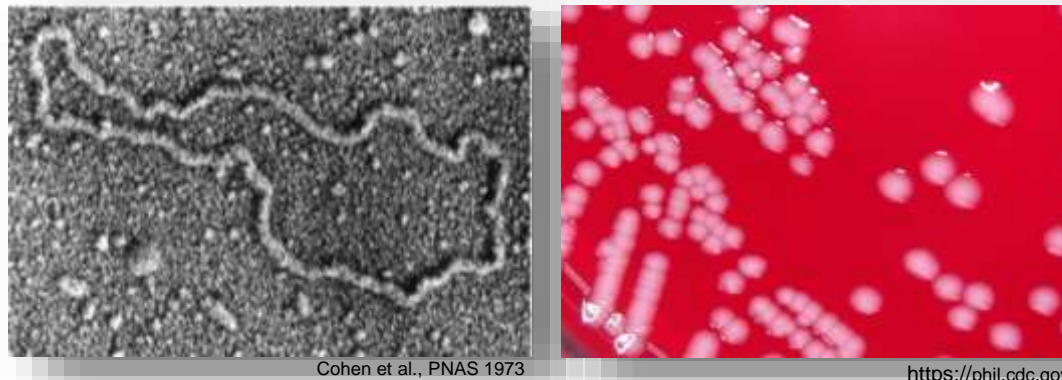


Diversity of OXA-235-like encoding plasmids in *Acinetobacter baumannii* clinical isolates



Kyriaki Xanthopoulou

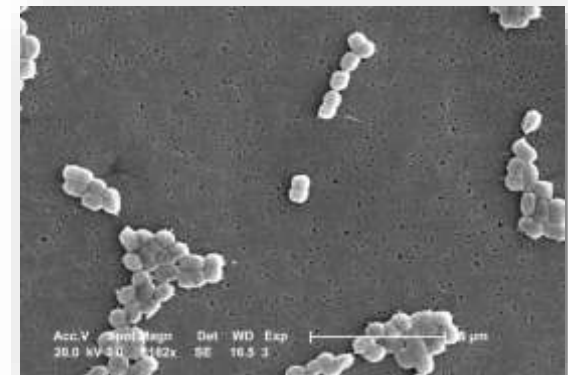
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Acinetobacter baumannii

- opportunistic nosocomial pathogen
- causing epidemic outbreaks mainly in the ICU-settings
- ability to survive on dry surfaces for extended time periods
- multidrug-resistance (MDR)
extensive drug-resistance (XDR)
pan drug-resistance (PDR)
- increasing incidence of carbapenem resistance



