Diversity of mutations in regulatory genes of RND-type efflux pumps in association with tigecycline resistance in *Acinetobacter baumannii*

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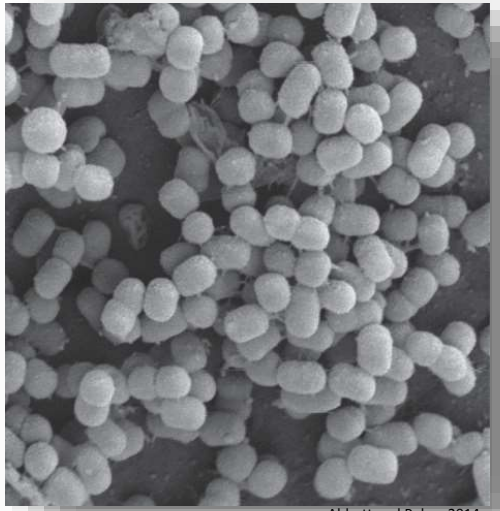
Acinetobacter baumannii

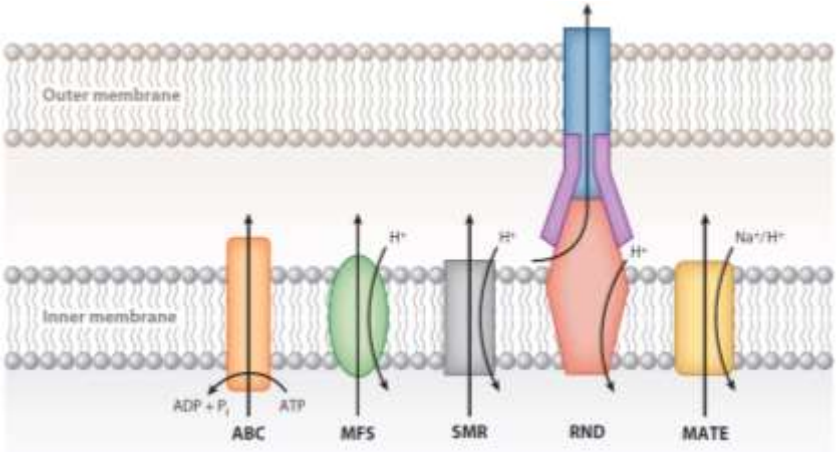
- hospital-acquired pathogen
- high frequency of multidrug-resistant strains
- important last-resort antibiotics: carbapenems, colistin and tigecycline
- tigecycline resistance through overexpression of resistance-nodulation-cell divison (RND)-type efflux pumps



Abbott and Peleg, 2014

Resistance-Nodulation-Cell Division pumps

- RND-type efflux pumps often associated with multidrug-resistant phenotype
- broad substrate specificity:
  - aminoglycosides, macrolides, tetracyclines, dyes, detergents etc.
- RND-type efflux pump systems in *A. baumannii*:
  - *adeRSABC*, *adeN-IJK*, *adeLFGH*



Delmar *et al.* 2014

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- RND-type efflux pump systems in *A. baumannii*:
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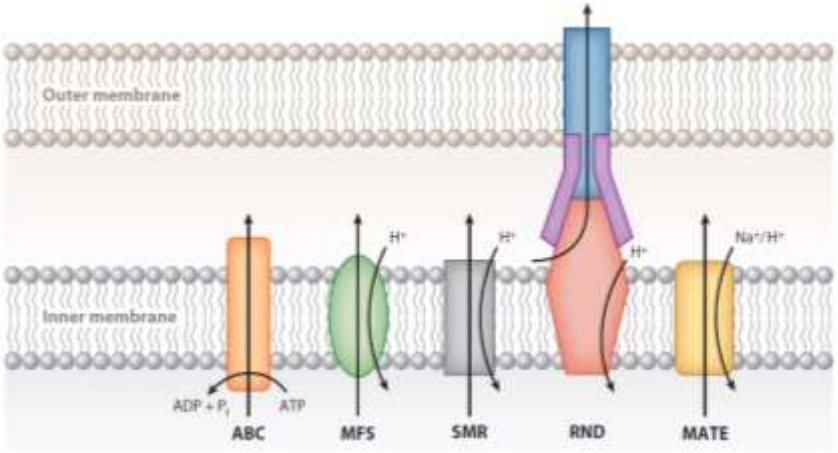
- different regulators:
  - two component system *adeRS*
  - TetR-like transcription regulator *adeN*
  - LysR transcription regulator *adeL*



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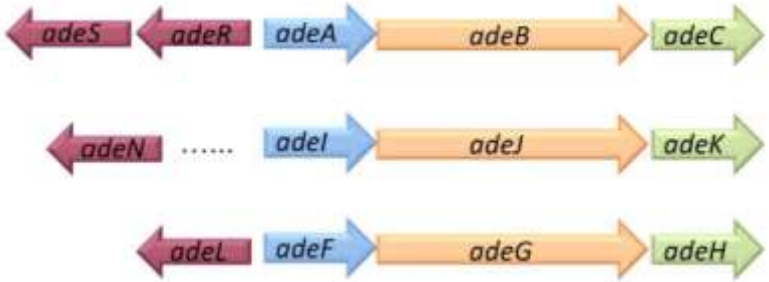
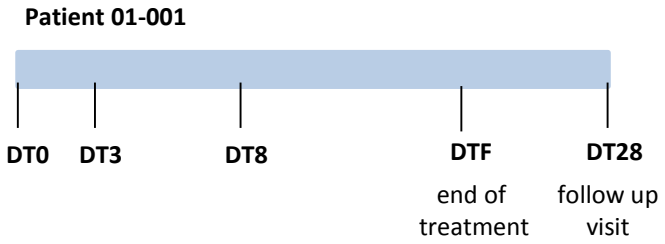


