KARIUS
clarity at speed®

AMR TECHNICAL OVERVIEW

KARIUS TEST® WITH ANTIMICROBIAL RESISTANCE (AMR) MARKER DETECTION

BACKGROUND

Since 2016, the Karius Test ® has reported microbial species causing infectious disease based on agnostic metagenomic sequencing (mNGS) of microbial cell-free DNA (mcfDNA) in plasma specimens. On April 2022, the Karius Test began testing for methicillin-resistant Staphylococcus aureus (MRSA). As of September 2023, Karius will also include automatic follow-on testing for 7 AMR markers associated with 4 classes of antimicrobial resistance across 18 AMR threat pathogens whenever one or more of these pathogens are reported by the Karius Test **(Table 1)**. Species identification will continue to be reported one day after specimen receipt at Karius, and the results of AMR will be reported as an addendum to the original report approximately 24 hours later. Results are intended to guide antimicrobial management within appropriate clinical context.

TABLE 1. TARGETED BACTERIA AND ASSOCIATED AMR MARKERS

Bacterium	AMR Marker						
	SCC*mec*	*mecA*	*mecC*	*vanA*	*vanB*	CTX-M	KPC
Methicillin-resistant *Staphylococci*							
Staphylococcus aureus	+	+	+				
Staphylococcus epidermidis		+	+				
Staphylococcus lugdunensis		+	+				
Vancomycin-resistant *Enterococci*							
Enterococcus faecalis				+	+		
Enterococcus faecium				+	+		
Carbapenemase and extended-spectrum beta-lactamase producing bacteria							
Enterobacter cloacae						+	+
Escherichia coli						+	+
Klebsiella aerogenes						+	+
Klebsiella pneumoniae						+	+
Klebsiella oxytoca						+	+
Proteus mirabilis						+	+
Proteus vulgaris						+	+
Salmonella bongori						+	+
Salmonella enterica						+	+
Serratia marcescens						+	+
Pseudomonas aeruginosa						+	+
Acinetobacter baumannii						+	+
Acinetobacter calcoaceticus						+	+

ANTIMICROBIAL RESISTANCE GENES

SCC*mec/mecA/mecC:* The staphylococcal cassette chromosome mec (SCC*mec*) is a mobile genetic element bearing the *mecA* and/or *mecC* gene which encodes for methicillin resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the *mecA* or *mecC* gene have alterations of penicillin-binding protein (PBP2a) leading to low affinity for beta-lactams and resistance to most beta-lactam antibiotics.[1-2]

vanA/vanB: Vancomycin resistance in *Enterococcus* spp. is mediated by the *vanA* and/or *vanB* gene clusters which encode for modifications of cell wall peptidoglycan precursors which serve as the drug binding site. Vancomycin-resistant *Enterococcus* (VRE) is most commonly *Enterococcus faecium* and less frequently *Enterococcus faecalis*. *Enterococci* carrying the *vanA* or *vanB* genes are highly resistant to vancomycin, and those harboring *vanA* are also resistant to other glycopeptides.[3]

CTX-M: The bla_{CTX-M} gene found in gram-negative bacteria encodes for production of CTX-M extended-spectrum beta-lactamase (ESBL), which hydrolyzes and inactivates most beta-lactams. CTX-M ESBL is most commonly found in *Enterobacterales*, however can also be present in non-enteric gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. ESBL-producing bacteria are resistant to penicillins, aztreonam, and most cephalosporins with the exception of the cephamycins.[4-5]

KPC: The bla_{KPC} gene found in gram-negative bacteria confers resistance to almost all beta-lactam antibiotics by production of *Klebsiella pneumoniae* carbapenamase (KPC). The KPC enzyme hydrolyzes and inactivates most beta-lactam antibiotics including carbapenems. Variants of the bla_{KPC} gene are frequently carried on mobile genetic elements and can spread resistance easily between different genera/species, including *Enterobacterales*, *Acinetobacter baumannii,* and *Pseudomonas aeruginosa*.[4-5]

TESTING METHODS

Plasma specimens undergo optimized microbial cell-free DNA extraction, library preparation, and enrichment, followed by mNGS. Sequencing reads are aligned to a database of curated reference genomes and compared to real-time no template and process controls to identify and quantify mcfDNA fragments of pathogens in each specimen. A reference interval derived from asymptomatic patient cohorts is provided to aid the clinician in assessing the relative importance of each microbe reported.

