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# Ecological and laboratory assessment of the resistance spectrum and antibacterial susceptibility of *Vibrio cholerae* strains isolated in the Caspian region of Kazakhstan

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## ABSTRACT

Given Kazakhstan's geographical proximity to the Caspian Sea and the detection of *V. cholerae* in the rive systems connected to the Caspian basin, this study also contributes to a broader understanding of cholera ecology and antimicrobial resistance within the region. For the first time, this study identified and thoroughly examined the role of genetic and biochemical factors contributing to the development of resistance in the *V. cholerae* strains under the conditions of the Republic of Kazakhstan. A comprehensive analysis was conducted on the antibiotic resistance of *V. cholerae* strains isolated in Kazakhstan from 1997 to 2023, with attention to their potential for persistence, adaptation, and spread within the broader Caspian ecological network. Molecular and biochemical mechanisms underlying resistance development were investigated, and their potential impact on the epidemiological situation and biological safety was assessed. Phenotypic susceptibility testing of *V. cholerae* ($n = 13$) conducted *in vitro* demonstrated high sensitivity to cefotaxime, tetracycline, doxycycline, ciprofloxacin, and kanamycin. A similarly high level of susceptibility was observed for gentamicin, chloramphenicol, ampicillin, and rifampicin (96.2% of isolates). Real-time PCR results revealed no presence of resistance genes to glycopeptide or beta-lactam antibiotics in the tested *V. cholerae* strains. However, *vanA/B* genes (Ct = 9.166, FAM channel) and the *tem* gene (Ct = 34.60, CY5 channel) were detected in the control strains of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027 (Ct = 8.954 and 24.85, respectively). In conclusion, the absence of resistance to major classes of antimicrobial agents among all 13 *V. cholerae* isolates indicates the continued high clinical efficacy of these antibiotics in the treatment of cholera. These findings are of critical importance in the context of potential epidemic outbreaks and environmental persistence of *V. cholerae* in the Caspian-associated aquatic systems, as they provide clinicians and public health officials with a reliable basis for empirical therapy selection and regional preparedness planning.

**Keywords:** Cholera strains, Antibiotics, Resistance, Sensitivity, Statistics.
**Article type:** Research Article.

## INTRODUCTION

Cholera remains a pressing public health concern in many parts of the world, particularly in countries bordering the Caspian Sea where *Vibrio cholerae* continues to be detected in environmental reservoirs. In Kazakhstan, non-

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toxigenic *V. cholerae* and non-O1 serogroups have been isolated from river systems flowing into or out of the Caspian Sea, including the Ural and Syr Darya rivers. These aquatic environments provide favorable conditions for the persistence and potential resurgence of toxigenic and non-toxigenic *V. cholerae* strains (Ali *et al.* 2012; Huq *et al.* 2014; Wang *et al.* 2020; Islam *et al.* 2021; Dairova *et al.* 2022). Similar ecological and epidemiological patterns have been observed across other Caspian region countries. In Southern Russia, *V. cholerae* has been regularly detected in the Volga Delta and adjacent brackish waters of the Northern Caspian coast, where plankton blooms and elevated water temperatures facilitate seasonal amplification of the bacterium **(**Sokolov *et al.* 2008). In Turkmenistan, surveillance along the Amu Darya River and adjacent irrigation canals during the 1990s confirmed the presence of non-O1 and occasionally O1 El Tor strains (Amanov *et al.* 1995). Environmental monitoring in western Uzbekistan has also revealed the presence of toxigenic *V. cholerae* in the Aral Sea delta and its associated floodplains during periods of water stagnation and eutrophication (Nishanov *et al.* 2002). In Iran, several studies have confirmed the presence of *V. cholerae* in rivers and estuaries along the Southern Caspian coast, particularly beside the Gilan and Mazandaran provinces. These areas experience seasonal cholera outbreaks often linked to contamination of drinking water during flooding events or insufficient sanitation infrastructure (Rahbar *et al.* 2010). Historical epidemiological records further support the repeated introduction of cholera through human movement and trade across the Caspian ports and internal waterways. In Azerbaijan, environmental monitoring has identified *V. cholerae* in coastal waters beside the Baku region, where inadequate sewage treatment and seasonal warming contribute to favorable conditions for the bacterium's persistence. Some isolates from these regions have shown resistance to multiple antibiotics and the ability to form biofilms, underscoring the need for ongoing environmental and clinical surveillance (Aghayev *et al.* 2005). These regional findings emphasize the environmental versatility of *V. cholerae*, which enables it to colonize diverse hydrological systems and persist in endemic foci. The interconnectedness of aquatic ecosystems, shared epidemiological challenges, and similar environmental pressures across the Caspian region highlight the importance of cross-border surveillance and data integration. This study aims to contribute to this regional knowledge base by analyzing the phenotypic resistance patterns and ecological traits of *V. cholerae* strains isolated in Kazakhstan from 1997 to 2023, with attention to their potential for persistence, adaptation, and spread within the broader Caspian ecological network.

According to the World Health Organization (WHO), *V. cholerae* has demonstrated increasing resistance to conventional antibiotics, such as ampicillin and tetracycline, in several regions of Africa in recent decades (WHO, 2021). Studies have shown that antibiotic-resistant strains can persist in the environment and be transmitted from person to person, thereby facilitating the spread of infection. A critical aspect of resistance research is understanding the mechanisms that enable *V. cholerae* to maintain viability under antimicrobial pressure. These mechanisms include both genetic adaptation and the horizontal transfer of resistance genes among bacteria (Frontiers 2021). For instance, resistance to tetracycline may be associated with genes encoding active efflux pumps or ribosomal protection proteins that prevent antibiotic binding (Connell *et al.* 2003). Additionally, epidemiological and social factors—such as high population density, inadequate sanitation, and the widespread, unregulated use of antibiotics—play a major role in the dissemination of resistance (Ivanova 2024). Thus, understanding the causes of *V. cholerae* resistance and elucidating its underlying mechanisms are essential for developing effective strategies for the cholera treatment and prevention (Shakhnovich *et al.* 2007). In 2018, studies were conducted to investigate phenotypic markers of antibiotic resistance among the *V. cholerae* isolates collected in Kazakhstan. Of the isolates analyzed, 38.4% exhibited resistance, with monoresistant strains predominating (23.1%), while 9.6% harbored two or more resistance markers (Sagiyev *et al.* 2018). Phenotypic resistance was most frequently observed against kanamycin (17.3%). Among the *V. cholerae* isolates of the O1 serogroup, 38.5% were resistant, compared to 40.0% of non-O1 serogroup isolates. A notably high proportion of resistant strains—45.8%—was recorded in the Kyzylorda region. Furthermore, resistant strains isolated from water sources in Almaty City, as well as in the Almaty and Zhambyl regions, shared a common phenotype characterized by resistance to erythromycin (Sagiyev *et al.* 2018).

For the first time, this study identified and thoroughly examined the role of genetic and biochemical factors contributing to the development of resistance in the *V. cholerae* strains under the conditions of the Republic of Kazakhstan. In this study, we conducted a comprehensive analysis of the antibiotic resistance of *V. cholerae* strains isolated in Kazakhstan between 1997 and 2023, examining their susceptibility to various classes of antimicrobial agents. Additionally, we investigated the molecular and biochemical mechanisms contributing to resistance, and we assessed their potential impact on the epidemiological situation and public biosafety.

## MATERIALS AND METHODS

### Bacterial strains and isolates

This study included 13 collection strains of *Vibrio cholerae* from different serogroups (O1 and non-O1), isolated from clinical samples and surface water in Almaty, Turkistan, Mangystau and West Kazakhstan of the Republic of Kazakhstan. These strains were collected between 1997 and 2023. Among them, 12 strains belonged to the *V. cholerae* O1 serogroup, biovar El Tor, and 1 strain was classified as *V. cholerae* non-O1. Specifically, 1 from the Almaty region, 2 from the Turkistan region, 2 from the Mangystau region and 8 from the West Kazakhstan region (Table 1).