Nowak, 2016

Methods

- *A. baumannii* isolates from 15 hospitals in Greece, Italy and Spain

- **MagicBullet clinical trial**
  phase IV clinical trial comparing colistin vs. meropenem for empirical treatment of ventilator-associated pneumonia (VAP)
  - 65 patients
  - respiratory samples collected at different time points during treatment



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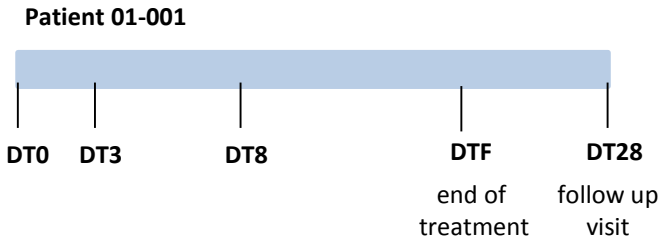


- **first available respiratory isolate** per patient was selected

Country	Number of cities (hospitals)	Number of hospitals	Number of isolates
Spain	4	5	14
Greece	4	7	37
Italy	2	3	14

65 isolates

Methods

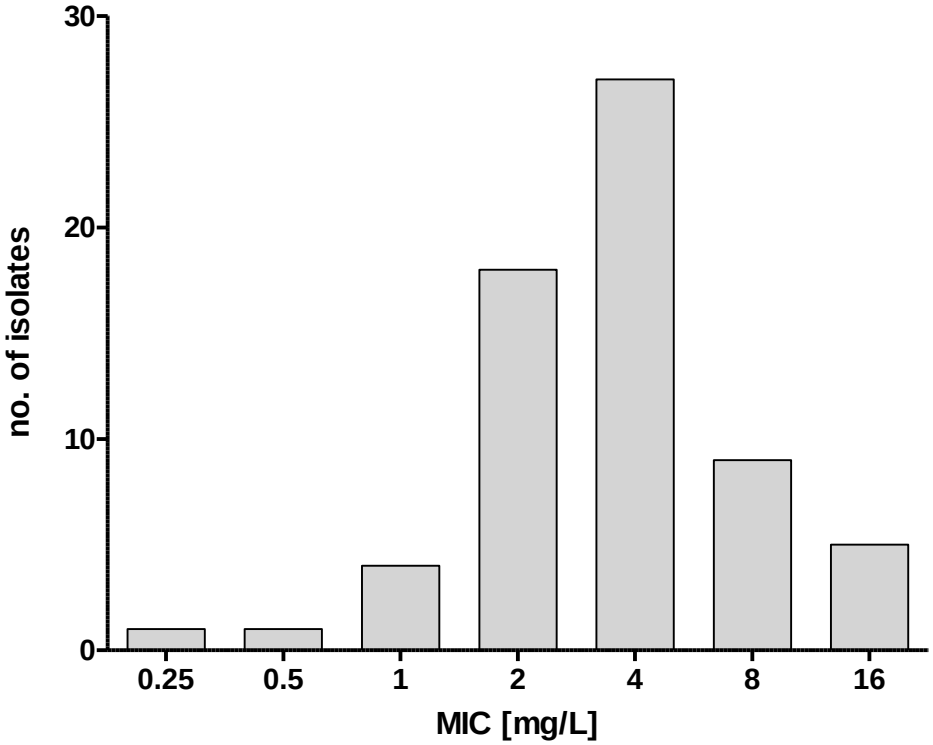
- determination of tigecycline MICs by **Etest**
  - EUCAST resistance breakpoint for *Enterobacteriaceae*: susceptible ≤ 1 mg/L
    non-susceptible ≥2 mg/L

- **whole-genome-sequencing** by MiSeq
  - assembly of draft genome by Velvet

- screening for insertion sequences in RND-type efflux pump regulators
  - IS-Mapper, Sanger sequencing and ISfinder database

- screening for amino acid substitutions and other changes (e.g. frameshift) by comparing the amino acid sequence of regulators with reference strain *A. baumannii* ACICU

Distribution of tigecycline MICs and clonal lineages



- 65 isolates in total: 59 TGC non-susceptible (90.8%)
  6 TGC susceptible (9.2%)

