial treatment or even sometimes without treatment. These untreated inhabitants were carrying the infection and there may have every possibility to spread the infection in that community.

Laboratory investigation

A total of 152 (one hundred and fifty two) numbers of suspected cholera/gastroenteritis cases were reported from Joyhing and koilamari TE under Boginodi block public health centre (BPHC) during the month of September, 2014. Male: Female ratio was 1:1.17. The symptoms of the patients are vomiting, watery diarrhoea, fever and abdominal pain. The initial case was detected on 4th September from Amguri line (Figure 2 & Figure 3). However during this outbreak, most numbers of gastroenteritis cases were observed from Tarbine line and Line no 15 (Figure 3). Both are neighboring lines. The trend reached at peak level on 8th September, and then declining slowly (Figure 2). The overall case fatality rate was 2.63%. People from all age groups were found to be suffering from gastroenteritis disease (Table 1). Ages ranged from 2 months to 68 years (median $\pm$ 23 years). Female were more vulnerable for infection. 23 (Twenty three) nos. of stool samples were collected for culture & sensitivity test. Culture showed growth of *Vibrio cholerae* O1 biotype El Tor serotype Ogawa in 13 (thirteen) samples. Normal growth of *E. coli* was detected in 2 (two) cases. However, no growth was observed from the remaining 8 (eight)

samples. Children below 15 years of age groups were more vulnerable for Cholera infection (Table 1). Our finding have shown similarity with many other previous results reported from other parts.^{11, 12} Out of total 13 (thirteen) *Vibrio cholerae* positive patients, 6 (six) cases were male and remaining 7 (seven) cases belong to female group. Few previous studies also reported that females were more risk groups for cholera vulnerability.¹³ The fatality rate of cholera was 7.69% and the remaining 92.31% patients were recovered after treatment. Most of the cholera positive cases (4 nos) were detected from Tarbine line. The initial case of *Vibrio cholerae* was also reported from the Tarbine line. Later on it was spreading in all other adjacent lines by fecal–oral route of transmission.

From many other previous finding, it has been established that cholera is mostly prevalent among the people of tea garden communities in different parts of Assam. There are large numbers of tea garden in Dibrugarh, Tinsukia, Sonitpur and Sivasagar, where *Vibrio cholerae* cases were reported frequently.^{14,15} This is due to the poor knowledge regarding sanitation and health hygiene, open air defecation, improper cooking of food, migration of people from other gardens etc.

A river namely “Dhekia” is running away through this garden. Due to high rainfall in August, flood like situation was occurred (water from river cross the threshold) in several houses of Tarbine line. Those may be identified as the source of infection. 55 (fifty five) numbers of water samples were collected from different drinking water sources for H₂S test, all were found contaminated. Water samples from 3 (three) numbers of drinking water sources were collected for most probable number (MPN) test. Result showed unsatisfactory for drinking purpose. Two samples have shown class III (suspicious) grade and the remaining one have shown class IV (unacceptable).

The antibiotic susceptibility test showed, Sensitive to: Cetotaxime, Ciprofloxacin, Ceftriaxone, Chloramphenicol and Norfloxacin and Tetracycline; intermediate sensitivity: Amoxiclav, Amikacin, Ampicillin, Gentamicin and resistance: Penicillin G, Bacitracin, Co-trimoxazole and Trimethoprim.

The district health authority has taken many steps to control the outbreak. Such measures includes, organizing medical camp on daily basis, IEC regarding personal hygiene and safe drinking water, leaflets distribution, disinfection of all well by bleaching powder, household distribution of ORS, halogen tablets and antidiarrheal to the affected people etc.

CONCLUSION

Poor hygiene practices among the villagers are associated with the transmission of the pathogen. It leads to cholera outbreak as well as resistant to commonly-used antimicrobials.