The presence or absence of SCC*mec* in S. *aureus* is determined by testing for 44 SCC*mec* variants and 4 non-*mec* SCC variants. Resulted Karius Tests that report one or more of the 18 targeted bacteria identified, have sufficient specimen volume for follow-on testing, and meet additional success criteria enter the AMR gene detection workflow. In this workflow, mcfDNA is extracted from plasma and is subjected to targeted sequencing using more than 300 primers to amplify ultrashort mcfDNA fragmentsfrom 6 AMR target genes, several species-specific housekeeping genes, and quality control oligos. Amplicons are aligned to AMR markers and synthetic sequence spike-ins. The number of unique templates is calculated and used as input into the AMR caller which also integrates information aboutpathogen abundance, reaction efficiency and abundance of housekeeping genes to output AMR results.

ANALYTICAL VALIDATION

Analytical validation showed high precision, inclusivity and exclusivity as demonstrated in **Table 2**.

TABLE 2. ANALYTICAL VALIDATION PERFORMANCE SUMMARY

Metric	Definition	Estimate	Note
Precision	Reproducibility of results within and across runs	100%	
Inclusivity	Sensitivity to detect variants of the AMR genes	100%*	Assessed in-silico# using gene sequences from CARD AMR database
Exclusivity	Specificity to avoid cross-reactivity with other DNA sequences	100%	Assessed in-silico# using bacterial, fungal, and archaeal reference genomes

CARD = Comprehensive Antimicrobial Resistance Database
*The CTX-M variants exhibit significant genetic diversity, which limits our test's ability to detect all of them and results in expected coverage of 67%-75% of CTX-M variants in clinical population.
#Comprised of 50 samples per AMR gene

CLINICAL VALIDATION

A total of 115 patient plasma samples with orthogonal culture and phenotypic antimicrobial susceptibility test results that had one or more of the 18 target pathogens detected by the Karius Test were included in the clinical validation study. Orthogonal data were collected from 7 study sites and included a wide variety of specimen types and 12 (66.7%) of the 18 target pathogens. Details on orthogonal data and isolated bacterial pathogens are shown in **Figures 1** and **2**, below. Clinical test performance results are shown in **Table 3**. Resolution of false-negative results was performed by further adjudication based on pathogen susceptibility and known phenotypic expression of alternative mechanisms of resistance. Results of resolutions are shown in **Table 4** and **Table 5**.

