CPHM02
Friday, June 14, 2024
#206

Rapid phenotypic ESBL confirmation testing using a holographic imaging system

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INTRODUCTION

Extended-spectrum β-lactamase (ESBL) Producing Enterobacterales

- Multiple Enterobacterales species have the potential to produce ESBL enzymes, which convey resistance to some of the most widely used antimicrobial classes targeting Gram-negative organisms.
- The occurrence rate of ESBL-producing Enterobacterales is increasing (e.g., 165 cases per 100,000 surveyed individuals in 2021, 5% of those organisms isolated from blood samples [1]; ca. 2.4% of Enterobacterales isolated from patients with blood-stream infections are ESBL producers [2,3]).
- Accurate Identification of ESBL-producing organisms is thus important for epidemiological surveillance.
- Rapid detection further allows for treatment escalation (e.g., to carbapenems), whereas rapid confirmation of ESBL absence may allow for de-escalation.

The Accelerate WAVE™ System*

- The Accelerate WAVE™ system* is an automated device producing antimicrobial susceptibility testing results directly from positive blood culture and eventually isolated colonies.
- An aliquot of the positive blood culture is filled into two test cards (at two different dilutions) containing 96 test chambers, each with dried-down antimicrobials at different concentrations.
- A sequence of images is taken of each test chamber over several hours, while the bacteria respond to the presence of the antimicrobials.
- The system uses in-line holography, i.e., light is transmitted through the test chamber and light patterns resulting from interference due to the presence of bacteria suspended in the chamber are recorded on a sensor, thus creating holograms that contain information from objects suspended in a volume without the need to focus.
- Images can be digitally focused on individual slices through space, allowing for quantification of intricate morphological reactions of individual bacterial cells to the tested antimicrobials in addition to overall growth responses.
- In this study, we used the Accelerate WAVE™ system* to create a phenotypic test for ESBL production directly from positive blood culture.

The Accelerate WAVE™ system comprised of (from right to left) the user interface, the analysis module, and up to five AST modules. Each AST module houses 10 bays accommodating a 96-chamber test card.*

REFERENCES

[1] CDC 2023; Emerging Infections Program, Healthcare-Associated Infections – Community Interface Surveillance Report, Multi-site Gram-negative Surveillance Initiative (MuGSI), Extended-spectrum β-lactamase-producing Enterobacterales Surveillance, 2021.
[2] Rhee C et al. CDC Prevention Epicenters Program. Prevalence of Antibiotic-Resistant Pathogens in Culture-Proven Sepsis and Outcomes Associated With Inadequate and Broad-Spectrum Empiric Antibiotic Use. *JAMA Netw Open* 2020; 3(4): e202899.
[3] Sader HS et al. Frequency of occurrence and antimicrobial susceptibility of bacteria isolated from patients hospitalized with bloodstream infections in United States medical centers (2015-2017). *Diagn Microbiol Infect Dis* 2019; 95(3): 114850.

METHODS

Study Design

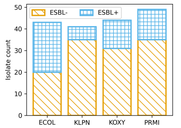
- 177 strains of *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* tested
- Each strain spiked into negative blood culture and incubated until positive
- Tested in parallel on Accelerate WAVE™ system and by broth microdilution (BMD)

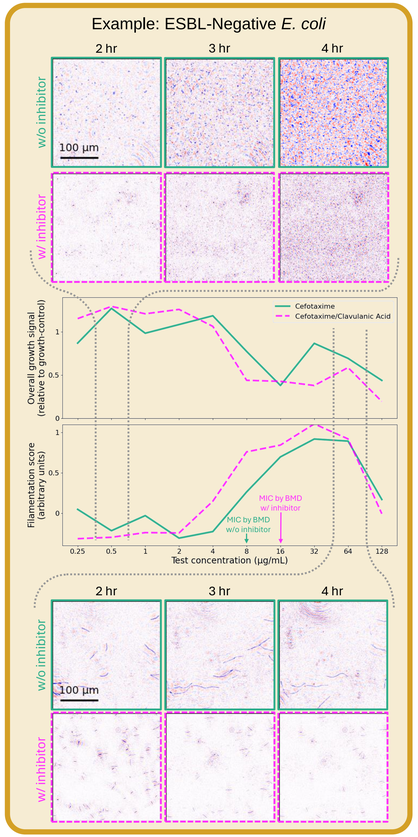
BMD Reference Testing (Per CLSI Guidelines)

- Performed minimum inhibitory concentration (MIC) testing for β-lactam drugs ceftazidime and cefotaxime, each with and without addition of clavulanic acid
- Occurrence of an MIC difference between pure β-lactam drug and combination drug of three or more doubling dilutions for either of the two drugs interpreted as detection of an ESBL-producing organism