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Table 1. Age group wise numbers of acute diarrheal disease and Cholera positive cases/deaths

Incidence					
Disease	0-5	6-15	16-30	31-50	51 above
Acute diarrheal disease	13	38	53	37	11
<i>Vibrio Cholerae</i> positive	0	6	3	4	0
Death cases					
Acute diarrheal disease	1	-	1	-	1
<i>Vibrio Cholerae</i> positive	-	1	-	-	-



Figure 1. Drinking water sources in the affected areas (Wells without brim or platform)

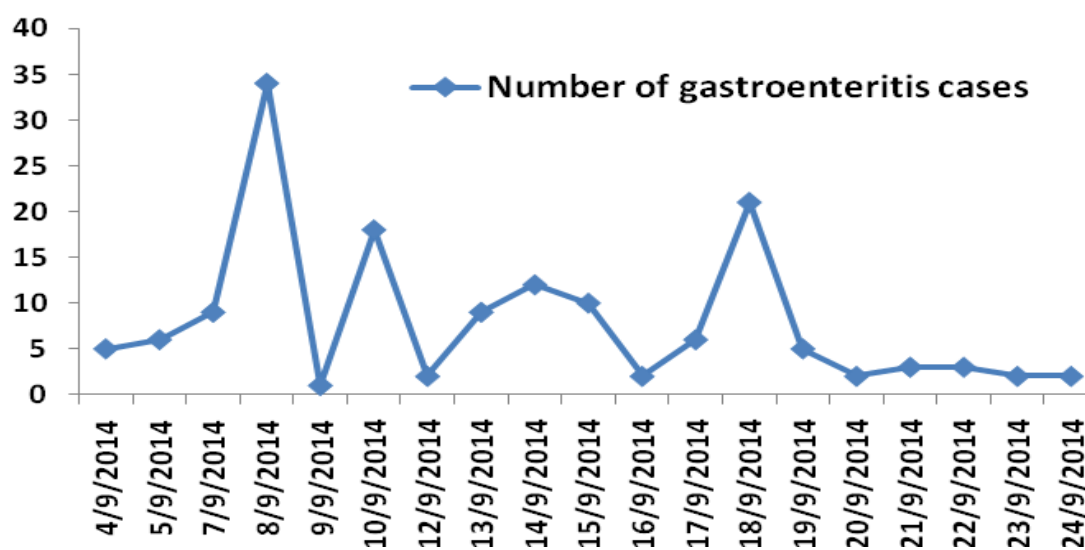


Figure 2. Numbers of gastroenteritis cases by date of onset (Number of cases in Y axis and date of onset in X axis)

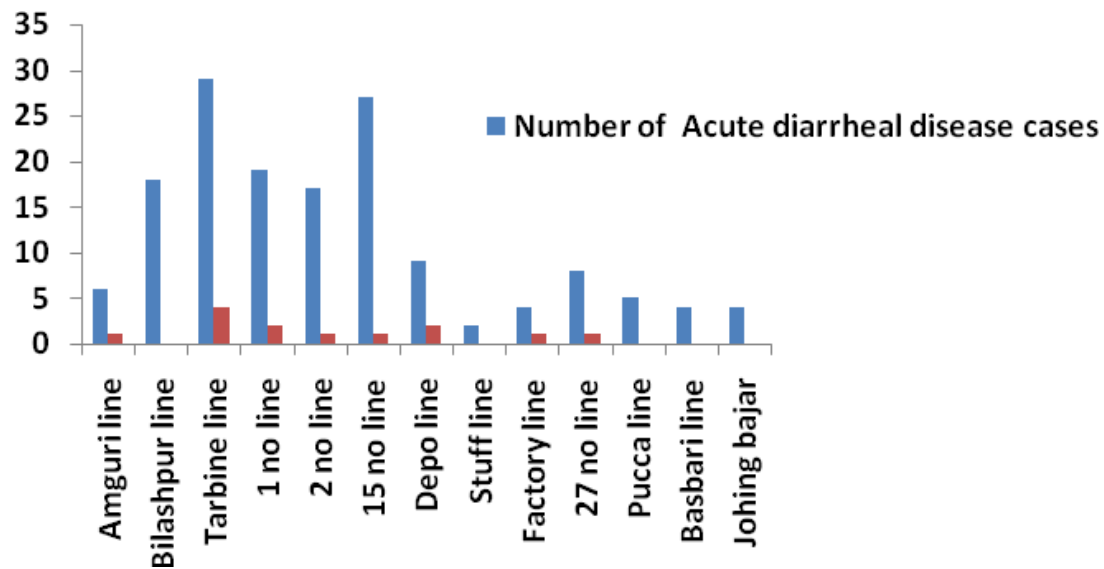


Figure 3. Numbers of gastroenteritis cases in different lines of Joyhing TE (Number of Acute diarrheal disease and Cholera cases in Y axis and reported areas in X axis)