



26th **ECCMID**

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Multidrug- and extremely drug-resistant Gram-negative bacilli: the storm is here

Resistance in *Acinetobacter baumannii* to non-beta-lactam agents



Luis Martínez-Martínez
Dpt. Molecular Biología
University of Cantabria
Service of Microbiology
Univ. Hosp. Marqués de Valdecilla
Santander, Spain



reipi-isciii CONSEJO REGULADOR
POR RESOLUCIÓN
2010/10/15

Amsterdam, April 10 2016

A. baumannii INTRINSIC RESISTANCE

AMP	AMX-CLV	FAZ	CTX	ETP	TMP	FOS
R	R	R	R	R	R	R

Acinetobacter spp. NON-β-LACTAMS BREAKPOINTS (1)

	S		I		R	
	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST
LIPOPEPTIDES						
Polymyxin B	≤2				≥4	
Colistin	≤2	≤2			≥4	>2
AMINOGLYCOSIDES						
Gentamicin	≤4	≤4	8		≥16	>4
Tobramycin	≤4	≤4	8		≥16	>4
Amikacin	≤16	≤8	32	16	≥64	>8
Netimicin	≤8	≤4	16		≥32	>4
[...cont.]

Acinetobacter spp. NON-β-LACTAMS BREAKPOINTS (2)

	S		I		R	
	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST
LIPOPEPTIDES						
Polymyxin B	≤2				≥4	
Colistin	≤2	≤2			≥4	>2
AMINOGLYCOSIDES						
Gentamicin	≤4	≤4	8		≥16	>4
Tobramycin	≤4	≤4	8		≥16	>4
Amikacin	≤16	≤8	32	16	≥64	>8
Netimicin	≤8	≤4	16		≥32	>4
TETRACYCLINES						
Doxycycline	≤4		8		≥16	
Minocycline	≤4	IE	8		≥16	IE
Tetracycline	≤4		8		≥16	
Tigecycline		IE				IE
FLUOROQUINOLONES						
Ciprofloxacin	≤1	≤1	2		≥4	>1
Levofloxacin	≤2	≤1	4	2	≥8	>2
Gatifloxacin	≤2		4		≥8	
Nalidixic acid (screen)		NA				NA
FOLATE PATHWAY INH.						
Trimethoprim-sulfamethoxazole	≤2/38	≤2			≥4/76	>2

**ACTIVITY OF ANTIMICROBIAL AGENTS AGAINST *A. baumannii*
IN SPAIN (MULTICENTER STUDY, 2010, 43 CENTERS)**

(Ceftazidime I+R: 99%; Imipenem I+R: 82%)

	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Not-susceptible isolates (%)
Gentamicina	32	> 64	70
Tobramicina	8	64	60
Amicacina	16	256	49
Netilmicina	32	> 64	ND
Tetraciclina	> 64	> 64	83
Doxiciclina	32	64	70
Minociclina	2	8	30
● Tigeciclina	1	2	24
Ciprofloxacino	64	> 64	94
Colistina	≤ 0,5	1	3
● Rifampicina	4	64	30

ACTIVITY OF ANTIMICROBIAL AGENTS AGAINST MULTIRESISTANT *A. baumannii*

N=215 strains

Hospitalised patients, Thailand

RESISTANT to amikacin, ceftazidime, meropenem, imipenem

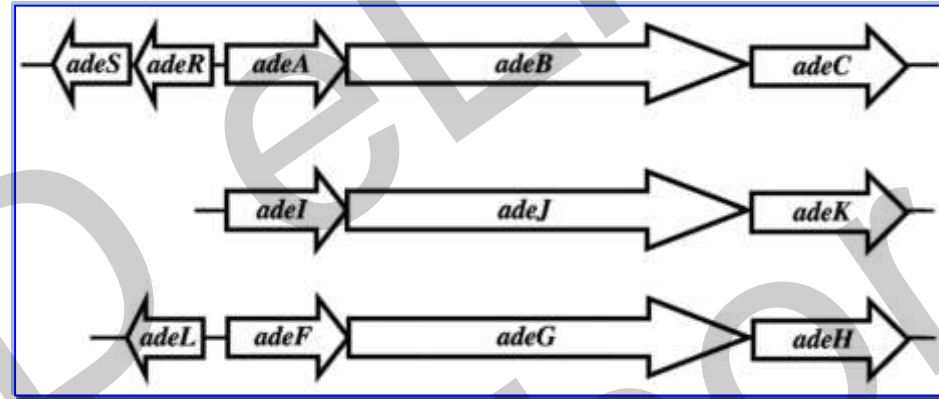
Antimicrobial agent	MIC (mg/L)	
	MIC ₅₀	MIC ₉₀
Minocycline	4	8
Tetracycline	>64	>64
Tigecycline	0.5	1
Polymyxin B	0.5	1
● Sitafloracin	0.5	1
Levofloxacin	4	8