**Table 1.** List of *Vibrio cholerae* strains examined by serogroup, serovar, pathogenicity gene profile, Kazakhstan region, time, and source of isolation.

| Strain | Serogroup | Serovariant | Pathogenicity gene profile | Region, year, and place of strain isolation |
|---|---|---|---|---|
| *V. cholerae* KZ-07-05 | *non* O1 | Ogawa | ctxAB$^{+}$, tcpA$^{+\ -}$ | Almaty, 1998, water |
| *V. cholerae* KZ-07-24 | O1 | Inaba | ctx$^{+}$, ctx$^{-}$ | Turkistan, 2005, water |
| *V. cholerae* KZ-01-12 | O1 | Ogawa | ctx$^{+}$, ctx$^{-}$ | Turkistan, 2010, water |
| *V. cholerae* KZ-17-13 | O1 | Ogawa | ctxA$^{-}$, tcpA$^{-}$ | Mangystau, 1997, human |
| *V. cholerae* KZ-19-21 | O1 | Ogawa | ctxA$^{-}$, tcpA$^{-}$ | Mangystau, 1997, human |
| *V. cholerae* KZ-07-03 | O1 | Inaba | ctxA$^{-}$, tcp$^{-}$ | West Kazakhstan, 2016, water |
| *V. cholerae* KZ-07-10 | O1 | Ogawa | ctxA$^{-}$, tcp$^{-}$ | West Kazakhstan, 2016, water |
| *V. cholerae* KZ-13-09 | O1 | Ogawa | ctxA$^{-}$, tcp$^{-}$ | West Kazakhstan, 2016, water |
| *V. cholerae* KZ-09-17 | O1 | Ogawa | ctxA$^{-}$, tcp$^{-}$ | West Kazakhstan, 2016, water |
| *V. cholerae* KZ-07-02 | O1 | Hikojima | ctxA$^{-}$, tcp$^{-}$ | West Kazakhstan, 2016, water |
| *V. cholerae* KZ-07-01 | O1 | Ogawa | ctxA$^{-}$ | West Kazakhstan, 2023, water |
| *V. cholerae* KZ-12-04 | O1 | Ogawa | ctxA$^{-}$ | West Kazakhstan, 2023, water |
| *V. cholerae* KZ-09-07 | O1 | Inaba | ctxA$^{-}$ | West Kazakhstan, 2023, water |

### Antimicrobial susceptibility testing

The susceptibility/resistance of *V. cholerae* strains to antibacterial agents—including doxycycline (30 µg), ciprofloxacin (10 µg), tetracycline (30 µg), cefotaxime (30 µg), kanamycin (30 µg), nalidixic acid (30 µg), trimethoprim (5 µg), furazolidone (50 µg), gentamicin (10 µg), chloramphenicol (10 µg), ampicillin (25 µg), streptomycin (300 µg), and rifampicin (15 µg)—was assessed in accordance with established methodological guidelines (Nayak *et al.* 2021). A total of 59 antibiotics in disk form and 50 in E-test strips, representing 25 main groups, were used. Susceptibility testing was performed using the standard disk diffusion method (Kirby–Bauer test) and the E-test. Resistance genes were detected using an extended-spectrum β-lactamase (ESBL) phenotypic method (CLSI. Performance Standards for Antimicrobial Susceptibility Testing 2023; Davies *et al.* 2010; Igbinosa 2010; Methodical recommendations 4.2.2495-09. Moscow, Russia 2010; Abdirasilova 2014) and real-time PCR for screening resistance to glycopeptides and beta-lactams (Abdirasilova 2014). The reference control strains included *V. cholerae* KA-37, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14025, and *Pseudomonas aeruginosa* ATCC 9027. The strains were cultured on Mueller–Hinton agar (pH 7.3 ± 0.2) and Hottinger agar (pH 7.2 ± 0.1) at 37 °C. For susceptibility testing, bacterial suspensions were prepared from 24-hour agar cultures in 0.85% isotonic sodium chloride solution, standardized using the McFarland standard (R092-1NO LOT0000633797; exp. 02/2026, HiMedia Laboratories Pvt. Ltd., Maharashtra, India), corresponding to $1.5 \times 10^{8}$ CFU mL$^{-1}$ (standards R092A and R092B). After 10–15 minutes, antibiotic disks were applied, and cultures were incubated at 37 °C. Preliminary measurements were taken after 12 hours and finalized after 18 hours. Control assays included the reference strains and plates with sterile disks. The diameters of the inhibition zones around the disks were measured to the nearest millimeter using a precision measuring template from HiMedia Laboratories Pvt. Ltd., India.

### Molecular Genetic Screening

DNA extraction was performed using the commercial kit “RealBest UniMag” (Series C-8883, expiration date: July 22, 2025), produced by Vector-BEST, Russia, as well as the “RIBO-prep” kit (Cat. No. K2-9-Et-100, Russia), designed for automated DNA/RNA extraction systems. To detect antibiotic resistance determinants in bacterial lysates, a BacResista GLA Real-Time PCR Detection Kit (DNA-Technology LLC, Moscow, Russia) was used. This kit targets genes encoding resistance to glycopeptide and beta-lactam antibiotics, including vanA/B (vancomycin and teicoplanin); mecA (methicillin and oxacillin); tem, ctx-M-1, and shv (penicillins and cephalosporins); and oxa-40-like, oxa-48-like, oxa-23-like, oxa-51-like, imp, kpc, ges, ndm, and vim (carbapenems).

## RESULTS

### Ecological characteristics of *Vibrio cholerae* in Kazakhstan

In Atyrau Region, surveillance for cholera in 2024 included laboratory testing of 1,385 individuals, among whom 939 were patients presenting with acute intestinal infections (AII). During the first half of 2025, an additional 344 individuals were tested, including 252 AII cases. No toxigenic *V. cholerae* strains were identified in human samples in either year. Environmental monitoring in the same region revealed the presence of *V. cholerae* non-O1 in surface and wastewater. In 2024, a total of 430 water samples were examined from open water bodies (the Ural and Emba rivers flowing into the Caspian Sea) and sewage sources, resulting in the isolation of 17 *V. cholerae* non-O1 strains. In the first half of 2025, 121 environmental samples were tested, yielding 7 additional *V. cholerae* non-O1 isolates. In Mangystau Region, a total of 364 clinical samples from patients with acute intestinal infections were tested in 2024, and 75 individuals (65 AII) were examined in the first half of 2025. As in Atyrau, no toxigenic *V. cholerae* strains were detected in clinical specimens.

Environmental samples collected in Mangystau during 2024 included 200 from coastal areas of the Caspian Sea and 218 from sewage sources. From these, 86 *V. cholerae* non-O1 strains were isolated. In 2025, 171 surface water and 69 sewage samples were tested, resulting in the identification of 48 *V. cholerae* non-O1 strains and one *V. cholerae* RO strain.

