Molecular mechanisms of resistance to daptomycin and tigecycline in *Enterococcus faecium*

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Introduction

Daptomycin and tigecycline are active antibiotics against gram-positive bacteria such as methicillin resistant Staphylococci (MRSA) and vancomycin-resistant Enterococci (VRE). The mechanisms of resistance to these molecules are not yet totally known especially for *E. faecium*, an important opportunistic bacteria responsible for serious nosocomial infections. The aim of the study was to investigate the molecular mechanisms of resistance towards these two antibiotics in *E. faecium* by sequencing the genomes of resistant strains obtained *in vitro*.

Methods

Mutants strains of *E. faecium* resistant to daptomycin or tigecycline were obtained *in vitro* (MIC of 128µg/mL and 1µg/mL, respectively) by increasing antibiotics concentration in plates as shown in Fig. 1. Their corresponding genomes were sequenced and the data were used for a comparative genomics study. Observed mutations have been then verified by re-sequencing of the region amplified by PCR. In addition, RT-qPCR experiments were performed in order to verify the transcription level of genes surrounding mutations identified into intergenic regions.

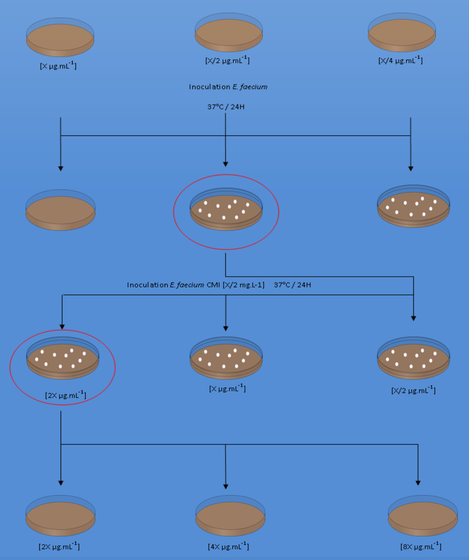

Fig. 1: Method used for obtaining antibiotic-resistant strains

E. faecium Strains

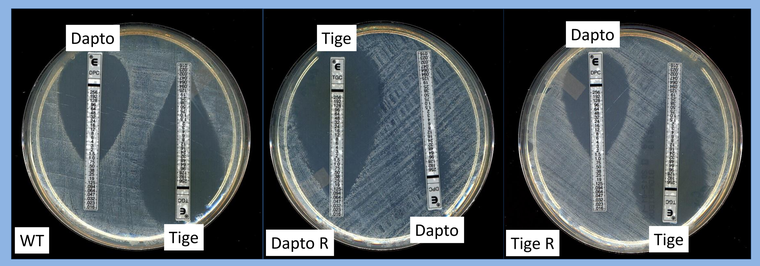

Fig. 2: Measurment of antibiotic sensitivity by E-test of *E. faecium* HM 1070 wild-type (Left), HM 1070 daptomycin MIC of 128µg/mL (Middle) and HM 1070 tigecycline MIC 1µg/mL

Table 1: Mutations indentified for *E. faecium* daptomycin resistant (MIC of 128µg/mL)

Mutation protein	Protein	AUS 0004 gene
R --> G	cation efflux family protein	GENE ID: 11956632 EFAU004_00980
EC*		
L --> I	xanthine/uracile/vitaminC permease	GENE ID: 11956968 EFAU004_01296 |
L --> F		
G --> G	Rh-like protein ammonium transporter	GENE ID: 11954753 EFAU004_00856
EC*	ORF 3--> Yit T family protein	GENE ID: 11956042 EFAU004_02649
EC*	ORF 1 <-- conserved hypothetical protein	GENE ID: 11955866 EFAU004_02479
W --> R	Metal dependent phosphohydrolase, HD subdomain	GENE ID: 11955313 EFAU004_01938
EC*	ORF 1 <-- acetyl transferase, gnat family	GENE ID: 11956042 EFAU004_02649
EC*	ORF 1 <-- transcriptional regulator	GENE ID: 11954475 EFAU004_00581
R --> R	HD domain protein	GENE ID: 11955369 EFAU004_01994
EC*	ORF 19 --> dephospho-coA kinase, putative	GENE ID: 11954921 EFAU004_01548
EC*	ORF 1 <-- peptide methionine sulfoxide reductase	GENE ID: 11954213 EFAU004_00321
V --> E	transcription repair coupling factor	GENE ID: 11955999 EFAU004_02606
L --> W		
EC*	ORF 54 <-- ornithine cyclodeaminase	GENE ID: 11955468 EFAU004_02090
EC*		
EC*	///	GENE ID: 11956894 EFAU004_01239
IGR*	ORF 8 <-- Sua 5/YciO/YrdC/Ywlc family protein	GENE ID: 11955742 EFAU004_02357
IGR*		
EC*	ORF 84 <-- regulator protein	
EC*		

