



Absence of Evidence Is Not Evidence of Susceptibility: Failure Modes of Determinant-Count Rules in Resistome-Guided Antibiotic Selection

Seshu Kumari K¹, Dr. Munesh Kumar Sharma²

¹Research Scholar, Department of Microbiology, Amaltas University, Dewas, Madhya Pradesh, India

²Research Supervisor, Department of Microbiology, Amaltas University, Dewas, Madhya Pradesh, India

Abstract- Frameworks that convert resistome content into antimicrobial guidance must decide what to do when no resistance determinant is found. The decision is easy to get wrong, and the error is invisible at the scale at which such frameworks are usually run. This paper audits, decision by decision, the 96 class-level outputs of a six-stage resistome-to-guidance framework applied to ten reference strains of the human gut microbiota carrying six determinants, against standard clinical microbiological references. Under a binary rule in which a determinant count of zero produces the decision preferred candidate, 89 of 96 decisions were positive recommendations. Eight classes of error were identified, several contradicting firmly established fact: cephalosporins were recommended for *Enterococcus faecalis* V583, which is intrinsically resistant to the entire class through low-affinity penicillin-binding proteins; aminoglycosides were recommended for both *Bacteroides* strains, which cannot accumulate the drug for want of oxygen-dependent uptake; and penicillins were recommended for both *Enterobacterales* despite a curated β -lactamase mapping to the penam ontology class, an implementation defect distinct from the conceptual one. Twelve of the sixteen clinical classes were unrepresented in the determinant panel and returned zero for every strain. A revised three-state rule, supplemented by a separately compiled intrinsic resistance register, removes every identified error; it converts 89 positive recommendations into 23 avoidance decisions on register evidence and 66 explicit statements of absence of evidence, and emits no positive recommendation at all. We argue that this last property is a requirement rather than a deficiency of any determinant-based guidance system, and that small auditable panels are a necessary stage in the validation of such systems.

Keywords- Antimicrobial resistance; antimicrobial stewardship; intrinsic resistance; genotype–phenotype prediction; decision rules; clinical decision support

I. INTRODUCTION

A resistance determinant detected in a genome is evidence that an organism can resist a drug. A determinant not detected is evidence of nothing in particular. The asymmetry is elementary and is stated in every careful discussion of genotype-based prediction, yet it is systematically discarded at the point



where resistome data are turned into advice, because advice that lists only what to avoid appears less useful than advice that also says what to use.

The pressure to close that gap is real. Empirical prescribing decisions are taken before susceptibility results are available, and a framework that ranks agents by determinant evidence looks like exactly the instrument such decisions need. The temptation is to treat a determinant count of zero as a positive signal, and the implementation that does so is a single line of code. Its consequences are not visible in aggregate output statistics, because a rule that recommends nearly everything produces confident-looking output across the board.

This paper makes those consequences visible by auditing every decision individually. The subject is a six-stage resistome-to-guidance framework, specified in a companion paper, applied to a panel of ten reference strains of the human intestinal microbiota carrying six well-characterised determinants across sixteen clinical antimicrobial classes. The panel is deliberately small: at ninety-six decisions, every output can be checked by hand against standard references, which is not possible for a survey of hundreds of metagenomes. The contributions are an error taxonomy derived from that audit, a revised three-state rule with an accompanying intrinsic resistance register, and an argument that the correct behaviour of such a rule is to make no positive recommendation at all.

II. RELATED WORK

Two bodies of work bear directly on the question. The first concerns the predictive value of a determinant call. Comparative studies of genotype-to-phenotype agreement report that prediction is strong where resistance follows from acquisition of a single well-characterised gene and weak where it depends on expression level, regulatory context or combinations of chromosomal mutations. Crucially, these studies measure agreement where a determinant is found; the complementary case, in which none is found, is rarely reported as a separate figure, so the error rate of the inference from absence is largely undocumented.

The second concerns intrinsic resistance, which clinical microbiology has catalogued in detail for decades and which susceptibility-testing standards encode as expected phenotypes used to detect laboratory error. This catalogue sits entirely outside determinant databases, whose curation model is the gene. A guidance system built on determinant data alone therefore has no access to it unless it is supplied separately, and the omission is silent: nothing in the determinant output signals that a class has been assessed without the relevant evidence.

