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MEDICAL SCHOOL
LABORATORY OF CLINICAL MICROBIOLOGY

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12. HRVATSKI KONGRES KLINIČKE MIKROBIOLOGIJE
9. HRVATSKI KONGRES O INFEKTIVNIM BOLESTIMA
s međunarodnim sudjelovanjem

UNIVERSITY GENERAL HOSPITAL "ATTIKON"

Antibiotic heteroresistance in *Acinetobacter baumannii*

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Transparency Declaration

- Nothing to declare for this presentation

Bacterial heterogeneous growth

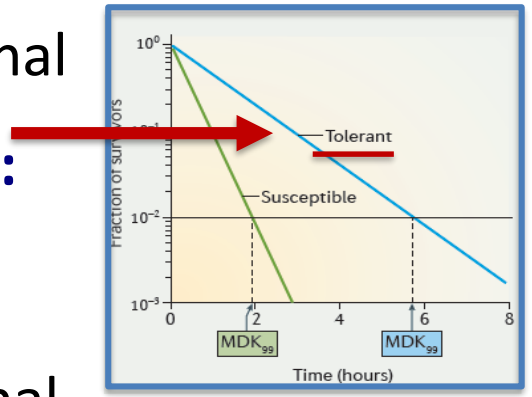
"Insufficient efficacy of antimicrobials"

Brauner et al, Nat Rev Microbiol 2016

It may be presented as:

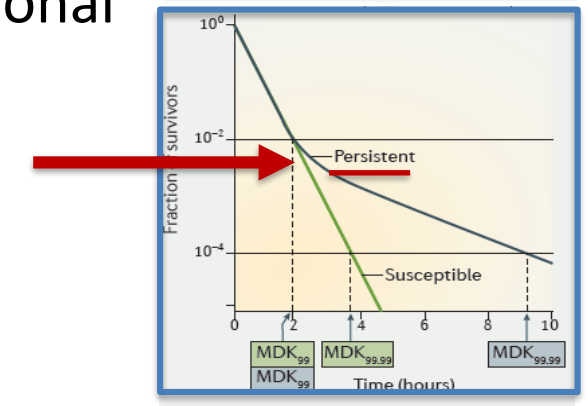
I. Tolerance. Whole bacterial population survives **transiently** exposure to lethal antibiotic concentrations, via **slowing down an essential bacterial process:**

No change in the MIC



II. Persistence. Phenotypic phenomenon, in which a **subpopulation** of a clonal bacterial population survives lethal antibiotic concentrations

No genetic changes → non-inherited



III. Heteroresistance. Heritable for sufficient generations

Stably elevated MICs, antibiotic concentration inhibiting all bacteria is **≥ 8-fold** the highest concentration not affecting **the dominant population**

Multiple Definitions/Descriptions for Heteroresistance

El-Halfawy and Valvano, Clin Microbiol Rev 2015;28:191–207

- Subset of the microbial population is resistant but the rest susceptible,

  based on (doubling or stepwise) concentrations in *in vitro* susceptibility testing

- Definitions based on **single cutoff concentrations**

- **Bimodal growth** in Population Analyses or diffusion assays: growth inhibition with a low concentration but another peak of growth at higher concentration

- **Clinical Descriptions:** "heteroresistance" is referred to infections with genetically related bacterial strains with different levels of resistance

Due to the lack of a uniform/consistent definition,

retrospective comparisons to assess its true clinical significance are impossible

Which are the Clinical Implications of Heteroresistance?

I. **Diagnostic implications**

a. Correct identification of susceptibility status

b. Correct identification of underlying resistance mechanisms

II. **Potential for selection and spread of resistant mutants**

III. **Implications for treatment outcome**

What do we know about heteroresistance and persistence?

Gram-positives:

- **Heterogeneous MRSA** (Ryffel et al AAC 1994)
- **Heterogeneous VISA** (Wootton et al JAC 2001)
- **Vancomycin-heteroresistant *E. faecium*** (Alam et al JCM 2001)
- **Teicoplanin-heteroresistant *E. faecium*** (Qu et al JCM 2009)
- **Heteroresistance to fosfomycin/penicillin in *S. pneumoniae*** (Engel et al AAC 2013)

Heteroresistance Issues in Gram-Negatives:

β-lactams

- **Heteroresistance to carbapenems in:**
  A. baumannii (Pournaras et al J Antimicrob Chemother 2005)
  P. aeruginosa (Pournaras et al J Med Microbiol 2007)
  E. aerogenes (Gordon et al J Clin Microbiol 2009)
  K. pneumoniae-KPC (Pournaras et al J Clin Microbiol 2010)

- **Heteroresistance to piperacillin/tazobactam in *P. aeruginosa***
  (Pournaras et al J Antimicrob Chemother 2008)

- **Heteroresistance to ampicillin/sulbactam in *A. baumannii***
  (Savini et al J Infect 2009)

- **Heteroresistance to cephalosporins and penicillins in *A. baumannii*** (Hung et al J Clin Microbiol 2012)

Heteroresistance in Gram-Negatives:

✓ Colistin

- Heteroresistance to colistin in ***A. baumannii*** (Li et al JCM 2006)
- Heteroresistance to colistin in *K. pneumoniae* (Meletis et al JAC 2011)
- Heteroresistance to colistin in *E. aerogenes* and *E. cloacae* (Landman et al JCM 2013)

✓ Aminoglycosides

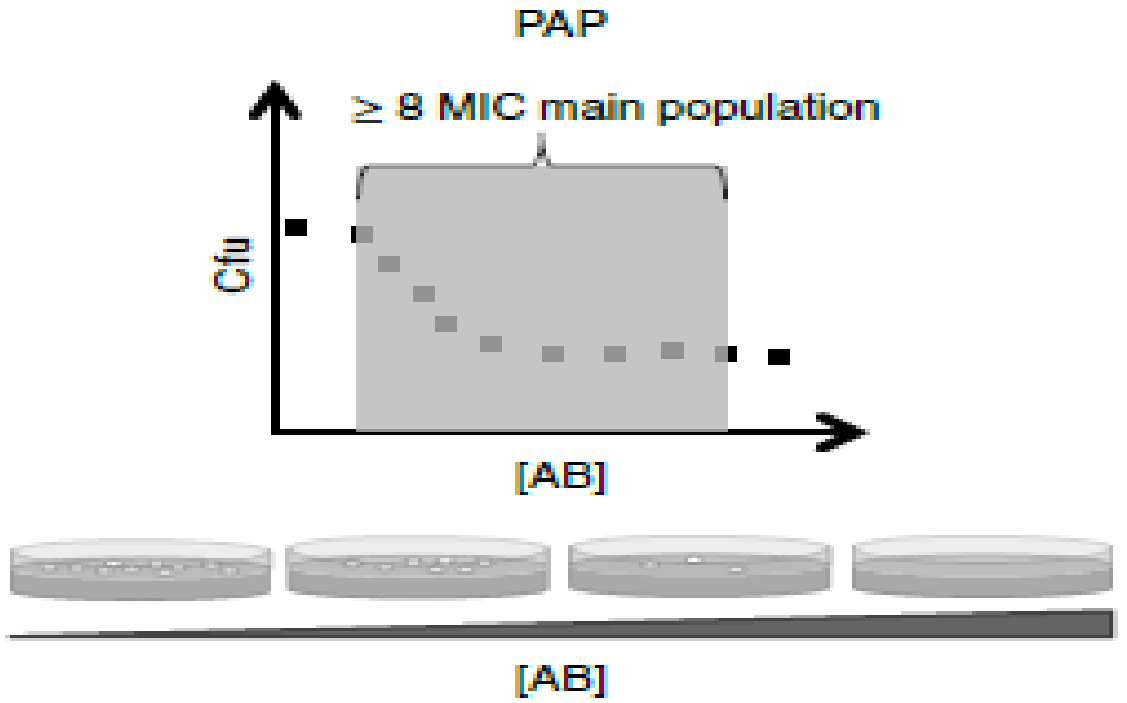
- Heteroresistance to aminoglycosides in ***A. baumannii*** (Anderson et al mSphere 2018)

Bacterial Heterogeneity
Microbiological Investigation:

- **Population analyses (gold standard):**

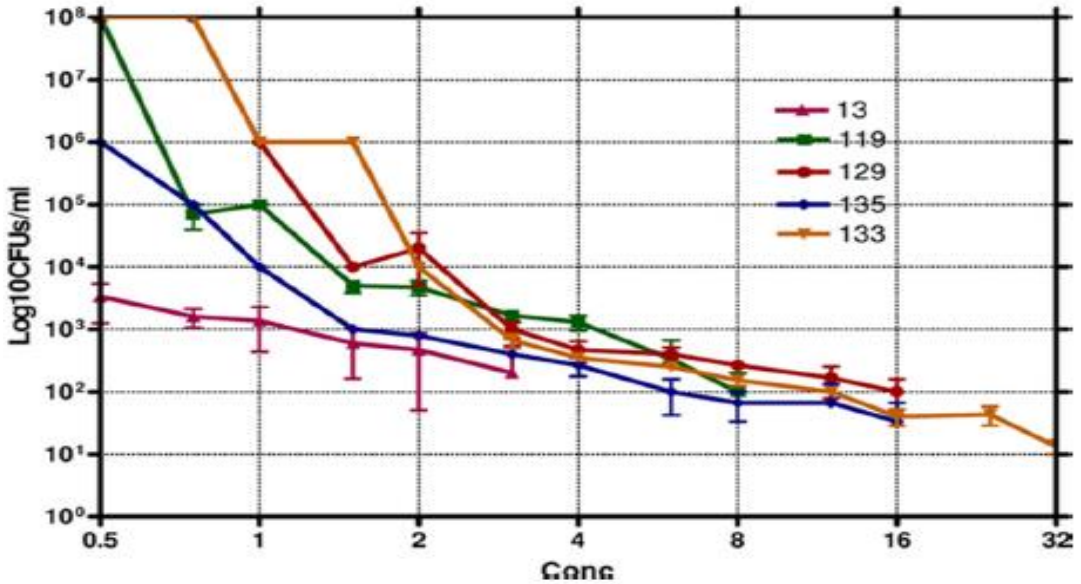
  quantification of bacterial growth in doubling antibiotic increments

