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A REVIEW ARTICLE

Phenotypic–Genotypic Discordance in Antimicrobial Susceptibility Testing of Staphylococcus aureus: Microbiological Mechanisms and Diagnostic Implications

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Abstract

Antimicrobial susceptibility testing (AST) is central to the management of Staphylococcus aureus infections because therapeutic efficacy depends on the selection of agents that effectively target this highly adaptable pathogen. Conventional phenotypic AST techniques, including broth microdilution, disk diffusion, and automated systems, directly measure bacterial growth inhibition and are considered laboratory reference methods. However, the increasing use of rapid genotypic methods, such as PCR-based resistance gene detection and whole-genome sequencing (WGS), has introduced additional complexity. Although molecular techniques provide faster turnaround times and high sensitivity for detecting known resistance determinants, they do not always correlate with phenotypic susceptibility. This phenomenon represents a major challenge in clinical microbiology and antimicrobial stewardship. The frequency of methicillin-related genotype-phenotype discordance (methicillin-susceptible but resistant) reported in the analyzed data ranged between 1–5% of the Staphylococcus aureus isolates, depending on the geographical location and testing methods. Discordance may arise from silent resistance genes, mutations conferring insignificant, conditional resistance, and phenotypic resistance mechanisms involving novel or poorly defined pathways that are not detected by genotypic testing. Conversely, phenotypic susceptibility may not reflect the genetic potential for resistance activation under selective pressure. These discrepancies can generate clinical uncertainty, potentially leading to inappropriate antibiotic selection, delays in therapeutic adjustment, and unnecessary use of broad-spectrum agents. These discrepancies highlight the need to understand the factors underlying discordance and their clinical implications. This review examines the microbiological and molecular mechanisms responsible for phenotypic–genotypic discordance in S. aureus, with particular emphasis on the limitations of diagnostic assays and the interpretation challenges associated with current AST techniques. Understanding these mechanisms is essential for improving laboratory reporting strategies and enhancing the reliability of antimicrobial susceptibility assessments.

1. Introduction

Staphylococcus aureus remains among the most clinically significant human pathogens. Its ability to rapidly obtain and regulate antimicrobial resistance determinants has rendered it a major challenge for both clinicians and microbiology laboratories [1]. Phenotypic AST has traditionally relied on observing bacterial growth in the presence of antimicrobial agents to determine susceptibility profiles. These phenotypic techniques, such as microdilution, disk diffusion, and automated testing systems, reflect the operational and physiologic implications of resistance mechanisms [2,3]. The rapid development and use of genotypic assays have introduced a new paradigm of susceptibility testing [4,5]. Genotypic assays have a major limitation, as they are not directly related to the expressed phenotype. This has also resulted in increased awareness of the phenomenon of phenotypic-genotypic discordance, which is defined as a situation in which the genetic profile and observable susceptibility pattern do not match [6,7]. There are two major types of such discordance: strains that carry resistance genes with the potential to be phenotypically susceptible, and those that do not express any known resistance determinants but are phenotypically resistant [8]. The overestimation of resistance could lead to the inappropriate use of broad-spectrum or second-line

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agents, which will adversely affect stewardship efforts. In contrast, resistance could be underestimated because of lack of known markers, leading to therapeutic failure, protracted infection, or worsening of clinical conditions [9]. Although a growing number of research articles have focused on antibiotic-resistant Staphylococcus aureus, a major gap still exists regarding phenotypic-genotypic discordant results; previous studies have generally been one-dimensional. They could either detect the presence of known resistance genes at the molecular level or use phenotypically based methods to determine whether an isolate is susceptible to an antibiotic [10]. The lack of standardization for definitions and diagnostic algorithms also complicates the interpretation of discrepancies from routine clinical use. This study will provide an overall analysis of the mechanisms that cause phenotypic-genotypic discrepancies in Staphylococcus aureus and highlight the challenges encountered during the diagnosis using current methods that determine the sensitivity of microorganisms against antibiotics and clinical approaches used to interpret discordant results.

2. Review Methodology

This narrative review was conducted using a structured literature search to identify relevant studies addressing phenotypic–genotypic discordance in antimicrobial susceptibility testing of Staphylococcus aureus. The current study was conducted as narrative review, and no study-selection process, quantitative screening flowchart or meta-analysis was carried out in accordance with the PRISMA guidelines. We systematically searched the electronic databases PubMed, Scopus, and Google Scholar. The search strategy combined the following keywords: "Staphylococcus aureus," "antimicrobial susceptibility testing," "phenotypic resistance," "genotypic resistance," "discordance," "heteroresistance," "molecular diagnostics," and "AST." Boolean operators were used to refine the searches. Studies published between 2018 and 2025 were considered, with particular emphasis on recent literature from 2021 to 2026, to ensure up-to-date coverage of emerging diagnostic technologies and resistance mechanisms. The inclusion criteria were original research articles, systematic reviews, and relevant clinical or microbiological studies focusing on Staphylococcus aureus and antimicrobial susceptibility testing. Studies addressing genotype–phenotype relationships, resistance mechanisms, and diagnostic interpretations were prioritized. The exclusion criteria were studies not directly related to Staphylococcus aureus, reports lacking relevance to antimicrobial susceptibility testing, and articles with insufficient methodological details. The selected literature was critically analyzed to synthesize the current knowledge on the microbiological mechanisms, diagnostic implications, and challenges associated with phenotypic–genotypic discordance.

