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SWITCHING OFF BACTERIAL RESISTANCE

The target evaluation platform revealed an AdeR unrelated tigecycline resistance mechanism in XDR *Acinetobacter baumannii*

Vincent Trebosc[1,2], Sarah Gartenmann[1], Kevin Royet[1], Pablo Manfredi[2], Birgit Schellhorn[1], Michel Pieren[1], Sergio Lociuro[1], Peter C. Sennhenn[1], Marc Gitzinger[1], Marcel Tigges[1], Dirk Bumann[2], and Christian Kemmer[1]

[1] BioVersys AG, Hochbergerstrasse 60c, CH-4057 Basel, Switzerland
[2] Biozentrum, University of Basel, Klingenbergstrasse 70, CH-4056 Basel, Switzerland

Acinetobacter baumannii – an increasing threat in health care units

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2007

An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*

Lenie Dijkshoorn, Alexandr Nemec‡ and Harald Seifert§*

2011

***Acinetobacter* Infection – an Emerging Threat to Human Health**

Paolo Visca[1], Harald Seifert[2] and Kevin J. Towner[3]
[1]*Department of Biology, University Roma Tre, Rome, Italy*
[2]*Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Germany*
[3]*Department of Clinical Microbiology, Nottingham University Hospitals, Nottingham, UK*

2016

German hospital gripped by outbreak of multiresistant bacteria

A team of experts has arrived in Kiel following an outbreak of a multiresistant pathogen at a university clinic. At this point, 27 patients have been infected with the strain. Eleven have died.

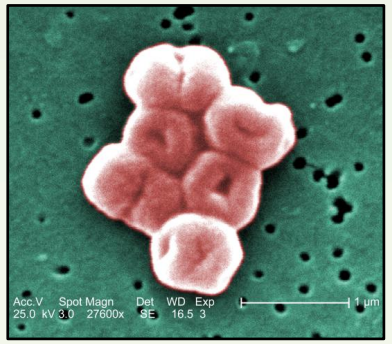

Photo: Janice Carr

- Mainly affects patients in the ICU
- Increasing emergence of A. baumannii outbreaks in hospitals
- MDR/XDR strains are emerging worldwide
- Remaining treatment options: a) polymyxins, b) minocycline, **c) tigeycycline**

AdeRS TCSs controls tigecycline efflux

AdeR as drug target for adjuvants?

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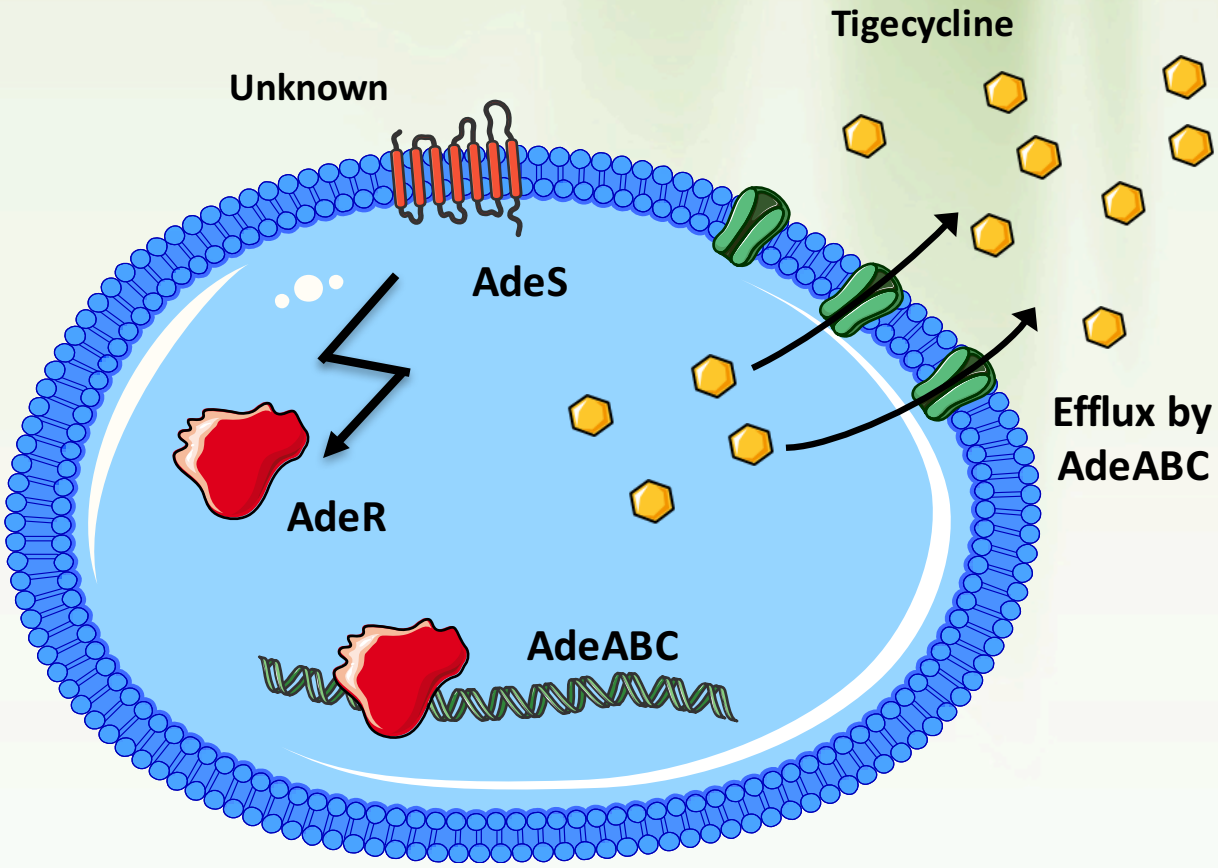

