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Rapid Molecular Diagnostic Methods for Detecting Antimicrobial Resistance in Clinical Microbiology

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Research Article

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Rapid Molecular Diagnostic Methods for Detecting Antimicrobial Resistance in Clinical Microbiology

A reproducible reanalysis of BIOFIRE BCID2 resistance-marker data with a focused narrative synthesis

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Abstract

Background: Rapid molecular methods can identify antimicrobial resistance determinants before conventional susceptibility results become available. Their clinical interpretation nevertheless depends on which determinants are covered, the organisms present and the relationship between genotype and drug-specific phenotype. This study examined how those distinctions affect the evidence supporting molecular resistance detection.

Methods: A reproducible secondary analysis reconstructed resistance-target agreement and selected genotype–phenotype cross-tabulations from a published multicentre evaluation of the BIOFIRE Blood Culture Identification 2 (BCID2) panel. Target-specific positive percent agreement (PPA), negative percent agreement (NPA) and Wilson confidence intervals were calculated from aggregate counts. An explicitly hypothetical model examined the effect of mechanism coverage on residual resistance after a negative result. A focused narrative synthesis contextualised PCR, isothermal amplification, CRISPR-assisted detection and sequencing.

Results: Reconstructed target-level totals were 325/332 comparator-positive detections and 2,465/2,467 comparator-negative results. Positive evidence was uneven: *mecA/C* represented 195/332 positive observations, while IMP, OXA-48-like and *mcr-1* had none. Among CTX-M-negative specimens in the drug-specific subset, cefepime resistance occurred in 2/216 and piperacillin–tazobactam resistance in 19/216. The scenario model showed that incomplete mechanism coverage can leave substantial residual resistance despite high target-detection sensitivity.

Conclusions: Strong molecular agreement supports the detection of specified determinants, but does not establish universal susceptibility prediction. Laboratories should report target coverage and uncertainty, preserve phenotypic confirmation and connect rapid results to validated stewardship pathways. Findings are limited to a single published aggregate dataset and should not be interpreted as independent clinical validation.

Keywords: antimicrobial resistance; molecular diagnostics; multiplex PCR; blood culture; genotype–phenotype discordance; diagnostic stewardship

1. Introduction

Antimicrobial resistance presents a diagnostic problem as well as a therapeutic one. A laboratory must establish which organism is present, whether a relevant resistance mechanism is detectable and whether an antimicrobial is likely to remain active under the intended conditions of use. Molecular methods can shorten the interval required to answer the second question, and some platforms answer the first simultaneously. They do not necessarily answer the third. Treating these questions as interchangeable can convert a technically correct result into an unsafe clinical inference [11, 28, 29].

Targeted assays detect predefined genes or mutations. These may encode a mechanism with a strong organism-specific relationship to resistance, but they may also capture only a subset of the routes to a particular phenotype. Additional routes include alternative enzymes, altered permeability, efflux, regulatory changes and combinations of determinants. Conversely, a detected sequence may not produce the expected phenotype if expression, gene integrity or the tested organism differs from the assumed context. Genome-based prediction studies demonstrate both the potential of sequence information and the importance of organism-specific interpretation [6, 14, 24].

A positive blood culture offers a useful clinical setting for examining these distinctions. Organisms have already grown, facilitating identification and molecular testing, while a substantial opportunity may remain to accelerate antimicrobial decisions. However, the preceding culture incubation means that an assay described as rapid is not equivalent to an equally rapid result measured from specimen collection. Screening rectal swabs for colonisation, testing isolated colonies and testing positive blood cultures are also different intended uses. Multisite investigations of carbapenemase PCR make this specimen dependence explicit [18, 25, 27].

The present article combines an original, reproducible reanalysis of published aggregate observations with a focused appraisal of molecular diagnostic approaches. Its contribution is methodological and interpretative: it separates target detection, the distribution of evidence across targets, drug-specific phenotype and the residual risk implied by incomplete coverage. The numerical analysis concerns one blood-culture panel and does not compare the clinical accuracy of all molecular platforms. The title describes the clinical microbiology problem; the subtitle and all empirical claims are restricted to the analysed setting. A companion reanalysis applies a similar denominator-focused approach to published paired genomic and phenotypic susceptibility results (Abdulwahab, manuscript in preparation); the present article concerns a targeted blood-culture panel.

