Use of Molecular Assays for Resistance Detection

Antimicrobial resistance and susceptibility are complex, and current *in vitro* methods have been developed to predict a microorganism's response to antibacterial therapy *in vivo*. Standardized phenotypic methods have evolved over many decades, but faster and potentially more reliable nucleic acid- and protein-based methods have been recently developed to detect antimicrobial resistance. The current challenge for clinical laboratories is to integrate molecular assays for antimicrobial resistance determinants with conventional antimicrobial susceptibility testing procedures, sometimes in spite of an incomplete understanding of test limitations.

The tables in this section provide a practical approach for testing and reporting results among clinical laboratories that routinely use molecular techniques (with or without a phenotypic test) for the detection of antimicrobial resistance. Antibacterial resistance is genetically complex, and based on available data, molecular methods are often used as a tool in the clinical laboratory for screening (e.g., MRSA from nasal swabs) or as a rapid adjunct to traditional phenotypic methods (e.g., KPC from instrument-flagged blood culture bottles). Interpretation requires critical thinking and an understanding of the dynamics between detection of "resistance" determinants and the testing of phenotypic "susceptibility." Detection of a resistance marker does not necessarily predict therapeutic failure of antibacterial agents. The gene may be non-functional or expressed at clinically insignificant levels. Conversely, the absence of the genetic marker does not necessarily indicate susceptibility, as technical issues may interfere with detection (e.g., inhibition of amplification, emergence of genetic variants, etc). In some cases, a molecular approach may be superior to traditional phenotypic methods, such as in the case of low *in vitro* expression, heteroresistance, or poor growth masking higher MICs. Overall, clinical laboratorians should attempt to apply a consistent approach to molecular-based methods and aim to resolve discordant results with repeat or supplementary testing, by referral to a reference laboratory, or by reporting both results in accordance with institutional policies.

As understanding of the molecular mechanisms of antimicrobial resistance continues to develop, more sophisticated approaches to molecular detection of antimicrobial resistance in the clinical microbiology laboratory will undoubtedly emerge. These tables will be updated as needed to ensure the provision of relevant guidance as methods evolve.

Table 1. Strategies for Reporting Methicillin (Oxacillin) Results When Using Molecular and Phenotypic AST Methods for *S. aureus*

Indication	Target(s)	Method	Specimen Type	Result		Suggestions for Resolution	Consider reporting as:	Footnotes*
				Genotype or Predicted Phenotype	Observed Colony Phenotype (if tested)			
Detection of methicillin resistance in *S. aureus*	PBP2a	Latex agglutination, immunochromatography	Colony	PBP2a positive	cefoxitin R	N/A	Methicillin R	1
				PBP2a negative	cefoxitin S	N/A	Methicillin S	1
				PBP2a positive	cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST and consider *mecA* colony NAAT if available.	If discrepancy is not resolved by suggested testing, report as methicillin R	1-2
				PBP2a negative	cefoxitin R	Confirm isolate identification, repeat latex agglutination and AST and consider *mecA* colony NAAT if available.	If discrepancy is not resolved by suggested testing, report as methicillin R	1
	mecA	NAAT, microarray hybridization, ISH	Colony, blood culture broth, surveillance specimen	*mecA* detected	cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin R) and consider reporting molecular result as per institutional protocol	3-6
				mecA not detected	cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin S) and consider reporting molecular result as per institutional protocol	3-6
				mecA detected	cefoxitin S	Confirm isolate identification, repeat AST and repeat or perform *mecA* colony NAAT if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin R	2-5, 8-9
				mecA not detected	cefoxitin R	Confirm isolate identification, repeat AST and repeat or perform *mecA* colony NAAT if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin R	3, 7
	SCC*mec-orfX* junctional regions ONLY	NAAT	Blood culture broth, surveillance specimen	SCC*mec* detected	cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin R) and consider reporting molecular result as per institutional protocol	*3-6*
				SCC*mec* not detected	cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin S) and consider reporting molecular result as per institutional protocol	3-6
				SCC*mec* detected	cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT if available. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	2, 10
				SCC*mec not* detected	cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT if available. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R	7, 12
	SCC*mec-orfX* junctional regions AND *mecA* and/or other targets	NAAT	Blood culture broth, surveillance specimen	SCC*mec* AND *mecA* or other target detected	cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin R) and consider reporting molecular result as per institutional protocol	3-6
				SCC*mec* AND *mecA* or other target *not* detected	cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin S) and consider reporting molecular result as per institutional protocol	3-6
				SCC*mec* AND *mecA* or other target detected	cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin R	2
				SCC*mec* AND *mecA* or other target *not* detected	cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin R	3, 11

