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ORIGINAL RESEARCH

Phenotypic and Genetic Analysis of Antimicrobial Susceptibility in *Vibrio cholerae* Isolates Collected Between 1970 and 2024

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Purpose: This study aimed to evaluate the antimicrobial susceptibility and identify genetic determinants of resistance in Vibrio cholerae strains maintained in the culture collection of the Masgut Aikimbayev National Scientific Center for Especially Dangerous Infections (NSCEDI, Republic of Kazakhstan). The analyzed isolates were previously obtained from various environmental and laboratory sources as part of microbiological and epidemiological surveillance conducted between 1970 and 2024.

Methods: Twenty-six V. cholerae isolates representing different serogroups were analyzed using phenotypic and molecular methods. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion test and E-test against 59 antibacterial agents from major pharmacological classes. The presence of resistance genes to β-lactams and glycopeptides was examined using the BacResista GLA Real-Time PCR Detection Kit (DNA-Technology LLC, Moscow, Russia).

Results: All V. cholerae isolates demonstrated high susceptibility to key antibiotics, including doxycycline, ciprofloxacin, tetracycline, cefotaxime, and kanamycin. Sporadic intermediate resistance was observed to nalidixic acid, trimethoprim, and streptomycin. Real-time PCR screening did not detect any β-lactamase or glycopeptide resistance genes among the isolates.

Conclusion: The Vibrio cholerae strains preserved in the NSCEDI collection and isolated during 1970–2024 remain highly susceptible to first-line antibiotics and lack molecular markers of resistance. These findings confirm the continued effectiveness of current antimicrobial regimens for cholera treatment and underscore the importance of ongoing national surveillance of antimicrobial resistance to ensure preparedness and biosafety in potential outbreak situations.

Plain Language Summary: Cholera remains a serious public health threat, especially in areas with limited access to clean water. The disease is caused by the bacterium Vibrio cholerae, which can develop resistance to antibiotics over time, making treatment and outbreak control more difficult. To better understand the current situation in Kazakhstan, this study examined how V. cholerae strains isolated between 1970 and 2024 respond to commonly used antibiotics.

We tested 26 bacterial strains obtained from both patients and environmental water sources. Standard laboratory methods, including disk diffusion and real-time PCR, were used to determine antibiotic susceptibility and to detect resistance genes.

The results showed that all strains remained sensitive to key antibiotics such as doxycycline, ciprofloxacin, tetracycline, cefotaxime, and kanamycin. No genetic determinants of resistance to β-lactam or glycopeptide antibiotics were found.

These findings confirm that the antibiotics currently used in Kazakhstan remain effective for treating cholera. Continued monitoring of V. cholerae antibiotic susceptibility is essential to detect emerging resistance early, support effective treatment strategies, and strengthen epidemic preparedness.

Keywords: *Vibrio cholerae*, cholera strains, antibiotics, resistance, sensitivity

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Introduction

Cholera is a rapidly progressing and potentially fatal disease that requires prompt intervention for effective control and treatment. Without timely treatment, cholera can lead to death within hours or days, with case fatality rates exceeding 50%. However, early rehydration and antibiotic therapy can reduce mortality to below 1%.[1–4] Antibiotic resistance is a global public health concern that affects not only well-known pathogens but also less-studied organisms such as *V. cholerae*. Cholera has traditionally been treated with antibiotics such as tetracycline, doxycycline, and fluoroquinolones. However, the increasing use of these agents has led to the emergence of *V. cholerae* strains resistant to these drugs, posing significant challenges for disease management and increasing the risk of transmission.[5]

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.[6] 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.[7] For instance, resistance to tetracycline may be associated with genes encoding active efflux pumps or ribosomal protection proteins that prevent antibiotic binding.[8] 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.[9]

Thus, understanding the causes of *V. cholerae* resistance and elucidating its underlying mechanisms are essential for developing effective strategies for cholera treatment and prevention.[10]

In the Republic of Kazakhstan, cholera is not considered an endemic infection; however, certain regions retain ecological and social conditions that enable the long-term persistence of *Vibrio cholerae* in environmental reservoirs and its potential spread among the population (particularly in the Caspian Sea region and rivers of the southeastern part of the country). The population density in Kazakhstan is among the lowest in the world—7.5 persons per square kilometer. Among the first-level administrative divisions, the highest population density is observed in the southern regions of the country.

Between 1993 and 2024, more than 400 cholera cases and carriers were registered in Kazakhstan, all of which were imported from abroad.During 2022–2024, a total of 64,420 cases of acute intestinal infections were recorded in the country, of which 39,392 (61.1%) patients were tested for cholera; all laboratory results were negative.

Throughout Kazakhstan, 76,180 water samples from open and wastewater sources were examined during the same period. *Vibrio cholerae* non-O1 was isolated from 6545 samples (8.59%), and toxigenic *V. cholerae* O1 was detected in five samples (0.01%)—three *Inaba* and two *Ogawa* serovars—from the Turkistan and Mangystau regions.

In 2018, studies were conducted to investigate phenotypic markers of antibiotic resistance among *V. cholerae* isolates collected in Kazakhstan. 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.[11]

For the first time, this study identified and thoroughly examined the role of genetic and biochemical factors contributing to the development of resistance in *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 1970 and 2024, 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 26 collection strains of *V. cholerae* from different serogroups (O1 and non-O1), isolated from diagnostic laboratory samples and surface water in Almaty city and the Almaty, Turkistan, Mangystau, West Kazakhstan, and Karaganda regions of the Republic of Kazakhstan. These strains were collected between 1970 and 2024. Among them, 25 strains belonged to the V. cholerae O1 serogroup, biovar El Tor, and 1 strain was classified as V. cholerae non-O1. Specifically, 5 strains were isolated in Almaty, 1 from the Almaty region, 6 from the Turkistan region, 2 from the Mangystau region, 8 from the West Kazakhstan region, and 4 from the Karaganda 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-07-23	O1	Ogawa	*ctxAB*$^{+}$, *tcpA*$^{+}$ $^{-}$	Almaty, 2009, human
V. cholerae KZ-07-22	O1	Ogawa	*ctxAB*$^{+}$, *tcpA*$^{+}$ $^{-}$	Almaty, 2024, human
V. cholerae KZ-07-05	*non* O1	Ogawa	*ctxAB*$^{+}$, *tcpA*$^{+}$ $^{-}$	Almaty, 1998, water
V. cholerae KZ-09-15	O1	Ogawa	*ctx*$^{+}$, *ctx*$^{-}$	Turkistan, 2003, water
V. cholerae KZ-07-08	O1	Inaba	*ctx*$^{+}$, *ctx*$^{-}$	Turkistan, 2005, water
V. cholerae KZ-07-26	O1	Inaba	*ctx*$^{+}$, *ctx*$^{-}$	Turkistan, 2005, water
V. cholerae KZ-07-24	O1	Inaba	*ctx*$^{+}$, *ctx*$^{-}$	Turkistan, 2005, water
V. cholerae KZ-05-27	O1	Ogawa	*ctx*$^{+}$, *ctx*$^{-}$	Turkistan, 2010, water
V. cholerae KZ-01-12	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Turkistan, 2023, 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
V. cholerae KZ-11-06	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Karaganda, 1997, human
V. cholerae KZ-07-18	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Karaganda, 1997, human
V. cholerae KZ-13-16	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Karaganda, 1997, human
V. cholerae KZ-12-14	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Karaganda, 2000, human
V. cholerae KZ-07-19	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Almaty, 1970, human
V. cholerae KZ-02-20	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Almaty, 1970, human
V. cholerae KZ-07-11	O1	Ogawa	*ctxA*$^{-}$, *tcpA*$^{-}$	Almaty, 1971, human