3. Mechanisms of Discordance

Discordance in AST is typically categorized into two categories.

3.1. Genotype-Positive / Phenotype-Susceptible (G+/P-)

The organism carries a known antimicrobial resistance (AMR) gene or mutation; however, laboratory susceptibility testing shows that the isolate is still susceptible to the antibiotic. This is also known as silent, cryptic, or latent resistance and is a potentially serious form of discordance [11]. This type of discordance is caused by:

3.1.1. Weak or Down-Regulated Promoters

The absence of even a resistance gene can prevent an organism from expressing resistance in vitro because the gene may be downstream of a weak promoter that produces low levels of mRNA, or the promoter may have point mutations that further decrease the level of transcription [12]. Moreover, some of the environmental or physiological conditions under which the promoter must be induced are not necessarily present in a standard susceptibility test; therefore, the gene is largely silent [13].

Consequently, resistance genes may be expressed at low levels to produce functional proteins, causing isolates to appear phenotypically susceptible in standard laboratory tests [14]. However, in other situations, such as during host-associated stress or nutrient limitation, or in response to certain signals, the level of gene expression may be elevated, and the isolate may acquire detectable resistance, despite being phenotypically susceptible originally [15].

3.1.2. Gene Truncation or Structural Degradation

When a resistance gene is maintained in the genome, it can be because the gene encodes a defective or incomplete protein, often by insertions or deletions that cause frameshifts, early stop codons that give rise to truncated proteins that lack essential functional domains, interruption by insertion sequences or transposons that disrupt normal gene expression and translation, lead to partial deletions of key catalytic or structural domains that are important for the resistance phenotype [16]. For instance, a mutation causing a deletion of part of the penicillin-binding domain (PBD) of the mecA gene may lead to a lack of a functional PBP2a protein and a deletion of part of an aminoglycoside-modifying enzyme (AME) gene will not produce an effective modification of the antibiotic [17]. Thus, phenotypic testing will show the organism to be susceptible, even when the gene is detected, and the organism is typically unable to resume functional resistance without rare compensatory mutations restoring function [18].

3.1.3. Regulatory Gene Mutations (Global Regulators or Local Regulators)

In many cases, resistance phenotypes rely on complex regulatory systems; although a resistance gene may be present, its expression or activation can be inhibited by mutations in the regulatory factors [19]. Essential systems, including *sigB*, regulate stress response pathways, and their reduced activity can reduce the expression of the agr quorum-sensing system, which regulates virulence and indirectly regulates the activation of resistance determinants in *S. aureus*. Two-component systems, such as vraSR and walKR, coordinate cell wall stress responses, and various repressor proteins can become overactive following mutations [20]. Examples of such regulatory disruptions include mutations that block transcription factor activation, loss-of-function mutations that block signal transduction, operon alterations that enhance repressor binding, deactivate gene expression, and silencing of regulatory RNAs [21]. As a result of such regulatory disruptions, the expression of resistance genes is blocked or not induced in standard laboratory testing; however, under certain conditions, such as host immune stress or changes in metabolic conditions, the regulatory pathway is re-adjusted, potentially allowing for induction [22].

3.1.4. Inducible Expression Not Triggered During Testing

Other genes are inducible and activated only under specific stimuli and may be silent when standard phenotypic tests are performed because the inducer molecule of the sensor-kinase system is not present, the time allowed in the standard phenotypic assay is short to trigger activation, or the sensor-kinase system may be mutated such that the organism no longer recognizes and responds to the inducing signal [23]. Consequently, the gene will not be expressed under laboratory conditions, and the organism will present a phenotypically susceptible appearance despite possessing the resistance determinant [24].

3.1.5. Epigenetic or Chromosomal Position Effects

The genomic environment of the resistance gene can modify the genome-wide expression of the resistance gene, be transcriptionally inactive, and be influenced by DNA supercoiling or nucleoid-associated proteins, genome-wide patterns of DNA methylation that expose the resistance gene promoter or promote the integration of the gene into inaccessible chromosomal locations, by gene silencing or activation of the resistance gene by other genes can reduce expression and make the gene functionally silent despite its presence in the genome [25].

3.2. Genotype-Negative / Phenotype-Resistant (G-/P+)

The organism is resistant to laboratory phenotypic testing, and molecular assays do not identify known resistance genes or mutations. This is generally a result of new, unknown, or novel resistance mechanisms [26]. This type of discordance is caused by the following factors.

3.2.1. Heteroresistance

Heteroresistance is a phenomenon in which a small but highly resistant subpopulation exists within an otherwise susceptible bacterial population. In some cases, standard testing may classify the isolate as sensitive; however, the resistant minority will grow in the presence of antibiotics, leading to treatment failure. These subpopulations vary in cell wall thickness, membrane charge, efflux activity,

and metabolic state [27]. This effect is due to resistant cells that may not attain the sensitivity limit of regular microculture incubation methods and may increase in number only by stepwise growth, sometimes detected by special supplementary tests such as population analysis profiling (PAP) or gradient diffusion [28].

