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Abstract

Background:

Rapid phenotypic antibiotic susceptibility tests (ASTs) may source inocula directly from complex samples, saving the time needed for solid media culturing. A fraction of these samples may prove to be polymicrobial, and it is important to assess their impact on AST results and potential consequences for therapy. A study by Snyder et al. (October 2024, Journal of Clinical Microbiology) analyzed AST outcomes of polymicrobial samples sourced from positive blood cultures (PBCs) and tested on the LifeScale AST System, finding that it detected the most resistant constituent within mixed cultures. This analysis has been expanded to a larger set of polymicrobial samples.

Methods:

PBCs positive for Gram-negative bacteremia were tested on LifeScale and the automated standard-of-care (SOC) AST platforms across multiple sites: University of Louisville, New York-Presbyterian - Columbia University Irving Medical Center, Baylor Scott and White Health (MicroScan WalkAway, Beckman Coulter), and Associated Regional and University Pathologists (Phoenix, Becton Dickinson). Polymicrobialsamplesidentifiedbymorphologywere excluded from enrollment. Organism identification (ID) was performed using Verigene or BioFire platforms. Subculture isolates were identified via Bruker MALDI-TOF and tested on the SOC AST platform.

Results

AST results for a total of 60 polymicrobial samples not detected by Gram stain were produced on the LifeScale AST system. Rapid ID platforms (Verigene or BioFire) failed to identify 28 of these samples as polymicrobial. LifeScale's results agreed with the SOC result for the most resistant constituent in > 95% of 477 organism/drug combinations.

Conclusions:

If the identification of resistant constituents proves reliable, probability would be very low that the LifeScale AST result would influence the administration of an ineffective antibiotic. Further work is needed for a complete assessment of the impact of polymicrobials on AST results.

Background

Rapid phenotypic antibiotic susceptibility tests (ASTs) can source inocula directly from complex samples, saving the time needed for solid media culturing. However, a small fraction of these samples may prove to be polymicrobial, and it is important to assess their impact on AST results and potential consequences for therapy.

A previous study by Snyder et al. (October 2024, Journal of Clinical Microbiology) analyzed AST outcomes of polymicrobial samples sourced from positive blood cultures (PBCs) and tested on the LifeScale AST System and found that the system reliably detected the most resistant constituent within mixed cultures¹. This analysis has now been expanded to a larger set of polymicrobial samples to further assess the system's performance in clinically relevant, mixed-organism scenarios.

Introduction

Rapid phenotypic antibiotic susceptibility testing (AST) platforms such as the LifeScale AST System enable direct-from-sample testing, potentially bypassing the time-intensive step of solid media subculturing. This approach is particularly valuable for positive blood cultures (PBCs), where timely results are critical for effective therapy.

However, direct testing from complex samples introduces the challenge of polymicrobial infections. Although the combination of Gram stain and rapid molecular identification (BCID) methods are very effective in identifying mixed cultures, there are a small fraction that bypass these methods. Failure to recognize mixed infections could lead to incomplete AST results and inappropriate therapy. A previous study demonstrated that the LifeScale AST System reliably reports the susceptibility of the most resistant organism within polymicrobial samples¹.

In this expanded study, we evaluated the frequency with which polymicrobial infections evade Gram stain and BCID detection and assessed whether LifeScale AST results for the identified organism remain accurate and clinically reliable in such cases.

Methods

Positive blood cultures (PBCs) showing a clean Gram stain for Gram-negative bacilli were tested using the LifeScale AST System at the following clinical sites:

- 1. University of Louisville
- 2. New York-Presbyterian / Columbia University Irving Medical Center
- 3. Associated Regional and University Pathologists (ARUP)
- 4. Baylor Scott & White Health

Organism identification (ID) was performed using Verigene (Diasorin) or BioFire (bioMérieux) platforms. Identifications were input into the LifeScale AST System to generate final AST results.

Polymicrobial samples that bypassed Gram stain and blood culture ID (BCID) screening and reached the LifeScale AST System were further analyzed. These were isolated, identified using Bruker MALDI-TOF, and tested with the MicroScan WalkAway (Beckman Coulter) or Phoenix (Becton Dickinson) per manufacturer protocols.

Any discordant results between SOC and LifeScale AST were adjudicated using broth microdilution according to CLSI guidelines^{2,3}. Samples identified as polymicrobial by initial morphology were excluded from enrollment.

Results

From 738 prospective patient samples enrolled onto the LifeScale AST System, 60 (8.13%) were not captured by Gram stain as being polymicrobial (Figure 1). Of these, 32 samples were correctly detected as polymicrobial by the rapid BCID system.

The remaining 28 out of 738 enrolled samples (3.79%) had only 1 organism identified by the BCID and were determined to be polymicrobial only after subculturing. LifeScale AST results were generated from these 28 samples using the organism identification provided by the rapid BCID system.

When compared to the standard-of-care (SOC) interpretations for all constituent organisms, the LifeScale S/I/R categorization was equivalent to or more resistant in 96.65% of the 477 organism/drug combinations evaluated (Table 2). All major discrepancies were resolved (Table 3), and 7 very major discrepancies remained after adjudication by BMD. Notably, 5 of these VMJs originated from a single patient sample (Table 4).



Figure 1, Detection of polymicrobial samples among Gram-negative PBCs

A total of 738 prospective PBC samples with pure Gram-negative bacilli observed on Gram stain were tested on the LifeScale AST. Subculture revealed 60 samples (8.13%) were confirmed polymicrobial, despite not being identified as such by the Gram stain.

