

Jordy Evan SULAIMAN, a PhD student of Prof. Henry LAM, has won the Top-Rated Poster Award at the 31st ECCMID 2021 and the Outstanding Poster Award at the WMF 2021


Jordy Evan SULAIMAN, a PhD student from Prof. Henry LAM's group, has won the Top-Rated Poster Award at the 31st European Congress of Clinical Microbiology & Infectious Diseases (ECCMID). The award was given to the top ~1% of the submitted abstracts & posters, based on rating by peer reviewers.



At the same time, he has also won the Outstanding Poster Award at the World Microbe Forum (WMF) 2021 (out of >3000 other posters). WMF 2021 is one of the largest microbiology conferences to date, putting together two of the largest meetings in the microbial sciences (American Society for Microbiology (ASM) Microbe 2021 and Federation of European Microbiological Societies (FEMS) 2021) under one digital platform.

In both of the conferences, he has presented his recent work on the proteomic study of tolerant and resistant MRSA strains generated through laboratory evolution.

Congratulations to Jordy Evan SULAIMAN and his supervisor Prof. Henry LAM.





31st ECCMID
Vienna, Austria
19-22 July 2021

Comprehensive Proteomic Analysis of Tolerant and Resistant Methicillin-Resistant *Staphylococcus aureus* Strains Generated through Laboratory Evolution

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Scan to see the references of this poster

Abstract

Recent discoveries indicate that tolerance and resistance could rapidly evolve in bacterial populations, under lethal-dose intermittent antibiotic treatment. In the present study, we applied antibiotic combinations in laboratory experiments to generate novel methicillin-resistant *Staphylococcus aureus* (MRSA) strains with distinct tolerance/resistance phenotypes that showed previously reported and other unreported single point mutations. The proteomic profiles of the ancestral and evolved strains with distinct phenotypes (tolerance, resistance, and suppressed tolerance) were then generated and their differential expression patterns were compared across strains to uncover the adaptation mechanisms. We observed that while the tolerant strains have very different proteomes than the susceptible ancestral strain, with significant differences in numerous biological processes and pathways, the resistant one largely resembles the ancestral in terms of their proteomes. Our proteomics data and other assays support the connection between the detected mutations to the observed phenotypes, confirming the general understanding of tolerance and resistance mechanisms. While resistance directly counteracts the action mechanism of the antibiotic, tolerance involves complex substantial changes in the cells' biological process to achieve survival advantages.

Methods

Evolution Experiment

First week: 1st scheme (DAP), 2nd scheme (DAP+RIF), 3rd scheme (DAP+RIF+PPF)

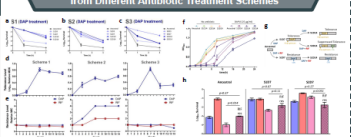
Second week: 1st scheme (DAP), 2nd scheme (DAP+RIF), 3rd scheme (DAP+RIF+PPF)

Shotgun Proteomics

- Sample Preparation: 150 µg proteins were processed with Anionion filter device, digested with sequencing grade modified trypsin (1.50 µg), and desalted by C18 reverse-phase ZipTip.
- LC-MS/MS: Processed through Bruker nanoflow UHPLC system coupled to TimsTOF Pro mass spectrometer via a nano-electrospray ion source.
- Database Searching: Search against the protein database.
- Database construction: Convert genome sequence of MRSA ATCC 43302 to protein database using GeneMark. Proteins were annotated using BLASTP (NCBI) using MRSA NCBI E232 as protein database. Add sequences of common contaminants & decoy sequences.
- Label-Free Quantification by Spectral Counting: Normalized spectral abundance factor (NSAF). Differentially expressed proteins: 1) p-value from Student's t-test <math>< 0.05</math>, 2) Fold change (higher/lower) > 1.5-folds.

Results

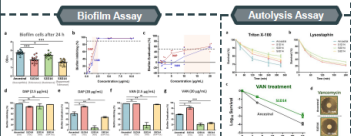
MRSA with Distinct Genotypes & Phenotypes Evolved from Different Antibiotic Treatment Schemes



1) Two weeks of intermittent DAP treatment led to DAP-tolerant strain (S1014), while DAP+RIF treatment led to a DAP-resistant strain (S2014). Interestingly, adding RIF to the treatment regime after one week of DAP treatment led to reduced tolerance (S2014).

2) RIF resistance is not allowed to evolve in the DAP-tolerant & resistant strains under drug combination treatment.

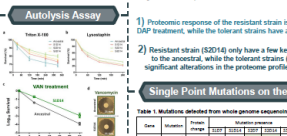
Biofilm Assay



1) In general, the evolved strains had lower amounts of biofilm cells after 24 h of growth than the ancestral one.

2) Antibiotics are unable to disrupt resistant strain biofilms but can eradicate biofilms of the ancestral and other evolved strains.

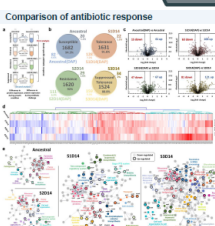
Autolysis Assay



1) The evolved strains possess cell membrane and other cell wall-related modifications.

Proteomics Analysis

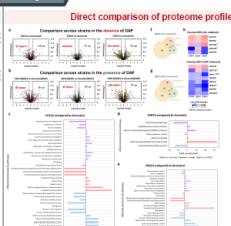
Comparison of antibiotic response



1) Proteomic response of the resistant strain is similar to the ancestral upon DAP treatment, while the tolerant strains have a more complicated response.

2) Resistant strain (S2014) only have a few key altered processes compared to the ancestral, while the tolerant strains (S1014 and S2014) have a significant alterations in the proteome profile, but both were very similar.


Direct comparison of proteome profile



1) Proteomic response of the resistant strain is similar to the ancestral upon DAP treatment, while the tolerant strains have a more complicated response.

2) Resistant strain (S2014) only have a few key altered processes compared to the ancestral, while the tolerant strains (S1014 and S2014) have a significant alterations in the proteome profile, but both were very similar.

Expression Level of Mutated Genes



The expression level of *sgpF* is higher on the resistant strain, while *pgpA* and *pgpB* are lower on the tolerant strains.

Conclusions

1) Laboratory evolution under different schemes led to strains with distinct tolerance/resistance phenotypes. Combination of DAP and RIF applied to a DAP-tolerant strain increase its susceptibility.

Overall, the apparent ease of evolution of distinct strains with different phenotypes in such a short time highlights the diverse evolutionary pathways available to the bacteria to develop tolerance and resistance, and the benefits and costs associated with the specific treatment conditions may bias the selection of one mutant strain over another, showing the complex competition dynamics among emerging mutants.


2) Novel tolerance-related proteins revealed by proteomics. Protein network of DAP-tolerant strain is more perturbed than the resistant strain.

Tolerance mutations are more upstream and may be better considered as the result of a perturbed biological network, while resistance mutations are directly related to the action mechanism of the antibiotic.

Acknowledgement

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CHAT with the TOP RATED POSTER presenters on the ECCMID platform



Comprehensive proteomic analysis of tolerant and resistant methicillin-resistant *Staphylococcus aureus* strains generated through laboratory evolution

Monday, 12 July 2021 - 16:00-17:00 CEST

No. 00046



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31st ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

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