Notes: *ctxA* and *ctxB* are the genes encoding the A and B subunits of the cholera toxin, respectively (cholera toxin A and B subunit genes). The *tcpA* gene encodes the main structural subunit of the toxin-coregulated pilus (toxin-coregulated pilus structural subunit gene). Symbols: + — gene present (positive for the gene, PCR positive result); $^{-}$ — gene absent or not detected (negative for the gene). *ctxAB*+ indicates that both *ctxA* and *ctxB* genes are present in the strain. *ctx*+, *ctx*$^{-}$ mean that the detection results of the *ctx* gene varied among different isolates or colonies of the same strain. The toxigenicity of a strain is assessed based on its gene profile: strains carrying *ctxA*+/*ctxB*+/*tcpA*+ genes are considered toxigenic, while those lacking *ctxA*$^{-}$/*ctxB*$^{-}$/*tcpA*$^{-}$ are classified as non-toxigenic.

Antibiotics Used in the Study

A total of 59 antibiotics belonging to different pharmacological groups were tested, including β-lactams (penicillins, cephalosporins, carbapenems, monobactams), aminoglycosides, tetracyclines, macrolides, lincosamides, fluoroquinolones, glycopeptides, sulfonamides, nitrofurans, and others. The complete list of antibiotics used in the study is presented in Table 2.

Table 2 List of Antibiotics Used in the Study (n = 59)

№	Antibiotic Group	Antibiotic Names
1	Penicillins (β-lactams)	Amoxicillinum, Oxacillinum, Piperacillinum, Ampicillinum, Azlocillinum, Ticarcillinum, Meticillinum
2	Cephalosporins (β-lactams)	Cefazolinum, Cefamandolum, Cefuroximum, Cefaclorum, Cefdinirum, Cefoperazonum, Cefotaximum, Ceftazidimum, Cefiximum, Cefepimum
3	Carbapenems (β-lactams)	Doripenum, Ertapenemum, Imipenemum/Cilastatinum, Meropenemum
4	Monobactams	Aztreonamum
5	Tetracyclines	Doxycyclinum, Oxytetracyclinum
6	Aminoglycosides	Amikacinum, Gentamycinum, Kanamycinum, Neomycinum, Sisomycinum, Streptomycinum, Tobramycinum, Netilmicinum
7	Macrolides	Erythromycinum
8	Lincosamides	Clindamycinum
9	Spectinomycins	Spectinomycinum
10	Fosfomycins	Phosphomycinum
11	Fluoroquinolones	Levofloxacinum, Ciprofloxacinum, Gatifloxacinum, Lomefloxacinum, Moxifloxacinum, Norfloxacinum, Pefloxacinum, Sparfloxacinum, Ofloxacinum
12	Glycopeptides	Vancomycinum
13	Amphenicols	Chloramphenicolum
14	Rifamycins	Rifampicinum
15	Sulfonamides and combination drugs	Sulfadiazinum, Co-trimoxazolum, Trimethoprimum
16	Nitrofurans	Furazolidonum, Nitrofuralum, Nitrofurantoinum
17	Nitroxolines	Nitroxolinum
18	Polymyxins	Polymyxinum
19	Polypeptide antibiotics	Bacitracinum
20	Quinolones (first generation)	Acidum nalidixicum
21	Other antibiotics	Novobiocinum

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.[12] 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,[13–17] and real-time PCR for screening resistance to glycopeptides and beta-lactams.[17] Reference control strains included *V. cholerae* KA-37, *E. coli* ATCC 25922, *Salmonella typhimurium* ATCC 14025, and *P. 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 1.5×10^8 CFU/mL (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

The results of the laboratory screening for the antimicrobial susceptibility and resistance profiling of *V. cholerae* strains (n = 26), isolated in the Republic of Kazakhstan between 1970 and 2024 from diagnostic laboratory and environmental sources, are presented in Table 3. Detailed data on inhibition zone diameters for 26 *Vibrio cholerae* isolates tested with 59 antibiotics on Mueller–Hinton agar are provided in Table 4.

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.

Of the 26 *V. cholerae* O1 and non-O1 strains examined, 100% (26/26) were susceptible to the following antimicrobial agents: doxycycline (30 μg), ciprofloxacin (10 μg), tetracycline (30 μg), cefotaxime (30 μg), and kanamycin (30 μg). With respect to nalidixic acid (30 μg), 65.4% (17/26) of the strains demonstrated high susceptibility, 30.8% (8/26) exhibited intermediate susceptibility, and 3.8% (1/26) showed resistance. For trimethoprim (5 μg), 73.1% (19/26) of the isolates were highly susceptible, while 26.9% (7/26) showed intermediate susceptibility.

For furazolidone (50 μg), 73.1% (19/26) of the *V. cholerae* strains were highly susceptible, while 26.9% (7/26) exhibited intermediate susceptibility. With respect to gentamicin (10 μg), 96.2% (25/26) of the isolates were highly susceptible, and 3.8% (1/26) showed intermediate susceptibility. Similarly, for chloramphenicol (10 μg) and ampicillin (25 μg), 96.2% (25/26) of the strains demonstrated high susceptibility, with 3.8% (1/26) showing intermediate susceptibility to each antibiotic.

Regarding streptomycin (300 μg), 88.5% (23/26) of the *V. cholerae* strains were highly susceptible, 7.7% (2/26) showed intermediate susceptibility, and 3.8% (1/26) exhibited low susceptibility. For rifampicin (15 μg), 96.2% (25/26) of the strains demonstrated high susceptibility, while 3.8% (1/26) showed 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 Figures 1 and 2, along with selected individual results in Figures 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 26 strains, with an average inhibition zone diameter of 11.1 mm (range: 10–18 mm).