The following are clinically relevant cases: hVISA, in which vancomycin-intermediate subclones survive because of thickened cell walls; early MRSA strains, in which heterogeneous expression of PBP2a generates low-level methicillin resistance that is not detected by mecA-targeted PCR tests; and certain strains of *Enterobacter*, in which variable expression of PBP2a generates carbapenem heteroresistance [29]. The clinical significance of heteroresistance is that, despite seemingly susceptible MICs, the risk of treatment failure is high, and careful interpretation of results is required [30].

3.2.2. Target Site Mutations

This may not result from inherited genes or point mutations in housekeeping genes but rather from changes that are missed by the current repertoire of PCR panels, which are used to detect only known resistance genes and not the entire range of potential mutations [31]. They tend to be single-nucleotide polymorphisms (SNPs) that affect antibiotic-binding affinity and do not require the acquisition of foreign DNA because they alter drug affinity with modest effects; sometimes, several small-effect mutations interact to produce higher levels of resistance [32].

Examples include *RpoB* mutations inducing rifampin resistance in *Staphylococcus* and *Mycobacterium* spp.*,gyrA/parC* mutations producing fluoroquinolone resistance in both Gram-negative and Gram-positive bacteria, 23S rRNA mutations contributing to linezolid resistance in *Enterococci,* and PBP mutations in *Streptococcus pneumoniae* with high penicillin resistance, despite lacking *mecA* or *mecC* [33]. These mechanisms are frequently missed by PCR because the commercial assays are based on popular resistance genes and hotspots; therefore, any new, unusual, or scattered mutations are not targeted by existing primers [34].

3.2.3. Novel or Emerging Resistance Genes

The mechanism of resistance can be carried by new or newly identified genes, resulting in PCR-negative but phenotypically resistant isolates because standard molecular tests are designed to amplify only known gene sequence sets; consequently, emerging variants can avoid detection because of sequence divergence [35]. A notable example is *mecC*, which is a ß-lactam resistance gene with homology to *mecA* of approximately 70%, but is sufficiently divergent to avoid detection by older mecA-specific primers and is now widely observed in humans and livestock [36].

Other emerging genes, including *optrA* and *poxtA*, which confer oxazolidinone (linezolid) resistance in *Enterococci*; various mcr variants (mcr-1 to mcr-10), which mediate plasmid-borne colistin resistance in Gram-negative bacteria; new bla alleles, such as ESBLs, and evolving carbapenemase variants such as NDM derivatives; and fosA variants, which confer fosfomycin resistance in *Enterobacterales*, also raise these concerns [37]. Such genes are frequently overlooked because of sequence divergence; the targets are mismatched by primers, novel or geographically isolated genes are not represented in commercial kits, and the genes present on plasmids may have a low copy number or variable expression under laboratory conditions, thus making the process of detection even more difficult [38]. The microbiological mechanisms and their diagnostic consequences are summarized in Figure 1.

The principal microbiological mechanisms contributing to phenotypic–genotypic discordance and their diagnostic implications are summarized in Table 1.

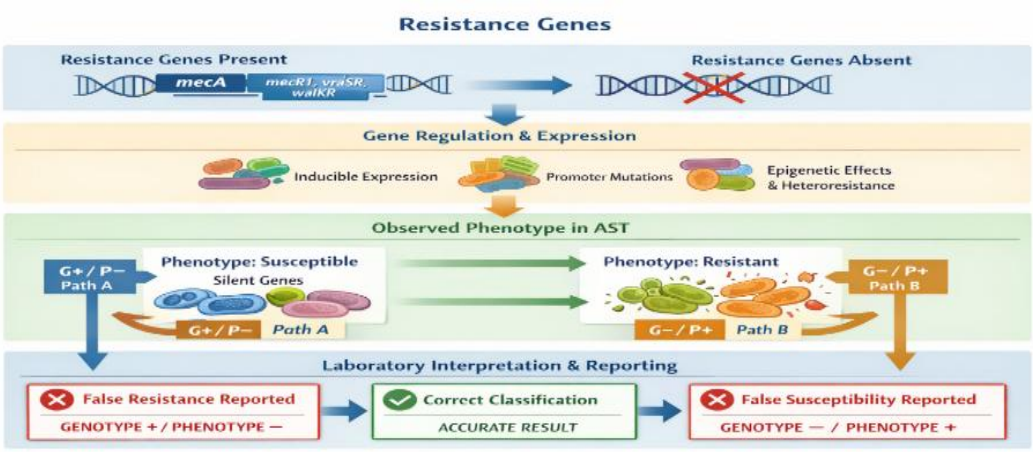

Figure 1. Microbiological mechanisms and diagnostic consequences of phenotypic–genotypic discordance in antimicrobial susceptibility testing of *Staphylococcus aureus*.