- **Time-Kill curves**
- **Growth rates**
- **Experimental infections**

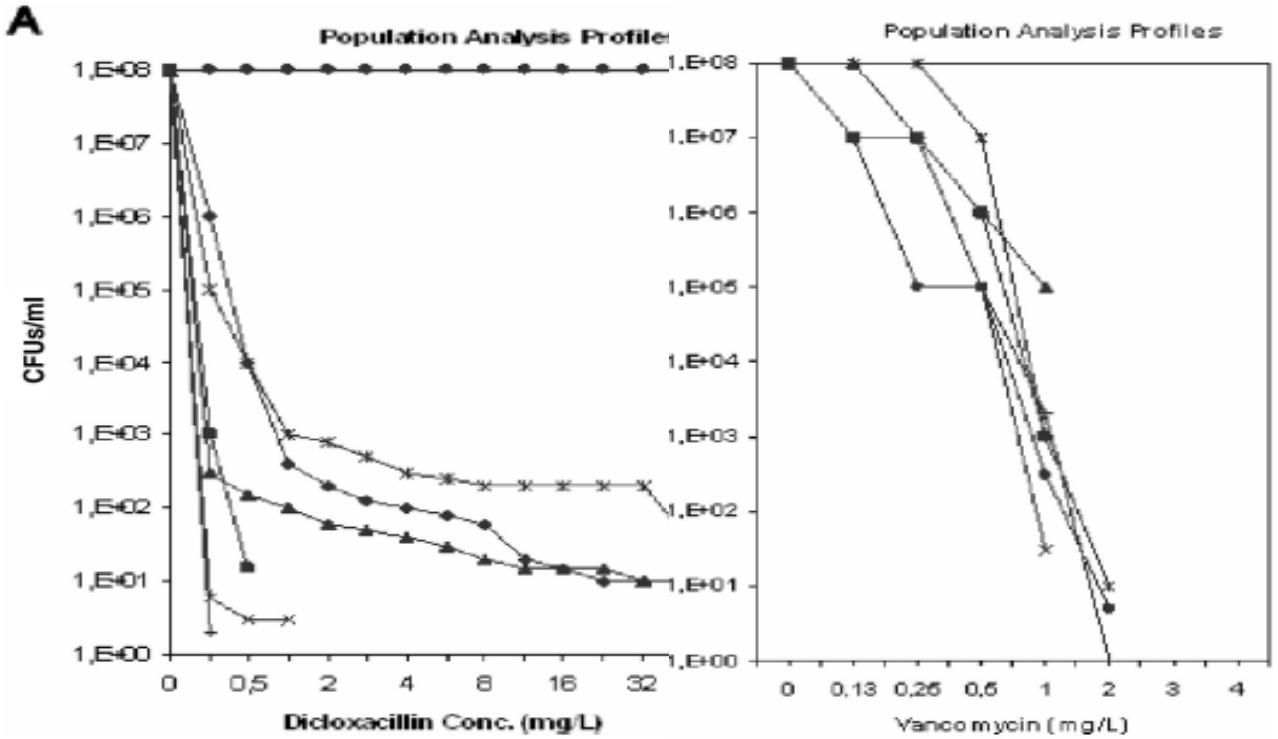


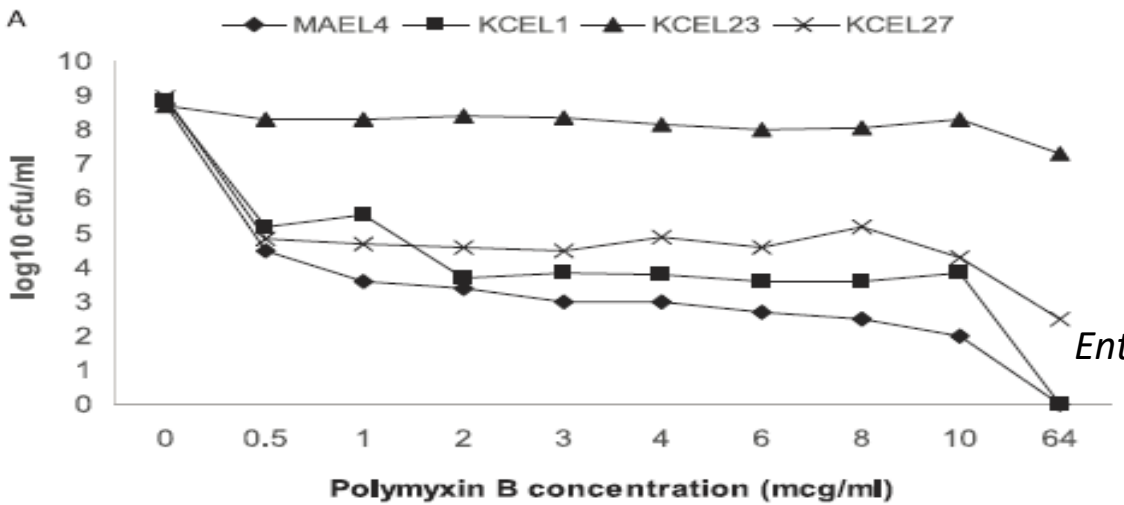
Population Analyses

Acinetobacter & Meropenem

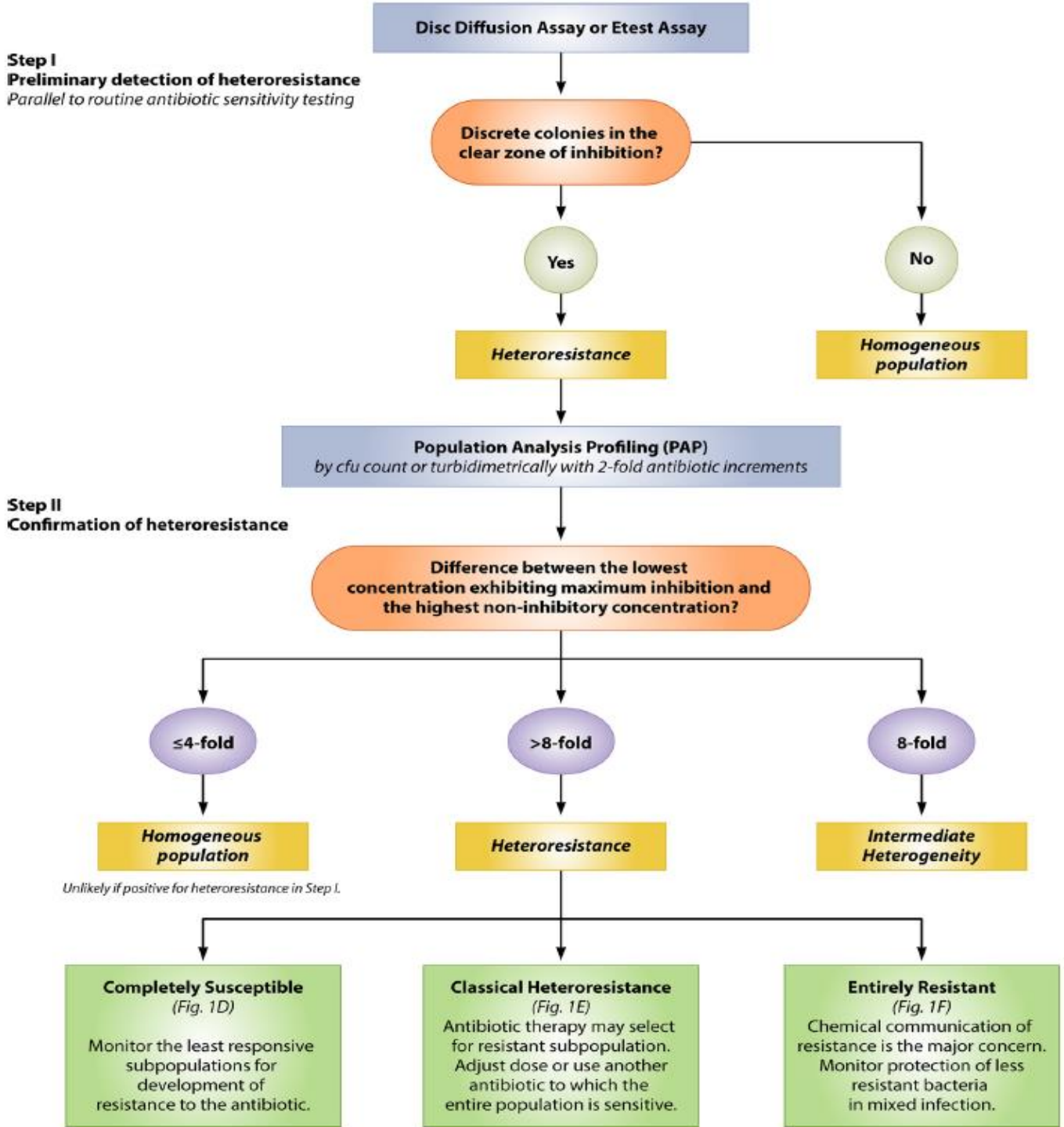


MRSA & Oxacillin / Vancomycin





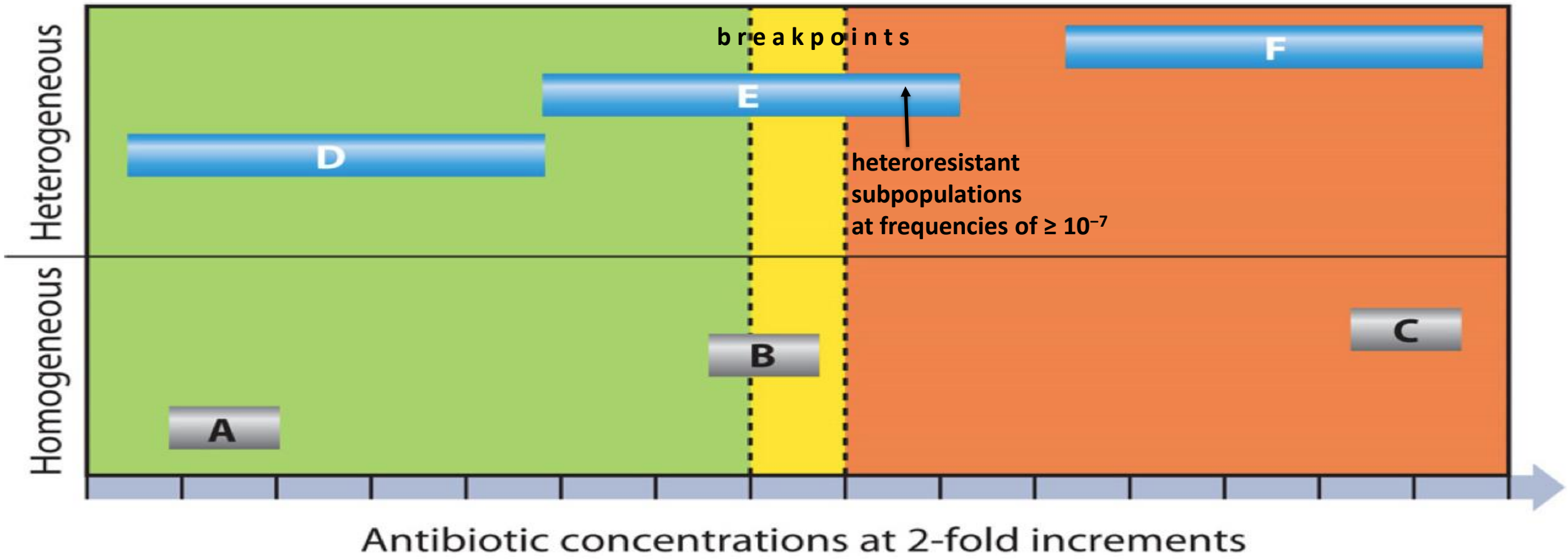
Enterobacter & polymyxin B



El-Halfawy and Valvano
Clin Microbiol Rev 2015

El-Halfawy and Valvano Clin Microbiol Rev 2015

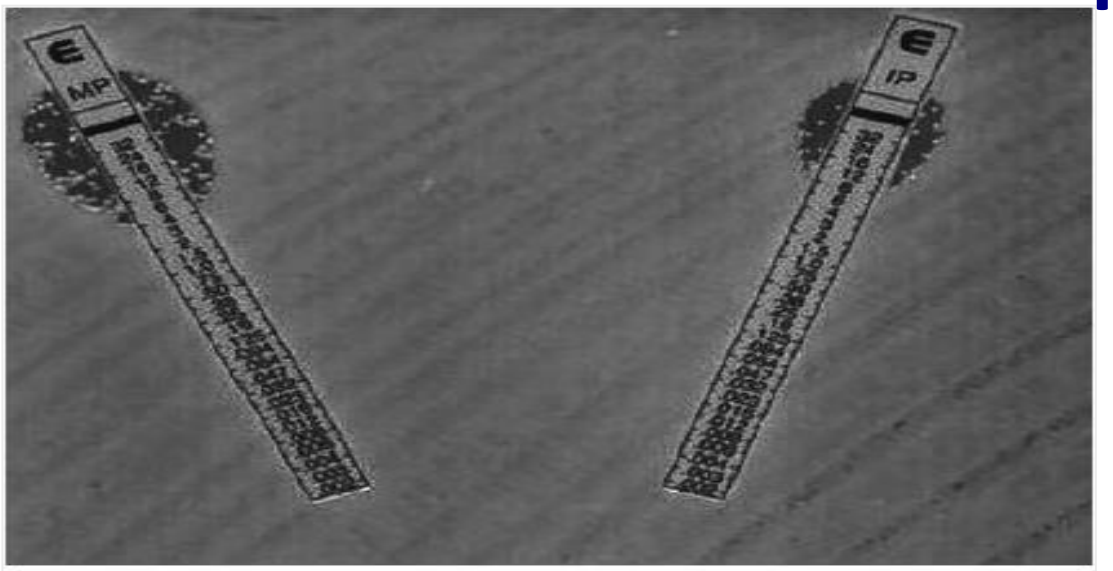
FIG 1 Heteroresistant versus homogeneous responses to antibiotics.



Why to focus on *Acinetobacter baumannii* ?

↓

Gradual evolution of *A. baumannii* to panresistance



Pournaras et al J Antimicrob Chemother 2005

The paradigm of carbapenem resistance in *A. baumannii*, Greece

Was carbapenem resistance developed step by step..(?)

Possible role of heteroresistance..

