Infection and Drug Resistance

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REVIEW

Recent Advances in Understanding the Clinical Implications of Heterogeneous Drug Resistance in *Klebsiella pneumoniae*

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Abstract: *Klebsiella pneumoniae* (KP), a clinically significant Gram-negative opportunistic pathogen, has emerged as one of the predominant causative agents of hospital-acquired infections (HAIs). This bacterium is responsible for various severe clinical manifestations, including pneumonia, urinary tract infections, bloodstream infections, and sepsis. In recent years, the global prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *K. pneumoniae* strains has been escalating rapidly. Epidemiological surveillance data reveal a persistent upward trend in infections caused by MDR microorganisms worldwide, a phenomenon disproportionately prevalent in resource-limited developing countries. This trend presents formidable challenges to clinical infection management and constitutes a critical threat to global public health security. In the context of bacterial antibiotic resistance, the phenomenon of heteroresistance (HR) has attracted growing scientific attention due to its unique clinical significance. HR is characterized by the coexistence of subpopulations within a clonal (ie, genetically similar) bacterial population that exhibit divergent susceptibility profiles to an antimicrobial agent. This subtle phenotypic heterogeneity is considered a crucial precursor to the development of stable, high-level antibiotic resistance, representing a pivotal transitional phase in the evolution of MDR. The clinical importance of HR is twofold: first, the resistant subpopulations are often missed by conventional antimicrobial susceptibility testing, potentially leading to unexpected treatment failure. Second, HR serves as an early warning indicator for the impending emergence of complete resistance.

Keywords: heterogeneous drug resistance, *Klebsiella pneumoniae*, clinical research

Introduction

Bacterial heteroresistance (HR) is driven by diverse mechanisms, including gene mutations, transcriptional regulation, variable cellular physiological states, and the horizontal transfer of resistance determinants.[1,2] As a major pathogen in healthcare environments, *Klebsiella pneumoniae* (KP) has become a focal species for HR research. Its genome is characterized by unique plasticity, featuring a high density of mobile genetic elements (MGEs; ≥12% of the genome) and highly variable capsular polysaccharide loci (resulting in K1-K78 serotypes).[3] Integrative whole-genome and transcriptomic analyses indicate that under antibiotic pressure, distinct *K. pneumoniae* subpopulations can upregulate resistance via porin modifications (eg, OmpK35/36 truncation) and efflux pump overexpression (eg, AcrAB-TolC). Conversely, other subpopulations may maintain low-level tolerance through metabolic dormancy, often mediated by regulatory systems like PhoPQ.[4] This highlights the complex interplay of genetic factors, environmental cues, and inter-subpopulation interactions. Furthermore, strains with an identical genetic background can exhibit divergent susceptibility

Received: 12 May 2025
Accepted: 16 November 2025
Published: 26 November 2025

patterns under different environmental conditions. This is particularly evident in a biofilm-embedded state, where the extracellular polymeric substance (EPS) matrix can increase antibiotic tolerance by 4- to 8-fold. KP heteroresistance has been documented for several clinically critical drug classes, including polymyxins (often mediated by *mgrB* mutations and LPS modification), tigecycline (associated with *ramA* overexpression), cephalosporins (linked to variable ESBL/AmpC production), and aminoglycosides (dependent on 16S rRNA methylation).[5,6]

The clinical threat of *K. pneumoniae* heteroresistance is compounded by the bacterium's high colonization potential (notably, a 30–60% intestinal carriage rate in hospitalized patients) and the inability of conventional antimicrobial susceptibility testing (AST) to reliably detect these low-frequency resistant subpopulations. Emerging technologies show great promise for the precise identification of heteroresistance.[7] These include single-cell transcriptomics (scRNA-seq), capable of identifying rare resistant clones at frequencies as low as 0.1%; whole-genome sequencing (WGS), for tracking plasmid-mediated resistance gene dynamics; and Raman spectroscopy, which can detect phenotypic changes within 2 hours. However, the full spectrum of resistance mechanisms across diverse clinical *K. pneumoniae* isolates remains incompletely understood. This is especially true for hypervirulent (hvKP) strains, where the characteristic hypermucoid phenotype and enhanced iron acquisition systems further complicate the resistance profile. Therefore, this review synthesizes the current understanding of *K. pneumoniae* heteroresistance, focusing on recent advances in detection methodologies, mechanistic insights, and therapeutic implications. By highlighting this phenomenon, we aim to enhance awareness among clinicians and researchers and to stimulate further in-depth investigation in this critical field.