EFFLUX SYSTEMS AND ANTIMICROBIAL RESISTANCE

1. MFS: *Major Facilitator Superfamily*
- 2. RND: *Resistance Nodulation-Division***
3. SMR: *Small Multidrug Resistance*
4. ABC: *ATP-Binding Cassette*
5. MATE: *Multidrug And Toxic Extrusion*
- 6. PACE: *Proteobacterial antimicrobial compound efflux***

EFFLUX SYSTEMS AND RESISTANCE IN *A. baumannii*

RND SYSTEMS



	B-Lact	AMG	FQui	CHLO	TET	TIG	RIF	ERY	CLIN	TRIM	STX
AdeABC	(FEP)	+	+	+	+	+					
AdeIJK	+	+		+	+	+	+	+	+		
AdeFGH			+	+	+	+			+	+	+

EFFLUX SYSTEMS AND RESISTANCE IN *A. baumannii*

MFS EFFLUX SYSTEMS

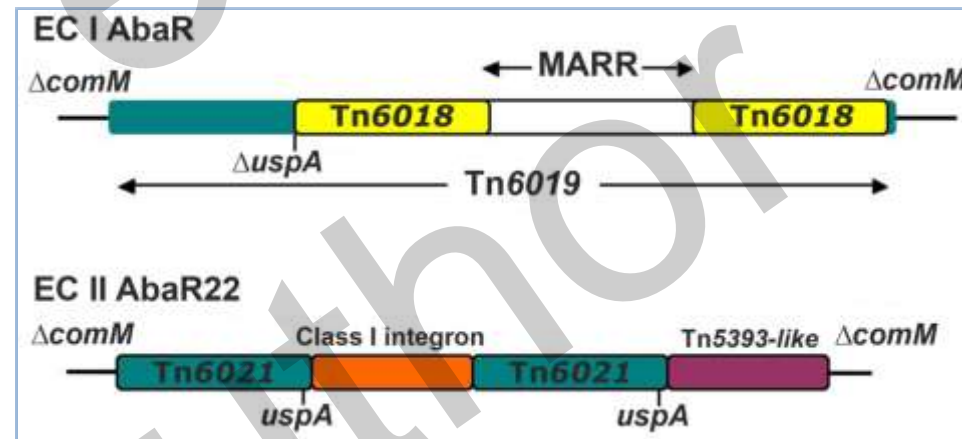
	EFFLUX FAMILY	TET	MIN	CHLO	ERY	Comments
TetA	MSF	+				
TetB	MSF	+	+			More frequent than TetA
CraA	MSF			+		Homologous to MdfA of <i>E. coli</i>
CmlA	MSF			+		
FloR	MSF			+		
AmvA	MSF				+	Also disinfectants and detergents