Multiresistant *Acinetobacter baumannii* isolates in intensive care units in Greece

A. N. Maniatis[1,], S. Pournaras[1], S. Orkopoulou[2], P. T. Tassios[2], N. J. Legakis[2] and the Bacterial Resistance Study Group*

Clin Microbiol Infect 2003; 9: 547–553

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range	Percentage of resistant isolates
Amikacin	8	64	1–64	29.8
Ampicillin–sulbactam	16	128	4–128	58.7
Aztreonam	128	128	1–128	93.4
Ceftazidime	32	32	4–128	95.9
Ciprofloxacin	4	4	0.5–128	92.6
Gentamicin	16	16	1–32	87.6
Imipenem	2	4	1–4	0
Netilmicin	16	32	1–128	56.2
Piperacillin	128	128	8–256	97.5
Ticarcillin–clavulanate	128	128	2–128	95.9
Tobramycin	16	16	1–32	72.7

In vitro activity of 11 antimicrobial agents against 121 *A. baumannii* isolates recovered throughout Greece between **1995-1998**

A. baumannii, nationwide data, **Greece**

% resistance to imipenem by hospital (Jan - Dec 2000)

A. Wards			
Hospitals	**No of Isolates**	**% R**	**% I**
GR027	17	0	6
GR040	177	0	1
GR009	2	0	0
GR046	4	0	0
GR035	25	0	16
GR015	40	0	0
GR012	30	0	0
GR024	13	0	0
GR026	40	0	0
GR007	28	4	0
GR018	25	4	0
GR005	42	5	0
GR042	30	20	10
GR038	12	25	0
GR033	65	26	11
GR013	14	36	14
GR044	2	50	0
GR008	2	50	0
Cumulative	**568**	**6,5**	**3,2**

B. ICU			
Hospitals	**No of Isolates**	**% R**	**% I**
GR027	24	17	4
GR040	203	1	1
GR046	7	0	0
GR035	23	4	17
GR015	5	20	0
GR012	31	0	0
GR024	7	0	0
GR007	27	4	0
GR018	17	0	0
GR005	42	2	0
GR038	19	26	5
GR033	46	15	9
GR013	26	42	12
Cumulative	**477**	**6,9**	**3,0**

A. baumannii bacteremias, Greece

2004

Drug	Medical Wards Isolates tested: 62		
	%NS	%R	%I
Ampicillin / Sulbactam	52,5	22,5	30,0
Piperacillin	90,9	76,4	14,5
Piperacillin / Tazobactam	76,4	56,4	20,0
Ceftazidime	80,0	67,7	12,3
Aztreonam	93,5	83,9	9,7
Cefepime	77,8	70,4	7,4
Imipenem	26,2	16,9	9,2
Meropenem	33,3	28,9	4,4
Gentamicin	67,7	61,5	6,2
Tobramycin	65,5	51,7	13,8
Amikacin	69,2	64,6	4,6
Netilmicin	69,2	61,5	7,7
Ciprofloxacin	80,0	80,0	0,0

2009

Drug	Medical Wards			
	Isolates Tested	%NS	%R	%I
Piperacillin	98	98,0	87,8	10,2
Piperacillin/Tazobactam	102	90,2	82,4	7,8
Ampicillin/Sulbactam	66	57,6	31,8	25,8
Aztreonam	94	97,9	92,6	5,3
Ceftazidime	104	87,5	84,6	2,9
Cefepime	103	87,4	69,9	17,5
Imipenem	103	75,7	67,0	8,7
Meropenem	103	59,2	30,1	29,1
Gentamicin	105	62,9	51,4	11,4
Tobramycin	101	54,5	20,8	33,7
Amikacin	98	65,3	58,2	7,1
Netilmicin	64	73,4	68,8	4,7
Cotrimoxazole	105	87,6	86,7	1,0
Ciprofloxacin	105	88,6	88,6	0,0

2013

Drug	Medical Wards			
	Isolates Tested	%NS	%R	%I
Cefepime	78	87,2	84,6	2,6
Imipenem	82	81,7	81,7	0,0
Meropenem	77	77,9	76,6	1,3

Increacing incidence

of *A. baumannii* infections

Incidence of *A. baumannii* infections, Europe

Annual Epidemiological Report 2016 – Healthcare-associated infections acquired in intensive care units. Stockholm: ECDC; 2016

***A. baumannii* bacteremias, Rank 9th in Europe overall**

Table 3. Number of isolates and percentages of the ten most frequently isolated microorganisms in ICU-acquired bloodstream infection (BSI) episodes by country, EU/EEA, 2014

Microorganism	Belgium (n=27)	Czech Republic (n=95)	Estonia (n=77)	France (n=1 300)	Germany (n=2 188)	Hungary (n=17)	Italy (n=85)	Lithuania (n=45)	Luxembourg (n=18)	Malta (n=22)	Portugal (n=207)	Romania (n=49)	Slovakia (n=15)	Spain (n=890)	United Kingdom (n=65)	Total (n=5 588)
Coagulase-negative staphylococci	18.5	36.4	32.5	20.2	27.5	29.4	38.8	43.9	22.2	0	13.5	6.1	26.7	27.6	20	25.3
Enterococcus spp.	14.8	15.6	18.2	12.4	17.7	0	7.1	5.3	27.8	4.5	11.6	16.3	0	15.1	13.8	15
Staphylococcus aureus	22.2	15.6	3.9	11.9	16.5	5.9	1.2	8.8	5.6	0	15	0	6.7	2.7	18.5	12.1
Klebsiella spp.	3.7	24.7	7.8	11.8	8.1	5.9	14.1	8.8	5.6	36.4	15	22.4	33.3	10.3	10.8	10.3
Escherichia coli	18.5	7.8	3.9	10.8	9.7	5.9	2.4	3.5	5.6	9.1	7.7	0	6.7	7.6	9.2	9.2
Candida spp.	7.4	7.8	18.2	9.2	8	0	9.4	5.3	5.6	9.1	4.8	4.1	6.7	8.4	12.3	8.4
Pseudomonas aeruginosa	3.7	10.4	3.9	9.8	4.4	29.4	8.2	3.5	5.6	18.2	11.1	8.2	13.3	13.7	4.6	8
Enterobacter spp.	0	0	9.1	10.4	4.9	17.6	3.5	3.5	11.1	13.6	8.7	0	0	6	6.2	6.7
Acinetobacter spp.	0	1.3	1.3	1.3	0.8	5.9	14.1	15.8	5.6	9.1	7.2	40.8	6.7	5.4	4.6	2.5
Serratia spp.	11.1	3.9	1.3	2	2.3	0	1.2	1.8	5.6	0	5.3	2	0	3.1	0	2.5

A. baumannii bacteremias, Greece: Rank 1st among carbapenem-resistant pathogens…

ICU (incidence per 10,000 patient days)	
Isolated organism	***Weighted Average**
Acinetobacter baumannii	39,70
» A. baumannii [Carbapenem resistant]	39,22
Pseudomonas aeruginosa	24,49
» P. aeruginosa [Carbapenem resistant]	11,80
Klebsiella pneumoniae	39,83
» K. pneumoniae [Carbapenem resistant]	35,03
Staphylococcus aureus	5,04
» MRSA	2,40

All ward types (incidence per 10,000 patient da	
Isolated organism	***Weighted Average**
Acinetobacter baumannii	3,10
» A. baumannii [Carbapenem resistant]	2,95
Pseudomonas aeruginosa	2,59
» P. aeruginosa [Carbapenem resistant]	1,05
Klebsiella pneumoniae	4,19
» K. pneumoniae [Carbapenem resistant]	2,86
Staphylococcus aureus	2,35
» MRSA	0,93

also... A. baumannii infections in Taiwan, 2015

Rank 1st (bacteremias) and 4th overall

Taiwan Nosocomial Infections Surveillance System

Table 14 Common pathogens of healthcare-associated infections in the ICUs of regional hospitals, 2015

Pathogens	Total		Types of Infection									
			Urinary tract		Bloodstream		Pneumonia		Surgical site		Others	
	Rank	No.	Rank	No.	Rank	No.	Rank	No.	Rank	No.	Rank	No.
Escherichia coli	1	561	1	397	7	91	6	37	4	24	10	12
Candida albicans	2	512	2	321	3	121	8	24	8	18	3	28
Klebsiella pneumoniae	3	511	4	155	2	160	3	143	2	28	5	25
Acinetobacter baumannii	4	504	8	85	1	177	2	177	7	20	1	45
Pseudomonas aeruginosa	5	499	3	158	7	91	1	188	1	35	4	27
Staphylococcus aureus	6	275	13	25	4	117	4	84	3	26	6	23
Enterococcus faecium	7	240	6	110	6	97	27	1	9	10	7	22
Other *Candida* spp. or NOS	8	216	5	127	9	79	15	4	16	2	14	4
Enterobacter species	9	192	10	43	10	70	5	42	4	24	9	13
E.cloacae		141		33		58		20		19		11
Other *Enterobacter* spp. or NOS		51		10		12		22		5		2
Coagulase negative staphylococci	10	176	12	26	5	102	20	3	9	10	2	35
Others		1,152		365		489		141		81		76
Total		4,838		1,812		1,594		844		278		310

and… *A. baumannii* infections in China, 2015:

Rank 2nd overall

A point-prevalence survey of healthcare-associated infection in fifty-two Chinese hospitals

Journal of Hospital Infection 95 (2017) 105–111

Reported causative pathogens, according to the major types of infection

Pathogen	All HCAIs (*N* = 2182)	Rank	Low respiratory tract infection (*N* = 1029)	Urinary tract infection (*N* = 268)	Upper respiratory tract infection (*N* = 240)	Surgical site infection (*N* = 135)	Skin and soft-tissue infection (*N* = 113)	Primary bloodstream infection (*N* = 96)
Pseudomonas aeruginosa	206 (9.4%)	1	147 (14.3%)	13 (4.9%)	3 (1.3%)	12 (8.9%)	16 (14.2%)	5 (5.2%)
Acinetobacter baumannii	172 (7.9%)	2	131 (12.7%)	4 (0.0%)	5 (2.1%)	8 (5.9%)	8 (7.1%)	4 (4.2%)
Klebsiella pneumoniae	160 (7.3%)	3	120 (11.7%)	16 (6.0%)	0	1 (0.7%)	7 (6.2%)	10 (10.4%)
Escherichia coli	145 (6.6%)	4	45 (4.4%)	53 (19.8%)	4 (1.7%)	11 (8.1%)	3 (2.7%)	11 (11.5%)
Staphylococcus aureus	110 (5.0%)	5	49 (4.8%)	5 (1.9%)	1 (0.4%)	15 (11.1%)	26 (23.0%)	5 (5.2%)
Candida spp.	95 (4.4%)	6	51 (5.0%)	19 (7.1%)	3 (1.3%)	3 (2.2%)	0	7 (7.3%)
Coagulase-negative *Staphylococcus* spp.	72 (3.3%)	7	23 (2.2%)	5 (1.9%)	2 (0.8%)	10 (7.4%)	7 (6.2%)	20 (20.8%)
Enterococcus species	68 (3.1%)	8	10 (1.0%)	36 (13.4%)	0	4 (3.0%)	6 (5.3%)	2 (2.1%)
Enterobacter cloacae	31 (1.4%)	9 (tie)	15 (1.5%)	1 (0.4%)	0	5 (3.7%)	4 (3.5%)	1 (1.0%)
Stenotrophomonas maltophilia	31 (1.4%)	9 (tie)	25 (2.4%)	1 (0.4%)	0	1 (0.7%)	1 (0.9%)	1 (1.0%)
Bacillus proteus	29 (1.3%)	11	14 (1.4%)	10 (3.7%)	0	1 (0.7%)	3 (2.7%)	2 (2.1%)
Serratia spp.	19 (0.9%)	12	5 (0.5%)	4 (1.5%)	0	4 (3.0%)	3 (2.7%)	1 (1.0%)
Streptococcus pneumoniae	12 (0.5%)	13	7 (0.7%)	2 (0.7%)	3 (1.3%)	0	0	0
Burkholderia cepacia	11 (0.5%)	14	7 (0.7%)	0	0	2 (1.5%)	1 (0.9%)	0

Current mechanistic data on heteroresistance...

