Impact of Delayed-Growth Phenotypes on Rapid Antibiotic Susceptibility Testing

Zachary Ruhe, Cynthia Schneider, John Tedesco, Peter Harris, Ken Babcock

Affinity Biosensors, LLC

Abstract

Background: Rapid antibiotic susceptibility tests (ASTs) rely on short incubation times to provide results in a matter of hours. However, these technologies often fail to detect antibiotic resistance as accurately as traditional methods. In this study, we develop a screen to identify resistance phenotypes which are difficult to detect using rapid phenotypic ASTs.

Methods: ASTs were performed on 2220 gram-negative bacterial isolates gathered from clinical sites and strain repositories via the broth microdilution (BMD) reference method according to CLSI M07 guidelines, and susceptibility was interpreted using CLSI M100 guidelines. In parallel, BMD samples were measured after 3 hours of incubation using a resonating microfluidic cantilever. This data was used to measure population growth and mass changes in response to antibiotic exposure. A subset of isolates was selected for a timecourse study in which BMD samples were measured periodically over 12 hours. A panel of 35 strains displaying a delayed-growth resistance phenotype were tested using different AST methods as part of a multi-site study. Performance was assessed by determining categorical agreement and very major errors relative to the BMD reference method.

Results: By comparing growth data acquired at 3 hours to the BMD reference method, we identified a large number of resistant isolates which fail to grow at early timepoints in concentrations of antibiotic below susceptible breakpoints. Timecourse studies revealed a delayed-growth resistance phenotype in which resistant organisms suspend growth for up to 11 hours in the presence of antibiotics. The delayed-growth phenotype was most prevalent in isolates resistant to β-lactams, with prevalence rates of 35% for meropenem/vaborbactam-, 25% for meropenem-, 14% for ertapenem-, 13% for piperacillin/tazobactam-, and 13% for ceftazidime/avibactam-resistant isolates. Mass profile analysis of several delayed-growth resistant samples revealed large increases in individual cell mass in response to antibiotic treatment and cell lysis coincident with MICs. Current AST technologies were assessed on a panel of delayed-growth resistant organisms. The LifeScale system, utilizing resonating microfluidic cantilever technology, outperformed other rapid AST methods in detecting resistance in delayed-growth organisms.

Conclusion: Delayed-growth resistance in response to β-lactams is prevalent among gram-negative bacteria. Population mass profiling can identify resistance in many of these samples at early timepoints.

Delayed-Growth Resistance

Growth of a meropenem-resistant isolate of *Klebsiella pneumoniae*

This figure illustrates the dose-dependent growth delay of a meropenem-resistant *K. pneumoniae* isolate (MIC value: 4 µg/ml). The strain was cultured in cation-adjusted MH broth at an inoculation concentration of 5×10^5 CFU/ml with varying meropenem concentrations. Biomass content was assessed at regular intervals using a resonating microfluidic cantilever. The observed growth delay highlights the challenges of using growth-based techniques to assess resistance at early time points.

Delayed-Growth Screen

Comparative Growth Analysis of *Klebsiella spp.* Isolates at 3 Hours

This figure presents the growth data of three K. pneumoniae isolates, including one meropenem-susceptible isolate and two meropenem-resistant isolates, in the presence of varying meropenem concentrations. The isolates were inoculated at 5×10^5 CFU/ml in cation-adjusted MH broth and incubated for three hours. Cell concentrations (cells/ml) were determined using a resonating microfluidic cantilever. For many isolates, growth inhibition at three hours coincides with the MIC determined through the reference method (red dashed line). Delayed-growth resistant organisms are characterized by discrepant growth patterns, in which measurements taken at three hours reveal growth inhibition at antibiotic concentrations below the susceptible breakpoint. Susceptible (S), intermediate (I), and resistant (R) concentrations of meropenem are indicated by shaded regions.

This observed growth discrepancy led to the development of a screening protocol, which compares growth data from a three-hour incubation period to the growth observed at 24 hours in the broth microdilution method. The purpose of this screening method is to identify resistant organisms that initially appear susceptible during early time points of incubation. These deceptive growth patterns could prevent the identification of resistance in ASTs with short incubation times.