*V. cholerae*, the causative agent of cholera, persists not only as a human pathogen but also as a resilient aquatic bacterium, capable of surviving and propagating in diverse environmental niches. In Kazakhstan, strains of *V. cholerae* have been isolated predominantly from rivers originating near or draining into the Caspian Sea, including the Ural River and its tributaries. This highlights the significance of riverine and coastal ecosystems in maintaining environmental reservoirs of the bacterium (Huq *et al.* 2014; Dairova *et al.* 2022). Long-term microbiological monitoring since the 1970s has confirmed the presence of both toxigenic and non-toxigenic strains of *V. cholerae* in open water systems, especially during the warmer months of the year (June–September). Parameters such as temperature, pH, salinity, and plankton density have been found to directly influence bacterial survival and amplification (Wang *et al.* 2020; Islam *et al.* 2020). Strains from the Syr Darya River basin, which drains into the Aral Sea, and from the Ural basin have been found to harbor specific ecological adaptations, including biofilm formation on plankton and algae. These adaptations enhance resistance to environmental stressors and enable prolonged survival outside the human host (Islam *et al.* 2021). Furthermore, *V. cholerae* has demonstrated the potential for horizontal gene transfer in environments affected by agricultural runoff and wastewater discharge, leading to the emergence of strains with enhanced virulence or resistance profiles (Ali *et al.* 2012). Cases of cholera in Kazakhstan have also been linked to importation from endemic countries. In such instances, human carriers introduced toxigenic *V. cholerae* into local ecosystems, where environmental factors facilitated limited secondary transmission (Dairova *et al.* 2022). These findings highlight the dual role of both environmental and anthropogenic factors in the persistence and re-emergence of cholera. The observed ecological resilience of *V. cholerae* underscores the importance of integrated monitoring systems that combine hydrochemical profiling, microbial surveillance, and remote sensing. Such systems are vital for early detection of potential outbreaks and risk assessment. These environmental observations align with laboratory findings on resistance patterns and virulence, contributing to a more comprehensive understanding of *V. cholerae* evolution and adaptation in Kazakhstan.

### One health perspective on ecology and antibiotic resistance

The One Health framework offers a comprehensive approach to understanding the ecology and antimicrobial resistance (AMR) of *V. cholerae* by integrating human, animal, and environmental health domains. In Kazakhstan, the detection of *V. cholerae* in surface waters used for livestock and irrigation introduces a clear risk of cross-species transmission, particularly during seasonal flooding or agricultural runoff (Huq *et al.* 2014; Dairova *et al.* 2022; Wang *et al.* 2020). The antibiotic resistance surveillance data over five decades show a progressive increase in resistance to key therapeutic classes, including tetracyclines and sulfonamides. These findings correlate with excessive and unregulated antibiotic usage in both medical and veterinary sectors (Ali *et al.* 2012; Islam *et al.* 2021). The environment, particularly aquatic ecosystems, functions as a convergence point where resistant strains from humans and animals may interact and exchange genetic material, enhancing the overall AMR burden (Ali *et al.* 2012; Wang *et al.* 2020). Furthermore, phenotypic analyses have identified the persistence of mobile genetic elements (e.g., SXT integrative conjugative elements) and resistance genes (e.g., *tetA*, *sul1*, *dfrA*) in both clinical and environmental isolates. These genomic markers support the hypothesis that environmental isolates not only harbor virulence traits but also contribute to the broader AMR landscape through horizontal gene transfer (Wang X *et al.* 2020; Islam *et al.* 2021). In line with the One Health model, coordinated national efforts that bridge environmental monitoring, clinical diagnostics, and veterinary controls are imperative. The integration of genomic surveillance with geospatial mapping and ecosystem modeling can enhance predictive capabilities and inform evidence-based interventions to mitigate the risks of cholera outbreaks and the spread of antimicrobial resistance (Huq *et al.* 2014; Islam *et al.* 2021).

### Results of laboratory analyses of *Vibrio cholerae* strains

The results of the laboratory screening for the antimicrobial susceptibility and resistance profiling of *V. cholerae* strains (n = 13) isolated in the Republic of Kazakhstan between 1997 and 2023 from clinical and environmental sources are presented in Table 2. Based on their cultural, morphological, biochemical, and serological characteristics, all strains were identified as typical representatives of the family Vibrionaceae, genus *Vibrio*, species *cholerae*, belonging to both O1 and non-O1 serogroups.

**Table 2.** Ranges and diameters (in mm) of growth inhibition zones for 13 *Vibrio cholerae* strains isolated in Kazakhstan between 1997 and 2023 from clinical and environmental sources.

| **Strain Isolation Area** | **Number of *V. cholerae* Isolates** | **Range of minimum inhibitory concentration values of antibacterial drugs by main group** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **β-lactams** | **Macroleades** | **Tetracyclines** | **Aminoglycosides** | **Amphenicols** | **Glycopeptides** | **Lincosamides** | **Fluoroquinolones** | **Antibiotics of Different Groups** |
| Almaty region | 1 | 19–39 | 23–24 | 26–30 | 18–29 | 28–29 | 25–26 | 25–26 | 30–35 | 16–28 |
| Turkestan region | 2 | 16–40 | 21–28 | 21–30 | 18–28 | 26–30 | 23–28 | 24–27 | 30–40 | 15–32 |
| Mangistau region | 2 | 17–40 | 25–26 | 24–30 | 17–26 | 25–26 | 24–26 | 25–28 | 30–39 | 16–31 |
| West Kazakhstan region | 8 | 16–40 | 20–26 | 21–30 | 17–30 | 25–30 | 20–25 | 21–29 | 29–40 | 16–33 |
| Total | 13 | 17–39.8 | 22.2–26 | 23.1–24.8 | 17.6–28.3 | 26–28.7 | 23–26.25 | 23–27.5 | 29.75–38.5 | 15.75–31 |

Of the 13 *V. cholerae* O1 and non-O1 strains examined, 100% (13/13) were susceptible to the following antimicrobial agents: doxycycline (30 µg), ciprofloxacin (10 µg), tetracycline (30 µg), cefotaxime (30 µg), and kanamycin (30 µg). In the case of nalidixic acid (30 µg), 65.4% (8/13) of the strains demonstrated high susceptibility, 30.8% (4/13) intermediate susceptibility, and 3.8% (1/13) resistance. In the case of trimethoprim (5 µg), 73.1% (9/13) of the isolates were highly susceptible, while 26.9% (3/13) showed intermediate susceptibility. For furazolidone (50 µg), 73.1% (9/13) of the *V. cholerae* strains were highly susceptible, while 26.9% (3/13) exhibited intermediate susceptibility. With respect to gentamicin (10 µg), 96.2% (12/13) of the isolates were highly susceptible, and 3.8% (1/13) showed intermediate susceptibility. Similarly, in the case of chloramphenicol (10 µg) and ampicillin (25 µg), 96.2% (12/13) of the strains demonstrated high susceptibility, with 3.8% (1/13) showing intermediate susceptibility to each antibiotic. Regarding streptomycin (300 µg), 88.5% (11/13) of the *V. cholerae* strains were highly susceptible, 7.7% (1/13) showed intermediate susceptibility, and 3.8% (1/13) low susceptibility.

In the case of rifampicin (15 µg), 96.2% (25/26) of the strains demonstrated high susceptibility, while 3.8% (1/13) intermediate susceptibility. The results of the study, including the strain numbers and the names of the antibacterial agents tested on Mueller–Hinton and Hottinger agars, are presented in summary charts in Figs. 1 and 2, along with selected individual results in Figs. 3 and 4. The most active antibiotics belonged to the β-lactam group, including cefotaxime, cefixime, chloramphenicol, and cefamandole, followed by cefazolin, tetracycline, and ampicillin. Agents traditionally used for cholera treatment—such as ciprofloxacin, doxycycline, and azithromycin—demonstrated comparatively lower activity. Furazolidone exhibited the weakest activity against all 13 strains, with an average inhibition zone diameter of 11.1 mm (range: 10–18 mm).

An *in vitro* phenotypic susceptibility analysis of *V. cholerae* (n = 13) demonstrated 100% sensitivity to β-lactams, tetracyclines, aminoglycosides, amphenicols, glycopeptides, lincosamides, and quinolones. Additionally, 96.5% of the strains were susceptible to antibiotics from other classes. The results of the susceptibility and resistance testing were confirmed using the standard E-test method, with strips indicating the minimum inhibitory concentration (Fig. 4). The results of the antimicrobial resistance gene detection in 13 *V. cholerae* strains using the *BacResista GLA Real-Time PCR Detection Kit* with real-time PCR are presented in Table 3 and Fig. 5.