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Table 3 Ranges and Diameters (in mm) of Growth Inhibition Zones for 26 *Vibrio cholerae* Strains Isolated in Kazakhstan Between 1970 and 2024 from Diagnostic Laboratory 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 city	5	16–40	20–27	24–29	19–28	25–28	23–28	21–29	30–40	0–33
Almaty region	1	19–39	23–24	26–30	18–29	28–29	25–26	25–26	30–35	16–28
Turkestan region	6	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
Karaganda region	4	16–40	23–28	23–0	17–29	25–29	24–25	22–27	29–40	11–33
Total	26	16.6–39.8	22.1–26.5	23.1–24.8	17.6–28.3	25.8–28.6	21.1–26.1	23–27.5	29.6–39	5.3–31.6

Table 4 Summary of Inhibition Zone Measurements Obtained During Susceptibility Testing of 26 *Vibrio cholerae* Isolates with 59 Antibacterial Agents on Mueller–Hinton Agar, in Millimeters

№	Antibiotics	Isolates																										
		KZ-07-01	KZ-07-02	KZ-07-03	KZ-12-04	KZ-07-05	KZ-11-06	KZ-09-07	KZ-07-08	KZ-13-09	KZ-07-10	KZ-07-11	KZ-01-12	KZ-17-13	KZ-12-14	KZ-09-15	KZ-13-16	KZ-09-17	KZ-07-18	KZ-07-19	KZ-02-20	KZ-19-21	KZ-07-22	KZ-07-23	KZ-07-24	KZ-07-25	KZ-07-26
1	Amoxicillinum	16	16	17	17	19	18	17	19	18	20	22	21	21	21	20	22	20	22	22	21	21	16	18	22	22	22
2	Oxacillin	21	21	21	20	22	20	22	22	21	21	16	18	22	22	22	16	16	17	17	19	18	17	19	18	20	22
3	Piperacillinum	21	21	16	18	22	22	22	16	16	17	17	19	18	17	19	18	20	22	21	21	21	20	22	20	22	22
4	Ampicillinum	21	21	21	20	22	20	22	22	21	21	16	18	22	22	22	16	16	17	17	19	18	17	19	18	20	22
5	Azlocillinum	20	22	22	21	21	16	18	22	22	22	16	16	17	17	19	18	17	19	18	20	22	21	21	21	20	22
6	Ticarcillinum	18	20	22	21	21	21	20	22	20	22	22	21	21	16	18	22	22	22	16	16	17	17	19	18	17	19
7	Meticillinum	21	21	20	22	20	22	22	21	21	16	18	22	22	22	16	16	17	17	19	18	17	19	18	20	22	21
8	Cefazolinum	30	30	30	32	31	31	33	33	33	35	36	36	36	38	39	31	31	37	37	38	36	36	32	40	40	40
9	Cefamandolum	31	31	37	37	38	36	36	32	40	40	40	30	30	30	32	31	31	33	33	33	35	36	36	36	38	39
10	Cefuroximum	33	33	33	33	35	30	30	36	36	38	39	34	40	40	39	38	35	36	32	31	30	30	30	37	38	39
11	Cefaclorum	39	34	40	40	39	38	35	36	32	31	30	30	30	37	38	39	33	33	33	33	35	30	30	36	36	38
12	Cefdinirum	39	30	38	37	37	40	30	38	38	35	35	36	34	32	32	30	30	31	31	33	33	33	32	32	36	36
13	Cefoperazonum	30	30	30	39	39	38	38	37	36	36	33	33	32	32	31	31	31	30	30	40	39	39	39	38	40	40
14	Cefotaximum	36	36	33	33	32	32	31	31	31	30	30	40	39	39	39	38	40	40	30	30	30	39	39	38	38	37
15	Ceftazidimum	38	38	36	36	36	35	35	35	32	32	32	34	34	31	31	30	30	30	30	30	34	35	36	36	39	39
16	Cefiximum	40	40	32	33	33	33	30	30	30	32	32	31	31	30	31	30	30	30	36	39	38	37	37	37	39	39
17	Cefepimum	31	31	31	32	32	36	36	35	32	33	33	33	34	38	39	38	38	37	37	40	40	40	33	30	30	30
18	Doripenemum	29	29	28	23	24	25	25	22	22	22	23	23	26	28	27	27	27	28	28	29	22	22	24	25	25	22
19	Ertapenemum	29	29	29	29	25	25	24	26	26	23	23	23	22	22	22	23	25	24	26	28	28	27	27	27	23	22
20	Imipenem/Cilastatin	22	22	22	23	25	26	28	27	28	29	22	22	24	24	24	24	25	26	26	27	27	28	28	23	22	22
21	Meropenum	22	25	25	25	26	26	23	23	23	28	27	29	29	29	27	27	28	28	28	24	22	25	25	26	28	22
22	Doxycyclinum	21	21	23	30	30	29	21	21	22	22	24	25	25	23	26	27	27	28	25	25	24	24	25	23	20	20
23	Oxytetracyclinum	25	25	25	26	26	26	27	28	28	28	29	29	30	30	25	25	25	25	25	26	26	27	27	28	25	30

(*Continued*)

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Table 4 (Continued).