<https://phil.cdc.gov>



Carbapenem resistance in *A. baumannii*

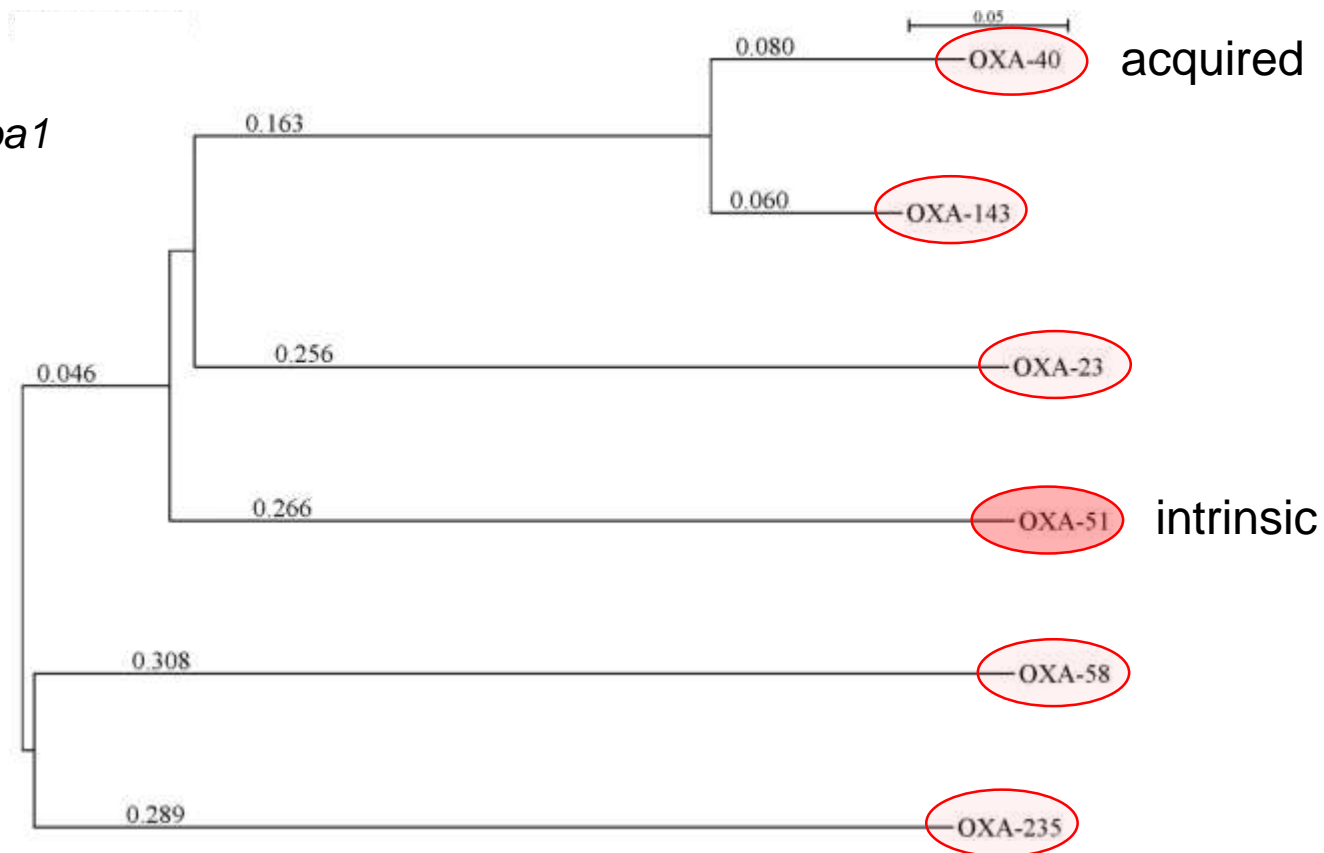
- porin loss or modification
- modification of penicillin-binding proteins
- nearly always associated with β -lactamase production
- **Carbapenem-hydrolyzing class D β -lactamases (CHDLs)**
 - oxacillinases (OXA)
 - serine β -lactamases
 - weak carbapenemases



OXA carbapenemases

- chromosomally encoded
- plasmid-encoded

- OXA associated with *ISAba1* leading to resistance
 - OXA-51
 - OXA-23
 - OXA-235





OXA-235, a Novel Class D β -Lactamase Involved in Resistance to Carbapenems in *Acinetobacter baumannii*

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We investigated the mechanism of carbapenem resistance in 10 *Acinetobacter baumannii* strains isolated from the United States and Mexico between 2005 and 2009. The detection of known metallo- β -lactamase or carbapenem-hydrolyzing oxacillinase (OXA) genes by PCR was negative. The presence of plasmid-encoded carbapenem resistance genes was investigated by transformation of *A. baumannii* ATCC 17978. Shotgun cloning experiments and sequencing were performed, followed by the expression of a novel β -lactamase in *A. baumannii*. Three novel OXA enzymes were identified, OXA-235 in 8 isolates and the amino acid variants OXA-236 (Glu173-Val) and OXA-237 (Asp208-Gly) in 1 isolate each. The deduced amino acid sequences shared 85% identity with OXA-134, 54% to 57% identities with the acquired OXA-23, OXA-24, OXA-58, and OXA-143, and 56% identity with the intrinsic OXA-51 and, thus, represent a novel subclass of OXA. The expression of OXA-235 in *A. baumannii* led to reduced carbapenem susceptibility, while cephalosporin MICs were unaffected. Genetic analysis revealed that *bla*_{OXA-235}, *bla*_{OXA-236}, and *bla*_{OXA-237} were bracketed between two IS*Aba1* insertion sequences. In addition, the presence of these acquired β -lactamase genes might result from a transposition-mediated mechanism. This highlights the propensity of *A. baumannii* to acquire multiple carbapenem resistance determinants.