Table 1. Main Microbiological and Molecular Mechanisms Leading to Phenotype-Genotype Incompatibility in *Staphylococcus aureus*

Category	Genetic element/gene	Underlying mechanism	Diagnostic impact
Genotype-positive/phenotype-susceptible (G+/P−)	mecA, mecC, mecI–mecR1	Reduced expression, regulatory defects, or structural gene disruption	False methicillin susceptibility may lead to inappropriate β-lactam therapy, delayed MRSA-directed treatment, and possible treatment failure. Conversely, gene-only interpretation may overclassify resistance and promote unnecessary broad-spectrum therapy.
Genotype-positive/phenotype-susceptible (G+/P−)	blaZ, blaI–blaR1	Weak, delayed, or inducible β-lactamase expression	False penicillin susceptibility may result in ineffective penicillin treatment and therapeutic failure; overcalling resistance may unnecessarily exclude narrow-spectrum β-lactams.
Genotype-positive/phenotype-susceptible (G+/P−)	ermA, ermC	Inducible resistance not activated during routine testing	Failure to perform an inducible-clindamycin D-test may produce false clindamycin susceptibility, with resistance emerging during therapy and possible clinical failure.
Genotype-positive/phenotype-susceptible (G+/P−)	vanA	Silent, incomplete, or poorly expressed operon	Genotypic positivity without an elevated vancomycin MIC may cause false classification as vancomycin resistant, unnecessary use of reserve agents, and avoidable infection-control alerts; phenotypic confirmation is required.
Genotype-negative/phenotype-resistant (G−/P+)	walKR, vraSR, graRS	Regulatory mutations, cell-wall thickening, and heteroresistance	Targeted gene assays may falsely report vancomycin susceptibility and miss hVISA/VISA, which can delay effective therapy and contribute to persistent infection or treatment failure.
Genotype-negative/phenotype-resistant (G−/P+)	blaZ, native pbp genes, gdpP	β-lactamase hyperproduction or altered penicillin-binding proteins	mecA/mecC-only assays may misclassify BORSA/MODSA as methicillin susceptible, risking inappropriate β-lactam selection, treatment failure, and inaccurate resistance surveillance.
Genotype-negative/phenotype-resistant (G−/P+)	gyrA, grlA, rpoB, 23S rRNA, or novel genes	Target-site mutations, sequence variation, or resistance determinants absent from targeted PCR panels	Repeat phenotypic AST or expanded sequencing might be necessary in cases of a false-negative molecular result, as this could underestimate resistance, result in improper antibiotic selection and failure to treat with the right antibiotic, and produce misleading surveillance results.

4. Frequency of Phenotypic-Genotypic Discordance Across Antibiotic Classes

The prevalence of genotype-phenotype discordance may differ among antibiotic classes, antibiotic resistance markers, bacterial species and geographical region [39]. Genotypic testing (e.g. PCR, WGS) and phenotypic susceptibility testing (AST) sometimes do not match, resulting in high incidence of resistance with no detectable resistance gene (G+/P–) or in the presence of resistance gene without detectable phenotypic resistance (G-/P +) [40]. These differences can complicate the interpretation of clinical data, and result in misclassification of resistance patterns in surveillance data, as well as delayed response to treatment. Furthermore, discordance may be caused by gene

silencing, variable expression, heteroresistance, low inoculum effects, and/or technical issues in laboratory detection methods [41]. The genetic diversity and antibiotic resistance of Staphylococcus aureus has been shown between different regions, in studies conducted at the regional level. A national survey conducted in Saudi Arabia revealed different circulating lineages and regional variations in resistance and demonstrated the different levels of genomic resistance prediction depending on the antimicrobial class studied [42]. Whole genome sequencing has demonstrated that there is a high level of strain variation in Egypt, with the identification of new sequence types and the common presence of the mecA gene [43]. Likewise, an Algerian study of the whole genome sequencing combined with susceptibility testing found that there were several clonal complexes, multidrug-resistant isolates and multiple resistance determinants [44]. The results indicate that geographical variation in the reported phenotype–genotype concordance rate is due to a combination of differences in the circulating clone, the epidemiology of the region, populations sampled, and testing methods. The frequency and patterns of phenotypic–genotypic discordance across major antibiotic classes in Staphylococcus aureus are summarized in Table 2.

Table 2. Patterns of phenotypic–genotypic discordance across antibiotic classes in ***Staphylococcus aureus***

Class	Marker	Discordance	Freq
Penicillins	mecA/mecC	G+ / P−	1–5%
Glycopeptides	vanA/vanB	G− / P+	10–20%
Aminoglycosides	aac(6')-Ie-aph(2'')	G+ / P−	2–4.5%
Fluoroquinolones	gyrA/grlA	Variable	Region-based

This discordance is typical of *S. aureus,* with the *mecA* gene possibly being active and silent because of regulatory mutations, including *mecR1* defects, and some strains are borderline OXA-resistant (BORSA) with phenotypic susceptibility despite genotypic detection [45]. Likewise, heteroresistant vancomycin-intermediate *S. aureus* (hVISA) strains may display phenotypic resistance without vanA/vanB, which is believed due to cell wall thickening and not the acquisition of van genes, with geographical variations in prevalence and a higher prevalence in certain parts of Asia [46].

Some aminoglycoside-resistant isolates carry the bifunctional modifying enzyme gene, aac(6)-Ie-aph(2)-like, but are phenotypically sensitive owing to low levels of gene expression or mutations that do not affect enzyme activity; this discordance is more commonly observed in *enterococci* [47]. The phenotypic expression of specific mutations affecting efflux pumps and the apparent genotype-phenotype discordance are due to mutations in the quinolone resistance-determining regions (QRDRs) of *gyrA* and *grlA* in some regions; specific mutations result in low-level or borderline resistance [48].

