Metabolic preference assay for rapid diagnosis of bloodstream infections

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ABSTRACT

In bloodstream infections, the use of culture directed antibiotics is crucial. However, existing leading platforms (VITEK, BioMerieux) take 2-5 days to identify pathogens and to determine their antimicrobial susceptibility. Here, it is shown that a rapid metabolic preference assay (MPA) is able to decrease testing time to under 20 hours.

INTRODUCTION

Time is crucial when it comes to identifying pathogens in bloodstream infections (BSI). A single day of receiving ineffective antimicrobials increases average BSI mortality by up to 5%. When BSI progresses to septic shock, treatment delays can increase mortality by over 7% per hour. As a result, clinicians have to make therapeutic decisions under time pressure. This encourages the widespread use of broad spectrum antimicrobials and selection for resistant organisms. The current microbiology testing timeline is dominated by three microbial culturing steps:

Growth of Microbe	Identification (ID)	Susceptibility (AST)
Patients blood is incubated for 7-43h to allow growth of microbes to detectable levels.	Once positive, aliquots are streaked onto agar plates and incubated for another 12-24h to obtain single colonies. Then, subcultured isolates are identified via mass spectrometry (MS)	Antimicrobial susceptibility is then performed by incubating a fixed number of microbes for 12-24h in a medium containing antimicrobials using an automated testing system

Metabolic preference assays (MPA) use patterns of consumed versus excreted metabolites of ex vivo microbial cultures for pathogen identification (ID), while metabolic inhibition assays (MIA) can be used for antimicrobial susceptibility testing (AST). The metabolic profile of 7 pathogens for ID and AST were analysed - *Candida albicans* [CA], *Klebsiella pneumoniae* [KP], *Escherichia coli* [EC], *Pseudomonas aeruginosa* [PA], *Staphylococcus aureus* [SA], *Enterococcus faecalis* [EF] and *Streptococcus pneumoniae* [SP]

METHODS

1. MPA as a tool for ID

- 3 clinical isolates (2 for PA) for each target species (n=7) incubated in triplet and analysed.
- Metabolite levels present in the cultures were analysed at 0 h and 4 h on a Thermo Q Exactive HF MS.
- Features were identified with untargeted analysis using MAVEN software, and were filtered using an in-house script.
- Signals were clustered into groups based on retention times, known adduct/fragment masses, and covariance of signal intensities across replicates. Data clustering and parent ion identification were completed using in-house software tools.
- Putative metabolite assignments were made via the Madison Metabolomics Consortium Database (MMCD) and Human Metabolome Database.

2. MIA as a tool for AST

- Changes in the metabolic composition of microbial culture supernatants after a 4h incubation period with and without antimicrobials were monitored using the same metabolomics analyses for general MPA testing.
- 3 patient isolates for each of the 7 target pathogens (two for SA and PA) were analysed.

3. Validation of MPA/MIA

- A cohort of isolates (n = 246) with varying antibiotic susceptibility profiles, (including EC (n = 50), SA (n = 63), KP (n = 35), SP (n = 49), EF (n = 23), and *Enterococcus faecium* (n = 24)) were challenged with commonly prescribed antibiotics at breakpoint concentrations. The results were then compared with those of the VITEK 2 platform.

4. Reduction of time to identification

- Head to head ID and AST race using MPA/MIA was performed against the VITEK 2 platform using EC and SA on 3 separate occasions, and time taken was recorded

RESULTS

1. MPA as a tool for ID - 203/210 markers had significant differences by one-way ANOVA. 7 metabolites (arabitol, urocanate, succinate, xanthine, mevalonate, N1,N12-Diacetylspermine, and lactate) were able to differentiate between prevalent species responsible for BSIs (p-values ranging from 1.6×10^{-136} to 1.1×10^{-279} by one-way ANOVA), and all were significantly linked to one or more species via pairwise post-hoc comparisons using Tukey-Kramer HSQ (see Figure 1)

2. MIA as a tool for AST - Antimicrobial sensitivity profiles determined by MIA were consistent with 98% of the profiles observed in traditional microbial growth assays. In most cases, the biomarkers used to identify microbes were also useful for differentiating drug-sensitive and resistant strains (see Figure 2 for the example of KP)

3. Validation of MPA/MIA - There was a 95.2% agreement between the MIA assay and results generated by the VITEK 2 platform with species-specific predictions ranging from 90.3% for KP to 97.6% for SA. The major error rates (susceptible by VITEK 2 and resistant by MIA) were 3.1%, whereas the very major error rates (resistant by VITEK 2 and susceptible by MIA) were only 1.7%.