- so far only reported four times in unrelated isolates



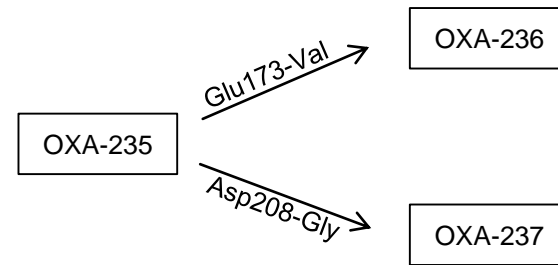
OXA-235-like

- three OXA-235-like variants

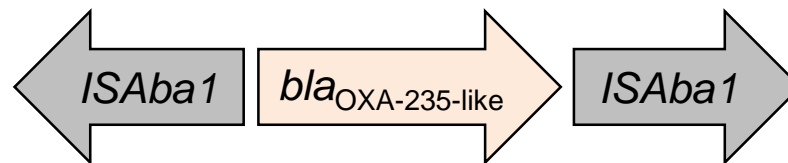
*bla*_{OXA-235}

*bla*_{OXA-236}

*bla*_{OXA-237}



- bracketed by two IS*Aba1* insertion sequences





OXA-235-like

- *bla*_{OXA-235-like} is chromosomally and plasmid-encoded

TABLE 1 Acquired OXA and strain information

Strain	Date of isolation (mo-yr)	Type of specimen	Location where isolate was recovered	Acquired OXA	Bacterial location of acquired <i>bla</i> _{OXA} gene	OXA-51-like variant	International clone ^a
BMBF 255	06-2005	Wound	USA ^c	236	Chromosome and plasmid	241	Unc
AF 684	03-2007	TS ^b	California	237	Plasmid	66	2
AF 670	06-2007	Blood	Arizona	235	Chromosome and plasmid	242	Unc
AF 401	07-2007	Small intestine	Mexico ^d	235	Chromosome and plasmid	65	5
AF 667	10-2007	Wound	Arizona	235	Chromosome and plasmid	242	Unc
AF 785	10-2007	TS	Nevada	235	Chromosome	66	2
AF 678	12-2007	Sputum	California	235	Chromosome	66	2
AF 707	01-2008	Wound	Illinois	235	Chromosome	66	2
AF 673	03-2008	Sputum	Arizona	235	Chromosome	66	2
AF 875	11-2009	Sputum	Utah	235	Chromosome	66	2

^a As determined by rep-PCR and *bla*_{OXA-51} typing. Unc, unclustered.

^b TS, tracheal secretion.

^c State not known.

^d Isolate recovered from unknown location in Mexico.



OXA-235-like

Aim of this study

Characterization of OXA-235-like encoding plasmids among *A. baumannii* clinical isolates from multiple geographical regions

- whole genome sequencing was performed on MiSeq (Illumina) sequencing by synthesis, paired-end reads, up to 250bp read length
- *de novo* assembly was performed by SPAdes and Velvet
- plasmid scaffolds were analyzed *in silico*
- plasmid assembly was completed by PCR-based gap closure using plasmid DNA