To our knowledge the behaviour of the absence case has not previously been audited decision by decision in a published resistome-to-guidance pipeline, which is what the present work does.

III. MATERIALS AND METHODS

A. The framework and panel under audit

The framework maps curated determinant calls onto drug classes of the Antibiotic Resistance Ontology, maps those onto sixteen clinical antimicrobial classes, and emits one decision per strain per clinical class. The panel comprises ten reference strains: *Escherichia coli* Nissle 1917, *Klebsiella pneumoniae* MGH78578, *Bacteroides fragilis* NCTC9343, *Bacteroides thetaiotaomicron* VPI5482, *Enterococcus faecalis* V583, *Clostridioides difficile* 630, *Faecalibacterium prausnitzii* A2165, *Bifidobacterium longum* NCC2705, *Lactobacillus rhamnosus* GG and *Akkermansia muciniphila* BAA835. Six determinants were curated from the primary genome literature: AmpC and SHV β -lactamases, the cepA cephalosporinase,



tetW, aac(6')-li and the vanB operon. Six strains carry at least one determinant, giving $6 \times 16 = 96$ decisions.

Because the determinant calls were curated rather than computed from sequence, the audit concerns the decision rule and not the sensitivity of any annotation tool. This is the appropriate isolation for the question at hand: the errors reported below would occur even if determinant detection were perfect.

B. The rule as originally implemented

For strain s and clinical class c , let $n(s,c)$ be the number of panel determinants mapping to c . The binary rule was: $n \geq 1 \rightarrow$ avoid or use with caution; $n = 0 \rightarrow$ preferred candidate. No other input was consulted.

C. Audit procedure

Each of the 96 decisions was compared against the species-level position recorded in standard clinical microbiological references and in the primary literature for the strain. A decision was recorded as erroneous where the reference position contradicts the decision, and as unsupported where the reference position is variable or silent and the decision is nonetheless positive. Errors were then grouped by cause. No statistical inference was applied; the audit is exhaustive rather than sampled, so error counts are complete for this panel and carry no sampling uncertainty, and equally no claim of generality beyond it.

D. Coverage of the clinical class space

Fig. 1 shows how many strains register a mapped determinant against each of the sixteen clinical classes. Four classes are represented in the panel; twelve are not, and return a count of zero for every strain in every case. This asymmetry is the structural precondition for the errors reported below, and it is not an artefact of the panel being small: any determinant set of any size omits classes whose resistance is conferred by mechanisms not represented as identifiable genes.

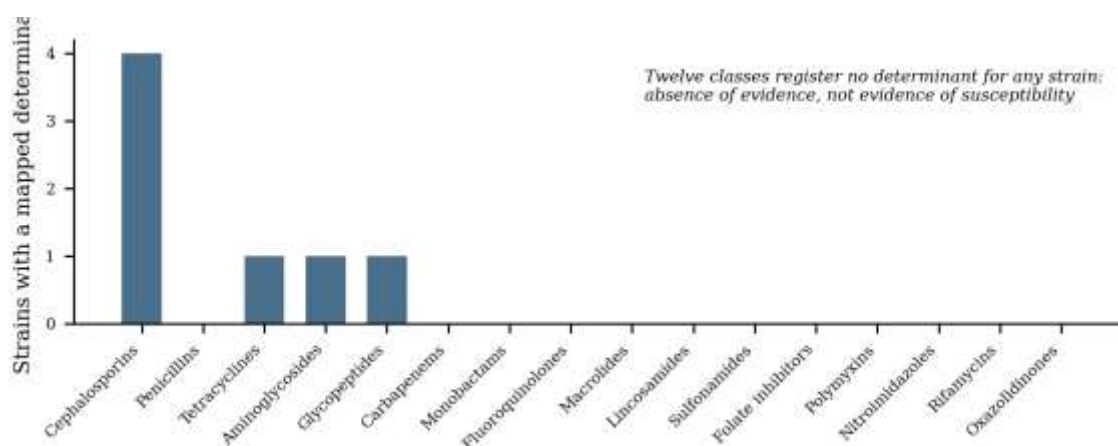


Fig. 1. Strains registering a mapped determinant against each of the sixteen clinical antimicrobial classes. Grey bars mark classes for which the determinant panel contains nothing at all.

