BMC Public Health

RESEARCH

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# Prevalence and antimicrobial susceptibility pattern of *Vibrio cholerae* isolates from cholera outbreak sites in Ethiopia

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## Abstract

**Background** Cholera is an acute infectious disease caused by ingestion of contaminated food or water with *Vibrio cholerae*. Cholera remains a global threat to public health and an indicator of inequity and lack of social development. The aim of this study was to assess the prevalence and antimicrobial susceptibility pattern of *V. cholerae* from cholera outbreak sites in Ethiopia.

**Methods** Across-sectional study was conducted from May 2022 to October 2023 across different regions in Ethiopia: Oromia National Regional State, Amhara National Regional State and Addis Ababa City Administration. A total of 415 fecal samples were collected from the three regions. Two milliliter fecal samples were collected from each study participants. The collected samples were cultured on Blood Agar, MacConkey Agar and Thiosulfate Citrate Bile Salt Sucrose Agar. A series of biochemical tests Oxidase test, String test, Motility, Indole, Citrate, Gas production, $H_2S$ production, Urease test were used to identify *V. cholerae* species. Both polyvalent and monovalent antisera were used for agglutination tests to identify and differentiate *V. cholerae* serogroup and serotypes. In addition, Kirby-Bauer Disk diffusion antibiotic susceptibility test method was done. Data were registered in epi-enfo version 7 and analyzed by Statistical Package for Social Science version 25. Descriptive statistics were used to determine the prevalence of *Vibrio cholerae*. Logistic regression model was fitted and *p*-value < 0.05 was considered as statically significant.

**Results** The prevalence of *V. cholerae* in the fecal samples was 30.1%. Majority of the isolates were from Oromia National Regional State 43.2% (*n* = 54) followed by Amhara National Regional State 31.2% (*n* = 39) and Addis Ababa City Administration 25.6% (*n* = 32). Most of the *V. cholerae* isolates were O1 serogroups 90.4% (*n* = 113) and Ogawa serotypes 86.4% (*n* = 108). Majority of the isolates were susceptible to ciprofloxacin 100% (*n* = 125), tetracycline 72% (*n* = 90) and gentamycin 68% (*n* = 85). More than half of the isolates were resistant to trimethoprim-sulfamethoxazole 62.4% (*n* = 78) and ampicillin 56.8% (*n* = 71). In this study, participants unable to read and write were about four times more at risk for *V. cholerae* infection (AOR: 3.8, 95% CI: 1.07–13.33). In addition, consumption of river water were about three times more at risk for *V. cholerae* infection (AOR: 2.8, 95% CI: 1.08–7.08).

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**Conclusion** our study revealed a high prevalence of *V. cholerae* from fecal samples. The predominant serogroups and serotypes were O1 and Ogawa, respectively. Fortunately, the isolates showed susceptible to most tested antibiotics. Drinking water from river were the identified associated risk factor for *V. cholerae* infection. Protecting the community from drinking of river water and provision of safe and treated water could reduce cholera outbreaks in the study areas.

**Keywords** Cholera outbreak, *Vibrio cholerae*, Antimicrobials

## Background

Cholera is an acute diarrheal disease of the intestine which is caused by *Vibrio cholerae (V. cholerae)* [1]. The transmission of the disease is due to ingestion of polluted food or water [2]. In the history of cholera, the first six cholera pandemics were caused by the *V. cholerae* O1 classical biotype, whereas the 7th pandemic was caused by El Tor biotype [3]. In 1992, a new epidemic strain, Bengal (*V. cholerae* O139) was reported [4]. *Vibrio cholerae* has more than 206 serogroups, however, majority of the disease is caused by two serogroups (O1 and O139) [5]. On the basis of phenotyping and genotyping, *V. cholerae* O1 is sub-divided in to two biotypes (classical and El Tor) and three serotypes (Ogawa, Hikojima and Inaba) [6].

Cholera is transmitted through fecal oral route, and the endemic cholera has been found to be associated with tidal seawater, whereas epidemic cholera often occurs near waterways when weather conditions are favorable for the growth of the organism [7, 8]. High magnitude and frequency of cholera outbreak are found around rivers, high population density, low deceitful lands and high absolute humidity environments [9]. The other very plausible cause for cholera outbreak is seasonal variations. High prevalence of the outbreak is occurred during pre-monsoon season of the year, and a second peak during the post-monsoon period [10] or at the end of the dry season or at the beginning of the rainy season, when water sources are limited and become brackish and/or highly polluted [11], since *V. cholerae* is a natural inhabitant of aquatic environment, particularly in brackish, estuarine, coastal, marine, river and lake waters [12].