2. Methods

2.1 Design, source selection and scope

This was a secondary analysis of published aggregate diagnostic data. The source was the multicentre evaluation by Rhoads and colleagues, which included 1,074 eligible positive blood culture samples and reported molecular resistance-marker agreement as well as selected phenotypic comparisons [23]. It was selected because the full-text tables expose the numerators

and denominators needed to distinguish target-level performance from phenotype prediction. No new specimens, patient records or laboratory experiments were collected. The source publication, rather than a newly recruited cohort, defines the sampling frame.

Table 6 of the source supplied ten resistance-marker rows. Tables 7–10 supplied selected Enterobacterales genotype–phenotype comparisons. The original reportability conditions and eligible-organism restrictions were retained conceptually: a denominator for one marker must not be treated as the denominator for another. The unit in the agreement analysis is an eligible target/comparator observation. Multiple target observations can arise within a study; they are not independent patients. No patient-level clustering adjustment or study-level meta-analysis was attempted.

The accompanying literature synthesis, presented in Section 3 before the results of the reanalysis, was purposive and narrative. It prioritised primary diagnostic evaluations, foundational method reports, genome-prediction studies, curated resistance resources and clinical implementation evidence available by 19 November 2026. It was not a systematic review: no exhaustive retrieval claim, screening flow diagram or pooled effect is presented. Diagnostic reporting principles informed the separation of index test, comparator, denominator and uncertainty, without claiming a completed formal risk-of-bias assessment [5, 30].

2.2 Extraction and statistical analysis

Counts were parsed from the public full-text XML (Europe PMC, PMC10281132). For a molecular target, PPA was calculated as TP/(TP + FN), and NPA as TN/(TN + FP), using the source comparator definitions. These are agreement measures against molecular comparators, not sensitivity and specificity for all phenotypic resistance. Two-sided 95% Wilson score intervals were recalculated for both PPA and NPA using $z = 1.95996398454$. The score interval remains informative when every observed positive specimen is detected; its lower limit is not 100% [19, 31].

For auditability, totals were reconstructed by summing Table 6 rows. This yields 325/332 positive and 2,465/2,467 negative observations. The source abstract prints the negative total as 2,465/2,767; because the ten table rows sum to 2,467, the abstract figure appears to be a typographical error. All calculations here use the table-level reconstruction. This is an arithmetic reconciliation, not a reclassification of source specimens. The reproduction script asserts the reconstructed counts before any output is produced (see Data and code availability).

For cefepime and piperacillin–tazobactam, resistant (R), susceptible–dose dependent (SDD) and susceptible (S) counts were retained separately. The source used broth microdilution and its contemporary CLSI interpretation. Six specimens with KPC, VIM and/or NDM were excluded from those two drug-specific tables, leaving 260 observations. Categories were not retrospectively reassigned to current breakpoints, and SDD was not combined with R. No significance test was used to imply a causal effect of CTX-M status or a treatment recommendation.

2.3 Analytical coverage scenario

An independent scenario illustrates a limitation not estimable from the aggregate molecular agreement table. Let *p* be the prevalence of phenotypic resistance, *c* the fraction of resistant cases attributable to mechanisms represented by the assay, and *a* the sensitivity for those represented mechanisms. Assuming specificity of 1 and no additional detection outside the represented mechanisms, the residual probability of resistance after a negative result is:

$$P(R \mid \text{negative}) = p(1 - ca) / [p(1 - ca) + (1 - p)]$$

The figure varies *p* from 0.01 to 0.50 and *c* from 0.40 to 1.00, with *a* fixed at 0.98. These are transparent assumed inputs; *a* is not fitted to the source cohort. Coverage is defined at the case level, including overlapping mechanisms, rather than as the fraction of names in a gene catalogue. Specificity of 100% is an optimistic simplifying assumption. The model does not incorporate uncertain gene expression, mixed organisms, invalid results, antimicrobial exposure or clinical severity.

3. Narrative synthesis: molecular diagnostic approaches

3.1 Targeted and multiplex PCR

Real-time PCR couples sequence-specific amplification with a measurable signal. Cartridge systems can reduce the manual handling needed for extraction, amplification and interpretation. The strength of such systems is their explicit target definition: a result can be evaluated against an independent molecular comparator for the same determinant. Their main interpretative limitation follows from that same definition. An undetected targeted carbapenemase is not equivalent to the absence of every carbapenem resistance mechanism.