FIGURE 1. ORTHOGONAL DATA SPECIMEN TYPES

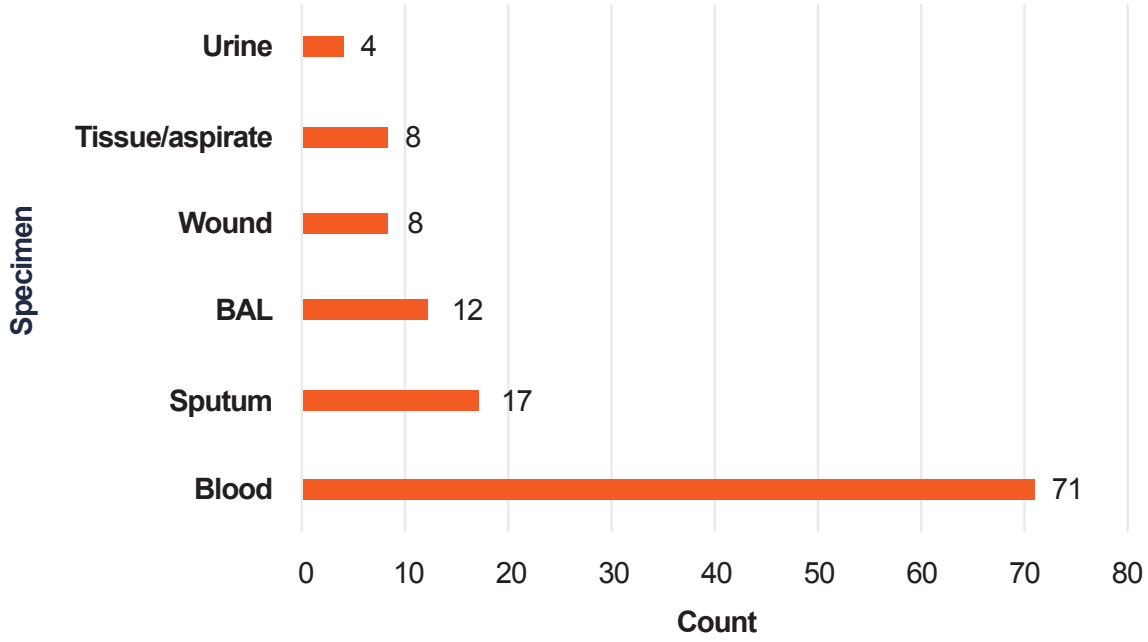


FIGURE 2. ORTHOGONAL DATA PATHOGENS ISOLATED BY CULTURE

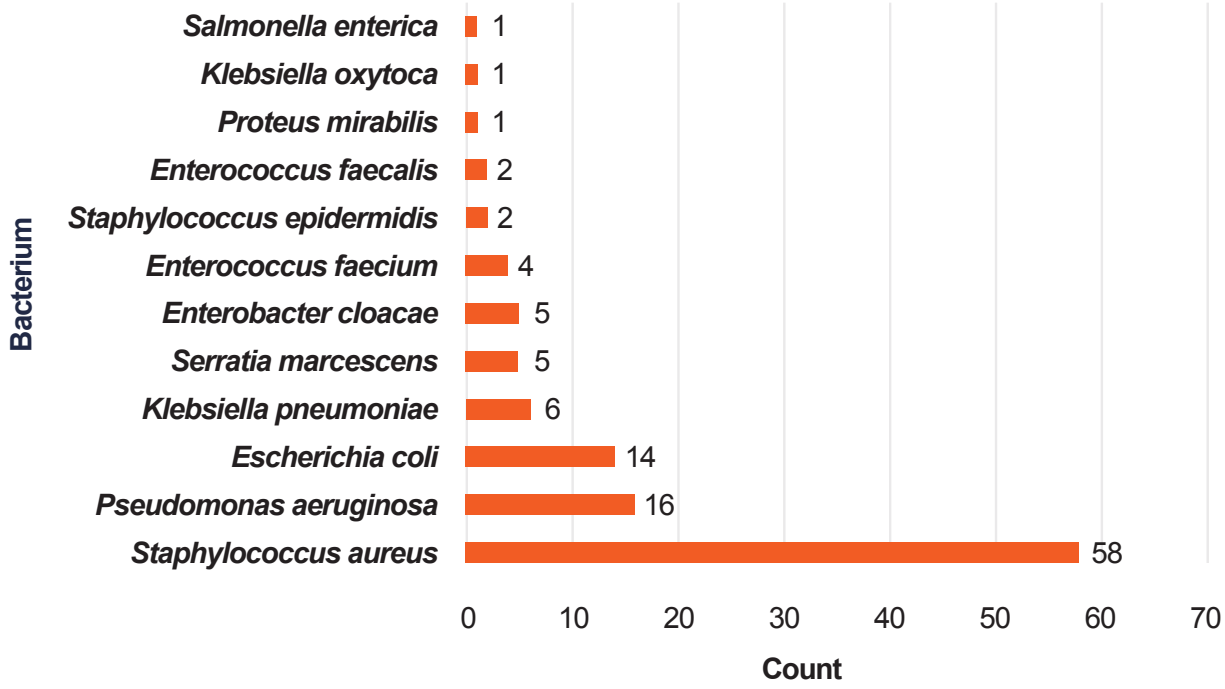


TABLE 3. CLINICAL VALIDATION PERFORMANCE SUMMARY*

			Karius Test AMR Performance[&]			
Species	**Drug / Class**	**AMR Marker**	**PPA**	**NPA**	**Accuracy**	**Total Validation Yield**
S. aureus, S. epidermidis (n=60)	Methicillin	*SCCmec, mecA, mecC*	19/20 (95.0%) [75.19%-99.9%]	21/22 (95.4%) [77.1%-99.9%]	40/42 (95.2%) [83.8%-99.4%]	42/60[#](70.0%) [56.8%-81.1%]
E. faecium, E. faecalis (n=6)	Vancomycin	*vanA, vanB*	3/3 (100%) [29.2%-100%]	2/2 (100%) [15.8%-100%]	5/5 (100%) [47.8%-100%]	5/6 (83.3%) [35.8%-99.5%]
Gram-negative bacteria (n=49)[%]	Extended-spectrum beta-lactams	bla_{CTX-M}	5/9 (55.5%) [21.2%-86.3%]	26/26 (100%) [86.7%-100%]	31/35 (88.5%) [73.2-96.8%]	35/49 (71.4%) [56.7%-83.4%]
	Carbapenems	bla_{KPC}	0/2 (0%) [0%-84.2%]	23/23 (100%) [85.1%-100%]	23/25 (92.0%) [73.9%-99.0%]	25/44 (56.8%) [41.0%-71.6%]

PPA=positive percent agreement; NPA= negative percent agreement
*No *mecC, vanB* or bla_{KPC} were detected.
#SCC*mec* and *mecA* combined
%44 of these samples passed criteria for bla_{KPC} detection.
&Clinical validation performance may differ from what is seen in production because metrics may be study-dependent.