IV. RESULTS OF THE AUDIT

A. Distribution of decisions

Under the binary rule, seven decisions returned avoidance and 89 returned preferred candidate. All seven avoidance decisions are correct and each corresponds to an established resistance: cepA-mediated cephalosporin resistance in both Bacteroides strains, AmpC in E. coli Nissle, SHV in K. pneumoniae, tetW in B. longum, and vanB and aac(6')-li in E. faecalis V583. The rule fails nowhere in the presence case. Of the 89 positive decisions, the audit classed 23 as erroneous against a documented



intrinsic resistance and the remaining 66 as unsupported, being assertions of suitability resting on the absence of a determinant from a six-member panel.

B. Error taxonomy

Table I lists the eight classes of error. The first six arise from the conceptual defect: a zero count was read as susceptibility where the species is in fact intrinsically non-susceptible for reasons that no resistance gene encodes. The seventh, the recommendation of penicillins to both Enterobacterales, arises differently and deserves separate comment. Both organisms carry a curated β -lactamase that the ontology maps to the penam class, and the ontology-to-clinical mapping assigns penam to Penicillins correctly. The guidance stage nonetheless counted determinants per clinical class after the ontology assignment had been collapsed, so the penam assignment was lost before the count was taken. This is an implementation defect independent of the conceptual one, and it was identified only because the panel was small enough to check by hand. The eighth entry covers the twelve unrepresented classes, in which every strain received a positive recommendation on no evidence whatever.

Table I. Erroneous and unsupported guidance produced by the binary rule

Strain	Class recommended	Rule output	Established position	Cause
<i>Enterococcus faecalis</i> V583	Cephalosporins	Preferred candidate	Intrinsically resistant, low-affinity PBPs	Conceptual
<i>Bacteroides fragilis</i> NCTC9343	Aminoglycosides	Preferred candidate	Intrinsically resistant, no oxidative uptake	Conceptual
<i>Bacteroides thetaiotaomicron</i> VPI5482	Aminoglycosides	Preferred candidate	Intrinsically resistant, no oxidative uptake	Conceptual
<i>Bacteroides fragilis</i> NCTC9343	Fluoroquinolones	Preferred candidate	Variable, frequently non-susceptible	Conceptual
<i>Bacteroides thetaiotaomicron</i> VPI5482	Macrolides	Preferred candidate	Frequently non-susceptible	Conceptual
<i>Enterococcus faecalis</i> V583	Carbapenems	Preferred candidate	Reduced activity, not first line	Conceptual
<i>E. coli</i> Nissle 1917; <i>K. pneumoniae</i> MGH78578	Penicillins	Preferred candidate	AmpC and chromosomal SHV confer aminopenicillin resistance	Implementation
All six determinant-carrying strains	Twelve unrepresented classes	Preferred candidate	No panel determinant maps to these classes	Structural

Conceptual: a zero determinant count read as evidence of susceptibility. Implementation: ontology-class assignment lost before counting. Structural: clinical classes absent from the determinant panel.

C. The revised three-state rule

The rule was revised to emit three decisions rather than two. Where one or more panel determinants map to the class, the decision is determinant detected — avoid or use with caution. Where no panel determinant applies but the species is documented as intrinsically non-susceptible, the decision is no panel determinant, intrinsic resistance recorded — avoid, generated from a register described below and not from the determinant panel. Where neither applies, the decision is no panel determinant, no intrinsic resistance recorded, which is reported as an absence of evidence within a narrow panel and is not a recommendation.



The intrinsic resistance register was compiled separately from standard clinical microbiological references. It records, for each panel species, the antimicrobial classes to which the species is intrinsically non-susceptible for reasons not represented by a resistance gene: the non-susceptibility of enterococci to all cephalosporins, that of obligate anaerobes to aminoglycosides arising from the absence of oxygen-dependent drug uptake, and the vancomycin resistance of *Lactobacillus rhamnosus* arising from a D-Ala-D-Lac peptidoglycan terminus, among others. That this register had to be built separately, and that its contents contradict the original output at twenty-three points, is itself a result: the information required to make a determinant-based rule safe is not present in determinant data.