Carbapenemase PCR studies have evaluated rectal swabs, colonies and other specimen types separately. Differences in bacterial load, specimen inhibitors, reference methods and selection of suspected resistant isolates affect the claims each study supports [7, 10, 16]. Surveillance detection of colonisation can support infection prevention, but does not independently establish the cause of a current infection. A laboratory should therefore validate both the analytical target and the clinical question attached to it.

Multiplex blood-culture panels add pathogen identification and multiple resistance markers to a shared run. Independent evaluations support their practical utility while reinforcing the need to understand panel composition and reportability [15]. In polymicrobial material, identifying a resistance sequence does not always establish which organism carries it. Composite interpretations, such as *mecA/C* together with an organism-associated junction target, are distinct analytical outputs and should not be merged with standalone *mecA/C* results.

3.2 Isothermal amplification and CRISPR-assisted detection

Loop-mediated isothermal amplification uses strand-displacing synthesis and multiple primer-binding regions to amplify a target without repeated thermal cycling [20]. This can reduce equipment requirements, but does not remove extraction, contamination prevention or result interpretation from the workflow. A visible signal may be convenient without being sufficient

evidence of clinical specificity. Prospective testing in the intended matrix and inclusion of related non-target variants remain necessary.

CRISPR-assisted methods employ programmable nucleic-acid recognition in several different assay designs. Some use collateral reporter cleavage after amplification; others enrich selected sequences before sequencing. FLASH, for example, demonstrated CRISPR-based enrichment for multiplexed resistance-sequence detection, a different architecture from a cartridge PCR assay [21]. A Cas13-assisted study developed fluorescence and lateral-flow approaches for carbapenem-resistant *Klebsiella pneumoniae* detection [8]. These examples establish technical possibilities within defined experiments; they do not justify assuming equivalent clinical readiness across every CRISPR implementation.

The clinically relevant performance belongs to the full workflow, including extraction, preamplification, recognition, readout and thresholding. Reporting only the most favourable component time or analytical detection limit obscures failures occurring elsewhere. Table 1 compares these approaches by the claims that require validation, rather than assigning unsupported universal rankings.

Table 1. Molecular approaches and the claims their validation must support

Approach and input	Analytical advantage	Boundary requiring validation
Targeted PCR; isolate or defined specimen	Rapid detection of a specified resistance family; established cartridge workflows	A negative result excludes only validated targets; specimen and variant coverage matter.
Multiplex PCR; positive blood culture	Concurrent identification and resistance markers in an actionable workflow	Culture incubation precedes the test; organism attribution may be ambiguous in mixed cultures.
LAMP; extracted nucleic acid	Isothermal amplification can reduce instrument complexity	Multiple primer sites, contamination control and clinical matrix effects require evaluation.
CRISPR-assisted detection; extracted or amplified nucleic acid	Programmable sequence recognition with fluorescence, strip or sequencing readout	Preamplification, guide mismatch and readout thresholds affect full-workflow performance.
Whole-genome sequencing; usually cultured isolate	Broad interrogation of known genes and mutations with epidemiological context	Database version, assembly quality and organism-specific genotype–phenotype mapping constrain interpretation.

3.3 Sequencing and resistance interpretation resources

Whole-genome sequencing can interrogate more resistance loci and provide epidemiological context. However, wider sequence coverage is not synonymous with complete phenotypic knowledge. Prediction depends on read quality, assembly, allele identification, mutation catalogues and the biological relationship between each determinant and the drug under consideration [11]. ResFinder, PointFinder and AMRFinderPlus illustrate complementary approaches to acquired genes and chromosomal changes [4, 13, 32].

Database maintenance is part of the diagnostic method. Curated updates can alter nomenclature, sequence assignments and interpretation, making software and database versions essential reproducibility fields [1, 12]. Comparisons of genotypic and phenotypic testing in clinical isolates further caution against extrapolating a well-performing rule across all organisms and drugs [22]. Responsible sharing of linked genotype–phenotype records is consequently a practical requirement for improving future prediction rather than an optional supplement [9].

4. Results of the secondary analysis

4.1 Target-specific agreement and unequal evidence

Seven of the ten marker rows had at least one comparator-positive observation. CTX-M agreement was 46/47, *mecA/C* agreement was 195/195, the *mecA/C*–MREJ composite was 52/57, and *vanA/B* agreement was 23/24. KPC and VIM each had four comparator-positive observations, all detected, while NDM had one. IMP, OXA-48-like and *mcr-1* had no positive observations. Their PPA is not estimable in this dataset; a negative-only series cannot demonstrate detection of positive specimens [23].