TABLE 4. RESOLUTION OF bla_{CTX-M} FALSE NEGATIVE DETECTIONS

Lab no.	Category	Bacterium	Phenotype[a]	Genotype	Resolution
DK-27435	bla_{CTX-M} FN	*E. cloacae complex*	Cefepime - S Ceftazidime - R Ceftriaxone - R	Not available	TN
LB-70865	bla_{CTX-M} FN	*E. cloacae complex*	Aztreonam - R Cefepime - R Ceftriaxone - R	Not available	FN
LB-75775	bla_{CTX-M} FN	*E. cloacae complex*	Cefepime - S Ceftriaxone - R	bla_{CTX-M} not detected by Verigene® BC-GN test	TN
LB-77110	bla_{CTX-M} FN	*E. coli*	Cefepime - S Ceftriaxone - R	Not available	FN

S= susceptible; R= resistant

[a]ESBL and AmpC β-lactamases can be distinguished phenotypically in *E. cloacae* complex based on differential susceptibility to cefepime. Cefepime is usually not hydrolyzed by AmpC β-lactamases.[6]

Following resolution of false negative result discrepancies, the PPA of bla_{CTX-M} producing gram-negatives increased from 55.5% to 71.4%.

TABLE 5. RESOLUTION OF *bla*$_{KPC}$ FALSE NEGATIVE DETECTIONS

Lab no.	Category	Bacterium	Phenotype[a]	Genotype	Resolution
LB-66877	*bla*$_{KPC}$ FN	*P. aeruginosa*[a]	Meropenem - R/S	Not available	TN
LB-68049	*bla*$_{KPC}$ FN (*bla*$_{CTX-M}$ TP)	*E. coli*[b]	Ertapenem - R Imipenem - S Meropenem - R	Not available	FN