In recent study, the persister phenotype of *V. cholerae* can survive about 700 days in nutrient-poor lake water [13]. From the environment, *V. cholerae* growth is aggravated by the presence of physical, chemical and biological factors like temperature shift, humidity, high pH, 1%NaCl, phytoplankton and zooplankton [14]. As a result of this environmental factors, cholera can remain dormant in water, attaching to plankton and the chitin in the shells of mollusks and other crustaceans by forming biofilms resulting seasonal cholera outbreaks [15]. In addition, typical aggravating factors for *V. cholerae* transmission are peri-urban slums, refugees, poor access to healthcare services areas, drinking polluted water and poor hygiene practices [16]. Clinically associated risk factors for *V. cholerae* infection are being O blood group, pregnant women, under five years age children, and immunocompromised peoples [17].

Cholera outbreak causes huge socioeconomic disruption as well as loss of life [18]. The global burden of cholera was 1.4 to 4.0 million cases and 21,000 to 143,000 deaths. The epidemicity remains yet to a global threat to public health and an indicator of inequity and lack of social development [19]. Cholera affects all age groups, however the magnitude increases among under five children [20]. The average cholera case fatality rate (CFR) was 0.4% and the highest CFR was 1.6% [21].

Historical evidences on cholera in Ethiopia showed that, the first case comes from Middle East India and the first epidemic was recorded in 1830 that persisted with a recrudescence case up to1998 [22, 23]. Currently, 70 million people are at risk of cholera in Ethiopia, of which an estimated of 275,221 cases and 10,458 deaths were recorded annually [24]. Oromia National Regional State, Amhara National Regional State, Addis Ababa City Administration were highly affected regions in Ethiopia. Since August 2022 as of 13 September 2023, a total of 23,449 cases and 317 deaths with case fatality rate of 1.4% were reported [25].

From the existing knowledge, we understood that the source of the organism is mostly environmental sources and the outcome of the outbreak is very devastating. Even though, the consequence of the outbreak is high, the prevalence, serotype and antimicrobial susceptibility pattern of *V. cholerae* in fecal samples were not well studied in Ethiopia.

Therefore, the findings of the present study are very essential for the prevention and control of cholera outbreaks in the country. Hence, this study was aimed to assess the prevalence and antimicrobial susceptibility pattern of *V. cholerae* in Ethiopia.

## Materials and methods

### Study design, period and area

A cross-sectional study was conducted from May 2022 to October 2023 in Oromia National Regional State, Amhara National Regional State, and Addis Ababa City administration. Majority of the outbreaks were raised at Bale zone, Guji zone, west Arsi and Madawolabo in Oromia National Regional State. Similar outbreaks were reported at West Gondar zone, Bahir Dar zuria and Awi zone in Amhara National Regional State. In addition, outbreaks of cholera were identified in Kolfe Qeranio

sub-City of the Addis Ababa City administration. Ethiopia has different areas that have different climatic changes including Desert, Highland and Lowland areas and four known seasons like winter, autumn, summer and spring.

The first 2022 cholera outbreak case was confirmed and reported at Bale Zone in eastern Oromia National Regional State on the 10th of October 2022, in autumn season. Thereafter, cholera outbreaks were observed other places in Oromia National Regional State, Somalia National Regional State, Southern Nation and Nationalities Regional State, Addis Ababa City Administration, and Amhara National Regional State (Fig. 1).

### Study participants

The study population was all cholera disease suspected individuals at different cholera outbreak sites in Ethiopia.

### Inclusion and exclusion criteria

Study participants with sign and symptoms of cholera infection at the outbreak sites were included. Patients who were on antimicrobial treatment for the past two weeks before sample collection were excluded from this study.

### Dependent variables

*Vibrio cholera* infection.

Antimicrobial susceptibility pattern.

### Independent variables

Sociodemographic and environmental data such as age, sex, religion, residence, occupation, educational status, frequency of toilet used per-day, types of drinking water and season of the outbreak were collected.

### Sample size and sampling technique

A total of 415 fecal samples were collected from Oromia National Regional State ($n$=277), Amhara National Regional State ($n$=73) and Addis Ababa City Administration ($n$=65) within the study period. Convenient sampling technique was used to collect data.

