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Integrated Phenotypic, Molecular, and Genomic Analysis of Antimicrobial Resistance in *Yersinia pestis* Isolates from Natural Plague Foci of Kazakhstan

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Abstract

Plague remains a globally significant zoonotic infection maintained in natural foci, with ongoing epizootic activity and periodic human cases reported in different regions of the world. Continuous monitoring of antimicrobial susceptibility of *Yersinia pestis* is essential due to the potential emergence and spread of resistant strains. A total of 75 *Yersinia pestis* isolates, including clinical and epizootic strains obtained from plague outbreaks in Kazakhstan, were analyzed. Antimicrobial susceptibility was evaluated using standard phenotypic methods, and molecular screening for resistance determinants was performed by real-time PCR. Genome-level analysis based on whole-genome sequencing (WGS) data from the NCBI BioProject PRJNA1249055 was conducted to assess the presence of acquired antimicrobial resistance genes and chromosomal mutations associated with resistance. All isolates demonstrated high susceptibility to clinically relevant antibiotics. No resistance genes were detected by molecular screening. Genome-based analysis confirmed the absence of acquired antimicrobial resistance determinants, resistance-associated mutations in key loci (*rpsL*, *gyrA*, *parC*), and plasmid-mediated resistance mechanisms. Minor lineage-associated variation in *phoP* was identified in a limited number of isolates and was not associated with antimicrobial resistance. These findings indicate a stable antimicrobial susceptibility profile of *Yersinia pestis* in Kazakhstan and confirm the absence of emerging resistance despite long-term circulation in natural plague foci. The results highlight the importance of integrated surveillance and support the continued effectiveness of current therapeutic strategies for plague.

Keywords: plague; *Yersinia pestis*; antimicrobial susceptibility; antimicrobial resistance; resistome; whole-genome sequencing

1. Introduction

Plague remains one of the most historically significant and epidemiologically important zoonotic infections caused by *Yersinia pestis* (Lehmann and Neumann, 1896) [1,2]. Despite advances in antimicrobial therapy and public health measures, the pathogen persists in natural foci across multiple regions, including Central Asia, Africa, and the Americas [3,4]. The infection is maintained in complex ecological systems involving wild rodents as reservoirs and fleas as vectors, forming long-term enzootic cycles with periodic epizootic activity [2,5].

Antimicrobial resistance (AMR) is recognized as one of the leading threats to global public health and socio-economic development. The World Health Organization (WHO) has identified AMR among the top ten global health risks facing humanity [6,7]. Despite the proven effectiveness of

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antibiotics in reducing mortality from infectious diseases, their widespread and often uncontrolled use has accelerated the emergence and dissemination of resistant microorganisms, posing a major global challenge [8]. According to current estimates, antimicrobial resistance is already responsible for approximately 1.27 million deaths annually, and its burden is expected to increase substantially in the coming years [9].

The global epidemiological situation of plague remains of concern, with ongoing activity in endemic regions and no clear trend toward reduction in incidence.

Kazakhstan represents one of the largest plague-endemic territories globally, with extensive natural foci occupying diverse ecological zones such as deserts, semi-deserts, and highland regions [10–12]. Historically, plague has had a significant impact on public health in Kazakhstan, with large outbreaks and epidemics reported in the past. The last human case was registered in 2003 [13,14], reflecting the effectiveness of long-term surveillance and preventive measures. However, ongoing epizootic activity in natural reservoirs indicates the continued circulation of the pathogen and the potential risk of re-emergence [15,16].

The emergence of antimicrobial resistance in *Yersinia pestis* remains a significant global concern. In endemic regions such as Madagascar, multidrug-resistant strains associated with plasmid-mediated resistance determinants have been reported, whereas in other regions, resistance has been linked to chromosomal mutations [17,18]. These findings underscore the need for continuous monitoring of antimicrobial susceptibility, particularly in countries with extensive natural plague foci.

In Kazakhstan, within the framework of national and international antimicrobial resistance surveillance programs (e.g., WHO, GLASS, EARS-Net), and with the support of the World Health Organization, a national AMR surveillance system has been established, along with the implementation of further measures in accordance with the One Health approach [19].

A recent study reported a meta-analysis of human plague cases in Kazakhstan covering the period 1926–2003 [14], providing a foundation for further investigations. Prior to the introduction of antibiotic therapy [20,21], major plague outbreaks were recorded in Kazakhstan up to 1948, accounting for 80.7% of all reported cases [14].

Modern surveillance systems increasingly rely on integrated approaches combining epidemiological monitoring, laboratory diagnostics, and genomic analysis. Whole-genome sequencing (WGS) has become a powerful tool for understanding pathogen evolution, tracking transmission, and identifying antimicrobial resistance determinants [22,23].