The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification

Nature Microbiology. 2019

Hervé Nicoloff[1,3], Karin Hjort[1,3], Bruce R. Levin[2] and Dan I. Andersson[1*]

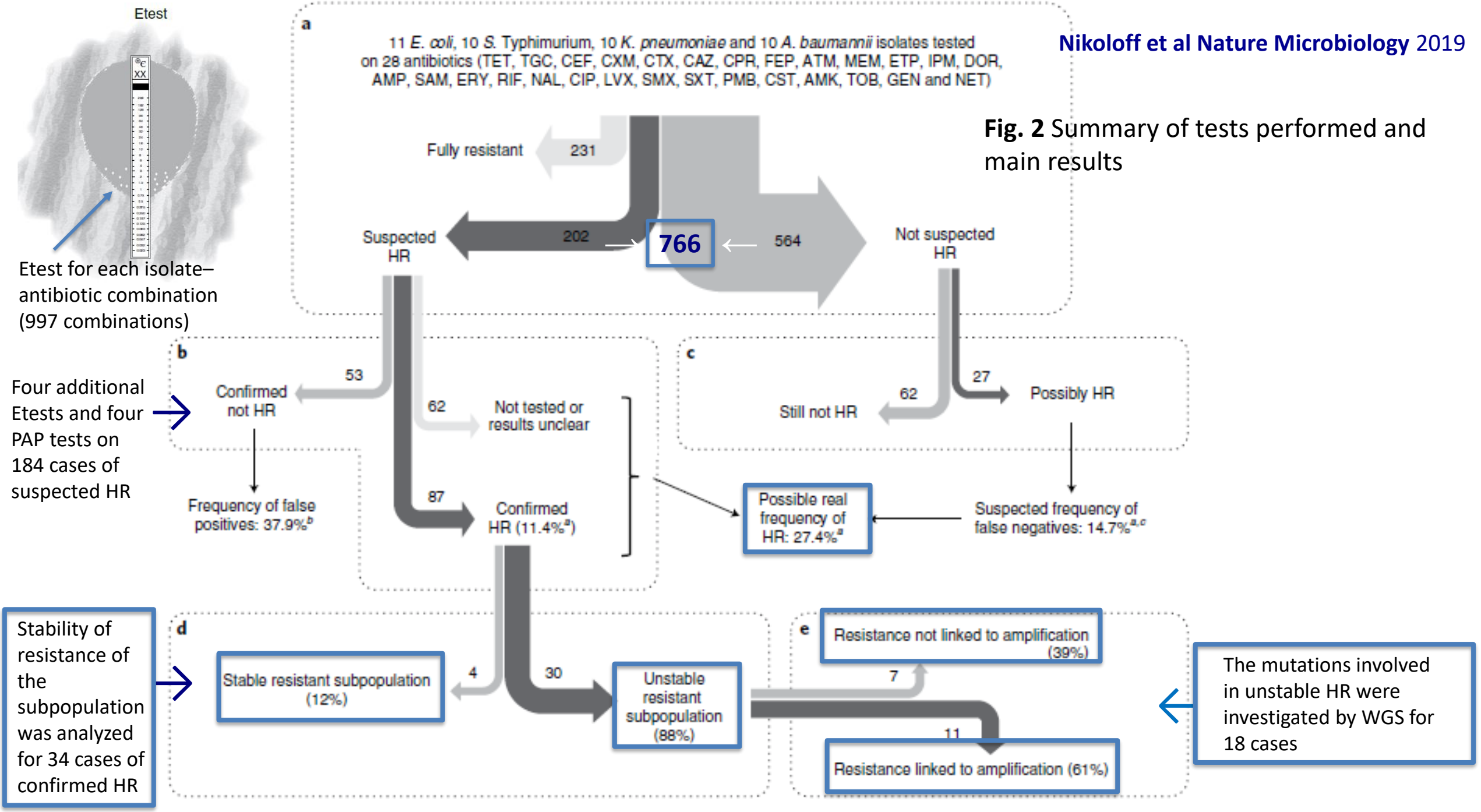
- **Prevalence and mechanisms of heteroresistance** (HR) investigated in 41 clinical isolates of *E. coli*, *S. enterica*, *K. pneumoniae* and ***A. baumannii*** **(10 isolates) against 28 different antibiotics**

- **27.4% of the 766 bacteria–antibiotic combinations was heteroresistant!**

- **Genetic analysis showed that most HR cases (88%) were unstable – 12% stable**

- Resistance in the subpopulations resulted from **spontaneous tandem amplifications**, typically including known resistance genes

- Limitations of MIC as sole criterion for susceptibility determinations

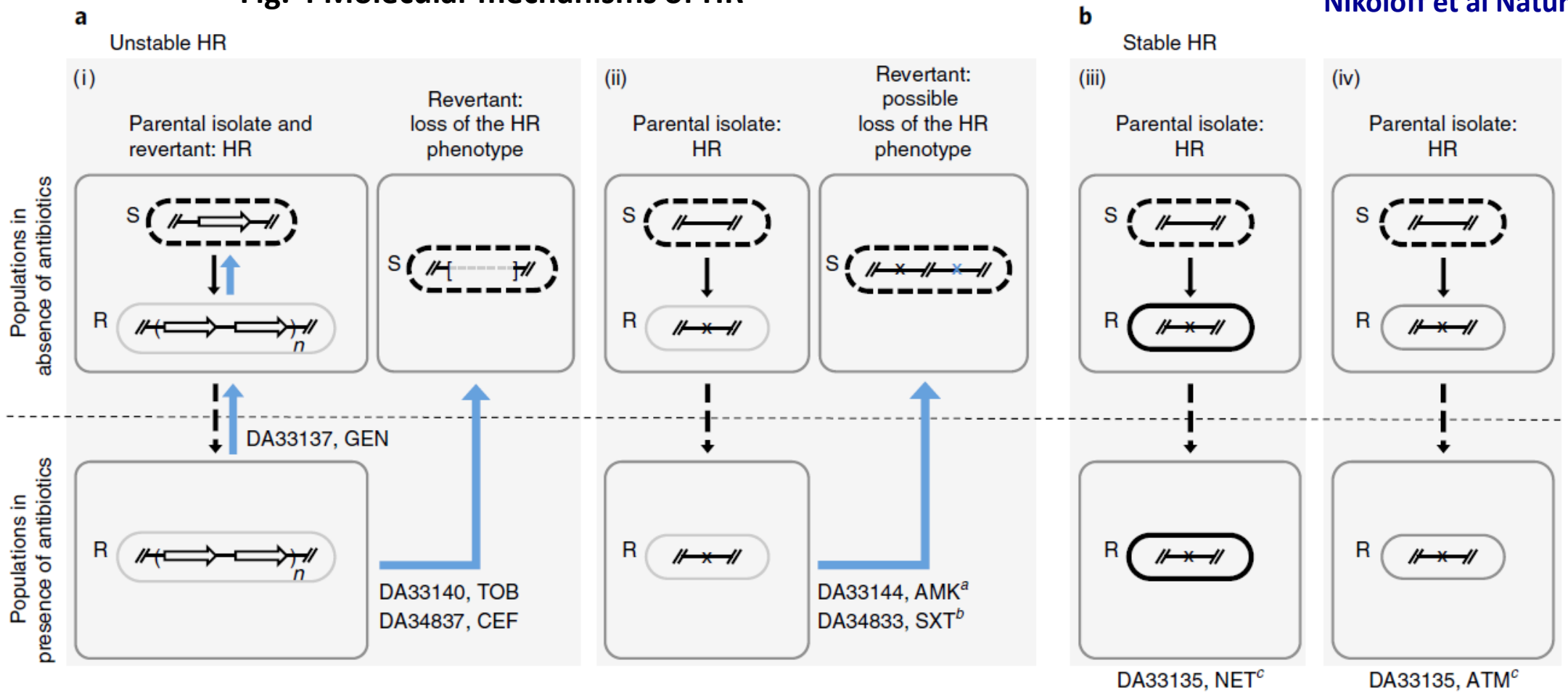


Nikoloff et al Nature Microbiology 2019

Fig. 2 Summary of tests performed and main results

Fig. 4 Molecular mechanisms of HR

Nikoloff et al Nature Microbiology 2019



a, (i) **Unstable HR, linked to genetic** amplifications or (ii) other types of mutations.

b, Stable HR. HR is stable if the mutation increasing resistance has no measurable effect on fitness.

HR is stable if (iii) the mutation increasing resistance has no measurable effect on fitness

or

(iii, iv) if the reversion rate or the effect on fitness is too low to allow revertants to take over rapidly during growth in the absence of antibiotic

Heteroresistance in *A. baumannii*:

Available descriptions in the literature…

Heteroresistance to carbapenems in *Acinetobacter baumannii*

Pournaras et al. J Antimicrob Chemother 2005

- *A. baumannii* with **subcolonies present in the zone of inhibition**
- When re-testing resistant colonies, carbapenem MICs were the same with those of the original clinical isolate
- Again, heterogeneous subpopulations were grown in the inhibition halo → **persistence**

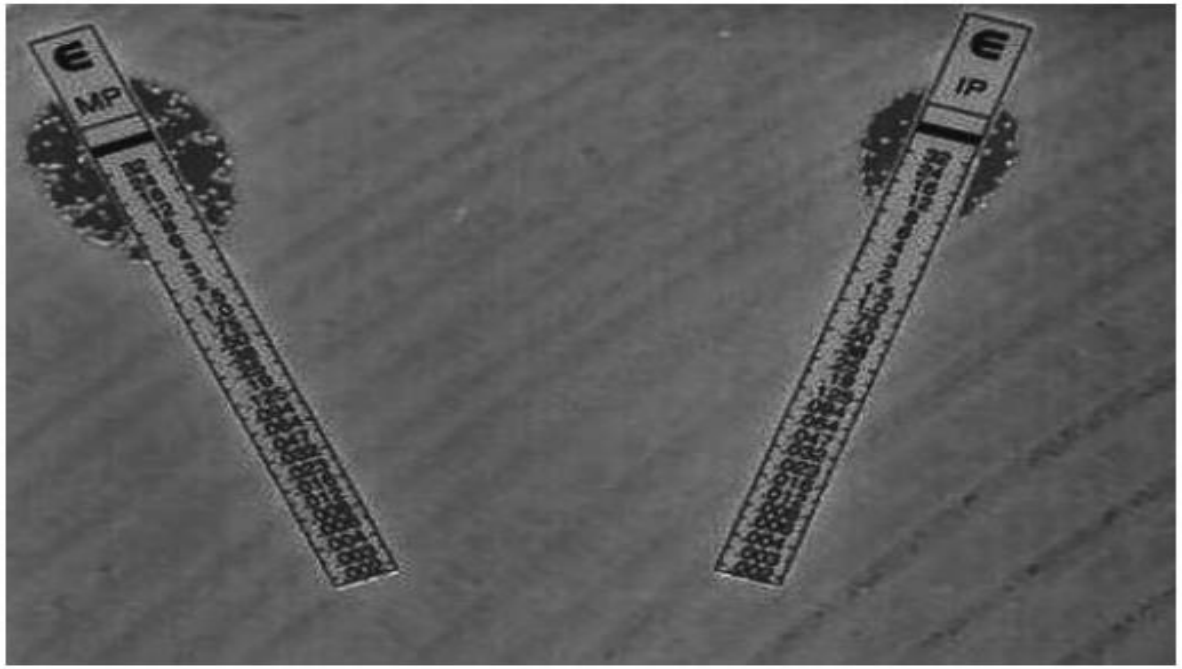


Figure 1. Imipenem and meropenem Etests on *A. baumannii* showing resistant subpopulation. In this particular instance, imipenem and meropenem MICs were 12 and 6 mg/L, respectively, but a subpopulation of resistant cells was grown at up to an imipenem or meropenem concentration of > 32mg/L.