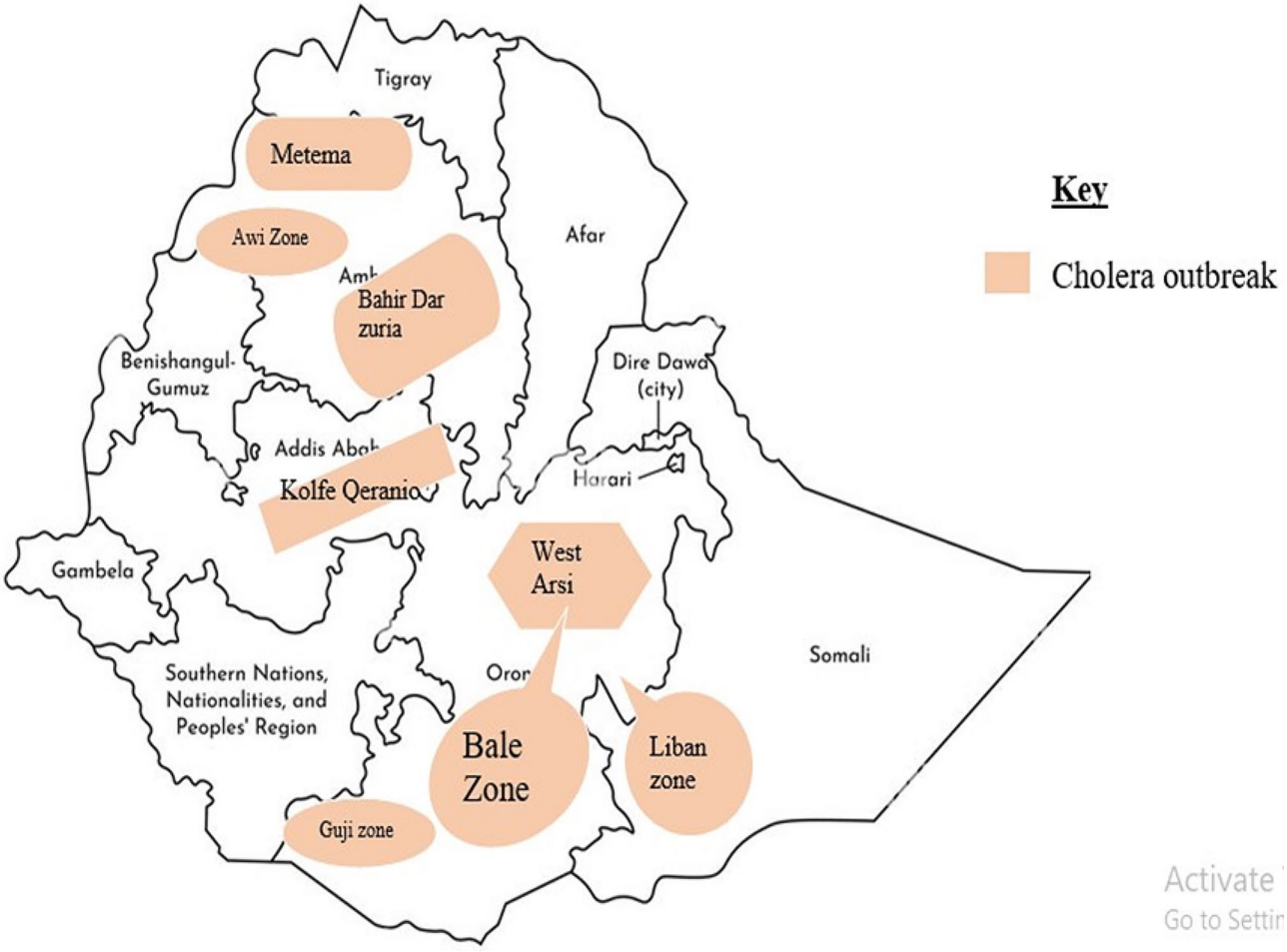


**Fig. 1** Cholera outbreak sites in Oromia National Regional state, Amhara National Regional state, and Addis Ababa City Administration from May 2022 to October 2023 in Ethiopia

### Demographic data collection

Sociodemographic data such as age, sex, religion, residence, occupation, educational status and toilet use were collected. In addition, environmental factors such as seasons of the outbreak and possible water sources for drinking were included in the questionnaire. Face-to-face interviews were made with the study participants by a trained and experienced medical laboratory professional's at each outbreak sites.