EFFLUX SYSTEMS AND RESISTANCE IN *A. baumannii*

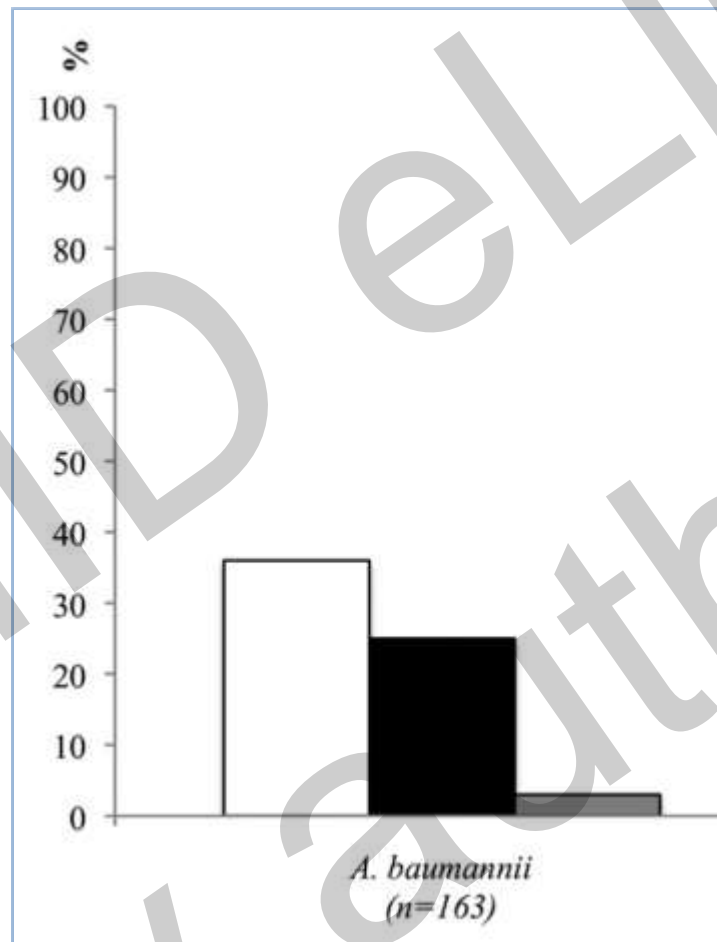
OTHER RELEVANT EFFLUX SYSTEMS

	EFFLUX FAMILY	AMG	FQui	CHLO	ERY	TRIM	NOV	Comments
AbeM	MATE	+	+	+		+		Also dyes
AbeS	SMR	+	+	+	+		+	Also dyes
QacE	SMR							Quaternary ammonium
AceI	PACE							Chlorhexidine [Resistance to biocides]

AbaR-type	Size (kb)	Strain	EC	<i>comM</i> Insertion
AbaR1	86	AYE	I	+
AbaR2	17	ACICU	II	+
AbaR3	63	AB0057	I	+
AbaR4	18	AB0057	I	-
AbaR5	56	3208	I	+
AbaR6	27	D2	I	+
AbaR7	20	A92	I	+
AbaR8	29	D13	I	+
AbaR9	39	AB056	I	+
AbaR10	30	AB058	I	+
AbaR11	20	NIPH470	I	+
AbaR12	38	LUH 6013	I	+
AbaR13	45	LUH6015	I	+
AbaR14	21	LUH5881	I	+
AbaR15	55	LUH6125	I	+
AbaR16	39	LUH7140	I	+
AbaR17	58	LUH8592	I	+
AbaR18	52	NIPH2713	I	+
AbaR19	30	NIPH2554	I	+
AbaR21	64	RUH875	I	+
AbaR22	39	MDR-ZJ06	II	+
n.a.	n.f.	A473	I	-
n.a.	n.f.	A473	I	-