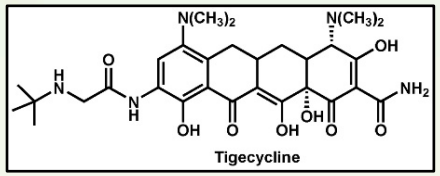

Tigecycline resistance:

1. Tigecycline triggers AdeABC efflux pump overexpression
2. AdeABC effluxes tigecycline to reduce intracellular concentration

AdeR plays a major role in tigecycline resistance but presence of alternative mechanism

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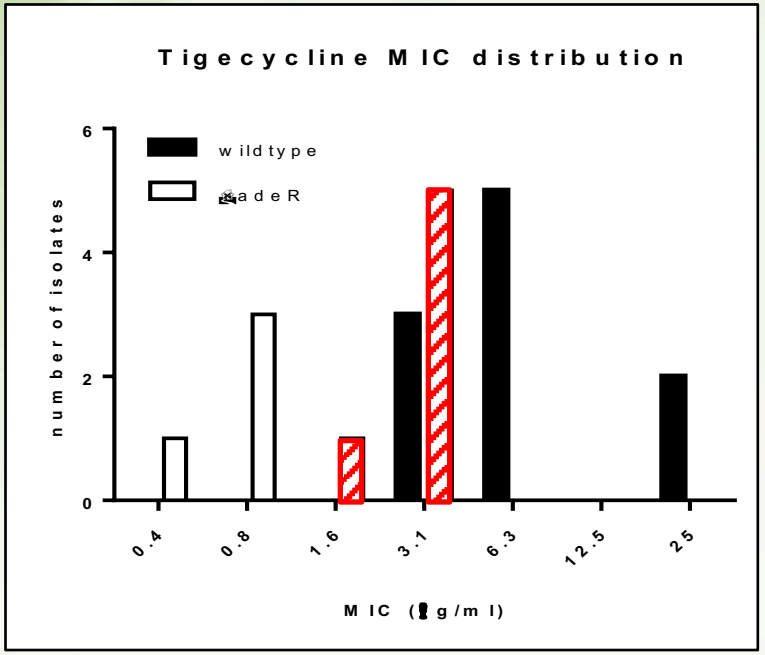
Strain	Tigecycline MIC	
	WT	*ΔadeR*
ATCC-17978	0.4	0.4
BV26	6.3	0.4
BV94	25	3.1
BV173	6.3	0.8
BV175	3.1	0.8
BV185	6.3	3.1
BV186	3.1	3.1
BV187	3.1	3.1
BV189	6.3	3.1
BV190	6.3	0.8
BV191	25	1.6