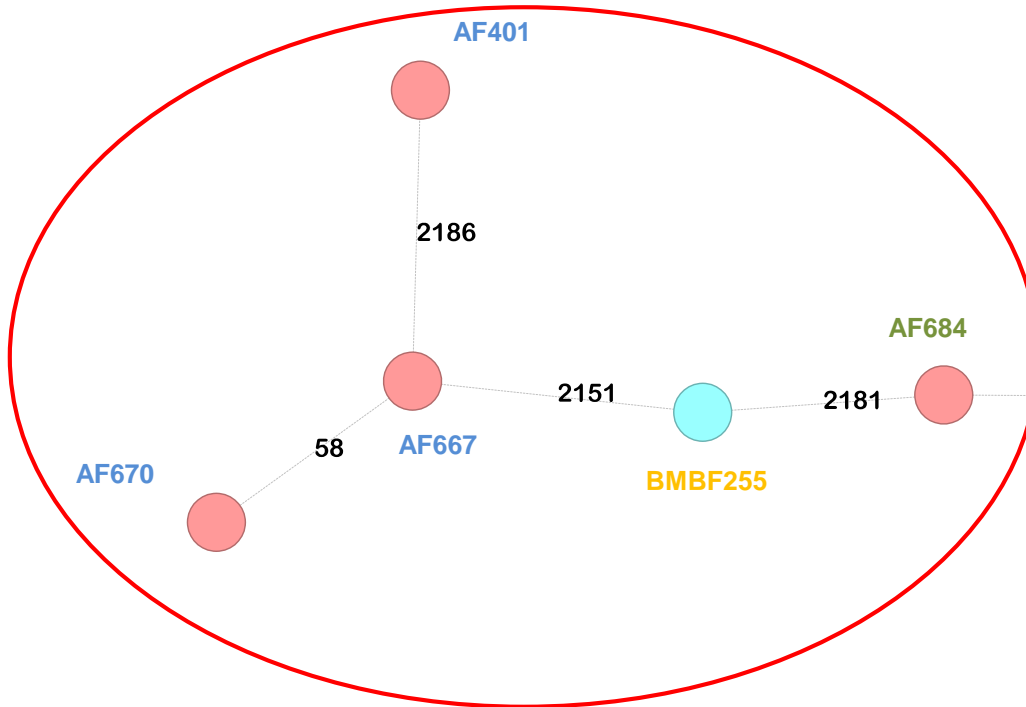


OXA-235-like isolates cgMLST analysis

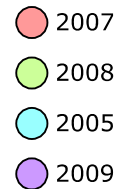
*bla*_{OXA-235}

*bla*_{OXA-236}

*bla*_{OXA-237}



Year of isolation

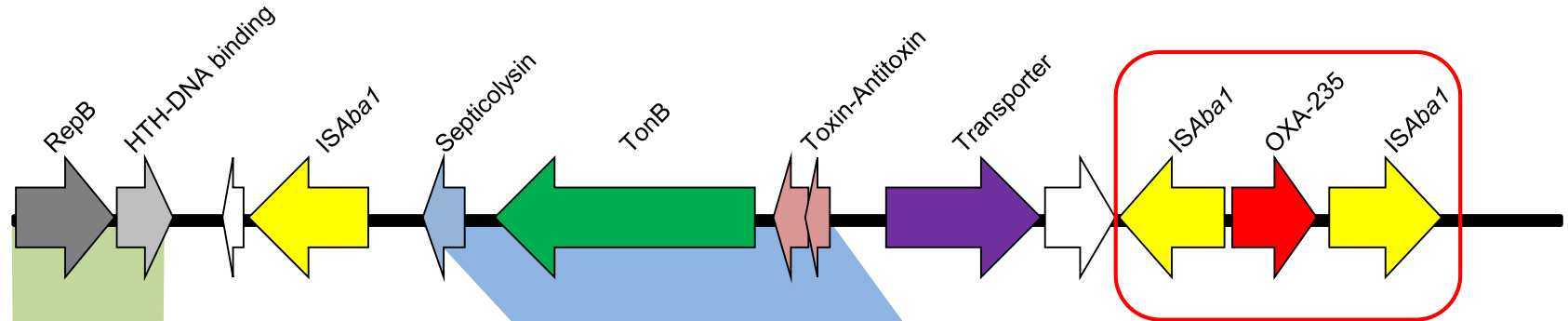




Replicase Group 2

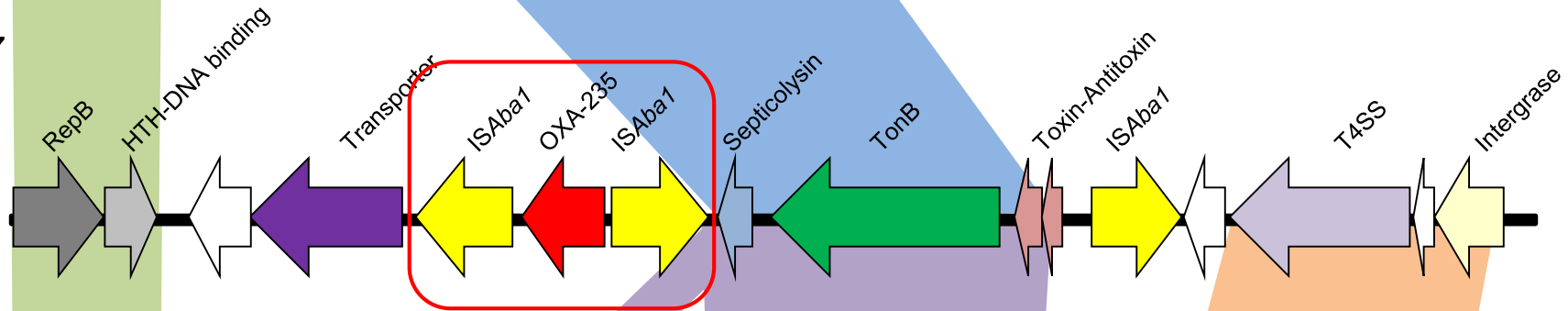
