Host metabolic consequences of intracellular infection by members of the ESKAPE pathogens

Blanca Lorente-Torres[1]; Helena Á. Ferrero[1]; Pablo Castañera[1]; Sergio Fernandez Martinez[1]; Steffen Braekling[2]; Mohamed Elsadig[3]; Eliska Ceznerova[4]; Sonja Klee[2]; Valesca Anschau[5]; Matthew R. Lewis[6]; Volker Behrends[7]; Michal Latek[1]

[1]Universidad de León, Leon, Spain; [2]TOFWERK, Thun, Switzerland; [3]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [4]Bruker Switzerland AG, Fällanden, Switzerland; [5]Bruker Scientific LLC, Billerica, MA; [6]Bruker UK Ltd., Coventry, United Kingdom; [7]University of West London, London, United Kingdom

Introduction

Members of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*) are leading causes of hospital-acquired infections. Several are facultative intracellular pathogens capable of invading both phagocytic and non-phagocytic cells. The emergence of multidrug-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), has significantly complicated treatment strategies and underscores the need for alternative therapeutic approaches. This study aimed to identify host-directed drugs that interfere with intracellular infection by MRSA.

Methods

We screened 5,599 approved host-directed drugs in A549 cells infected with *S. aureus* USA300 LAC and evaluated transcriptional and metabolic effects of promising candidates that affected intracellular bacterial proliferation (Figure 1).

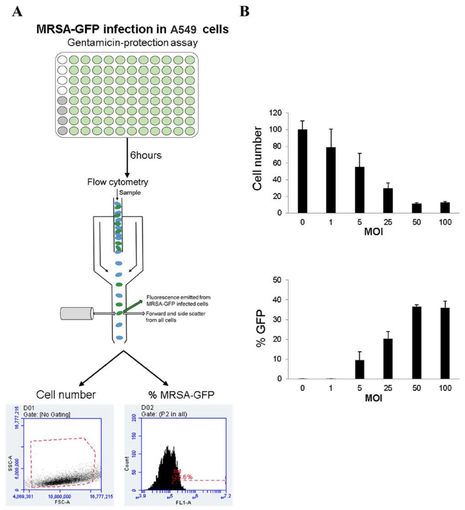


Fig. 1 Drug screening approach. We used GFP-tagged MRSA and mCherry-tagged host cell for differential quantification of host cell and bacterial viability via flow cytometry (A). The approach enabled simultaneous semi-quantitative examination of FDA-approved host—targeting drug efficacy against MRSA invasion in a high-throughput 96 well-based assay (B). Figure modified from [1].

To further elucidate physiological consequences of MRSA invasion and generate hypotheses on drug mechanism of action for promising candidates, we employed GC/MS-based profiling to assess the impact of MRSA infection on host central carbon metabolism with or without treatment. In addition to standard GC-single quad-MS analysis, we employed the novel ecTOF (Bruker Daltonics, Bremen, Germany).

For GC/MS-based assay, A549 cells were infected via a gentamicin protection assay (1h exposure time before gentamicin addition at MOI (Multiplicity of Infection) 33, 6h total invasion time). Cells were harvested in 1 ml + 0.5 ml acidified (0.1% formic acid) methanol:acetonitrile:water (2:2:1, *v/v/v*), dried and derivatized using a two-step methoximation-*tert*-butyl-trimethylsilylation as described previously [2].

Table 1 GC-ecTOF Method

GC Method		ecTOF Method	
Injection Volume	1 µL	Heated Transfer Lines Temperature	180 °C
Injector Type	Splitless	Ion Source Temperatures	EI 250 °C CI 250 °C
GC Column	Rxi-5MS 30 m, 0.25 mmID, 0.25 µmdf	Ion Source Types	EI StarBeam 70eV CI HRP using NH_4^+ Reactant
Carrier Gas Flow	1.0 mL/min He, constant flow	Mass Range	1-1000 m/z
GC oven programm	60 °C for 1 min 10 °C/min to 325 °C hold for 10 min	EI/CI Switching Speed	10 Hz