- ***adeR* deletion reduced MIC_{90} from 25µg/ml to 3.1µg/ml**
- Clinically accepted susceptibility breakpoint: 1ug/mL

→ **60% of *ΔadeR* mutants remained tigecycline non-susceptible**

WT: wildtype
MIC: minimal inhibitory concentration

AdeR plays a major role in tigecycline resistance but presence of alternative mechanism

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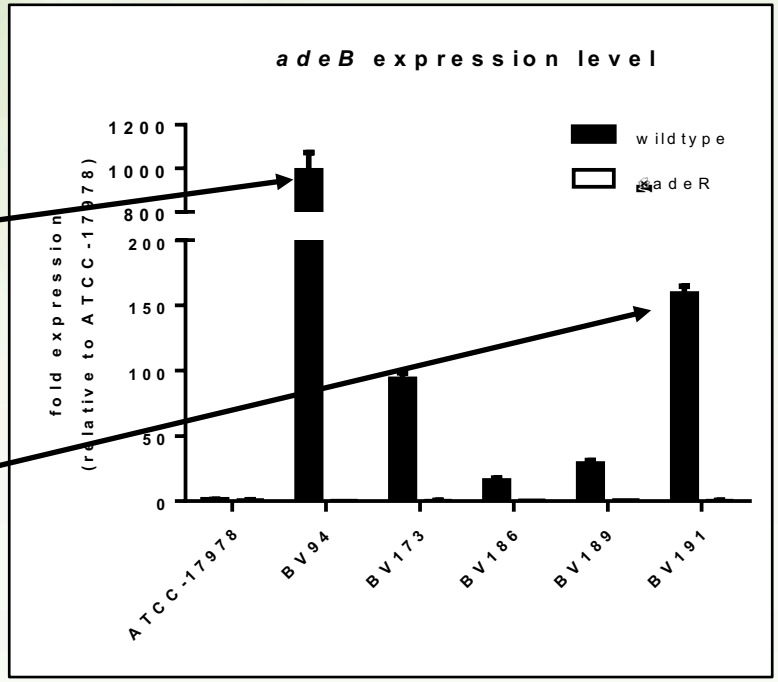
Strain	Tigecycline MIC		AdeRS mutations (amino acid substitutions)
	WT	*ΔadeR*	
ATCC-17978	0.4	0.4	reference
BV94	25	3.1	F170S in AdeS
BV173	6.3	0.8	D21V and D26N in AdeR
BV186	3.1	3.1	No mutation
BV189	6.3	3.1	No mutation
BV191	25	1.6	G79S in AdeS



- **adeR is essential for AdeABC expression**
- Tigecyline resistance is mediated by adeABC drug efflux
- Sequencing of TCS identified no mutation in strains not responding to loss of adeR

→ **Alternative resistance mechanism(s), independent of AdeR**

WT: wildtype
MIC: minimal inhibitory concentration

Artifical resistance development and whole genome sequencing

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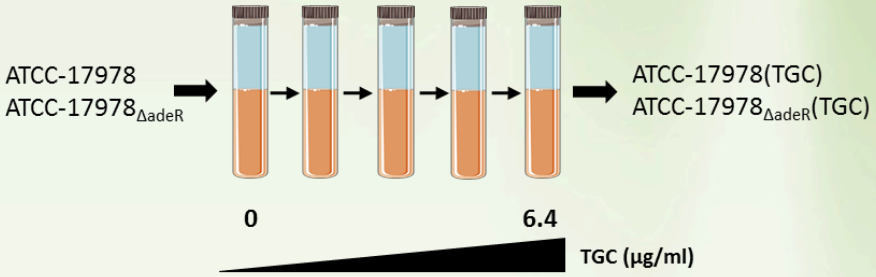