pAF670

14,4 Kb



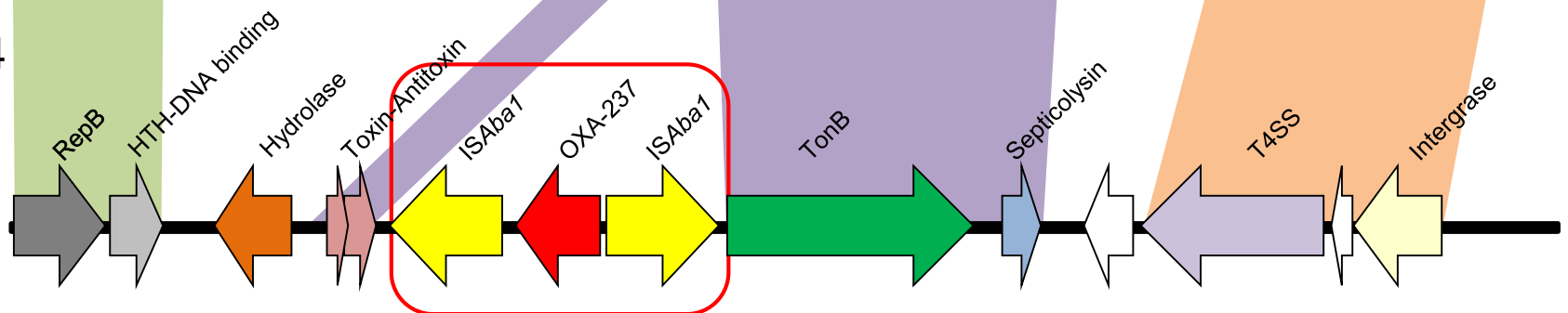
pAF667

18,3 Kb



pAF684

15,1 Kb

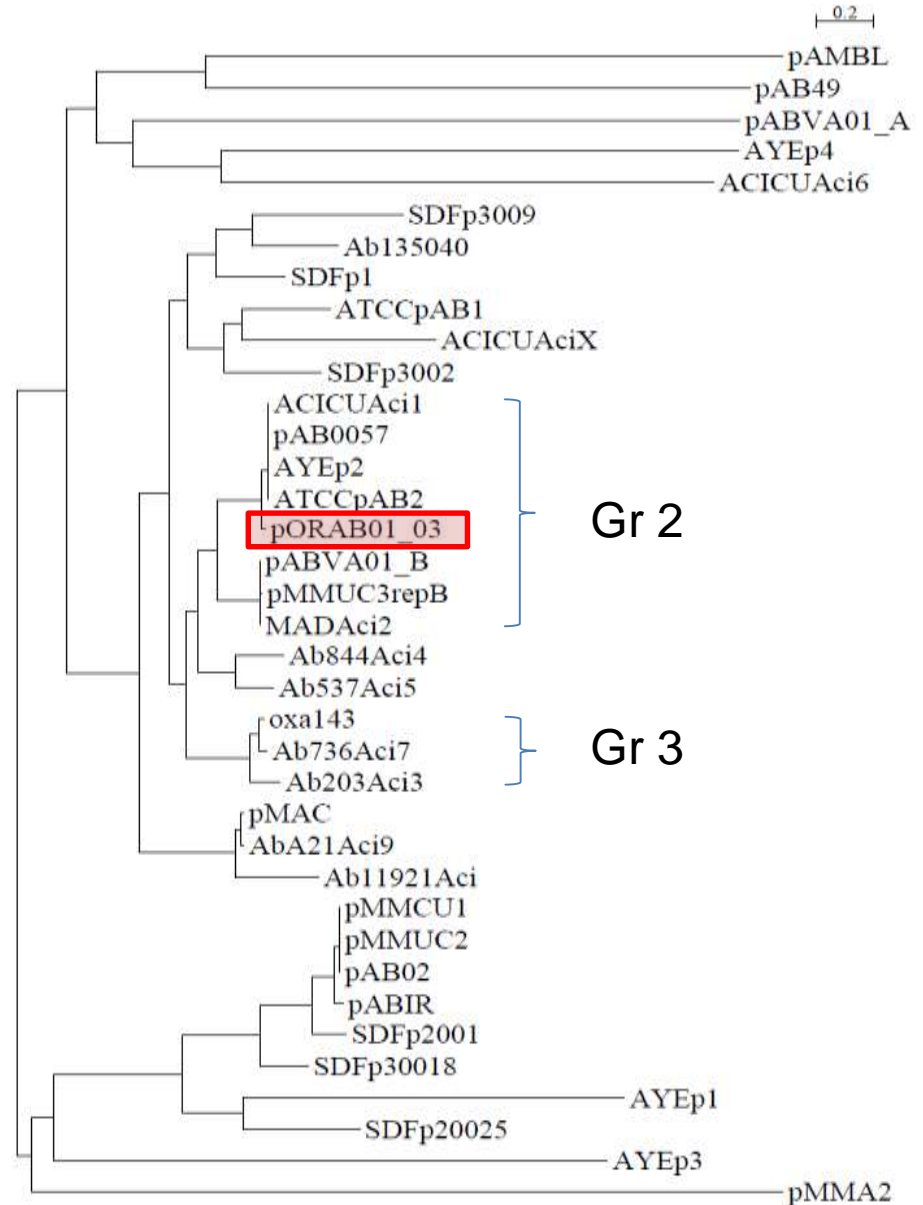




Replicase Group 2

- the same replicase was identified in pORAB01-3 harboring OXA-237 detected in *A. baumannii* isolate from Oregon, USA

