Colistin in Clinical Practice: Synergy studies and combination treatment in clinical practice

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The species of main interest

Pathogen causing infection	
Acinetobacter baumannii	107 (53.5)
Klebsiella pneumoniae	104 (52)
Pseudomonas aeruginosa	31 (15.5)
other Gram-negative bacteria	43 (21.5)

Paul M. JAC 2010

The combinations of main interest

- Various beta-lactams, particularly carbapenems
- Rifampin
- Tigecyclin
- Vancomycin
- Aminoglycosides
- Quinolones
- Fosfomycin
- Sulbactam

Combination therapy, why?

- Improve clinical success
  - via better killing or inhibiting of the pathogen
    - » Inhibiting or killing at lower drug concentration
    - » More rapid killing
    - » Preventing selection or emergence of resistance
- To allow using of lower drug dose to prevent toxicity

Why not to use combination therapy

- Agents may be antagonistic, thus, reducing the efficacy of the treatment
  - Penicillin and tetracycline
- Toxicities may add-up or even augment each other
  - Estimated 10% side effects with each agent and more when using certain drugs
  - Renal toxicty of colistin and aminoglycosides

Emergence of resistance

- A susceptible strain antibiotic and a similar but resistant strain is subsequently isolated
- Result in severe patient outcome
  - Failure to eradicate the causative organism
  - Prolonged period of ineffective therapy
- Emergence of resistance is the end result of two processes
  - Acquisition of a new resistance mechanism (mutation)
  - Selection of pre-existing resistant sub-populations
    - Which are not detected initially by routine susceptibility testing

Colistin Heteroresistance in *Acinetobacter* and Its Association with Previous Colistin Therapy[▽]

Joshua S. Hawley,[1] Clinton K. Murray,[1]* and James H. Jorgensen[2]

TABLE 1. Prior colistin treatment of patients who contributed study isolates

Isolate	Cumulative dose (mg of colistin base activity)	No. of doses	No. of days since last dose
1	900	7	0
2	325	2	12
3	325	2	18
4	260	2	13
5	375	3	2
6	450	4	4
7	450	4	1

Colistin	n	Mean number of colonies growing at 8mcg/ml	Resistant CFU/million
exposed	7	307	2.1
Not-exposed	12	97	0.53

AAC 2008

Selection of resistant population by exposure to colistin is reduced by combination in hetero-resistant strain

Table 3: Proportion of colistin-resistant subpopulations in *P. aeruginosa* ATCC 27853 at various times in the *in vitro* PK/PD model

Inoculum (cfu/mL)	Time (h)	Proportion of colistin-resistant subpopulations in the presence of 4 mg/L colistin							
		Control	Col 0.5 mg/L	Col 2 mg/L	Col 5 mg/L	Col 0.5 mg/L + Dor 2.5 mg/L	Col 0.5 mg/L + Dor 25 mg/L	Col 2 mg/L + Dor 2.5 mg/L	Col 2 mg/L + Dor 25 mg/L
10^6	0	ND[§§]	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND	ND
	24	ND	3.08×10^{-1}	ND	ND	ND	ND	ND	ND
	48	ND	2.82×10^{-1}	ND	ND	ND	ND	1.12×10^{-3}	ND
	72	ND	1.80×10^{-2}	8.58×10^{-3}	ND	ND	ND	2.67×10^{-3}	ND
	96	ND	3.67×10^{-2}	7.37×10^{-1}	ND	1.83×10^{-5}	ND	1.75×10^{-5}	8.78×10^{-5}
10^8	0	1.19×10^{-7}	1.72×10^{-7}	ND	4.05×10^{-7}	3.51×10^{-7}	9.60×10^{-7}	4.43×10^{-7}	7.38×10^{-6}
	6	5.81×10^{-8}	1.75×10^{-5}	3.67×10^{-5}	ND	ND	ND	ND	ND
	24	1.01×10^{-7}	8.22×10^{-6}	1.25×10^{-1}	1.29×10^{-2}	ND	ND	ND	ND
	48	1.83×10^{-7}	4.90×10^{-3}	2.65×10^{-1}	8.74×10^{-1}	3.70×10^{-6}	2.51×10^{-5}	4.57×10^{-5}	ND
	72	2.95×10^{-8}	3.18×10^{-3}	3.01×10^{-1}	9.49×10^{-1}	3.14×10^{-4}	ND	4.77×10^{-3}	ND
	96	8.92×10^{-8}	3.22×10^{-3}	2.72×10^{-1}	9.71×10^{-1}	7.78×10^{-4}	5.66×10^{-5}	6.55×10^{-3}	ND