	MIC (µg/ml)	Tigecycline
WT	ATCC-17978	0.4
	ATCC-17978(TGC)	25
ΔadeR	ATCC-17978$_{\Delta adeR}$	0.4
	ATCC-17978$_{\Delta adeR}$(TGC)	6.3

Mutated genes or regions	Mutations		Gene function
	ATCC-17978(TGC)	ATCC-17978$_{\Delta adeR}$(TGC)	
***trm* (A1S_2858)**	**IS-17 like transposon disruption**	**Loss of adenine at position 311 leads to premature stop codon**	**Methyltransferase potentially involved in rRNAs methylation**
***RNase E* (A1S_0403)**	58 bp deletion	-	Ribonuclease involved in rRNAs processing and RNA decay
Intergenic region between *RNase E* (A1S_0403) and *23S rRNA pseudouridylate synthase* (A1S_0404)	ISaba11 element inserted at position 439,336	-	RNase E see above. 23S rRNA pseudouridylate synthase involved in modification of 23S rRNA
***adeN* (A1S_1979)**	-	IS transposon disruption	Transcriptional repressor of the AdeIJK efflux pump
***abeM* (A1S_0395)**	-	C->T mutation (position 429,259) in the promoter region of abeM	MATE family efflux pump

- ***ΔadeR* mutant were able to develop tigecycline resistance**
- *trm* was the only mutated gene in both strains

→ **Is *trm* disruption a general tigecycline resistance mechanism?**

trm disruption is not the only trigger for the alternative resistance mechanism

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Strain	Isolation		Tigecycline MIC		*trm* mutations (nucleotide)
	Location	Date	WT	*ΔadeR*	
ATCC-17978	France	1951	**0.4**	**0.4**	No mutation
BV26	Switzerland	**1979**	**6.3**	**0.4**	**No mutation**
BV94	USA	2011	**25**	**3.1**	G1065
BV173	Greece	2012	**6.3**	**0.8**	A240
BV175	Turkey	2012	**3.1**	**0.8**	A240
BV185	Mexico	2013	**6.3**	**3.1**	A240
BV186	USA	2013	**3.1**	**3.1**	G1065
BV187	USA	2013	**3.1**	**3.1**	G1065
BV189	Spain	2013	**6.3**	**3.1**	A240
BV190	Greece	2012	**6.3**	**0.8**	T1050
BV191	China	2013	**25**	**1.6**	A240

→ **isolated before tigecycline approval**

***trm* disruption cannot be the only driver of resistance**

- Correlation between *trm* disruption and tigecycline exposure
- However, loss of *adeR* in renders 3 strains tigecycline susceptible while *trm* was disrupted

→ ***trm* disruption cannot be the only driver of resistance**

Facts that devalidated AdeR as a drug target

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1. **AdeABC-mediated tigecycline efflux is the major resistance mechanism**
   - loss of AdeR reduces tigecycline MIC_{90} from 25µg/ml to 3.1µg/ml
   - the *adeABC* efflux pump expression is abolished in *ΔadeR* mutants
   - we found a correlation between tigecycline MICs, AdeRS mutations and AdeABC expression level
2. **60% of *ΔadeR* mutants remained resistant to tigecycline**
3. **We successfully developed tigecycline resistance in a *ΔadeR* strain**
4. **We identified a candidate protein involved in an alternative resistance mechanism**
   - whole genome sequencing identified *trm* as the only gene mutated in the two independently evolved tigecycline resistant strains
   - *trm* gene was disrupted in 9 of 10 primarily tested tigecycline resistant clinical isolates
   - loss of *adeR* renders 3 strains tigecycline susceptible while *trm* is disrupted
   - → ***trm* disruption cannot be the only driver for an alternative resistance mechanism**

AdeR inhibition by small molecule drug will not re-sensitize *A.baumannii* to tigecycline

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For further information, please contact Christian.Kemmer@bioversys.com

backup

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Overview Drug Discovery

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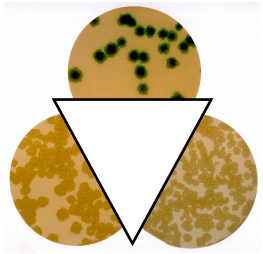
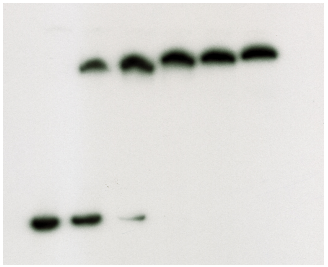
Target Identification and Validation