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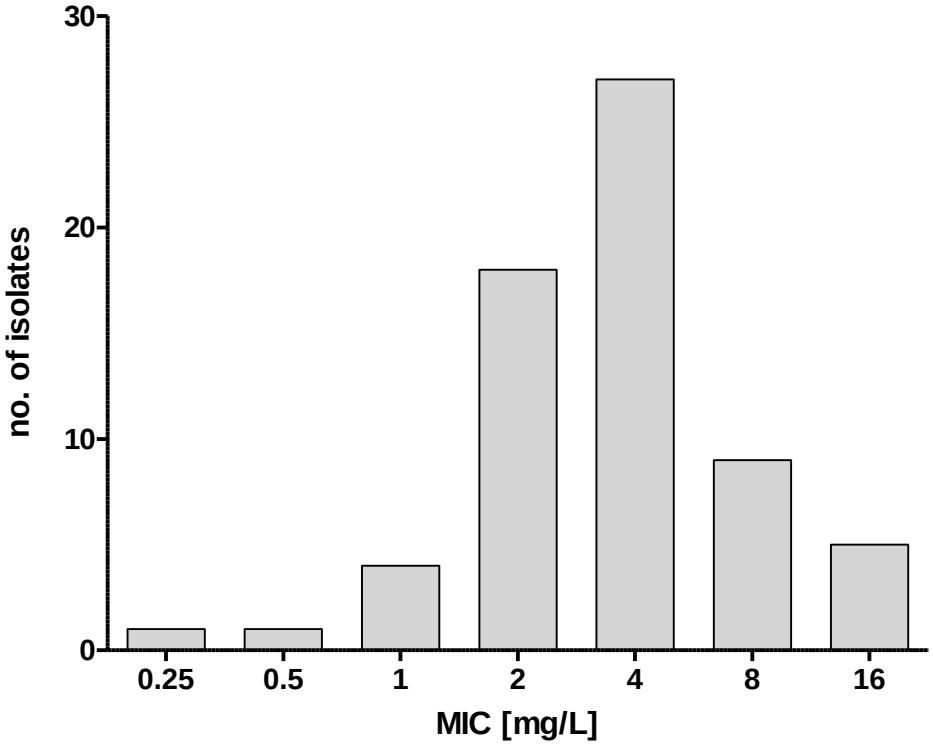

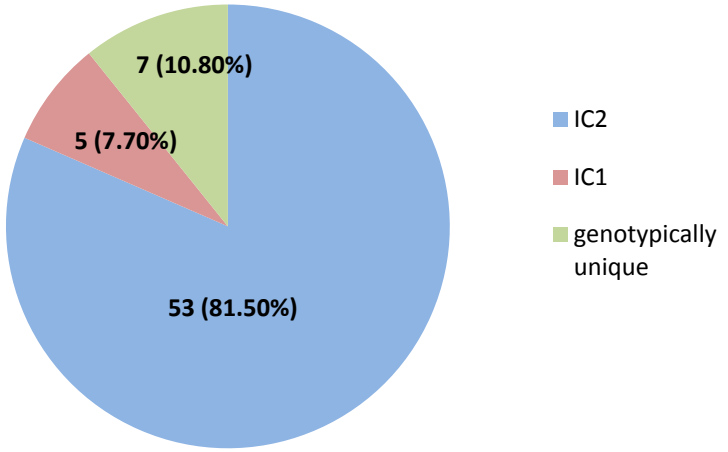

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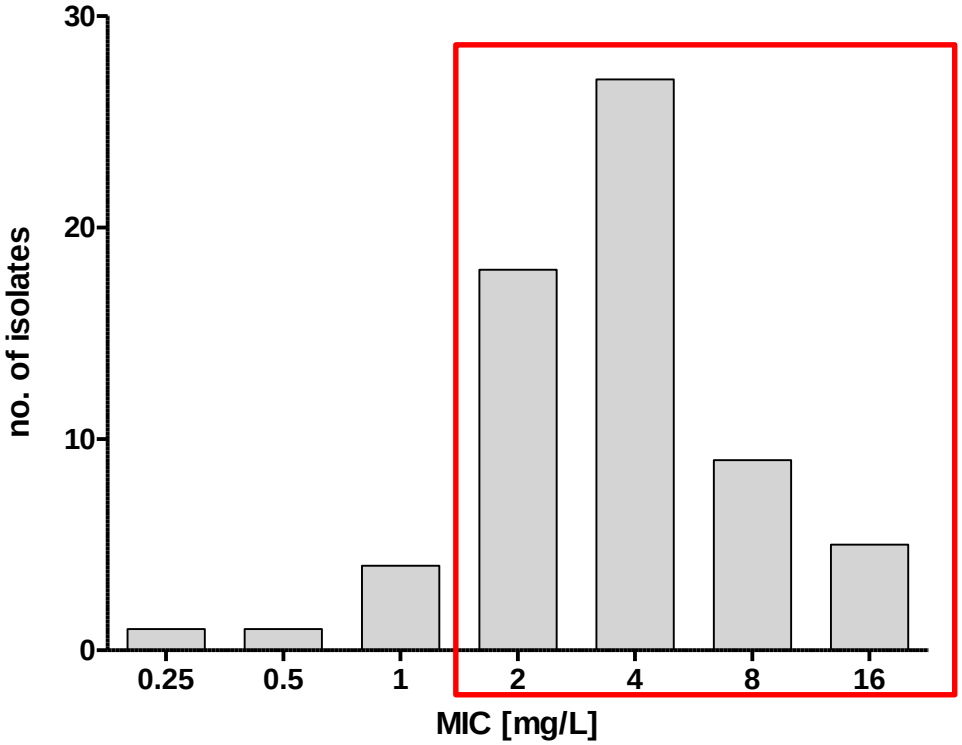

