

ORIGINAL ARTICLE

Cholera: An Outbreak Causing Diarrhoea; A Serious Threat to PopulationROMAH ISHFAQ¹, S. M. ABBAS NAQVI², MEHVISH JAVEED³, ATIF ZULFIQAR⁴, HUBZA RUATT KHAN⁵, AHSANULLAH M MIRBAHAR⁶¹Women Medical officer of Pathology Dept. Nishtar Medical University and Hospital Multan²Professor of Pathology Dept. Nishtar Medical University and Hospital Multan³Microbiologist of Pathology Dept. Nishtar Medical University and Hospital, Multan.⁴Demonstrator of Oral and Maxillofacial Surgery Dept. Nishtar Institute of Dentistry, Multan⁵Research Scholar of Microbiology and Molecular Genetics Dept. The Women University, Multan⁶Research Officer, Health Research Institute/National Institute of Health Dept. Nishtar Medical University and Hospital, Multan.Corresponding author: Romah Ishfaq, Email: dr_romahishfaq@yahoo.com, Cell: 0333 6138231**ABSTRACT****Objective:** To evaluate the association of *Vibrio cholera* infection with age and gender. To develop the antibiotic sensitivity pattern for *Vibrio cholera* against commercially available preparations in market.**Study Design:** Descriptive cross-sectional**Place and duration:** Study was conducted at department of Pathology, Nishtar Medical University and Hospital Multan, in the months from April to September, 2022.**Methodology:** Total 191 Stool samples were sent to Microbiology laboratory, Pathology department of hospital which was included in this study. These samples were from Nishtar Hospital various wards and from CEO office. After successful isolation of bacteria the antibiotic sensitivity were tested by Kirby-Baur Disc Diffusion Method.**Results:** During the outbreak of *Vibrio cholera* in our area, 10.99% cases were detected in whole diseased population. Mean age of patients who developed *Vibrio cholera* was 7.68 + 0.66 years. Gender showed no discrimination as both males and females were almost equally affected. Age and gender were not found statistically significant ($p=0.740$ and $p=0.329$). The sensitivity was measured for three antibiotic drugs (Ampicillin, Erythromycin and Tetracycline). Among them only Ampicillin showed 50% sensitivity while other two antibiotics were 100% sensitive.**Conclusion:** Unhygienic environment especially in pediatrics group has showed to be the main risk factor in the outbreaks of cholera. Proper disposal of waste, especially from infected persons, to keep it segregated from drinking water along with awareness programs are the need of hour to prevent further outbreaks.**Keywords:** Kirby-Baur Disc Diffusion Method, *Vibrio cholera*, Outbreak, Pediatrics, Ampicillin, Erythromycin and Tetracycline**INTRODUCTION**

Cholera, a disease of acute watery diarrhoea has been defined by World Health Organization as increased frequency of loose milky white watery stools due to *Vibrio cholera* bacterial infection through contamination of food and water¹. The symptoms commonly develop are vomiting and diarrhoea. Diarrhoea of this infection has special characteristic presentation of "rice water stools". Appearance of stool is milky white, looks as if rice has been rinsed in water². The frequency of stools is much high which leads to severe dehydration in very short time. Dehydration brings more and more electrolyte imbalance which mostly leads to shock, a critical emergency situation³. Many other gastrointestinal infections causing diarrhoea are also problematic but their effects are confined to host in which they caused disease. The main problem of cholera diarrhoea is its transmissibility to other human and epidemic outbreaks⁴.

According to estimation almost 3-5 million people suffer from cholera disease worldwide, annually⁵. Among these patients the estimated deaths are almost 100,000 in endemic countries⁶. In this high death percentage children <5 years age take half of the place⁷. Major pathogen involved in this disease is gram negative curved bacterium i.e. *Vibrio cholera*. It has many serotypes but the most important and notorious for causing epidemic are 2 types, O1 and O139⁸. O1 is further categorized as E1 tor and Classical. Other serotypes O2-O138 does not cause epidemics. Epidemic behaviour of a disease requires multiple modes of transmission. Among these modes *Vibrio cholera* mostly uses the feco-oral route. Food and water are main reservoirs of this bacterium for contamination⁹. In addition to this mode, the infected individuals also shed this bacterium in their stools for first 7-14 days after infection exposure¹⁰. For this reason, improper hand washing is the most dominant reason behind its outbursts.