The present study aimed to assess the antimicrobial resistance profile of *Y. pestis* isolates from natural plague foci of Kazakhstan using a comprehensive approach integrating phenotypic testing, molecular screening, and whole-genome sequencing data. The results provide insights into the current status of antimicrobial susceptibility and contribute to the development of effective surveillance strategies.

2. Materials and Methods

2.1. Bacterial Strains and Study Design

A total of 75 *Yersinia pestis* strains were included in the study. The strain collection comprised 61 clinical isolates obtained from patients and deceased individuals during plague outbreaks in Kazakhstan between 1926 and 2003, as well as 14 isolates recovered from animal hosts and vectors in natural plague foci in more recent years. All strains were obtained from the National Working Collection and the Microorganism Depository of the M.Aikimbayev National Scientific Center for Especially Dangerous Infections.

The study was designed as a comprehensive analysis integrating previously obtained phenotypic and molecular data with whole-genome sequencing (WGS)-based resistome profiling.

The overall study design integrating phenotypic, molecular, and genomic approaches is presented in Figure 1.

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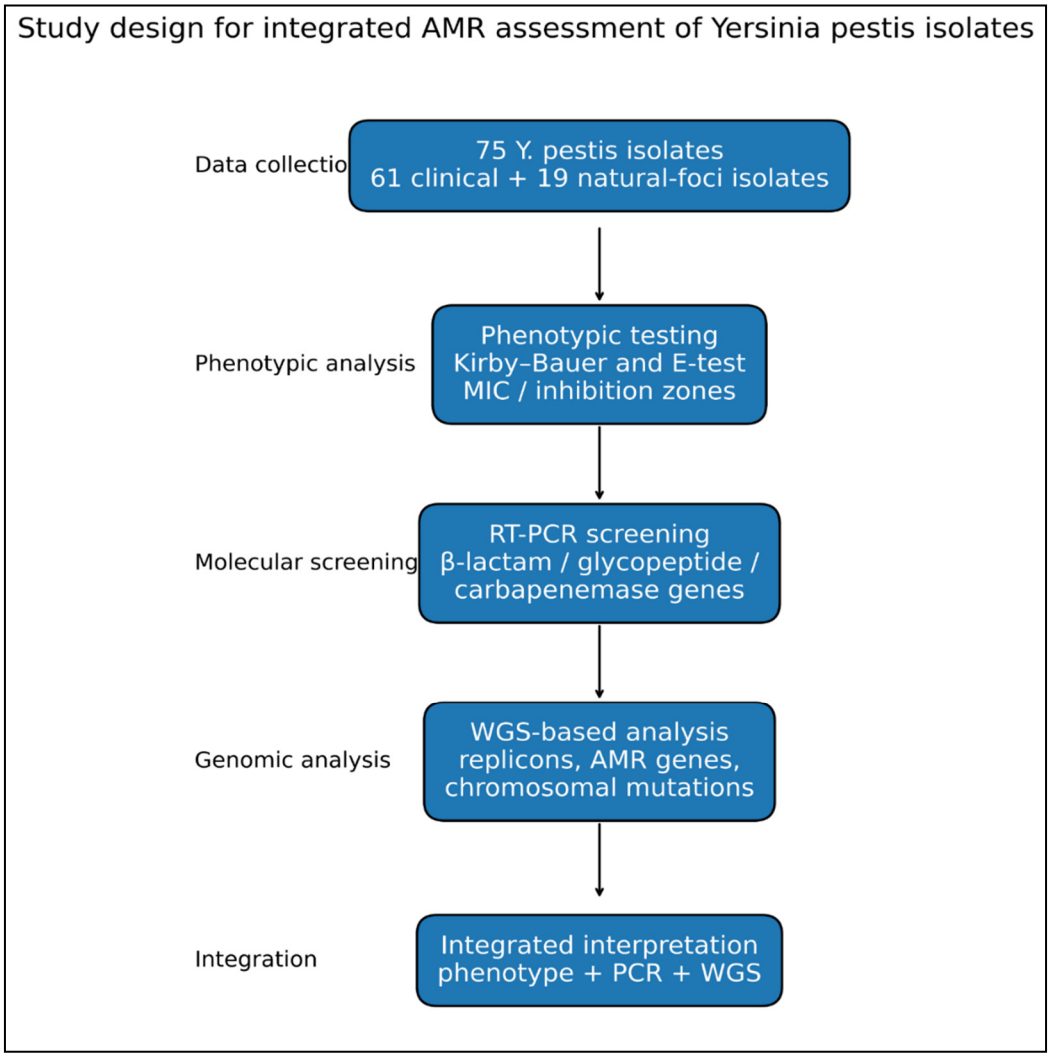

Figure 1. Study design for integrated antimicrobial resistance assessment of *Yersinia pestis* isolates. The workflow includes data collection, phenotypic susceptibility testing (Kirby–Bauer and MIC determination), molecular screening by RT-PCR, whole-genome sequencing-based resistome analysis, and integrated interpretation of phenotypic, molecular, and genomic data.