*In addition the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets

1. False positive and false negative PBP2a latex bead agglutination results have been observed (J Clin Microbiol. 2005 Sep;43(9):4541-4).
2. Rare *mecA* positive *S. aureus* isolates will test susceptible to cefoxitin (Curr Microbiol. 2007 Dec;55(6):473-9; J Clin Microbiol. 2005 Aug;43(8):3818-23)
3. *mecC* or *mecA* variant gene mediated methicillin resistance may not be detected by *mecA* PCR (Antimicrob Agents Chemother. 2011 Aug;55(8):3765-73; Lancet Infect Dis. 2011 Aug;11(8):595-603).
4. The presence of *mecA* positive CoNS and MSSA may result in falsely positive MRSA molecular results (J Clin Microbiol. 2008 Oct;46(10):3285-90; Antimicrob Agents Chemother. 2008 Dec;52(12):4407-19).
5. Strains harboring unstable SCC*mec* insertions may lose *mecA* during culture (J Clin Microbiol. 2010 Oct;48(10):3525-31).
6. Compared to culture, the sensitivity of molecular methods may be higher while the specificity may be lower.
7. Occasional false negative *mecA* results have been reported for direct blood culture molecular assays (J Clin Microbiol. 2013 Dec;51(12):3988-92).
8. For ISH assays with a cefoxitin induction step, false positive *mecA* results should be rare (J Clin Microbiol. 2014 Nov;52(11):3928-32).
9. In polymicrobial cultures, the presence of *mecA* cannot be attributed to a specific isolate.
10. Strains harboring a SCC*mec* remnant lacking the *mecA* gene (*mecA* dropout) or mutant *mecA* allele may test positive in assays that only target SCC*mec-orfX* junctional regions. Laboratories using molecular tests that only detect SCC*mec-orfX* junctional region targets may consider adding a disclaimer to the report stating the proportion of false positives related to *mecA* dropouts observed in isolates from the patient population served.. (J Clin Microbiol. 2011 Apr;49(4):1240-4).
11. Multiple SCC*mec* types exist; depending on the design of the assay, some SCC*mec* variants may not be detected (Clin Microbiol Infect. 2007 Mar;13(3):222-35).

Table 2. Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic AST Methods for *Enterococcus* spp.

Indication	Target(s)	Method	Specimen Type	Result		Suggestions for Resolution	Report as:	Footnotes*
				Genotype or Predicted Phenotype	Observed Phenotype (if tested)			
Detection of vancomycin resistant enterococci	*vanA*, *vanB*	NAAT or array hybridization technology	Blood culture broth or surveillance cultures	*vanA* and/or *vanB* detected	Vancomycin R	N/A	Report phenotypic result as found (if available), consider reporting presence of molecular target per institutional protocol	1-3
				vanA and/or *vanB not* detected	Vancomycin S	N/A	Report phenotypic result as found (if available), consider reporting presence of molecular target per institutional protocol	
				vanA and/or *vanB* detected	Vancomycin S	Confirm isolate identification to species level (e.g. *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R	1-3
				vanA and/or *vanB not* detected	Vancomycin R	Confirm isolate identification to species level (e.g. *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R	4
	vanA	NAAT	Surveillance cultures	*vanA* detected	Vancomycin R	N/A	Report phenotypic result as found (if available), consider reporting presence of molecular target per institutional protocol	1-2
				vanA not detected	Vancomycin S	N/A	Report phenotypic result as found (if available), consider reporting presence of molecular target per institutional protocol	5
				vanA detected	Vancomycin S	Confirm isolate identification to species level (e.g. *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R	1-2
				vanA not detected	Vancomycin R	Confirm isolate identification to species level (e.g. *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R	4-5

*In addition to the specific possibilities referenced, genotype/phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

References

1 vanA may be present in nonenterococcal species (Patel R.2000 Apr 1;185(1):1-7).

