

# Survey of Cholera Outbreak and its Resistant Pattern in Baluchistan, Pakistan

About the Author(s)

Ashiq Hussain<sup>1\*</sup>, Yasmeen Lashari<sup>1</sup>, Muhammad Mazhar<sup>2</sup>

<sup>1</sup>Associate Professor, Department of Pathology, Bolan Medical College Quetta

<sup>2</sup>Assistant Professor, Department of Pathology, Bolan Medical College Quetta

<sup>3</sup>Assistant Professor, Department of Pathology, Loralai Medical College Loralai

\*Correspondence: dr.shah73@gmail.com  
Bolan Medical College Quetta

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## Abstract

**Objective:** The present study was conducted to identify the isolates of *V. cholerae* as causative agent for the current outbreak in Baluchistan and evaluate the antibiotic sensitivity profile of the isolates

**Methodology;** This cross-sectional study was conducted at Bolan Medical Complex Hospital Quetta from March to October 2022. Suspected stool samples were received with a history of untreated severe diarrhea within 12-hour duration from different affected districts of Baluchistan Province, Suspected colonies were subjected to gram staining, biochemical analysis and antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method.

**Results;** Total 483 diarrheal samples were collected out of which 43 (8.90 %) were positive and 440 (91.09%) were negative. The all isolates 100 % were resistant to common antibiotics were whereas Ciprofloxacin 100%, Levofloxacin 100% and Azithromycin 100% were sensitive to all isolates.

**Conclusion:** Culture methods and biochemical identification of *V. cholerae* from suspected cholera may be helpful. There needs to be systematic surveillance of commonly used antibiotics and encourage the design and implementation of the cholera control policy advised by the WHO

**Key Words:** Cholera, Outbreak, *Vibrio cholerae*, Baluchistan.

**Conflict of Interest:** None

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## Introduction

Cholera is a serious life-threatening infection that has very high morbidity and mortality.<sup>1</sup> Cholera is an acute disease of the intestine spread by contaminated water and food with the Gram-negative facultative anaerobic rod-shaped *V. cholerae* bacterium. This illness is characterized by severe diarrhea like rice water stool with rapid loss of fluid and salts.<sup>2</sup> *V. cholerae* O1 and O139 serogroups are the causative agents of the secretory diarrheal disease. On the basis of O antigen of cell wall of lipopolysaccharide, certain subgroups of *V. cholerae* O1 are further distinguished into two biotypes, classical and El Tor on the basis of phenotypic traits.<sup>3</sup>

Cholera outbreaks were categorized in endemic or epidemic by occurrence.<sup>4</sup> In cholera endemic population, out breaks usually repeated seasonally where children below five years of age are more vulnerable due to the lack of sufficient pre-existing natural anti-cholera immunity.<sup>5</sup> *V. cholerae* has proven to be transmitted via fecal-oral route, commonly observed around the world,

the infection is now specifically confined to developing countries.<sup>6</sup> The leading factor in the occurrence of cholera is the deficiency of clean drinking water and the absence of suitable hygienic facilities. Compromised sanitary conditions like over population, townships with inadequate health facilities, momentary refugee camps or internally displaced people, and humanitarian or environmental predicaments with disturbed settlements are also said to be major risk factors.<sup>7</sup>

The cholera disease remains endemic in around ninety-six countries, causing about 2.86 million cases and 95,000 deaths a year.<sup>8</sup> Pakistan has a high prevalence of cholera, with Baluchistan appearing to be a key area of the disease spread. Over 13000 suspected cases of cholera have been recorded in the province of Baluchistan. This is an unusual spike in the disease, according to the latest reported, there might be ten deaths in the affected districts of Khuzdar and Zhob, making a serious risk to public health.<sup>9</sup> In Baluchistan province cholera is specially found constantly which add up a

large number of deaths every year. Episodic diarrhea repeatedly happened by drinking contaminated water resulting in malnutrition causing an increase in the susceptibility to disease.<sup>10</sup>

The leading causes of death from severe diarrhea are dehydration, electrolyte disorders and related complications.<sup>11</sup> Cholera is treated with rehydration and antibiotics. Antibiotics are used as a supplement in rehydration therapy because they help reduce the duration of the disease and vibrio excretion, as well as help in prophylaxis to prevent epidemics.<sup>12</sup>

## Methodology

This study was conducted for characterization of *V. cholerae* in department of Pathology (Microbiology) Bolan Medical Complex Hospital Quetta from March 2022 to October 2022.