5. Diagnostic and Interpretative Implications of Phenotypic-Genotypic Discordance

Phenotypic–genotypic discordance represents an important challenge in clinical microbiology laboratory environments, particularly in the interpretation and reporting of antimicrobial susceptibility testing (AST) results [49]. The main impact of this discordance is that it affects diagnostic accuracy and laboratory-based decision-making rather than direct patient outcomes. As the key to successful treatment is to accurately and timely identify resistance, genotype–phenotype mismatches introduce an element of uncertainty that may lead clinicians to make either unsafe or excessively aggressive choices [50]. The diagnostic workflow and interpretative pathways for concordant and discordant phenotypic and genotypic AST results are shown in Figure 2.

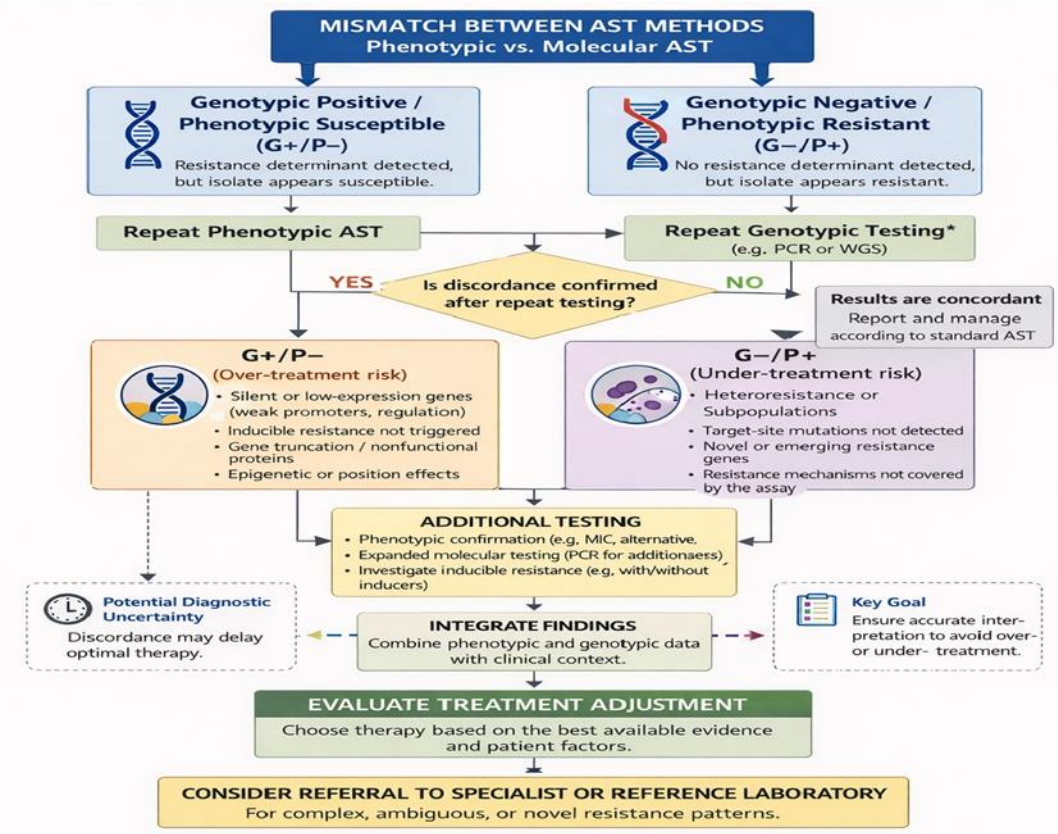

Figure 2. Diagnostic algorithm for the interpretation and management of phenotypic–genotypic discordance in antimicrobial susceptibility testing of Staphylococcus aureus.

5.1. Under-treatment (G– / P+)

False-susceptibility interpretation occurs when the expression of resistance determinants phenotypically in the process of antimicrobial susceptibility testing (AST) is not detected by molecular tests [51]. The limitations common to genotypic panels that are manifest in this modality of discordance include the inability to identify new or unusual mutations, regulatory changes that modify gene expression, heteroresistant subpopulations, or resistance mechanisms that are not reflected in the target assays. Consequently, the application of genotypic data could lead laboratories to declare susceptibility in the presence of evidence of phenotypic resistance [52]. Laboratories must recognize situations in which phenotypic resistance suggests functional resistance strategies undetectable by molecular techniques and should institute carefully considered interpretation measures, reflex testing, or additional tests [53]. It is necessary to address false-susceptibility interpretation to maximize the accuracy, reliability of AST reporting and prevent the misclassification of antimicrobial susceptibility at the laboratory level [54].

5.2. Over-treatment (G+ / P–)

False resistance interpretation occurs when resistance genes or molecular markers are observed in phenotypically susceptible Staphylococcus aureus isolates during antimicrobial susceptibility testing [55]. This phenotypic genotypic discordance is frequently associated with silent or weakly expressed resistance genes, mutations in the regulatory genes, undiscovered inducible genes, and mutations in structural genes that affect the production of functional protein [56]. Without context, genotypic positivity may lead to overreporting of resistance at the reporting level by diagnostic laboratories [57]. Various studies have shown that the presence of resistance determinants is not always equivalent to expressed resistance, especially when regulatory RNA interference is involved, in promoter mutations, or signal transduction pathway disruption. In such cases, the exclusive use of molecular detection may skew laboratory reporting to classify isolates as resistant, even when they are phenotypically susceptible [58,59]. Diagnostic laboratories should consider combined phenotypic confirmation and genotypic results to reduce the incidence of false resistance reporting and clearly present methodological limitations in AST reporting [60,61].