Detection of Heterogeneous Drug Resistance

Homogeneous and heterogeneous resistance represent two fundamentally distinct manifestations of bacterial drug tolerance. Although both involve reduced antibiotic susceptibility, they diverge sharply in the laboratory and at the bedside. Homogeneous resistance presents a stable, uniform phenotype reliably captured by standard assays. Heteroresistance, by contrast, is a cryptic phenomenon where a resistant subclone—often comprising only 0.1–1% of the total population—coexists with a susceptible majority. Multiple studies now show that infections initially reported as "susceptible" *Klebsiella pneumoniae* (KP) and associated with clinical failure retrospectively harbor these hidden resistant minorities.[8,9]

Detecting such low-frequency subpopulations remains a major diagnostic challenge. Conventional antimicrobial susceptibility testing (AST) is designed to measure the bulk phenotype of a bacterial population, and its analytical sensitivity is insufficient to detect resistant clones present at these low proportions. Consequently, these subpopulations are effectively invisible to routine platforms, leading to false-susceptible reports and delayed, inappropriate therapeutic adjustments.[8–10]

A new generation of technologies is emerging to close this sensitivity gap. Single-cell transcriptomics, for example, can resolve gene-expression heterogeneity at the individual-cell level; Raman spectroscopy offers label-free, real-time chemical fingerprinting of antibiotic stress responses; and refined drug-gradient assays can selectively enrich and quantify minority resistant variants (Figure 1). Collectively, these advanced tools promise to elucidate the full scope of heteroresistance, fundamentally transforming the surveillance, diagnosis, and treatment of bacterial infections.

Population Analysis Profiling (PAP)

Population Analysis Profiling (PAP) remains the in vitro gold standard for quantifying heteroresistance in *Klebsiella pneumoniae* (KP). By testing a high-density inoculum of a single clinical isolate across a wide antibiotic gradient (eg, spanning from 0.1 to 100 $\mu g \cdot mL^{-1}$), PAP can resolve minority resistant subclones present at frequencies as low as 10^{-7}. This sensitivity is well below the detection limit of any conventional antimicrobial susceptibility testing (AST) platform. These covert populations, invisible during routine testing, are precisely the reservoirs that can later expand under therapeutic pressure and precipitate clinical failure. Methodologically, PAP is deceptively simple yet analytically rigorous. Following standardized inoculation onto (or into) media containing each antibiotic concentration, bacterial growth is quantified, typically by colony enumeration or spectrophotometrically (OD_{600}). The resulting dose-response profile—the "PAP curve"—reveals inflection points, plateaus, and tail fractions that delineate the proportion and phenotypic distribution of resistant cells within the population.[11] The technique is broadly applicable: it can be adapted

