Annotation of the *Staphylococcus aureus* metabolome using liquid chromatography coupled to high resolution mass-spectrometry (LC-HRMS) : Application to the study of methicillin resistance

Sandrine AROS-CALT[1,2], Florence CASTELLI[1], Patricia LAMOURETTE[1], Bruno H. MULLER[2], Gaspard GERVASI[2], Christophe JUNOT[1], François FENAILLE[1]

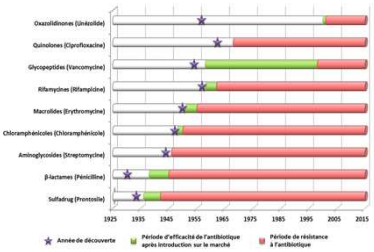
1. *Commissariat à l'Energie Atomique, DSV / IBiTec-S / SPI, Laboratoire d'Etude du Métabolisme du Médicament, 91191 Gif-sur-Yvette, France;*
2. *bioMérieux S.A, Innovation, Chemin de l'Orme, 69280 Marcy l'Etoile, France*

Correspondence: florence.castelli@cea.fr; francois.fenaille@cea.fr

Introduction

- *Staphylococcus aureus* is a major human pathogen and represents an ever growing medical challenge.
- Antibiotic resistance often appears shortly after the introduction of a new molecule as a direct consequence of its intensive use, we are running out of treatment options for many infections.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a major cause of hospital- and community-acquired bacterial infections.
- Understanding of resistance mechanisms is required to develop new effective therapies against pathogens such as *S. aureus*.

Antibiotic and resistance



Overview

1/ A few metabolic pathways have been described to be modified in an antibiotic resistance context but this aspect is still poorly documented.

2/ Broadening metabolome coverage could permit to identify new metabolic pathways involved in antibiotic-resistance. We have already implemented a robust and reliable LC-HRMS method to study up to 210 distinct metabolites in clinical MSSA and MRSA strains exposed (or not) to an antibiotic.

3/ Metabolic pathways mainly impacted : Krebs cycle, peptidoglycan biosynthesis, amino acids and nucleotides metabolism.

Metabolomic study

I- An optimized method to detect a maximum of bacterial metabolites

II- *Staphylococcus aureus* metabolome detection and annotation

S. aureus clinical strains

MSSA (n=13)

MRSA (n=11)

Efficient and bias-free metabolite extraction

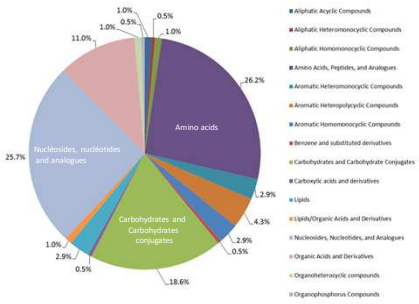
Thermo scientific Exactive mass spectrometer

Metabolomic analysis MS/MS validation

210

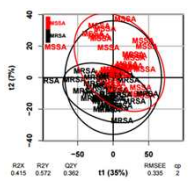
metabolites characterized

(~20% of total detected metabolites)

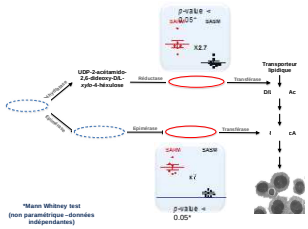


III - MRSA and MSSA metabolome comparison

Without antibiotic pressure, the bacterial metabolome detected by our LC-MS method discriminates clinical MRSA and MSSA strains



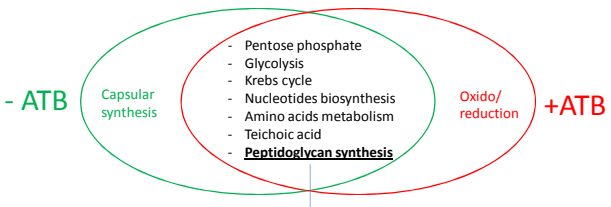
Example



Resistance phenotype affects the capsular polysaccharide biosynthesis (without antibiotic exposure)

+ Antibiotic (cefoxitin)

Metabolic pathways implicated with and w/o antibiotic :



Ex: peptidoglycan biosynthesis

- **UMP** — - ATB
- N-Acetylmuramic 6-P
- UDP-MurNAc-Ala-Glu-Lys-Ala-Ala — +/- ATB
- N-Acetyl-galactosamine/glucosamine
- **UDP-N-acetylmuramoyl-Ala**
- **D-Ala-D-Ala/L-Ala-L-Ala** — +ATB
- **N-Acetylneuraminic acid**

Conclusion

- ✓ Development of **a specific optimized sample preparation** to obtain a reliable snapshot of bacteria metabolism.
- ✓ **Up to 210 intracellular metabolites** from *S. aureus* characterized.
- ✓ Metabolomics rather efficiently **discriminates MRSA from MSSA** strains under specific conditions (*i.e.* given growth stage and rate).
- ✓ Metabolic profiling allows to distinguish MSSA and MRSA strains without antibiotic exposure (especially capsular biosynthesis).
- ✓ Addition of cefoxitin reinforces the differences observed without antibiotic treatment.

Perspectives

- → Continue to interpret raw data to expand metabolite coverage and the number of covered metabolic pathways.
- → Applying this protocol to other bacterial species (ex: gram- *E.coli*)
- → Applying this protocol to other microbiological questions (ex: efflux pumps)