Table II. Extract from the separately compiled intrinsic resistance register

Species	Class	Basis of intrinsic non-susceptibility
<i>Enterococcus faecalis</i>	Cephalosporins	Low-affinity penicillin-binding proteins
<i>Bacteroides</i> spp.	Aminoglycosides	No oxygen-dependent drug uptake
<i>Bifidobacterium longum</i>	Aminoglycosides	Obligate anaerobe; no oxidative uptake
<i>Lactobacillus rhamnosus</i>	Glycopeptides	D-Ala-D-Lac peptidoglycan terminus
<i>Clostridioides difficile</i>	Cephalosporins	Documented non-susceptibility; selected by exposure
<i>Klebsiella pneumoniae</i>	Penicillins (amino-)	Chromosomal SHV β -lactamase
<i>Akkermansia muciniphila</i>	Aminoglycosides	Obligate anaerobe; no oxidative uptake

The register is consulted at the guidance stage as an input independent of the determinant panel. Its entries contradict the binary rule at twenty-three of ninety-six decisions.

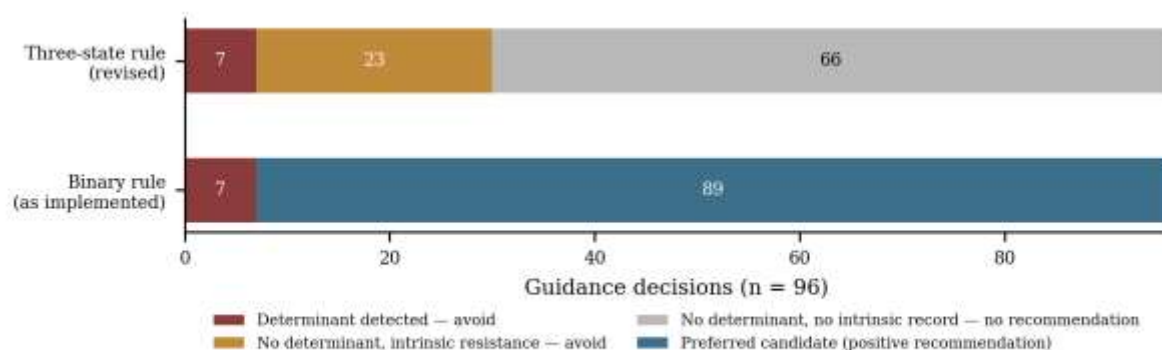


Fig. 2. Distribution of the 96 class-level decisions under the binary rule as implemented and under the revised three-state rule.

Table III. Decision distribution before and after revision (n = 96)

Decision category	Binary rule	Revised rule	Clinical meaning
Determinant detected — avoid or use with caution	7	7	Positive evidence of resistance
No determinant, intrinsic resistance recorded — avoid	0	23	Avoid, on register evidence
No determinant, no intrinsic resistance recorded	0	66	Absence of evidence; not a recommendation



Preferred candidate	89	0	Not emitted under the revised rule
Decisions contradicting a documented position	23	0	—

D. Why the audit required a small panel

Every error in Table I was found by reading decisions against references, not by any automated check. At ten strains and sixteen classes the reading takes an afternoon. At the scale at which resistome pipelines are normally run, it is infeasible, and the aggregate statistics such pipelines report would not have revealed a single one of these errors: the binary rule produces a confident, complete and internally consistent output table in every case.

The same small scale sets a hard ceiling on the statistical machinery that might otherwise be used to validate such a framework, and the ceiling is worth stating because it is often crossed silently. Fig. 3 shows two instances. No Fisher exact test of association between a determinant and Gram reaction can attain $p < 0.05$ with ten strains divided four to six between the groups, whatever the underlying biology; the strongest observed association reached $-\log_{10} p \approx 0.35$ against a threshold of 1.30. And a Shannon index computed across determinants can take only two values when no strain carries more than two determinants, zero and $\ln 2$, so the diversity figure records which strain carries two determinants rather than describing community structure. A classifier trained and evaluated on the same ten observations returned a training accuracy of 0.90 that estimates nothing about generalisation.