The distribution of positive observations materially changes interpretation. *mecA/C* accounts for 58.7% of the 332 positive target observations. The apparent pooled PPA therefore represents the source mixture of targets, rather than an equal assessment of each resistance mechanism. It must not be described as a transportable 97.9% probability of detecting any clinically relevant resistance. A setting with a different gene distribution could have a different effective performance even before local preanalytical factors are considered.

Recalculated Wilson lower confidence limits are approximately 51.0% for 4/4 and 20.7% for 1/1. Perfect observed detection is therefore compatible with substantial uncertainty when positive support is sparse. For *mecA/C*, the larger 195/195 series produces a much narrower PPA interval, although its negative support is small: 60/60 comparator-negative results give an NPA lower limit of 94.0%. Table 2 and Figure 1 show these differences directly; Figure 2 exposes the denominator imbalance that an aggregate accuracy statement conceals.

Table 2. Reconstructed target agreement against molecular comparators (source Table 6)

Marker	Positive tests	PPA, % (95% CI)	Negative tests	NPA, % (95% CI)
CTX-M	46/47	97.9 (88.9–99.6)	312/312	100.0 (98.8–100.0)
IMP	0/0	Not estimable	359/359	100.0 (98.9–100.0)
KPC	4/4	100.0 (51.0–100.0)	328/328	100.0 (98.8–100.0)
NDM	1/1	100.0 (20.7–100.0)	358/358	100.0 (98.9–100.0)
OXA-48-like	0/0	Not estimable	323/323	100.0 (98.8–100.0)
VIM	4/4	100.0 (51.0–100.0)	355/355	100.0 (98.9–100.0)
mecA/C	195/195	100.0 (98.1–100.0)	60/60	100.0 (94.0–100.0)
mecA/C and MREJ (MRSA)	52/57	91.2 (81.1–96.2)	92/94	97.9 (92.6–99.4)

Marker	Positive tests	PPA, % (95% CI)	Negative tests	NPA, % (95% CI)
mcr-1	0/0	Not estimable	240/240	100.0 (98.4–100.0)
vanA/B	23/24	95.8 (79.8–99.3)	38/38	100.0 (90.8–100.0)
Total (row sum)	325/332	97.9 (pooled; see text)	2,465/2,467	99.9 (pooled; see text)

Intervals are two-sided 95% Wilson score intervals recalculated from the counts. Pooled totals are descriptive and are not transportable performance estimates.