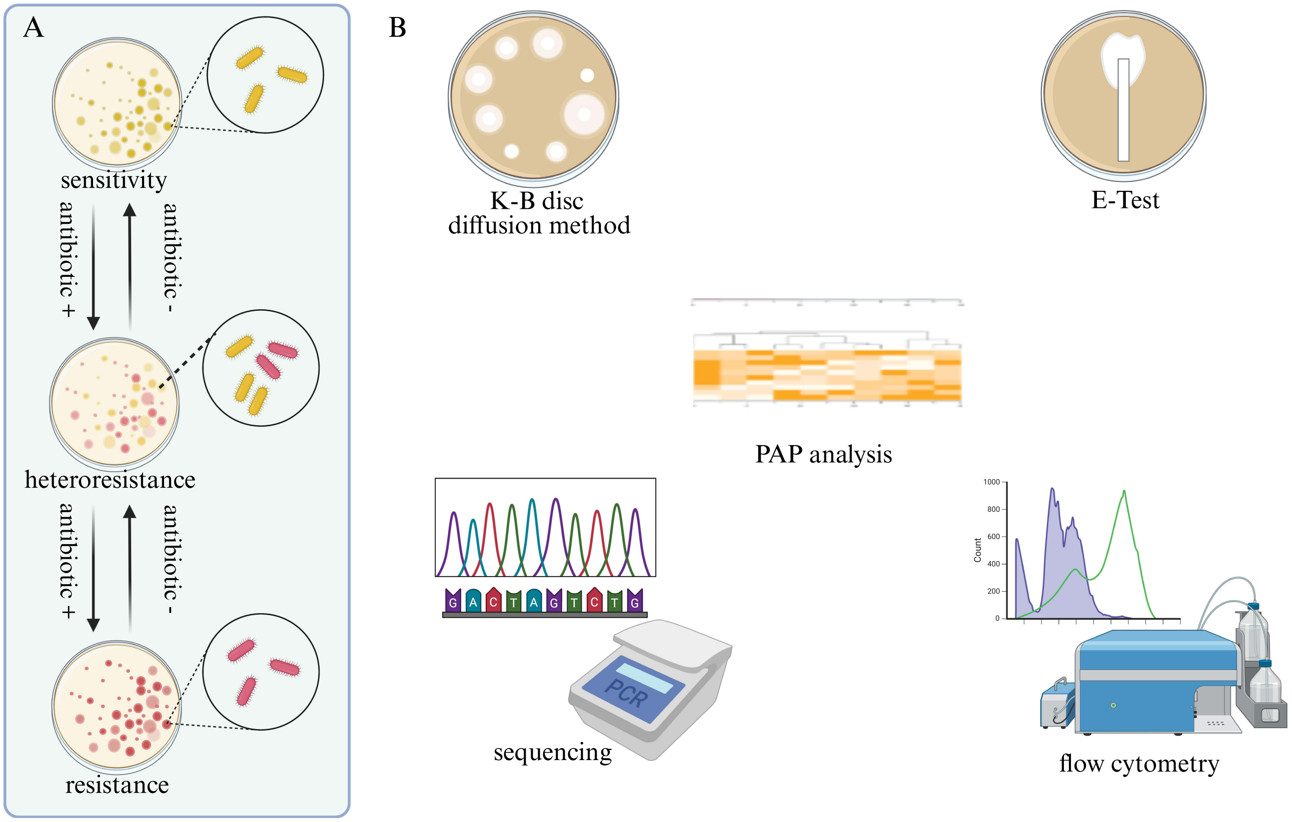

Figure 1 Definition and detection methods of heterogeneous drug resistance. (**A**) Heterogeneous resistance is an intermediate state in the transition from sensitivity to resistance in the presence of antibiotics. (**B**) Common assays for heterogeneous drug resistance include PAP, K- B method, E-test, sequencing, flow cytometry, etc. Created in BioRender. Lai, H. (2025) https://BioRender.com/aofy965.

for colistin, ceftazidime-avibactam, carbapenems, or virtually any antibiotic-organism pair of clinical interest. This exquisite sensitivity translates into actionable clinical intelligence. Early detection of heteroresistance could, in principle, trigger a timely escalation to combination regimens or guide the use of next-generation β-lactam/β-lactamase inhibitor therapies, potentially averting breakthrough bacteremia and curtailing nosocomial transmission. However, the very precision that makes PAP powerful also renders it impractical for routine diagnostics. A single PAP assay requires ≥48 hours from subculture to final interpretation, necessitates the precise preparation of antibiotic gradients from analytical-grade stocks, and remains vulnerable to contamination. These factors inflate cost and labor far beyond the capacity of most clinical laboratories. Consequently, the field faces a translational imperative: to re-engineer PAP into a clinically tractable format. Emerging solutions include microfluidic gradient generators that reduce reagent volumes by orders of magnitude, single-cell Raman phenotyping coupled with convolutional neural networks (CNNs) capable of reconstructing PAP curves in <4 hours, and decision-support algorithms that integrate such surrogate data with electronic health record (EHR) risk scores. Realizing these hybrid workflows will be pivotal for moving heteroresistance detection from the research bench to the bedside, transforming this occult liability into a routinely monitored therapeutic target.

Single-Cell Transcriptomics

Single-cell transcriptomics (scRNA-seq) offers a powerful, high-resolution approach for mapping bacterial heteroresistance. The protocol, while conceptually straightforward, is technically exacting: individual cells are first isolated from a clinical sample—typically using microfluidic droplet generators or fluorescence-activated cell sorting (FACS)—to ensure single-cell partitioning. Total RNA is then extracted, ribosomal RNA (rRNA) is depleted, and the remaining messenger RNA (mRNA) is reverse-transcribed and amplified to generate full-length cDNA libraries. These libraries are subjected to massively parallel sequencing, yielding a high-resolution atlas of gene expression across the population. Dimensionality-reduction algorithms, such as t-SNE, UMAP, or graph-based embeddings, are then used to partition cells into distinct transcriptional clusters, revealing gene-expression signatures that demarcate resistant, susceptible, and intermediate subpopulations.[12]

Despite its exquisite granularity, scRNA-seq remains largely confined to the research realm. The workflow is time-consuming (often >48 h), reagent-intensive (costing hundreds of dollars per isolate), and demands specialized expertise in microfluidics, molecular biology, and computational biology. Moreover, a critical limitation is that resistance is inferred from transcriptional proxies rather than being phenotypically measured under direct antibiotic challenge. Therefore, the technique cannot yet displace conventional phenotypic susceptibility testing.