Bergen PJ. AAC 2012

Doripenem-Colistin combination: role of inocula and resistance

Isolate	Inoculum (cfu/mL)	Time (h)	Log change (= $\log_{10}$(CFU) - $\log_{10}$(CFU_0))									
			Col 0.5 mg/L	Col 2 mg/L	Col 5 mg/L	Dor 2.5 mg/L	Dor 25 mg/L	Dor 50 mg/L	Col 0.5 + Dor 2.5	Col 0.5 + Dor 25	Col 2 + Dor 2.5	Col 2 + Dor 25
ATCC 27853††	10^6	6	-1.71	-6.18	-6.29	-1.55	-1.71	-2.36	-6.27	-4.97	-6.16	-6.04
		24	1.49	-2.96	-6.29	0.34	-0.34	-0.30	-2.26	-1.37	-2.27	-4.57
		48	1.12	-0.81	-6.29	1.20	0.43	-0.05	-2.14	-0.21	-2.97	-3.35
		72	1.41	0.69	-3.99	1.47	1.05	0.19	-1.97	-0.67	-2.11	-2.20
		96	1.20	1.10	-1.97	1.92	1.31	0.92	0.07	-0.43	-0.14	-0.03
	10^8	6	-0.36	-1.85	-4.69	-1.25	-2.79	-3.12	-2.45	-3.82	-4.66	-8.17
		24	-0.42	-1.53	-2.75	-1.17	-2.16	-2.54	-2.97	-2.68	-3.53	-4.91
		48	-0.75	-1.54	-1.91	-1.10	-2.07	-2.98	-1.32	-1.78	-2.79	-5.83
		72	-0.73	-1.50	-0.80	-0.74	-1.78	-1.86	-0.75	-1.82	-1.81	-3.73
		96	-0.82	-1.53	-0.79	-0.54	-1.66	-1.16	-0.88	-1.31	-1.53	-3.67
19147 n/m‡‡	10^6	6	-	-	0.83	-0.67	-2.39	-3.14	-1.42	-2.12	-2.89	-3.58
		24	-	-	1.47	-0.39	-3.26	-2.89	-3.08	-6.52	-3.90	-6.22
		48	-	-	1.21	1.28	-1.58	-0.45	-1.45	-6.52	-2.09	-6.22
		72	-	-	1.07	1.53	-0.63	0.21	-0.08	-6.52	0.47	-6.22
		96	-	-	0.47	1.69	-0.14	0.35	1.38	-6.52	1.59	-6.22
	10^8	6	-	-	0.01	-1.04	-2.79	-2.50	-0.95	-2.91	-1.82	-4.32
		24	-	-	-0.37	-0.58	-2.94	-1.96	-0.53	-3.67	-2.42	-2.73
		48	-	-	-0.59	-0.05	-1.37	-1.68	-0.51	-8.06	-2.38	-3.47
		72	-	-	-0.65	-0.04	-1.48	-1.28	-0.49	-8.06	-0.29	-4.13
		96	-	-	-0.99	-0.01	-1.35	-1.57	-0.69	-6.46	-0.27	-4.24