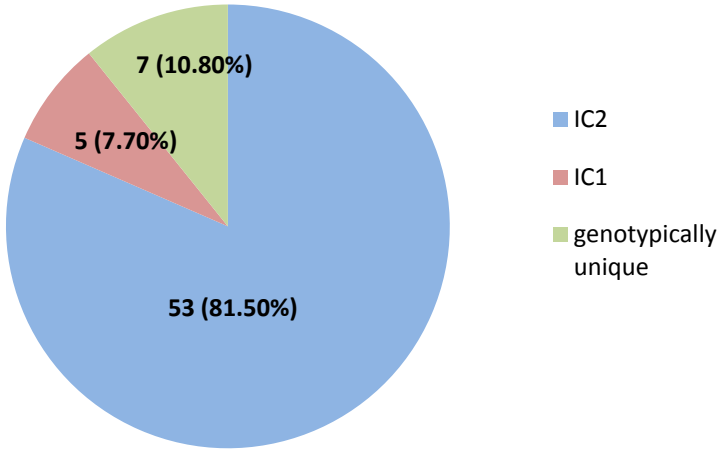

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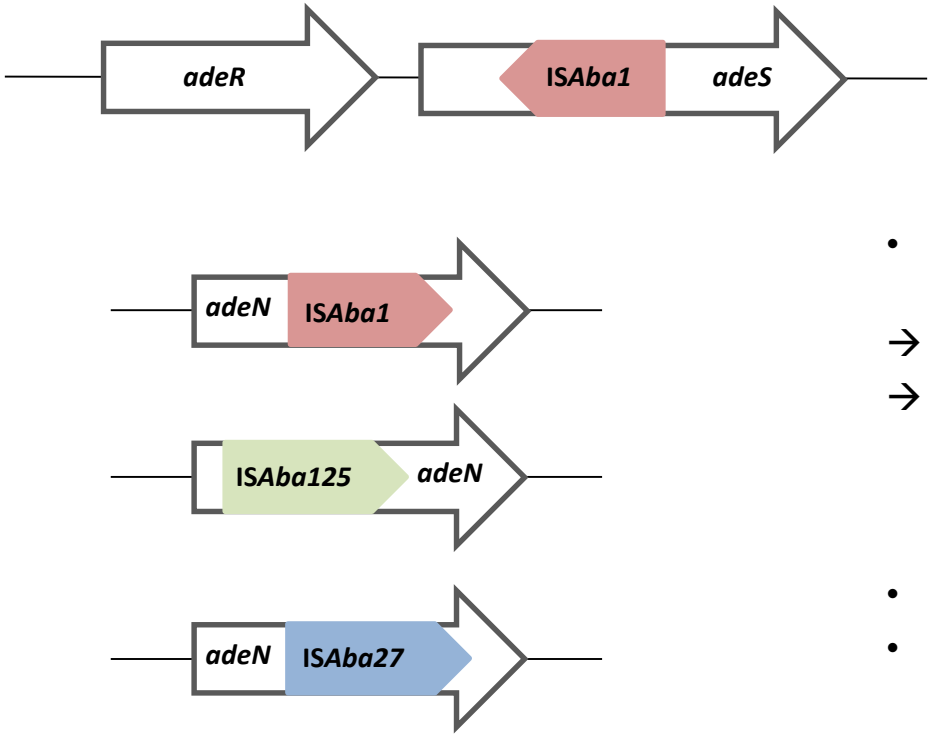
Insertion sequences in regulatory genes *adeS* and *adeN*

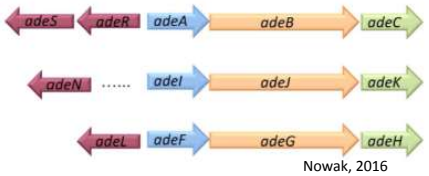


- in total 23 isolates with disrupted *adeS* or *adeN*

→ *adeS* was disrupted by IS*Aba1* (n=3)

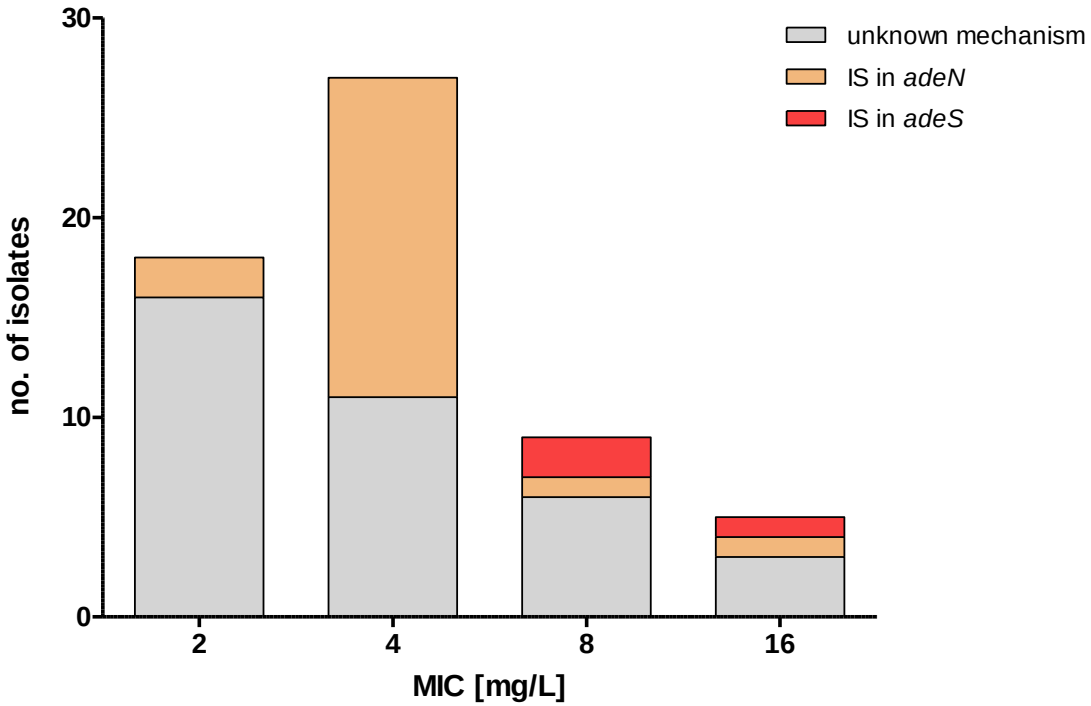
→ *adeN* was disrupted by IS*Aba1* (n=18), IS*Aba27* (n=1) or IS*Aba125* (n=1)