Nevertheless, future prospects are promising. Convergent advances—including sub-microliter microfluidic chips that slash reagent volumes, simplified "one-pot" library preparation chemistries that significantly reduce hands-on time, and cloud-native analytical pipelines that broaden access to data interpretation—promise to compress both cost and turnaround time, potentially by an order of magnitude. Should these innovations mature, scRNA-seq could graduate from a specialized research tool to a frontline diagnostic, equipping clinicians not only with the detection of heteroresistance but also with the mechanistic insights required to design precision combination therapies.

Raman Spectroscopy

Raman spectroscopy, a label-free analytical technique, provides a distinct chemical fingerprint of cells based on their molecular vibrational modes, offering insights unattainable through traditional culturing methods.[13] It has been increasingly applied to the study of bacterial drug resistance, particularly for the detection of heteroresistance.

The typical workflow involves the isolation of bacteria at the single-cell level, often utilizing flow cytometry or microfluidic chips. A focused laser is then directed onto these individual cells via a microscope, and the resulting scattered Raman light is collected. By recording spectra from cells exposed to different antibiotic concentrations or following treatment, researchers can compare spectral variations and extract characteristic peaks associated with a resistant phenotype. High-throughput Raman platforms enable the analysis of large cell populations, and the resulting high-dimensional data are typically processed using machine learning algorithms—such as cluster analysis, principal component analysis (PCA), and support vector machines (SVM)—to identify and classify distinct subpopulations that respond differently to antibiotic pressure.[14]

However, the widespread clinical adoption of this technique has been limited by its reliance on high-precision instrumentation, the need for specialized personnel for operation, and the complexity of data interpretation.

Other Detection Methods

High-throughput screening (HTS) platforms, particularly those based on nanoliter-droplet microfluidics, are powerful tools for investigating heteroresistance. By orchestrating tens of thousands of parallel micro-cultures, these systems monitor population growth dynamics and stress responses with high temporal resolution.[15] When coupled with molecular barcoding and combinatorial antibiotic matrices, HTS can resolve subtle shifts in the minimum inhibitory concentration (MIC) distribution that are otherwise masked in bulk assays. Critically, these micro-reactors often permit the retrieval of specific subclones for downstream characterization, directly linking population-level statistics to single-cell fates.

Single-cell analytical techniques provide the granular detail required to characterize the outlier phenotypes flagged by HTS. Time-lapse microfluidics, for instance, enables the direct observation of individual bacterial trajectories under antibiotic pressure, quantifying lag-phase heterogeneity, persister resuscitation rates, and morphological adaptations. Complementary molecular methods dissect the underlying drivers. Single-cell RNA sequencing (scRNA-seq) can quantify the transcriptional noise that seeds phenotypic escape; for example, stochastic upregulation of the AcrAB-TolC efflux pump, even without canonical resistance mutations, may enable survival in a small subpopulation (eg, 0.1%)—a phenomenon invisible to bulk transcriptomics. Whole-genome sequencing (WGS) of these retrieved clones can then identify the specific mutations or regulatory changes responsible for resistance. Integrating these data with single-cell metabolomics (eg, via Raman spectroscopy or mass spectrometry) can generate a multi-omics atlas that links metabolic state to transcriptional initiation and, ultimately, to phenotypic resistance.[16]

While these advanced methods excel at pinpointing minor resistant fractions that elude conventional susceptibility tests, they face significant hurdles. Major challenges include technical complexity, high costs, potential for false-positive or false-negative results, and limited scalability for routine clinical use. Future efforts must therefore focus on streamlining these detection platforms, reducing their cost, and validating their clinical utility. Bridging the gap from research to routine diagnostics is essential to better confront the escalating threat posed by antibiotic-resistant pathogens.

Mechanisms of Heteroresistance

Despite its crucial role as a key intermediate stage facilitating the evolution from full susceptibility to stable resistance, the mechanisms underlying heteroresistance (HR) in *Klebsiella pneumoniae* (KP) remain incompletely understood.[4] The mechanisms driving this phenomenon are complex and diverse, encompassing gene mutations, dynamic regulation of gene expression, shifts in cellular physiological states, and various environmental factors[17] (Figure 2).

Gene Mutation and Amplification

Gene amplification and mutation are primary drivers of heteroresistance. This can involve the tandem amplification of resistance genes, such as the *bla*$_{\text{SHV-12}}$ gene in *K. pneumoniae*, which has been shown to confer heteroresistance to cefotaxime.[18] In the absence of selection pressure, these amplified copies may be lost, leading to phenotypic instability.