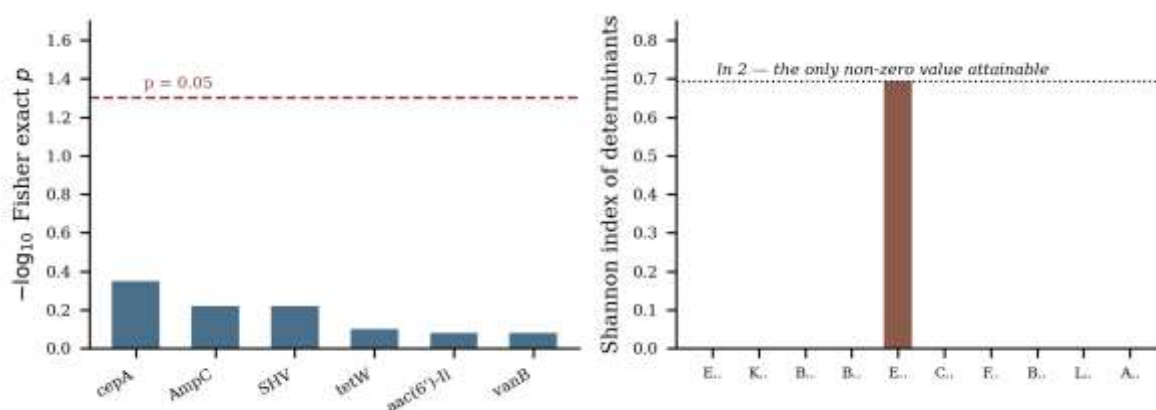


Fig. 3. Two statistical ceilings at this panel size. Left: Fisher exact association between each determinant and Gram reaction, with the $p = 0.05$ threshold. Right: Shannon index per strain, attaining only zero or $\ln 2$.

E. A check that tests transmission, not prediction

The framework output was also compared against the phenotype described in the source publication for each of the nine strains with a documented phenotype, returning complete agreement. That figure carries no evidential weight regarding predictive accuracy and is reported here to say so. The determinant calls supplied as input were curated from the same publications against which the output was compared; complete agreement was guaranteed by construction, and anything less would have indicated a transcription error. What the check does establish is that the mapping and guidance stages transmit input to output without corruption across all nine strains, including the four whose expected output is an empty determinant set. That is a genuine test of the implementation, and it is passed. Reporting such a figure as validation, which the arrangement invites, would be the same category of error as the one this paper audits.



V. DISCUSSION

The central finding is that the failure of a determinant-count rule is not a matter of accuracy but of logic. The rule is correct wherever evidence exists and wrong wherever it does not, and since evidence is absent for most strain-class pairs in any realistic panel, most of its output is wrong in the same way. No improvement in determinant detection addresses this. A complete and perfectly sensitive annotation of every resistance gene in a genome would still leave intrinsic non-susceptibilities that no gene encodes, expression-level and regulatory effects that a gene call does not capture, and classes for which resistance is not gene-mediated at all.

The revised rule is therefore conservative by design rather than by caution. It emits no positive therapeutic recommendation under any circumstances. This is likely to be read as a limitation, and we argue it is the correct behaviour: a genotype-based system is epistemically entitled to say that an agent is unlikely to work, and is not entitled to say that one will. Positive recommendation requires phenotypic evidence, and the framework that pretends otherwise is more dangerous than the one that declines, because its errors are confident and uniform.

Three practical recommendations follow for others building such systems. Distinguish absence of evidence from evidence of absence in the output vocabulary itself, so the distinction cannot be lost downstream by a reader or by a subsequent processing step. Maintain intrinsic resistance as a separate, independently curated input, since it cannot be derived from determinant data. And audit a small panel by hand before scaling, because the failure modes that matter are structural and are therefore fully visible at ten strains, while being wholly invisible at ten thousand.

The limitations of this work follow from its design. The panel is small and its taxonomic coverage narrow; the determinant calls were curated rather than computed; the audit standard is the reference literature rather than paired phenotypic susceptibility testing on the same isolates. The error taxonomy of Table I is complete for this panel and is not claimed to be exhaustive for any other. What generalises is not the error count but the mechanism producing it, which is a property of the rule and not of the panel.

VI. CONCLUSION

An exhaustive audit of 96 class-level guidance decisions found that a determinant-count rule treating zero as evidence of susceptibility produced 23 decisions contradicting documented intrinsic resistance and 66 further decisions resting on no evidence at all, while being entirely correct in the seven cases where a determinant was present. A revised three-state rule with a separate intrinsic resistance register removes every identified error and makes no positive recommendation. Resistome-guided antibiotic selection can presently tell a prescriber what to avoid; it cannot tell them what to use, and systems that appear to do so should be audited decision by decision on a panel small enough to permit it.

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