Though, cholera is a preventable and treatable disease still its pandemics in history were always considered as disasters. During 19th and 20th century, the continent Asia faced cholera pandemic almost seven times. It was first started in 1817 and after that substantial pandemics occurred in 1829, 1852, 1863, 1881, 1889 and 1961. First six pandemics were caused by O1 serotype of cholera in which biotype was "Classical". In 7th pandemic the

dominant biotype was "E1 tor" of serotype O1 *Vibrio cholera*. During these seven waves of pandemic in 19th and 20th century the major interlinking hub for spread was Bay of Bengal. Most recent in 2017, 1.2 million cholera cases worldwide were reported by WHO. Out of these, 84% cholera positive cases were from Yemen¹¹. Like other countries Pakistan has also faced problem of Cholera outbreaks many times. An outbreak in Central Jail Lahore was reported in 1881, in which 90 persons died out of 127 positive reported cases¹² (Dickson WP., 1882). Similarly, in 1937 cholera epidemic hit the Swat state of Pakistan. During that epidemic 202 cases were reported positive out of which 100 patients could not survive. In July 2002 and June 2003 an outbreak was reported in a village outside the Karachi. According to the report 69 cases were positive in that village at the time of surveillance¹³ (Khan HA, Masood W et al., 2022). In recent times, Karachi has again faced a surge of cholera outbreak in 2022. Sindh health department has set a surveillance program to control and prevent the further spread of Cholera. Cholera pandemic has been declared by health authorities after analysing the positive cases that were 5 in January 2022, 14 in February 2022, 54 in March 2022 and 56 in April 2022 (World Health Organization¹⁴. Weekly Bulletin on Outbreaks and other Emergencies, 2022)

Risk factors which are responsible for exaggerated transmission of cholera infection include multiple elements. Among these causes, poverty and poor sanitary conditions are a major problem. A densely crowded area where chances of water and food contamination are high in warm humid places is most significant cause of its spread and outbreak¹⁵ (Deen J et al., 2020). The people having high quality of life and educated environment can also get the outbreaks. Among these people, the persons on Ant-acids or PPIs are at risk to develop the disease. Reason behind is that *Vibrio cholera* cannot survive in acidic medium. Stomach act as direct defence against this bacterium. But use of PPIs or presence of *Helicobacter pylori* infection can lead to less acidity in stomach which serves as medium for growth of *Vibrio cholera*¹⁶ (Bavishi C, Dupont HL., 2011)

METHODOLOGY

The study was carried out in Microbiology section of Nishtar Medical University Hospital, Multan. In this study samples of watery stools from CEO Office and Nishtar hospital wards were received. Stool samples were collected in a special container and were transported to Microbiology laboratory with completely filled Performa's (containing complete information). The age limit was categorized in 2 i.e. <12 years (Paediatrics) and >12 years (Adults). In case of delay of sample transport for more than 8 hours, it was requested to send sample in Cary Blair medium. If less than 8 hours then sample should be refrigerated. As soon as the samples were received, they were incubated in Alkaline Peptone Water (APW) medium for 4-6 hours at 37°C. After that, the sterilized wire loop is dipped and touched at superficial layer of APW tube. The dipped wire loop was then used for streaking on TCBS agar (selective agar for growth of *Vibrio cholera* species) and Blood agar (growth sample used for biochemical tests). Agar plates were incubated at 37°C for 24 hours. Next day growth was observed. Blood agar showed greenish colonies showing clear areas around heavy growth. Selective media does not allow growth of any other bacteria within 24 hours. The characteristics of *Vibrio cholera* on TCBS include yellowish, lactose fermenter, flattened dull colonies. *Vibrio cholera* para-hemolyticus produce dull, flat, green, non-lactose fermenter colonies. After isolation of *Vibrio cholera* species successfully, gram staining and biochemical identification was done by some tests. On gram staining, small gram negative curved rods were identified. Tests performed, included Oxidase (+), Catalase (+), Indole (+), Citrate (+), String test (+), Urease (-), TSI (A/A, - gas, - H₂S). After confirmation of *Vibrio cholera* from multiple tests, antibiotic sensitivity was checked. For that purpose Kirby-Baur disc diffusion method was applied.

RESULTS

191 suspected patients' loose stool samples were received in a period of 6 months. Out of them 21 were confirmed as *Vibrio cholera*. According to the results 10.99% of positive cases were found. Among these samples, 10 were from males and 11 from females. It was observed that gender was not statistically significant (p=0.329). All the samples were from paediatric age group except 2 samples (one aged 16 years and second aged 93 years). Mean age of patients who developed *Vibrio cholera* was 7.68 + 0.66 years with 95% confidence level. Median age calculated was 8 years. Standard deviation was 2.88 and statistically not significant (p=0.740).

