

Correlation Between Some Risk Factors and the Incidence of Diarrhea Outbreak in Kosar Industrial Estate, A Case-Control Study in 2013

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Background: The most important diarrheal disease of the year warm seasons is cholera with *Vibrio cholera* bacteria that some cases are observed in the country every year.

Objectives: The purpose of this study was to identify the outbreak factors of diarrheal disease and prevention ways of its mortality rate among Gonabad city workers.

Patients and Methods: This study is a case-control study conducted in 29 June 2013 to 6 August 2013, followed by diarrhea outbreak occurred in Brick kilns of Kosar industrial unit in Gonabad city. The data were collected by a questionnaire from patients and healthy people. Cholera sample and Stool exam were taken from all the patients. To determine the source of disease, water microbial sampling, chlorine metering and chlorine chlorination was conducted.

Results: A total of 120 workers were studied (60 cases and 60 controls) who were the same from age and sex point of view. Clinical signs observed in 60% of cases were abdominal cramping and 11.6% had nausea. Human test results showed 1 sample of Eltor ogawa serotype and 4 Nag strains. No significant differentiation was observed in hand washing between the patients and the healthy ones ($P = 0.15$). The risk factor of using from water storage was 3 times more in case than the control group.

Conclusions: Health disobedience and neglect of local residents were the causes of water pollution that was controlled with preventional measures, with performing daily chlorination of Brick kilns drinking water and diarrheal disease treatment of the patients.

Keywords: Disease Outbreaks; Epidemiology; Diarrhea

1. Background

V. cholerae is a Gram-negative bacillus of the Vibrionaceae family, which has features in common with the family of Enterobacteriaceae (1, 2). Filippo Pachiny, Italian scientists discovered *Vibrio* bacteria for the first time in 1854 (3). Koch isolated *Vibrio cholerae* in the 19th century for the first time, from the culture medium and reported it (2). Today, cholera is such an acute diarrheal disease that affects all the age groups and sex, and if untreated, it can cause death within a few hours. 75% of cholera patients are asymptomatic and the said microbes can be identified 7 - 14 days after entering into the intestine in stool from which many people can be infected by contact with an infected person (4).

20% of the people show symptoms such as mass watery diarrhea without straining, abdominal ache and fever, crystal clear water stool with a mild smell of fish. Children who are malnourished, HIV-positive patients and O blood group (its pathophysiology is not yet known) are more involved in the disease (4). It is estimated that each year 3-5 million people have been infected with cholera worldwide and 100000 - 120000 people have died of the

disease (4). Oral cholera vaccine is made, but 80% of people with symptoms are treated with oral saline fluids and this vaccine has not been an alternative treatment yet (4).

In Cholera outbreak in history, the human has experienced 7 pandemic from 1800. The seventh pandemic which is still ongoing began in Indonesia in 1961 and has affected 100 countries, so far (4, 5). Developed countries severely affected have reported the attack on the country's population more than 1% (4). The disease is transmitted through contaminated drinking water (water of wells and aqueducts that are not chlorinated), eating foods left over night without heating, consumption of raw sea foods such as oysters and non-washed vegetables and fruits that infect millions of people every year. The disease germs can survive in fresh water for 7 days and in sewage-contaminated water for more than 1 month and on the vegetables and fruit for 5 days but quickly disappears in acidic environments and 100C water (5).

Vibrio cholerae has two Serogroups of O1 and O139 (6). Serogroups members O1 are divided into two parts. Serotype Inaba, Ogawa. The Eltor biotype and classic was described

in the 20th century by Saw (6). Seventh pandemic *Vibrio cholerae* strain was Eltor biotype O1 that was replaced for O1 biotype strain strains in the last pandemic (6). At present *V. cholerae* serotype includes 206 serogroups (7). The strains belonging to serogroups of non-O1 and non-O139 is known as non-O1 and non-O139 that exist in abundance in the living environment water that can cause mild diarrhea, with no power of epidemic.

So, recent studies results showed that in most countries, particularly India, the incidence of watery diarrhea are associated with *Vibrio cholerae* non-O1 and non-O139 strains (8-13). In cases of diarrhea in Thailand (1993-1995) the study showed that the prevalence of non-O1 and non-O139 was comparable with O1 *Vibrio cholerae* (9). Furthermore, these strains were often belonged to serogroups O6 and O14. Acute watery diarrhea that was caused by *V. cholerae* in most countries belonged to different serogroups, Serogroups O10 and O12 in Peru (8), serogroup O10 in India (14), serogroup O37 in Sudan and Czechoslovakia (15) and O141 serogroups were identified in many parts of the world (16).