Novel GC-ecTOF Principle

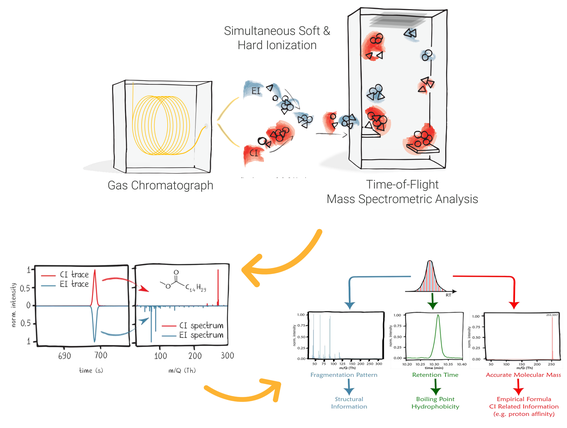


Fig. 2 Working principle of the novel dual ionization GC-ecTOF. Within a single sample injection both molecular information (Chemical Ionization, CI) and structural information (Electron Ionization, EI) in generated simultaneously.

MS Data Review Example

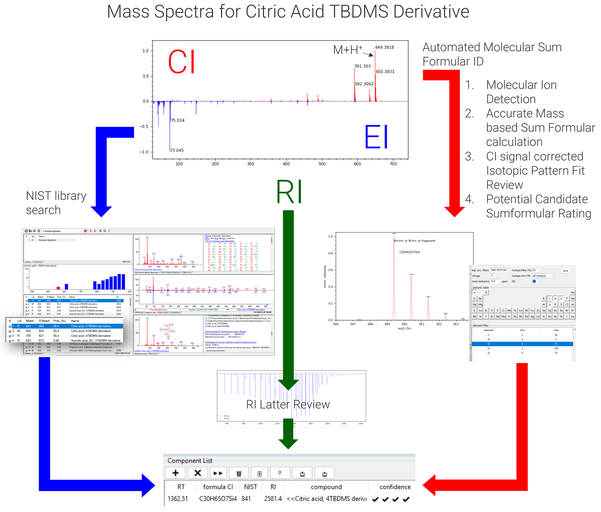


Fig. 3. Compound ID workflow for the combined usage of EI, CI and RI data after statistical investigation of the data set.

Results

MRSA infection induced marked changes in central carbon metabolism, affecting amino acid levels and TCA flux. The nucleoside analogue 5-fluoro-2'-deoxycytidine (5-FdC) modulated these pathways and significantly reduced intracellular bacterial load. The metabolic signatures suggested interference with nutrient acquisition and stress adaptation mechanisms. In combination with known antibiotics, 5-FdC reduced bacterial burdens in murine lungs and spleen. Our integrative pipeline, from screening to *in vivo* validation, identifies 5-FdC as a promising repurposable drug against intracellular S. aureus and provide a basis for further development of host-directed therapeutic strategies.

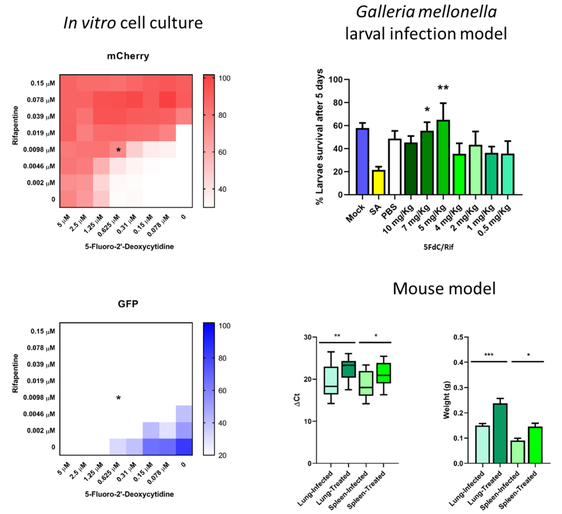
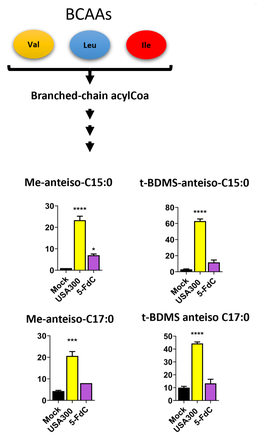


Fig. 4 Treatment with 5FdC leads to decreased pathogen survival and increased animal model health



Branched chain fatty acids (BCFAs) are made from branched chain amino acids (BCAAs) and constitute the main component of the cell membrane in *S. aureus*, making up up to 80% of the lipid-bound fatty acids, depending on growth medium. The most common species are *anteiso*-C15:0 (12-methyltetradecanoic acid) and *anteiso*-C17:0 (14-methylhexa-decanoic acid), made from isoleucine, though *iso* species (13-methyl and 16-methyl, respectively), made from leucine also occur.