2 Vancomycin-variable *E. faecium* isolates have been recently revealed in Canada. They carry wildtype *vanA*, but initially test as vancomycin-susceptible with culture based method. They are able to convert to a resistant phenotype during vancomycin treatment (Gagnon S et al. 2011. J Antimicrob Chemother 66:2758–2762.; Thaker MN et al. 2015. Antimicrob Agents Chemother 59:1405–1410).

3 *vanB* gene has been found in several commensal nonenterococcoal bacteria which may lead to misclassification of vancomycin susceptible enterococci as resistant in surveillance cultures containing mixed bacterial species *(*Ballard SA et al., Antimicrob Agents Chemother 2005;49:77-81).

4 Constitutive low-level vancomycin resistance can be detected phenotypically (2-32µg/ml) from the presence of vanC, an intrinsic resistance characteristic of *E. gallinarum* (vanC1) and *E. casseliflavus* (vanC2-4) (Courvalin P. 2006. Clin Infect Dis 42:S25-34).

5 Targeting *vanA* only may miss regional *vanB*-carrying VRE (Nebreda T et al. J Antimicrob Chemother 2007; 59:806-7).

Table 3. Reporting Results from ESBL and Carbapanemase Molecular Tests for *Enterobacteriaceae*

Indication	Target(s)	Method	Specimen Type	Result		Suggestions for Resolution	Report as:	Footnotes*
				Molecular Target Result	**Observed Phenotype (if tested)**			
Detection of Extended Spectrum β-Lactam resistance in *Enterobacteriaceae* (in an isolate susceptible to all carbapenems)	ESBL Type CTX-M, SHV, TEM	NAAT, Microarray	Colony, blood culture	Detection of any ESBL target	R to all 3rd and 4th generation cephalosporins tested, e.g. ceftriaxone R cefotaxime R ceftazidime R cefepime R	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol	1-12
				Detection of any ESBL target	S to all 3rd and 4th generation cephalosporins tested, e.g. ceftriaxone S cefotaxime S ceftazidime S cefepime S	Repeat molecular and phenotypic tests; If blood culture, check for mixed culture; If mixed, test isolates individually and report phenotypic results as found	If discrepancy is not resolved, repeat susceptibility testing should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported	1-12
				Detection of CTX-M ESBL target	Variable resistance to 3rd and 4th generation cephalosporins e.g. ceftriaxone R cefotaxime R ceftazidime R or S cefepime R or S	Expected phenotype for some CTX-M strains; Check cefepime using a reference method if S	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol	1-12
				Detection of TEM or SHV ESBL target	Variable resistance to 3rd and 4th generation cephalosporins, e.g. ceftriaxone R or S cefotaxime R or S ceftazidime R or S cefepime R or S	Expected phenotype for some TEM/SHV strains; Check cefepime using a reference method if S	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol	1-12
				No detection of ESBL targets	Resistance to 3rd generation cephalosporins and variable resistance to 4th generation cephalosporins e.g. ceftriaxone R cefotaxime R ceftazidime R cefepime R or S	Likely non-tested broad spectrum β-lactamase (e.g. AmpC, carbapenemase or other ESBL); consider repeating molecular tests and checking cefepime using reference method if S	Report phenotypic results as found, including reference cefepime result if tested	1-12

