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HETERORESISTANCE: THE HIDDEN DRIVER OF 'SUSCEPTIBLE-BUT-FAILS' INFECTIONS AND RAPID EVOLUTION TO FIXED RESISTANCE, A PUBLIC HEALTH ISSUE

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Abstract
Antimicrobial heteroresistance (HR)—reproducible minority subpopulations that grow at antibiotic concentrations inhibiting the dominant clone—offers a unifying explanation for "susceptible-but-fails" infections and for rapid, on-therapy evolution to fixed resistance. We narratively reviewed evidence (1997–30 Sept 2025) across MEDLINE, Embase and Web of Science on definitions, epidemiology, mechanisms, detection, clinical impact and translational tools for HR. HR is best established for glycopeptide heteroresistance in *Staphylococcus aureus* (hVISA) and for polymyxin HR in carbapenem-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*, where links to persistent bacteraemia, treatment modification and on-therapy emergence of high-level resistance recur despite MICs that read "susceptible." Mechanistically, unstable architectures (gene amplification, regulatory toggling, plasmid copy-number variation) and early fixed mutations generate minority survival, with spatial drug gradients and biofilms amplifying selection in vivo. Emerging triage solutions—semi-automated PAP derivatives, direct-from-specimen ddPCR/WGS, and machine-learning assisted image/genome analysis—can increase sensitivity at scale if validated and explicitly positioned as screening, not diagnosis. We propose HR-aware stewardship triggers (high-risk organism–drug pairs; persistent bacteraemia at 48–72 h) coupled to PK/PD verification, source control and mechanism-aware therapy, and call for prospective, assay-anchored trials testing HR-guided strategies with time-to-clearance and failure endpoints.

Keywords: Heteroresistance, hVISA, colistin, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, population analysis profiling (PAP), antimicrobial stewardship

1. Introduction

Antimicrobial heteroresistance (HR) describes a bacterial population in which minority subpopulations can grow at antibiotic concentrations that inhibit the dominant clone, producing the clinical pattern of "susceptible-but-fails." Routine antimicrobial-susceptibility testing (AST)

collapses this heterogeneity into a single MIC, so clinically important subpopulations often go undetected until failure declares itself. [1-4] HR is present when reproducible minority subpopulations within a single isolate form colonies at drug concentrations substantially exceeding the isolate's modal MIC (for example, by ≥8-fold) on population analysis profiling (PAP) or an equivalent confirmatory approach. [2,5] In practice, laboratories encounter HR as microcolonies within inhibition zones, trailing or "tailing" growth around gradient strips, or discordant results across methods or inocula that resolve to subpopulation growth on confirmatory testing; the point of this working definition is to detect risk states that a binary MIC will miss.[2,5] Conflating these categories inflates prevalence estimates and muddies clinical decisions. We therefore reserve "HR" for within-isolate heterogeneity demonstrable by growth at higher drug concentrations and at least partly heritable on short time scales.[6-8] Why should clinicians care? Across organism–drug pairs, HR has been linked—most consistently for glycopeptide HR in *Staphylococcus aureus* (hVISA)—to persistent bacteraemia, delayed clearance, and higher rates of treatment modification, although mortality effects are context-dependent.[1-9] In Gram-negative pathogens, colistin HR in *Klebsiella pneumoniae* and *Acinetobacter baumannii* is frequently reported and biologically plausible as a driver of failure; however, outcome data are heterogeneous and assay-dependent, underscoring the need for standardised definitions and prospective studies. [3,10-12] Mechanistically, HR can arise from unstable gene amplifications, reversible regulatory states, or early fixed mutations—differences that matter because they shape the probability that on-therapy selection will convert HR into fixed, high-level resistance. Recognising HR therefore serves two purposes: (i) it explains AST–outcome discordance and guides immediate treatment adjustments, and (ii) it flags patients at heightened risk of on-therapy evolution to stable resistance.[8, 13-14]

2. Methods

This is a narrative Review. We searched MEDLINE (PubMed), Embase, and Web of Science for articles from Jan 1, 1997 to Sept 30, 2025 using terms including "heteroresistance OR hetero-resistance OR subpopulation*" combined with "antibiotic* OR antimicrobial*" and organism–drug pairs (for example, vancomycin *Staphylococcus aureus*; colistin *Klebsiella pneumoniae*/*Acinetobacter baumannii*; carbapenems *Pseudomonas aeruginosa*). We additionally screened reference lists and relevant guidance (for example, CLSI, EUCAST). English-language reports were included if they (i) defined or demonstrated HR within a single isolate using an accepted method (for example, population analysis profiling, PAP–AUC; gradient or disk "tailing" with confirmation), or (ii) examined clinical implications of HR (microbiological failure, persistent bacteraemia, time-to-clearance, treatment modification, or short-term mortality). Two reviewers independently screened titles/abstracts and full texts; disagreements were resolved by consensus. We prioritised outcome-linked human studies, then animal models, then mechanistic bench work, and graded certainty qualitatively by study design, sample size, assay reproducibility, and indirectness. Preprints were cited only when subsequently peer-reviewed or where clearly labelled. This Review does not undertake a meta-analysis, was not registered (for example, PROSPERO), and follows narrative-review conventions rather than PRISMA reporting.