- *adeL* undisrupted in all isolates
- *adeRS*, *adeN* and *adeL* undisrupted in susceptible isolates



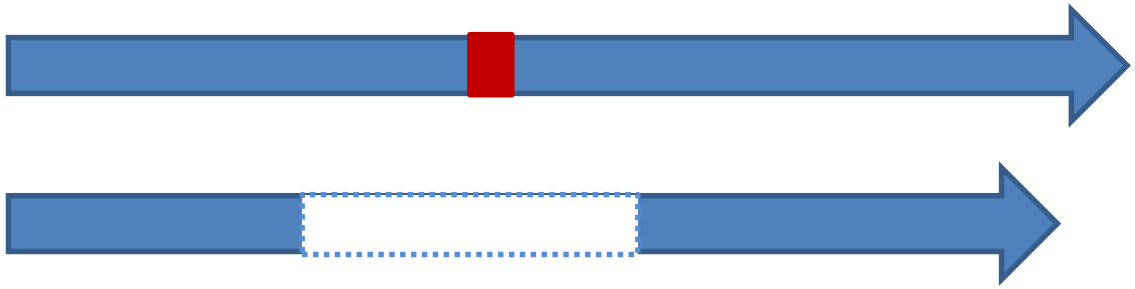
Nowak, 2016

Distribution of MICs of tigecycline non-susceptible isolates



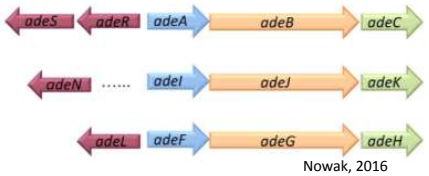
- insertion in *adeN* more prevalent
- disruption of *adeS* associated with higher tigecycline MICs

Differences in *adeN*



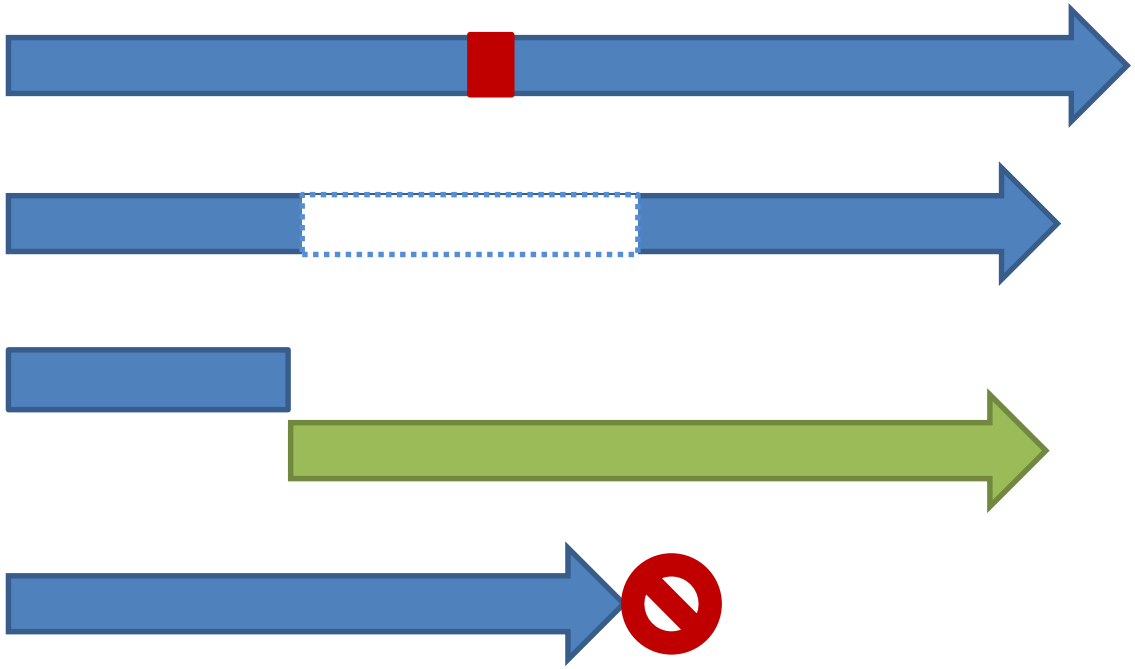
6 nucleotide insertion (n=5)

87 nucleotide deletion (n=1)



Nowak, 2016

Differences in *adeN*

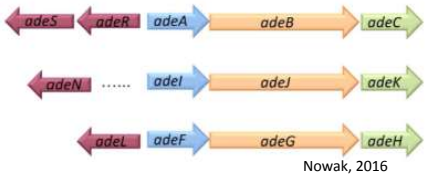


6 nucleotide insertion (n=5)

87 nucleotide deletion (n=1)

frameshift due to one nucleotide deletion (n=6)

premature stop codon (n=3)