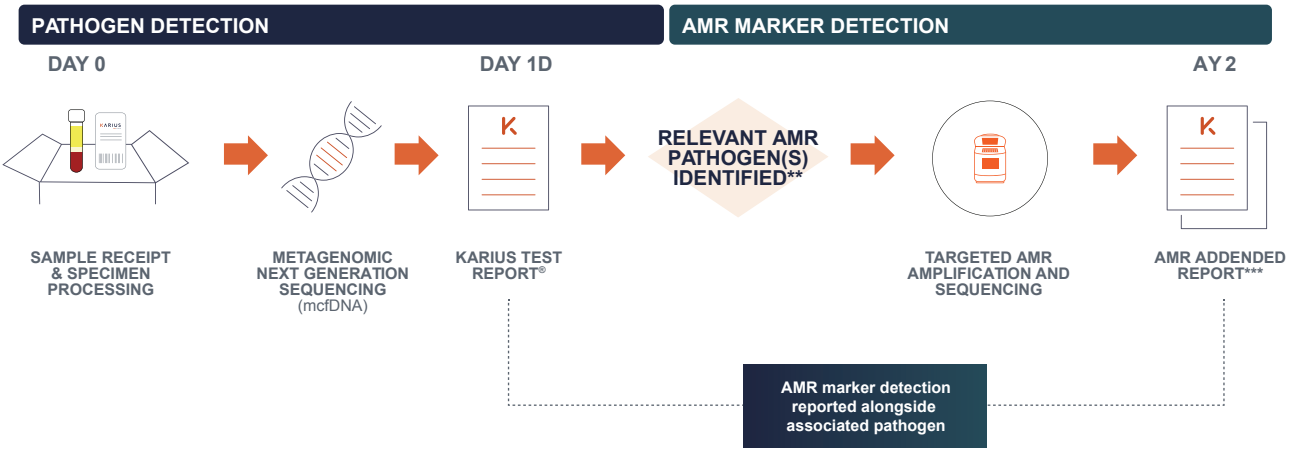
S= susceptible; R= resistant

[a]This plasma sample was associated with two *P. aeruginosa* strains (sputum and BALF), of which only one was carbapenem-resistant (sputum). The most resistant strain was selected to label the specimen, but the strain from the BALF was deemed more likely to be associated with disease.
[b]The failure to detect a *bla*$_{KPC}$ positive strain is likely to represent a combination of the small sample size, rarity of carbapenem resistance, and diverse mechanisms of carbapenem resistance[7]. This discrepancy was unable to be resolved.

AMR WORKFLOW

Reporting of AMR results occurs one day after microorganism identification results by the Karius Test (**Figure 4**) via an AMR Addended Report, with the exception of SCC*mec* results, which are reported the same day as microorganism tests results on the Karius Test Report.

FIGURE 4. SAMPLE PROCESSING TO KARIUS TEST AND AMR ADDENDED REPORT



*** Karius Test Report Notes**
1. >85% of specimens received by 8:30 AM (PT) Monday through Saturday are reported the next day
2. When *S. aureus* is detected, SCC*mec* AMR marker result may also be provided at this time; indeterminate results will undergo testing for *mecA/mecC*

AMR Marker Detection Notes
**If specimen volume is sufficient for testing and additional analytical conditions are met
***Samples with Karius Test reported on Sunday would have AMR marker detection addended on Tuesday

ANTIMICROBIAL RESISTANCE MARKER INTERPRETATION*

AMR Marker	DETECTED	NOT DETECTED	INDETERMINATE
SCCmec, mecA, mecC[1]	Consistent with resistance to methicillin and all other penicillins, most cephalosporins, and carbapenems	Consistent with susceptibility to methicillin and other β-lactamase resistant penicillins, cephalosporins, and carbapenems	Antimicrobial susceptibility or resistance of the microorganism cannot be determined. Causes of an indeterminate result include: insufficient sequence data, ambiguous linkage to specific species due to the presence of confounding microorganisms, insufficient species specific markers observed, or ambiguous/ insufficient AMR marker abundance observed
vanA[3]	Consistent with resistance to vancomycin and other glycopeptides	Consistent with susceptibility to vancomycin and other glycopeptides	
vanB[3]	Consistent with resistance to vancomycin		
CTX-M[4]	Consistent with resistance to extended-spectrum and most other cephalosporins, penicillins, and aztreonam	Unable to determine susceptibility or resistance to extended spectrum cephalosporins and the other drug classes due to multiple other mechanisms of resistance	
KPC[4]	Consistent with resistance to carbapenems, penicillins, cephalosporins, and aztreonam	Unable to determine susceptibility or resistance to carbapenems and the other drug classes to the left due to other potential mechanisms of resistance	

*Antimicrobial resistance can occur via multiple mechanisms including a broad spectrum of beta-lactamases, target site modifications, decreased cell permeability by porin mutations, and/or efflux pumps.[5] Not all mechanisms can be detected with the Karius Test. A "Not detected" result for a genetic marker of antimicrobial resistance may not indicate susceptibility to the associated antimicrobial drugs or drug classes. A "Detected" result for a genetic marker of antimicrobial resistance may not be definitively linked to the microorganism(s) detected in some cases. Confirmation of in-vitro susceptibility is recommended.

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