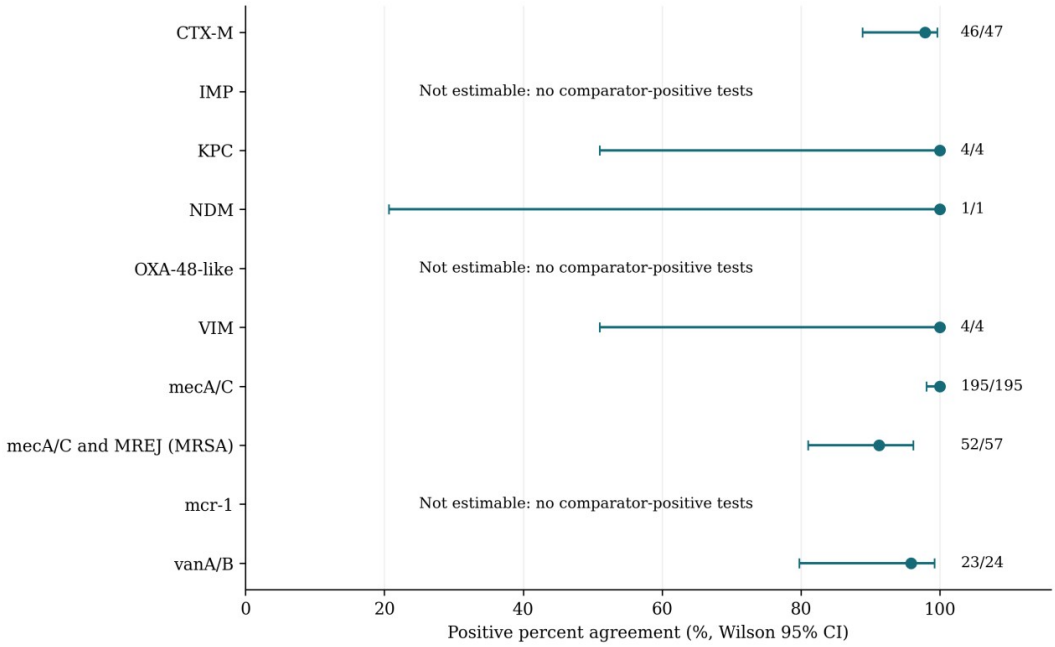


Figure 1. Target-specific positive percent agreement and recalculated Wilson 95% confidence intervals. Counts derive from Rhoads et al. (2023), Table 6. Empty positive denominators are not plotted as zero accuracy. This is a single-study reanalysis, not a meta-analysis.

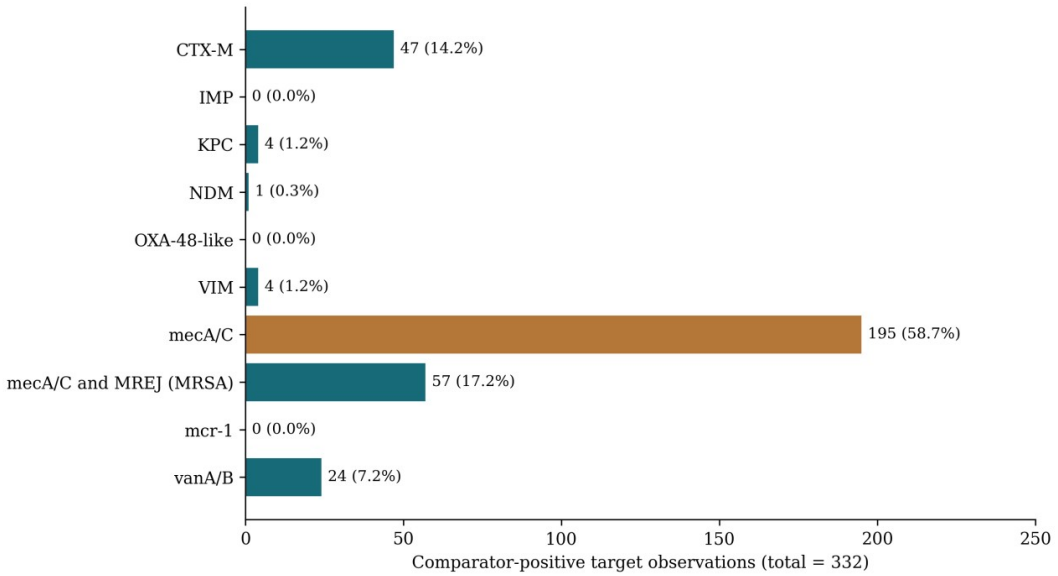


Figure 2. Unequal evidence supporting resistance targets. Comparator-positive observations are target-level tests, not unique patients. *mecA/C* contributes 195/332 observations; three targets have no positive observations. Source: Rhoads et al. (2023), Table 6.

Across the reconstructed rows there were seven false-negative molecular comparisons and two false-positive comparisons. Five false negatives and both false positives arose in the composite MRSA output. These aggregate counts locate where agreement was reduced, but cannot establish the biological or technical cause of each discrepancy. Source investigation and specimen-level evidence would be necessary before attributing them to target variation, low abundance or mixed organisms.

4.2 Molecular markers and drug-specific phenotype

The selected phenotypic comparisons answer a different question. In source Table 7, CTX-M-positive specimens included 43 phenotypically ESBL-positive and three ESBL-negative observations; CTX-M-negative specimens included three ESBL-positive and 217 ESBL-negative observations. This supports a close association in that subset without making the gene and phenotypic endpoint identical. Other mechanisms can produce relevant phenotypes, and molecular presence and measured expression need not coincide.

The drug-specific tables contain 260 rather than 266 observations because the source excluded the six carbapenemase-positive specimens from them. The CTX-M-positive group therefore falls from 46 in Table 7 to 44, and the CTX-M-negative group from 220 to 216. For cefepime, 36/44 CTX-M-positive observations were R, six were SDD and two were S. Among 216 CTX-M-negative observations, two were R, seven were SDD and 207 were S. Piperacillin–tazobactam produced a different pattern: 13/44 CTX-M-positive observations were R, three were SDD and 28 were S; among negative observations, 19 were R, seven were SDD and 190 were S. Thus, resistance among marker-negative observations was 0.9% for cefepime and 8.8% for piperacillin–tazobactam in this selected subset.