Alternatively, stable point mutations can arise in key regulatory or structural genes, imposing a potential fitness cost but conferring resistance. A prominent example in KP is heteroresistance to polymyxins, which is frequently associated

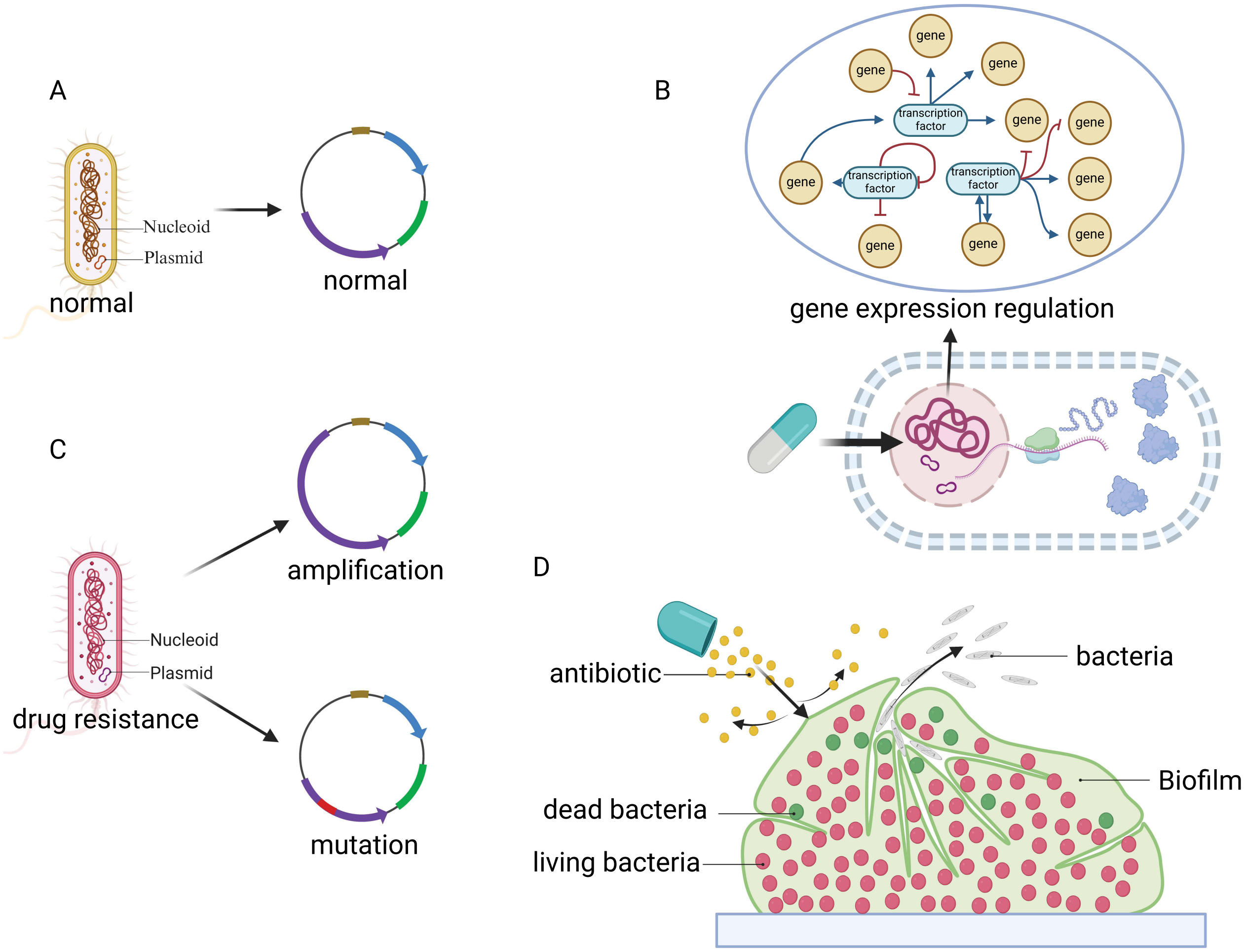

Figure 2 Mechanistic studies of heterogeneous drug resistance.(**A**) Normal bacteria harbouring a native nucleoid and plasmid (without mutations or amplifications) exhibit baseline antibiotic susceptibility.(**B**) Upstream-downstream interactions through multiple genes and transcription factors in the strain may lead to the development of heterogeneous drug resistance.(**C**) The mutation or amplification of drug resistance genes (such as β-lactamase genes, aminoglycoside modifying enzyme genes, etc.) is an important mechanism leading to heterogeneous drug resistance.(**D**) Bacteria in biofilms can develop heterogeneous resistance by reducing drug permeability, altering drug targets, or enhancing drug efflux. Created in BioRender. Lai, H. (2025) https://BioRender.com/mkbjr36.

with mutations in genes like *phoP*.[19] Similarly, mutations within the *acrAB* efflux pump operon[20] or in various β-lactamase and aminoglycoside-modifying enzyme genes[21] are strongly associated with the heteroresistant phenotype.

