A LOCALIZED OUTBREAK OF CHOLERA DUE TO VIBRIO CHOLERAE O1, OGAWA RESISTANT TO TETRACYCLINES

Shahid Ahmed, Raja Kamran Afzal*, Umar Arif Mian*

Combined Military Hospital Gilgit, Pakistan, *Combined Military Hospital Lahore, Pakistan

ABSTRACT

Objective: To study the clinical and laboratory parameters of a localized Cholera outbreak and determine the sensitivity pattern of the subtype involved.

Study Design: A descriptive study.

Place and Duration of Study: Combined Military Hospital, Lahore.

Duration of Study: Two weeks.

Patients and Methods: The study is about a localized outbreak of cholera in a group of soldiers, who consumed water from a single contaminated source of water. We are presenting here an account of the clinical and laboratory parameters of 39 hospitalized cases of cholera, who presented with profuse watery diarrhoea and vomiting. There vital signs, hydration status and systemic examination findings were recorded. Stool samples were sent for routine and microscopic examination and bacteriological culture. Blood samples were taken for complete blood count, serum sodium, potassium, urea and creatinine examination. SPSS 18 was used for statistical analysis of the results.

Results: The average age of thirty nine men studied in this outbreak was 24.9 ± 6.9 years. There was no statistically significant difference between confirmed and suspected cholera cases on descriptive analysis of the clinical and laboratory parameters. Majority of patients showed pre-renal azotemia which improved within 48 to 72 hours of hospitalization. Stool cultures isolated Vibrio cholerae O1, subtype Ogawa, which was resistant to tetracyclines, cotrimoxazole and nalidixic acid but sensitive to fluoroquinolones and third generation cephalosporins. The outbreak was controlled when the contaminated water source was sealed and rectified.

Conclusion: Multiple drug resistance strains of Vibrio cholera are causing large outbreaks which should be controlled by prevention of the disease and avoiding inappropriate use of antibiotics.

Keywords: Cholera, Ogawa, Serogroup O1, Tetracyclines, Vibrio cholerae.

INTRODUCTION

Cholera is an acute diarrhoeal disease that can kill within hours if left untreated[1]. There are an estimated 3–5 million cholera cases and 100 000–120 000 deaths due to Cholera every year[1]. The current (seventh) pandemic of Cholera started in South Asia in 1961, and reached Africa in 1971 and the America in 1991. Cholera is now endemic in many countries[1]. Well known risk factors for cholera outbreaks include poverty, lack of development, poor sanitation, dense population, lack of education, and lack of previous exposure[2]. However, the most dominant risk factor for Cholera outbreaks is contaminated drinking water[2]. The causative organism is Vibrio cholerae which has two biotypes – El Tor and classical. It is divided into serogroups based on the somatic O antigen. Only O1 and O139 serogroups are known to cause epidemic and pandemic disease. The O1 serogroup is further divided into subtypes of Ogawa, Inaba and rarely, Hikojima[3]. In cholera management, antibiotics are essential for disease treatment in severe cases. However, Vibrio cholera strains from endemic and outbreaks situation within the last decade or so revealed interesting patterns of antibiotic resistance to commonly used antimicrobial agents. Mobile genetic elements are able to transfer multiple drug resistance among Vibrio cholera strains have also been described in numerous studies and are considered a major public health problem[4].

We are reporting an outbreak of Cholera in a group of soldiers who consumed contaminated water from a single source. The outbreak was controlled when that water

Correspondence: Dr Shahid Ahmed, Classified Medical Specialist and Endocrinologist, MH Rawalpindi, Pakistan

Email: shahidahmed833@gmail.com

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source was sealed and purified water supply was thereby ensured for the rest of the troops.

PATIENTS AND METHODS

This descriptive study was conducted at Combined Military Hospital Lahore over a period of two weeks, based on an outbreak of cholera caused in a group of soldiers who consumed water from a single contaminated source of water. The cluster of cases presented at Combined Military Hospital Lahore, in a period of two weeks and the outbreak was controlled when contaminated water source was identified and sealed. We are presenting here a descriptive analysis of the clinical and laboratory parameters of 39 hospitalized cases of cholera. A modified WHO clinical case definition was used for suspected and confirmed cases of cholera[5]. A suspected case was defined as any person suffering from acute watery diarrhea with or without vomiting, from the same area. A confirmed case was defined as any suspected case with a positive stool sample to Vibrio cholera. All these cases were hospitalized during the outbreak with profuse watery diarrhoea and associated vomiting in some cases. There vital signs, hydration status and systemic examination findings were recorded. Stool samples were sent for routine and microscopic examination and bacteriological culture. The stool samples were inoculated in alkaline peptone water for enrichment. From alkaline peptone water, the samples were cultured on thiosulphate citrate bile salt agar (TCBS) and MacConkey agar for aerobic incubation at 37 ^{o}C. Blood samples were taken for complete blood count, serum sodium, potassium, urea and creatinine examination. Hematological profile was done on Sysmex kx-21 haematology analyzer. Serum creatinine was done on Vitalab Selectra E Chemistry analyzer and electrolytes were measured through Easylyte Na/K Electrolytes analyzer.