5.3. Diagnostic Latency

Diagnostic latency is a major interpretive difficulty in antimicrobial susceptibility testing (AST) that arises from the inherent differences in turnaround time between fast genotypic and traditional phenotypic tests [62]. Although molecular diagnostics facilitate the early detection of certain resistance determinants, the respective findings often precede phenotypic validation by 18–48 h, and laboratory interpretation can rely on incomplete or tentative data. This lag increases the chances of misclassification at the reporting juncture in cases of phenotypic–genotypic discordance [63]. From a laboratory perspective, initial genotypic outcomes are likely to have a disproportionate effect on the initial reports on susceptibility, particularly when phenotypic mechanisms of resistance are inducible, heteroresistant, or conditional upon regulatory pathways that are not directly manifested under normal testing conditions [64]. However, subsequent phenotypic resistance can be inconsistent with pre-existing genotypic inferences and reports or reflex testing plans must be revised. These delays highlight the inadequacy of the sequential testing process, which does not incorporate phenotypic and genotypic information in a comprehensive manner [65]. Reducing diagnostic latency requires the implementation of structured laboratory algorithms to define the situations in which provisional reports are appropriate, those in which phenotypic confirmation is required, as opposed to molecular predictions, and those require ancillary testing, such as repeat AST, population analysis profiling, or expanded molecular assays [66,67].

6. Knowledge Gaps in Understanding Phenotypic-Genotypic Discordance

The development of new methods for antimicrobial susceptibility testing and molecular-based resistance detection has led to significant improvements in the ability to detect antimicrobial resistance in bacteria. However, important gaps remain in our understanding of the mechanisms that contribute to the observed phenotypic and genotypic discordance in Staphylococcus aureus are important for improving diagnostic test interpretation and developing more accurate susceptibility prediction models [68]. First, although the regulation of resistance gene expression has been extensively studied at the level of individual regulators, such as two-component systems and stress response pathways, little is known about how the overall regulatory architecture controls resistance activation in Staphylococcus aureus under clinically relevant conditions [69].

Most studies address the roles of regulatory components one at a time and with other components of the cell regulatory network largely ignored, and few mechanisms of regulatory functions are known [70]. Second, although many studies have reported phenotypic–genotypic discordance in Staphylococcus aureus, standardized definitions or classification criteria for this concept do not exist [71]. Differences in laboratory techniques and assays employed for the detection of specific resistance genes make comparisons between study results difficult, and contribute to the lack of reliable prevalence estimates. These differences also prevent any meaningful meta-analysis and obscure epidemiological trends related to resistance occurrence [72]. Third, the relationship between clinical outcomes and genetic–phenotypic discordance in Staphylococcus aureus remains unclear. Although it can be inferred that discrepancies in antimicrobial susceptibility test (AST) results will influence treatment decisions, very few investigations have evaluated the association between AST result discordance and clinical endpoints. Thus, the actual clinical impact resulting from this type of discrepancy remains unknown[70]. Fourth, detecting heteroresistance is generally less sensitive than detecting antibiotic resistance in traditional clinical cultures. Most detection methods, such as population analysis profiling or single-cell-based methods, have been limited to use in research laboratories because of their cost, complexity, and difficulty of application in a clinical setting; therefore, clinically relevant resistant subpopulations that are present in culture may be undetected when using typical diagnostic practices [73]. Fifth, the integration of multi-omics data into susceptibility interpretation frameworks is still in its early stages. Typically, diagnostic models developed using molecular data are based on a single type of data; consequently, the translation of molecular data into actionable phenotypic predictions is limited. Closing this gap presents a significant opportunity to advance precision medicine. [74]. Addressing these limitations will require standardized methodologies for diagnosing resistance in clinical isolates, increased collaboration among researchers from different disciplines, and the application of systems biology approaches to understand the complex interplay between bacterial physiology and antimicrobial susceptibility [75].

7. Fututre Prespectives in Resolving Phenotypic- Genotypic Discordance

Overall, the evidence reviewed suggests that a phenotypic–genotypic discordance cannot be attributed to the existence or absence of antimicrobial resistance determinants in S. aureus. Rather, it is a consequence of the interplay between gene expression, genomic and structural polymorphisms, heteroresistance, test conditions and limitations of both phenotypic and molecular assays. Thus, the phenotypic and genotypic results should not be used independently. In order to minimize diagnostic uncertainty and increase the reliability of the reporting of antimicrobial susceptibility, an integrated interpretation of the functional susceptibility data and its molecular and regulatory context is needed.

7.1. Emerging Diagnostic and Technological Perspectives

The integration of advances in technology and analysis will help us better understand and manage the relationship between genetic and phenotypic variations in antimicrobial susceptibility testing. Many emerging technologies and methodologies are expected to dramatically alter our ability to predict antibiotic susceptibility using diagnostic microbiology [76]. An integrated multi-omics strategy for future susceptibility prediction is conceptually illustrated in Figure 3.