3. Epidemiology of heteroresistance

3.1 Cross-cutting patterns

Associations with persistent bacteraemia and treatment modification are consistent, while mortality effects vary by illness severity and source control.[1,4] Non-fermenters and colistin. Colistin HR is repeatedly documented in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, with estimates highly assay-dependent: gradient-strip "tailing" or population screens report higher rates than confirmatory PAP; within-patient selection during therapy has been described.[2,5,6] Enterobacterales and polymyxins/β-lactams. In *Klebsiella pneumoniae*—especially carbapenem-resistant lineages—HR to polymyxins and selected β-lactams has been observed in multicentre series, with occasional progression from HR to fixed resistance during ongoing therapy.[2,5,7]

3.2 What drives the spread

Rates are typically higher in ICU and ventilated populations, in chronic respiratory specimens, and among previously exposed patients—settings with steep antibiotic gradients and biofilm niches that favour minority survival.[5,10] Finally, temporal antibiotic pressure shapes what is measured; cohorts sampled before and after formulary or stewardship changes often show prevalence shifts that track with use intensity, consistent with on-therapy selection amplifying pre-existing minorities.[10,11,13]

3.3 Reporting pitfalls and take-home message

Refrain from implying ubiquity; prefer phrasing such as "reported across multiple regions and settings, with prevalence contingent on the method used.[13] Overall, heteroresistance is neither curiosity nor inevitability: it is sufficiently common in specific organism–drug pairs and clinically consequential in defined contexts to warrant routine consideration—provided detection is standardised, assay-anchored, and explicitly tied to decision-relevant outcomes.[14]

Table 1. Epidemiology of heteroresistance—assay-anchored estimates and clinical signals

Organism	Drug/class	Setting / specimen	Assay (screen → confirm)	Prevalence (confirmed %)	Outcome link (endpoint)
Staphylococcus aureus (MRSA; hVISA)	Vancomycin (glycopeptide)	Bacteraemia cohorts	Gradient-strip tailing or disk microcolonies → **PAP–AUC**	~5–20% in Asian series; decline Korea 25%→2.2% (2006–2013) [4,5,13]	Persistent bacteraemia; treatment modification; mortality context-dependent [1,4]
Klebsiella pneumoniae(CRKP)	Colistin/polymyxin B	Bloodstream & mixed clinical isolates	Screen (tailing) → **PAP**	Multicentre Enterobacterales: **~6%**[5]; single-centre CRKP up to ~70%	On-therapy evolution HR→fixed resistance; pre-existing HR in most who became resistant [11,14]
Acinetobacter baumannii	Colistin	MDR cohorts; ICU/respiratory enriched	Screen (tailing) → **PAP**	Often **>50%**; some series **~90–100%** (assay-dependent) [3,12,15]	Signal strengthened by prior colistin exposure; clinical concern repeated[3,12,15]
Acinetobacter baumannii	Tigecycline	Clinical isolates	Screen → confirm (varied; **PAP**where used)	Up to **~56%** in some series [16,17]	Outcome data limited/heterogeneous [16,17]
Pseudomonas aeruginosa	Aminoglycosides/carbapenems/polymyxins	Mixed clinical isolates	Screen → **PAP**(single-centre series)	Heterogeneous; strongly assay/site-dependent	Emerging outcome signals; not uniform[2]
Enterobacterales (mixed)	Carbapenems (imipenem/meropenem)	VIM-producers (Spain); Sweden/USA/China	**PAP** or confirmatory profiling	Spain (VIM): **~17%**; imipenem **0–25%**, meropenem **up to ~30%** across centres [2,8]	Limited outcome linkage; assay-dependent [2,8]
K. pneumoniae(KPC-KP)	Ceftazidime–avibactam	Bloodstream/varied	Screen → **PAP/confirmation**	**~12%** of CZA-susceptible isolates harboured HR minorities [18]	Outcome linkage emerging (treatment modification/failure case series [18]
Enterobacterales (mixed)	Fosfomycin	Urinary & other isolates (multicentre)	Screen (double-disk/gradient) → **PAP** when used	**~10%** confirmed HR [3]	Limited outcome data [3]
*Enterobacter cloacae*complex	Colistin	Clinical isolate studies	**PAP** + exposure experiments	High HR prevalence in targeted series; context-specific [19]	Cross-resistance to host lysozyme; plausible failure mechanism[19]
Staphylococcus aureus (clinical)	Multiple (gentamicin, oxacillin, daptomycin, teicoplanin)	Blood/clinical	Screen → confirm	HR detected for several drugs; **none for vancomycin** in one 2024 study[9]	Drug-specific heterogeneity; calibrates expectations [9]
Enterococcus faecium	Linezolid	Blood/clinical	Poorly standardised; **PAP-confirmed HR under-reported**	Unknown (true HR prevalence unclear)	Failures mainly fixed resistance mechanism [2]
K. pneumoniae(CRKP)	Colistin (mechanistic & outcome anchor)	**Animal models**reflecting human therapy	**PAP** (bench) with in vivo failure	HR → **treatment failure in vivo**despite AST "susceptible"	Direct causal evidence (pre-clinical) [20,21]