2.2. Culture Conditions and Identification

Strains were cultured on Mueller–Hinton agar (pH 7.3 ± 0.2) and Hottinger agar (pH 7.2 ± 0.1) at incubation temperatures ranging from 28 °C to 37 °C [24].

Taxonomic identification was performed using the automated system VITEK 2 Compact 30 (bioMérieux, France), which обеспечивал высокую точность идентификации штаммов *Y. pestis*.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was evaluated using two complementary phenotypic methods:

– Kirby–Bauer disk diffusion method
– E-test gradient diffusion method

Testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Bacterial suspensions were standardized to 0.5 McFarland (≈1.5 × 10^8 CFU/mL), inoculated onto agar plates, and incubated at 28 °C. Inhibition zones were measured after 24–48 hours. The tested antibiotic classes included: β-lactams, tetracyclines, aminoglycosides, amphenicols, glycopeptides,

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quinolones, lincosamides, macrolides. Minimum inhibitory concentrations (MICs) were determined using E-test strips [24–26].

2.4. Phenotypic Detection of Resistance Mechanisms

Extended-spectrum β-lactamase (ESBL) production was assessed using standard phenotypic methods based on inhibition zone analysis and confirmatory tests. Quality control strains included: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Staphylococcus aureus* ATCC 372, *Pseudomonas aeruginosa* ATCC 377 [24–26].

2.5. Molecular Detection of Resistance Genes (RT-PCR)

Screening for antibiotic resistance genes was performed using real-time PCR (RT-PCR) with the BacResista GLA Detection Kit (DNA-Technology LLC, Russia). The following gene groups were targeted: β-lactam resistance (*tem, ctx-M-1, shv*), carbapenemases (*kpc, ndm, vim, imp, oxa variants*), glycopeptide resistance (*vanA, vanB*), methicillin resistance (*mecA*). Amplification was monitored in FAM, HEX, and CY5 channels, and Ct values were used for interpretation of results [27,28].

2.6. Whole-Genome Sequencing Data and Resistome Analysis

Whole-genome sequencing (WGS) data for 75 Yersinia pestis strains were obtained from the NCBI BioProject PRJNA1249055. Genome assemblies and annotated files (FASTA, GBFF, FAA) were used for downstream resistome analysis. Genome-based analysis was performed using a bioinformatic approach combining annotation screening and sequence-based comparison with established antimicrobial resistance databases.

2.6.1. Detection of Acquired AMR Genes

Screening for acquired antimicrobial resistance genes was performed using sequence similarity-based approaches with reference databases, including the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder. Genome annotations were screened for known resistance determinants, and sequence comparison was performed using BLAST-based methods to identify homologs of characterized antimicrobial resistance genes.

2.6.2. Identification of Chromosomal Resistance-Associated Mutations

Key chromosomal loci associated with antimicrobial resistance were analyzed, including:

- rpsL (streptomycin resistance)
- gyrA and parC (fluoroquinolone resistance)
- regulatory genes (pmrA, pmrB, phoP, phoQ)

Amino acid sequences of these genes were extracted from annotated genomes and aligned to assess sequence conservation and to detect substitutions at canonical resistance-associated positions.

2.6.3. Plasmid Analysis

Plasmid content was evaluated based on genome assembly annotations to identify known virulence-associated plasmids (pCD, pMT1, pPCP) and to screen for potential plasmid-mediated antimicrobial resistance determinants. Additional plasmid replicons were assessed for similarity to known resistance-associated plasmids.

2.6.4. Comparative Analysis

Comparative genomic analysis was performed to assess the distribution of resistance-associated determinants across isolates from different natural plague foci, host species, and time periods.

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2.7. Statistical Analysis

Descriptive statistics were used to summarize phenotypic susceptibility data, including mean values, standard deviations, and MIC50/MIC90.

Comparisons between groups were performed using non-parametric tests (Mann-Whitney U test), with statistical significance set at $p < 0.05$.

3. Results

3.1. General Characteristics of the Studied Strains

A total of 75 *Yersinia pestis* isolates originating from diverse natural plague foci of Kazakhstan were included in the analysis. The collection comprised both historical clinical isolates (1926–2003) and more recent strains obtained from animal reservoirs and vectors.