SPSS 18 was used for statistical analysis. Data was presented in mean ± standard deviation and median (range). Independent sample t test was used to compare the difference of means. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

The average age of thirty nine men studied in this outbreak was 24.9 ± 6.9 years. The descriptive analysis of the clinical and laboratory parameters of the two groups of confirmed and suspected cholera cases is given in table-1. There was no significant statistical difference in the means of various parameters between two groups except in diastolic blood pressure on presentation and length of hospital stay which was more in confirmed cholera group. Due to insignificant difference in descriptive analysis between the two groups, a descriptive analysis for the total number of cases has also been presented. The duration of symptoms when the patient presented to hospital was 17.3 ± 11.4 hours, systolic blood pressure 101.6 ± 13.3 mm Hg and diastolic blood pressure was 65.8 ± 8.8 mm Hg. The total leucocyte count was (9.8 ± 3.0) x 109/l, serum creatinine 131.4 ± 45.5 μmol/l, sodium 137.6 ± 3.5 mmol/l and potassium 3.9 ± 0.3 mmol/l. Duration of hospital stay was 4 (1-7) days. Twenty six patients were moderate to severely dehydrated on presentation and thirteen patients were mildly dehydrated. Serum urea and creatinine levels on presentation were more elevated in the moderate to severely dehydrated patients and their hospital stay was relatively longer (table-2). Stool cultures, after 24 hours of incubation revealed yellow coloured sucrose fermenting colonies on TCBS agar and colourless colonies on MacConkey agar. Gram staining showed gram negative rods, which tested positive for oxidase test. The characteristic colonies were subcultured on nutrient agar for performing biochemical profile. After 24 hours incubation at 37 ^{o}C, biochemical reactions confirmed the isolates to be Vibrio cholerae. Serotyping revealed serogroup O1, subtype Ogawa. Antibiotic susceptibility showed that all the isolates were resistant to tetracyclines, nalidixic acid and cotrimoxazole but susceptible to fluoroquinolones and third generation cephalosporins. All patients were treated with oral ciprofloxacin 750 mg 12 hourly for three days. A few patients with severe vomiting were started on intravenous ciprofloxacin 400 mg 12 hourly but switched over to oral ciprofloxacin

when vomiting settled after rehydration. All the patients recovered fully with normalization of their renal functions. Length of maximum hospital stay was 7 days.

DISCUSSION

Cholera is an important public health concern and one of the main causes of morbidity and mortality in poorest areas of the world. In these circumstances, the burden of cholera is underestimated or non-estimated and many countries face recurrent epidemics[6,7]. Globally, the three most common risk factors were water source contamination (29%), rainfall, flooding (25%), and refugee settings (13%). The outbreak which we are reporting, occurred in a group of soldiers who consumed water from a single water point. When investigated later, this water was found to have a very high coliform count suggesting gross contamination of water through a crack in the water pipe in its underground course. The outbreak stopped when that water point was sealed and taken care of. No clinical case of cholera was recorded after rectification, from the same area and the surrounding military units. The isolate from stool cultures was Vibrio cholerae O1 subtype Ogawa. The strain was resistant to tetracyclines, nalidixic acid and cotrimoxazole but responsive to fluoroquinolones and third generation cephalosporins. A few years ago cases of cholera due to Vibrio cholera O1 subtype Ogawa were reported from Mirpur Khas of Sind province, which showed a mixed sensitivity pattern but all were resistant to nalidixic acid[8]. Another cholera outbreak was reported from Baluchistan in 2009, which was also water-borne, due to contamination of water supply. The causative organism in this outbreak was Vibrio cholera O1, subtype Inaba, sensitive to tetracyclines and ciprofloxacin and resistant to cotrimoxazole and nalidixic acid[9].

This outbreak was localized in a military unit so all of the patients were men. Majority of those patients who reported to the hospital were hospitalized within twenty four hours but still most of them had significant pre-renal azotemia. That is why, the cholera is included in the differential diagnosis of patients with severe watery diarrhea and vomiting, especially those with rapid and severe dehydration[10].

Table-1: Descriptive analysis of cholera cases.

