Resistance Mechanisms of Tigecycline in *Enterococcus faecalis*

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Background

Enterococcus faecalis have been regarded as one of the leading causes of the nosocomial infections worldwide. Tigecycline (TGC) is considered as a choice of last resort for the treatment of infections caused by multidrug-resistant *E. faecalis*, however, the emergence of TGC non-susceptibility has posted the therapeutic challenge. Non-susceptibility in clinical strains could be due to resistance (MIC>0.5 mg/l) or heteroresistance. Therefore, this study aimed to understand the underlying molecular mechanisms of TGC resistance and heteroresistance in *E. faecalis*.

Methods

In vitro induction experiments were carried out under TGC pressure with two TGC- sensitive *E. faecalis* strains. Heteroresistance was evaluated by population analysis profiling (PAP) in 270 clinical TGC- sensitive *E. faecalis* strains. TGC susceptibility was determined by the agar dilution method. Resistance and heteroresistance mechanisms were investigated by identifying genetic mutations in tetracycline (Tet) target sites and susceptibility testing in the presence of the efflux protein inhibitors phenylalanine-arginine-β-naphthylamide (PaβN) and carbonyl cyanide m chlorophenylhydrazine (CCCP). Comparison of single nucleotide polymorphism in the whole genome between the parental isolate and two TGC-resistant strains were investigated by next-generation sequencing.

Results

No mutations in Tet target sites in seven TGC heteroresistant strains were present, whereas the mutations in Tet target sites of seven TGC-resistant *E. faecalis* were frequently found (Table 1). TGC MICs in heteroresistant strains were reduced by CCCP (Table 2). Whole genome sequencing revealed the same non-synonymous mutations and transcoding deletions in the exons of several genes encoding for various enzymes or transfer systems (Table 3).

Table 1. The characteristics of the antimicrobial susceptibility, resistance mechanism of TGC-induced resistant isolates

NO.	MIC（mg/L）	Mutation in the individual copies of 16S rRNA				30S ribsome protein	30S ribsome protein
		RR1	RR2	RR3	RR4	S30	S10
Deng	0.03125	W	W	W	W	W	W
Deng-T4-3	2	A927G A931T	W	W	W	W	Ala54Glu
Deng-T10-3	2	W	W	W	G771C	Arg155Ser	Ala54Glu, His56Asn
Deng-T60-3	16	C945T	W	W	W	Arg155Ser	Ala54Glu, His56Asn
F4	0.0625	W	W	W	W	W	W
F4-T2-1	8	W	W	W	W	W	Del52IRATH56
F4-T4-1	16	W	W	W	W	W	Del52IRATH56
F4-T10-1	16	W	W	G9741T G771C	W	W	Del52IRATH56
F4-T30-1	32	W	W	W	W	Arg155Cys	Del52IRATH56

Table 2. Characteristics of clinical heteroresistant mother *E. faecalis* strains and heteroresistance-derived *E. faecalis* clones.

NO.	TGC MIC (mg/L)	NO.	TGC MIC (mg/L)	TGC+CCCP	TGC+PABN
EF16C186	0.0625	Tig0.5-EF16C186-1	2	<=0.06	2
		Tig0.5-EF16C186-2	2	<=0.06	2
NEFA53	0.125	Tig0.5-NEFA53-1	1	<=0.06	2
		Tig0.5-NEFA53-2	1	<=0.06	2
NEFA5	0.25	Tig0.5-NEFA5-1	2	<=0.06	2
		Tig0.5-NEFA5-2	2	<=0.06	2
NEFA37	0.25	Tig0.5-NEFA37-1	2	<=0.06	2
		Tig0.5-NEFA37-2	2	<=0.06	2
NEFA26	0.5	Tig0.5-NEFA26-1	2	<=0.06	2
		Tig0.5-NEFA26-2	2	<=0.06	2
NEFA27	0.5	Tig0.5-NEFA27-1	2	<=0.06	1
		Tig0.5-NEFA27-2	2	<=0.06	1
NEFA32	0.5	Tig0.5-NEFA32-1	2	<=0.06	2
		Tig0.5-NEFA32-2	2	<=0.06	2

Table 3. Mutation-related genes, amino acids and proteins

Name of proteins	Locus name Deng	Gene	Amino acid	
			ZHOU-T10-3	ZHOU-T60-1
Non-synonymous SNP				
Hypothetical protein	Deng_159		A54E H56N	A54E H56N
Amidase	Deng_487		E926D D929E	E926D D929E
Ferrichrome ABC transporter, ATP-binding protein	Deng_166		R155S	R155S
Glycosyl transferase, group 2 family protein	Deng_2227			E113G
Synonymous SNP				
Excinuclease ABC, subunit A	Deng_816	uvrA	G261G	G261G
Menaquinone-specific isochorismate synthase, putative	Deng_433	menF	G32G	G32G
Tunicamycin resistance protein	Deng_1190		A446A, T438T, L437L, P428P, Y422Y,E421E, G32G, G419G, N399N, S355N	A446A, T438T, L437L, P428P, Y422Y,E421E, G32G, G419G, A400A, N399N
Gene deletion				
Glutamyl-tRNA synthase	Deng_44	gltX1	267_268del	267_268del

Conclusion

Our data indicated that the main mechanism of TGC heteroresistance in *E. faecalis* might be associated with the efflux pumps. TGC resistance in *E. faecalis* was associated with mutations in the 16SrRNA site or 30S ribosome protein S10. The genetic mutations in several enzymes and transfer systems might also participate in the resistance development to TGC in *E. faecalis*.