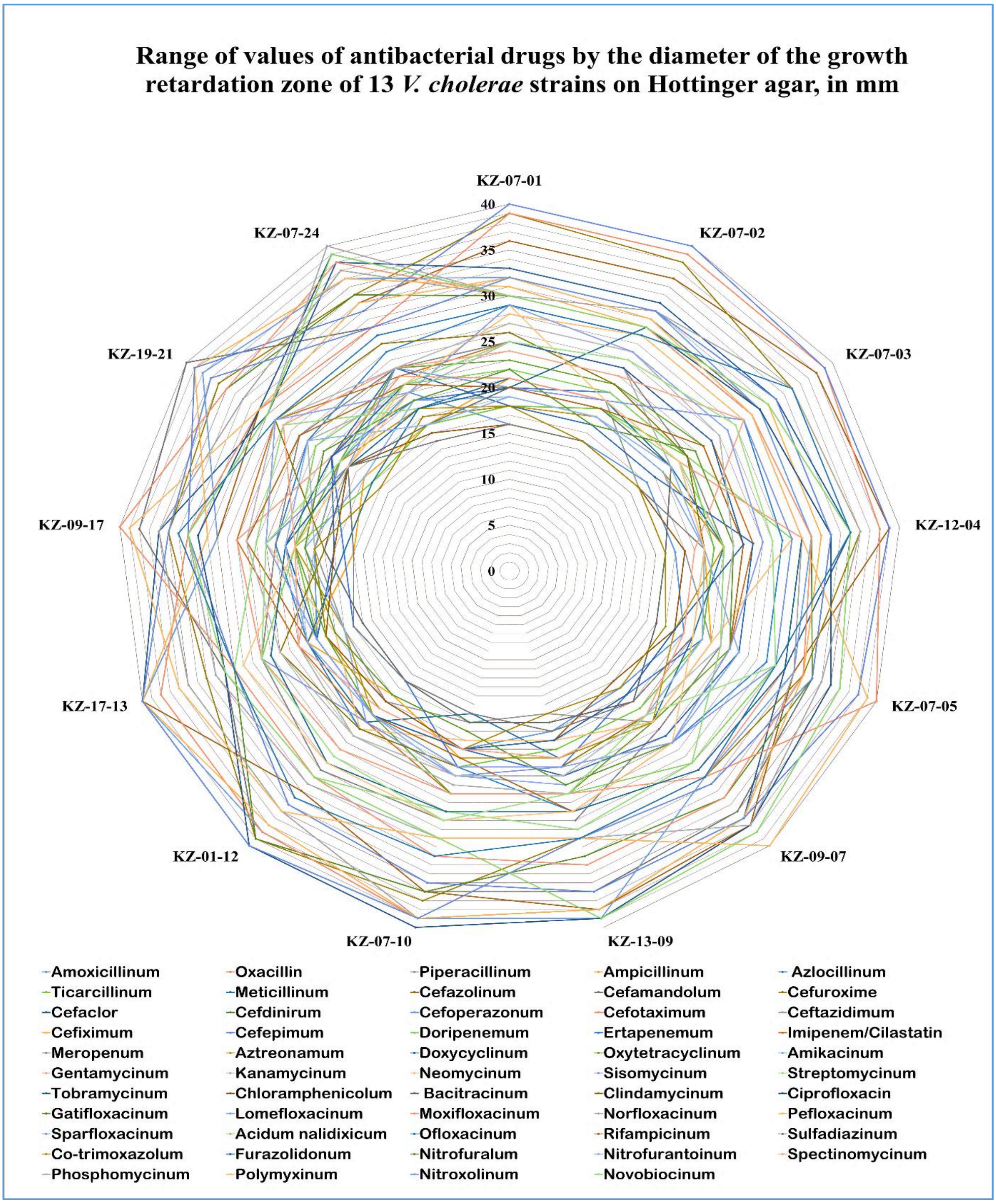

**Fig. 1.** Range of inhibition zone diameters for 13 *Vibrio cholerae* strains on Hottinger agar, in millimeters.

Currently, research on the genetic determinants of bacterial resistance to glycopeptide and beta-lactam antibiotics is receiving significant attention, as these classes of antimicrobial agents are widely used for the treatment of complicated and/or severe infections. Investigating the most common types of beta-lactamases produced by various pathogenic bacteria can aid in interpreting their antibiotic susceptibility profiles, informing therapeutic decision-making, and enhancing infection control practices at the local level. A molecular genetic analysis aiming to detect antibiotic resistance determinants in the genomes of 13 *V. cholerae* isolates, including clinical strains collected in Kazakhstan from 1997 to 2023, revealed no presence of resistance genes to glycopeptides (*vanA/B*—vancomycin and teicoplanin) or beta-lactam antibiotics (*mecA*—methicillin and oxacillin; *tem*, *ctx-M-1*, and *shv*—penicillins and cephalosporins; *oxa-40-like*, *oxa-48-like*, *oxa-23-like*, *oxa-51-like*, *imp*, *kpc*, *ges*, *ndm*, and *vim*—carbapenems). The control strains used in this study included *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14025, and *Pseudomonas aeruginosa* ATCC 9027, in which the *vanA/B* gene (Ct = 9.166, FAM channel) and the *tem* gene (Ct = 34.60, CY5 channel) were detected in *E. coli* ATCC 25,922 and in *P. aeruginosa* ATCC 9027 (Ct = 8.954 and 24.85, respectively).