№	Antibiotics	Isolates																										
		KZ-07-01	KZ-07-02	KZ-07-03	KZ-12-04	KZ-07-05	KZ-11-06	KZ-09-07	KZ-07-08	KZ-13-09	KZ-07-10	KZ-07-11	KZ-01-12	KZ-17-13	KZ-12-14	KZ-09-15	KZ-13-16	KZ-09-17	KZ-07-18	KZ-07-19	KZ-02-20	KZ-19-21	KZ-07-22	KZ-07-23	KZ-07-24	KZ-07-25	KZ-07-26
24	Amikacinum	21	21	20	20	18	18	18	21	21	19	19	19	19	19	20	25	25	25	24	24	23	22	22	18	18	21
25	Gentamycinum	21	21	25	25	25	23	23	25	24	24	24	22	22	21	21	25	25	25	22	21	21	21	23	23	25	23
26	Kanamycinum	20	20	20	20	21	21	25	25	23	22	22	22	25	25	20	20	21	21	21	22	25	24	24	24	23	23
27	Neomycinum	19	19	21	21	25	25	26	20	20	20	23	23	24	28	28	29	29	29	22	22	22	21	19	19	21	23
28	Sisomycinum	28	28	26	23	27	19	21	21	20	20	20	25	24	24	24	24	28	28	28	21	21	21	19	19	19	19
29	Streptomycinum	17	20	20	20	17	17	19	18	18	17	17	17	17	18	19	19	20	20	20	20	20	17	17	17	17	19
30	Tobramycinum	20	20	25	25	25	25	24	24	24	22	21	21	21	23	23	23	23	20	22	22	22	21	21	20	20	20
31	Clindamycinum	18	18	19	20	20	29	29	29	28	28	28	26	26	23	22	20	21	20	21	21	22	22	23	23	25	25
32	Spectinomycinum	19	30	29	22	22	20	20	28	27	26	25	23	23	24	24	29	19	19	19	21	21	21	20	20	20	23
33	Phosphomycinum	21	22	22	29	29	25	25	26	28	27	27	26	23	23	22	21	21	23	23	24	24	25	25	26	28	27
34	Chloramphenicolum	25	25	25	30	29	29	30	30	27	27	27	26	26	26	26	28	28	25	25	26	25	26	28	28	30	30
35	Levofloxacinum	29	35	35	29	31	32	32	33	34	34	34	35	35	35	35	29	29	29	31	30	30	30	31	31	31	35
36	Ciprofloxacinum	31	33	33	32	32	32	30	30	31	38	38	39	36	34	30	30	31	33	35	36	37	38	39	40	40	40
37	Gatifloxacinum	39	39	40	40	30	30	32	35	36	34	38	37	39	30	30	30	31	32	32	33	33	33	32	31	30	30
38	Lomefloxacinum	30	30	30	31	32	32	33	33	33	32	31	30	30	39	39	40	40	30	30	32	35	36	34	38	37	39
39	Moxifloxacinum	33	33	32	31	30	30	39	39	40	40	30	30	32	35	36	34	38	37	39	30	30	30	31	32	32	33
40	Norfloxacinum	30	31	32	32	33	33	33	32	31	30	30	35	35	35	35	30	30	32	35	34	30	31	31	32	35	34
41	Pefloxacinum	33	33	32	31	30	30	35	35	35	35	30	30	32	35	32	31	31	31	33	33	30	30	31	32	32	33
42	Sparfloxacinum	37	39	30	30	30	31	32	32	33	33	33	32	31	30	30	39	39	40	40	30	30	32	35	36	34	38
43	Ofloxacinum	32	34	34	34	35	35	31	39	39	39	30	31	32	35	35	35	36	36	30	30	30	32	33	33	30	32
44	Erythromycinum	20	24	25	26	24	28	25	21	20	20	20	21	25	25	23	23	24	28	27	27	26	26	26	28	28	28
45	Vancomycinum	20	21	21	21	25	25	25	25	25	23	23	23	24	24	24	24	25	25	25	25	26	26	28	27	27	28
46	Acidum nalidixicum	25	32	32	32	25	25	26	26	27	29	30	30	30	31	32	32	33	33	33	32	31	30	30	26	29	25
47	Netilmicinum	21	21	21	25	25	24	24	23	22	22	22	21	21	21	23	23	24	24	25	25	25	24	24	23	23	21
48	Rifampicinum	21	21	22	27	27	27	25	26	26	26	23	23	23	22	22	24	24	24	25	25	25	27	21	21	21	22

49	Sulfadiazinum	17	17	17	16	20	20	20	20	16	16	16	20	20	16	16	16	20	20	19	19	18	18	16	16	16	17
50	Co-trimoxazolum	20	20	20	20	16	16	16	20	20	16	16	16	20	20	19	19	18	18	16	16	16	17	17	17	17	16
51	Furazolidonum	25	25	24	18	18	18	19	19	20	20	21	21	21	22	23	23	21	21	22	22	22	18	23	23	24	25
52	Nitrofuralum	22	18	23	23	24	25	25	25	24	18	18	18	19	19	20	20	21	21	21	22	23	23	21	21	22	22
53	Nitrofurantoinum	21	21	21	22	23	23	21	21	22	22	22	18	23	23	24	25	25	25	24	18	18	18	19	19	20	20
54	Novobiocinum	22	22	21	21	21	23	24	24	25	26	28	27	29	21	21	22	25	28	29	27	27	26	24	25	25	23
55	Polymyxinum	29	27	27	22	22	21	20	20	20	23	22	22	21	21	29	21	21	20	20	23	25	26	27	28	28	28
56	Nitroxolinum	21	21	22	25	28	29	27	27	26	24	25	25	23	22	22	21	21	21	23	24	24	25	26	28	27	29
57	Bacitracinum	16	16	18	18	19	19	20	20	20	20	18	18	19	17	20	20	20	16	16	18	19	17	17	17	16	16
58	Aztreonamum	22	22	21	21	21	21	21	20	20	18	18	18	19	19	22	18	18	22	22	21	21	21	21	18	18	18
59	Trimethoprimum	18	18	19	20	20	21	22	22	25	25	25	23	23	23	24	24	24	18	19	19	19	19	20	21	21	22