Delayed-Growth Prevalence

Prevalence of Delayed-Growth Resistance Phenotypes Among Gram-Negative Bacteria

This figure illustrates the percentage of resistant isolates exhibiting the delayed growth phenotype, comparing the prevalence of this phenotype among different antibiotics and common gram-negative bacterial species, including *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Citrobacter spp*, *Proteus spp.*, and *Serratia spp*. The screening, which involved 2220 gram-negative bacterial isolates, was conducted for meropenem, ertapenem, piperacillin/tazobactam (Pip/Taz), ceftazidime/avibactam (Ceft/Avi), meropenem/vaborbactam (Mero/Vab), and cefepime. The overall prevalence of the delayed growth phenotype ranged from 6% of isolates resistant to cefepime, to 35% of isolates resistant to meropenem/vaborbactam, highlighting its significant occurrence across various antibiotics.

Proteus species displayed the highest rate of delayed growth, but all species investigated showed substantial levels of this phenotype. This illustrates that the delayed growth resistance phenotype is not a rare occurrence, warranting further investigation into its underlying mechanisms and potential implications for antibiotic resistance detection and treatment strategies. The development of screening protocols, such as the one described in this study, can aid in the identification of resistant organisms that initially appear susceptible, improving the accuracy of antimicrobial susceptibility testing and informing better clinical decisions.

Population Mass Profiling

Population Mass Profiling of Klebsiella spp. Isolates with Different Resistance Phenotypes

This figure compares the population mass profiles of three *Klebsiella spp.* isolates, including a susceptible isolate, a resistant isolate, and a delayed-growth resistant isolate. The isolates were inoculated in cation-adjusted MH broth at 5×10^5 CFU/ml and incubated for three hours in the presence of varying concentrations of meropenem. Following incubation, cultures were measured using resonating microfluidic cantilever technology, generating population mass distributions for each isolate. The mass distributions reveal nearly complete lysis of bacterial cells at their respective minimum inhibitory concentrations: 0.5 µg/ml for the susceptible isolate, and 16 µg/ml for both the resistant and delayed growth resistant isolates.

This figure highlights the potential of population mass profiling, utilizing resonating microfluidic cantilever technology, to identify delayed growth resistant organisms at early time points. The ability to distinguish between susceptible, resistant, and delayed-growth resistant isolates using mass distribution data can lead to more accurate antimicrobial susceptibility testing and better-informed treatment strategies.

Delayed-Growth Challenge Panel

AR Bank Strain #	Species	MEM	ETP	PTZ	CZA	MEV	FEP
0034	*Klebsiella pneumoniae*	DGR*	DGR	SDD*	R	S	DGR*
0043	*Klebsiella pneumoniae*	S*	R	DGR	S	S	R
0048	*Escherichia coli*	DGR	R	R	R	DGR	R
0055	*Escherichia coli*	DGR	R	R	R	R	R
0058	*Escherichia coli*	S	I	DGR	S	S	R
0061	*Escherichia coli*	DGR	DGR	R	S	S	R
0069	*Escherichia coli*	DGR	R	R	R	R	R
0080	*Klebsiella pneumoniae*	DGR	R	R	R	DGR**	R
0114	*Klebsiella pneumoniae*	DGR	R	R	R	R	R
0126	*Klebsiella pneumoniae*	DGR	R	R	R	R	R
0135	*Klebsiella pneumoniae*	I*	DGR	R	R	S	R
0147	*Klebsiella oxytoca*	I*	DGR*	R*	S	S	R
0149	*Escherichia coli*	DGR	DGR	R	R	R	R
0276	*Acinetobacter baumannii*	DGR	NR	DGR	NR	NR	R
0312	*Acinetobacter baumannii*	DGR	NR	NR	NR	R	R
0364	*Klebsiella pneumoniae*	I*	DGR	R	R	S	SDD
0434	*Escherichia coli*	S	S	DGR	S	S	R
0435	*Escherichia coli*	S	S	R	DGR	S	S