### Fecal sample collection and laboratory analysis

Fecal samples were collected from cholera suspected patients following the national cholera sample collection protocol. Two ml of fecal sample was collected from each study participant using a leak proof, clean, and dry container. Following collection, the sample were transported using Kari Blair transport media to Shashemene General Hospital, Armauer Hansen Research Institute and Amhara Public Health Institute for laboratory analysis [26]. The samples were inoculated on Blood Agar (BAP), MacConkey Agar (MAC) and Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar and incubated at 37 °C for 24 h aerobically. The incubation condition was used to optimize the growth, detection and isolation of *V. cholerae* [27]. A series of biochemical tests, namely Oxidase test, String test, Motility, Indole, Citrate, Gas production, H2S production, Urease test were used to identify *V. cholerae* species [28]. Both polyvalent and monovalent antisera were used for slide agglutination tests to identify and differentiate *V. cholerae* serogroup and serotypes [29].

### Antimicrobial susceptibility pattern

The antimicrobial susceptibility test was done by using Kirby-Bauer Disk diffusion method on Muller Hinton agar. Three to five pure colonies were taken and mixed with 5 ml sterile normal saline and compared with 0.5% McFarland standard. The homogeneous solution was inoculated on Mueller-Hinton agar by using sterile swab evenly. Then, the inoculums were allowed to dry for 5–15 min. Antibiotic Disks were placed 15 mm away from the edge and ≥24 mm apart from each other. Antimicrobials such as ampicillin (10 µg), cefotaxime (30 µg), meropenem (10 µg), gentamycin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg) and chloramphenicol (30 µg) were used. The criteria for antimicrobial selection was based on the availability of the antibiotic disks in the study area laboratory and Clinical and based on the guideline provided by Laboratory Standards Institute (CLSI) recommendations to *V. cholerae.* Interpretive criteria was based on the guideline provided by CLSI. These criteria was categorized based on inhibition zone sizes into different categories (e.g., susceptible, intermediate and resistant) [30].

### Statistical analysis

Data were coded and checked, then entered to Epi-info version 7 and exported to Statistical Package for Social Science (SPSS) version 25 software for analysis. Descriptive statistics was computed and odds ratios was used as a measure of the strength of the association and reported with 95% confidence intervals. *P*-value ≤ 0.05 was considered to be statistically significant. Categorical variables were compared using the Pearson $x^2$ test. The bivariate logistic regression model was fitted to identify possible associated factors with *V. cholerae* infection and multivariate logistic regression model was fitted for those variables with *p*-value ≤ 0.2 in the bivariate logistic regression. Figures and tables were used to present the findings.

### Quality control

Data quality was maintained on daily bases during the collection and registration of data. Laboratory protocols were prepared and the SOPs of the AHRI were strictly followed. All steps in data collection and recording were monitored by the principal investigator. The reagents were checked for expiry date and appropriate storage of temperature and humidity. Positive and negative controls were included and run in parallel. *Vibrio cholerae* reference strains; N16961 (O1 El Tor) and MO45 (O139) were used as controls. The quality assurance was ensured with good practice in preparing and reading [31].

## Results

### Socio-demographic characteristics of the study participants

A total of 415 fecal samples were collected from cholera suspected patients visited the cholera treatment center (CTC). Most of the samples 66.7% (*n*=277) were from Oromia National Regional State. Data also showed that more than half of the study participants were females 54.9% (*n*=228) and nearly half of the study participants were in the age category 6–18 years 47.2% (*n*=196). The majority of the study participants were rural dwellers, completed Elementary schooling, married and Self-employed (Table 1).

### Cholera outbreak seasonality and drinking water sources

Data also showed that, majority of the study participants 82.7% (*n*=343) had used river water for drinking and 36.6% of the outbreaks occurred during the autumn followed by the summer season (34.2%) (Table 2).

### Prevalence of *V. Cholerae*

Out of the 415 fecal samples collected from suspected cholera patients, 30.1% (*n*=125) were positive for *V. cholerae*. The prevalence varied across different regions, with 43.2% (*n*=54) from Oromia National Regional State, 31.2% (*n*=39) from Amhara National Regional State,

**Table 1** Socio-demographic characteristics of study participants at cholera outbreak sites in Ethiopia, November 2022 to October 2023