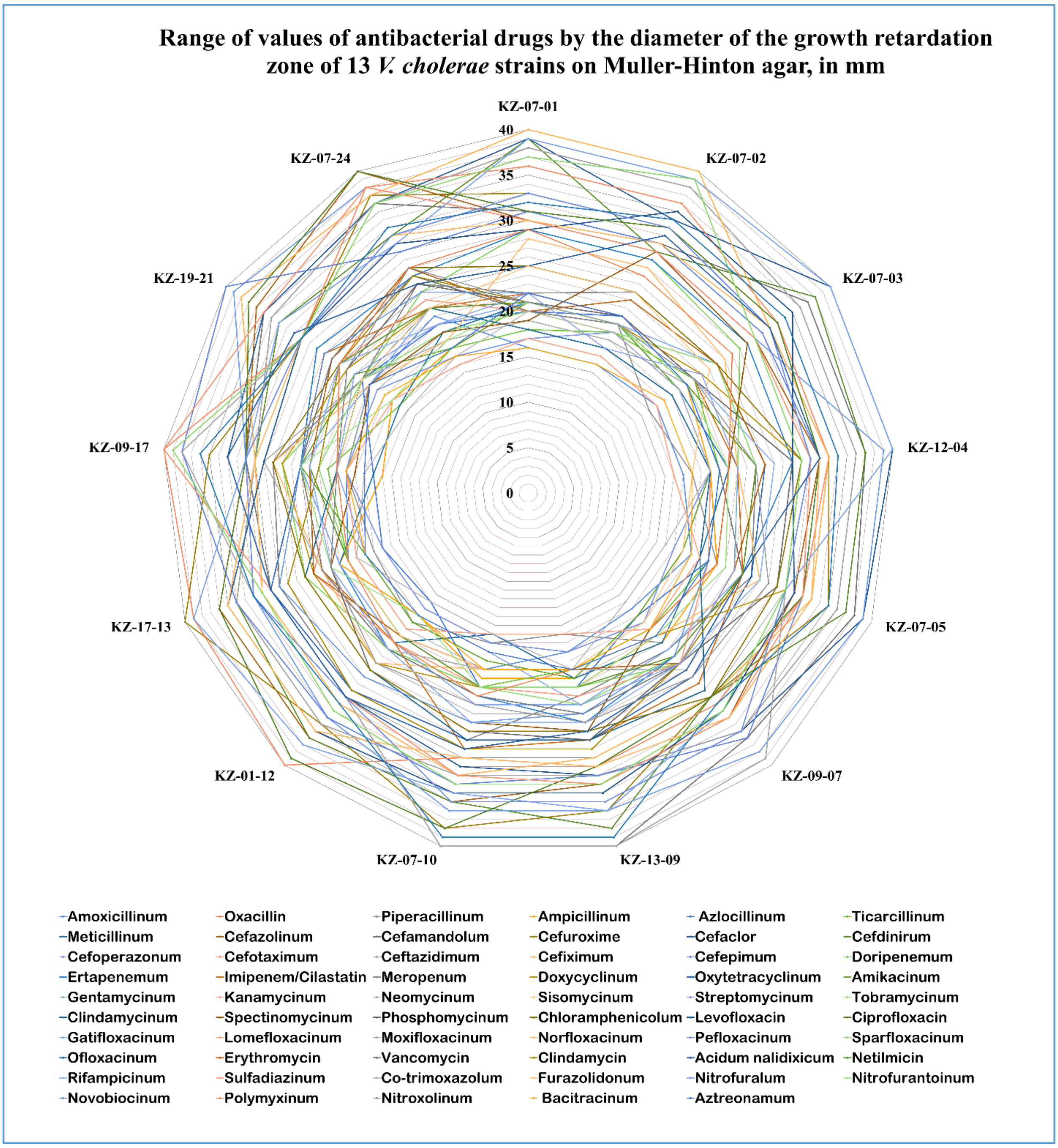

**Fig. 2.** Range of inhibition zone diameters for 13 *Vibrio cholerae* strains on Mueller–Hinton agar, in millimeters.

Thus, the results confirm the absence of genetic markers of resistance to 59 antimicrobial agents in all examined *V. cholerae* isolates. No resistance was detected to the following major classes of antibiotics: extended-spectrum beta-lactams (penicillins, cephalosporins, and carbapenems), monobactams, macrolides, tetracyclines, aminoglycosides, amphenicols, glycopeptides, lincosamides, fluoroquinolones, and other antibiotic groups. These findings highlight the importance of the regular surveillance of *V. cholerae* susceptibility to antimicrobial agents, which enables timely adjustments to therapeutic strategies and effective responses to changes in the epidemiological situation.

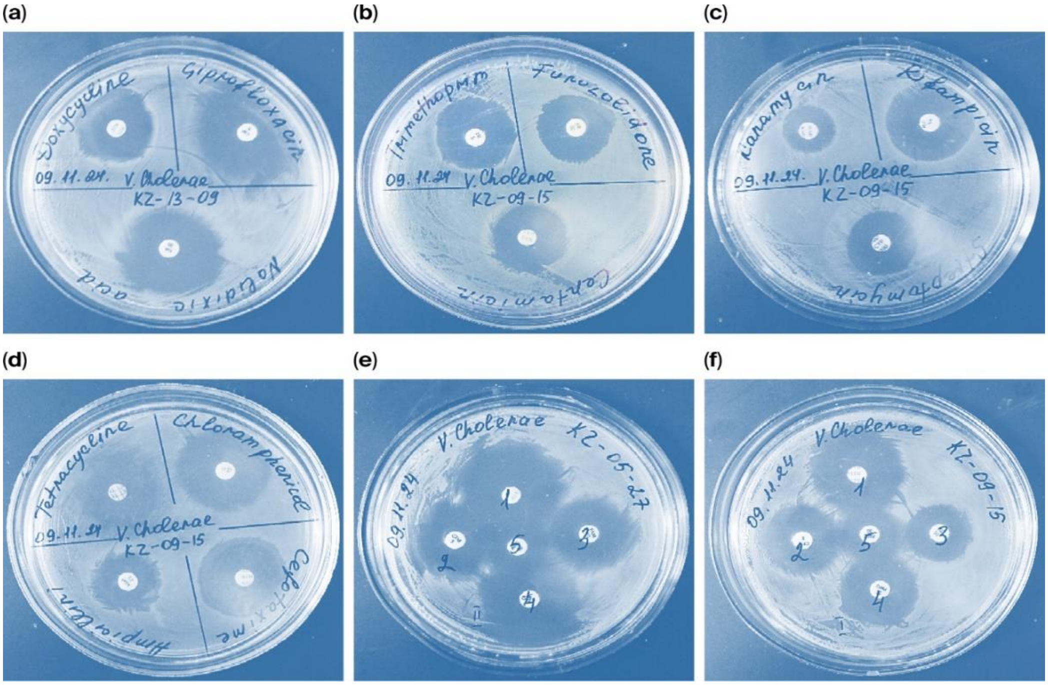

**Fig. 3.** Inhibition zone diameters determined using the disk diffusion method and the detection of ESBL resistance genes using the phenotypic method: (a–d)—susceptibility to antibiotics; (e, f)—absence of resistance genes; Image shows a Petri dish with a visual diameter of 11 cm.

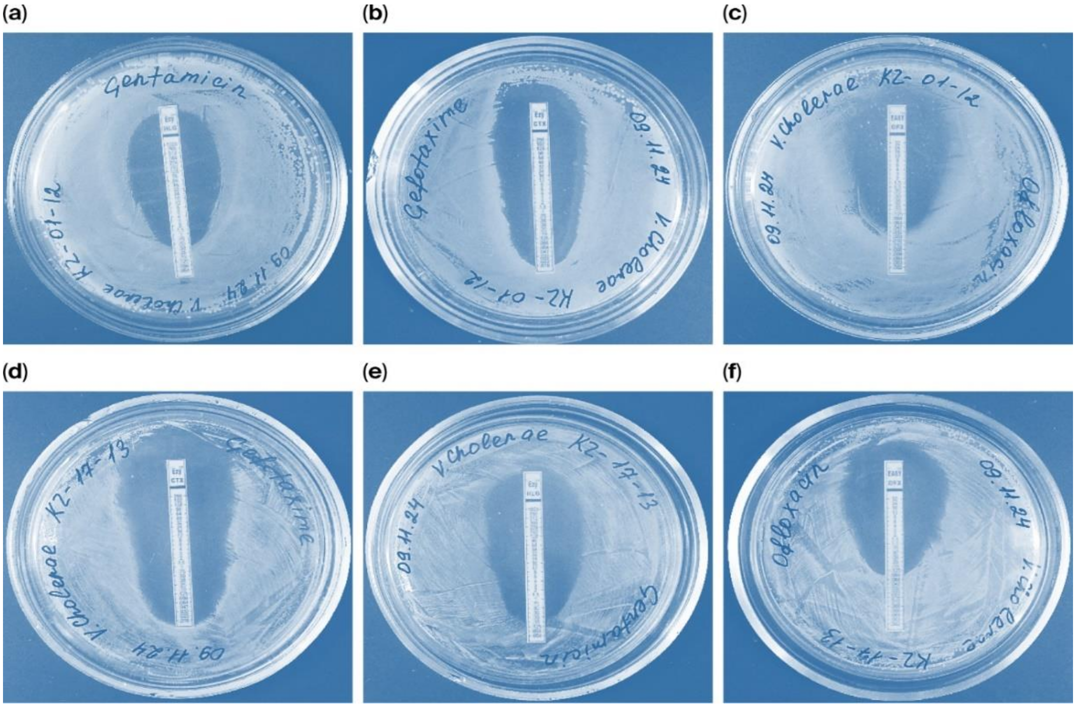

**Fig. 4.** Results of susceptibility testing using the E-test method: panels (a–f) show the susceptibility of two different *Vibrio cholerae* strains to gentamicin, cefotaxime, and ofloxacin. Panels (a), (b), and (c) correspond to Strain 1, while panels (d), (e), and (f) correspond to Strain 2.