Fig. 5 BFCAs are made from BCAAs and constitute a major component of the cell membrane of S. aureus. Increased BFCAs indicate elevated infection in A549 cells. Reduced BFCAs are a marker of treatment success in 5-FdC treated cells..

We were able to detect several BCFA species inside A549 host cells. Their relative abundance was greatly increased in cells exposed to MRSA USA300 and greatly reduced after 5-FdC exposure (Fig 5). This suggests that BCFA measurement is a viable method for intracellular S. aureus/MRSA detection/quantification.

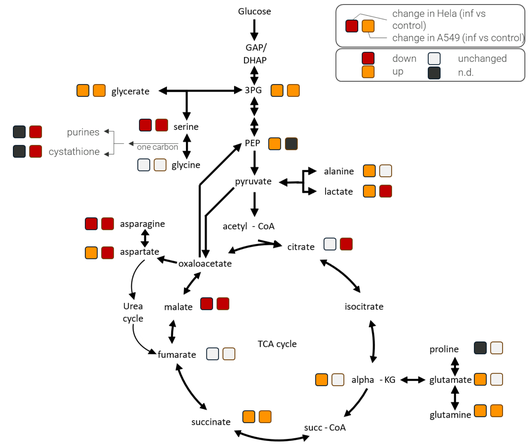


Fig.6 Comparison of metabolic changes in central carbon metabolism in HeLa vs A549 cells infected by MRSA.

We had previously investigated the impact of MRSA on HeLa cells [3]. To compare physiological perturbations introduced by MRSA invasion in these different cell models, we looked at up- and down-regulation of pool sizes of metabolites of the extended central carbon metabolism. Some metabolites (e.g. purines and cystathione) were only detected by the ecTOF.

Summary

GC-ecTOF metabolomics reveals major host metabolic shifts during intracellular MRSA infection and identifies BCFA markers of infection. Treatment with 5-FdC reduces bacterial burden and alters host metabolism, highlighting its potential as a host-directed therapy.

Literature

1. Sci Rep. 2019. 9(1):4876. doi: 10.1038/s41598-019-41260-8.
2. Arch Toxicol. 2019. 93(2):341-353. doi: 10.1007/s00204-018-2371-0.
3. mSphere. 2018. 3(4):e00374-18. doi: 10.1128/mSphere.00374-18.

Conclusions & Outlook

- Simultaneous EI/CI GC-ecTOF enabled confident metabolite annotation through combined molecular ion and structural information in a single injection.
- The integrated EI, CI, and RI workflow improved detection of host–pathogen metabolic alterations compared to conventional GC-MS approaches.
- MRSA infection caused significant changes in host central carbon metabolism, particularly in amino acid and TCA-cycle related pathways.
- Branched-chain fatty acids (BCFAs) were identified as potential markers for intracellular S. aureus infection and treatment response.
- 5-FdC significantly reduced intracellular MRSA burden and altered metabolic pathways linked to bacterial adaptation and survival.
- The combined screening and GC-ecTOF metabolomics workflow highlights 5-FdC as a promising host-directed therapeutic candidate against intracellular MRSA infection.
- Some metabolites such as purines and cystathione were only detected by the ecTOF.

Conflict of Interest Disclosure

Sonja Klee and Steffen Bräkling are employees of Tofwerk AG. Mohamed Elsadig, Eliska Ceznerova, Matthew Lewis and Valesca Anschau are employees of Bruker Daltonics GmbH & Co KG. Tofwerk AG/Bruker provided access to the ecTOF GC/MS setup. This study was conducted independently, and the results presented reflect the authors' objective assessment of the technology.