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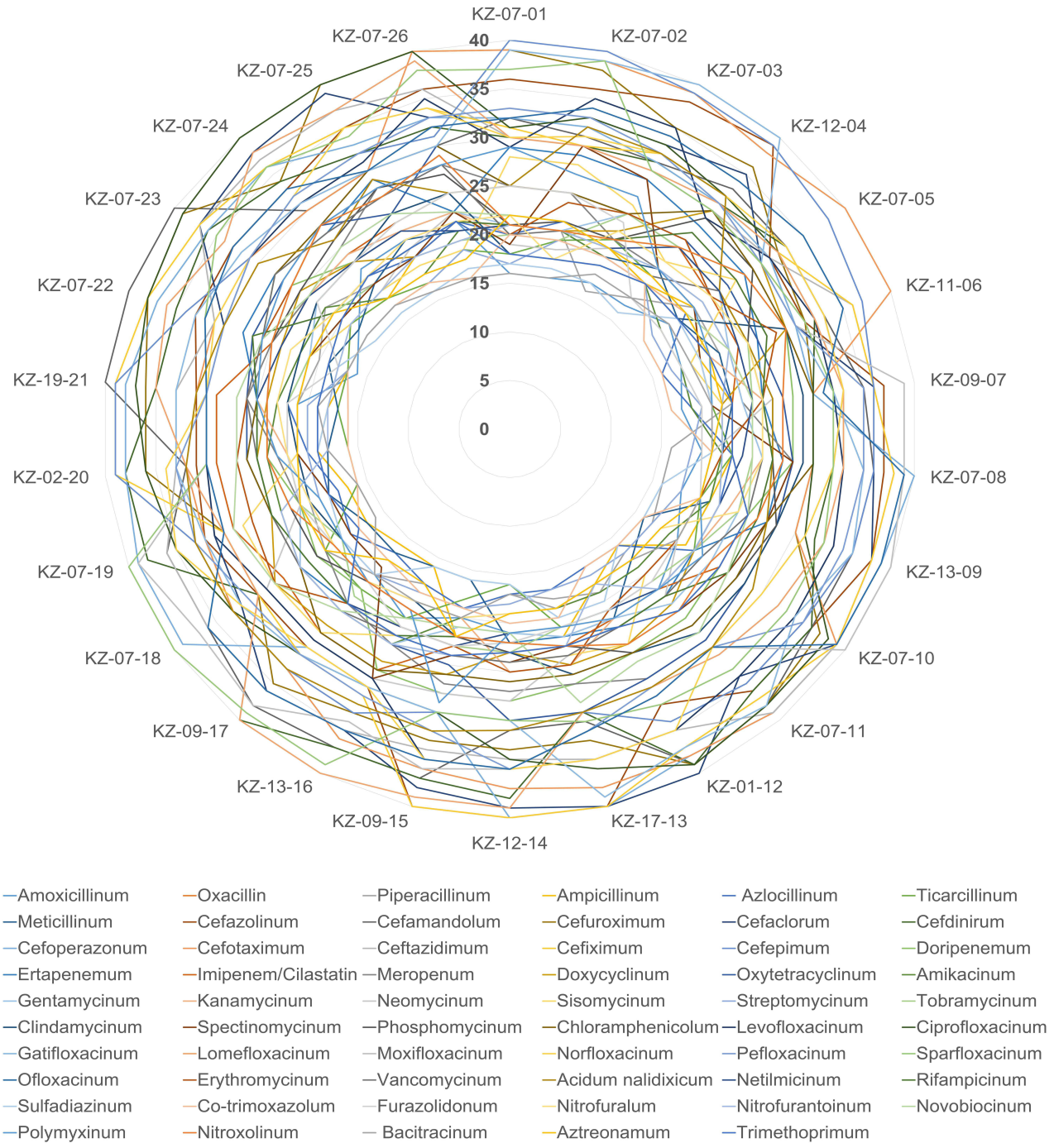

Figure 1 Inhibition zone diameters for 26 *Vibrio cholerae* isolates tested against 59 antibiotics on Hottinger agar, in millimeters.

An in vitro phenotypic susceptibility analysis of *V. cholerae* (n = 26) 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 (Figure 4).

The results of the antimicrobial resistance gene detection in 26 *V. cholerae* strains using the BacResista GLA Real-Time PCR Detection Kit with real-time PCR are presented in Table 5 and Figure 5.

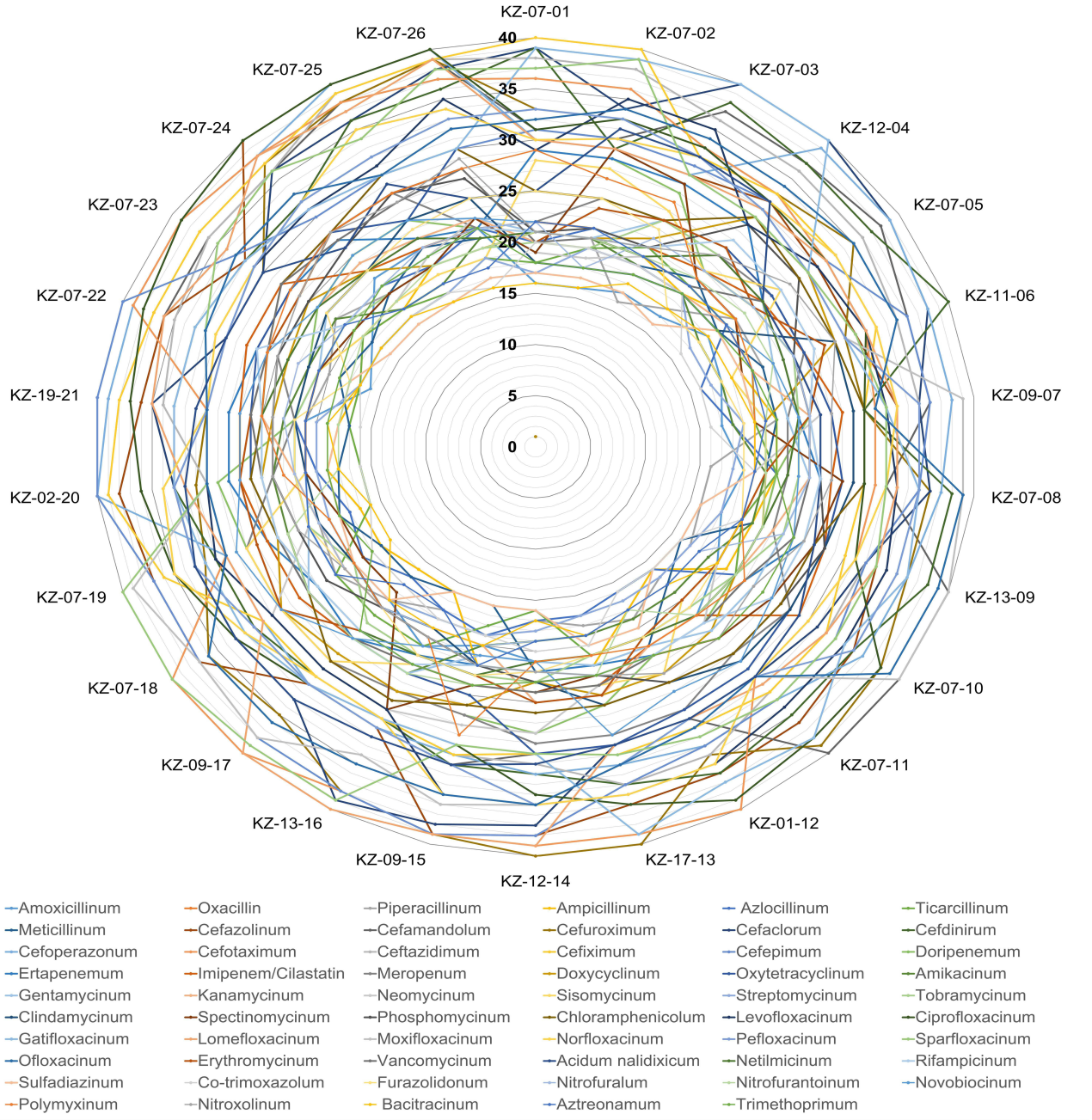

Figure 2 Inhibition zone diameters for 26 *Vibrio cholerae* isolates tested against 59 antibiotics on Mueller–Hinton agar, in millimeters.