4. Reduction of time to identification - The MPA/MIA workflow reduced total testing time by an average of 24.3h for SA and 22.4 h for EC, corresponding to a 2.2- and 2.3-fold decrease in total testing time, respectively. The time required for strain identification and antibiotic susceptibility post-blood bottle flagging alone decreased by 4.7- and 5.0-fold for SA and EC, respectively

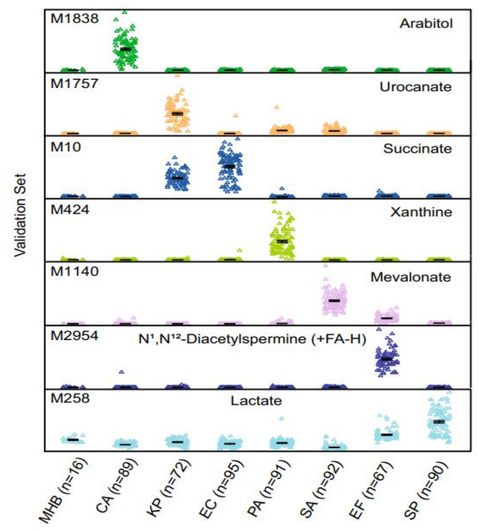

Figure 1. A validation dataset consisting of 596 clinical isolates demonstrates that top seven biomarkers that can robustly differentiate between the seven species studied

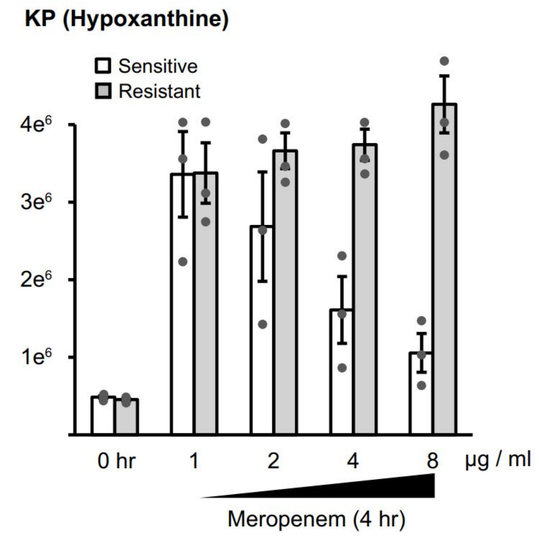

Figure 2. Drug-sensitive strains of K. pneumoniae show an incremental reduction in hypoxanthine production proportional to meropenem concentrations whereas resistant strains remain unaffected within the clinically relevant antibiotic concentrations

DISCUSSION

Macroscopically, rapid diagnostic tools for identifying microbes and measuring antibiotic susceptibility profiles could have a significant impact on morbidity and mortality rates from BSI. These tools could also help promote antibiotic stewardship by allowing clinicians to more efficiently transition patients off of broad-spectrum antibiotics and onto more precise therapies.

Metabolic boundary fluxes (MBF) have sufficient resolution to distinguish the major taxa seen in clinical settings. This study demonstrated the differentiation of seven BSI-causing organisms, which could be extended to the full spectrum of BSI pathogens as well as a wide range of other microbes in future applications. Additionally, MBF are altered in response to both bacteriostatic and bactericidal antimicrobial agents, which provide a flexible diagnostic approach that could be applied to potentially any antimicrobial agent.

On a practical level, the MPA/MIA workflow can integrate microbial identification and antimicrobial susceptibility testing onto a single instrument. It requires minimal sample handling, allows samples to be taken directly from the blood bottle, and does not require the subculturing of isolates. Furthermore, there are already commercially available mass spectrometers certified as medical devices, which could simplify the transition of MPA/MIA into clinical practice. The high sensitivity of mass spectrometry, along with the high abundance of metabolites relative to cells or macromolecules, also make the MPA/MIA workflow inherently amenable to rapid diagnostics.

For future applications, expanding this workflow to less prevalent BSI pathogens will likely result in the discovery of additional biomarkers and improved species coverage. Optimizing chromatography time and transitioning the MPA/MIA workflow from a high-resolution quadrupole orbitrap mass spectrometer to existing clinical-grade triple quadrupole mass spectrometers could also support the high sample throughputs needed in real-world clinical laboratories and promote uptake in laboratories with existing platforms.

In conclusion, this ex vivo metabolomic workflow can (i) accurately identify the most common bloodstream pathogens, (ii) empirically determine antibiotic susceptibility profiles with high accuracy, and (iii) decrease diagnostic testing timelines of bloodstream infection by more than 20 h.