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**RESEARCH ARTICLE**
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# CONCURRENT OUTBREAKS OF AEROMONAS SPECIES AND CHOLERA IN PERI-URBAN AREAS OF ISLAMABAD: A CASE CONTROL STUDY FROM MAY TO JULY 2022

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**Abstract**

**Background:** Cholera outbreaks had re-emerged with unusual trend in 2022 in most of the African and Asian countries.

**Methods:** A case control study was conducted from May to July 2022 in peri-urban population of Islamabad, Pakistan by using purposive sampling technique. Case was defined as a resident belonging to any age group with 03 or more episodes of watery, non-bloody diarrhea (rice water stools) with or without severe dehydration or any person irrespective of symptoms with history of contact/dining with a suspected case or with laboratory confirmation of Cholera/Aeromonas while control was a resident belonging to any age group with no diarrheal illness. A standard questionnaire regarding acute watery diarrhea, designed according to the protocols of U.S. Centre for Disease Control, was used for this study. Data regarding sociodemographic details, clinical profile and relevant risk factors were collected, frequencies and percentages were computed, and inferential statistical analysis was done using Epi info version 7.2.

**Results:** Total 07 cases were identified through active case finding; afterwards, 35 controls were enrolled. Median age of cases was 07 years (IQR=13) with male to female ratio 2:1. Overall attack rate (AR) was 2%, most affected age-group was <05 years (AR=11%). Out of 42 stools samples 17% (n=07) were positive for *Vibrio cholerae*, while 33% (n=14) of 42 total water samples were positive for Aeronomas sp. and 5% (n=2) for Cholera sp. Contact with case (OR: 10.3, 95%CI: 1.7-64), and attending social gathering (OR: 8.3, 95%CI: 1.4-47) remained signification risk factors.

**Conclusion:** Contact transmission was the main driver for the current Cholera outbreak. Clean water, sanitation and hygiene practices are recommended for the affected community.

**Keywords**: Aeromonas, Outbreak, Case-control Study, Cholera, Pakistan

**Introduction:**

Food and water borne diseases are a global health concern in both developed and developing countries making millions of people ill annually.[1,2]Although they do not have a lasting impact but in some cases chronic and lifelong health problems can occur. Quality of work and health economics at individual and organizational level can be affected resulting in financial losses.[3]In order to ensure food safety, rapid detection of these pathogenic organisms is of crucial importance.[4]There are various causes of infectious gastrointestinal diseases ranging from very small viruses, bacteria and/or their toxins to large and multicellular protozoans like *Amoeba hystollitica* and *Guardia lamblia*[a] while non-infectious diarrheal illnesses are mostly caused by certain food allergens and drugs.[b]

Bacterial diarrheal outbreaks are usually caused by *Vibrio cholerae* which can transmit from one person to other through feco-oral route by consuming water or food contaminated with the microorganism. Most of the patients after contracting cholera infection usually are asymptomatic but about 20-30% of patients develop symptoms as more than three episodes of watery stools in last 24 hours accompanied by fever and abdominal pain. Patient may present with severe dehydration and sunken eyes, anuria and shock which may result in death within hours if not promptly and properly.[c] The disease trend is highly associated with poor socio-economic status and poor water and sanitation practices. With the unique characteristics of *Vibrio cholerae* to rapidly divide and potential for genetic manipulations,[d] Cholera outbreaks have emerged with unusual trend in 2022 from most of the African and Asian countries. Like many other African countries, Cholera is endemic in Pakistan with outbreak threshold of one confirmed case. More than 500 confirmed cholera cases have been reported from all over Pakistan till so far with loss of 15 human lives as per National Institutes of Health, Islamabad, Pakistan.