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 26 *V. cholerae* isolates, including strains originally obtained during diagnostic procedures and from environmental sources in Kazakhstan between 1970 and 2024, 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

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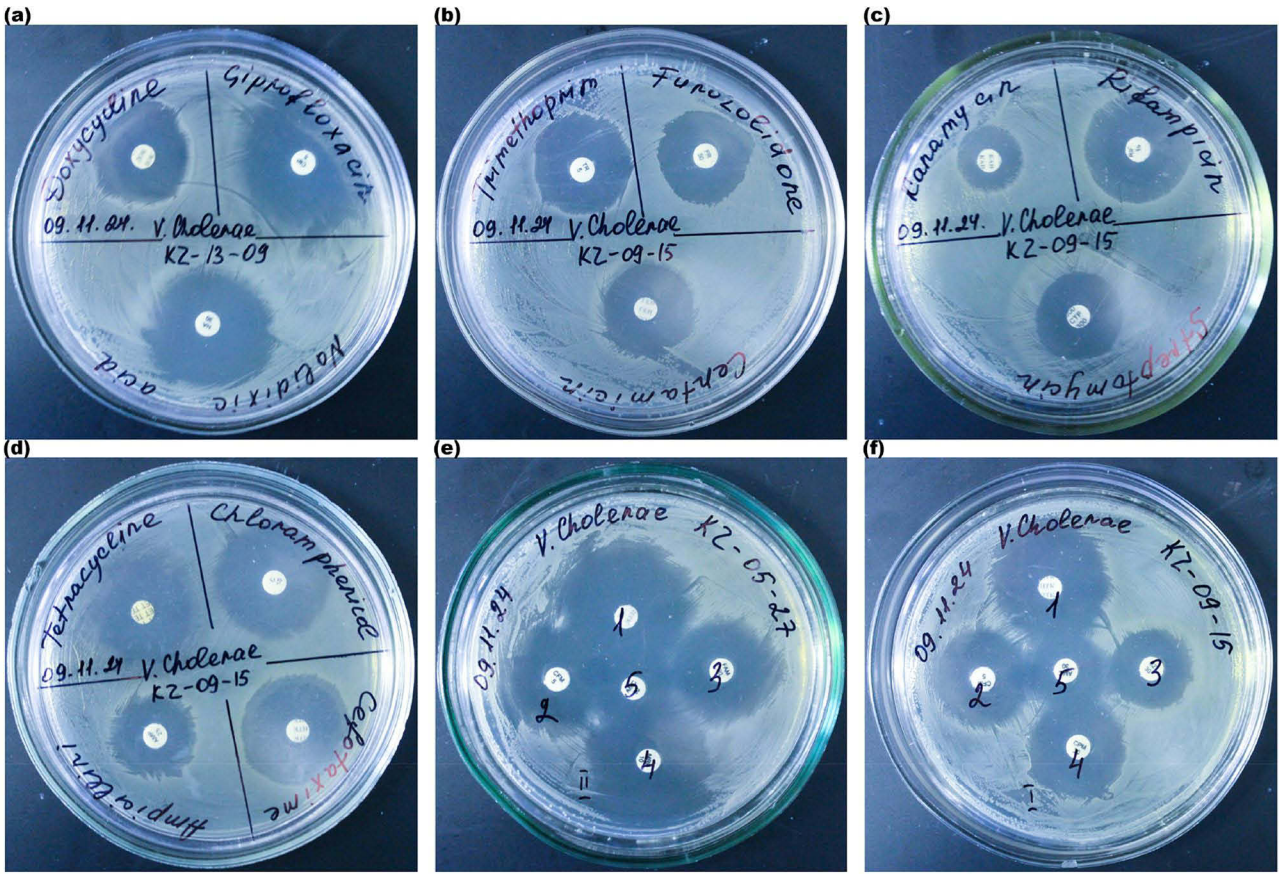

Figure 3 Inhibition zone diameters obtained using the disk diffusion assay and phenotypic detection of ESBL resistance. (**a**) Inhibition zones for *Doxycycline, Ciprofloxacin*, and *Nalidixic acid*. (**b**) Inhibition zones for *Trimethoprim, Furazolidone*, and *Gentamicin*. (**c**) Inhibition zones for *Kanamycin, Rifampicin*, and *Streptomycin*. (**d**) Inhibition zones for *Tetracycline, Chloramphenicol, Cefotaxime*, and *Ampicillin*. (**e**) Phenotypic evaluation showing no detectable ESBL resistance genes; assessed with *Trimethoprim, Furazolidone, Gentamicin, Tetracycline*, and *Chloramphenicol*. (**f**) Phenotypic evaluation showing no detectable ESBL resistance genes; assessed with *Cefotaxime, Ampicillin, Streptomycin, Kanamycin*, and *Rifampicin*. The Petri dish has a visual diameter of 35 cm.

control strains used in this study included *E. coli* ATCC 25922, *S. typhimurium* ATCC 14025, and *P. 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).

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.

Discussion

This study provides a comprehensive phenotypic and molecular assessment of antimicrobial susceptibility in *Vibrio cholerae* isolates preserved in the reference collection of the Masgut Aikimbayev National Scientific Center for Especially Dangerous Infections (NSCEDI). The isolates, obtained between 1970 and 2024 during long-term microbiological surveillance in Kazakhstan, demonstrated consistently high sensitivity to major antibiotic classes and complete absence of β-lactamase or glycopeptide resistance genes. These findings contrast with global patterns of increasing multidrug resistance in *V. cholerae* and emphasize the unique epidemiological context of Central Asia.