These conditional proportions cannot be converted into a universal therapeutic rule. They reflect selected organisms, the source study's breakpoints and exclusion of six carbapenemase-positive specimens. They nevertheless demonstrate why one molecular signal cannot be assigned a single drug-independent meaning. Table 3 preserves the absolute counts, while Figure 3 displays the different category distributions without hiding SDD.

Table 3. CTX-M status and drug-specific phenotype: published counts (source Tables 8–9)

Drug	CTX-M	R	SDD	S	Total
Cefepime	Positive	36	6	2	44
Cefepime	Negative	2	7	207	216
Piperacillin–tazobactam	Positive	13	3	28	44
Piperacillin–tazobactam	Negative	19	7	190	216

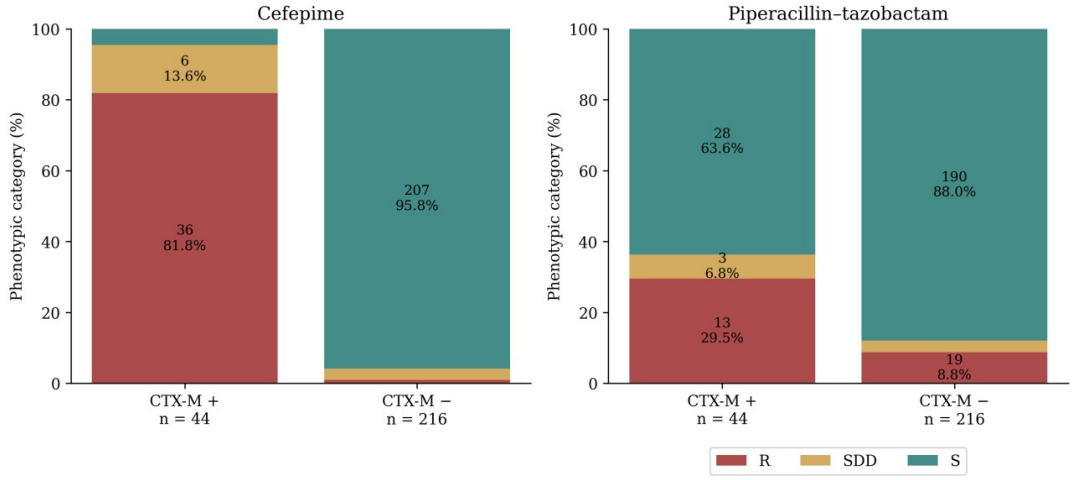


Figure 3. Drug-specific phenotypes conditional on CTX-M detection. Stacks show published counts and percentages; SDD denotes susceptible–dose dependent under the source study's CLSI interpretation. Six carbapenemase-positive specimens were excluded by the source analysis. Source: Rhoads et al. (2023), Tables 8–9.

For context, the source carbapenemase-versus-phenotype table contained five marker-positive resistant observations, one marker-positive susceptible observation, one marker-negative resistant observation and 259 marker-negative susceptible observations. The six phenotypically resistant observations provide limited support for estimating phenotype prediction. This cross-tabulation must remain separate from Table 2's gene-versus-gene agreement; combining the two would conflate endpoints and denominators.

4.3 Consequences of incomplete coverage

The analytical scenario illustrates how high detection sensitivity for represented mechanisms can coexist with appreciable residual resistance. At $p = 0.20$ and $c = 0.80$, the assumed effective resistance sensitivity is 0.784, yielding approximately 5.1% residual resistance after a negative result. Increasing coverage to 1.00 under the same assumptions reduces that probability to approximately 0.5%. At $p = 0.50$ and $c = 0.60$, it is approximately 29.2%.

These values are mathematical consequences of stated assumptions, not estimates for the source hospital populations. Figure 4 is intended to identify the missing information a laboratory needs: local prevalence and the fraction of clinically resistant cases its targets represent. It cannot select a de-escalation threshold or account for the consequences of missing resistance in an individual patient.