Abbreviations: HR, heteroresistance; PAP, population analysis profiling; PAP–AUC, area-under-the-curve method; CRKP, carbapenem-resistant *K. pneumoniae*; CZA, ceftazidime–avibactam; ICU, intensive care unit.

4. Defining heteroresistance

4.1 Historical perspective (brief)

Early descriptions in *Staphylococcus aureus* exposed to β-lactams noted that a small fraction of cells survived otherwise inhibitory concentrations, presaging today's concept of heteroresistance (HR). Reports of paradoxical ("Eagle-type") survival at higher β-lactam concentrations and temperature-sensitive heterogeneity highlighted that resistance phenotypes within clonal populations could be unstable and easily missed by routine methods. These observations foreshadowed modern diagnostic blind spots.[22,23]

4.2 Operational definition and distinctions

For the purposes of this Review, heteroresistance denotes within-isolate heterogeneity in which reproducible minority subpopulations form colonies at drug concentrations substantially exceeding the isolate's modal MIC (for example, by ≥8-fold) when assessed by population analysis profiling (PAP–AUC) or an equivalent confirmatory approach. [2,24] Tolerance slows killing without a resistant subpopulation sweep; persistence reflects rare dormant cells that survive transiently but do not grow at higher concentrations; mixed infection denotes co-isolation of genetically distinct strains or species. Conflation of these categories inflates prevalence estimates and muddles clinical interpretation; we therefore reserve "HR" for demonstrable growth of minority subpopulations at higher drug concentrations, with at least short-term heritability.[2,24,26] Figure 1 illustrates how subpopulations broaden/bifurcate MIC distributions, explaining AST–outcome discordance.

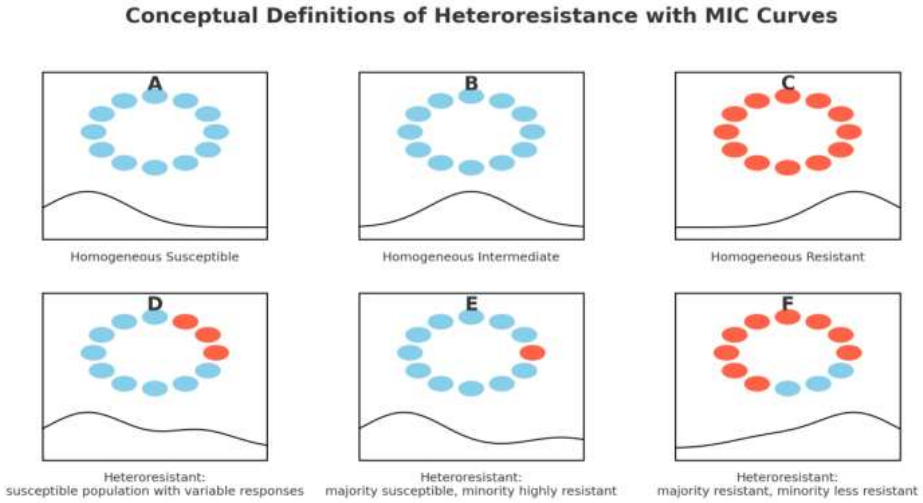

Figure 1. Conceptual definitions of heteroresistance with MIC distributions

Panels A–C show homogeneous states: all cells susceptible (A), uniformly intermediate (B), or resistant (C), each with a unimodal MIC curve. Panels D–F illustrate heteroresistance, where subpopulations with differing susceptibility coexist within a clonal isolate: (D) susceptible majority with resistant minority; (E) majority susceptible with a small, highly resistant minority; (F) majority resistant with a residual susceptible minority. Heteroresistance produces broadened or bimodal MIC distributions and can underlie clinical non-response despite apparent susceptibility.