Dynamic Gene Expression and Regulatory Mechanisms

Heteroresistance is often driven by dynamic, non-genetic changes in gene expression.[22] This regulation allows subpopulations to transiently adapt to antibiotic pressure.

Overexpression of Drug Efflux Pumps

A critical and well-documented mechanism in KP is the overexpression of drug efflux pumps. Studies have shown that increased expression of pumps like AcrAB-TolC and OqxAB is a key driver of tigecycline heteroresistance.[23] This phenomenon arises when, under antibiotic pressure, a subpopulation upregulates efflux pump expression while the rest of the population remains sensitive. The role of efflux is confirmed by the use of inhibitors, such as carbonyl cyanide m-chlorophenylhydrazone (CCCP), which can significantly reduce the MICs of these resistant subpopulations.[24,25]

Transcriptional and Post-Transcriptional Regulation

More broadly, differential gene expression is controlled by a complex, multi-level regulatory system. This includes the action of transcription factors that can activate resistance-associated genes in a subset of cells,[26] as well as epigenetic modifications (eg, DNA methylation) and non-coding RNAs (ncRNAs).[27,28] These regulatory networks can control metabolic states, signaling pathways, and even induce dormancy in certain subpopulations, allowing them to evade antibiotic killing and contribute to the overall heteroresistant phenotype.[29–33]

Biofilm Formation and Phenotypic Heterogeneity

Biofilm formation is another key factor contributing to heteroresistance. As a community lifestyle, biofilms possess high structural complexity. Within the biofilm matrix, steep chemical and physiological gradients promote phenotypic diversity. Subpopulations can reduce drug permeability, limiting antibiotic absorption.[34] Furthermore, bacteria within biofilms may alter antibiotic targets[35] or enhance efflux pump activity to expel antibiotics.[36] These mechanisms interact, enabling resistant subpopulations to persist and increasing the difficulty and complexity of treatment.

Physiological State and Environmental Factors

Beyond specific genetic changes, the general physiological state of the bacteria, including metabolic activity and growth rate, significantly affects antibiotic susceptibility. This can cause subpopulations to exhibit different resistance phenotypes under varying conditions.[37] Environmental factors within the infection microenvironment, such as temperature and pH, also play a critical role.[38] For instance, acidic environmental stress can alter the expression of outer membrane proteins in Gram-negative bacteria, thereby affecting antibiotic influx and efflux.[39] When these stressors are removed, the bacteria may revert to a susceptible state.

In conclusion, a deeper understanding of these diverse and often overlapping mechanisms of heteroresistance in *K. pneumoniae* is essential for guiding effective clinical anti-infective therapy and understanding the evolution of bacterial resistance.

Clinical Anti-Infective Therapy

The heteroresistance (HR) of *Klebsiella pneumoniae*, a leading opportunistic pathogen in hospital-acquired infections, has become a critical focus in antibiotic resistance research.[40] Studies consistently demonstrate that upon antibiotic exposure, *K. pneumoniae* populations can segregate into subpopulations, with some exhibiting enhanced resistance while others remain susceptible.[41,42] This instability poses a significant clinical challenge, as these low-frequency resistant subpopulations are difficult to detect with conventional diagnostics. The presence and amplification of these subpopulations are strongly associated with clinical anti-infective treatment failure, recurrent infections, and the accelerated spread of resistance. Addressing this phenomenon requires a multi-pronged strategy that integrates precision diagnostics, combination drug regimens, novel therapeutic tools, and stringent infection control (Figure 3).