| S.no | Variables | Category | Percentage % |
|---|---|---|---|
| 1. | Age | 1–5 | 95 (22.9) |
| | | 6–18 | 196 (47.2) |
| | | ≥ 19 | 124 (29.9) |
| 4. | Sex | Male | 187 (45.1) |
| | | Female | 228 (54.9) |
| 6. | Region | Oromia | 277 (66.7) |
| | | Amhara | 73 (17.6 ) |
| | | Addis Ababa | 65 (15.7) |
| 9. | Residence | Urban | 196 (47.2) |
| | | Rural | 219 (52.8) |
| 11. | Educational status | Unable to read and write | 166 (40) |
| | | Elementary | 217 (52.3) |
| | | Secondary | 4 (0.9) |
| | | Higher | 28 (6.7) |
| 15. | Occupational status | None | 66 (15.9) |
| | | Student | 83 (20) |
| | | House wife | 80 (19.3) |
| | | Daily laborer | 58 (14) |
| | | Self-employee | 116 (28) |
| | | Government employee | 12 (2.9) |
| 21. | Marital status | Married | 190 (45.8) |
| | | Unmarried | 210 (50.6) |
| | | Divorced | 11 (2.7) |
| | | Widowed | 4 (0.9) |
| 25. | Toilet use frequency | 1–3 | 38 (9.2) |
| | | ≥ 3 | 377 (90.8) |

**Table 2** Seasonality of Cholera outbreaks and the water sources of the study participants at cholera outbreak sites in Ethiopia, November 2022 to October 2023

| sno | Variable | Category | Percentage |
|---|---|---|---|
| 1 | Outbreak season | Summer | 142 (34.2) |
| | | Spring | 46 (11.1) |
| | | Winter | 75 (18.1) |
| | | Autumun | 152 (36.6) |
| 2 | Types of water source | River | 343 (82.7) |
| | | Pond | 5 (0.9) |
| | | Spring | 24 (5.8) |
| | | municipality water | 43 (10.4) |

and 25.6% ($n$=32) from Addis Ababa City Administration. Serological testing revealed that the majority of culture-positive *V. cholerae* isolates belonged to the O1 serogroup, accounting for 90.4% ($n$=113) of cases, predominantly of the Ogawa serotype (86.4%, $n$=108). A lesser proportion were identified as Inaba serotypes (4%, $n$=5) and O139 serogroup (0.8%, $n$=1). Non-O1 and non-O139 isolates constituted 8.8% ($n$=11) of the cases.

**Table 3** Antimicrobials susceptibility of *V. cholerae isolate* in Ethiopia, November 2022 to October 2023

| Antibiotics tested | Susceptibility pattern | |
|---|---|---|
| | Susceptible (%) | Resistant (%) |
| Ampicillin | 54 (43.2) | 71(56.8) |
| Cefotaxime | 38 (30) | 87 (70) |
| Meropenem | 71 (56.8) | 54 (43.2) |
| Gentamycin | 85 (68) | 40 (32) |
| Tetracycline | 90 (72) | 35 (28) |
| Ciprofloxacin | 125 (100) | 0 (0) |
| Trimethoprim-sulfamethoxazole | 47 (37.6) | 78 (62.4) |
| Chloramphenicol | 106 ( 84.8) | 19(15.2) |

### Antimicrobial susceptibility patterns of *V. Cholerae*

The antimicrobial susceptibility pattern of *V. cholerae* showed that most of the isolates were susceptible to ciprofloxacin 100% ($n$=125), chloramphenicol 84.8% ($n$=106), tetracycline 72% ($n$=90) and gentamycin 68% ($n$=85). However, more than half of the *V. cholerae* isolates were resistant to cefotaxime 70% ($n$=87), trimethoprim-sulfamethoxazole 62.4% ($n$=78) and ampicillin 56.8% ($n$=71) (Table 3).

### Factors associated with *V. Cholerae* infection

The associations between *V. cholerae* and demographic and environmental factors were assessed using both bivariate and multivariate logistic regression models. Participants unable to read and write were 3.8 times more at risk of infection with *V. cholerae* as compared to participants who have higher education (AOR: 3.8, 95% CI: 1.07–13.33, $p$=0.039). In addition, Participants who drunk river water were 2.8 times more likely to be infected with *V. cholerae* compared to those consumed municipal water (AOR: 2.8, 95%CI: 1.08–7.08, $P$=0.035). Furthermore, the summer season was significantly associated with *V. cholerae* infection as compared with autumn ($P$=0.000) (Table 4).

## Discussion

Global cholera report showed that about 53,327 new cholera cases and 482 new deaths have been reported between the 26th September and the 31st October 2023. The five countries reporting most cases were Afghanistan, Haiti, Democratic Republic of the Congo, Ethiopia, and Somalia, [25]. These counties are often struggle with a combination of factors that makes them more prone to *V. cholerae* infectious disease and outbreaks. These factors include poverty, having inadequate healthcare system, poor sanitation, lack of clean water, political instability. As a result they frequently report high number of cholera cases compared to the developed regions having strong healthcare system and infrastructure [32].