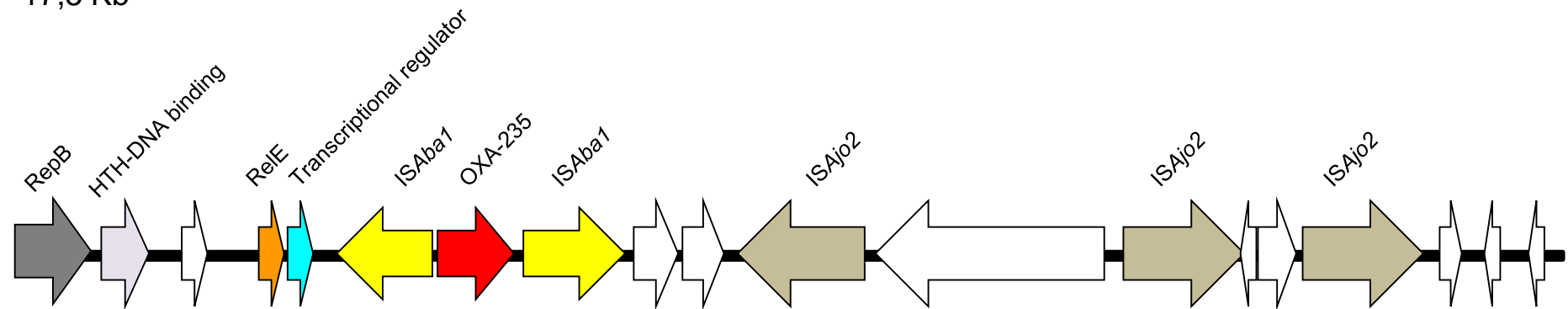
Replicase genes





Replicase Group 3

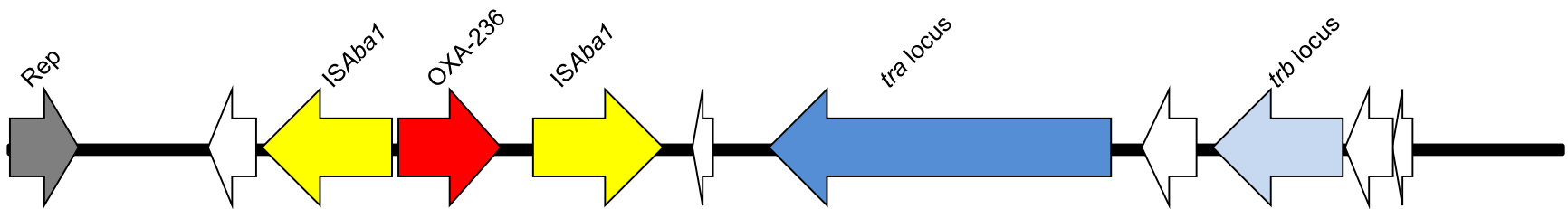
pAF401
17,5 Kb



- a completely different plasmid scaffold including a replicase gene of GR3 type
- three copies of an insertion sequence from *Acinetobacter johnsonii*