An acute watery diarrhea outbreak was also reported in 1996 with a non-O1 and non-O139 strains from Calcutta, India (17). World Health Organization (WHO) declared in 2006 that 236896 cases of cholera have occurred in 52 countries that had increased 79% compared to 2005 (18). Compared to the reported cases occurred in various countries during the period 2000 - 2004 years with 2004 - 2008 showed 24% increase in the incidence of cholera, that only in 2008, 190130 cases of acute diarrhea cases and 5143 deaths were reported from 56 countries (4).

Gonabad city with an area of 10,000 square kilometers is located in south of Khorasan Razavi province, 260 km far from Mashhad. It is located in east longitude of 46 - 57 to 27 - 59 and latitude of 30 - 34 to 34 - 54 with a hot and dry climate and prone to tropical diseases prevalence including vector borne diseases.

Brick kilns of Kosar industrial town, located 20 kilometers far from Gonabad city that non-native seasonal workers are working with their families for the first six months of the year. Family size is about 3 to 7 and each family has a room, but their bathrooms are shared. Region's population is estimated about 1,300 people. A team of medical staff was present at the site daily and patient records were completely controlled.

2. Objectives

Then, this study aimed to identify foci of disease risk, identify high-risk groups, the evaluation of health systems in response to the diarrheal diseases outbreak and strengthen of the strengths cases in Gonabad city.

3. Patients and Methods

3.1. Study Design

This study was a case-control study conducted from 29 June 2013 until 6 August 2013 in Kosar Industrial Township on patients and healthy people. From 1,300 workers at risk 60 cases had diarrhea. Control group in this study were all humans living in the ovens area who were not known as diarrheal patients in the time range according to themselves.

In this study diarrhea means that the stools be watery with or without blood more than 3 times a day. 60 workers were selected as control group randomly from the list based on the furnace number. The volunteer of health workers referred to their homes in Mend village to fill out the pre-designed questionnaires.

If a healthy person was not observed in the referred house, the house next door was selected to fill out the questionnaire for the control group. Diarrheal epidemic began on 29 June 2013 and continued for one month. 5 peaks was observed in disease process (Figure 1).

The data analysis was performed by Chi-square test and Odds Ratio using SPSS 16 software. All tests were considered significant at the 5% level or lower. To identify disease pathogens and prevent drug resistance stool test and Eltor sample on diarrheal specimens and the area water samples were performed.

3.2. Laboratory Study

3.2.1. Water Analysis

Chemical and microbiological quality control of drinking water of brick kilns piping system was performed daily. During the epidemics on average 52 water samples were taken from different parts of the furnace. Water samples were cultured in blood lactose, Bremen Green and lactose Broth. For coliform contamination of water, water chemistry parameters in health laboratory were tested.

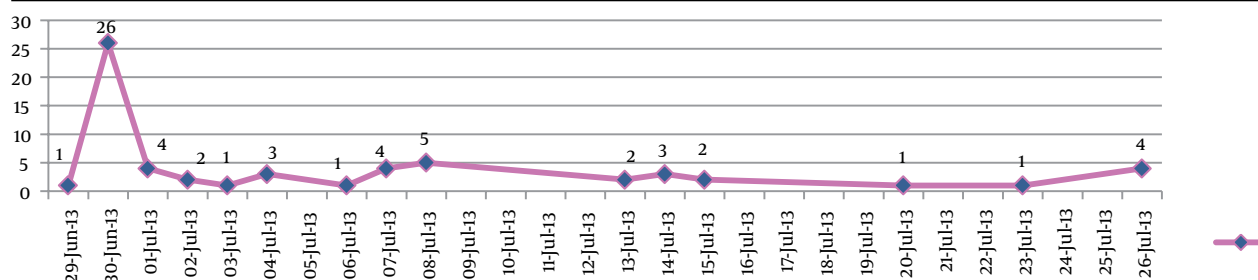


Figure 1. The Incidence of Diarrheal Cases in Brick Kilns of Gonabad City in 2013