In Ethiopia, the recent cholera outbreak was first reported at Bale zone of Oromia National Regional state

**Table 4** Bivariate and multivariate analysis of factors associated with *V. Cholerae* infection in Ethiopia, November 2022 to October 2023

| Characteristics | Cholera infection | | COR (95%CI) | *P*-value | AOR (95%CI) | *p*-value |
|---|---|---|---|---|---|---|
| | Positive (*n* = 125) | Negative (*n* = 290) | | | | |
| **Age in years** | | | | | | |
| 1–5 | 55 (57.3) | 40 (42.7) | 0.2 (0.11, 0.35) | 0.000* | 0.1 (0.02, 0.14) | 0.000* |
| 6–18 | 44 (22.2) | 152 (77.8) | 0.9 (0.63, 2.89) | 0.755 | 0.9 (0.39, 1.91) | 0.713 |
| ≥ 19 | 26 (21) | 98 (79) | 1 | | 1 | |
| **Sex** | | | | | | |
| Male | 60 (32.1) | 127 (67.9) | 0.8 (0.55, 1.29) | 0.430 | 1.2 (0.71, 2.10) | 0.473 |
| Female | 65 (28.5) | 163 (71.5) | 1 | | 1 | |
| **Region** | | | | | | |
| Oromia | 54 (19.5) | 223 (80.5) | 4.0 (2.27, 7.08) | 0.000* | 4.5 (2.27, 7.08) | 0.000* |
| Amhara | 39 (53.4) | 34 (46.6) | 0.9 (0.433, 1.651) | 0.623 | 0.4 (0.43, 1.65) | 0.623 |
| Addis Ababa | 32 (49.2) | 33 (50.8) | 1 | | 1 | |
| **Residence** | | | | | | |
| Urban | 60 (30.6) | 136 (69.4) | 1.0 (0.63, 1.46) | 0.836 | 2.0 (0.96, 4.33) | 0.066 |
| Rural | 65 (29.7) | 154 (70.3) | 1 | | 1 | |
| **Educational status** | | | | | | |
| Unable to read and write | 55 (33.5) | 109 (66.5) | 1.1 (0.48, 2.55) | 0.822 | 3.8 (1.07, 13.33) | 0.039* |
| Elementary | 59 (26.9) | 160 (73.1) | 1.5 (0.66, 3.45) | 0.332 | 1.5 (0.51, 4.35) | 0.465 |
| Secondary | 1 (25) | 3 (75) | 1.7 (0.15, 18.22) | 0.675 | 9.9 (0.69, 141.47) | 0.091 |
| Higher | 10 (35.7) | 18 (64.3) | 1 | | 1 | |
| **Marital status** | | | | | | |
| Married | 43 (22.6) | 147 (77.4) | 1 | | 1 | |
| Unmarried | 77 (36.7) | 133 (63.3) | 3.4 (0.47, 24.99) | 0.226 | 0.3 (0.14, 0.70) | 0.003* |
| Divorced | 3 (27.3) | 8 (72.7) | 1.7 (0.24, 12.51) | 0.588 | 0.4 (0.08, 2.01) | 0.263 |
| Widowed | 2 (50) | 2 (50) | 2.7 (0.25, 28.44) | 0.417 | 0.7 (0.06, 8.140) | 0.778 |
| **Outbreak season** | | | | | | |
| Summer | 72 (50.7) | 70 (49.3) | 0.2(0.13, 0.37) | 0.000* | 0.1(0.01, 0.65) | 0.017* |
| Spring | 16 (34.8) | 30 (65.2) | 0.4(0.20, 0.88) | 0.021* | 0.2(0.06, 0.570) | 0.003* |
| Winter | 9 (12) | 66 (88) | 1.7(0.74, 3.72) | 0.221 | 1.2(0.42, 3.22) | 0.772 |
| Autumun | 28 (18.4) | 124 (81.6) | 1 | | 1 | |
| **Types of water** | | | | | | |
| River | 86 (25.1) | 257 (74.9) | 2.4 (1.24, 4.53) | 0.009* | 2.8 (1.08, 7.08) | 0.035* |
| Pond | 1 (25) | 4 (75) | 3.3 (0.33, 30.73) | 0.320 | 0.2 (0.02, 2.82) | 0.957 |
| Spring | 19 (79.2) | 5 (20.2) | 0.2 (0.07, 0.66) | 0.008 | 0.3 (0.08, 1.15) | 0.079 |
| Municipal | 19 (44.2) | 24 (55.8) | 1 | | 1 | |
| **Toilet use frequency** | | | | | | |
| 1–3 | 12 (31.6) | 26 (68.4) | 1.1 (0.45, 1.90) | 0.827 | 2 (0.77,5.19) | 0.155 |
| > 3 | 113 (30) | 264 (70) | 1 | | 1 | |