All ages and both genders were included in this study. Total 483 fecal / rectal swab samples were collected from different affected districts (Zhub, Dera Bugti, Khuzdar, Loralai, Lasbela and Nasirabad) of Baluchistan where cholera outbreak reported. Suspected stool samples collected with a history of untreated severe (rice water) diarrhea of less than 12 hour's duration and with evidence of significant dehydration. Patients who had taken antibiotics were excluded from the study. Cary-Blair (Oxoid, Hampshire —U K) transport medium was used for collection of stool samples and immediately transported to Microbiology laboratory for further analysis.

The suspected stool samples were cultured primarily in alkaline peptone water with a pH of 8.0 and 2% NaCl, (Oxoid, UK) followed by incubation at 37 °C for six hours for the detection of *V. cholerae* from suspected diarrheal samples. Later the culture was streaked on thiosulfate citrate bile salts sucrose agar (TCBS). Incubated the plate for 24 hour at 37°C. Growth of *V. cholerae* colonies were gram stained and the characteristic observation of bacterial isolates were based on standard methods. Selected colonies were further cultured on medium such as nutrient agar for its non-selective characteristics at 37°C for 24 hours, biochemical tests such as oxidase, catalase, indol, Simmon citrate utilization tests and motility tests were performed. The antimicrobial susceptibility of *V. cholerae* isolates to different antibiotics was performed on Mueller Hinton agar (MHA) by Kirby-Bauer agar diffusion method.<sup>13</sup> The antibiotics panel testing includes Ampicillin (10µg), Nalaxic acid (30µg), Sulfonamides-Trimethoprim (251.25+23.75µg), Tetracycline (30µg), Doxycycline (30µg), Ciprofloxacin (5µg), Ceftriaxone

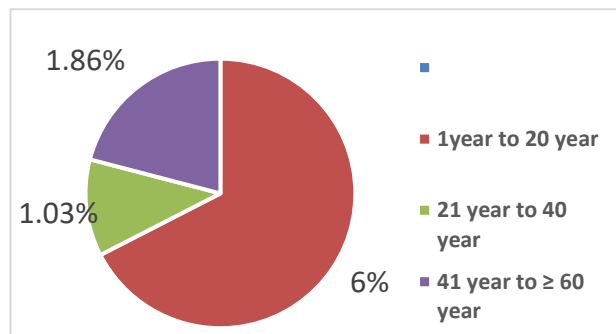
(30µg), Amikacin (30µg), Levofloxacin (5µg) and Azithromycin (15µg) according to guidelines of the clinical laboratory standards.<sup>14</sup>

A SPSS version 22 was used to analyze the data. The P value was calculated through *t*-test and 0.05 or lower was considered significant. The frequency of the antibiotic susceptibility test was also analyzed by using SPSS. Ethical approval for the research was obtained from the ethics review committee of Bolan Medical Complex Hospital Quetta (No. Supdt: /BMCH/465/49/2022) and was carried out in accordance with the Helsinki Declaration

## Results

All 483 suspected diarrheal samples, out of which 8.90% were positive during cholera outbreak. *V. cholerae* O1 serotype Ogawa as responsible for current outbreak which affected six districts Zhob, Dera bugti, Khuzdar, Loralai, Lasbela and Nasirabad of Balochistan province as shown in Table- I.

Affected districts	Percent Positive	P. value
Zhub	2.07%	< 0.001
Dera Bugti	1.86%	
Khuzdar	1.65%	
Loralai	1.44%	
Lasbela	1.0%	
Nasirabad	0.82%	



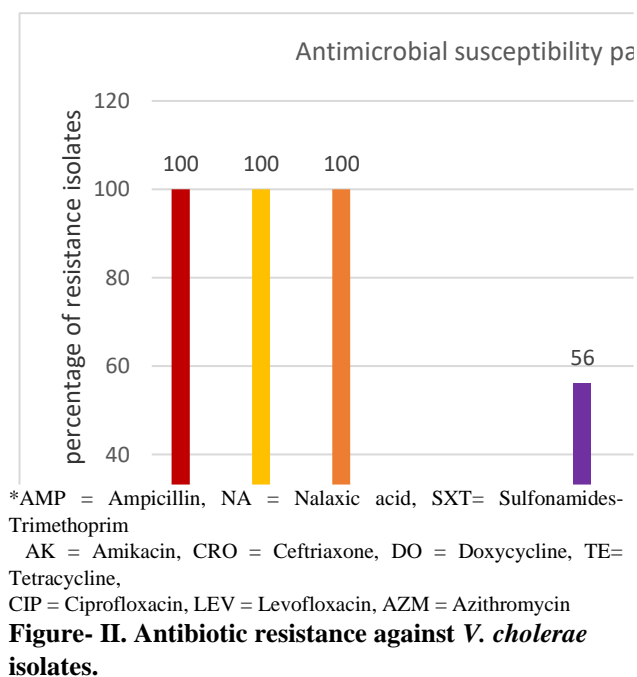
**Figure I. Cholera Incidence in different age groups.**