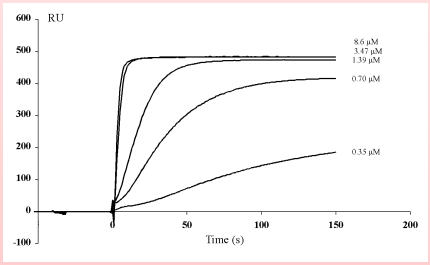
Genetic, cellular and *in vivo* experimental models to identify and validate targets

Compound Screening

High throughput (HTP) primary screenings

Constant, iterative compound synthesis to improve compound properties

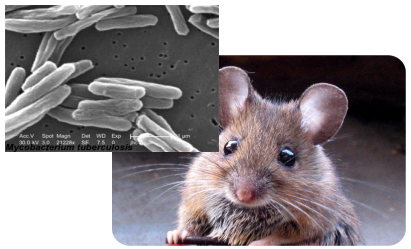
Secondary Assay



In vitro and *ex vivo* secondary assays (mode of action, selectivity)

In vitro assessment of pharmacological properties of the compounds

In vivo



In vivo assessment of pharmacological properties

Infection efficacy model

Early safety and toxicity studies

Adapted from Hughes *et al.* BJP, 2011

Urgent unmet medical need in Gram-negative bacteria

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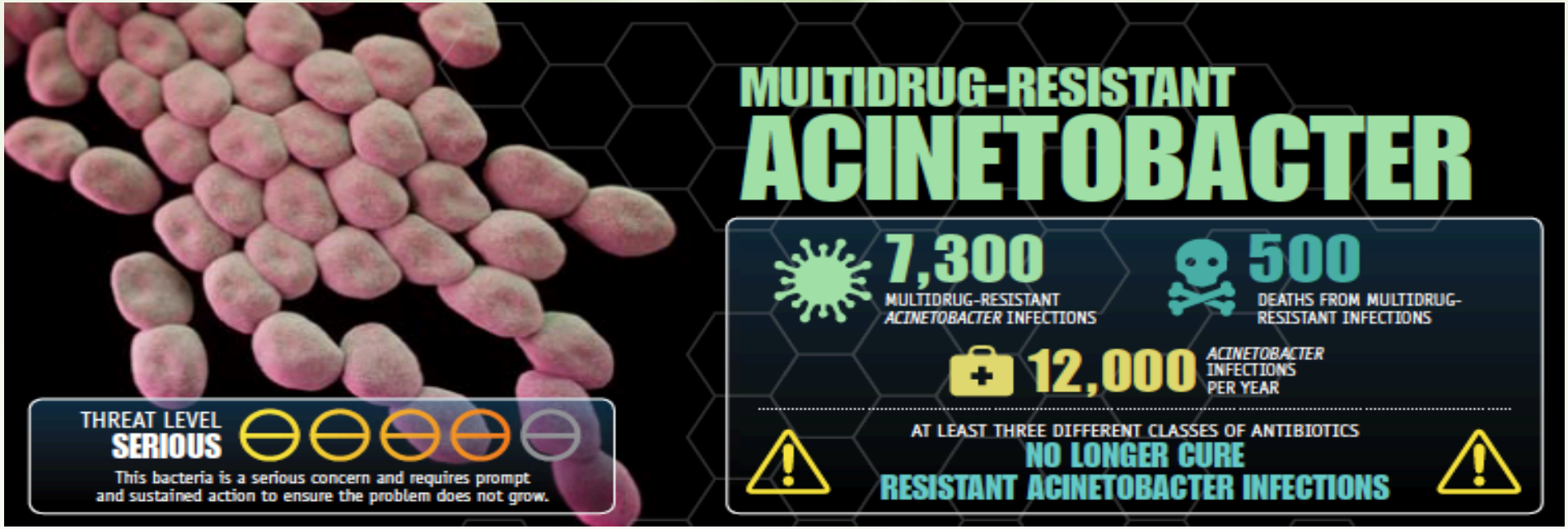