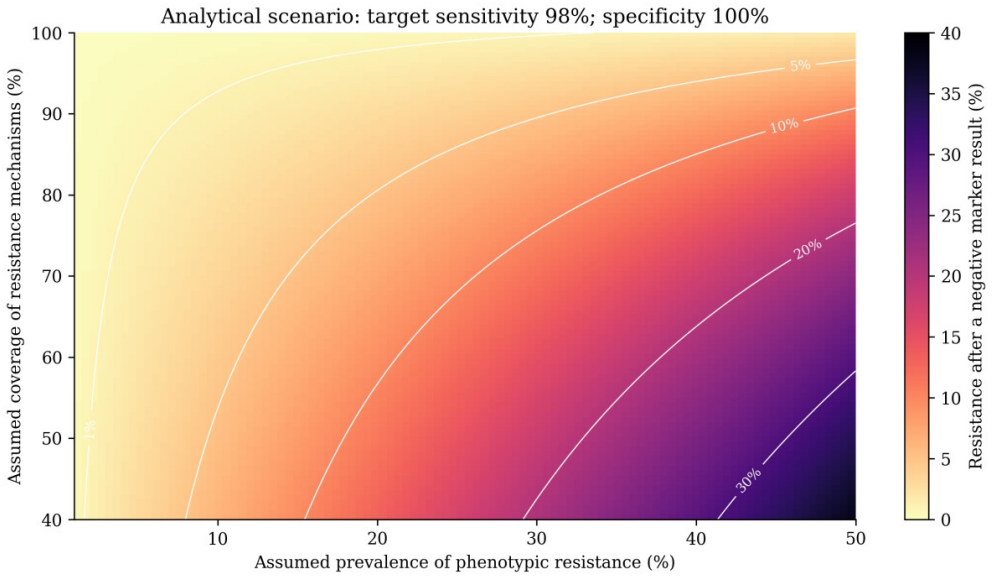


Figure 4. Analytical scenario for residual phenotypic resistance after a negative marker result. Target sensitivity is fixed at 98% and specificity at 100%; prevalence and mechanism coverage are assumed inputs. These are calculated scenarios, not observed patient data or validated clinical thresholds.

5. Discussion

5.1 What constitutes a useful rapid result?

The analysis supports a hierarchy of claims. First, a method may accurately detect its specified nucleic-acid target. Second, that target may predict a particular phenotype in a defined organism–drug context. Third, acting on the result may improve patient management. Each claim needs different evidence. Strong performance at the first level cannot substitute for validation at the other two.

Timing is similarly layered. Instrument run time excludes transport, batching, positive-culture incubation, result verification and communication. A result that reaches the clinician after an effective regimen has already been selected may add less immediate treatment value than the same result delivered during a prescribing decision. Laboratory and stewardship studies should therefore retain timestamps from specimen collection to clinical action, with pre-specified definitions of escalation, optimisation and de-escalation.

Randomised evidence provides an essential counterweight to purely analytical studies. A 2026 trial of rapid molecular bloodstream diagnosis reported earlier effective antimicrobial changes, but did not establish significant reductions in mortality or length of stay [17]. Earlier molecular diagnostic

evidence also highlights the importance of implementation and stewardship [26]. These findings support measuring clinical consequences directly, without assuming that every reduction in reporting time must produce a survival benefit.

Rapid phenotypic susceptibility testing offers a complementary route: it measures drug response rather than inferring it from a selected sequence. The RAPIDS-GN trial illustrates why the clinical impact of a rapid diagnostic pathway should be evaluated separately from the technology's analytical promise [2]. The subsequent multinational FAST trial, conducted in settings with high resistance prevalence, found that rapid phenotypic testing directly from positive blood cultures led to earlier antibiotic escalation or de-escalation but was not superior to standard testing for the day-30 ranked clinical outcome [3]. Molecular and phenotypic tests can therefore be assigned different responsibilities within one workflow: early recognition of actionable mechanisms, followed by broader drug-specific assessment.

5.2 Reporting and implementation

A useful report distinguishes "marker not detected" from "susceptible". It specifies relevant targets, eligible organisms and limitations of attribution where multiple organisms are detected. Positive results should be linked to locally validated interpretative rules and appropriate stewardship or infection-prevention review. Negative results should retain a route to phenotypic confirmation when resistance remains plausible.