Phenotypic testing confirmed that all *V. cholerae* isolates remained fully susceptible to doxycycline, ciprofloxacin, tetracycline, cefotaxime, and kanamycin — agents recommended by the WHO for cholera treatment.[18] Only sporadic

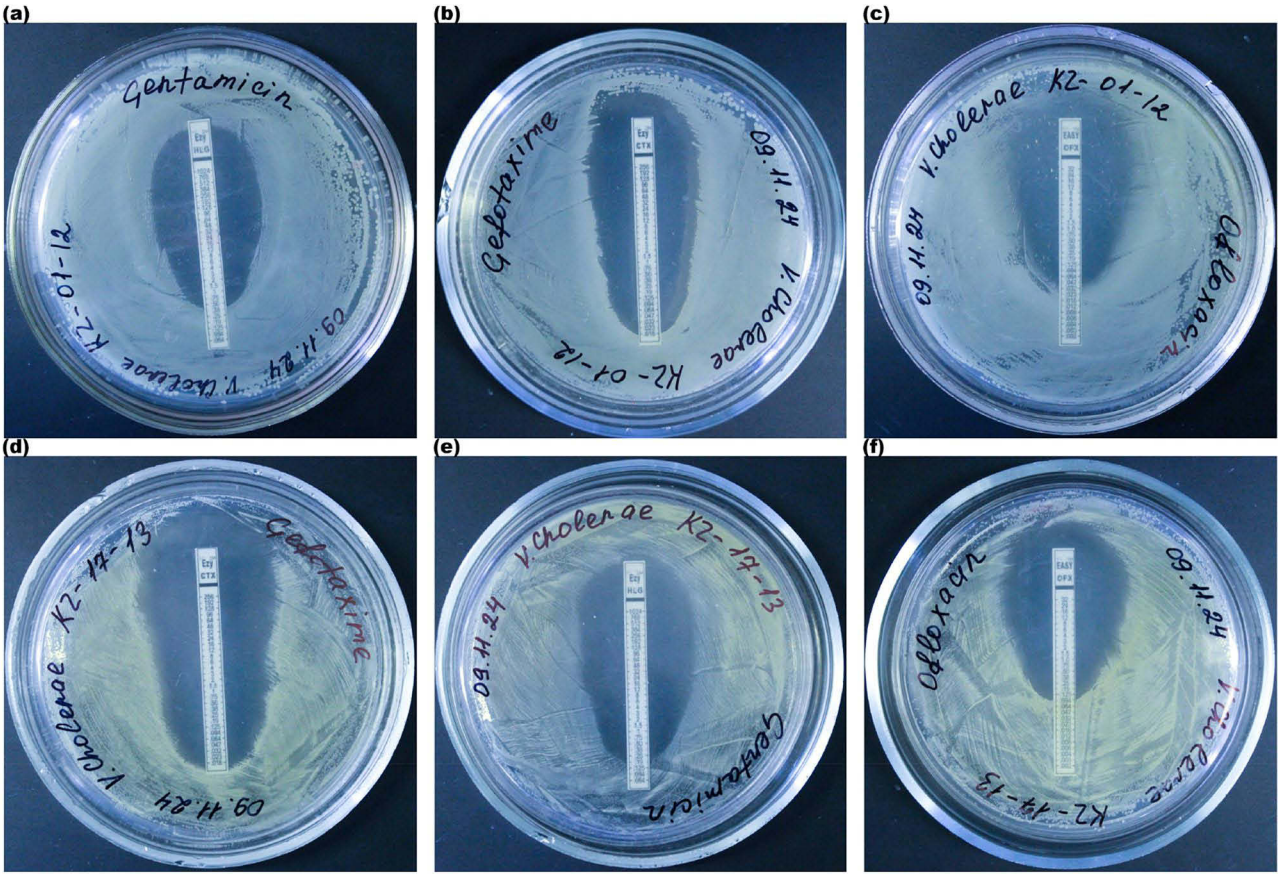

Figure 4 Results of susceptibility testing using the E-test method. (**a–f**) show the susceptibility of two different *Vibrio cholerae* strains to gentamicin, cefotaxime, and ofloxacin. (**a–c**) correspond to Strain 1, while (**d–f**) correspond to Strain 2. Multiple strains were tested using the same antibiotic strips, which explains the repeated use of gentamicin, cefotaxime, and ofloxacin. (**a**) Susceptibility of Strain 1 to *Gentamicin*. (**b**) Susceptibility of Strain 1 to *Cefotaxime*. (**c**) Susceptibility of Strain 1 to *Ofloxacin*. (**d**) Susceptibility of Strain 2 to *Gentamicin*. (**e**) Susceptibility of Strain 2 to *Cefotaxime*. (**f**) Susceptibility of Strain 2 to *Ofloxacin*.

intermediate susceptibility to nalidixic acid, streptomycin, and trimethoprim was detected. Molecular screening revealed no resistance determinants, including vanA/B, mecA, tem, ctx-M-1, shv, oxa, imp, kpc, or ndm, indicating that these isolates lack mobile genetic elements associated with extended-spectrum β-lactamases (ESBLs) and carbapenemases.

Comparable studies from endemic regions show markedly different results. Investigations in India and China have reported the widespread dissemination of SXT integrative elements, class I integrons, and resistance genes such as sulII, dfrA1, and strB, conferring resistance to trimethoprim–sulfamethoxazole, nalidixic acid, and tetracycline.[19–21] Similarly, in sub-Saharan Africa and Haiti, multidrug-resistant *V. cholerae* O1 El Tor lineages have emerged, carrying ctxB7, sul2, and floR genes.[4,22,23] These differences may reflect contrasting selective pressures: while clinical environments in endemic regions expose bacteria to continuous antibiotic use, the Kazakhstani isolates were predominantly environmental, recovered from surface water, sediments, and monitoring sites with minimal human antibiotic influence.

The absence of resistance genes in our isolates suggests that *V. cholerae* populations in Kazakhstan have retained their ancestral antibiotic susceptibility. This may be due to limited antibiotic exposure in aquatic ecosystems, low rates of horizontal gene transfer, and the relatively isolated character of natural foci in arid and semi-arid zones. Comparable findings have been reported from environmental isolates in the Ganges Delta and East Africa, where non-O1/non-O139 strains showed high sensitivity to most antibiotics.[24,25] Such ecological stability supports the hypothesis that environmental *Vibrio* populations act as reservoirs of sensitive genotypes, contrasting with epidemic clones under strong selective pressure.[7]

Clinically, these results confirm the sustained efficacy of standard first-line agents—doxycycline, ciprofloxacin, and cefotaxime—in cholera management.[26] Epidemiologically, the findings highlight the importance of continued laboratory-

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Table 5 Results of Antimicrobial Resistance Gene Detection in 26 *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	***E. coli* ATCC 25922**	***S. typhimurium ATCC 14,025***	***P. 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

Notes: * TBM—total bacterial mass; ** IC—internal control; *** KZ-00-00—average value based on results from 26 *V. cholerae* strains.
Abbreviations: TBM, total bacterial mass; IC, internal control.

based resistance monitoring, particularly in regions at risk of pathogen reintroduction through transboundary water systems or human mobility. Although no resistance determinants were detected, vigilance remains essential, as international travel and food trade can introduce resistant *Vibrio* strains from endemic areas.[27,28]