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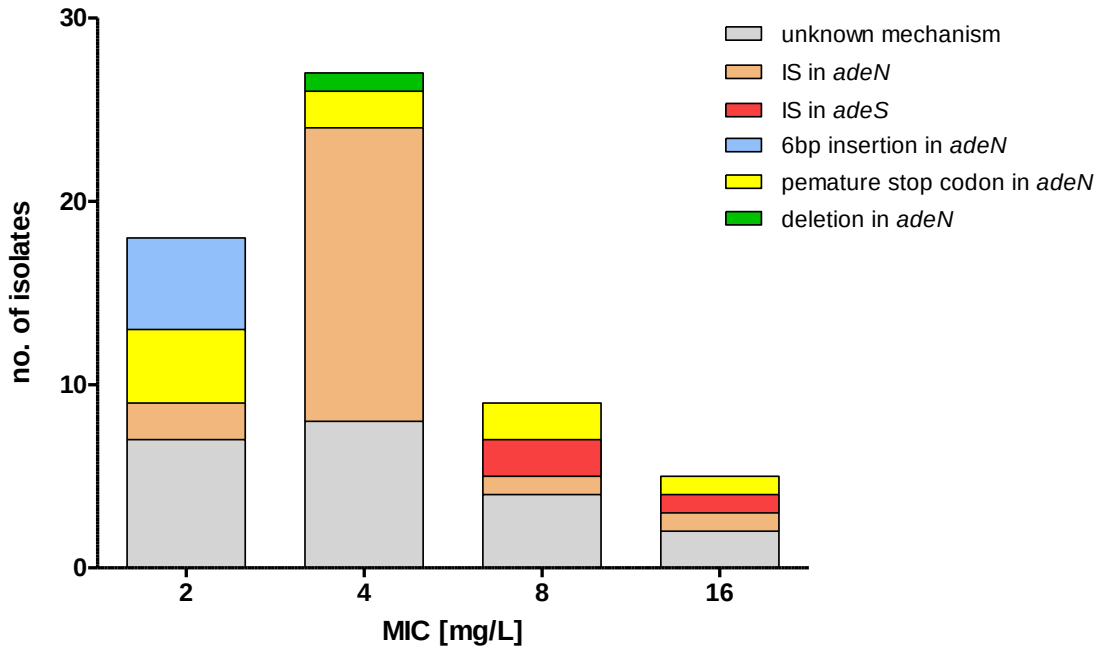

- premature stop codon and frameshift associated with higher tigecycline MICs
  → repressor function is impaired

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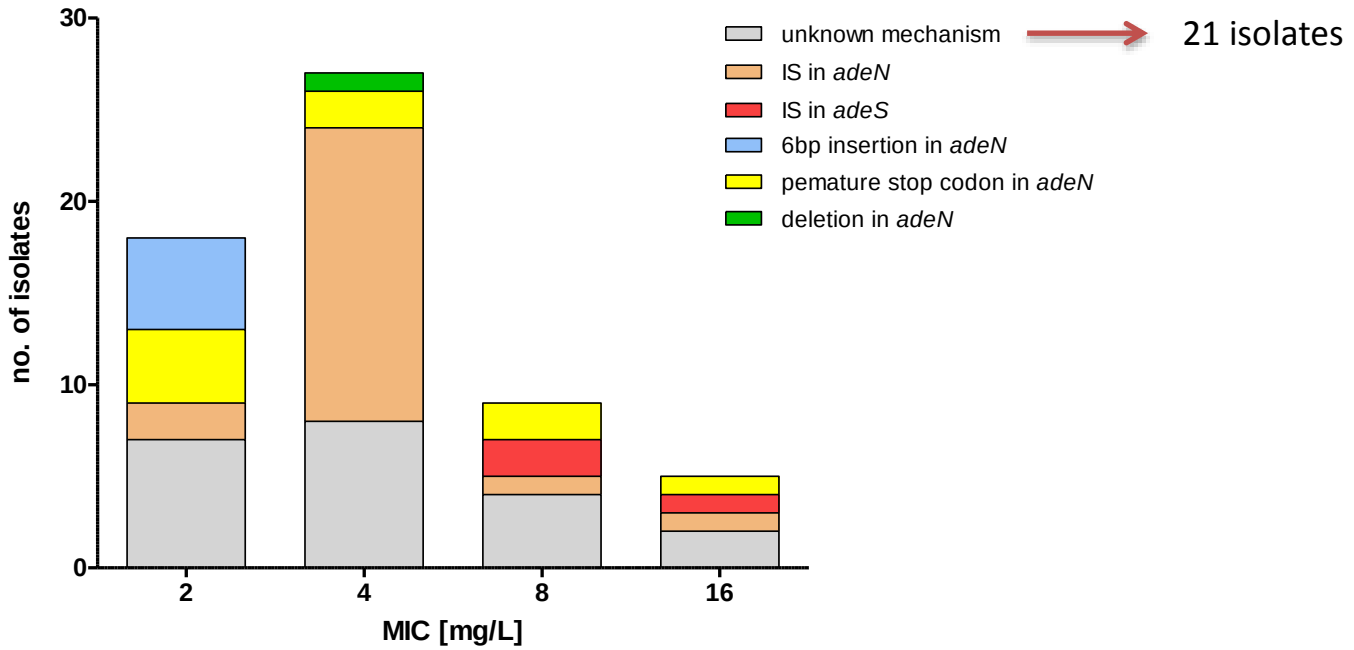