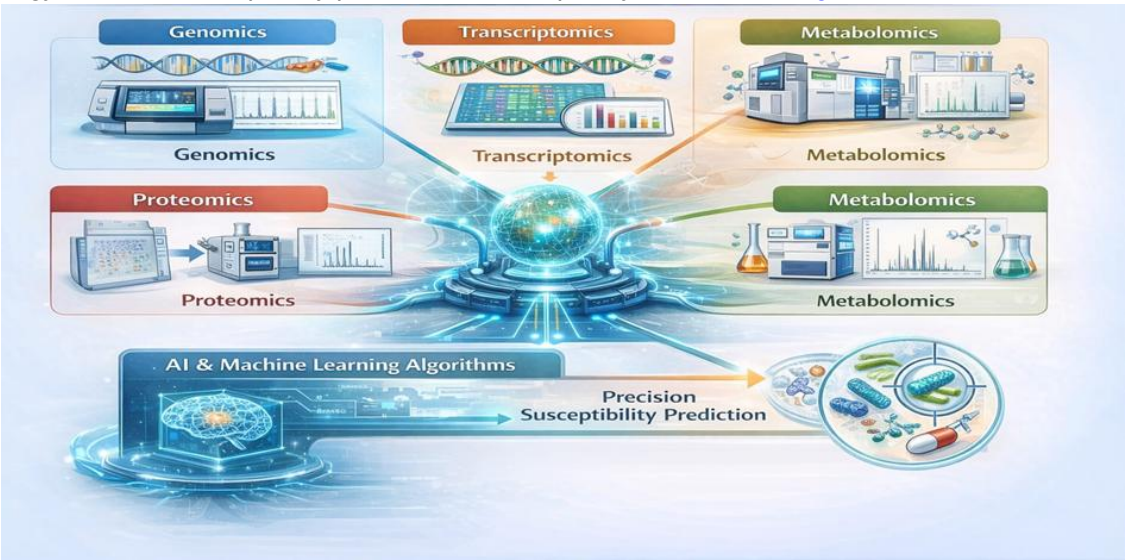

Figure 3. Multi-omics framework for AI-assisted precision antimicrobial susceptibility prediction.

For example, artificial intelligence and machine learning models can integrate both genomic and phenotypic data to predict resistance. The training of such predictive algorithms utilizing data generated through whole-genome sequencing and clinical outcome data is expected to generate probabilistic resistance forecasts that exceed traditional rule-based interpretations of resistance markers [77].

The second area of great promise is multi-omics integration, which involves combining various types of omics data, including transcriptomic, proteomic, metabolomic, and genomic data, into a single dataset to potentially go beyond simply identifying genes associated with resistance and instead predict functionally active resistance mechanisms. These integrated approaches would allow researchers to identify regulatory activation states and metabolic adaptations that ultimately lead to the expression of resistance phenotypes [78].

Additionally, rapid sequencing technologies and point-of-care molecular diagnostics are expected to decrease diagnostic delay and improve clinical decision-making at the bedside. Further development of nanopore sequencing and the detection methods based on the CRISPR system could enable the identification of resistance determinants and regulatory variants at the point of care [79]. In addition, the single-cell and microfluidic phenotypic testing are technologies that can identify the subpopulation of heteroresistant bacteria, which are typically not detected using traditional susceptibility testing. These technologies should provide greater resolution in susceptibility classification and enable a greater level of understanding of population level resistance dynamics [80]. Finally, future diagnostic frameworks are expected to feature integrated reporting approaches that combine phenotypic and molecular contextualization, such as standardized algorithms to interpret results, taking into account

the possibilities of gene expression, the context of regulation, and the environment, which would lead to a more reliable laboratory reporting and successful implementation of antimicrobial stewardship programs. The new techniques suggest a shift toward more dynamic susceptibility evaluations based on data for precision microbiology rather than static evaluations, the latter of which will take into account the complexity of genotype-phenotype associations.

7.2. Practical Recommendations for Laboratory Management of Discordant AST Results

Phenotypic–genotypic discordance during antimicrobial susceptibility testing is an important issue in routine clinical microbiological laboratories. Increasing the precision of diagnosis and the reproducibility of reports can be achieved through the implementation of the following recommendations:

7.2.1. When Genotypic Results Can Be Relied Upon

Genetic testing is reliable for detecting known resistance genes associated with a clear drug-resistant phenotype such as mecA-mediated methicillin resistance in Staphylococcus aureus. In serious clinical situations, laboratories can quickly report positive results based on genetic data. However, laboratories must exercise caution when detecting genes with inconsistent phenotypic expression and unknown clinical significance.

7.2.2. When Phenotypic Confirmation Is Essential

Phenotypic antimicrobial susceptibility testing (AST) should be routinely performed to verify genotypic results in patients with a history of inducible resistance, heteroresistance, or regulatory-dependent gene expression. The interpretation of discordance between molecular-based data and phenotypically resistant isolates should never be made solely based on molecular data; rather, the presence of phenotypic resistance should prevail over molecular data.