Heteroresistance to Meropenem in Carbapenem-Susceptible *Acinetobacter baumannii*

Ikonomidis et al. J Clin Microbiol 2009

- **Characteristics of carbapenem heteroresistance were studied in**

  14 apparently carbapenem-susceptible *A. baumannii:*

  imipenem and meropenem MICs of the native isolates = 0.25-4 mg/L

- PAP: subpopulations grown in the presence of imipenem up to 8 mg/L and **meropenem up to 32 mg/L**

- **Meropenem-heteroresistant subpopulations of 11 isolates exhibited**

  stable resistance: MICs 16 to >32 mg/L

Heteroresistance to Meropenem in Carbapenem-Susceptible *Acinetobacter baumannii*[▽]

Ikonomidis et al. J Clin Microbiol. 2009; 47:4055-96

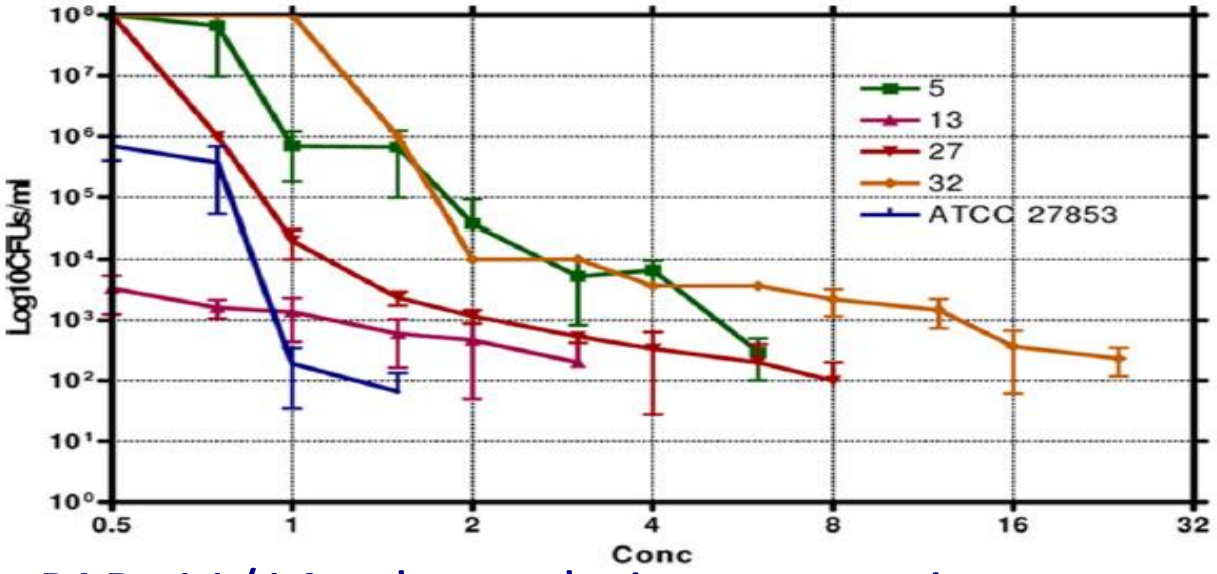
TABLE 2. Characteristics of the tested isolates

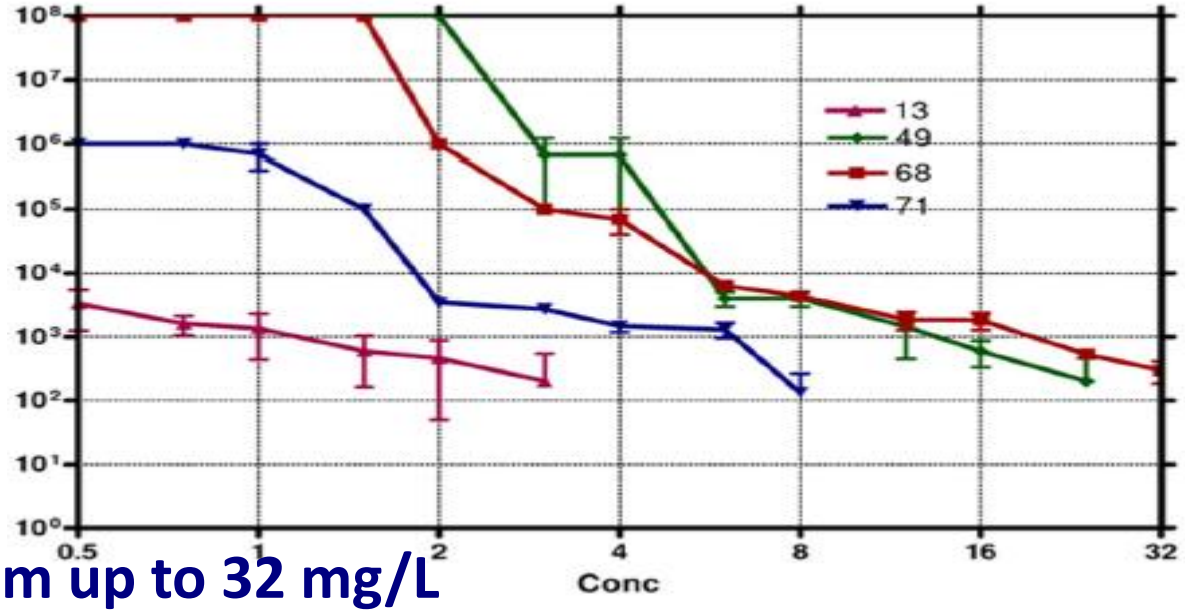
Isolate	Date of isolation (day/mo/yr)	Ward	Specimen	*bla*$_{OXA-51-like}$ allele	Allelic profile[a]	ST type[b]	PFGE type	Agar dilution MIC (mg/liter)		Highest concn of growth in population analyses (mg/liter)		Agar MIC (mg/liter) of heterogenous subpopulations in[c]:			
												IPM		MEM	
								IPM[d]	MEM[e]	IPM	MEM	IPM	MEM	IPM	MEM
AB5	09/10/07	Cardiology	Sputum	66	1-1-1	1	I	2	4	4	8	4	2	2	32
AB13	28/09/07	Medical	Decubitus ulcer	66	1-1-1	1	VI	2	0.5	2	2	2	0.5	4	4
AB27	15/09/07	ICU[f]	Bronchial	91	1d-4-1c*	NA	VII	0.25	1	1	8	0.5	1	0.5	>32
AB32	31/10/07	ICU	Bronchial	66	7-4-1a*	NA	V	1	2	1	8	2	1	1	16
AB49	26/11/07	Oncology	Blood	66	1a-1-1	1	I	4	4	8	16	4	2	1	32
AB68	19/12/07	Oncology	Sputum	66	1a-1-1	1	I	2	2	8	32	2	8	2	>32
AB71	28/11/07	Medical	Blood	66	1a-1-1	1	I	0.25	0.25	4	8	1	1	1	32
AB72	02/12/07	Orthopedic	Pus	94	7-2-7*	NA	II	1	2	2	8	0.5	1	1	32
AB78	07/01/08	Medical	Blood	94	7-2-10*	NA	II	1	2	2	8	2	1	2	32
AB79	16/01/08	Medical	Decubitus ulcer	69	2-2-2	2	III	0.5	1	2	8	0.5	1	1	8
AB119	07/02/08	Neurosurgery	Sputum	66	4-2-7*	NA	VIII	0.25	1	1	8	0.5	1	0.5	8
AB129	28/02/08	Neurosurgery	Blood	69	2-2-2	2	III	2	1	8	16	1	1	2	>32
AB133	27/03/08	Orthopedic	Pus	66	1-1-1	1	IX	2	2	4	32	2	1	1	>32
AB135	31/03/08	Oncology	Sputum	69	2-2-2	2	IV	1	1	4	16	1	1	1	16

[c] MICs estimated after 7 daily subcultures in antibiotic-free medium

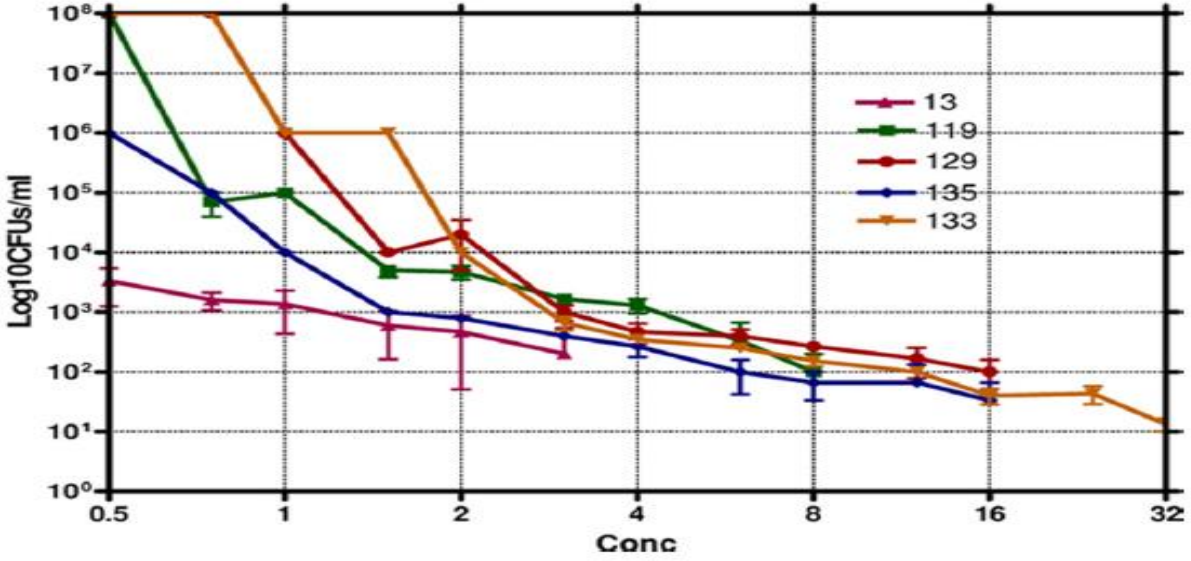
Heteroresistance to Meropenem in Carbapenem-Susceptible *Acinetobacter baumannii*

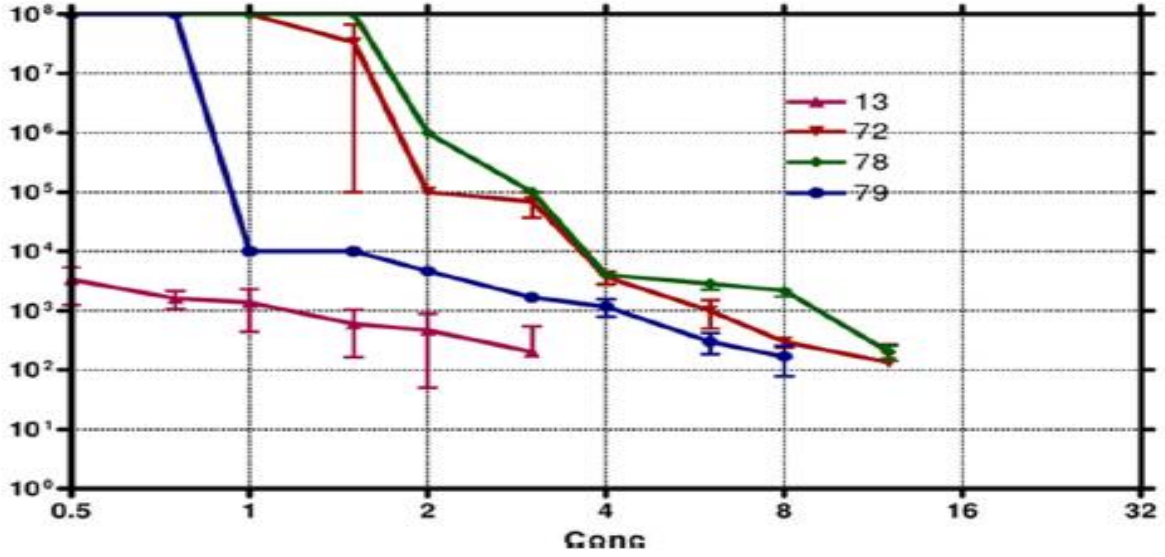
Ikonomidis et al. J Clin Microbiol. 2009; 47:4055-96





PAP: 11/14 subpopulations grown in **meropenem up to 32 mg/L**





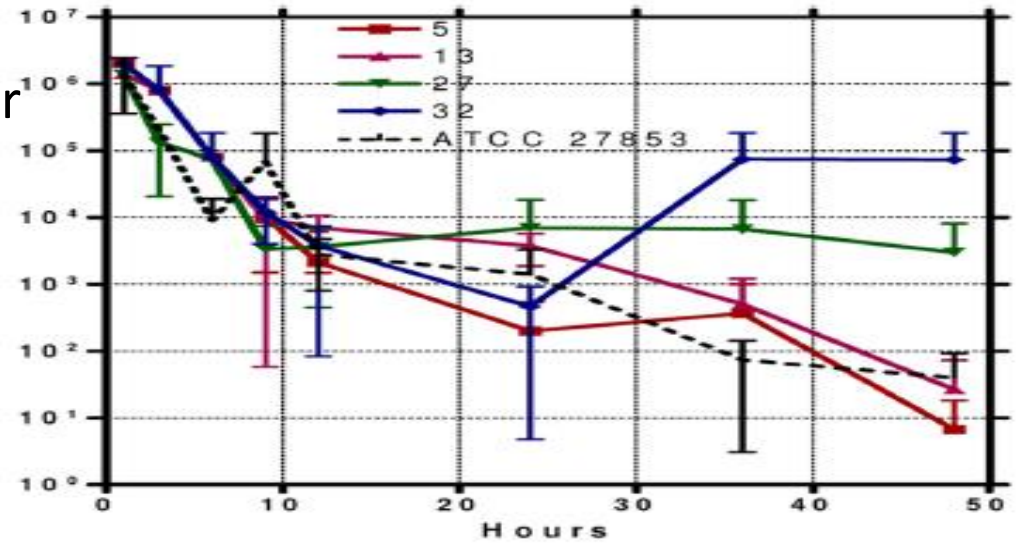
Heteroresistance to Meropenem in Carbapenem-Susceptible *Acinetobacter baumannii*