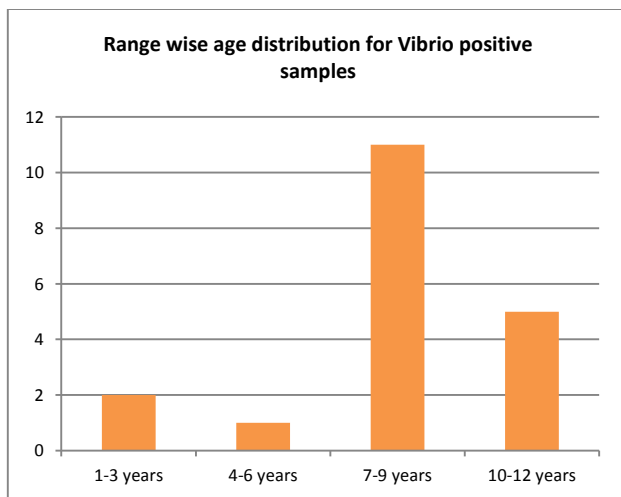


Figure 1: Shows older age maximum involvement in *Vibrio cholera* infection

The other variable of "Gender" was expected to be influential in *Vibrio cholera* research as mentioned in some publications. But this study results showed 11 females and 10 males. These results

confirm that there is no special impact of gender in this *Vibrio cholera* related infection.

Other important perspective which was observed during the study was educational status of head of families. Almost all the patients received were from low educational background. Some were labourers showing uneducated background. Few gave the occupational history as drivers.

In Disc Diffusion Method, antibiotics used for *Vibrio cholera* were Ampicillin, Erythromycin and Tetracycline. Ampicillin showed 50% sensitivity to the strain of *Vibrio cholera* existing in our society. Erythromycin and Tetracycline are 100 % in-vitro sensitive to *Vibrio cholera* infection in our region.

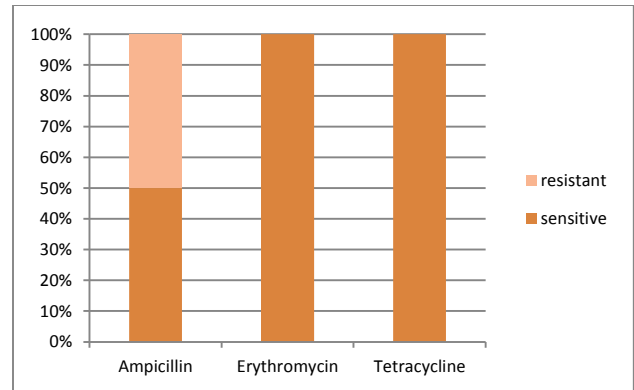


Figure 2: shows Sensitivity and Resistance percentage of Antibiotics against *Vibrio cholera*

DISCUSSION

In our study, the incidence of cholera in district of Multan has been observed with a value of 10.99%. 191 samples were considered from which 21 (10.99%) were confirmed *Vibrio cholera*. In 2021 a study was conducted in Baluchistan in which 444 samples were taken. Out of such huge sample size, 33 samples i.e. 7.43% were positive. In that study males were slightly affected more than females. 3.37% females and 4.07% males were infected with *Vibrio cholera*. Study conducted for *Vibrio cholera* in Baluchistan showed involvement of 1.12 % higher class, 2.25% middle class and 4.05% lower class¹⁷ (Hussain, A et al., 2021). Our study revealed the association of Cholera only among lower class families.

In an article the age relation to cholera was explained as children under age 5 were 2.5 times more prone to get *Vibrio cholera* infection because of immature immunity and less hygienic behavior¹⁸ (Chac D, et al., 2021). Similarly, in our study the age which was affected more ranged in 7-12 years. Only 2 adult samples were found to be positive in suspected 191 samples. Under 5 years children were also affected by *Vibrio cholera*. This age limit of paediatrics shows dependent hygienic self-care practices. In age of 7-12 years a child usually tries to get independent in his daily cleaning habits and chance of being exposed to infections are high. Hand washing is a major tool to combat the infectious and water borne diseases¹⁹ (Pal M, et al., 2018).

CONCLUSION

It is now the need of hour to improve the awareness among the patients of cholera and general population for safe necessary treatment and protective measures to control the spread. Social media campaigns should be programmed for encouraging local population towards better hygiene practices. This will help in policy making of empirical therapy to avoid unnecessary use of antibiotics and decrease the financial burden on patients.