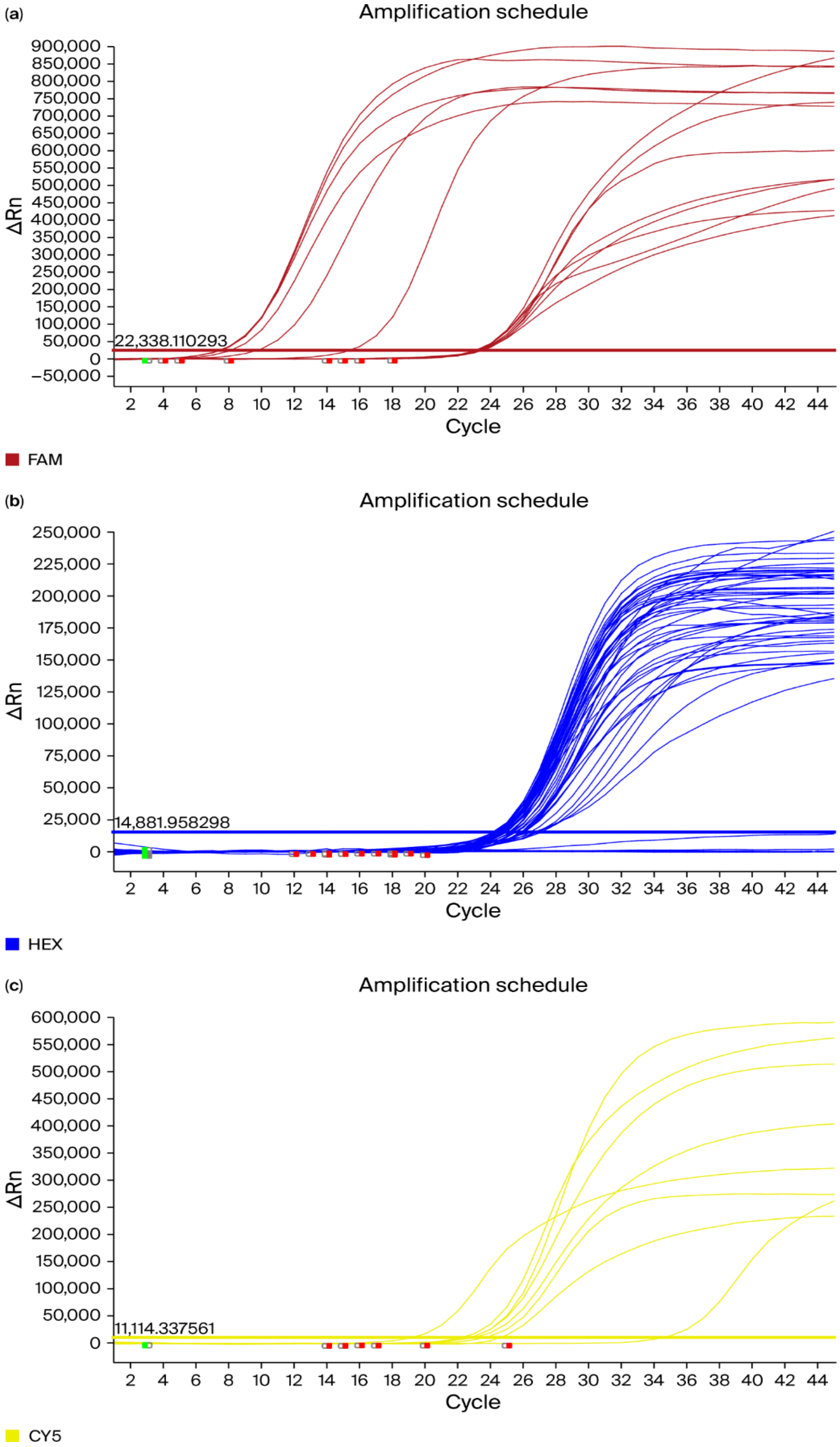

**Fig. 5.** Amplification curves by fluorophore detection channels: (**a**) FAM; (**b**) HEX; (**c**) CY5. Red and green dots represent PCR amplification curves of different samples. Red dots indicate positive amplification signals, while green dots indicate negative or no amplification.

**Table 3.** Results of antimicrobial resistance gene detection in 13 *Vibrio cholerae* strains using the *BacResista GLA Real-Time PCR Detection Kit* and real-time PCR.

| **Name of genetic determinants and verification** | | **Controls and indicators by species** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ***V. cholerae KA-37*** | ***Escherichia coli* ATCC 25922** | ***Salmonella typhimurium ATCC 14.025*** | ***Pseudomonas aeruginosa ATCC 9027*** | ***V. cholerae* KZ00-00 ***** | **Control « – »** | **Control « + »** |
| FAM | TBM * | 7.507 | 8.029 | 15.33 | 7.648 | $\bar{x}$= 7.17 | – | 23.27 |
| | imp | – | – | – | – | – | – | 23.63 |
| | ctx-M-1 | – | – | – | – | – | – | 22.20 |
| | van A/B | – | 9.166 | – | 8.954 | – | – | 24.40 |
| | oxa-48-like | – | – | – | – | – | – | 24.07 |
| | vim | – | – | – | – | – | – | 24.31 |
| | oxa-23-like | – | – | – | – | – | – | 23.43 |
| | shv | – | – | – | – | – | – | 23.61 |
| HEX | IC ** | 25.73 | 25.99 | 25.87 | 25.99 | $\bar{x}$= 25.78 | 26.21 | 26.70 |
| | IC | 26.91 | 26.79 | 25.94 | 26.79 | $\bar{x}$= 24.43 | 25.58 | 25.81 |
| | IC | 24.57 | 25.24 | 25.13 | 25.24 | $\bar{x}$= 24.56 | 25.13 | 25.27 |
| | IC | 24.37 | 24.39 | 24.93 | 24.39 | $\bar{x}$= 24.60 | 24.94 | 25.10 |
| | IC | 24.44 | 24.39 | 24.67 | 24.39 | $\bar{x}$= 24.45 | 24.98 | 25.12 |
| | IC | 24.62 | – | 24.96 | – | $\bar{x}$= 24.68 | 25.15 | 25.03 |
| | IC | 24.50 | 24.37 | 24.56 | 24.37 | $\bar{x}$= 24.63 | 24.67 | 24.65 |
| CY5 | oxa-51-like | – | – | – | – | – | – | 24.69 |
| | Tem | – | 34.60 | – | 24.85 | – | – | 20.20 |
| | mec A | – | – | – | – | – | – | 24.40 |
| | oxa-40-like | – | – | – | – | – | – | 24.79 |
| | Kpc | – | – | – | – | – | – | 24.12 |
| | Ndm | – | – | – | – | – | – | 23.53 |
| | Ges | – | – | – | – | – | – | 23.24 |

* TBM—total bacterial mass; ** IC—internal control; *** KZ-00-00—average value based on results from 13 *V. cholerae* strains.