*EC : Extremity of contig
*IGR : Intergenic Region

Table 2: Mutations affecting transport system identified for *E. faecium* tigecycline resistant strain (MIC of 1µg/mL)

Mutation protein	Protein	AUS 0004 Gene
IGR	ORF 17 --> pts system fructose - specific component ORF 22 --> phosphoenol pyruvate-dependent sugar phosphotransferase system EIIA2	GENE ID: 11955327 EFAU004_01952
Y --> H	beta - ketoacyl - acyl carrier protein synthase III	GENE ID: 11956996 EFAU004_01324
E --> K	sodium transporting two - sector ATPase	GENE ID: 11955472 EFAU004_02094
S --> N	prolipoprotein diacylglyceryl transferase	GENE ID: 11955398 EFAU004_02023
A --> T	sugar specific permease	GENE ID: 11954262 EFAU004_00370
T --> C	glucitol/ sorbitol permease IIC component	GENE ID: 11954893 EFAU004_01520
S --> N	hemolysin	GENE ID: 11957009 EFAU004_01337
T --> I	formate/ nitrite transporter**	GENE ID: 11954476 EFAU004_00582

*IGR : Intergenic Region

Results

***E. faecium* resistant to daptomycin or tigecycline have been selected *in vitro* as described in Fig. 1. The genomic sequence of the 3 strains: HM1070, HM1070 resistant to daptomycin (128µg/mL) and HM1070 resistant to tigecycline (1µg/mL) have been obtained (Fig. 2). Some mutations observed were not taken into account when they were present in both mutants or when they did not alter the amino-acid sequence of the protein.**

For the daptomycin resistant strain, 22 mutations were identified in 16 chromosomal regions (Table 1). Moreover, contrarily to published data for *E. faecalis*, the measurement of the thickness of the cell wall of the mutant by electron microscopy showed only a small increase compared with that of the wild type strain. Furthermore, analysis of genes known to be involved in daptomycin resistance in other Gram-positive bacteria did not reveal any mutation.

Surprisingly we identified 111 mutations in 93 chromosomal regions in the sequence of the strain resistant to tigecycline, with the majority located in genes involved in regulatory functions and transport. Because mechanisms of tigecycline resistance identified in other bacterial species involved efflux pumps, genes listed in Table 2 could be interesting candidates to explain the resistance in *E. faecium*.

Finally, analysis of the expression of genes flanking the mutations located in intergenic regions showed no changes.

Perspectives

All these data highlight the existence of new mechanisms of resistance to daptomycin and tigecycline in *E. faecium*. Among these new candidates, further investigations are needed to more precisely identify genes involved in the resistance.

Moreover, we used the completely sequenced strain *E. faecium* AUS0004 to select new resistant strains to daptomycin and tigecycline with MIC of 128 µg/mL and 0.3 µg/mL, respectively (Fig. 3). Genome sequencing is in progress and the comparison of the mutations that will be observed with the presented data will probably provide us pertinent candidates likely implicated in the resistance towards the two antibiotics.

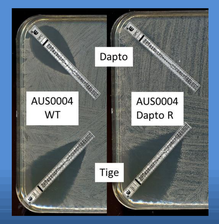

Fig. 3: Measurment of antibiotic sensitivity by E-test of *E. faecium* AUS0004 wild-type (Left), AUS0004 daptomycin resistant MIC of 128µg/mL (Right)