Detection of Carbapenem resistance in *Enterobacteriaceae*	KPC, OXA-48-like, VIM, NDM or IMP	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target	Resistance to all carbapenems, e.g. meropenem R imipenem R doripenem R ertapenem R	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol	1-4, 12-14
				Detection of any tested carbapenemase target	Susceptible to all carbapenems except ertapenem (variable), e.g. meropenem S imipenem S doripenem S ertapenem R or S	Repeat molecular and phenotypic tests; if blood culture, check for mixed culture; if mixed, test isolates individually and report phenotypic results as found; consider a phenotypic test for carbapenemase activity (such as Carba NP or mCIM)	If discrepancy is not resolved, repeat susceptibility testing should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective, or whether the molecular assays are completely accurate.	1-4, 12-15
				No detection of tested carbapenemase targets	Susceptible to all carbapenems except ertapenem, e.g. meropenem S imipenem S doripenem S ertapenem R	Likely ESBL/AmpC and porin alteration, especially for *Enterobacter*; consider a phenotypic test for carbapenemase activity (such as Carba NP or mCIM); carbapenemase unlikely if negative although rare carbapenemases, e.g. GES-types, are still possible	If carbapenemase activity is detected, repeat susceptibility testing should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective, or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-15
				No detection of tested carbapenemase targets	Resistance to any carbapenems except ertapenem, e.g. meropenem R imipenem R doripenem R ertapenem R or S	Possible other carbapenemase; if blood culture, check for mixed culture; if mixed, test isolates individually and report as found; consider repeating molecular and susceptibility tests and performing a phenotypic test for carbapenemase activity (such as Carba NP or mCIM)	If carbapenemase activity is detected, repeat susceptibility testing should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective, or whether the molecular assays are completely accurate. Otherwise report phenotypic results as found.	1-4, 12-16

*In addition the specific possibilities listed, genotype/phenotype discrepancies could arise as a consequence of mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

Footnotes

1. Multiple β-lactamases may be carried by individual bacterial isolates. Most carbapenemase-producing bacteria are resistant to 3rd and 4th gen cephalosporins, although bacteria with OXA-48 enzymes may not be unless they co-produce an ESBL or AmpC enzyme.
2. Molecular assays can detect the presence of specific β-lactamase genes but cannot exclude the presence of other beta-lactamase genes or resistance mechanisms, or novel variants with changes in primer / probe annealing sites. Therefore, phenotypic resistance should always be reported.
3. Isolates with phenotypic susceptibility despite the presence of a resistance determinant may indicate the potential for resistance to emerge during therapy.
4. These are provisional guidelines based on general principles; however, the performance characteristics of many individual RUO assays are presently unknown.
5. Susceptibility of TEM/SHV-carrying strains to β-lactam/inhibitor combinations is variable.
6. Susceptibility of ESBL-carrying strains to cefepime is variable.
7. Susceptibility of ESBL-carrying strains to β-lactam/inhibitor combinations is variable.
8. Some strains carrying CTX-M ESBLs remain susceptible to ceftazidime.
9. Some strains carrying TEM/SHV-derived ESBLs remain susceptible to cefotaxime/ceftriaxone.
10. Some molecular assays for *ampC* may not reliably distinguish between chromosomal and plasmid-encoded genes in some bacterial species.
11. Most strains with de-repressed AmpC expression remain susceptible to cefepime.
12. These recommendations are based on cephalosporin and carbapenem breakpoints in M100S, 26th edition.
13. The susceptibility to other carbapenems of ertapenem-resistant strains with ESBL or AmpC enzymes and reduced porin expression that do not contain carbapenemase genes or express carbapenemase activity may be reported as measured in phenotypic susceptibility assays.
14. Rapid tests for carbapenemase activity (e.g., CarbaNP) may not detect OXA-48-like and some other carbapenemases.
15. Caution is advised. Current clinical evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective.
16. Some isolates of Enterobacteriaceae, in particular but not exclusively *Morganella, Proteus* spp. and *Providencia* spp., may exhibit intrinsic low-level resistance to imipenem on a non-carbapenemase-mediated basis.