One of the emerging bacterial pathogen associated with FBDs is *Aeromonas hydrophilia* although its role as a causative agent of human disease is controversial, some estimated that they may cause 13 % of gastroenteritis cases in United States.[5] Aeromonas species are commonly found in ground water; drinking water at treatment plants, lakes, rivers, fresh produce, meat, and dairy products. Furthermore, they can also be found in soil,[6] birds,[7] fish, and cold-blooded marine and freshwater reptiles.[e] The risk of infection from oral ingestion is 7.3/billion for low exposure while infectious dose for humans and animals is greater than 10 $^{10}$ microorganisms. Infection is spread via fecal-oral transmission during direct ingestion of contaminated water or foods and contact with infected wounds.[8] The reported incubation period for Aeromonas-associated diarrhea is 1 to 2 days.[9] The length of time from entry in body to bacteremia ranges from 1 to 38 days.[10]

A greater risk of infection is reported in young children, elderly and immunocompromised patients.[11] In the last decades, Aeromonas has been increasingly recognized as the etiological agent of gastrointestinal illness[12,13,14,15] in humans due to virulence through extracellular toxins (heat labile enterotoxins associated with hemolysin and cytotoxin production,[16] protease, phospholipase, hydrolytic enzymes), structural features (pilli, S-layer, lipopolysaccharide), adhesion and

[a] Hoefer HL, Fox JG, Bell JA. Gastrointestinal diseases. Ferrets, Rabbits, and Rodents. 2012:27

[b] Chassany O, Michaux A, Bergmann JF. Drug-induced diarrhoea. Drug Safety. 2000 Jan; 22(1):53-72

[c] https://www.cdc.gov/cholera/index.html

[d] Hun Yoon S, Waters CM. *Vibrio cholerae*. Trends in microbiology. 2019 Sep 1;27(9):806-7

[e] Fulton, M. The bacterium *Aeromonas hydrophila* from lizards of the genus Anolis in Puerto Rico. Louisiana State University Medical Center.

invasion.[17,18,19] A. hydrophila are Gram-negative, rod-shaped facultative an5yaerobes[f] ranging in size from 0.3-1.0 µm wide by 1.0-3.5 µm long and motile by a single polar flagellum.[g]

Other than gastrointestinal symptoms, non-gastrointestinal complications that may arise subsequent to A. hydrophila infection include hemolytic syndrome, kidney disease, cellulitis, wound and soft-tissue infection, meningitis, bacteremia, septicemia,[20,21] ocular infections, pneumonia,[22]urinary tract infection in neonates,[23] osteomyelitis, peritonitis and acute cholecystitis. Severe infection can result from non-resolved intermittent diarrhea, which can occur months after the initial infection.[h]

On 17th June 2022, a list of 06 confirmed cholera cases of Islamabad was shared by Public Health Lab Division NIH, for investigation of subsequent cases. All the cases were contacted and requested for visiting their houses in order to investigate the source of transmission of the disease, contain the outbreak and prevent recurrence in the future.

**Materials and Methods:**

**Study design:** This is a case control study conducted from May 2022 to July 2022.
**Study population:** Peri-urban population of Islamabad was under investigation.
**Study duration:** This study was conducted from 1st May 2022 to 31st July 2022.
**Study setting:** This study was conducted in the peri-urban areas (in and around Lehtrar Road) of Islamabad.
S**ampling technique:** The participants were recruited using purposive sampling technique.
**Study tool:** A standard questionnaire regarding acute watery diarrhea was designed according to the protocols of U.S. Centre for Disease Control.

**Case definitions:**
**Suspected case:** A resident of peri-urban area of Islamabad belonging to any age group with 03 or more episodes of watery, non-bloody diarrhea (rice water stools) with or without severe dehydration.

**Probable case:** A resident of peri-urban area of Islamabad belonging to any age group irrespective of symptoms with history of having contact/dining with a suspected case.

**Confirmed case:** A resident of peri-urban area of Islamabad belonging to any age group with laboratory confirmation of Cholera/Aeromonas.

**Control:** A resident of peri-urban area of Islamabad belonging to any age group with non-diseased outcome.

**Data Collection and analysis:** Data regarding sociodemographic details, clinical profile and relevant risk factors were collected and compiled in Microsoft Excel. Frequencies and percentages were computed and inferential statistical analysis was done in Epi Info version 7.2.