3.2.2. Culture of Diarrhea Samples Method

Rectal swabs were transported to the laboratory (which had been placed in Cary Blair) immediately on TCBS medium first, and were cultured for 18 - 24 hours was incubated at a temperature of 35-37°C. Then, the swab was transferred in to APW and after 6-8 h incubation at 35-37°C picked up from the surface and top portion to be harvest in the second TCBS. After incubation time, if smooth and shiny yellow colonies with 2 - 4 mm in diameter were observed on TCBS, the colonies were cultured in medium KIA and it was incubated for 18 - 24 hours at temperature of 35-37°C. If alk/acid reaction were observed without gas, oxidase test was performed. If oxidase test was positive the following steps were performed: Bacteria were cultured on SIM environment and if positive, the following reactions were observed:

SH2: negative, indole: positive, Motility: positive. For performing serological tests new colonies grown in KIA or SIM medium was used. At first it was checked with polyvalent O1 antiserum and if the result was positive, it was surveyed with single valence Ogawa and Inaba antiserum. If the result was negative and in the case of suspecting an epidemic, strain of O139 with O139 single valence antiserum were tested..

4. Results

From a total a total of 120 workers were examined. Clinical signs observed in case group was lethargy 50%, headache 38.3%, abdominal cramps 60%, fever 33.3%, anorexia

26.6%, 33.3% nausea and 11.6% had vomiting. In The case group included 33 males and 27 females. also, 32 males and 28 females in the control group, and two groups were also similar in age item. The case group age range was 1 to 60 years and the control group was 2 to 55 years. The most diarrheal statues were reported between the ages of 1 and 10 years (30%). highest cases of diarrhea were observed in patients aged 1-10 years (32.1%).

From 52 water sampling, testing microbiology of 9 samples, was coliform and *E.coli*. A total of 60 human samples cultured in the laboratory, 4 non-O1 samples (Nag), and one case of Ogawa was reported. Laboratory results of 4 samples with dysentery diarrhea were *Asentobacteria baumannii* and *Serratia marcescens*. The most important risk factor was pond water for washing dishes and drinking compared to other factors, and a significant difference ($P = 0.0$) was observed between Disease and Health groups (Table 1).

Based on Odds Ratio drinking from water storage in infected cases was 33 times more than healthy ones. Risk factor of using from water storage in infecting the samples were 3 times more than the control group ($P = 0.005$). Also on hand washing between the patients and healthy volunteers, no significant differences were observed and in terms of disease among males, also no significant differences were observed ($P = 0.15$), but among females in both case and control groups a significant difference was observed ($P = 0.00$).

Temperature recorded during the course of the disease varied between 39 to 43 degrees, which somehow had influenced the disease process (Figure 2).

Table 1. Potential Risk Factors That Were Evaluated in the Outbreak Incidence in Bricks Kilns-Gonabad-2013 ^a

Variables	Disease	Health	OR (CI 95%)	P Value
Pond water			33.0 (11.7-97.7)	0.00
Use	55	15		
Unuse	5	45		
Reservoir water			3 (1.3-6.4)	0.005
Use	30	15		
Unuse	30	45		
Hand washing with soap after wc			2.5 (1.05-5.9)	0.35
Use	20	10		
Unuse	40	50		
Gender				
Male			2.8 (0.65-11.9)	0.15
Use	30	25		
Unuse	3	7		
Female			16.6 (3.8-72.0)	
Use	20	8		0.00
Unuse	7	20		

^a Abbreviation: CI, confidence interval.