INTEGRONS IN MULTIRESISTANT *A. baumannii*



□ *intI1* ■ *intI2* ▒ *intI1* and *intI2*

RESISTANCE TO AMINOGLYCOSIDES

Decreased intracellular concentration

Decreased uptake

Increased active efflux

Enzymatic drug modification

Aminoglycoside modifying enzymes

Target modification

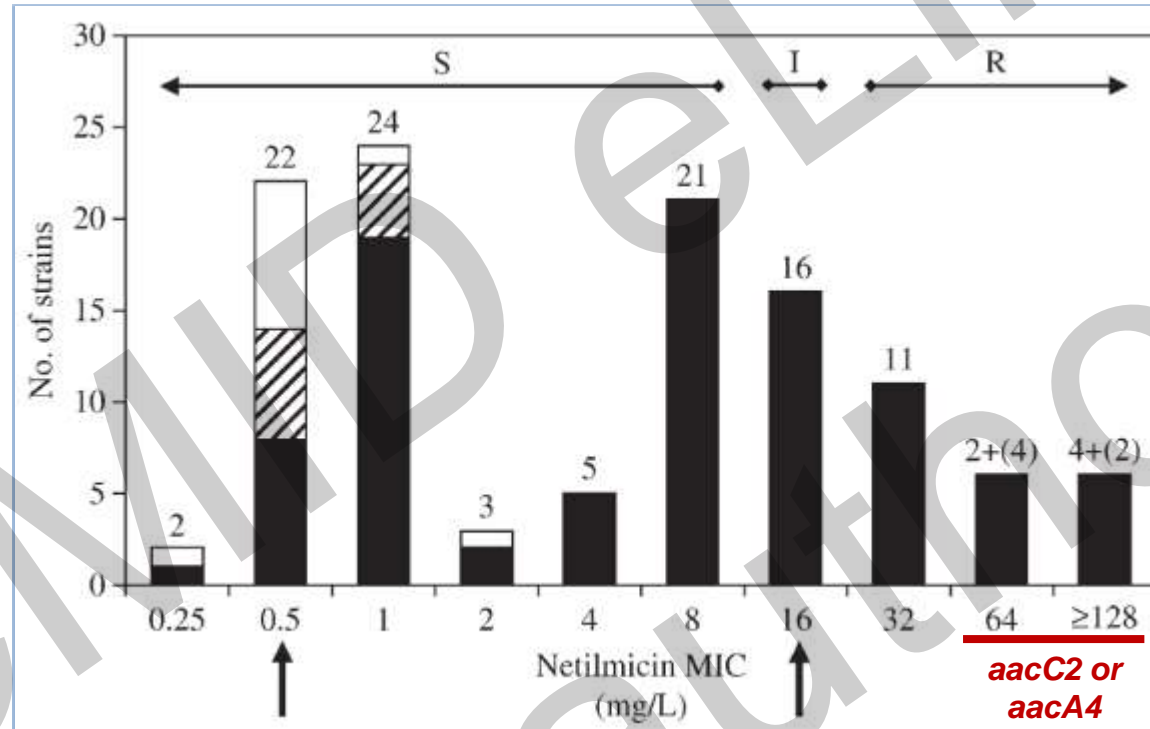
Ribosomal (proteins/16S rRNA) mutation

16S rRNA Methyltransferases (methylases)

Others

(swarming, drug sequestration, biofilm growth)

MICs of NETILMICIN AGAINST *A. baumannii* (n=116)

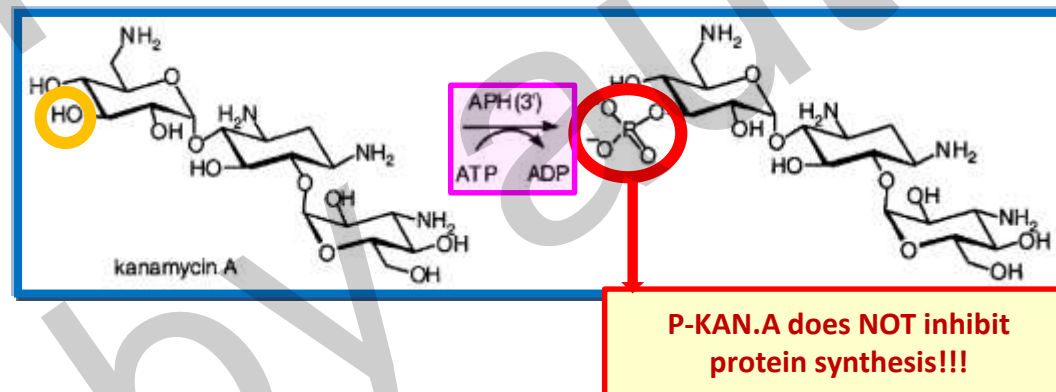


Black bars: Positive for *adeA*, *adeB*, *adeR*, *adeS*; [*adeC*]
 White bars: negative reactions for all five genes
 Shaded bars: Other combinations