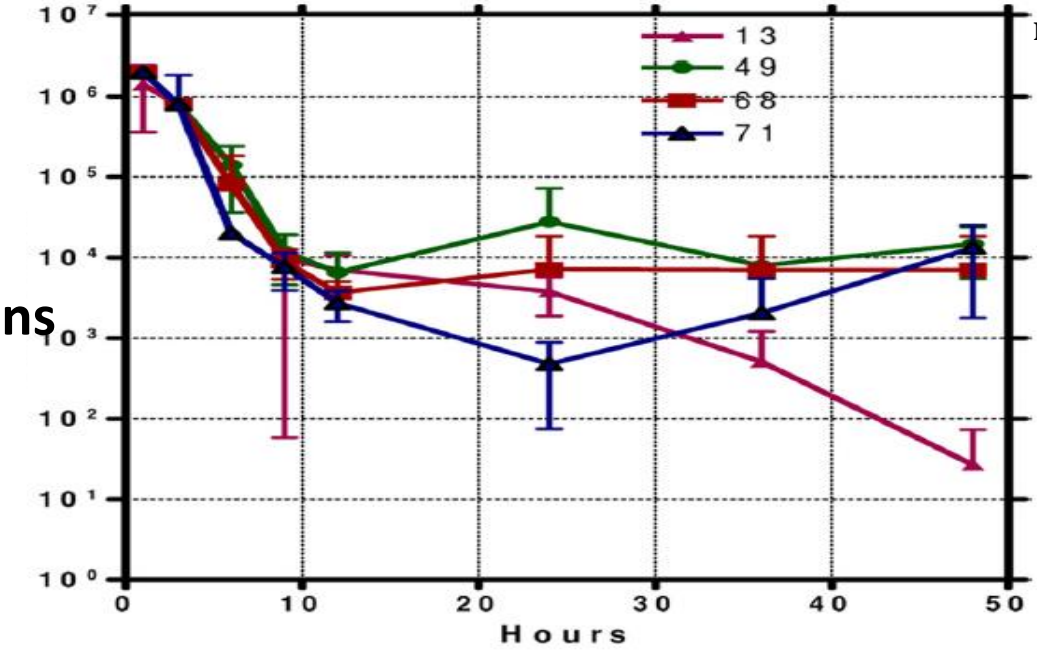
Ikonomidis et al. J Clin Microbiol 2009; 47:4055-96

- **Time-kill assays** with **meropenem:**
  4/14 isolates were killed in time-dependent manner

less pronounced killing for 10/14 isolates

- substantial regrowth after 9-12h for 3 isolates
- substantial regrowth after 24h for 7 isolates





Time-kill assays:
- inoculum 5 x 10^6 CFU/ml
- meropenem 4 mg/L

Conclusions:

- Apparently meropenem-susceptible *A. baumannii* may contain **resistant subpopulations**

- **Meropenem pressure may select highly resistant strains via heteroresistant subpopulations**

- **Potential for selection by suboptimal therapeutic dosages?**

Imipenem heteroresistance induced by imipenem in multidrug-resistant *Acinetobacter baumannii*: mechanism and clinical implications

Lee et al Int J Antimicrob Agents 2011

Retrospective case–control study, involving **clinical cases with subsequent clinical imipenem-non-susceptible MDR-AB isolates of the same genotype** and matched controls with imipenem-susceptible MDR-AB isolates

- *In vitro* experiments indicated that **imipenem heteroresistance**, associated with overexpression of bla_{ADC-29}, **could be induced by imipenem**
- **Carbapenem use was the only risk factor** identified for the **emergence of carbapenem-heteroresistant MDR-AB**

Table 3
Comparison of the length of use (days) of various antibiotics between cases and controls.

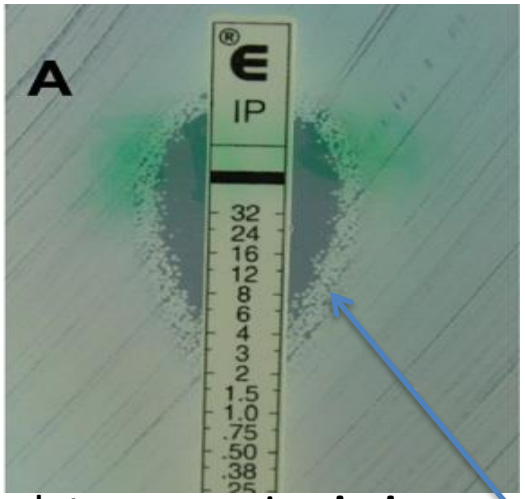
Antibiotic	Controls (n = 10)	Cases (n = 14)	P-value
Imipenem	5.33 ± 4.80	10.86 ± 6.48	0.02
Ciprofloxacin	2.33 ± 4.95	5.71 ± 6..83	0.22
Ceftriaxone	2.33 ± 4.95	2.71 ± 5.01	0.86
Ceftazidime	1.33 ± 3.04	1.00 ± 3.74	0.83
SAM	3.44 ± 5.45	3.07 ± 6.13	0.88
Moxifloxacin	0.78 ± 2.33	0.29 ± 10.60	0.49
Cefepime	0.33 ± 1.00	0.71 ± 1.98	0.60
Vancomycin	6.78 ± 6.18	11.43 ± 8.01	0.15
Amikacin	1.33 ± 4.00	1.50 ± 4.05	0.92
Ampicillin	0.00 ± 0.00	0.50 ± 1.87	0.44

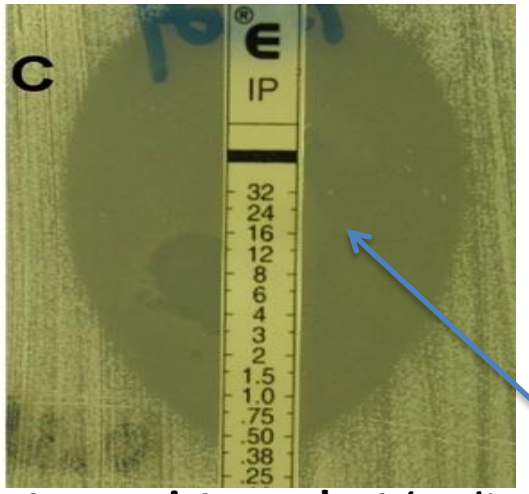
Imipenem heteroresistance induced by imipenem in multidrug-resistant *Acinetobacter baumannii*: mechanism and clinical implications

Lee et al Int J Antimicrob Agents 2011

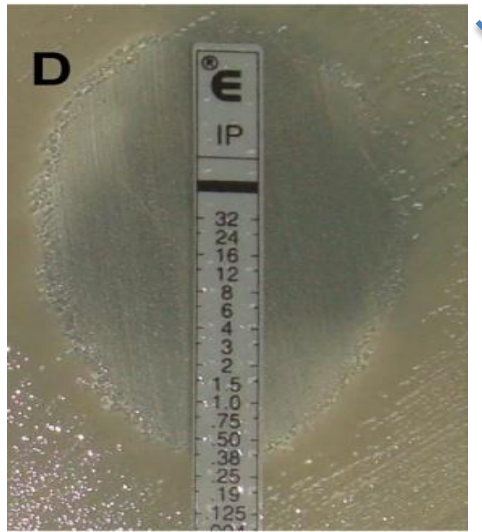
- Imipenem MICs 6–32 mg/L
  – resistant cells in the inhibition zone of Etest strips or disks
- Switch from **susceptibility** to **heteroresistance** more likely after exposure to imipenem
- Physicians should weigh risks-benefits of carbapenem treatment for carbapenem-susceptible AB infection

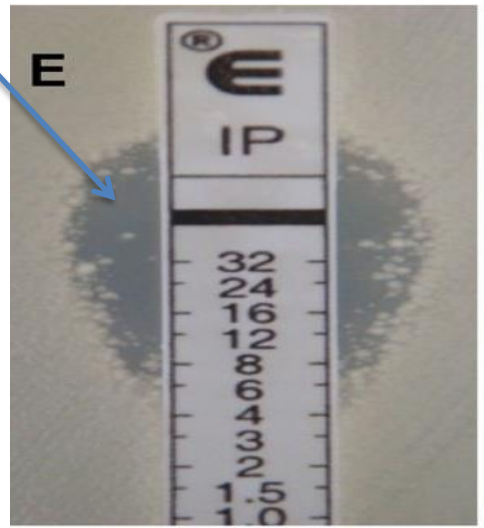
→ **Need for combination treatment to prevent heteroresistance?**





- Isolate expressing **imipenem heteroresistance lost** *in vitro* by Day 7 after serial passage in medium without imipenem
- When cultured in medium containing imipenem, the isolate **regained the heteroresistant phenotype** by Day 3





Persistence

Prevalence and analysis of microbiological factors associated with phenotypic heterogeneous resistance to carbapenems in *A. baumannii*

Fernández Cuenca et al Int J Antimicrob Agents 2012

- **Rate of phenotypic heterogeneous resistance (PHR):**
  20% to imipenem
  24% to meropenem

- Susceptibility to imipenem in 39% isolates with PHR
- Susceptibility to meropenem in 7% isolates with PHR

- MICs of carbapenems for heterogeneous colonies similar (±1 log2 dilution) to those of their parental isolates

- Colonies growing inside the inhibition halo also reproduced PHR to carbapenems
  → **persistence**

Heteroresistance to ampicillin/sulbactam

Misidentification of ampicillin/sulbactam heteroresistance in *A. baumannii* strains from ICU patients

Savini et al J Infect 2009

- Twenty genetically-unrelated MDR *A. baumannii* strains: all were susceptible to ampicillin/sulbactam (MICs 4 mg/L)
- Plates were **incubated for 48 h** (rather than 24 h)
- Interestingly, in five strains, few (≤5) colonies were observed in the amp/sul inhibition zones
- Heterogeneous colonies inside inhibition halos **had full amp/sul resistance**…
  → **heteroresistance**
- Heteroresistant colonies were **misidentified** both by automatic method (Vitek2) and by the CLSI BMD test, **having 18-24 h as endpoint for reading**

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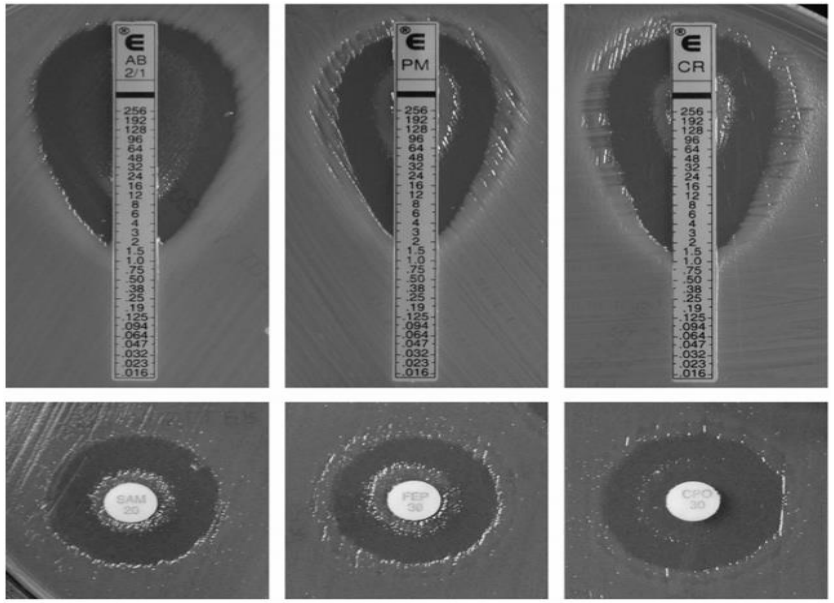
Hung et al J Clin Microbiol. 2012

Heteroresistance to Cephalosporins and Penicillins in *Acinetobacter baumannii*



Heteroresistance to ampicillin-sulbactam (AB; SAM), cefepime (PM; FEP), and cefpirome (CR; CPO) showing a distinct colony morphology of circular rings within the inhibition halos detected by the disk diffusion and Etest