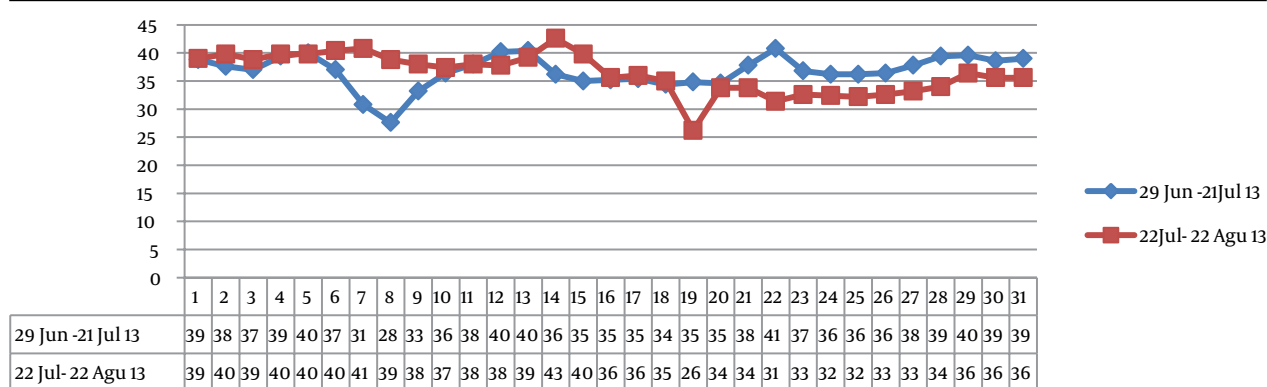


Figure 2. Gonabad Temperature Changes on 21 Jun to 22 Aug 2013

5. Discussion

Increasing of world population and also the increase of greenhouse gases resulted from industrial heating installations has decreased precipitation in industrialized countries, followed by tropical diseases, particularly diarrheal diseases rate are raising. The drought in these countries due to increased environmental temperature and lack of adequate rainfall has caused in reducing drinking-water sources and forced people to use wells water and aqueducts that are unsanitary water that in turn has increased cases of diarrheal diseases, particularly cholera. Then, Elnino phenomenon that causes a sudden warming of cold water in the Pacific that will result in increasing of phytoplankton and zooplankton populations that cholera stick to them and proliferate and will pollute the ocean's water resources around the world (5).

The third factor of rainfall in the rainy months is also a cause of filling the rivers with water and increase of phytoplankton and zooplankton (19). Then, the health care system must be alert more than any time in the summer to prevent the mortality rate of the diseases in age groups who are mainly children under 5 years. Gonabad city with an area of 10000 square kilometers located in South of Khorasan-e-Razavi due to its vast wilderness that makes up a whole part of desert is at the risk of diarrheal diseases that each year and with the beginning of summer, we are faced with cases of diarrhea. Due to this topography, the area water is saline and non-potable and necessary water is usually provided from the installed valves water in rural and urban areas. Warehouse and subterranean water resources in rural and some urban areas is of risk sources of diarrheal diseases that monitoring of chlorination on subterranean water resources and residual chlorine in the water storages and network water based on environment sanitation standard (0.5 ppm) is so important.

The survey results also reveal that with the temperature changes occurred diarrhea cases also has ascending -descending process that is in consistent with scientific findings. Temperature increase decreased workers drinking

water storage that this caused them to use polluted water ponds that was connected to the network for vegetables watering and washing dishes. Most cases of diarrhea of 3 was in children under 10 years that based on study and according to field observations, the reason could be due to the availability of water sources, such as contaminated water non-chlorination used for adobe making and continuous use of that water for drinking and bathing by children without the supervision of their parents leading to diarrhea among them. The survey of illnesses outbreak among adults in an epidemiologic questions form showed that the cause has been determined the distance between the place they live and work, and drinking of water for adobe making. Cholera patients suffering from Ogawa strains according to the literature (75% of patients are without signs of acute watery diarrhea) had no signs and or only had mild diarrhea with slight nausea that the sample was taken as outpatient in Cary Blair transport and its laboratory results was reported positive. Other pathogens include:

Acinetobacter species are aerobic gram-negative bacteria that have been spread widely in soil and water, and it is possible that be separated sometimes of the skin, mucous membranes, secretions and hospital environment. This bacterium is seen as the form of coccobacillus or Coccoci. Considering its spread this bacterium is like Neisseria. Baumannii is a species that commonly are separated.

5.1. *Serratia marcescens*

marcescens is a species of gram-negative, rod-shaped bacterium in the family of Enterobacteriaceae. A human pathogen, *S. marcescens* is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections.

Over the last 30 years, *S. marcescens* has become an important cause of nosocomial infection. There have been many reports concerning the identification, antibiotic

susceptibility, pathogenicity, epidemiological investigations and typing of this organism. Accurate identification is important in defining outbreaks. The growth of *S. marcescens* in the environment has been investigated in relation to water, disinfectants and plastics such as blood bags. Certain extracellular products are unique to *S. marcescens*. Pigment (prodigiosin) biosynthesis by *S. marcescens* has been investigated fully since the emergence of the organism as a cause of infection (20).