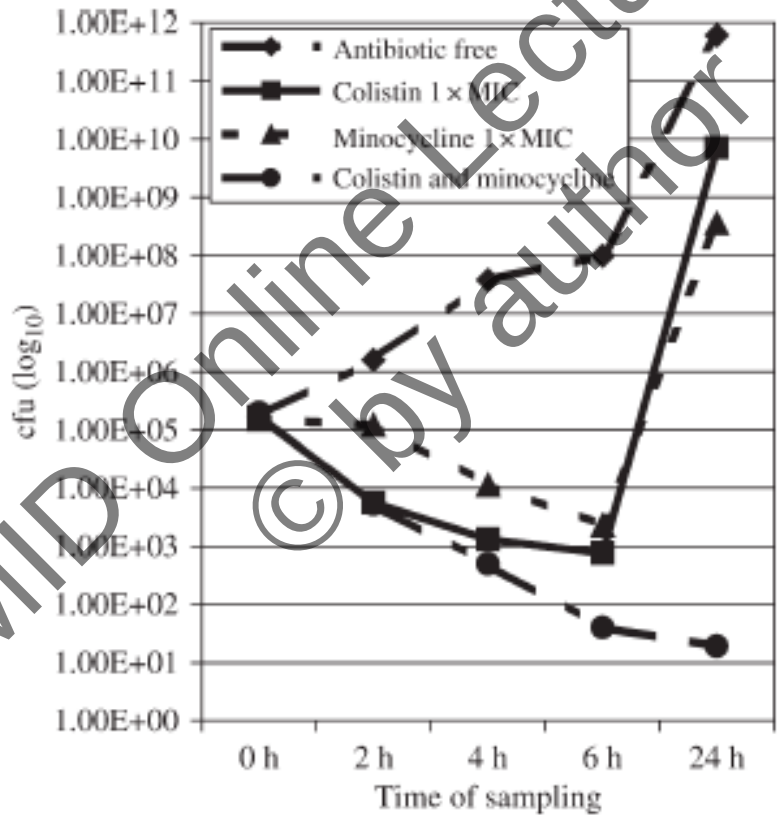
Hetero-resistant strain

Resistant strain

Grey = acitivity; Green= synergy Red= additive effect

Bergen PJ. AAC 2012

Killing at MIC result in late growth which can be prevented by Colistin-minocycline combination



Tan TY. JAC 2007

In Vitro Effects of Carbenicillin Combined with Gentamicin or Polymyxin B Against *Pseudomonas aeruginosa*[1]

THEODORE C. EICKHOFF

Department of Medicine, University of Colorado Medical Center, Denver, Colorado 80220

Received for publication 20 June 1969

TABLE 1. *Distribution of responses of 27 strains of P. aeruginosa to combinations of carbenicillin with gentamicin sulfate or polymyxin B sulfate, determined by agar-dilution tests*

Carbenicillin-polymyxin B	Carbenicillin-gentamicin		
	Synergy	Addition	Antagonism
Synergy	0	0	0
Addition	11	10	0
Antagonism	4	2	0

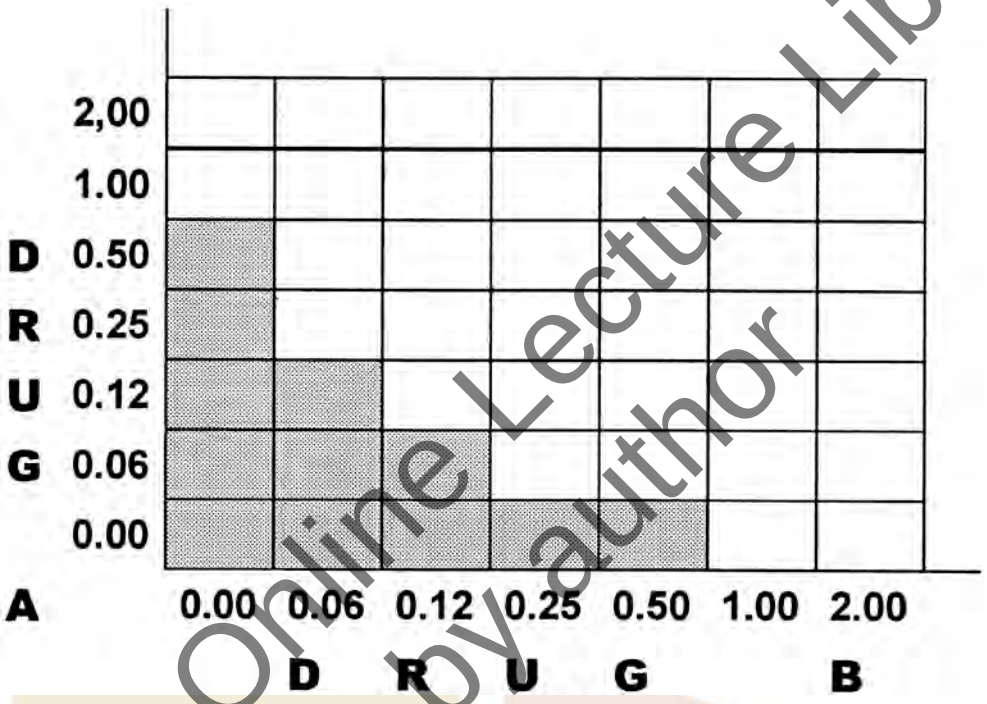
Effectiveness of combination therapy

- Is it effective?
- Is combination effective even when the organism is resistant to one of the antibiotic agents? To both?
- At what level of resistance (distance of MIC from breakpoint)