7.2.3. Interpretation of Discordant Results

For genotype-positive/phenotype-susceptible (g +/p −) cases, laboratories should consider the possibility of silent or poorly expressed resistance genes and avoid overreporting of resistance without phenotypic evidence. In contrast, for genotype-negative-phenotype-positive (g−/p+) cases, phenotypic resistance should be prioritized, as it represents functional antimicrobial resistance.

7.2.4. Reporting Recommendations

Laboratory reports should clearly indicate the presence of discordant results and provide interpretive statements regarding the possible mechanisms or limitations of the testing methodologies. The use of integrated reports combining phenotypic and genotypic information is highly recommended to aid in clinical decision-making.

7.2.5. Implementation of Diagnostic Algorithms

Standardized diagnostic algorithms for interpreting discordant results should be implemented. These algorithms should rely on molecular data and phenotypic confirmation tests, and additional tests may be required for their implementation. The implementation of structured approaches will improve the consistency of test interpretation, reduce diagnostic errors, and enhance antimicrobial stewardship practices.

8. Conclusion

Phenotypic–genotypic discordance in antimicrobial susceptibility testing presents a challenge. The presence of an antibiotic resistance gene does not provide sufficient predictive information about the expression of such a gene; therefore, phenotypic antibiotic resistance can be expressed via pathways that are undetectable by targeted molecular methods. Studies have suggested that reliance on testing methods is insufficient for assessing AMR. Therefore, integrated diagnostic approaches should be used to increase the reliability of reports related to the susceptibility of microorganisms to antimicrobials. Finally, improving the interpretation of phenotypic–genotypic discordance will improve the reliability of laboratory reports, strengthen antimicrobial stewardship, and enable more rational strategies for managing infections.

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التباين بين الأنماط الظاهرية والجينية في اختبار الحساسية للمضادات الحيوية لدى المكورات العنقودية الذهبية: الآليات الميكروبيولوجية والتداعيات التشخيصية

الملخص

يُعد اختبار الحساسية للمضادات الحيوية (AST) عنصرًا أساسيًا في إدارة عدوى المكورات العنقودية الذهبية، إذ تعتمد فعالية العلاج على اختيار العوامل الدوائية القادرة على استهداف هذا العامل الممرض عالي القدرة على التكيف. تُستخدم تقنيات AST الظاهرية التقليدية، بما في ذلك التخفيف المجهري في المرق(broth microdilution) ، واختبار الانتشار بالأقراص(disk diffusion) ، والأنظمة الآلية، لقياس تثبيط نمو البكتيريا بشكل مباشر، وتُعد قيمًا مرجعية مخبرية معتمدة. ومع ذلك، فإن التوسع المتزايد في استخدام الطرق الجينية السريعة، مثل الكشف عن جينات المقاومة المعتمد على تفاعل البوليميراز المتسلسل (PCR) وتسلسل الجينوم الكامل(WGS) ، قد أضاف مزيدًا من التعقيد. فعلى الرغم من أن التقنيات الجزيئية توفر زمن استجابة أسرع وحساسية عالية للكشف عن محددات المقاومة المعروفة، إلا أنها لا تتوافق دائمًا مع الحساسية الظاهرية. تمثل هذه الظاهرة تحديًا رئيسيًا في علم الأحياء الدقيقة السريري وبرامج ترشيد استخدام المضادات الحيوية. وتشير البيانات التي شملتها المراجعة إلى أن التباين المرتبط بمقاومة الميثيسيلين، ولا سيما وجود جيني mecA أو mecC مع بقاء العزلات حساسة ظاهريًا، قد سُجل بنسبة تقارب 1–5% من عزلات المكورات العنقودية الذهبية، مع اختلاف النسبة بحسب المنطقة الجغرافية ومنهجية الاختبار. وقد ينشأ عدم التوافق نتيجة وجود جينات مقاومة صامتة، أو طفرات تمنح مقاومة غير مؤثرة أو مشروطة، بالإضافة إلى آليات مقاومة ظاهرية تتضمن مسارات جديدة أو غير محددة بشكل كافٍ لا يمكن للاختبارات الجينية اكتشافها. وعلى العكس من ذلك، قد لا تعكس الحساسية الظاهرية الإمكانات الجينية الكامنة لتنشيط المقاومة تحت الضغط الانتقائي. ويمكن أن تؤدي هذه التناقضات إلى حالة من عدم اليقين السريري، مما قد يسبب اختيارًا غير مناسب للمضادات الحيوية، وتأخرًا في تعديل العلاج، واستخدامًا غير ضروري للمضادات واسعة الطيف. وتستعرض هذه المراجعة الآليات الميكروبيولوجية والجزيئية المسؤولة عن التباين بين النمط الظاهري والنمط الجيني في المكورات العنقودية الذهبية، مع التركيز على محدودية الاختبارات التشخيصية والتحديات المرتبطة بتفسير تقنيات اختبار الحساسية الحالية، بما يسهم في تحسين استراتيجيات التقارير المخبرية وتعزيز موثوقية تقييم الحساسية للمضادات الحيوية.

الكلمات المفتاحية: المكورات العنقودية الذهبية؛ مقاومة المضادات الحيوية؛ المقاومة غير المتجانسة؛ عدم التوافق بين النمط الظاهري والنمط الجيني؛ التشخيص الجزيئي؛ اختبار الحساسية للمضادات الحيوية.