AMINOGLYCOSIDE MODIFYING ENZYMES

ENZYMES = TRANSFERASES		MODIFIED GROUP	TRANSFERED GROUP	MODIFIED POSITIONS*
NUCLEOTIDYLTRANS. (ADENYLYLTRANS.)	ANT	Hydroxyl	AMP (from ATP)	<u>4'</u> , <u>2''</u> 3'', 6, 9
PHOSPHOTRANS.	APH	Hydroxyl	γ-Phosphate (from ATP or, in some cases, GTP)	<u>3'</u> , <u>2''</u> 3'', 7'', 4, 6, 9
ACETYLTRANS.	AAC	Amino	Acetyl-CoA	<u>3</u> , <u>2'</u> , <u>6'</u> 1

*Relevant positions related to modification of most commonly used aminoglycosides are underlined



AMINOGLYCOSIDE RESISTANCE PHENOTYPES RELATED TO AMES

	APR	FOR	GEN	TOB	AMK	NET	ISE	KAN	NEO
AAC(2')-I			+	+		+			+
AAC(3)-I		+	+						
AAC(3)-II			+	+		+		+/-	
AAC(3)-III			+	+				+	
AAC(3)-IV	+		+	+		+			
AAC(3)-VI			+	+/-		+/-		+/-	
AAC(6')-I			+/-	+	+/(-)	+	+/-	+	
AAC(6')-II			+	+		+		+	
ANT(2'')-I			+	+				+	
ANT(4')-II				+	+		+	+	
APH(3')-VI					+		+	+	+



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16S-RMTases

Intrinsic enzymes in Aminoglycoside-producing bacteria

Modification of specific residues in the Amglc-binding site of 16S rRNA

Plasmid-mediated in clinically relevant gram-negative bacteria

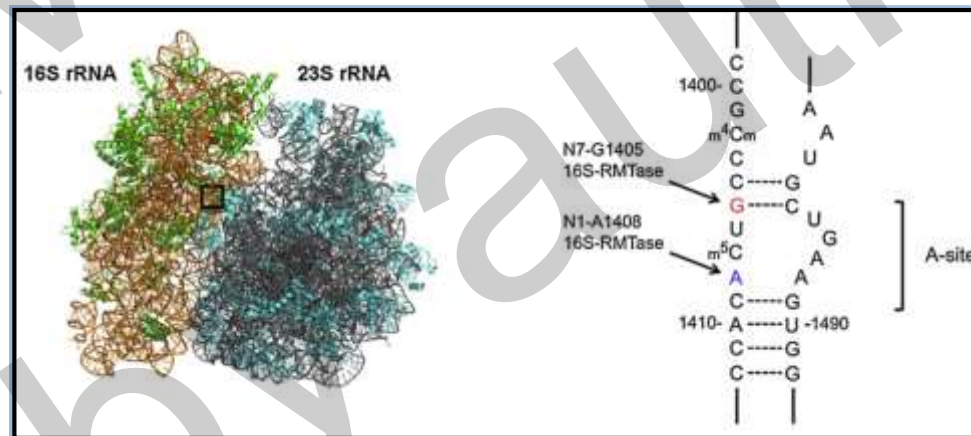
TWO MECHANISTICALLY DEFINED FAMILIES:

N7-G1405 family:

ArmA, RmtA, RmtB, RmtC, RmtD, RmtE, RmtF, RmtG, RmtH

N1-A1408 family:

NmpA



METHYLASES. PHENOTYPES

	N7-G1405 Family	N1-A1408 Family
4,6 Disubstituted DOS (Gentamicin, Amikacin,...)	↑R	R
4,5 Disubstituted DOS (Neomycin...)	S	↑R
Monosubstituted DOS (Apramycin)	S	↑R
No DOS ring (Streptomycin)	S	S

Difficult phenotypic detection (Interference of AMEs!!!)

Genetic detection: PCR detection (...Correa LL et al. IJAA 2014, 43:474)

METHYLASES IN *A. baumannii*

armA

Plasmidic location; Pittsburg (USA), 2007

....

India, 2010

[Norway] [2011 (2007-2009)]

....