Variable	Percent Positive	P value
<i>V. cholerae</i> O1 serotype Ogawa	8.90%	< 0.001
Gender Male	5.17%	
Gender Female	3.72%	

## Discussion

The cholera outbreak continues to be a very important public health problem in Baluchistan, where access to safe water and sufficient hygiene is still challenging. Due to high-occurrence diseases, health care workers are unreachable due to remote areas and overworked, which

often increases the possibility of cholera outbreaks in these environments. In 1994 there was cholera outbreak occurred in Sibi in which *V. cholerae* O139 were isolated.<sup>15</sup>The *V. cholerae* serotype O1 Ogawa has been isolated from other district of Balochistan in 2005.<sup>10</sup>



The age wise result showed that cholera infection was higher in individuals below 20-years-old patients as compared to above 20 years age group as shown in Figure I. Motaweq, (2018)<sup>16</sup>Hasoon et al., (2012)<sup>16</sup> reported that the *V. cholerae* affected all age population but the incidence rate was more in 1- 20-years population. Furthermore, gender wise *V. cholerae* infection was high in male patients as compared to female patients as shown in Table II. The gender wise associated findings of the current study were similar to those reported previous.<sup>18</sup> and the same case were with gender-related findings.<sup>19,20,16</sup>

The susceptibility of all isolates to ten commonly used antimicrobial agents as shown in Figure 1.2. The results showed that 100% isolates were resistance to Ampicillin, Nalidixic acid and Sulfonamides-Trimethoprim. Our finding was almost similar as described by Wang et al., (2012).<sup>21</sup>In current study Tetracycline was 93.18% resistance to isolates but previous study by Karki et al., (2010)<sup>22</sup> contrast to our study the isolates were found to be 100% sensitive to Tetracycline.

*V. cholerae* isolates were 56% resistance to Doxycycline. All *V. cholerae* strains were identified as susceptible to Doxycycline (100 %) by Gupta et al., (2016)<sup>18</sup> However, in contrast to our study.

This study revealed the resistance with Amikacin, Ceftriaxone as 24%, 14% respectively. On the contrary, in a study conducted in Iraq 2017<sup>16</sup>, strain of *V. cholerae* was found to be resistant to Amikacin (41%) and Ceftriaxone (93.3%). In the present study, the isolates showed utmost sensitive to Amikacin. High sensitivity of *V. cholerae* isolates to Amikacin has also been reported by many other researchers (Uppal et al., 2017; Kumar et al., 2014; Taneja et al., 2003).<sup>23, 24,25</sup>

All isolate were 100% sensitive to Ciprofloxacin, Levofloxacin and Azithromycin. Previous study by (Sack et al., 2006; Igere et al., 2020)<sup>26,27</sup> 100% percent sensitivity was observed for Levofloxacin, ciprofloxacin and Azithromycin which was similar to the findings of our study.

## Conclusion

In this study, commonly believed methods for isolation, detection, and characterization of *V. cholerae*, provided more extensive knowledge of the epidemiology of *V. cholerae*. We concluded that serological identification is also a good tool. We also conclude that *V. cholerae* O1 persist in developing country and is still causing incidence of cholera.

This study confirmed that *V. cholerae* has resistance to many antibiotics utilized in hospitals. The antimicrobial susceptibility pattern of the *V. cholerae* is essential to start an appropriate treatment.

**Recommendation:** Safe water supply and adequate sanitation, hygiene are the important factor for the control and prevention of cholera infection. Encourage the design and implementation of the cholera control policy advised by the WHO.

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#### Authors Contribution:

<sup>1</sup>Substantial contributions to the conception or design of the work;

<sup>2,3</sup>The acquisition, analysis, or interpretation of data for the work;

<sup>1,2</sup>Final approval of the version to be published;

<sup>1,2,3</sup> Drafting the work or revising it critically for important intellectual content;