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Amino acid substitutions in the regulators*

AdeR	No. of isolates	AdeS	No. of isolates	AdeN	No. of isolates	AdeL	No. of isolates
I120V, V136A, V243I	1	D167N, V186G, H268N, I348V, S357P	1	N58T, H170Y	1	Q262R	4
D26N	2	A325T	2	N58T	8		
V119I	2	I62M	1	N58T, G215V	2		
D21V, D26N	5	V27I, V32I, A94V, V186G, F214L, H268N, S280A, Q281D, Q299R, Q339K, I348V	3	N58T, D181N			
N115K	1	V27I, V32I, A94V, V137F, V186G, F214L, H268N, S280A, Q281D, Q299R, Q339K, I348V	1				
G25S, D26N, E147K	2						
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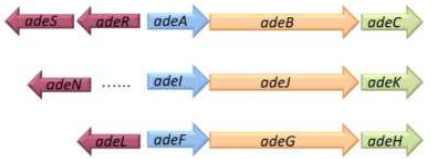
*compared to ACICU



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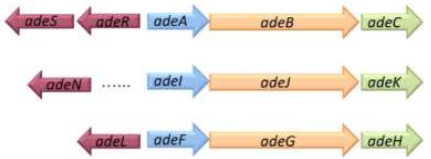

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genotypically unique
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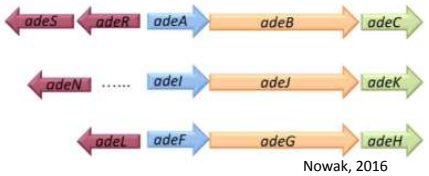

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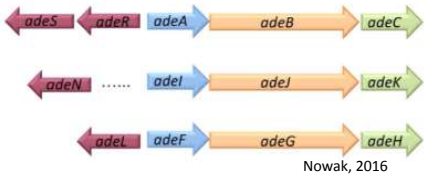

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The Asp20-to-Asn Substitution in the Response Regulator AdeR Leads to Enhanced Efflux Activity of AdeB in *Acinetobacter baumannii*

Jennifer Nowak,[a] Thamarai Schneiders,[b] Harald Seifert,[a,c] Paul G. Higgins[a]
Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany[a]; Division of Infection and Pathway Medicine, Edinburgh, United Kingdom[b]; German Centre For Infection Research, Bonn-Cologne, Germany[c]

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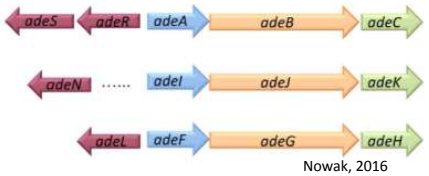

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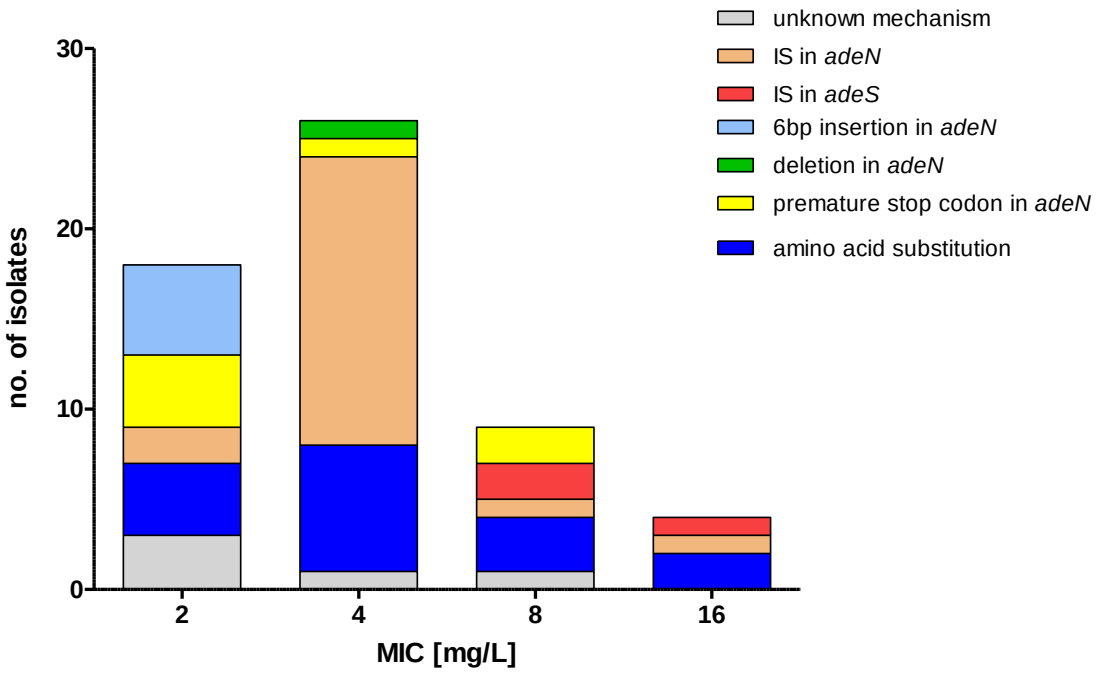