Plasmidic location; Egypt, 2015

MECHANISMS OF QUINOLONE RESISTANCE

- 1. Target (Type II topoisomerases) alteration**
2. Decreased permeability (porin-related)
- 3. (Over-) Expression of active efflux**
- 4. Target protection (Qnr proteins)**
- 5. [Production of inactivating enzyme (acetylase)]**
6. Other mechanisms (poorly characterized and... to be discovered!)

SUSCEPTIBILITIES OF *A. baumannii* TO QUINOLONES

Antibiotic	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)
Ciprofloxacin	32	128
Clinafloxacin	1	2
Trovafloxacin	2	16
Nalidixic acid	>32	>32
Norfloxacin	>32	>32
Ofloxacin	16	32
Pefloxacin	32	>32
Sparfloxacin	4	8

MUTATIONS IN *gyrA* AND *parC* IN QUINOLONE-RESISTANT *A. baumannii*

Strains	MIC (mg/L) ^a		Amino acid change ^b				
	CIP	NAL	Gly81	GyrA ^c Ser83	Ala84	ParC Ser80	Glu84
167 ^d	0.125	2	-	-	-	-	-
58 ^e , 201 ^f	0.125	8	-	-	-	-	-
88 ^f	0.5	8	-	-	-	-	-
77 ^e	1	8	-	-	-	-	-
93 ^e	1	8	Val	-	-	-	-
33 ^e , 661 ^f	4	256	-	Leu	-	-	-
175 ^e	8	64	-	Leu	-	-	-
34 ^e	8	512	-	Leu	-	-	-
29 ^e	16	256	-	Leu	-	-	-
547	32	>1024	-	Leu	-	Leu	-
31 ^e	64	>1024	-	Leu	-	-	Lys
13, 24, 30, 2545	64	>1024	-	Leu	-	Leu	-
65	64	>1024	-	Leu	Pro	Leu	-
14, 522, 1543	128	>1024	-	Leu	-	Leu	-

HIGH-LEVEL CIPROFLOXACIN RESISTANCE IN *A. baumannii* LACKING *parC* MUTATIONS

Strain	MIC (mg/L)							Amino acid substitutions		OMP 20 kDa band
	CIP	IPM	TET	TMP	GEN	CHL	CTX	<i>gyrA</i>	<i>parC</i>	
ATCC 19606 ^a	1	0.06	2	4	1	128	8	no mutation	no mutation	+
C1	8	0.12	2	4	2	256	8	Ser-83→Leu	no mutation	-
C2	32	0.12	2	8	2	256	32	Ser-83→Leu	no mutation	-
C3	64	0.12	4	32	4	256	32	Ser-83→Leu	no mutation	-
C4	128	0.12	4	32	8	256	32	Ser-83→Leu	no mutation	-

PMQR GENES IN *A. baumannii*

Yang R, et al. Detection of *qnr* gene [...] in clinical isolates of *A. baumannii*. Chin J Antibiot 2009;34:747–9.
(in Chinese)

39 *A. baumannii*
Anhui, China.

qnrB: 3 (7.7%)
qnrS: 1 (2.6%)

110 *A. baumannii*
Henan, China

qnrB4: 2 (1.8%) isolates



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POLYMYXIN RESISTANCE IN *A. baumannii*

LPS modification:

Mutations in the PmrA/B two-component system
Addition of phosphoethanolamine to lipid A

Glycosylation of lipidA with galactosamine at 1-P group

[*A. baumannii* lacks the machinery for L-Ara4N synthesis]

LPS biosynthesis genes mutation/inactivation:

lpxA, *lpxC*, *lpxD*

LPS loss

High level resistance

[Unknown mechanism]

Mutants vs wt: Differential expression of 35 proteins

OM proteins, chaperones, biosynthesis factors, metabolic enzymes

COLISTIN -R *A. baumannii*:

Reduced in vivo fitness; Decreased virulence
Increased susceptibility to other agents



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TETRACYCLINE RESISTANCE IN *A. baumannii*

Efflux Pumps (TetA, TetB: RND systems)

TetM (Ribosomal Protection)

trm mutation (S-adenosyl-L-methionine-dependent methyltransferase)
Decreased susceptibility to tigecycline, minocycline, doxycycline