The phenotypic antimicrobial susceptibility data presented in this study have been previously reported and are further analyzed here in the context of integrated resistome assessment [29]. Biochemical profiling confirmed that all isolates exhibited a stable and characteristic phenotype consistent with *Y. pestis*, supporting the reliability of subsequent antimicrobial susceptibility and molecular analyses.

The geographic distribution and characteristics of the studied isolates are summarized in Table 1.

Table 1. Geographic origin, plague natural foci, principal hosts and vectors, years of human plague case registration, and *Yersinia pestis* isolates included in the screening for glycopeptide and β-lactam resistance genes.

Region	**Natural Plague Focus**	**Years of Human Case Isolations**	***Yersinia pestis* Isolates**
Atyrau	Ural–Emba	1956, 1958, 1964, 1968, 1986, 1988, 1989, 1990, 1992, 1993	KZ-23-18, KZ-24-18, KZ-25-18, KZ-26-18, KZ-22-18, KZ-19-18, KZ-20-18, KZ-21-18
Atyrau	Volga–Ural Sand	1997	KZ-30-22, KZ-29-17, KZ-50-17, KZ-39-15
Atyrau, Mangystau	Pre-Ustyurt	1958, 1959, 1961, 1967, 1975	KZ-27-19, KZ-48-19, KZ-49-19
Mangystau, Aktobe	Ustyurt	1926, 1974, 1975, 1999	KZ-51-16, KZ-28-20, KZ-40-20, KZ-41-20
Aktobe, Kyzylorda	North Pre-Aral	1945,1993, 1999, 2002	KZ-32-21, KZ-33-21, KZ-34-21, KZ-35-21, KZ-54-21, KZ-55-21, KZ-36-21, KZ-37-21, KZ-38-21, KZ-31-21
Mangystau	Mangystau	1926, 1927, 1948, 1964, 1973, 1974, 2003	KZ-42-23, KZ-43-23, KZ-44-23, KZ-53-22, KZ-45-23, KZ-46-23, KZ-47-23
Aktobe, Kyzylorda	Pre-Aral–Karakum	1947, 1948, 1955, 1959, 1966, 1967, 1969, 1971, 1972, 1979, 1990, 1991, 1999, 2001, 2003	KZ-10-24, KZ-11-24, KZ-12-24, KZ-13-24, KZ-14-24, KZ-15-24, KZ-61-24, KZ-16-24
Kyzylorda	Kyzylkum	1966, 1971,1993, 1999	KZ-04-27, KZ-05-27, KZ-03-27, KZ-06-27, KZ-60-27, KZ-07-27, KZ-08-27, KZ-09-27

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Almaty	Pre-Balkhash	1947, 1948, 1989	KZ-57-30, KZ-58-30, KZ-59-30, KZ-01.30, KZ-56-30, KZ-02-30,	
Almaty	Ili Intermountain	1929	KZ-17-46, KZ-52-46, KZ-18-46	
Region	**Natural Plague Focus**	**Source of isolation of *Yersinia pestis***	**The years of isolation of *Yersinia pestis***	**Isolates *Yersinia pestis***
Zhambyl	Moyinkum	*R. opimus*	2004, 2012	KZ-66-28, KZ-67-28
Zhambyl	Betpak–Dala	*M. meridianus*	2005, 2009	KZ-68-42, KZ-69-42
Almaty	Taukum	*R. opimus*	2004, 2010	KZ-70-29, KZ-71-29
Kyzylorda	Aryskum–Dariyalyk	*R. opimus*	2007, 2011	KZ-72-22, KZ-73-22
Almaty	Prialakol	*R. opimus*	2005, 2008	KZ-74-45, KZ-75-45
Almaty	Sarydjaz highland	*Marmota baibacina*	2007, 2009	KZ-62-31, KZ-63-31
Zhambyl	Talas highland	*M. caudata*	2011, 2012	KZ-64-40, KZ-65-40

The dataset includes isolates from both human cases and natural reservoirs, reflecting the diversity of ecological niches and long-term circulation of *Y. pestis* in Kazakhstan.

3.2. Phenotypic Susceptibility to Antimicrobial Agents

Phenotypic testing demonstrated a uniformly high level of susceptibility of *Y. pestis* isolates to the majority of clinically relevant antibiotic classes.

All strains (100%) were susceptible to: β-lactam antibiotics, tetracyclines, aminoglycosides, amphenicols, glycopeptides, lincosamides end fluoroquinolones.

Overall susceptibility across all tested antimicrobial groups reached 97.5%, indicating the preservation of antibiotic effectiveness across the studied population.

In contrast, markedly reduced activity was observed for macrolides, with effectiveness ranging from complete inactivity to moderate inhibition (0–58%), reflecting the intrinsic resistance of Gram-negative bacteria to this class.