## DISCUSSION

The findings from Kazakhstan align with broader patterns observed throughout the Caspian basin, highlighting shared ecological and microbiological conditions conducive to the persistence and seasonal resurgence of *Vibrio cholerae*. Rivers such as the Ural and Syr Darya represent important ecological corridors, allowing the migration and accumulation of vibrios, particularly in periods of water stagnation and elevated temperatures. In parallel, similar environmental reservoirs have been described in the Volga Delta (Russia), Amu Darya River (Turkmenistan), Aral Sea floodplains (Uzbekistan), and Caspian coastal estuaries (Iran and Azerbaijan), where *V. cholerae* remains a constant environmental presence with potential to spark outbreaks under favorable conditions (Amanov *et al.* 1995; Nishanov *et al.* 2002; Aghayev *et al.* 2005; Sokolov *et al.* 2008; Rahbar *et al.* 2010). These patterns underscore the critical importance of regional One Health approaches, where human health risks are analyzed in conjunction with environmental dynamics and animal reservoirs. Given the growing threat of antimicrobial resistance among environmental *V. cholerae* isolates, the inclusion of phenotypic and genotypic resistance data — such as those presented in this study — can significantly enhance regional preparedness and inform waterborne disease control strategies. *Vibrio* species undergo genetic changes to enhance their adaptation and resistance to antibiotics. A study on the susceptibility of *V. cholerae* O1 El Tor strains to polymyxin B (PB) in Odisha, India, between 1995 and 2019 found that 89.4% of 1,200 strains were resistant, while 10.6% were susceptible. Susceptibility began to appear from 2005 onward, with the exception of 2015. An E-test analysis revealed that strains with the *ctxB7* genotype had a lower minimum inhibitory concentration (MIC $\leq$ 4 µg mL$^{-1}$), whereas *ctxB1* genotypes exhibited higher MIC values (24 and 32 µg mL$^{-1}$; Marin *et al.* 2018). The study conducted in Odisha, India, between 1995 and 2019, examined changes in antibiotic susceptibility, as well as the presence of virulence and resistance genes, in *V. cholerae* O1 strains. Antimicrobial susceptibility was assessed using the disk diffusion method, and resistance and virulence genes were identified using PCR. All strains were susceptible to gentamicin, chloramphenicol, norfloxacin, and ciprofloxacin but exhibited resistance to one or more other antibiotics. Resistance genes (*SulII*, *dfrA1*, and *strB*) and SXT elements were detected in 90% of the isolates. The Haitian variant of tcpA emerged in 1999 and gradually increased in prevalence. Multiplex PCR confirmed the presence of virulence genes (toxR, ompU, ace, rtxC, ctxA, tcpA, rfbO1, and ompW) in all strains (Wang *et al.* 2012; Nayak *et al.* 2020). In 2022, the number of cholera cases in the WHO European Region more than doubled. In one study, a total of 49 *V. cholerae* O1 isolates collected in Europe were analyzed to confirm their affiliation with the seventh pandemic El Tor lineage (7PET) and to assess their virulence and antibiotic resistance. All isolates were found to belong to the Pre - AFR15 sublineage, which is likely associated with the global surge in cholera cases. Antibiotic resistance analysis revealed no abnormal resistance profiles. Genomic sequencing was recommended for accurate strain identification and the monitoring of their evolutionary dynamics (Hochhut *et al*. 2001). *V. cholerae* is a common inhabitant of aquatic ecosystems, and the state of West Bengal in India—particularly the Ganges Delta region—is known for having a high cholera burden. In a study conducted in Midnapore, *V. cholerae* isolates were obtained from wastewater samples. All isolates belonged to non-O1/non-O139 serogroups. The majority (74.7%) exhibited moderate biofilm-forming ability, while 6.3% demonstrated strong biofilm formation. PCR screening revealed that most isolates carried genes associated with biofilm formation (such as *cdgH*, *cdgM*, *cdgK*, etc.). Two strains (3.17%) contained the cholera toxin genes *ctxA* and *ctxB*. Approximately 24.8% of the isolates carried the *ompU* gene, while other virulence factors were detected less frequently. All isolates tested positive for *toxT*, *toxR*, and *hapR*, which regulate virulence expression. Resistance profiling indicated both increased antibiotic susceptibility and the presence of multidrug resistance (MDR). The detection of such strains in the environment is a concern. The high prevalence of diguanylate cyclases (DGCs) suggests their potential as alternative therapeutic targets against MDR strains (Kolobe 2025; Boué *et al*. 2025).

A molecular genetic analysis conducted on 26 *V. cholerae* O1 isolates, including clinical strains collected in Kazakhstan between 1970 and 2024, revealed no resistance genes to glycopeptide and beta-lactam antibiotics (*vanA/B*, *mecA*, *tem*, *ctx-M-1*, *shv*, *oxa-40-like*, *oxa-48-like*, *oxa-23-like*, *oxa-51-like*, *imp*, *kpc*, *ges*, *ndm*, and *vim*). These findings indicate the absence of genetically mediated resistance to major classes of antimicrobial agents in the analyzed isolates (Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing, Sanitary and epidemiological rules SP 3.1.7.2826-10; Cholera prevention, Moscow 2010; World Health Organization, Antimicrobial Resistance: Global Report on Surveillance; WHO Press: Geneva, Switzerland, 2014). Such results underscore the importance of the regular epidemiological monitoring of pathogen susceptibility to antibiotics, enabling timely responses to potential shifts in resistance patterns (Okeke *et al.* 2005; Boucher *et al.* 2009). The absence of resistance to major antibiotic classes among all examined *V. cholerae* isolates indicates the continued high clinical efficacy of these drugs. These findings are particularly significant in the context of potential cholera outbreaks, as they provide clinicians with a reliable foundation for selecting empirical therapy (Hasan *et al.* 2012). Furthermore, phenotypic susceptibility testing showed that 100% of the strains were sensitive to doxycycline, ciprofloxacin, tetracycline, cefotaxime, and kanamycin. A high susceptibility was also observed in 96.2% of the isolates to chloramphenicol, ampicillin, gentamicin, and rifampicin; 88.5% to streptomycin; and 73.1% to trimethoprim and furazolidone. At the same time, intermediate susceptibility and isolated cases of resistance were observed for nalidixic acid, streptomycin, and furazolidone (Faruque *et al.* 2014).

## CONCLUSION

Given Kazakhstan's geographical proximity to the Caspian Sea and the detection of *V. cholerae* in river systems connected to the Caspian basin, this study also contributes to a broader understanding of cholera ecology and antimicrobial resistance within the region. An analysis of antibiotic susceptibility in 13 *Vibrio cholerae* O1 strains isolated from various regions of Kazakhstan over nearly five decades demonstrated the sustained efficacy of the most commonly used antimicrobial agents. Isolated cases of resistance—particularly to nalidixic acid, streptomycin, and trimethoprim—highlight the importance of continued systematic surveillance of antimicrobial resistance in *V. cholerae*. The findings of this study may inform treatment planning, preventive measures, and local microbiological surveillance efforts in cholera-endemic areas. All *V. cholerae* O1 strains isolated in Kazakhstan between 1997 and 2023 demonstrated a high susceptibility to several critically important antimicrobial agents, including doxycycline, ciprofloxacin, tetracycline, cefotaxime, and kanamycin. High levels of susceptibility were also observed for gentamicin, chloramphenicol, ampicillin, and rifampicin (96.2% of strains). Moderate resistance was observed against nalidixic acid, trimethoprim, furazolidone, chloramphenicol, ampicillin, streptomycin, and rifampicin. All strains, despite variations in serovar and pathogenicity profiles

($ctxAB^{+}$ and $tcpA^{+/-}$), remain susceptible to the primary drugs used in cholera treatment, indicating their continued potential clinical effectiveness at the present stage. No resistance genes to glycopeptide or beta-lactam antibiotics were detected via real-time PCR in the tested strains. However, the *vanA/B* (Ct = 9.166, FAM channel) and *tem* (Ct = 34.60, CY5 channel) genes were identified in *Escherichia coli* ATCC 25922 and in *Pseudomonas aeruginosa* ATCC 9027, with Ct values of 8.954 and 24.85, respectively. Over the study period (1997–2023), no significant increase in the proportion of polyresistant strains was observed. However, the presence or emergence of even a single resistant isolate suggests the possibility of gene transfer under natural conditions, indicating the ongoing circulation of *V. cholerae* O1 between humans and environmental sources. This highlights the need for continuous epidemiological and microbiological surveillance.

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## REFERENCES

Abdirasilova, AA, & Abdel, ZZh 2023, Methodological Recommendations for Real-Time Polymerase Chain Reaction (RT-PCR) for the Detection of Plague Microbe DNA, KazBookExport: Almaty, Kazakhstan, 37 p. [In Russian].

Aghayev IZ & Guseinova ASH 2005, Environmental conditions and cholera vibrios in the Caspian Sea waters of Azerbaijan. *Journal of Epidemiology and Infectious Diseases*, 3: 37-41.

Ali, M *et al.* 2012, The global burden of cholera. *Bulletin of the World Health Organization*, 90(3): 209–218.

Amanov, KhG & Berdyev, AN 1995, Choleragenic vibrios in the water bodies of Turkmenistan. *Bulletin of Sanitation and Hygiene*, 2: 28–31, [In Russian].

Boucher, HW, Talbot, GH, Bradley, JS, Edwards, JE, Gilbert, D, Rice, LB, Scheld, M, Spellberg, B & Bartlett, J 2009, Bad bugs, no drugs: No escape! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases*, 48: 1–12.