4.3 Panel: Operational definition and edge cases (for consistent reporting)

The core definition is "heteroresistance confirmed by PAP–AUC, defined as reproducible minority subpopulations capable of growth at antibiotic concentrations at least eight-fold higher than the isolate's modal MIC, within a single isolate." When the phenotype has not been formally confirmed, reports should instead describe the isolate as "screen-positive for features suggestive of HR—such as microcolonies within inhibition zones, trailing around gradient strips, or method discordance—with confirmatory testing recommended" in which heterogeneous resistance arises through transient expression changes rather than stable minority survival.[25-27]

5. Mechanisms of heteroresistance

5.1 Reversible (unstable) architectures

5.1.1. Gene amplification (copy-number bursts). Tandem or episomal duplications transiently increase expression of resistance loci—β-lactamases, lipid-modification enzymes, targets or efflux systems—creating HR minorities that expand on-therapy and **collapse when pressure is removed** because amplified arrays are costly or segregationally unstable [28,29] .

5.1.2. Regulatory toggling and bistability. Two-component systems and global regulators (for example, PhoPQ/PmrAB; MarA/SoxS/Rob) can switch a **fraction** of cells into high-expression states (lipid-A modification, efflux, envelope stress programmes). These phenotypes are **heritable over short spans** but revert as signals abate, producing assay- and inoculum-dependent HR calls.[30,32]

5.1.3. Low-copy plasmid dynamics. Variable plasmid copy number/partitioning yields subpopulations with different resistance-gene dosage (e.g., *mcr-1* carriage in *Escherichia coli*; plasmid-borne OXA carbapenemases in *Acinetobacter*). These HR minorities shrink off-drug unless maintenance systems stabilise the plasmid.[29,33]

5.1.4. Stress-induced physiological states. Sub-MIC antibiotic exposure or host pressures (oxidative stress, cation limitation) can transiently reduce drug entry or enhance repair—creating **short-lived** HR minorities (e.g., aminoglycoside uptake changes in *E. coli*; neutrophil-derived ROS effects in *K. pneumoniae*).[31,34]

5.2 Fixed (stable) architectures

5.2. Point mutations in subpopulations. Rare clones acquire resistance-determinant mutations (e.g., *gyrA*/*gyrB* in *Mycobacterium tuberculosis*; *mexR* or target mutations in *Pseudomonas aeruginosa*), generating **MIC "tails"** that are **stably heritable** and may coexist with susceptible majority cells. This is HR that borders on early fixed resistance.[1,5,35]

Clinical/readout implication*:* Stable HR is **more reproducible** on repeat testing; early **class switch** or **synergy** (e.g., daptomycin ± β-lactam for non-VISA hVISA physiology) and **close microbiological follow-up** are advisable.[36]

5.3 Spatial ecology and biofilms

Drug gradients across **biofilms, abscesses, prosthetic material** and poorly perfused tissue generate protected microniches where partially adapted subclones persist and reseed infection when systemic levels dip.

6. Measurement of heteroresistance

Purpose and scope. Measurement aims to detect **reproducible minority growth at higher drug concentrations** and report it in a way that is clinically interpretable and comparable across studies. The practical sequence is **specimen-aware sampling → sensitive screening → specific confirmation → assay-anchored reporting**, with quality controls that minimise over-calling.[4,37,38]

6.1 Challenges in detection

Heteroresistance (HR) is often **unstable and reversible**, so minority subpopulations can **collapse during subculture or routine AST**, yielding false "susceptible" calls and underestimation of prevalence. A second barrier is the absence of **harmonised diagnostic criteria** beyond hVISA, for which **PAP–AUC** has semi-standardised thresholds; there is no global consensus for other organism–drug pairs.[39]

6.2 Phenotypic methods

6.2.1 Disc diffusion and E-test (screening)

Microcolonies within inhibition zones and **gradient-strip "trailing"** are **screen-positive** cues; false-negatives versus PAP are common.[37] E-test GRD (glycopeptide resistance detection) shows **≈82% sensitivity and ≈60% specificity** against PAP for hVISA and has been linked to vancomycin non-response when standard MICs are "susceptible". [6,37]These tools are widely available and useful **as screens**, not confirmatory tests. Culture based and flow cytometry method.