COR: crud odds ratio, AOS: adjusted odds ratio

on the 27th of August 2022 and confirmed on the 9th of September 2022 [33]. This outbreak was disseminated rapidly to other Oromia National Regional State districts and other Regions. Ethiopia has low sanitation coverage and has been frequently affected by acute watery diarrhea [34]. The general populations have poor access to safe drinking water and sanitation facilities, and the situation is worse for those in rural areas. The national sanitation coverage in Ethiopia is only 57% which translates to more than 45 million people without access to improved sanitation facilities [35]. Healthcare service records and community based surveys indicate that diarrheal diseases are major causes of morbidity and mortality in Ethiopia because of low access to safe drinking water and adequate sanitation [36]. According to the WHO Weekly Bulletin report on cholera outbreaks and other emergencies issued on the 18th August 2019, 1,005 cholera cases had been reported from five regions and two city administrations. The affected administrative towns and regions were Oromia (437 cases, 43.5%), Amhara (202 cases, 20%), Afar (164 cases and 1 death, 16%), Addis Ababa (146 cases, 14.5%), Dire Dawa (1 case, 0.001%), Somali (33 cases, 3%), and Tigray (22 cases, 2%) (*The WHO Weekly Bulletin on cholera outbreaks and other emergencies issued on 18 August 2019*).

In the current study, the prevalence of confirmed *V. cholerae* infectious was 30.1%. The predominant serogroup responsible was *V. cholerae* O1, accounting for 90.4% of the cases. This is supported by different reports around the world. For example, the sero-prevalence of *V. cholerae* O1 was 60 to 86% in Bangladesh [37], 100% in Pakistan) [38, 39], and 59% in Nigeria [40]. In the current study, the positivity rate of *V. cholerae* from fecal sample was more than 50% (50.7%) during the summer season. Previous studies describe the temporal variation of cholera in localized study areas and many investigators postulate that the temporal variation of the disease is due to environmental and climatic factors that affect the seasonal patterns of infection [41, 42]. In Bangladesh, studies described a regular seasonal cycle for cholera outbreaks, including specific studies on the different strains: classical, El Tor, and O139 [43]. In the current study, the most prevalent serogroup was the *V. cholerae* O1 followed by the Ogawa serotype. Seasonality and serotype distribution of cholera outbreaks was previously reported [44]. The switching of serotype from Ogawa to Inaba and back to Ogawa has been observed temporally in *V. cholerae* O1, which was responsible for seasonal outbreaks of cholera in Dhaka, Bangladesh during the period 2015 to 2018. Study on sero-specificity is key for effective intervention and for preventing cholera [45]. The combination of environmental adaptability, enhanced colonization abilities in human hosts, efficient transmission and antibiotic resistance contributes to the higher prevalence of the O1 serogroup, El Tor biotype of *V. cholerae* [46]. In addition, *V. cholerae* O1 serogroup, El Tor biotype can produce more apparent infection due to its ability to persist longer time and multiply rapidly in the environment. Additionally, it induce less competent immunity compared to the classical biotype. This advantage is partly due to the JSF9 phage, which selectively target the classical biotype and favors the survival and proliferation of El Tor biotype from the environment [47].

The antimicrobial susceptibility patterns of *V. cholerae* isolates of the current study demonstrated that 70%, 62.4% and 56.8% were resistant to cefotaxime, trimethoprim-sulfamethoxazole and ampicillin, respectively. Drug resistance to different antimicrobials by *V. cholerae* isolates were also previously reported by different research works [48]. Report from a systematic study conducted on 131 articles showed that 50% drug resistance to cefoxitin and 94.6% to trimethoprim-sulfamethoxazole [49]. On the contrary, low antibiotic resistance isolates were reported by a systematic study conducted in Sub-Saharan countries trimethoprim-sulfamethoxazole (50%) and ampicillin (43.3%) [50]. By another study, a 60% and 89% drug resistance to Ampicillin was reported in South-Africa [51] and in Ethiopia [52], respectively. *Vibrio cholerae* isolates of the current study were 100%, 84.8%, 90%, and 68% susceptible to ciprofloxacin, chloramphenicol, tetracycline, and Gentamycin. Previous studies from some African countries reported *V. cholerae* isolates have relatively similar drug sensitivity patterns. For example, 93% of the *V. cholerae* isolates in Ghana were susceptible to tetracycline [53], 99.6% to ciprofloxacin and Gentamycin [54]. Antibiotic resistance in *V. cholerae*, has a significant implications in public health and treatment strategies, these implications are reduction of treatment options, increased mortality and morbidity, increase the spread of resistance gene, increase healthcare cost, and increase global health impact [55]. To reduce these implications, efforts are needed to strengthen surveillance system, improve sanitation infrastructure, promote vaccination, and ensure the use of susceptible antibiotics to *V. cholerae* and other pathogens [56].