AR Bank Strain #	Species	MEM	ETP	PTZ	CZA	MEV	FEP
0452	*Escherichia coli*	DGR	R	R	R	DGR	R
0506	*Klebsiella pneumoniae*	R	R	R	R	DGR	R
0543	*Escherichia coli*	R*	R	DGR	R	DGR	R
0549	*Klebsiella pneumoniae*	DGR	R	R	R	DGR	R
0553	*Klebsiella pneumoniae*	DGR*	R	R	R	R	R
0555	*Klebsiella pneumoniae*	DGR	R	R	R	R	R
0569	*Escherichia coli*	DGR	R	R	R	DGR	R
0601	*Klebsiella pneumoniae*	S	S	DGR	S	S	R
0602	*Klebsiella pneumoniae*	DGR	R	R	R	R	R
0603	*Klebsiella pneumoniae*	S	S	DGR	S	S	R
0612	*Escherichia coli*	DGR	R	R	R	R	R
0616	*Escherichia coli*	DGR	R	R	R	R	R
0837	*Klebsiella oxytoca*	R	R	R	R	DGR	R
0854	*Escherichia coli*	S	I	R	S	S	R
0860	*Klebsiella pneumoniae*	DGR	R	R	R	R	R
1057	*Escherichia coli*	DGR	R	R	R	I	R

*minor and **major discrepancies compared to AR Bank public data

Antibiotic Susceptibility of AR Bank Strains Exhibiting Delayed-Growth Resistance Phenotype

This table presents the antibiotic susceptibility profiles of 35 AR Bank strains which were identified to exhibit the delayed-growth resistance (DGR) phenotype using the screening method described earlier. The susceptibility of each strain was assessed for meropenem (MEV), ertapenem (ETP), piperacillin/tazobactam (PTZ), ceftazidime/avibactam (CZA), meropenem/vaborbactam (MEV), and cefepime (FEP) using the broth microdilution method according to CLSI guidelines. The results are categorized as susceptible (S), intermediate (I), susceptible dose-dependant (SDD), resistant (R), or delayed-growth resistant (DGR). Each strain was tested in at least three independent assays, and the susceptibility determinations represent the mode of these results.

The table provides detailed information about the susceptibility profiles of the selected strains exhibiting the DGR phenotype. It is important to note that there are some discrepancies between the susceptibility data presented in this table and the publicly-available data published by the AR Bank.

AST Performance

Evaluation of Current AST Technologies on Delayed Growth Resistant Panel

A panel of delayed-growth resistant organisms were tested on a number of AST platforms as part of a multi-site study. Performance metrics, including categorical agreement (CA), very major error rates (VME), and no result rates (NR), for various AST technologies were evaluated through discrepant analysis compared to the reference broth microdilution method. The technologies evaluated include disk diffusion, the Microscan WalkAway, the BD Phoenix, the Biomerieux Vitek, and Accelerate Pheno systems. Additionally, the LifeScale AST system, which employs resonating microfluidic cantilever technology and population mass profiling, was evaluated. The figure displays metrics for both the overall panel and the delayed-growth resistant samples exclusively.

The results indicate that delayed-growth resistant samples posed a challenge for many AST technologies. The Pheno system was most impacted, exhibiting a VME rate of over 22% and a NR rate of over 30% for delayed growth organisms. Technologies utilizing longer incubation times, such as disk diffusion and Phoenix, were less impacted with VME rates of 10% and 8%, respectively. The data suggests that the LifeScale system, with its resonating microfluidic cantilever technology, can correctly identify resistance in the majority of delayed-growth organisms at early time points.

Conclusion

The delayed-growth resistance phenotype presents a significant challenge to the clinical management of antibiotic resistance. A screening method designed to identify delayed-growth resistant organisms revealed significant prevalence of this phenotype across various gram-negative bacterial species and β-lactam antibiotics. The identification of delayed growth resistant organisms has important implications for improving antimicrobial susceptibility testing and informing better clinical decisions.

A panel of delayed-growth resistant organisms was used to evaluate current AST technologies, including disk diffusion, WalkAway, Phoenix, Vitek, and Pheno systems. The LifeScale system, with its resonating microfluidic cantilever technology, was the best-performing technology for detecting delayed-growth resistant organisms. Further investigation into the application of population mass profiling in clinical settings is warranted, as it may provide valuable insights into the early detection of delayed-growth resistance phenotypes and improve the overall management of antibiotic resistance. The development of accurate and rapid diagnostic tools is crucial in the face of increasing antibiotic resistance and the continuous emergence of new resistance mechanisms.