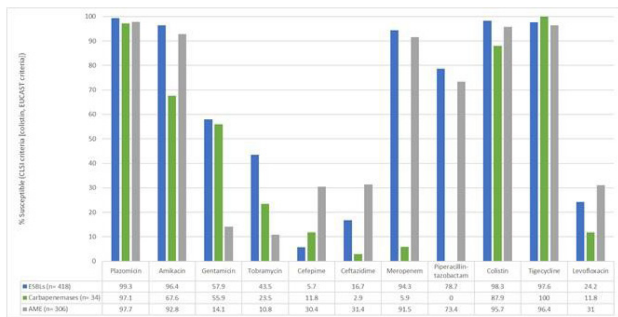
Figure 1



Disclosures. Mariana Castanheira, PhD, 1928 Diagnostics (Research Grant or Support)A. Menarini Industrie Farmaceutiche Riunite S.R.L. (Research Grant or Support)Allergan (Research Grant or Support)Allergan (Research Grant or Support)Amplyx Pharmaceuticals (Research Grant or Support)Cidara Therapeutics (Research Grant or Support)Cidara Therapeutics (Research Grant or Support)Cipla Ltd. (Research Grant or Support)Cipla Ltd. (Research Grant or Support)Fox Chase Chemical Diversity Center (Research Grant or Support)GlaxoSmithKline (Research Grant or Support)Melinta Therapeutics, Inc. (Research Grant or Support)Melinta Therapeutics, Inc. (Research Grant or Support)Melinta Therapeutics, Inc. (Research Grant or Support)Merck (Research Grant or Support)Merck (Research Grant or Support)Merck & Co, Inc. (Research Grant or Support)Merck & Co, Inc. (Research Grant or Support)Paratek Pharma, LLC (Research Grant or Support)Pfizer (Research Grant or Support)Qpex Biopharma (Research Grant or Support) Rodrigo E. Mendes, PhD, A. Menarini Industrie Farmaceutiche Riunite S.R.L. (Research Grant or Support) Allergan (Research Grant or Support)Allergan (Research Grant or Support)Basilea Pharmaceutica International, Ltd (Research Grant or Support)Cipla Ltd. (Research Grant or Support)Department of Health and Human Services (Research Grant or Support)GlaxoSmithKline (Research Grant or Support)Melinta Therapeutics, Inc. (Research Grant or Support)Merck (Research Grant or Support)Merck (Research Grant or Support)Pfizer (Research Grant or Support) Timothy B. Doyle, Allergan (Research Grant or Support)Allergan (Research Grant or Support)Cipla Ltd. (Research Grant or Support)Melinta Therapeutics, Inc. (Research Grant or Support)Pfizer (Research Grant or Support)Qpex Biopharma (Research Grant or Support) Valerie Kantro, n/a, Cipla Ltd. (Research Grant or Support) Helio S. Sader, MD, PhD, A. Menarini Industrie Farmaceutiche Riunite S.R.L. (Research Grant or Support)Allergan (Research Grant or Support)Allergan (Research Grant or Support)Allergan (Research Grant or Support) Cipla Ltd. (Research Grant or Support)Cipla Ltd. (Research Grant or Support)Melinta (Research Grant or Support)Merck (Research Grant or Support)Merck (Research Grant or Support)Paratek Pharma, LLC (Research Grant or Support)Pfizer (Research Grant or Support) Jaideep Gogtay, n/a, Cipla Ltd. (Employee) Sandhya Das, n/a, Cipla Ltd. (Research Grant or Support)

1455. Potential Mechanisms of Cefiderocol MIC Increase in Enterobacterales in *In Vitro* Resistance Acquisition Studies

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Session: P-66. Resistance Mechanisms

Background. Cefiderocol (CFDC) is a novel siderophore, iron-chelating cephalosporin, which is transported into bacteria via iron transporters. CFDC has potent *in vitro* and *in vivo* activity against all aerobic Gram-negative bacteria, including carbapenem-resistant strains. To date, clinical isolates with cefiderocol MIC >4 μg/mL have been found infrequently, in which the presence of a few β-lactamases or altered iron transport was found. We investigated potential new mechanisms causing CFDC MIC increases in non-clinical studies.

Methods. The mutation positions were determined by whole genome sequencing using four *K. pneumoniae* mutants including two KPC producers and one NDM producer that had shown CFDC MIC increases in previous *in vitro* resistance-acquisition studies. The mutant strains were obtained at the frequency of 10^{-7} to < 10^{-8} by spreading bacteria on standard Mueller−Hinton agar medium containing CFDC at concentrations of 10× MIC, with or without apo-transferrin (20 μg/mL). CFDC MIC was determined by broth microdilution using iron-depleted cation-adjusted Mueller-Hinton broth based on Clinical and Laboratory Standards Institute guidelines. The emergence of MIC increase mutants was also assessed by *in vitro* chemostat models under humanized plasma pharmacokinetic exposures of CFDC.

Results. The possible resistance mechanisms were investigated. Mutation of *baeS* or *envZ*, sensors of two-component regulation systems, were found in three or two mutants among the tested four isolates, respectively, and caused the MIC to increase by 4–32-fold. The altered expression level of specific genes by the *baeS* or *envZ* mutation could affect CFDC susceptibility, but the specific genes have not been identified. In addition, the mutation of *exbD*, an accessory protein related to iron transport, was identified in one case and caused the MIC to increase by >8-fold. *In vitro* chemostat studies using two isolates (one NDM producer and one KPC producer) showed no resistance acquisition during 24-hour exposure.

Table. Overview of mutation emergence in five isolates of K. pneumoniae

Species	Beta-lactamase	CFDC MIC of the original isolate	CFDC MIC of the mutant isolate	Mutation
K. pneumoniae	None	0.063	2	baeS
K. pneumoniae	NDM	4	>32	exbD
			>32	baeS
K. pneumoniae	KPC	4	16	envZ
K. pneumoniae	KPC	4	16	envZ
			16	baeS

KPC: *Klebsiella pneumoniae* carbapenemase; NDM: New Delhi metallo-β-lactamase

Conclusion: The mutation of two-component regulation systems (BaeSR and OmpR/EnvZ) and iron transport-related proteins were shown to be possible mechanisms causing CFDC MIC increases, but these mutants did not appear under human exposures.

Disclosures. Yoshinori Yamano, PhD, Shionogi & Co., Ltd. (Employee) Rio Nakamura, BSc, Shionogi & Co., Ltd. (Employee) Miki Takemura, MSc, Shionogi & Co., Ltd. (Employee) Roger Echols, MD, Shionogi Inc. (Consultant)

1456. Resistance Mechanisms of Tigecycline in *Enterococcus faecalis*

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Session: P-66. Resistance Mechanisms

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

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 S30	30S ribsome protein S10
		RR1	RR2	RR3	RR4		
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