Analysis of inhibition zone diameters revealed a narrow and consistent range across isolates, with no statistically significant deviations or outliers. This homogeneity indicates the absence of subpopulations with reduced susceptibility or emerging resistance phenotypes.

The overall distribution of phenotypic susceptibility across major antimicrobial classes is summarized in Table 2. The results indicate a uniformly high level of susceptibility, with no detectable shifts in inhibition zone diameters or evidence of emerging resistant subpopulations.

Table 2. Summary of phenotypic susceptibility of *Yersinia pestis* isolates to major antimicrobial classes.

Antibiotic class	Phenotypic result	Quantitative summary	Interpretation
β-lactams	100% susceptible	Inhibition zone range 23.2–39.8 mm	High activity preserved
Tetracyclines	100% susceptible	21.0–27.3 mm	Retained activity of first-line drugs

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Aminoglycosides	100% susceptible	18.8–27.8 mm	No phenotypic evidence of resistance
Amphenicols	100% susceptible	23.1–26.3 mm	Preserved susceptibility
Glycopeptides	100% susceptible	21.2–25.9 mm	No evidence of resistance determinants in screened panel
Lincosamides	100% susceptible	21.5–25.9 mm	Uniform susceptibility pattern
Quinolones / fluoroquinolones	100% susceptible	28.8–36.7 mm	High activity, including ciprofloxacin
Other antibiotic classes combined	97.5% overall susceptibility	14.2–38.9 mm	Broadly preserved activity
Macrolides	Low activity	0.0–58.0% activity	Consistent with expected low efficacy against Gram-negative bacteria

Overall, the phenotypic data indicate a stable susceptibility profile of *Y. pestis* isolates across all major antibiotic classes. The absence of variability in inhibition zones and MIC values suggests a lack of emerging resistance within the studied population.

As shown in Figure 2, inhibition zone diameters demonstrated low variability across all antibiotic classes, indicating a homogeneous susceptibility profile among the analyzed isolates.

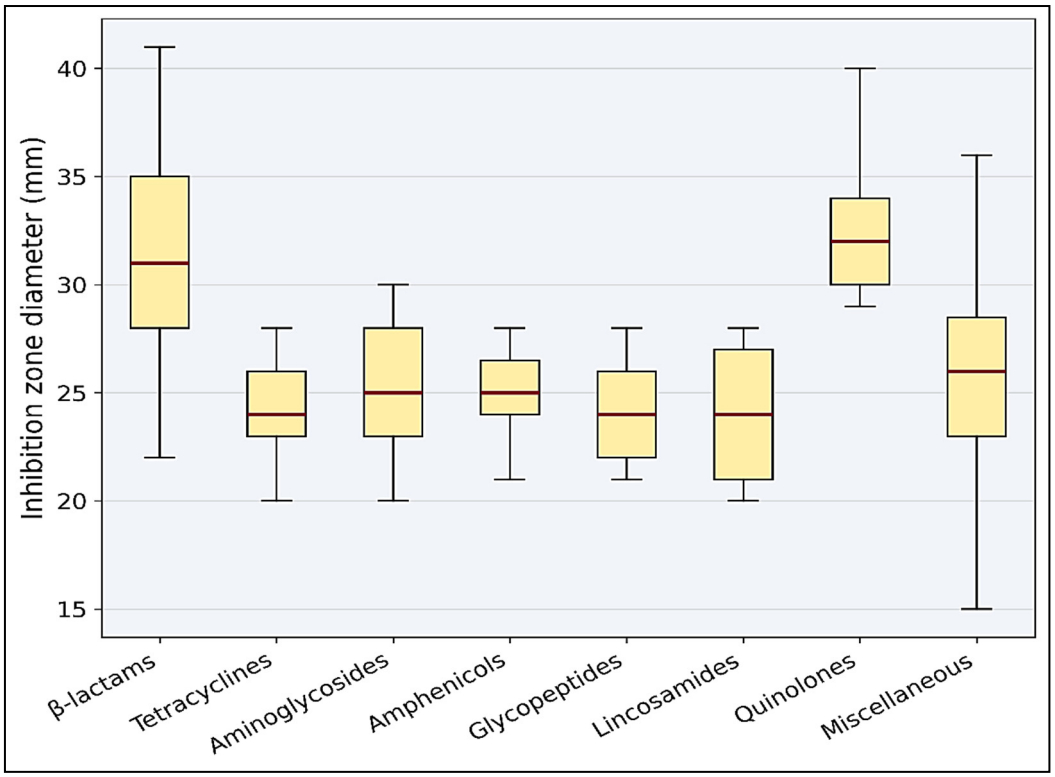

Figure 2. Distribution of inhibition zone diameters (mm) across major classes of antimicrobial agents for *Yersinia pestis* isolates (n = 75) tested on Hottinger agar.