Evidence on the effectiveness of combination therapy

- In-vitro studies
  - Checkerboard
  - E-test synergy testing
  - Time-kill curve
  - PK/PD models
- In-vivo
  - models of infections
  - Clinical data

Checkerboard interpretation



ΣFIC = FIC A + FIC B
FIC A =MIC of drug A in the combination/MIC of drug A alone
FIC B =MIC of drug B in the combination/MIC of drug B alone

ΣFIC ≤0.5 - Synergy
ΣFIC is >0.5 to <2 - Indifference
ΣFIC is ≥2 - Antagonism

Orhan J. JCM 2005

Using Etest method

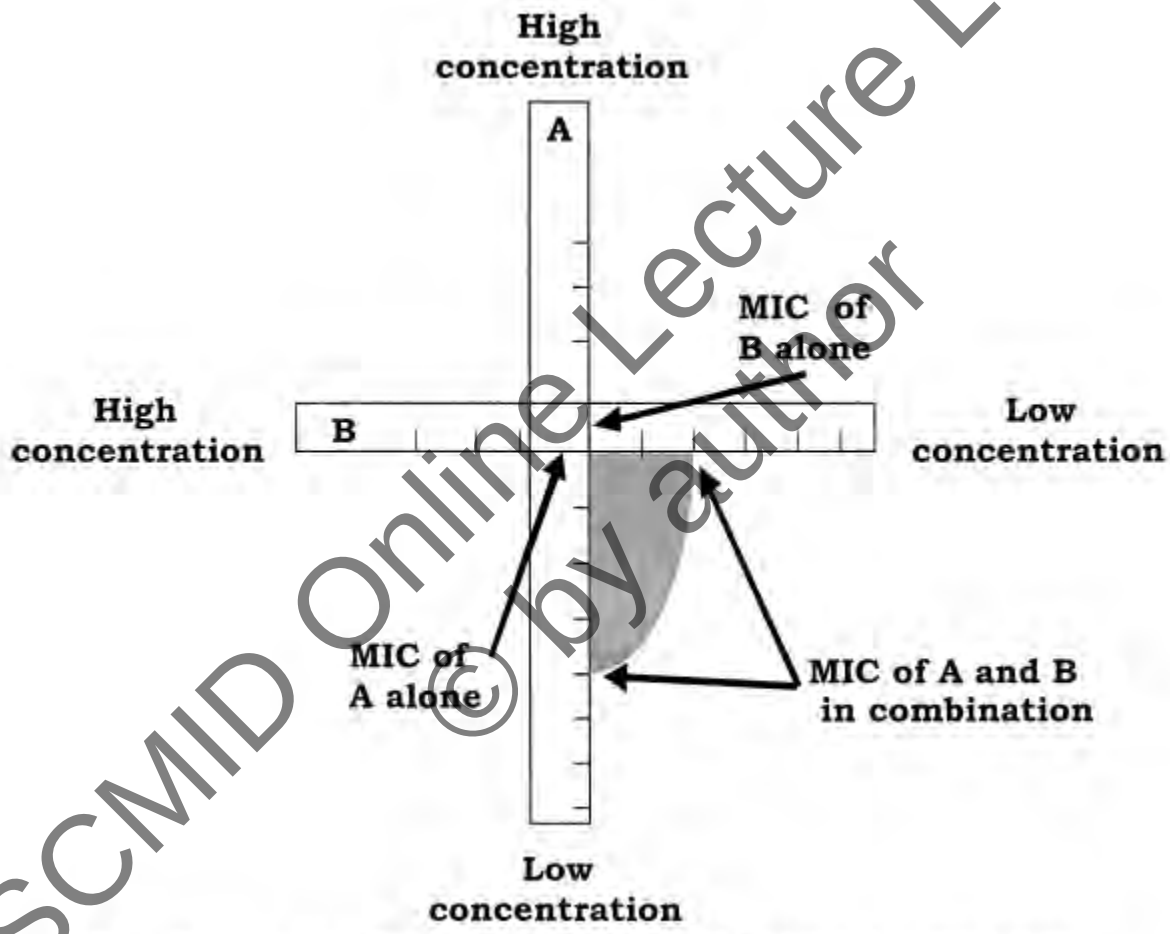
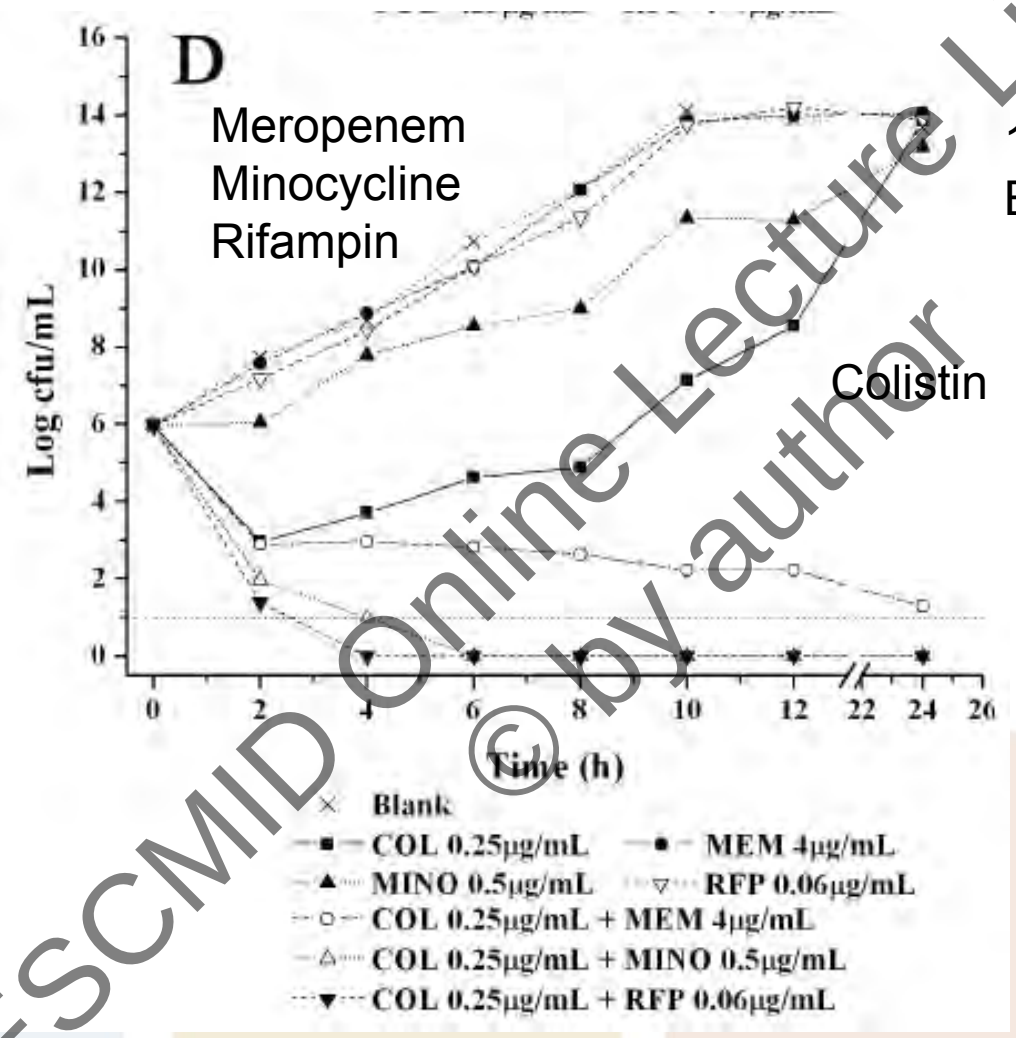


Fig. 1. Placement and interpretation of Etest strips for the Etest method of synergy testing