[f] Collier, L., Balows, A., & Sussman, M. (Eds.). (1998). Microbiology and Microbial Infection :Systematic Bacteriology (9th ed.). USA:
[g] Horneman, A. J., Ali, A., & Abbott, S. L. (2007). Aeromonas. In P. R. Murray, E. J. Baron, M. L. Landry, J. H. Jorgensen & M. A. Pfaller (Eds.), Manual of Clinical Microbiology (9th ed., pp. 715-722). Washington, D C: ASM Press.
[h] Collier, L., Balows, A., & Sussman, M. (Eds.). (1998). Microbiology and Microbial Infections: Systematic Bacteriology (9th ed.). USA:

**Results:**

A total of 42 participants, with 5 controls against each case, were recruited; and male to female ratio was 2:1 and median age was 07 years with Inter Quartile Range (IQR) 12.8 years. The most specific age attack rate was observed in age group from 0 to 4 years 52% (n= 22) followed by 5 to 8 years 21% (n= 9), 9 to 12 years 19% (n= 8) and 13 to 16 years 07% (n= 3). Mostly parents/guardians of the participants were uneducated 45% (n= 19), followed by primary 24% (n=10), Intermediate 12% (n=5), graduation 12% (n=5) and Matric 7% (n=3) education. From a total of 42 participants, 86% (n= 36) had one or more symptomatic persons at home. The most common symptom observed was more than 3 non-bloody watery stools in last 24-hrs 64 % (n=27) followed by abdominal pain 38% (n=16), nausea 38% (n=16), vomiting 36% (n=15), fever 24% (n=10), muscle cramps 14% (n=6), shock 07% (n=3) and others 5% (n=2). Among all recruited cases of acute watery diarrhea *Vibrio cholera* was confirmed in 17% (n=07), and Aeromonas species in 7% (n=03). Out of 42 participants 5% had history of hospitalization (n=2). From a total of 42 participants, 14% (n=6) used unwashed fruits/improperly cooked meat or vegetables, 41% (n=17) used unpasteurized milk, 12% (n=5-) attended social gathering, 19% (n=8) visited or dined with person with AWD, 57% (n=24) had shared toilet, 40% (n=17) used municipal bore water for drinking, 36% (n=15) used community filter water for drinking, 24% (n=10) used private bore water for drinking, and 69% (n=29) used municipal bore water for other purposes , 26% (n=11) used private bore water for other purposes. 5% (n=2) used community filter water for other purposes.

*Vibrio cholerae* was detected in 07 out of 42 stool samples (17% (n=07), and Aeromonas species in 7% (n=03). Among 42 Environmental samples for drinking water 33% (n=14) turned out be Aeronomas positive and 5 %( n=2) cholera positive.

The following risk factors were found to be associated with cholera: consumption of raw food/unwashed uncooked vegetables and fruits (OR, 3.10; 95% confidence interval [CI]: 0.44-21.62, P-value= 0.23), consumption of unpasteurized/unboiled milk (OR, 4.79; 95% CI: 0.80-28.4, P-value=0.06, history of visiting/dining-out with the infected persons in the previous 5-days (OR,10.33; 95% confidence interval [CI]: 1.66-64.00, P-Value=0.004), history of attending any social gathering in the previous 5-days (OR,8.00; 95% confidence interval [CI]: 1.36-47.02, P-Value=0.01), Shared Toiletries (OR, undefined ; 95% confidence interval [CI] undefined, P-Value=0.01), history regarding water use for drinking purposes ( Chi Square=2.82, df=2, Probability=0.24, Fischer's Exact =0.2543), and water used for other HH purposes (Chi Square=3.7655, df=2, Probability=0.1522, Fischer's Exact =0.22).

**Conclusion:**
Contact transmission was the main driver for the current Cholera outbreak. Clean water, Sanitation and Hygiene practices are recommended for the community.

***Keywords:*** Aeromonas, Outbreak, Case-control Study, Cholera, Pakistan

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