Boxplots represent the median, interquartile range, and overall distribution of inhibition zone diameters for each antibiotic class. The absence of extreme variability and outliers indicates a homogeneous susceptibility profile across all isolates.

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3.3. Quantitative Assessment of Antibiotic Activity (MIC Analysis)

Minimum inhibitory concentration (MIC) analysis confirmed the high sensitivity of the strains to key antimicrobial agents.

Field isolates demonstrated:

– MIC values as low as 0.023 μg/mL (moxifloxacin)
– upper MIC values up to 4 μg/mL (amikacin)
– mean MIC ≈ 1.06 μg/mL

Reference strains showed slightly higher MIC variability, but remained within susceptibility thresholds.

These findings confirm the retained efficacy of first-line and reserve antibiotics used in plague treatment, including: streptomycin, gentamicin, doxycycline, ciprofloxacin end chloramphenicol.

3.4. Phenotypic Detection of Resistance Mechanisms

No phenotypic evidence of extended-spectrum β-lactamase (ESBL) production was identified among the tested isolates.

Repeated testing confirmed the absence of resistance-associated phenotypes, reinforcing the conclusion that the studied population does not exhibit clinically relevant antimicrobial resistance traits.

3.5. Molecular Screening of Antibiotic Resistance Genes (RT-PCR)

Molecular analysis using real-time PCR did not detect any of the targeted resistance genes in the examined *Y. pestis* strains.

The following resistance determinants were absent in all isolates:

– β-lactam resistance genes: *tem, ctx-M-1, shv*
– carbapenemases: *kpc, ndm, vim, imp, oxa variants*
– glycopeptide resistance genes: *vanA, vanB*
– methicillin resistance gene: *mecA*

No amplification signals were observed in diagnostic channels, while internal controls confirmed the validity of the assay.

These findings indicate the absence of horizontally acquired antimicrobial resistance determinants in the studied strain collection, consistent with the phenotypic susceptibility profiles (Table 2) and the molecular screening results summarized in Table 3.

Table 3. Summary of molecular screening and preliminary resistome findings in 75 *Yersinia pestis* isolates.

Determinant / feature	Method	Result	Interpretation
tem	RT-PCR	Not detected	No evidence of common acquired β-lactam resistance determinant
ctx-M-1	RT-PCR	Not detected	No ESBL-associated signal
shv	RT-PCR	Not detected	No ESBL-associated signal
oxa-type targets	RT-PCR	Not detected	No carbapenemase-associated signal in screened panel
imp	RT-PCR	Not detected	No metallo-β-lactamase signal
kpc	RT-PCR	Not detected	No carbapenemase signal
ndm	RT-PCR	Not detected	No carbapenemase signal
vim	RT-PCR	Not detected	No carbapenemase signal

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vanA/B	RT-PCR	Not detected	No glycopeptide resistance determinant detected
mecA	RT-PCR	Not detected	No methicillin resistance determinant detected
ESBL phenotype	Phenotypic confirmatory testing	Not detected	No phenotypic evidence of extended-spectrum β-lactamase production
Acquired AMR plasmids	Preliminary WGS/plasmid review	Not identified	No additional plasmid replicons associated with antimicrobial resistance were identified at the assembly level

The molecular screening results further support the phenotypic findings, confirming the absence of key antimicrobial resistance determinants. Together, these data indicate that the analyzed *Y. pestis* isolates do not harbor known horizontally acquired resistance mechanisms.

To further validate these findings at the genomic level, whole-genome sequencing (WGS) data were analyzed.

3.6. Whole-Genome Sequencing (WGS) and Resistome Analysis

To further validate the phenotypic and molecular findings at the genomic level, whole-genome sequencing (WGS) data were analyzed to assess the presence of acquired antimicrobial resistance genes and chromosomal mutations associated with antibiotic resistance. Whole-genome sequencing data for 75 *Yersinia pestis* isolates were obtained from the NCBI BioProject PRJNA1249055, originally generated for studies on genetic diversity and biovar classification of Central Asian *Y. pestis* isolates [30].

A comprehensive genome-wide screening approach was applied to all annotated GBFF files to identify both acquired antimicrobial resistance determinants and chromosomal mutations associated with antibiotic resistance.

Key chromosomal loci known to be involved in antimicrobial resistance in *Y. pestis* and related Enterobacteriaceae were systematically extracted and compared across all genomes, including *rpsL, gyrA, parC, pmrA, pmrB, phoP,* and *phoQ*. Amino acid sequences were aligned to assess conservation and to detect substitutions at canonical resistance-associated positions.