Organism Distribution

Distribution of reference testing result for the 177 enrolled strains of *E. coli* (ECOL), *K. pneumoniae* (KLPN), *K. oxytoca* (KOXY), and *P. mirabilis* (PRMI)





ESBL Confirmation on the Accelerate WAVE™ System*

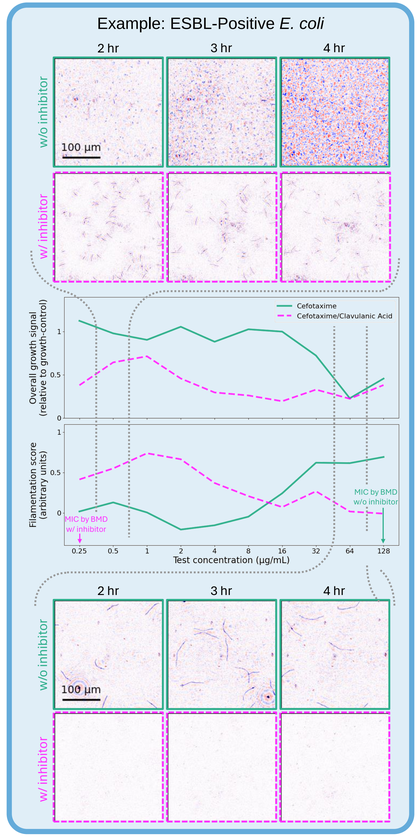
1) **Three β-lactam drugs** (ceftazidime, cefotaxime, cefepime) tested over a range of concentrations, from 0.25 µg/mL to 128 µg/mL, **with and without the addition of clavulanic acid as β-lactamase inhibitor**

2) **Time series** of holographic images obtained, containing information on bacterial **growth** and **cell morphology**, accessible via image reconstruction (see examples)

3) **Response** to each test concentration **quantified** via summary statistics, image reconstruction, computer vision techniques,… (see examples)

4) **Difference** in responses to β-lactam drugs **with and without inhibitor** was quantified

5) **Species-specific algorithm** combining all extracted differences into an ESBL-positive/negative prediction was fit to a subset of the data (training data) and its performance assessed on the remainder of data (test data)



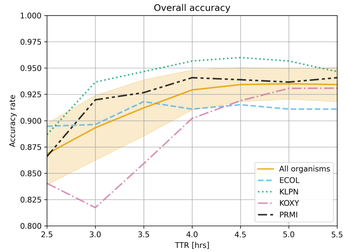
RESULTS

Bootstrapping

- Species-specific ESBL confirmation algorithms were fit to training data (ca. 75% of strains) and their concordance with BMD reference testing assessed on test data (ca. 25% of strains).
- To quantify the utility of the technology and algorithmic framework to generalize patterns learned from a training data set to predict results on an independent data set, 20 different, random splits of the sample into training and test sets were performed.
- Accuracy rates of the ESBL result as compared to BMD reference testing were averaged across the 20 bootstrapping iterations.

Accuracy

Average accuracies of the Accelerate WAVE™ system* ESBL results across 20 bootstrapping iterations separately for *E. coli* (ECOL), *K. pneumoniae* (KLPN), *K. oxytoca* (KOXY), and *P. mirabilis* (PRMI), as well as combined. The shaded region around the combined result indicates the variation across bootstrapping iterations.
Results are shown as a function of time to result (TTR), i.e., the length of the time series of holographic images on which the ESBL result was based.



CONCLUSIONS

- ESBL predictions with accuracies greater than 90% were obtained for all four species tested.
- Optimal results were reached after ca. 4 hours (slightly later for *K. oxytoca*), with minimal improvements from prolonged imaging beyond that time point.
- It should be noted that the study population contained a far higher fraction of ESBL-producing organisms than expected in a clinical setting. Thus, the resulting algorithms may not be optimally tuned for application in a general context.

This study **demonstrates the utility of the Accelerate WAVE™ system's*** holographic imaging technology and highly multiplexed test setup to

- **characterize the bacterial response** to an antimicrobial over time, using both coarse overall growth metrics and cell-morphological quantities,
- **infer the minimum inhibitory concentration** of an antimicrobial for a bacterial sample, directly from positive blood culture,
- use comparisons of the quantified responses to different test conditions to classify bacterial samples, e.g., as ESBL-positive/negative,
- **produce highly accurate, phenotypic ESBL confirmation within a few hours**.

*Product under development and not available for sale. Subject to regulatory review and clearance.