IN-VITRO ACTIVITY OF TIGECYCLINE AGAINST *A. baumannii*

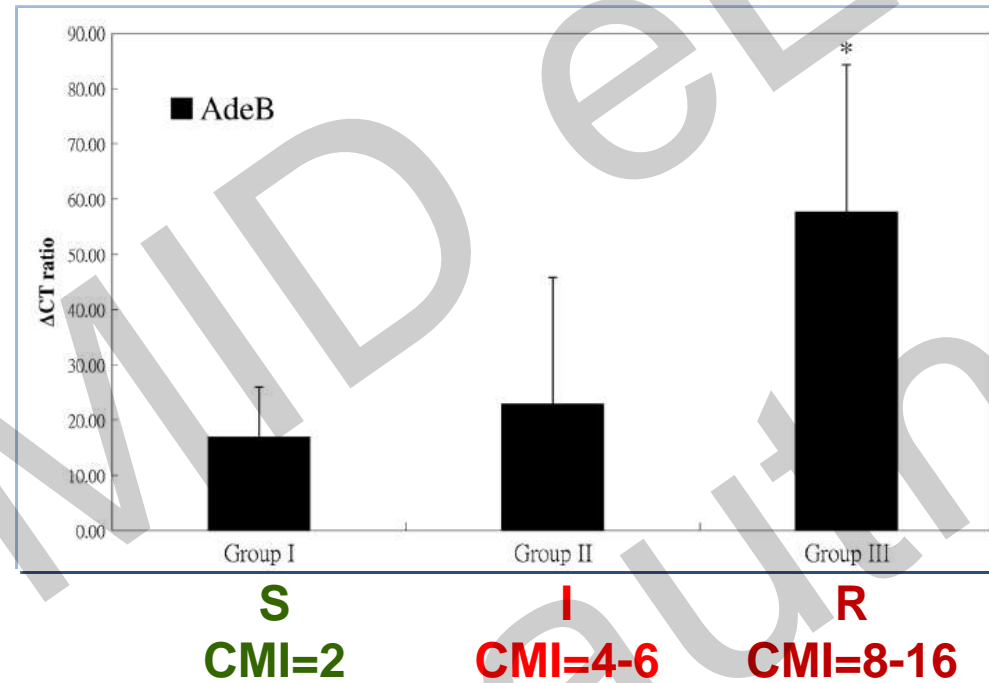
Coutry	Year	TIG Not Susceptible (%)
Taiwan	2006, 2008, 2012	18; 45.5; 18-29
Thailand	2006	2.7
Germany	2006	14.9
Spain	2007	12
Israel	2007	78
USA	2007	5
Italy	2008, 2009	27.5; 50
Turkey	2008	25.8
India	2009	58

PERCENTAGES OF TIG-R STRAINS WHEN TESTED (E-TEST) ON MUELLER-HINTON AGAR FROM DIFFERENT MAUFACTURERS

Organism	Difco	Oxoid	Merck
Manganese content (ppm)	2.5	2.5	680
ESBL(+) <i>E. coli</i>	0	0	10
ESBL(+) <i>K. pneumoniae</i>	0	0	30
<i>A. baumannii</i>	10	10	70

TIGECYCLINE RESISTANCE IN *A. baumannii*

ROLE OF AdeABC



adeB expression (RT-PCR) in *A. baumannii*

RIFAMPICIN RESISTANCE IN *A. baumannii*

[Modified permeability (PA β N effect)]

Enzymatic modification of the target

Chromosomal mutations in the RNA polymerase subunit *rpoB*

Enzymatic modification by Arr-2 (RIF. ADP-ribosyltransferase)

CONCLUSIONS

A. baumannii is frequently resistant to multiple agents, and this is related with the presence of multiple genes (chromosome/plasmid)

Resistance island and integrons are key genetic elements related to multidrug resistance in this pathogen

(Overexpression of) efflux pumps from several families (RND!) contribute to moderate levels of resistance to multiple agents

Additionally...

Resistance to aminoglycosides is related to AME and/or methylases

Resistance to quinolones is related to topoisomerase mutations (...and PMQR genes)

Resistance to polymyxins is related to modified/lost LPS