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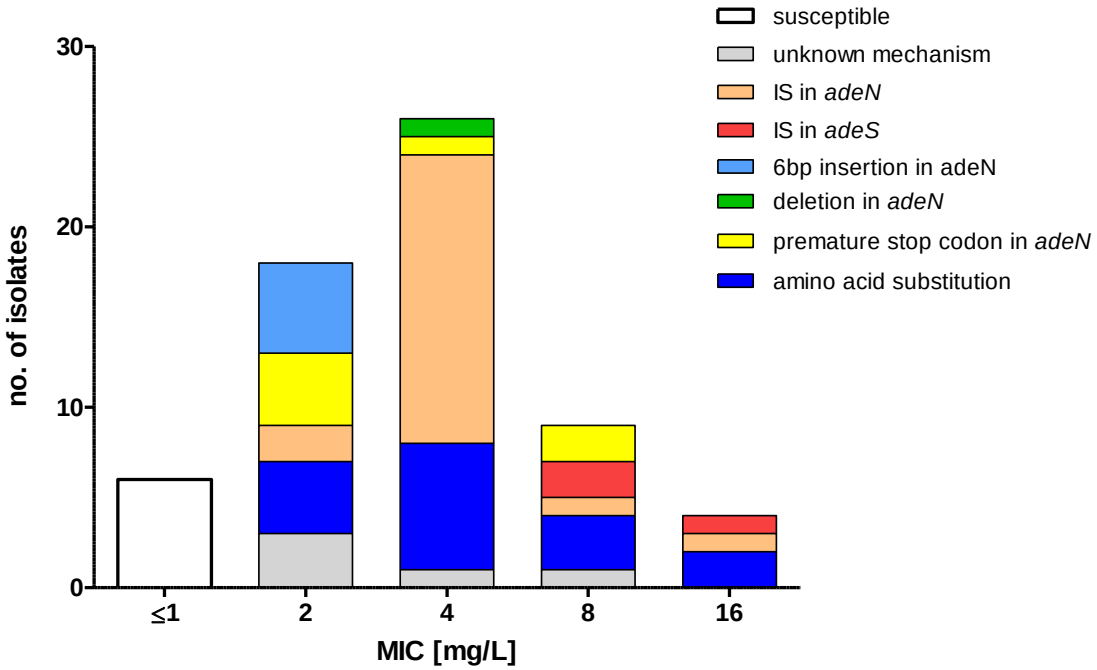

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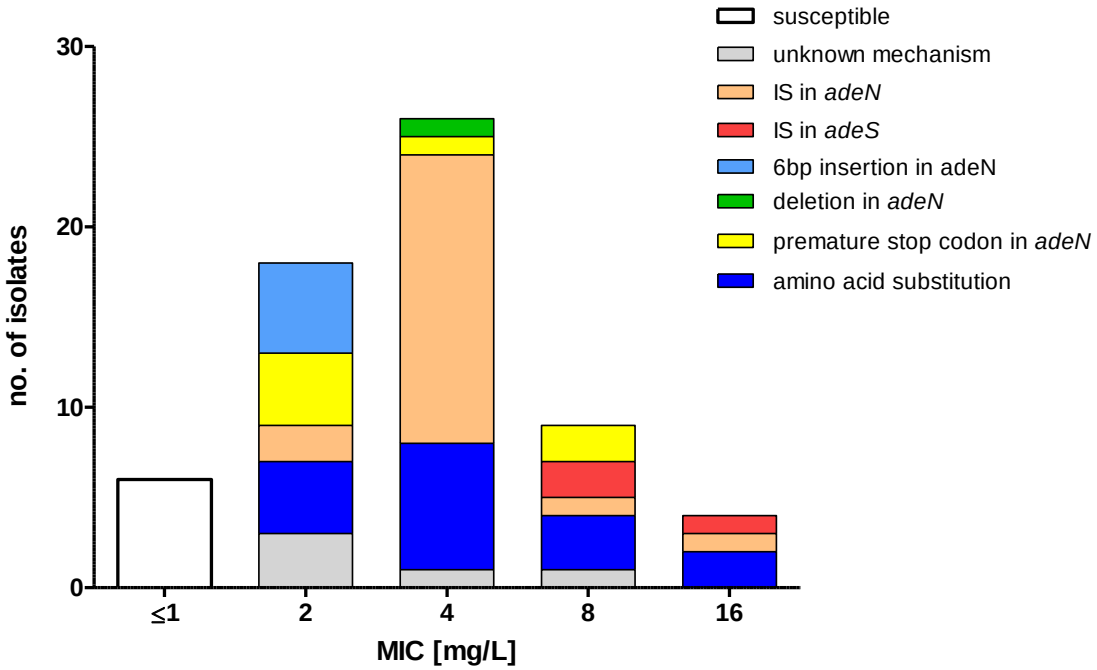

→ 5 isolates with unknown tigecycline resistance mechanism

Conclusion

- IS-elements were found to be associated with TGC non-susceptibility
  - IS*Aba1* most prevalent insertion sequence
  - disruption of *adeN* more frequent (about 30% of isolates)

- various mutations in *adeN* that might impair the repressor function leading to elevated *adeJ* expression

- amino acid substitutions diverse
  - polymorphisms belonging to different strains and clonal lineages have to be taken into account

→ **differences in RND-type efflux pump regulators are diverse**

→ **gene disruptions, deletions, frameshifts and premature stop codons are associated with tigecycline resistance**

Acknowledgement

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Julia Ertel
Lea Biniossek
Danuta Stefanik

Dr. Oleg Krut

DFG · FOR 2251 Acinetobacter
MagicBullet
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