New Replicase Group

pBMBF255
13 Kb

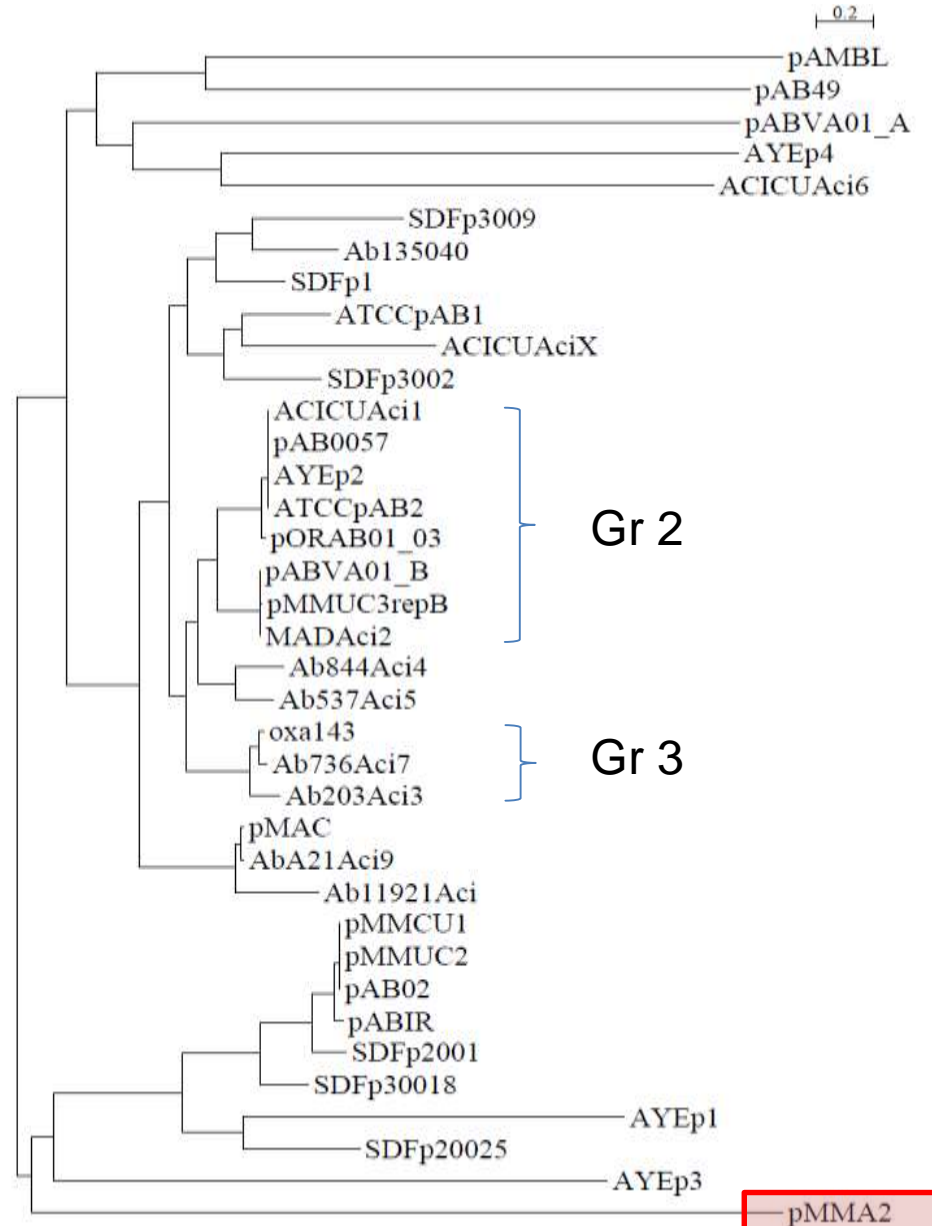


- Rep was identified in *bla*_{OXA-24}-carrying pMMA2 plasmid in an *A. baumannii* isolate from Spain
- Rep has also been detected in *A. baumannii* isolates recovered from Mexico and Italy
- conjugative transfer loci *tra* and *trb*



Novel Replicase Group

Replicase genes





Conclusions

- OXA-235-like was found on three different plasmid types discernable by the replicase genes
- three plasmids presented a similar genetic environment for OXA-235-like including:
 - replicase gene
 - TonB-dependent receptor
 - toxin-antitoxin system
 - septicolysin
- unrelated *A. baumannii* isolates from different geographical regions harbored similar OXA-235-like encoding plasmids
- these plasmids may support the dissemination of the OXA-235-like carbapenemases



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