In vivo selection of multidrug resistance in Enterobacter cloacae complex by a unique romR deletion

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In *vivo* selection of multidrug resistance in *Enterobacter cloacae* complex by a unique *romR* deletion

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Background

Members of the *Enterobacter cloacae* complex (ECC) are major opportunistic pathogens, especially among intensive care unit (ICU) patients.

The aim of this study was to decipher the phenotypic and genomic evolution of an ECC clone that acquired a multidrug resistance phenotype in an ICU patient.

Material and methods

- ✓ During a four-month period, 8 multi-drug resistant (MDR) ECC strains were recovered from the same patient: 5 from clinical samples and 3 from rectal swabs.
- ✓ The patient benefited from an escalation of antibiotic treatment (daptomycin, meropenem, fluconazole, erythromycin, amikacin, linezolid, and caspofungin) until he died.
- ✓ MIC values of 15 antibiotics (piperacillin-tazobactam (PTZ), temocillin (TEM), ceftazidime (CAZ), cefotaxime (CTX), cefepime (FEP), aztreonam (AZT), ertapenem (ERT), meropenem (MER), imipenem (IPM), amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), chloramphenicol (CHL) and tigecycline (TIG) were determined by the broth microdilution method.
- ✓ Whole-genome sequencing was performed for the 8 strains and a Single-nucleotide polymorphism (SNP) analysis was performed after alignment against the ECC reference genome (ATCC 13047).
- ✓ Mutagenesis : Each mutant was created by using the method described by Datsenko et al. (1) and adapted by Derbise et al. (2).

Results

- ✓ In strains isolated from the 3rd month, a significant increase in MICs (at least 4 fold change) was observed for TEM, CAZ, FEP, ERT, CHL and TIG.
- ✓ The 8 MDR ECC belonged to the same cluster 6 (C-VI) and the same ST (ST66) and were genetically related.
- ✓ All assembled genomes harbored the same panel of acquired antibiotic resistance genes : $bla_{\text{CTX-M-15}}$, bla_{TEM-1B}, bla_{OXA-1}, *strA, strB, aac(6')-Ib-cr, aac(3)-IIa, qnrB1, catB3 and dfrA14* according to center for genomic epidemiology and Comprehensive Antibiotic Resistance Database databases.
- ✓ No genetic evolution was found in chromosomal genes usually involved in β-lactam resistance (i.e. *ampD*, *ampR, nagZ, dacA, dacB, acrAB-tolC, ompK35, ompK36*). Nevertheless, the following strains FY6, FY8, FY9 and FY10 harbored a mutation in *gyrB* (T2205C), resulting to an amino acid substitution S626P that could play a role in increase of fluoroquinolone MICs.

Table I: Demographic data , antibiotic susceptibility and *romR* characteristics of the eight *Enterobacter cloacae* strains

	Demography		MICs (mg/l)							Genomic Information		
	Source	Isolation date	TEM	ETP	MER	GEN	CIP	CHL	TIG	Cluster	ST	*romR*
FY-1	Stools	05/02/2018	4	0.25	0.125	32	2	8	0.5	C6	ST66	256_277_ins
FY-2	Blood culture	13/02/2018	8	0.25	0.125	32	2	4	0.25	C6	ST66	256_277_ins
FY-3	Urines	13/02/2018	4	0.25	0.125	32	2	4	0.25	C6	ST66	256_277_ins
FY-6	bronchoalveolar fluid	30/03/2018	16	0.5	0.125	32	32	32	2	C6	ST66	256_277_ins, 495_507_del
FY-7	Stools	15/04/2018	16	2	0.25	256	32	64	0.5	C6	ST66	256_277_ins
FY-8	Peritoneal liquid	26/04/2018	16	0.5	0.125	64	32	32	2	C6	ST66	256_277_ins, 495_507_del
FY-9	Stools	07/05/2018	32	8	4	>256	32	32	2	C6	ST66	256_277_ins, 495_507_del
FY-10	Blood culture	20/05/2018	16	0.5	0.125	64	32	32	2	C6	ST66	256_277_ins, 495_507_del

Notes: Highlighted in yellow and green, MICs modifications that are related to *romR* mutations and to unknown mechanism(s) respectively.

- ✓ The isolates differed by 5 SNPs located in metabolism genes and a 13-bp deletion in *romR*, which coded for TetR-type transcriptonal regulator (3). This latter is known to play a repressor role on the expression of porins such as OmpK35, whose the downregulation might be involved in TIG, CHL TEM and CIP MIC increases observed here (**Table 1**).
- ✓ For 2 strains (Fy-7 & Fy-9), other antibiotic resistance mechanisms not detected by NGS are probably involved.

Table 2: MICs (mg/L) of antibiotics for ECL13047 (wild type) and mutants Δ*romR,* Δ*romA* and Δ*romR-A.*

Strains	MICs (mg/l)						
	TEM	ETP	MER	GEN	CIP	CHL	TIG
E. cloacae ATCC13047	4	0.25	0.125	0.5	0.016	4	0.25
13047Δ03155 (*romR*)	8	0.5	0.125	0.5	0.06	16	2
13047Δ03154 (*romA*)	4	0.25	0.125	0.5	0.016	4	0.125
13047Δ03154/55 (*romR-A*)	2	0.25	0.125	0.5	0.016	4	0.125

- ✓ We experimentally confirmed that the *romR*-deleted mutant was significantly more resistant to TEM, TIG, CHL and CIP (**Table 2**).

Conclusions

- ➢ **Consequent and long-term antibiotic treatment in one patient indicated the selection of a unique MDR ECC6 population where antibiotic resistance evolved more by chromosomal modifications than acquired antibiotic resistance genes.**
- ➢ **The analysis of the different genetic features through all the eight MDR ECC6 strains allowed us to highlight a candidate gene that could be implicated in TEM, CIP, CHL and TIG resistance.**
- ➢ **Further phenotypic and molecular characterization by transcriptomic are in progress in order to explain resistance to other antibiotic classes.**

References:

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