6.2.2 Molecular and genomic approaches (adjuncts)

Droplet digital PCR (ddPCR) can quantify **minority resistance alleles** (e.g., macrolide resistance in *Helicobacter pylori*; carbapenemase variants in *Klebsiella pneumoniae*) at low frequencies, but adoption is limited by cost, turnaround, and a lack of outcome-linked thresholds.[40] **Whole-genome sequencing (WGS)** with deep coverage detects **mixed alleles/copy-number bursts** and has identified polymyxin HR architectures in *K. pneumoniae*/*Pseudomonas aeruginosa* and minority resistant clones in *Mycobacterium tuberculosis*.[41,42]

7. Machine learning (ML) in the detection and interpretation of heteroresistance

7.1 Rationale and problem framing

Heteroresistance (HR) is typically low-frequency and transient, so routine AST (disc diffusion, gradient strips, broth microdilution) often misses it or yields discordant calls, whereas PAP/PAP–AUC—although the reference—remains labour-intensive and variably implemented across centres.[43] Clinical and modelling data indicate that minorities present at ~10^{-2}–10^{-6} can be enriched on therapy and precipitate failure, motivating earlier, more sensitive detection and risk stratification. ML contributes by: (i) extracting weak HR-linked signals from images/growth curves; (ii) quantifying minority genotypes from sequencing; and (iii) fusing phenotype with patient context to estimate failure risk.[46,47]

7.2 Data modalities and ML applications

Plate images / growth dynamics (computer vision). Convolutional/transformer models trained on Etest or agar-plate images can detect trailing and microcolonies within inhibition zones—features that proxy PAP without the full workflow—and improve sensitivity and reproducibility over unaided reads, particularly in Gram-negatives.[48,49]

Targeted genotyping (ddPCR) with ML. ddPCR quantifies minority resistance alleles (e.g., *mgrB/pmr* axis) and, coupled with calibrated ML, yields probabilistic HR calls when allele fractions sit below conventional thresholds.[51,52,53]

7.3 What ML changes (and what it does not)

Sensitivity & scale. Automated image/WGS pipelines can approximate PAP-level insight at bench speed, enabling screening at scale that is infeasible manually.[50,51]Mechanistic inference. Model features frequently recover known biology (e.g., gene amplification, plasmid copy-number), supporting interpretability.[54]

8. Clinical impact.

In practice, clinicians should suspect HR when bacteraemia persists 48–72 hours on a reported-susceptible agent in high-risk organism–drug pairs, particularly in deep-seated or biofilm-prone

infections, ventilated/ICU settings, or after recent exposure to the index class. [64,65] Pharmacokinetic/pharmacodynamic exposure and penetration[66,67] institute mechanism-aware therapy (e.g., daptomycin ± β-lactam for hVISA-type physiology; combination therapy for suspected polymyxin HR per genotype and site) [60,63], ensure source control [62,65], and arrange early repeat cultures (48–72 h) with reflex confirmation when risk is high, adjusting therapy while confirmation proceeds.[58-60] At the programme level, HR-aware stewardship pathways—locally defined triggers for reflex confirmation in units with high CR-*K. pneumoniae*/*A. baumannii* burden—can shorten time-to-effective therapy and reduce selective exposure.

9. Future directions and research gaps

9.1 Refine PK/PD for HR risk states.

Studies should quantify how target attainment (e.g., vancomycin AUC/MIC 400–600) and site penetration modulate minority outgrowth within the mutation-selection window, and test combinations/dosing schedules that suppress HR minorities without undue toxicity; guidance for vancomycin and polymyxins provides a template for endpoint selection and exposure targets.[1-3,66,67]

9.2 Surveillance and burden estimation.

Regional networks and stewardship programmes should add HR modules that track assay-defined, confirmed HR alongside classical resistance and capture denominators (specimen, syndrome, prior exposure) to estimate attributable failure and trigger reflex-confirmation policies for high-risk organism–drug pairs.[55,57,61]

10. Conclusion

Routine MIC-centric AST under-calls this risk because minority subpopulations are averaged away; PAP/PAP–AUC remains the reference but is impractical at scale. A pragmatic path forward is HR-aware diagnostics and stewardship: sensitive screens (disc/gradient, colony-frequency or semi-automated PAP derivatives) funnelling positives to confirmation; reflex testing triggered by high-risk organism–drug pairs or persistent bacteraemia; and mechanism-aware therapy (e.g., daptomycin±β-lactam for hVISA-type physiology; rational combinations for suspected polymyxin HR) alongside PK/PD verification and source control. Recognising, reporting, and acting on HR is thus not an academic nuance but a necessary update to susceptibility interpretation—key to preserving last-line agents and improving patient outcomes.

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