In parallel, genome annotations were screened for the presence of acquired antimicrobial resistance genes representing major clinically relevant classes, including aminoglycoside resistance genes (*strA, strB, aadA, aac, aph*), tetracycline resistance genes, chloramphenicol resistance genes (*cat-family*), sulfonamide resistance genes (*sul1*/*sul2*/*sul3*), β-lactamases (*bla-family*), carbapenemases (*kpc, ndm, vim, imp, oxa-type*), as well as other resistance determinants (*vanA, vanB, mecA, qnr, dfrA, erm,* and *mph*). Additionally, plasmid content was evaluated to identify the presence of known virulence-associated plasmids and to screen for potential plasmid-mediated antimicrobial resistance determinants.

Genome-wide screening did not identify any known acquired antimicrobial resistance determinants across the analyzed isolates. In particular, no genes associated with resistance to aminoglycosides (*strA, strB, aadA, aac, aph*), tetracyclines, amphenicols, sulfonamides (*sul1*/*sul2*/*sul3*), or β-lactams were detected. Likewise, no genes encoding carbapenemases (*kpc, ndm, vim, imp, oxa-type*) or other clinically relevant resistance determinants (*qnr, dfrA, erm,* and *mph*) were identified. The only annotation related to the "sul" family corresponded to the intrinsic *sulA* gene, which is not interpreted as an acquired sulfonamide resistance determinant.

Analysis of chromosomal loci associated with antimicrobial resistance demonstrated a high level of conservation across all isolates. No substitutions were observed at canonical resistance-associated positions in *rpsL* (Lys43 and Lys88) or *gyrA* (Ser83 and Asp87), indicating the absence of mutations linked to streptomycin and fluoroquinolone resistance, respectively. The *parC* gene was also fully

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conserved among the analyzed genomes. Similarly, regulatory loci (*pmrA*, *pmrB*, and *phoQ*) showed no detectable variation. Minor variation was observed only in *phoP*, limited to two isolates from highland foci, and is not currently associated with clinically relevant resistance phenotypes.

Plasmid analysis confirmed the presence of the core virulence plasmids (*pCD*, *pMT1*, and *pPCP*) in all 75 isolates. The cryptic plasmid *pCKF* was detected in three isolates, while no additional plasmid replicons associated with antimicrobial resistance were identified.

A summary of WGS-based resistome findings is presented in Table 4. Overall, the genomic data are fully consistent with phenotypic and molecular results, indicating the absence of acquired antimicrobial resistance genes, major resistance-associated chromosomal mutations, and plasmid-mediated resistance mechanisms in the analyzed *Y. pestis* population.

Table 4. Whole-genome sequencing (WGS)-based resistome analysis of *Yersinia pestis* isolates (n = 75).

Category	Feature	Result	Interpretation
Acquired AMR genes	Aminoglycoside resistance genes (*strA*, *strB*, *aadA*, *aac*, *aph*)	Not detected	No evidence of acquired aminoglycoside resistance
	Tetracycline resistance genes (*tet-family*)	Not detected	No acquired tetracycline resistance
	Chloramphenicol resistance genes (*cat-family*)	Not detected	No acquired amphenicol resistance
	Sulfonamide resistance genes (*sul1*/*sul2*/*sul3*)	Not detected	No acquired sulfonamide resistance
	β-lactamase genes (*bla-family*)	Not detected	No acquired β-lactam resistance
	Carbapenemases (*kpc*, *ndm*, *vim*, *imp*, *oxa-type*)	Not detected	No carbapenem resistance determinants
	Other AMR genes (*qnr*, *dfrA*, *erm*, *mph*)	Not detected	No additional resistance determinants
Chromosomal loci	*rpsL* (Lys43, Lys88)	No mutations	No streptomycin resistance-associated substitutions
	gyrA (Ser83, Asp87)	No mutations	No fluoroquinolone resistance-associated substitutions
	parC	No variation	Conserved across isolates
	pmrA, *pmrB*, *phoQ*	No variation	No adaptive resistance-related changes
	phoP	Minor variation (2 isolates)	Likely lineage-associated, not linked to AMR
Plasmid content	Core virulence plasmids (*pCD*, *pMT1*, *pPCP*)	Detected in 75/75 isolates	Typical plasmid profile of *Y. pestis*
	Cryptic plasmid (*pCKF*)	Detected in 3/75 isolates	Not associated with AMR

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	MDR-associated plasmids	Not detected	No plasmid-mediated resistance

These findings provide comprehensive genomic confirmation of the absence of antimicrobial resistance in the studied *Y. pestis* population and highlight the stability of susceptibility profiles despite long-term circulation in diverse natural plague foci. The implications of these results in the context of global reports of antimicrobial resistance in *Y. pestis* and other zoonotic pathogens are discussed below.