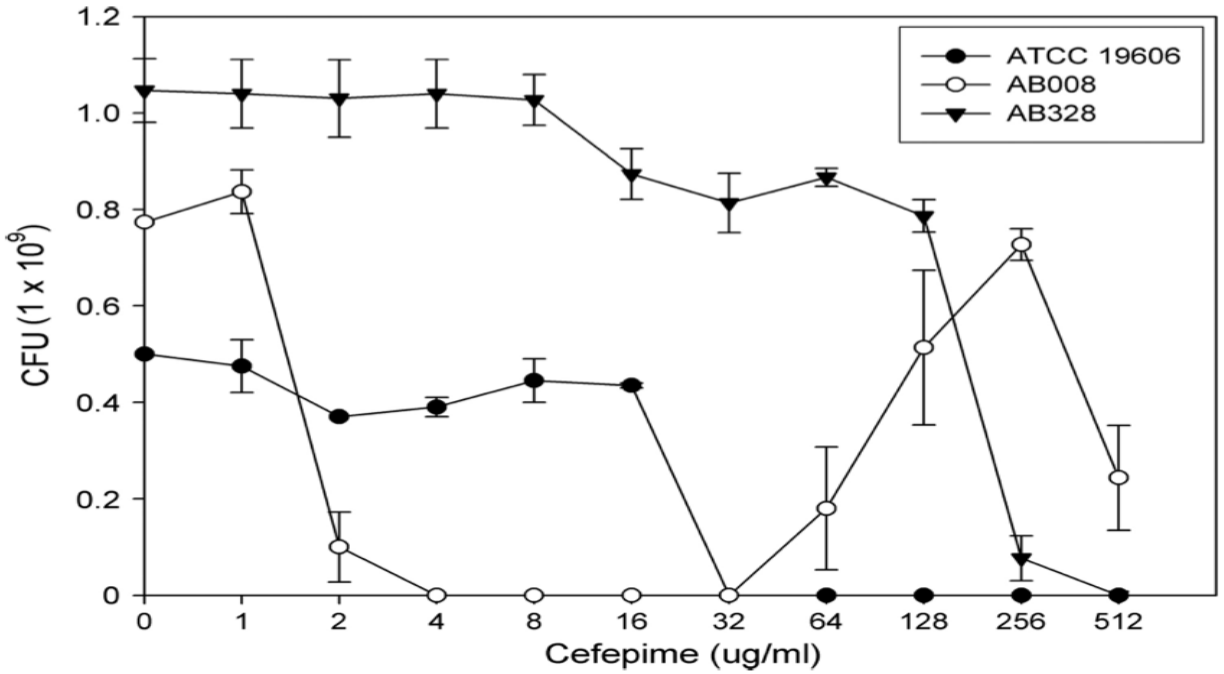


FIG 3 Population analysis profiles (PAP) for *A. baumannii* strains. Cefepime was used to select subpopulations with higher resistance levels.

High-level cefepime-resistant subpopulations of *A. baumannii* were stable:
retained their high-level resistance trait upon repeated passages in medium without cefepime

Isolate AB008 exhibited **bimodal growth in PAP with cefepime**:
- initial peak of growth at 1 µg/ml
- growth inhibition at 2--32 µg/ml
- peak of growth at 256 µg/ml

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Aminoglycoside Heteroresistance in *Acinetobacter baumannii* AB5075

Anderson et al, mSphere. 2018

- The **tobramycin-resistant subpopulation was cross-resistant to gentamicin** but not amikacin

- The increased tobramycin resistance phenotype was highly unstable: cells reverted to a less resistant population after growth on nonselective media

- Mechanistic basis of heteroresistance:
  At least two different mechanisms:
  - *aadB* gene → increased expression of *aadB*
  - *aadB*-independent mechanism

- Clinical effects of aminoglycoside heteroresistance are unknown

Persistence

Anderson et al mSphere 2018

Heteroresistance and Resistance to Colistin

a long story…

Heteroresistance to Colistin in MDR *A. baumannii*

Li et al Antimicrob Agents Chemother 2006

- Heteroresistance to colistin in 16 clinical *A. baumannii* isolates, apparently susceptible to colistin on the basis of MICs

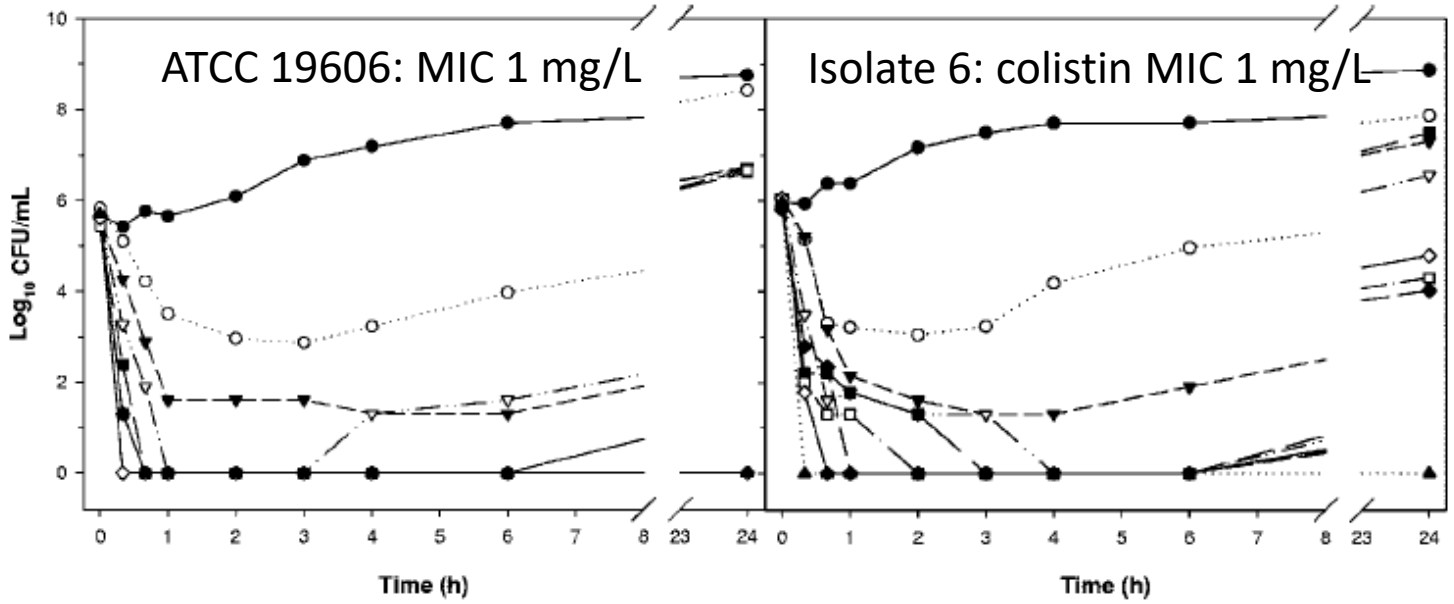
- Colistin showed early concentration-dependent killing, but **bacterial regrowth** was observed at 24 h

- PAPs revealed that **heteroresistance to colistin was prevalent: 15/16** clinical isolates

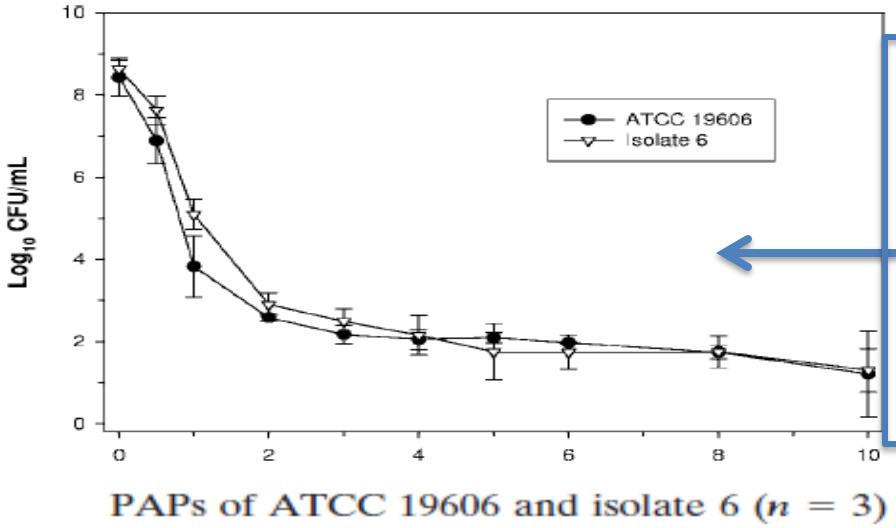
- Colistin-resistant *A. baumannii* may be observed more frequently due to potential suboptimal dosage of colistin methanesulfonate

Heteroresistance to Colistin in MDR *A. baumannii*

Li et al Antimicrob Agents Chemother 2006

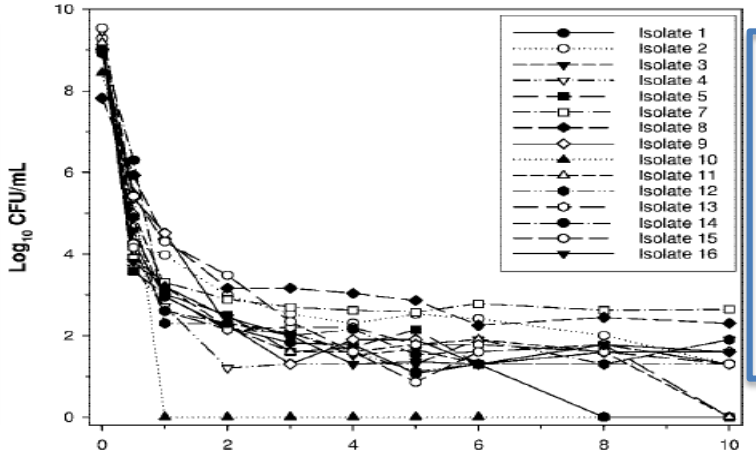


Killing curves for 2 isolates: Substantial regrowth at 24 h, up to 32xMIC, for both isolates



PAPs of ATCC 19606 and isolate 6 ($n = 3$)

Colistin MICs of subpopulations of ATCC 19606 and isolate 6 on plates with 10 mg/L colistin: 128 and 32 mg/L, respectively



Co PAPs of 15 clinical isolates

Heteroresistance is predominant (almost all isolates)

Comparative evaluation of colistin susceptibility testing methods in colistin-heteroresistant *E. cloacae* and *A. baumannii*

Lo-Ten-Foe et al Antimicrob Agents Chemother 2007

- **Colistin heteroresistance observed in *E. cloacae* and *A. baumannii***
- Resistance to colistin induced upon colistin exposure rather than caused by stable mutations **[not known in 2007!]**
- **Heteroresistance could be detected** by broth microdilution, agar dilution, Etest, or disk diffusion test, **not by VITEK 2**
- "In species known to exhibit heteroresistance, susceptibility methods capable of detecting heteroresistance should be used"

Colistin Heteroresistance in *Acinetobacter:* Its Association with Previous Colistin Therapy

Hawley et al Antimicrob Agents Chemother 2008

- Population analysis profiles identified resistant *Acinetobacter* subpopulations among colistin-susceptible clinical isolates
- **The proportion of cells exhibiting heteroresistance was significantly higher among isolates from patients treated with colistin**

Detection of colistin heteroresistance in *A. baumannii* from blood and respiratory isolates

Srinivas et al Diagn Microbiol Infect Dis 2018

- The majority of colistin-susceptible *A. baumannii* blood and respiratory isolates demonstrated colistin heteroresistance (20/24, 83%)

- Colistin heteroresistance was **not associated with suboptimal clinical outcomes** possibly due to the use of aggressive colistin dosing regimens and combination therapy

but…

- The previous use of colistin was not associated with the subsequent development of heteroresistance…

Colistin heteroresistance and the involvement of the PmrAB regulatory system in *A. baumannii*

Charretier et al Antimicrob Agents Chemother 2018

- From 3 unrelated *A. baumannii* clinical strains (ST 2, 3, and 20), 8 colistin-resistant mutants were selected
- Using **PAP, half of the mutants showed unstable HR to colistin**, associated with several point mutations within *pmrA* and *pmrB*
- The other half mutants exhibited stable resistance, associated with point mutations within *pmrB*

Virulence of *A. baumannii* exhibiting phenotypic heterogeneous growth against meropenem in a murine thigh infection model

Neou E et al, Antibiotics 2013; 11: 73-82

Investigation of the virulence of 5 *A. baumannii* isolates grown heterogeneously in the presence of meropenem

Clinical importance?