Of these, 28 (3.79% of total) were also not detected by rapid blood culture identification (BCID) methods (28.6% of the time with BioFire and 56.4% with Verigene). These 28 represent real-world IVD scenario in which mixed cultures could be inadvertently tested and reported by rapid AST systems such as LifeScale.

Results

Table 1, LifeScale AST Results Evaluated in Polymicrobial Samples Missed by Initial Screening

Total No. Polymicrobials	Total No. Evaluated	No. Susceptible	No. Resistant		
28	477	384	87		

From the 28 polymicrobial samples that were not detected by Gram stain or rapid BCID, LifeScale AST results were generated using the organism identified by BCID. These results were compared to the standard-of-care (SOC) categorical susceptibility interpretations (S/I/R) for each constituent of the polymicrobial sample. A total of 477 organism-drug combinations were evaluated.

Table 2, Concordance of LifeScale AST with SOC in Polymicrobial Samples Missed by Initial Screening

Metric	Count	Notes			
Concordant / More Resistant than SOC	461/477 (96.65%)	After adjudication via BMD			
Very Major Discrepancies (VMDs)	7/86 (8.14%)	5 of 7 VMDs from a single sample			
Major Discrepancies (MDs)	0/385 (0.00%)	All resolved or excluded			
Minor Discrepancy (MIN)	13/477 (2.73%)	1 VMD became a MIN post BMD			

Of the 477 S/I/R categorical results reported by LifeScale for the organism identified by the rapid BCID, 461 (96.65%) were either concordant with, or more resistant than the most resistant interpretation reported by SOC for each constituent organism within the polymicrobial sample. Discrepant results were adjudicated using broth microdilution (BMD) in accordance with CLSI guidelines.

Table 3, Major Discrepancies (MDs) in LifeScale AST vs SOC

Antibiotic	Sample	Organism 1		LS S/I/R of PBC 1	Organism 2	SOC S/I/R	LS S/I/R of PBC 1	Resolution
Aztreonam		8 Pseudomonas aeruginosa	S	R		NR	R	
Cefepime	LV-100128		S	R	Stenotrophomonas maltophilia	NR	R	Excluded: SOC did not report an S/I/R result for the secondary isolate
Piperacillin/Tazobactam			S	R		NR	R	
Cefazolin	LV-100251	Klebsiella oxytoca	S	R	Staphylococcus capitis	NR	R	BMD results in favor of LifeScale per CLSI BMD Guidelines; BMD for isolate 1 > 16 R

A total of 4 major discrepancies (MDs) were adjudicated among the 477 organism-drug combinations evaluated. All were resolved. The SOC did not report an S/I/R for organism 2 of sample LV-100128 in the antibiotics listed thus was excluded due to the lack of SOC comparator. Cases resolved by detecting resistance in other polymicrobial constituents were considered clinically appropriate and excluded.

Results

Table 4, Very Major discrepancies (VMDs) in LifeScale AST vs SOC

Antibiotic	Sample	Organism 1	SOC S/I/R	LS S/I/R of PBC 1	Organism 2	SOC S/I/R	LS S/I/R of PBC 1	Discrepancy After Analysis		
Piperacillin/Tazobactam	LV-100170	Escherichia coli	R	s	Proteus mirabilis	S	S	Expert rule		
Aztreonam	LV 100217	Psoudomonas agruginosa	R	s	Enterococcus faecalis	NR	S	MIN		
Cefepime	LV-100214	Pseudomonas aeruginosa	R	s		NR	S	None		
Trimethoprim/Sulfamethoxazole	LV-100211	Acinetobacter Variabilis	R	s	Enterobacter cloacae	S	S	None		
Ertapenem	CMB-100100	Escherichia coli	s	s	Pseudomonas aeruginosa	R	S	VMJ		
Piperacillin/Tazobactam			s	s		R	S	None		
Trimethoprim/Sulfamethoxazole			S	s		R	S	VMJ		
Ampicillin	CMB-100147	Escherichia coli	R	s	Proteus mirabilis	s	s	NW1		
Aztreonam			R	s		S	S	LWA		
Cefepime			R	s		s	s	ΛW1		
Ceftazidime			R	s		S	s	ΛW1		
Gentamicin			R	s		s	s	NW1		
Amikacin	CMB-100158	B-100158 Escherichia coli R S Klebsiella pneumoniae S S	S	s	Klebsiella pneumoniae	R	S	None		
Gentamicin			R	s		S	s	None		
Levofloxacin				R	S	None				
Ampicillin	ADUD 4000/0	Fach seighin!i	S	S			Wishels Harassia	R	S	None
Trimethoprim/Sulfamethoxazole	ARUP-100069	0069 Escherichia coli		S	Klebsiella oxytoca	R	S	None		

Following adjudication with broth microdilution (BMD) per CLSI guidelines, 7 very major errors (VMJs) remained. Notably, 5 of the 7 VMJs originated from a single sample (CMB-100147).

Conclusions

The combination of Gram staining and rapid BCID correctly identified 710 out of 738 samples (96.21%). Among the remaining 3.79% of samples that were not detected by either method, we assessed the impact of reporting the LifeScale AST result based on the organism detected by BCID. In 96.65% of these cases, LifeScale AST reliably identified the most resistant constituent, suggesting a low risk of guiding ineffective therapy in undetected polymicrobial cases.

In summary:

- 1. Gram stain + rapid BCID is highly effective in detecting polymicrobial samples prior to testing on rapid ASTs such as LifeScale AST.
- 2. The LifeScale AST reliably reports the resistance of a mixed culture's constituents.

Further investigation is warranted to more fully assess the clinical impact of polymicrobial infections on AST outcomes and therapeutic decision-making.

- Acknowledgment-

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References

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