This study has certain limitations. The dataset included 26 archival isolates representing multiple decades, yet it may not capture all genetic diversity of *V. cholerae* circulating in Central Asia. Whole-genome sequencing of both historical and newly isolated strains would allow for finer resolution of evolutionary trends, virulence determinants, and potential resistance acquisition pathways. Future work should also investigate environmental variables—temperature, salinity, and organic matter content—that influence *V. cholerae* persistence and potential adaptation to antibiotic stress.[14,29]

In conclusion, the *Vibrio cholerae* isolates collected in Kazakhstan between 1970 and 2024 exhibit no genetic markers of antimicrobial resistance and remain highly susceptible to key therapeutic drugs. These data provide essential reference

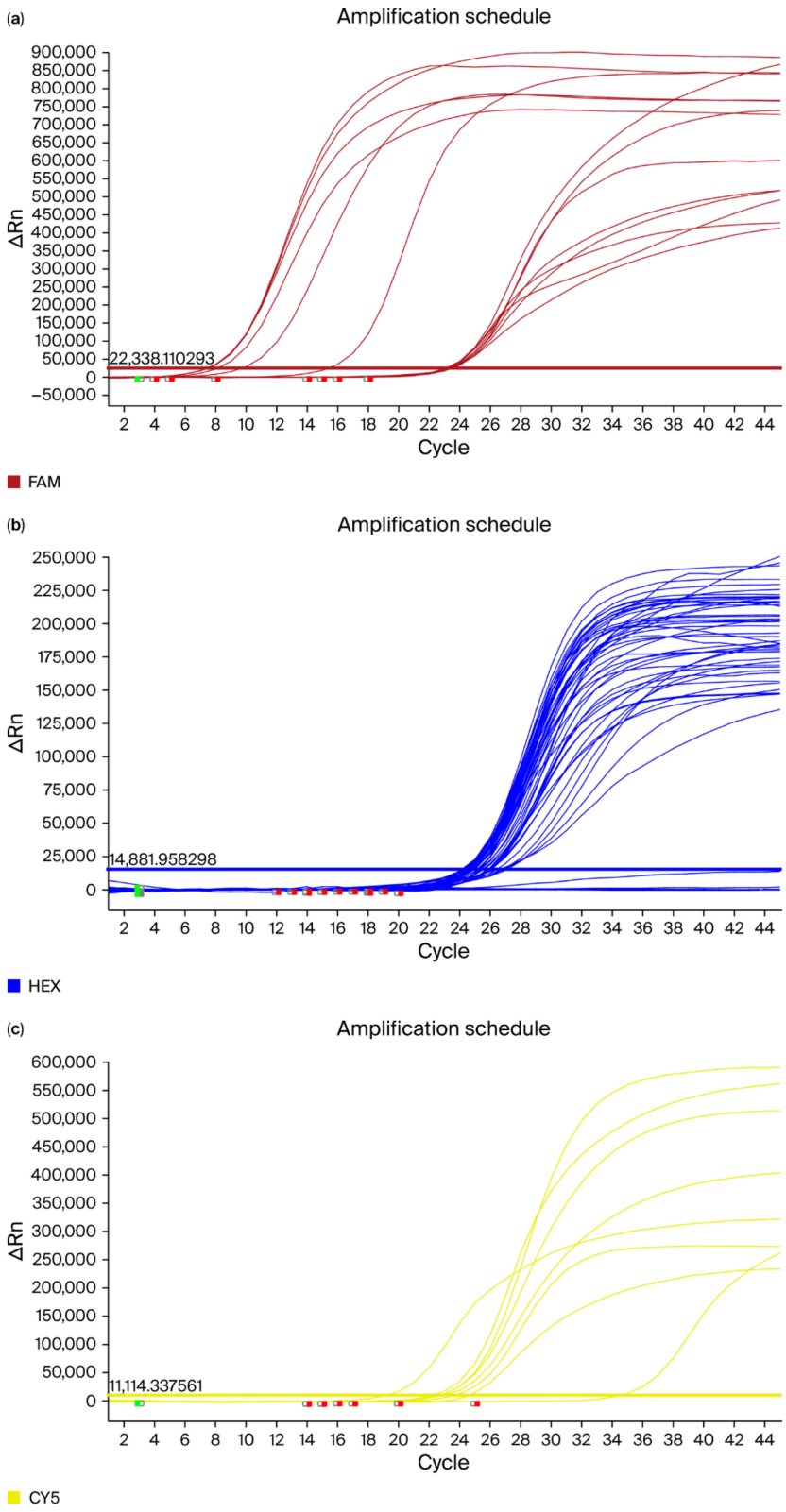

Figure 5 Amplification curves by fluorophore detection channels: (**a**) FAM; (**b**) HEX; (**c**) CY5. Red and green dots represent PCR amplification curves of different samples. Red dots indicate positive amplification signals, while green dots indicate negative or no amplification.

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values for national and regional surveillance programs and reinforce the global need for sustained monitoring to prevent the emergence of resistant *V. cholerae* lineages.

Conclusions

This study provides the first comprehensive phenotypic and molecular characterization of *Vibrio cholerae* O1 isolates collected in Kazakhstan between 1970 and 2024. The analysis demonstrated a generally high level of antimicrobial susceptibility, with all isolates remaining sensitive to key therapeutic agents such as doxycycline, ciprofloxacin, tetracycline, cefotaxime, and kanamycin. Occasional intermediate susceptibility was observed to nalidixic acid, trimethoprim, furazolidone, and streptomycin.

No resistance genes to β-lactam or glycopeptide antibiotics were detected using real-time PCR, suggesting that, within this limited sample, genetic determinants of resistance were not present. These findings indicate that the studied isolates largely retained susceptibility to standard cholera treatment drugs; however, the results should be interpreted with caution given the restricted number of strains analyzed and their archival origin.

Although no multidrug-resistant or gene-bearing isolates were identified, the possibility of resistance emergence through horizontal gene transfer or importation from endemic regions cannot be excluded. Therefore, sustained epidemiological and molecular surveillance remains essential to ensure early detection of resistance trends and to support evidence-based cholera control strategies.

Ethics Approval and Informed Consent

In accordance with the Declaration of Helsinki (2013), the CIOMS/WHO International Ethical Guidelines (2016), and the Order of the Ministry of Health of the Republic of Kazakhstan No. ҚР ДСМ-16 (2020) "On the approval of the rules for conducting biomedical research", the use of anonymized archival microbial strains obtained during routine diagnostics does not require additional ethics committee approval.

Acknowledgment

This paper has been uploaded to ResearchSquare as a preprint: https://www.researchsquare.com/article/rs-6902069/v1

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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