Characteristics of the study isolates

Isolate	[a] Susceptibility status to antimicrobials	PFGE	Agar dilution MEM MIC (mg/L)	Highest MEM concentration of growth in population analyses (mg/L)	[b] Agar dilution MIC (mg/L) of MEM grown subpopulations
Ab1	SAM, CIP, COL, MEM	I	4	16	32 ← stable HR
Ab2	GEN, TOB, CIP, SAM, MEM, COL	II	1	8	1
Ab3	GEN, TOB, SAM, MEM, COL	I	2	8	1 ←persisters
Ab4	GEN, SAM, MEM, COL	I	4	16	2
Ab5	GEN, TOB, SAM, MEM, COL	III	2	32	> 32 ← stable HR

[b] MICs estimated after 7 subcultures in antibiotic-free medium

Table 2.
Experimental infections treated with meropenem

Strain		Treatment regimen	Mean bacterial lung concentration (log CFU/g thigh)	Mortality (n)
Ab1	Clinical isolate	Untreated control	10.468	3/3
		Meropenem 20 mg /kg/8 h	10.250	1/3
		Meropenem 100 mg/kg/12 h	7.992	1/3
		Meropenem 400 mg/kg/8 h	8.480	0/3
Ab1h	Heterogeneous subpopulation	Untreated control	10.079	0/3
		Meropenem 20 mg /kg/8 h	10.010	0/3
		Meropenem 100 mg/kg/12 h	7.599	0/3
		Meropenem 400 mg/kg/8 h	6.135	0/3
Ab2	Clinical isolate	Untreated control	10.568	3/3
		Meropenem 20 mg/kg/8 h	10.450	2/3
		Meropenem 100 mg/kg/12 h	10.508	0/3
		Meropenem 400 mg/kg/8 h	7.393	0/3
Ab2h	Heterogeneous subpopulation	Untreated control	10.560	0/3
		Meropenem 20 mg /kg/8 h	10.450	0/3
		Meropenem 100 mg/kg/12 h	10.508	0/3
		Meropenem 400 mg/kg/8 h	7.393	0/3
Ab3	Clinical isolate	Untreated control	10.370	3/3
		Meropenem 20 mg/kg/8 h	10.250	2/3
		Meropenem 100 mg/kg/12 h	10.032	0/3
		Meropenem 400 mg/kg/8 h	8.100	0/3
Ab3h	Heterogeneous subpopulation	Untreated control	10.365	3/3
		Meropenem 20 mg /kg/8 h	10.125	0/3
		Meropenem 100 mg/kg/12 h	10.032	1/3
		Meropenem 400 mg/kg/8 h	8.124	0/3
Ab4	Clinical isolate	Untreated control	10.280	3/3
		Meropenem 20 mg/kg/8 h	10.145	1/3
		Meropenem 100 mg/kg/12 h	10.055	0/3
		Meropenem 400 mg/kg/8 h	7.950	0/3
Ab4h	Heterogeneous subpopulation	Untreated control	10.412	3/3
		Meropenem 20 mg /kg/8 h	10.275	1/3
		Meropenem 100 mg/kg/12 h	10.032	0/3
		Meropenem 400 mg/kg/8 h	8.116	0/3
Ab5	Clinical isolate	Untreated control	10.350	3/3
		Meropenem 20 mg/kg/8 h	10.150	2/3
		Meropenem 100mg/kg/12 h	10.035	1/3
		Meropenem 400 mg/kg/8 h	8.066	0/3
Ab5h	Heterogeneous subpopulation	Untreated control	10.128	0/3
		Meropenem 20 mg /kg/8 h	10.078	0/3
		Meropenem 100 mg/kg/12 h	10.032	0/3
		Meropenem 400 mg/kg/8 h	8.299	0/3
E. coli ATCC 25922		Untreated control	9.077	0/3
		Meropenem 20 mg/kg/8 h	8.015	0/3
		Meropenem 100 mg/kg/12 h	7.979	0/3
		Meropenem 400 mg/kg/8 h	2.015	0/3

MIC 4 mg/L

MIC 32 mg/L

MIC 2 mg/L

MIC 32 mg/L

Neou E et al, Antibiotics 2013; 11: 73-82

Virulence of *A. baumannii* exhibiting phenotypic heterogeneous growth against meropenem in a murine thigh infection model

Neou E et al, Antibiotics 2013; 11: 73-82

- No treatment:
  Heteroresistant *A. baumannii* subpopulations killed considerably fewer mice → **reduced virulence**

- Meropenem treatment:
  Similar outcome in infections caused by heterogeneous vs. parental isolates [irrespective **much higher MICs of the HR isolates**]
  → **further evidence of reduced virulence**

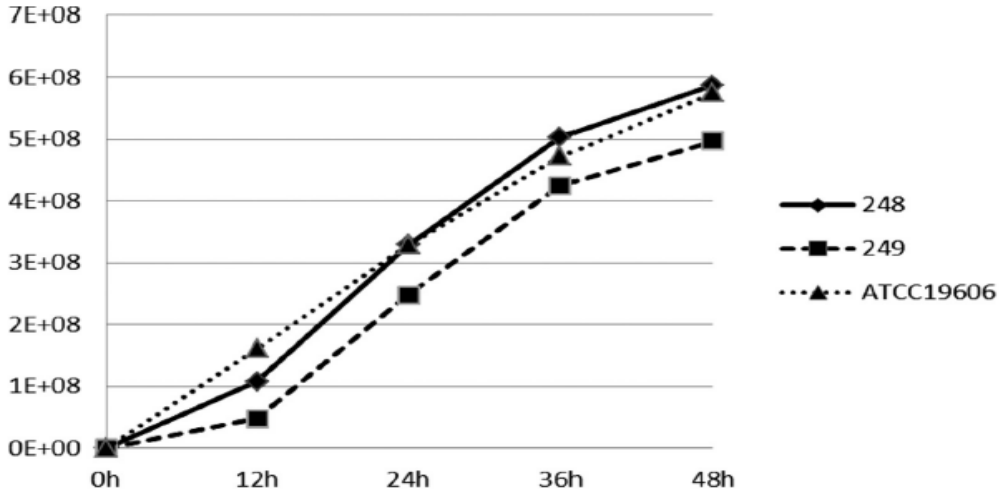
Clinical importance of heteroresistance in *A. baumannii* questionable…

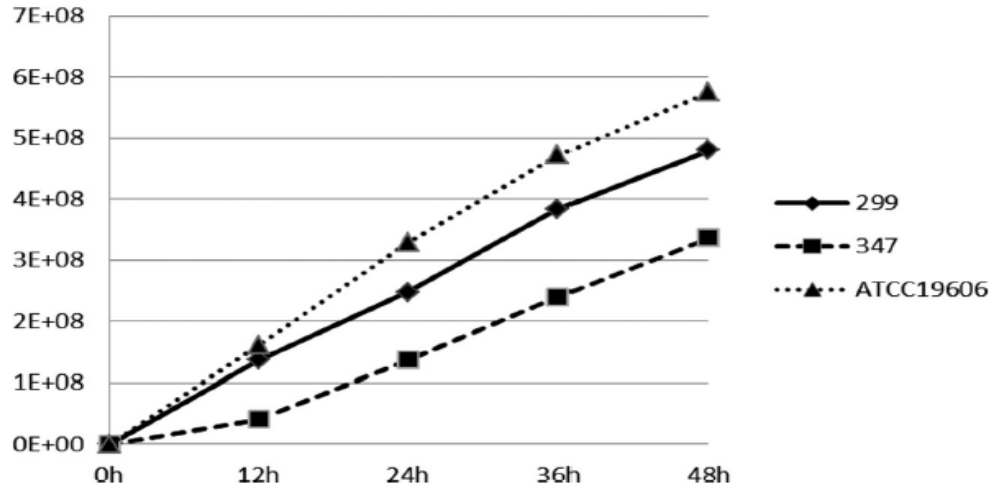
REPORT OF POSSIBLE CLINICAL HETERORESISTANCE

Growth Retardation, Reduced Invasiveness, and Impaired Colistin-Mediated Cell Death Associated with Colistin Resistance Development in *Acinetobacter baumannii*

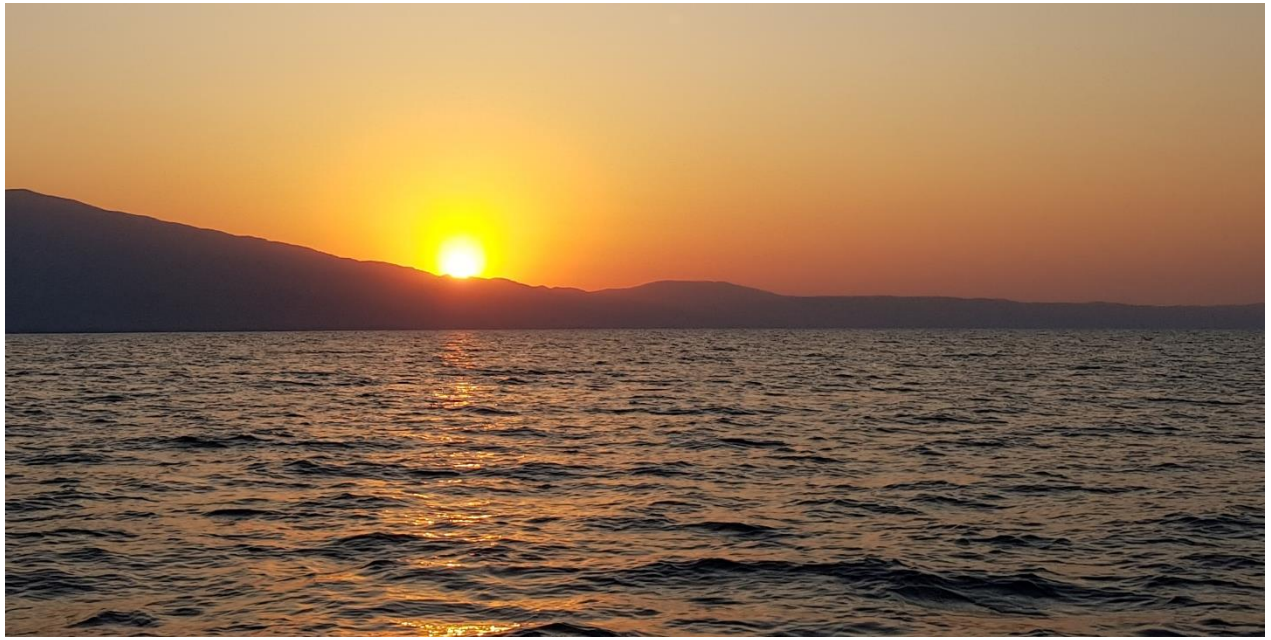
Pournaras et al *Antimicrob Agents Chemother* 2014

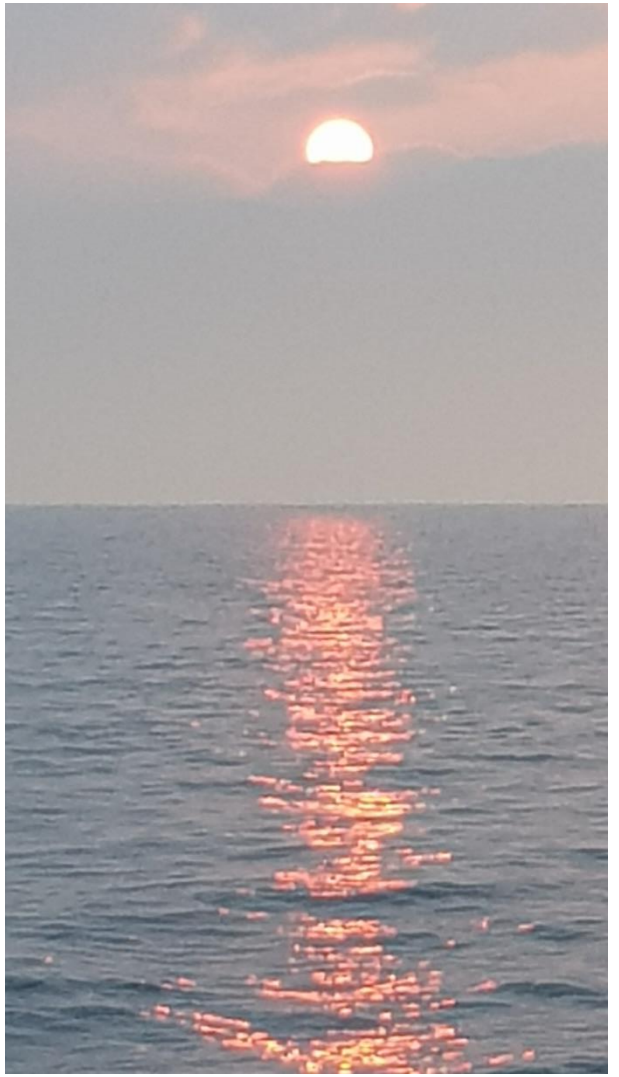
- **Colistin-susceptible** isolates caused severe **bloodstream or soft tissue infections**
- **Colistin-resistant** isolates were **mainly colonizers; they had significantly slower growth**





These observations overall suggest that changes contributing to colistin (hetero)resistance **confer a considerable fitness cost and affect the capacity to produce clinical infections**





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