An outbreak of nosocomial infections caused by non-pigmented *S. marcescens* is described. There were 655 bacterial isolates from 374 patients during a 10-month period; 50.4% of isolates were from sputum, 24.5% from urine, and the remainder from wounds, blood, and miscellaneous sites. Opened bottles of aerosol inhalation therapy medications were implicated. Forty-three percent of a random sampling of opened bottles was contaminated with *S. marcescens*, and viable bacterial counts reached 107 organisms per milliliter (20).

5.2. *Vibrio cholera*

cholerae, the causative agent of severe watery diarrheal disease of cholera, comprises 206 serogroups (O1-O206) based on antigenic diversity of their outer membrane lipopolysaccharides 1, 2. Strains of the O1 serogroup are divided into two biotypes i.e. classical and El Tor, according to their phenotypic differences. Cholera is endemic in southern Asia and parts of Africa and Latin America, where seasonal outbreaks occur widely and are particularly associated with poverty and poor sanitation. Serogroups other than O1 and O139 are designated as *V. cholerae* non-O1, non-O139, or nonagglutinating vibrios (NAGs) (21).

Toxigenic and nontoxigenic NAGs have caused several diarrheal outbreaks in India and other countries, including Haiti (3-6). Non-O1, non-O139 *V. cholerae* strains is generally considered to be non-pathogenic. However, intestinal and extra-intestinal infections with non-O1, non-O139 strains have occurred both sporadically as well as in outbreaks. There are 154 known serogroups of non-O1 vibrios (21).

It seems that workers low education and their lifestyle is effective in their disease outbreak. Non-educability and also lack of attention to health advices of health team predispose them to diarrheal conditions. Water storage without cover that is sometimes connected to drinking water network and its contamination with human wastes, as well as fish production in water resources affects the residents to be infected. Other factors are such as summer winds and scattering of soils contaminated with human wastes because of wastewater that is open and in the area and workers food that is contaminated with infected soils can be effective in causing disease. Due to lack of drinking water chlorination that is stored by water tankers and alga growth in water storage floor due to lack of regular cleaning can also contribute to the incidence of diarrheal diseases.

Solving the problems it means that when health team put a cover on the water storage tanks, chlorinated the water supplies, did disinfection for bathing, distributed cholera, installed washing hands liquid soap in place, cleaning water tanks, advising to boil drinking water before using, learning how to disinfect fruits and vegetables and eventually let the workers to go on leave to return back to their mother town, diarrheal cases declined and the patients were treated.

The study findings of this research are roughly consistent with the findings of the other studies. In 2004, in Sarbaz city in Sistan and Baluchestan province diarrheal epidemic occurred and lasted for one month. 2242 cases of patients susceptible to cholera were identified from a population of 41,000 people. 90 cases of vibrio cholera O1 and 29 cases of Nags were diagnosed. The age range was from 9 months to 70 years (median 9 years) and 34 (37.6%) of total 90 cultures were less than 5 years (22). The cholera epidemic that occurred during 2002 and 2004 in India, all laboratory cultures of diarrheal patients from Eltor *Vibrio cholerae* was Ogawa strains O1 and drug resistance metering showed that the said strains were sensitive to Ciprofloxacin and Cefixime (23).

In one case-control study in china at 2012, compared the food sources and examined the food and water items consumed between the probable and confirmed cases and the asymptomatic control students. Among the 87 cases, 62 (71%) experienced vomiting and other common symptoms, including nausea (66%), diarrhea (60%), abdominal pain (46%), fever (25%), headache (5%), and dizziness (2%) but none were hospitalized. 61% (53/87) were male, and the median age was 20 years (range from 18 - 23 years) the outbreak lasted for 12 days, starting at 08:00 hour on December 11. According to the incubation period of Nov, the epidemic curve in the affected classes appeared to be that of a continuous common source pattern with seven peaks (24).

The results of this epidemic indicate that drinking water supply system for bricks kilns for drinking used by the workers is to be more modern and more hygienic and we must prevent connection of non-drinking waters to the water network of the workers. Otherwise, each year should be faced with diarrheal outbreak in our bricks kilns.

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Authors' Contributions

Study concept and design: Mr. Hamed Ramazani, Analysis and interpretation: Dr. Reza Ahmadi and Ali Reza Atarodi.

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