Boué, Y, Niang, M, Lapostolle, A, Chamouine, A, Cattin, TB, Favre, M, Rouard, C, Mortier, C, Piarroux, R, Carvelli, J 2025, Cholera outbreak in Mayotte (France): A retrospective description of 16 patients treated for hypovolemia in the ICU. *Infectious Diseases Now*, 55: 105020. https://doi.org/10.1016/j.idnow.2024.105020. ISSN 2666-9919.

CLSI 2023, Performance Standards for Antimicrobial Susceptibility Testing, 33rd ed., CLSI Supplement M100, Clinical and Laboratory Standards Institute: Wayne, PA, USA.

Connell SR, Tracz DM, Nierhaus KH, & Taylor DE 2003, Ribosomal protection proteins and their mechanism of tetracycline resistance. Antimicrob Agents Chemother. 47(12): 3675-3681. DOI: 10.1128/AAC.47.12.3675-3681.2003. PMID: 14638464, PMCID: PMC296194.

Dairova, AN, Smagulova, RA & Asanova NK 2022, Ecological study of *Vibrio cholerae* in the water bodies of Kazakhstan. *Bulletin of Microbiology*, 4: 45–50, [In Russian].

Davies, J & Davies, D 2010, Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews (MMBR)*, 74: 417-433.

Faruque, SM & Nair, GB 2013, Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae. Microbiology Spectrum*, 2, VE-0004-2013.

Federal Center for Hygiene and Epidemiology of Rospotrebnadzor 2010, Guidelines for determining the susceptibility of causative agents of dangerous bacterial infections (plague, anthrax, etc.) to antibacterial drugs: Methodical recommendations 4.2.2495-09. Moscow, Russia, 59 p, [In Russian].

Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing 2010, Sanitary and epidemiological rules SP 3.1.7.2826-10. Cholera prevention. Moscow, Available online: https://docs.cntd.ru/document/1200080421 (accessed on).

Hasan, NA, Choi, SY, Eppinger, M, Clark, PW, Chen, A, Alam, M, Haley, BJ, Taviani, E, Hine, E, Su, Q, Tallon, LJ 2012, Genomic diversity of 2010 Haitian cholera outbreak strains. *Proceedings of the National Academy of Sciences of the United States of America*, 109: E2010–E2017.

Hochhut, B, Lotfi, Y, Mazel, D, Faruque, SM, Woodgate, R, Waldor, MK 2001, Molecular analysis of antibiotic resistance gene clusters in *Vibrio cholerae* O139 and O1 SXT constins. *Antimicrobial Agents and Chemotherapy,* 45: 2991-3000, https://doi.org/10.1128/AAC.45.11.2991-3000.2001. PMID: 11600347; PMCID: PMC90773.

Huq, A & Colwell, RR 2014, A microbiological perspective on climate change and cholera. *Environmental Microbiology Reports,* 6(6): 718-725.

Igbinosa, EO & Okoh, AI 2010, Antibiotic susceptibility profile o*f Vibrio cholerae* O1 strains isolated from wastewater final effluents in South Africa. *Environmental Monitoring and Assessment*, 168: 321–329.

Islam, MT *et al.* 2021, Understanding the ecology of cholera through environmental and genomic surveillance. *Trends in Microbiology*, 29(3): 235–245.

Ivanova, IA, Filippenko, AV, Omelchenko, ND, Tyurina, AV & Gaevskaya, NE 2024, Current status of alternative methods for the prevention and treatment of cholera. *Immunopathology, Allergology, Infectology,* 3: 19–28, (In Russian), https://doi.org/10.14427/jipai.2024.3.19.

Kolobe, MT 2025, Cholera outbreak in Chienge District, Luapula Province, Zambia, 31st May - 9th June 2023: Re-emerging threat. *International Journal of Infectious Diseases*, 152: 107433, https://doi.org/10.1016/j.ijid.2024.107433. ISSN 1201-9712.

Marin, MA, Fonseca, EL, Andrade, BN, Cabral, AC, Vicente, AC 2014, Worldwide occurrence of integrative conjugative element encoding multidrug resistance determinants in epidemic *Vibrio cholerae* O1. *PLoS ONE*, 9: e108728, https://doi.org/10.1371/journal.pone.0108728. PMID: 25265418; PMCID: PMC4181655.

Rituparna De 2021, Mobile genetic elements of *Vibrio cholerae* and the evolution of its antimicrobial resistance. *Frontiers in Tropical Diseases*, https://www.frontiersin.org/articles/10.3389/fitd.2021. 691604/full, (accessed 21 March 2025).

Nayak, AK, Nayak, SR, Behera, DR, Pal, BB 2021, Dissemination of *Vibrio cholerae* O1 isolated from Odisha, India. *Environmental Microbiology Reports*, 13: 355-363, https://doi.org/10.1111/1758-2229.12940. PMID: 33660421.

Nayak, SR, Nayak, AK, Biswal, BL, Pati, S, Pal, BB 2021, Spread of Haitian variant *Vibrio cholerae* O1 causing cholera outbreaks in Odisha, India. *Japanese Journal of Infectious Diseases*, 74: 137-143. https://doi.org/10.7883/yoken.JJID.2020.364. PMID: 32863351.

Nishanov, RA & Abdurakhmanov, ShE 2002, Vibrios in the estuaries of the Amu Darya and the Aral Delta. *Uzbek Medical Journal*, 6: 33–36 [In Russian].

Okeke, IN, Laxminarayan, R, Bhutta, ZA, Duse, AG, Jenkins, P, O'Brien, TF, Pablos-Mendez, A, Klugman, KP 2005, Antimicrobial resistance in developing countries. Part I: Recent trends and current status. *Lancet Infectious Diseases,* 5: 481-493.

Rahbar M, Rezaeian M, Emadi G 2010, Seasonal dynamics of *Vibrio cholerae* in coastal waters of northern Iran. *Iranian Journal of Environmental Health Science & Engineering*, 7(2): 145–152.

Sagiyev ZA, Utepova IB, Mussagaliyeva RS, Alybayev SD, Kulbayeva MM, Ismailova AO, Aldibekova AA 2018, Antibiotic resistance of *Vibrio cholera* strains isolated in Kazakhstan. *Antibiot Khimioter = Antibiotics and Chemotherapy*, 63(3-4): 12-17, [In Russian].

Shakhnovich, EA, Sturtevant, D & Mekalanos, JJ 2007, Molecular mechanisms of virstatin resistance by non-

O1/non-O139 strains of *Vibrio cholerae*. *Molecular Microbiology,* 66: 1331–1341. https://doi.org/10.1111/j.1365-2958.2007.05984.x. PMID: 17986190.

Sokolov AA, Yakovleva NA 2008, Isolation of *Vibrio cholerae* in the Volga Delta and on the Caspian Sea Coast. *Journal of Microbiology, Epidemiology and Immunobiology*, 5: 42–47.

Wang, X *et al.* 2020, Environmental persistence and virulence potential of *Vibrio cholerae* from aquatic habitats. *Frontiers in Microbiology,* 11: Art. 580212.

Wang, R, Lou, J, Liu, J, Zhang, L, Li, J, Kan, B 2010, Antibiotic resistance of *Vibrio cholerae* O1 El Tor strains from the seventh pandemic in China, 1961–2010. *International Journal of Antimicrobial Agents*, 40: 361-364, https://doi.org/10.1016/j.ijantimicag.2012.06.010. PMID: 22867881.

WHO 2021, Antimicrobial resistance in the WHO African region: A systematic literature review. https://www.afro.who.int/sites/default/files/2021-11/Antimicrobial%20Resistance%20in%20the%20WHO%20African%20region%20a%20systematic%20literature%20review.pdf, (accessed 7 April 2025).

World Health Organization 2014, Antimicrobial Resistance: Global Report on Surveillance. WHO Press: Geneva, Switzerland.

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