	Total cases of cholera (n = 39)	Confirmed cholera (n = 22)	Suspected cholera (n = 17)	*p*-value
Age (years)	24.9 ± 6.9	24.6 ± 7.4	25.2 ± 6.3	0.73
Duration (hours)	17.3 ± 11.4	18.0 ± 12.1	16.4 ± 12.8	0.71
Fever (degree fahrenheit)	98.9 ± 1.3	98.9 ± 1.3	98.9 ± 1.2	0.93
Systolic blood pressure (mm Hg)	101.6 ± 13.3	98.8 ± 11.7	105.2 ± 14.7	0.14
Diastolic blood pressure (mm Hg)	65.8 8.8	63.4 ± 8.1	69.1 ± 9.0	0.04
Total leucocyte count (x 109/l)	9.8 ± 3.0	9.4 ± 2.9	10.2 ± 3.2	0.47
Serum Urea (mmol/l)	6.8 ± 2.7	6.3 ± 2.8	7.5 ± 2.3	0.18
Serum Creatinine (µmol/l)	131.4 ± 45.5	126.5 ± 46.9	137.7 ± 44.1	0.54
Serum Sodium (mmol/l)	137.6 ± 3.5	138.6 ± 3.0	136.2 ± 3.7	0.04
Serum Potassium (mmol/l)	3.9 ± 0.3	3.9 ± 0.4	3.9 ± 0.3	0.82
Hospital stay (days)	4 (1-7)	4 (1-7)	3 (1-4)	0.03

Table-2: Statistical difference of various variables in mild and severely dehydrated cholera cases.

	Mildly dehydrated (n=13)	Moderate to severely dehydrated (n=26)	*p*-value
Urea (mmol/l)	5.9 ± 1.8	7.3 ± 2.9	.06
Creatinine (µmol/l)	117.9 ± 46.1	138.1 ± 44.5	.27
Hospital stay (days)	3 (1-5)	4 (1-7)	.41

Patients with suspected cholera must be treated prior to laboratory confirmation, since death by dehydration can occur within hours. Despite early presentation and hospitalization, 66% of our patients were moderate to severely dehydrated and 33% showed mild dehydration despite profuse diarrhoea as they had been using some kind of oral rehydration. Pre-renal azotemia was obviously more severe in the moderate to severely dehydrated group. Azotemia almost completely resolved in most patients within 48 to 72 hours of hospitalization and institution of intravenous rehydration. We administered oral ciprofloxacin 750 mg 12 hourly for 3 days. In a few patients with severe vomiting, an initial dose of ciprofloxacin 400 mg intravenously was given. Antibiotics can lead to reductions of more than 50% in stool volume and in the duration of diarrhea, from more than 4 days to 2 days, thereby reducing the morbidity and duration of hospital stay. Antibiotics also reduce the shedding of viable V. cholerae from more than 6 days to about 1 day[11]. The outcome was good in all of our patients. There was no fatality and median hospital stay was 4 days with a range of 1-7 days. Pre-renal azotemia resolved in 48 to 72 hours of hospitalization.

Many outbreaks of cholera due to contaminated public water systems have been reported from various countries[12-14], e.g., Bhunia et al. (2009) found that the cholera outbreak in West Bengal was due to contamination of a municipal piped water supply by V. cholerae O1 Inaba[12]. Cholera is still a major public health concern in poor and developing countries of the world. It can be rapidly fatal if appropriate medical care is not provided early. A major reason for these outbreaks is contamination of drinking water source as was in our case, due to poor sanitation and improper disposal of excreta. It is recommended that people at risk should be educated to adopt appropriate hygienic practices. Water sources and sanitation should be improved. Public health measures should be expanded and appropriate vaccines may be used if a major outbreak is anticipated.

Antibiotics play a significant role in the management of severe cases of Cholera, so emerging multiple drug resistance among various strains of Vibrio cholera is a major public health concern nowadays. We isolated the strain, Vibrio cholerae O1 subtype Ogawa, which was resistant to tetracyclines, nalidixic acid and cotrimoxazole but responsive to fluoroquinolones and third generation cephalosporins. V. cholerae O1 strains associated with endemic cholera in Dhaka between 2006 and 2011 were analysed for major phenotypic and genetic characteristics. Of 54 representatives V. cholerae isolates tested, all were phenotypically El Tor and showed uniform resistance to co-trimoxazole and furazolidone. Resistance to tetracycline and erythromycin showed temporal fluctuation, varying from year to year, while all isolates were susceptible to gentamicin and ciprofloxacin[15].

In a recent large outbreak at Haiti, the strain introduced from Asia to Haiti (V. cholerae El Tor, Ogawa) showed greater virulence than the strain that caused the epidemic from Peru, causing more severe cases, offering greater resistance to antibiotics and generating a wider spread by the feces[16].

CONCLUSION

Cholera is an important public health problem in developing and poor countries. Contamination of drinking water is the major source of this infection. Multiple drug resistant strains of Vibrio cholera are causing large outbreaks which should be controlled by prevention of the disease and avoiding inappropriate use of antibiotics.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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