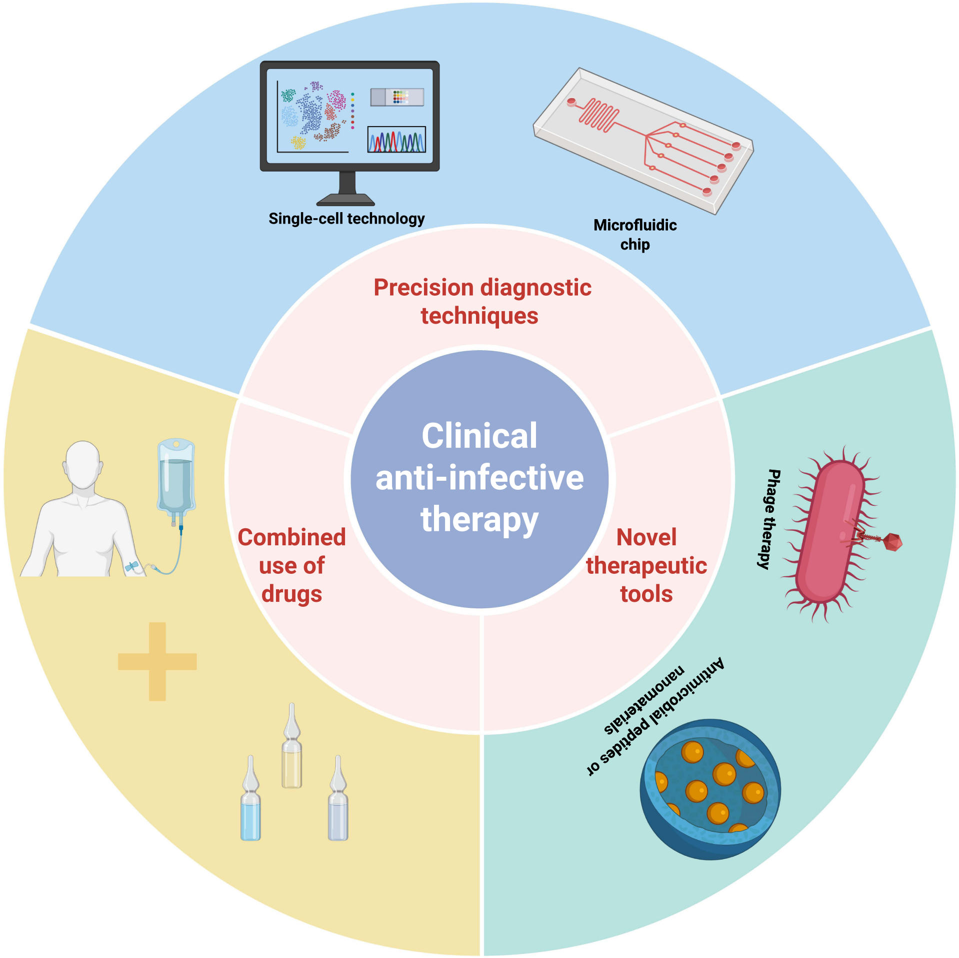

Figure 3 Clinical impact of heterogeneous drug resistance. The potential impact of heterogeneous drug resistance on clinical care is multifaceted, mainly in terms of treatment, diagnosis, prognosis, and public health resources. Created in BioRender. Lai, H. (2025) https://BioRender.com/7tbzmnu.

Precision Diagnostic Techniques

A major barrier to managing heteroresistance is the inadequacy of current clinical diagnostics. Standard methods, including disk diffusion (Kirby-Bauer) and gradient diffusion (E-test), lack the sensitivity to reliably detect low-frequency resistant subpopulations, leading to false-susceptible reports.[43,44]

To overcome this limitation, next-generation technologies are being adapted for high-resolution detection.

- Mass Spectrometry (MALDI-TOF MS): Already a clinical standard for rapid microbial identification, MALDI-TOF MS is being developed for detecting resistance markers and can, in some assays, infer resistance.[45]
- High-Throughput Sequencing (NGS): NGS is widely used for detecting known resistance genes, such as mecA in MRSA or rpoB and katG mutations in Mycobacterium tuberculosis.[46–48] Its application in HR involves quantifying gene copy number variations or identifying minority single-nucleotide polymorphisms (SNPs) associated with resistance.
- Flow Cytometry: This technology can rapidly assess cell viability and metabolic states (eg, using fluorescent dyes like FUN-1) on a single-cell basis, allowing for the quantification of subpopulations that respond differently to antibiotic stress.[49]
- Single-Cell Technologies: While still primarily a research tool, single-cell genomics and transcriptomics offer the ultimate resolution for detecting rare resistant mutants and characterizing their unique transcriptional profiles.[12,50–52]

While NGS and MALDI-TOF MS are increasingly integrated into clinical workflows for specific applications, a key future goal is the clinical translation of microfluidic and single-cell platforms. Such systems could one day enable dynamic, AI-driven predictions of antimicrobial susceptibility that account for heteroresistance.