4. Discussion

An essential component of molecular epidemiological surveillance is the monitoring of both phenotypic resistance profiles of pathogens and the underlying mechanisms of antimicrobial resistance that have clinical and epidemiological significance [31–33].

In the present study, an integrated approach combining phenotypic susceptibility testing, molecular screening, and whole-genome sequencing analysis consistently demonstrated the absence of antimicrobial resistance in *Yersinia pestis* isolates from natural plague foci of Kazakhstan [29].

The high susceptibility of all analyzed isolates to major classes of antimicrobial agents—including aminoglycosides, tetracyclines, β-lactams, amphenicols, glycopeptides, lincosamides, and quinolones—is consistent with previously reported data on *Y. pestis* populations and confirms the continued efficacy of antibiotics traditionally used for plague treatment [34–36].

These findings indicate that first-line therapeutic agents, such as streptomycin, gentamicin, doxycycline, ciprofloxacin, and chloramphenicol, remain effective against circulating strains.

At the same time, the emergence of antimicrobial resistance in *Y. pestis*, although rare, has been documented in several regions worldwide. The first multidrug-resistant (MDR) strain was identified in Madagascar in 1995 and exhibited plasmid-mediated resistance to multiple antibiotic classes, including streptomycin, tetracycline, and chloramphenicol [6,7,37,38].

Subsequently, resistant strains have been reported in other regions. In particular, a strain isolated in China demonstrated resistance to streptomycin due to a point mutation in the *rpsL* gene [39].

These observations confirm that *Y. pestis* is capable of acquiring antimicrobial resistance through both horizontal gene transfer and chromosomal mutations, although such events remain infrequent.

According to recent global analyses, only a limited number of resistant *Y. pestis* isolates have been identified worldwide between 1995 and 2021, indicating that antimicrobial resistance in this pathogen remains sporadic [39,40].

In this context, the results obtained in the present study are fully consistent with the global pattern of low prevalence of resistance.

Importantly, the incorporation of whole-genome sequencing data provides an additional level of validation for these findings. Genome-wide analysis of 75 isolates did not reveal any known acquired antimicrobial resistance genes, including determinants associated with aminoglycoside, β-lactam, tetracycline, or sulfonamide resistance.

Furthermore, no resistance-associated mutations were identified in key chromosomal loci, such as *rpsL* and *gyrA*, which are commonly implicated in resistance to streptomycin and fluoroquinolones, respectively. The absence of variation in regulatory genes (*pmrAB*, *phoPQ*) further supports the genomic stability of the studied isolates.

Plasmid analysis confirmed the presence of typical virulence-associated plasmids, while no additional plasmids associated with antimicrobial resistance were detected. These findings indicate the absence of plasmid-mediated resistance mechanisms in the analyzed population.

The complete concordance between phenotypic, molecular, and genomic data provides strong evidence that the studied *Y. pestis* population remains fully susceptible to clinically relevant antimicrobial agents.

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The absence of resistance in Kazakhstani isolates may be explained by several ecological and epidemiological factors. First, the limited use of antibiotics within natural plague foci likely reduces selective pressure for the emergence of resistant strains. Second, the relative ecological isolation of natural reservoirs may limit opportunities for horizontal gene transfer from other bacterial species.

Nevertheless, previously reported cases of resistant *Y. pestis* strains highlight the potential for the emergence of resistance under favorable conditions. These findings emphasize the importance of continuous surveillance and early detection of resistance determinants.

From a public health perspective, the results of this study are of considerable importance. The confirmed susceptibility of *Y. pestis* supports the continued use of standard treatment regimens and reinforces preparedness strategies for potential outbreaks.

At the same time, the integration of genomic approaches into routine surveillance systems represents a critical advancement, enabling comprehensive monitoring of pathogen evolution and the early identification of emerging resistance within the framework of modern biosafety and One Health strategies.

5. Conclusions

The present study demonstrates the absence of acquired antimicrobial resistance determinants and resistance-associated chromosomal mutations in *Yersinia pestis* isolates circulating in natural plague foci of Kazakhstan. The complete concordance between phenotypic, molecular, and genomic data confirms a stable susceptibility profile across both historical and recent isolates.

These findings indicate that current therapeutic regimens for plague remain effective in Kazakhstan. At the same time, the results highlight the importance of sustained integrated surveillance, including the application of whole-genome sequencing, to detect potential emergence of resistance at an early stage.

The study underscores the value of a One Health approach, integrating epidemiological, ecological, and genomic data to monitor zoonotic pathogens. Continued surveillance is essential to preserve the current favorable antimicrobial resistance situation and to mitigate future public health risks.

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