The evidence ledger in Table 4 translates these principles into operational requirements. It includes rare positive determinants, reportability, mixed cultures, versioning and clinical timestamps. Such a ledger supports an auditable implementation decision, but is not a validated scoring system. Costs should also be measured over the pathway: cartridges, labour, repeat tests, confirmatory work, training, quality assurance and the availability of staff to act on results. No cost-effectiveness estimate can be inferred from the present counts.

Table 4. Minimum evidence for a clinically interpretable molecular resistance report

Decision point	Evidence to retain	Reporting or implementation consequence
Specimen eligibility	Validated matrix, pretreatment, identification result and reportability rules	Do not extrapolate performance from isolates to direct specimens.
Target coverage	Gene families, variants, mutation sites and local resistance epidemiology	State precisely what "not detected" covers.
Rare determinants	Positive counts and uncertainty by target; reference materials when needed	Do not describe unobserved positive targets as clinically proven.
Mixed organisms	Culture, identification and marker linkage evidence	Flag uncertain organism attribution; obtain isolate-level confirmation.
Phenotypic discordance	Isolate recovery, reference AST, sequencing and repeat testing	Investigate systematically; do not automatically overwrite the phenotype.
Clinical pathway	Collection, positivity, reporting, communication and treatment timestamps	Evaluate treatment changes alongside safety, stewardship and resource use.

Decision point	Evidence to retain	Reporting or implementation consequence
Version changes	Assay, database, software and breakpoint versions	Revalidate affected claims and maintain an auditable change record.

In laboratories with constrained resources, simpler instruments may reduce one barrier while leaving procurement, specimen preparation or quality control unresolved. A staged introduction should therefore choose the clinical question first, then verify the assay in the intended matrix and assess the ability to act on the result. An inexpensive unlinked result may be less useful than a targeted service with reliable communication and confirmation. This is an implementation inference, not a claim of comparative economic superiority.

5.3 Limitations and future validation

This reanalysis uses one published aggregate dataset and cannot establish independent external validity. There are no patient-level covariates, organism-specific paired records or treatment histories with which to adjust for clustering, case mix or prior antimicrobial exposure. The narrative synthesis is selective and cannot establish a comprehensive comparative ranking of all methods. The original evaluation's sponsor relationships, selection procedures and comparator definitions remain relevant when interpreting its findings [23].

The data were generated under historical testing and interpretative conditions. Reanalysis preserves those conditions rather than assuming current software, panel coverage or breakpoints. The coverage scenario deliberately simplifies biology and clinical decision-making; it is neither a calibrated prediction model nor a substitute for local validation. Wilson intervals quantify binomial sampling uncertainty for individual proportions, but do not account for all sources of design or comparator bias.

Future work should prospectively enrol consecutive intended-use specimens across different resistance ecologies, document excluded and invalid results, and ensure adequate positive support for rare determinants. Linked organism, gene, MIC and treatment data would permit drug-specific assessment and investigation of discordance. Independent multicentre validation should then evaluate prespecified clinical actions and balancing outcomes, including missed resistance and unnecessary broad-spectrum treatment. Transparent access to such linked records would make a stronger contribution than repeating an aggregate accuracy percentage.

6. Conclusions

Rapid molecular diagnostics can provide clinically valuable early information about antimicrobial resistance mechanisms. Their interpretation requires explicit separation of target agreement, evidence support, phenotype prediction and clinical effect. The published blood-culture data examined here show high aggregate agreement alongside sparse evidence for several targets and drug-dependent phenotypes among CTX-M-negative specimens. Laboratories should communicate what the assay has tested, preserve confirmation where needed and evaluate the full

route from specimen to prescribing decision. These conclusions support disciplined implementation; they do not validate a new assay or establish a universal treatment rule.

Declarations

Data and code availability. All source data are publicly available in Rhoads et al. (2023) [23] (Europe PMC full text, PMC10281132). The reproduction script, which downloads the source XML, extracts Tables 6–10, asserts the reconstructed totals and regenerates every number in Tables 2–3 and Section 4.3, is available at [GitHub repository URL] and archived at [Zenodo DOI].

Ethics statement. This study used only published, aggregate, de-identified data. No human participants were recruited and no individual-level records were accessed; ethical approval and informed consent were therefore not required.

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Competing interests. [Confirm: The author declares no competing interests.]

Author contributions. F.A. conceived the study, extracted and analysed the data, prepared the figures and wrote and revised the manuscript.

Use of AI tools. [State here whether and how AI tools were used, if the target journal requires it.]

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