Drug target evaluation with knockout in relevant clinical strains

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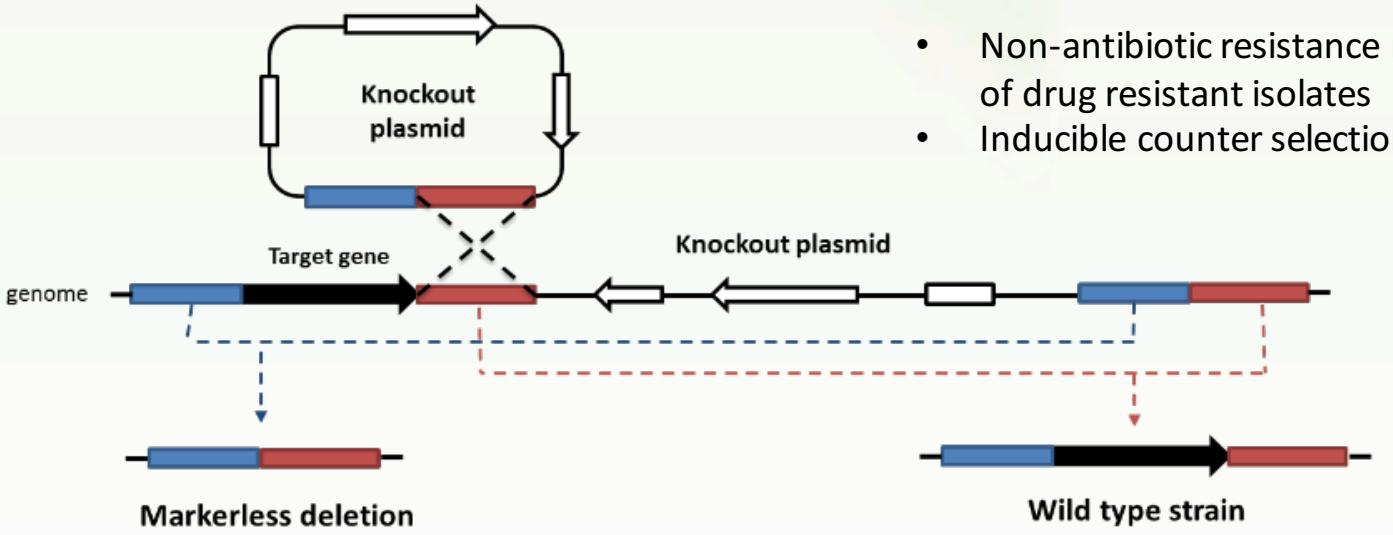
Strain designation	isolation		MLST	Resistance profile for XDR (non-susceptible to ≥1 antibiotic in all but ≤2 categories)									
	location	date		GENT	MERO	CIP	T/C	TZP	CTX	SXT	SAM	COL	TET
ATCC 17978	France	1951	77	S	S	S	S	S	I	R	S	S	S
BV26	Switzerland	1979	1	R	S	R	R	S	I	R	R	S	R
BV94	USA	2011	2	R	R	R	R	R	R	R	I	R	R
BV173	Greece	2012	2	R	R	R	R	R	R	R	R	R	R
BV175	Turkey	2012	2	R	R	R	R	R	R	R	R	R	R
BV185	Mexico	2013	2	R	R	R	R	R	R	R	R	R	R
BV186	USA	2013	2	R	R	R	R	R	R	R	R	R	I
BV187	USA	2013	2	R	R	R	R	R	R	R	I	R	I
BV189	Spain	2013	2	R	R	R	X	R	R	R	R	R	R
BV190	Greece	2012	1	R	R	R	X	R	R	R	R	R	R
BV191	China	2013	2	R	R	R	X	R	R	R	R	R	R



- Non-antibiotic resistance marker enables manipulation of drug resistant isolates
- Inducible counter selection cassette