REFERENCES

- 1 Taylor, D.L., Kahawita, T.M., Cairncross, S. and Ensink, J.H., 2015. The impact of water, sanitation and hygiene interventions to control cholera: a systematic review. *PLoS one*, 10(8), p.e0135676.
- 2 Drancourt, M., 2010. Acute diarrhea. *Infectious Diseases*, p.381
- 3 Cholera - symptoms and causes - Mayo clinic. <https://www.mayoclinic.org/diseases-conditions/cholera/symptoms-causes/syc-20355287>. (Accessed 6 May 2022).
- 4 Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor MK, Qadri F, Martinez-Urtaza J. *Vibrio cholera* spp. infections. *Nature Reviews Disease Primers*. 2018 Dec; 4(1):1-9.
- 5 Zuckerman, J. N., Rombo, L. & Fisch, A. The true burden and risk of cholera: implications for prevention and control. *Lancet Infect. Dis.* 7, 521–530 (2017).
- 6 World Health Organization. Cholera. WHO <http://www.who.int/mediacentre/factsheets/fs107/en/> (2017).
- 7 Ali, M. et al. Le fardeau mondial du choléra. *Bull. World Health Organ.* 90, 209–218 (2012). 14.
- 8 E.J. Nelson, J.B. Harris, J.G. Morris, S.B. Calderwood, A. Camilli, Cholera transmission: the host, pathogen and bacteriophage dynamic, *Nat Rev Microbiol* 7 (10) (2009) 693–702.
- 9 Zereen F, Akter S, Sobur MA, Hossain MT, Rahman MT. Molecular detection of *Vibrio cholera cholerae* from human stool collected from SK Hospital, Mymensingh, and their antibiogram. *Journal of Advanced Veterinary and Animal Research*. 2019 Dec;6(4):451.
- 10 Adagbada AO, Adesida SA, Nwaokorie FO, Niemogha MT, Coker AO. Cholera epidemiology in Nigeria: an overview. *Pan African Medical Journal*. 2012; 12(1).
- 11 Deen J, Mengel MA, Clemens JD. Epidemiology of cholera. *Vaccine*. 2020 Feb 29; 38:A31-40.
- 12 Bavishi C, Dupont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Alimentary pharmacology & therapeutics*. 2011 Dec; 34(11-12):1269-81.
- 13 Chac D, Dunmire CN, Singh J, Weil AA. Update on Environmental and Host Factors Impacting the Risk of *Vibrio cholera cholerae* Infection. *ACS Infectious Diseases*. 2021 Apr 12; 7(5):1010-9.
- 14 Cholera - symptoms and causes - Mayo clinic. <https://www.mayoclinic.org/diseases-conditions/cholera/symptoms-causes/syc-20355287>. (Accessed 6 May 2022).
- 15 Dickson WP. Report on an Outbreak of Cholera in the Lahore Central Jail in August 1881. *The Indian Medical Gazette*. 1882 Aug 8; 17(8):204.
- 16 Drancourt, M., 2010. Acute diarrhea. *Infectious Diseases*, p.381. <https://doi.org/10.1038/NRMICRO2204>.
- 17 Harris JB, LaRocque RC, Qadri F, Ryan ET, Calderwood SB. Cholera. *Lancet* 2012; 379(9835):2466–76
- 18 Hussain, A., Taj, M.K., Khosa, M.S., Baloch, B., Tahir, M. and Sadiq, A., 2021. Epidemic and PCR-based identification of *Vibrio cholera cholera* through *OmpW* gene from diarrheal patients admitted at different hospitals of Baluchistan. *Journal of the Pakistan Medical Association*, 71(4), pp.1189-1192.
- 19 Khan HA, Masood W, Siddiqui A, Ahmad S, Salman Y, Yasir M. The Cholera outbreak in Karachi, Pakistan: Challenges, efforts and recommendations. *Annals of Medicine and Surgery*. 2022 May 31:103873.
- 20 Pal M, Ayele Y, Hadush M, Panigrahi S, Jadhav VJ. Public health hazards due to unsafe drinking water. *Air Water Borne Dis*. 2018;7(1000138):2.
- 21 Pal P. Role of cholera toxin in *Vibrio cholera cholerae* infection in humans-A Review. *International Letters of Natural Sciences*. 2014;17.
- 22 Taylor, D.L., Kahawita, T.M., Cairncross, S. and Ensink, J.H., 2015. The impact of water, sanitation and hygiene interventions to control cholera: a systematic review. *PLoS one*, 10(8), p.e0135676.
- 23 World Health Organization. Weekly Bulletin on Outbreaks and other Emergencies: Week 17: 18-24 April 2022.