Combined Use of Drugs

Given that monotherapy provides a strong selective pressure that favors the amplification of resistant subpopulations, combination therapy is a cornerstone of managing heteroresistant infections.[52,53]

- Clinically, this strategy aims to achieve synergistic killing and prevent the emergence of resistance. For example, the combination of tigecycline and polymyxin can inhibit resistant subpopulations at lower drug concentrations, potentially preventing the full emergence of HR.[54,55]
- The combination of ceftazidime-avibactam (CZA) and meropenem has shown high synergism against KPC-producing isolates. This is particularly relevant for HR, where an isolate may appear susceptible to CZA (eg, MICs of 4 or 8 $\mu g \cdot mL^{-1}$), but monotherapy fails. The addition of meropenem can suppress the amplification of this resistant subpopulation.[56]
- Similarly, colistin (mucomycin) combined with meropenem, amikacin, gentamicin, or fosfomycin has been shown to inhibit the emergence of heteroresistant *K. pneumoniae*.[57] For highly virulent (hvKP) strains, triple combinations (eg, colistin, tigecycline, and CZA) have been explored.[58]

Combination therapy is demonstrably superior for many HR infections as it provides multi-target pressure, reducing the probability of selecting for resistant mutants.[59,60] However, the clinical selection of a combination regimen is complex, requiring careful consideration of the patient's clinical status, the specific infection, in vitro synergy data (if available), potential drug toxicities, and drug-drug interactions.

Novel Therapeutic Tools

Phage Therapy

Bacteriophage (phage) therapy presents a highly specific alternative. Phage cocktails can be designed to cover diverse bacterial subpopulations, including those with different resistance profiles, without damaging the patient's normal microbiota.

- Advantages: Phages are effective against MDR and carbapenem-resistant *K. pneumoniae* (CRKP)[61,62] and are unaffected by conventional antibiotic resistance mechanisms. They can also act synergistically with antibiotics; for example, some phages degrade the bacterial cell wall, enhancing the permeability of β-lactams.[63,64]
- Challenges: The primary limitation is specificity. Therapeutic phages must match the receptors (eg, capsular polysaccharide type) of the infecting strain, which can be costly and time-consuming to screen.[65,66]
- Future Directions: Solutions include establishing curated phage libraries covering common KP serotypes (eg, K1, K2) and resistance profiles. Furthermore, combining phages with antibiotics (eg, meropenem) or immunomodulators (eg, interferon-γ) may enhance clearance and delay the emergence of phage-resistant bacterial mutants.[16,67] Encapsulation in nanocarriers (eg, liposomes) can also improve stability and targeting.[68]

Antimicrobial Peptides or Nanomaterials

AMPs and nanomaterials offer novel mechanisms of action that can bypass conventional resistance.

- Antimicrobial Peptides (AMPs): These molecules typically work by disrupting bacterial cell membranes (eg, cationic peptides binding to negatively charged LPS) or interfering with intracellular targets.[69]
- Nanomaterials: Metal nanoparticles (eg, Ag^+, Zn^{2+}) exert bactericidal effects by disrupting membrane integrity and generating reactive oxygen species (ROS).[70,71] Carbon-based materials like graphene oxide can induce physical or photodynamic damage.[72,73]

These approaches are less susceptible to failure from single-point mutations. Their greatest potential may lie in combination. For example, nanoparticles can be used as delivery vehicles for antibiotics or AMPs, targeting them to the infection site and overcoming biofilm barriers.[74–76] Zinc oxide (ZnO) nanoparticles have been shown to interact with porin proteins, enhancing the permeability and efficacy of β-lactams against resistant *K. pneumoniae*.[77] Through mechanisms of synergy, precise delivery, and immunomodulation, these novel tools represent a promising frontier for treating heteroresistant infections.[78,79]

Conclusion

Heteroresistance (HR) in *Klebsiella pneumoniae* represents a critical, yet often occult, challenge in clinical practice, and its precise molecular mechanisms remain incompletely defined. This phenomenon, characterized by the coexistence of resistant subpopulations within a predominantly susceptible bacterial population, is strongly associated with clinical anti-infective treatment failure, recurrent infections, and the accelerated dissemination of resistance.

Despite these severe consequences, the clinical impact of HR is frequently overlooked, largely due to a lack of standardized definitions and the inability of routine susceptibility testing to reliably detect these low-frequency populations. Given that heteroresistance is a pivotal intermediate stage in the evolution toward stable, high-level resistance, its early identification is a major priority. The development of rapid, reliable phenotypic or genomic tools to detect HR before this transition occurs would represent a significant advancement in antimicrobial stewardship.

Ultimately, enhanced clinical surveillance, coupled with the rational implementation of combination therapies, represents a key strategy to slow the development and spread of heteroresistance. Future research into bacterial resistance must increasingly focus on this critical transitional state to fully understand and effectively counteract this cryptic threat.

Funding

Funding sources for this study were offered by Hospital science and technology innovation team cultivation project[No. YFYGJPY20240901], Key Project of the Key Research and Development Program of Jiangxi Province[No.20232BBG70020].

Disclosure

Jianhua Fang, Hongyi Lai, and Huade Chen share first authorship.

The authors report no conflicts of interest in this work.

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