One of the risk factors associated with *V. cholerae* infection in the current study was drinking river water. Use of an unimproved water source conferred > 3-fold increase in the odds of cholera in meta-analysis, highlighting the importance of a safe water source in cholera control [57]. The odds of drinking river water were 2.8 times more likely associated with *V. cholerae* infection as compared to drinking municipal water. Increased water surface runoff organic sediments such as faecal wastes from land to nearby river causing contamination and aggravating *V. cholerae* infection [58]. Evidences shown that in recent times, Ethiopia's conflict has erupted in Oromia and Amhara National Regional States. Examination of data sources and recent reviews indicate that cholera occurs in countries during war and civil unrest, in neighboring countries, where temporary camps accommodate masses of political refugees under poor conditions, during the postwar period when large numbers of repatriated persons return home and consequently place undue pressure on an eroded and fragile national infrastructure [59]. In the present study, almost all *V. cholerae* confirmed study participants suffered with acute rice watery diarrhea more than 3 times per day and dehydration, this is because of the biological nature of *V. cholerae* enterotoxin that activates the adenylate cyclase which helps the production of cyclic-AMP that helps the outpouring of fluids which is rich in electrolytes [60].

## Conclusion

The prevalence of *V. cholerae* was notably high, with the O1 serogroup and Ogawa serotypes predominating. Majority of the *V. cholerae* isolates were resistant to ampicillin and trimethoprim-sulfamethoxazole. Study participants who were unable to read and write were significantly association with *V. cholerae* infection. Furthermore, consumption of river water increased the likelihood of cholera infection by 2.8 times. Moreover, *V. cholerae* transmission was increased during the summer

season. These findings emphasize the critical importance of ongoing caution in monitoring and implementing public health interventions to diminish the risk of *V. cholerae* outbreaks and safeguard community health. Enhanced sanitation practices in drinking water sources are mandatory to prevent contamination and transmission of *V. cholerae*.

**Abbreviations**

| | |
|---|---|
| NCBIs | National Center of Biotechnology Institutes |
| APW | Alkaline Peptone Water |
| BAP | Blood Agar Plate |
| CFU | Colony Forming Unit |
| CLSI | Clinical Laboratory Standard Institute |
| TCBS | Thiosulfate citrate bile salt sucrose |
| WHO | World Health Organization |

**Acknowledgements**
We would like to thank the University of Gondar and College of Medicine and Health sciences, DebreMarkos University, and the Armauer Hansen Research Institute for the financial and material support. We also like to address our gratitude to the School of Biomedical and Laboratory sciences and the Department of Medical Microbiology of the CMHS, UOG. We also thank the study participants for their welling to give the necessary data and stool samples.

**Author contributions**
A.B and B.G designed the study; A.B collected the data and performed laboratory investigation, statistical analysis, interpretation of the results and manuscript writing. B.G, A.G and Y.W involved in manuscript writing, statistical analysis, approved quality of the data and interpretation of the results. B.Y, M.T, A.M and G.T.B contributed in the manuscript writing, statistical analysis and interpretation of the results. Z.A, M.Y, W.T, T.G, M.G involved in the data collection, laboratory analysis. All authors participated during manuscript writing. B.G, A.G, A.M edited the final manuscript. All authors read and approved the final manuscript.

**Funding**
The Authors received no fund.

**Data availability**
The data used and analyzed during the current study are available from the corresponding author on reasonable request. All relevant data are found in the manuscript.

## Declarations

**Ethics approval and consent to participate**
Ethical clearance was obtained from the University of Gondar Institutional Research Ethics review Committee (IRERC) (*VP/RTT/05/20/2022*). A written informed consent was obtained from participants after explaining the purpose and objective of the study. In addition, informed consent was obtained from the parents or legal guardians of participants under the age of 16 years and those unable to read and write. Participants had a full right to continue or withdraw from the study. All information were kept confidential by assigning code and assessed by the principal investigator and supervisors. *Vibrio cholerae* positive results were communicated health professionals to provide patient management in accordance with WHO cholera management guideline.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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Received: 23 January 2024 / Accepted: 29 July 2024
Published online: 31 July 2024

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