Time-kill synergy studies



1-2 Specific concentrations
Extensive work

Colistin+ Meropenem
Colistin +Minocycline
Colistin + Rifampin

Liang W. BMC ID. 2011

LABORATORY TESTS FOR ANTIBIOTIC SYNERGISM

Method	Medium	Concentration	Incubation	Effect observed
Checkerboard method	Broth dilution	Multiple concentration of A and B (including MIC)	18 h 37°C	Bacteriostatic*
Time-Killing curve	Broth dilution	One or two concentration of A and B	Sampling at various times (0, 2, 4, 8, 18h)	Bactericidal (count on each sample)
Disk diffusion/E-test	Agar	Variable	18 h 37°C	Bacteriostatic

•Bactericidal by adding sub-culture step

Static tests, not taking into account changing drug concentrations over time

Acar JF. Med Clin North Am 2000

How reliable are these methods?

Evaluation of antibiotic synergy against *Acinetobacter baumannii*: a comparison with Etest, time-kill, and checkerboard methods

Charles R. Bonapace, Roger L. White, Lawrence V. Friedrich, John A. Bosso,*

Agreement between Time-Kill and Checkerboard	51%
Agreement between Time-Kill and Etest	72%
Agreement between Etest and Checkerboard	63%

Variability in agreement between agents and concentrations relative to MIC

Table 3
Agreement between methods of synergy testing (%)

Method	Checkerboard	Time-Kill by Concentration				Time-Kill by Drug[c]			
		0.25 × MIC + 0.25 × MIC	0.25 × MIC + 2 × MIC[a]	2 × MIC + 0.25 × MIC[b]	2 × MIC + 2 × MIC	TV + CM	TV + PI	TO + CM	TO + PI
Etest	63	97	42	67	70	75	85	59	80
Checkerboard	-	67	37	47	45	30	55	54	60

[a] Combination of 0.25 × MIC of TV or TM and 2 × MIC of CF or PI
[b] Combination of 2 × MIC of TV or TM and 0.25 × MIC of CF or PI
[c] Abbreviations for antibiotics: CM, cefepime; PI, piperacillin; TM, tobramycin; TV, trovafloxacin

DMID 2000

Synergy studies checkerboard method colistin or Polymixin B

Organism	Agent combination	% isolates with synergy
A. baumannii	Rifampin	54-100%
	Carbapenems	38-60%
P .aeruginosa	Rifampin	0-100%
	Carbapenems	-
	Azythromcin	0-60%

Landman D. CMR 2008

IN VITRO ACTIVITY OF COLISTIN OR SULBACTAM IN COMBINATION WITH FOSFOMYCIN OR IMIPENEM AGAINST CLINICAL ISOLATES OF CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* PRODUCING OXA-23 CARBAPENEMASES

Testing of coupled antimicrobial agents against carbapenem-resistant *A. baumannii* (CRAB) clinical isolates by checkerboard technique.

Strains	FICI for combined antimicrobial agents[a]				
	Colistin + sulbactam	Colistin + imipenem	Colistin + fosfomycin	Sulbactam + imipenem	Sulbactam + fosfomycin
AB23	0.96 (I)	0.94 (I)	1.17 (I)	0.63 (I)	0.32 (S)
AB54	1.54 (I)	0.56 (I)	1.04 (I)	0.52 (I)	0.49 (S)
AB164	0.87 (I)	0.89 (I)	1.38 (I)	0.62 (I)	0.48 (S)
AB167	1.10 (I)	0.62 (I)	0.94 (I)	0.85 (I)	0.37 (S)
AB198	0.94 (I)	0.52 (I)	0.85 (I)	0.75 (I)	0.28 (S)
AB307	0.71 (I)	0.99 (I)	1.08 (I)	1.27 (I)	1.04 (I)
AB313	0.75 (I)	0.71 (I)	0.49 (S)	0.51 (I)	0.47 (S)
AB315	0.92 (I)	1.04 (I)	1.08 (I)	0.92 (I)	1.08 (I)

FICI, fractional inhibitory concentration index; ≤ 0.5, synergistic, S; > 0.5-4.0, indifferent, I; and > 4, antagonistic effect, AT

No synergy of colistin with sulbactam, imipenem, fosfomycin

South Asia J Trop Med Pub Health 2011

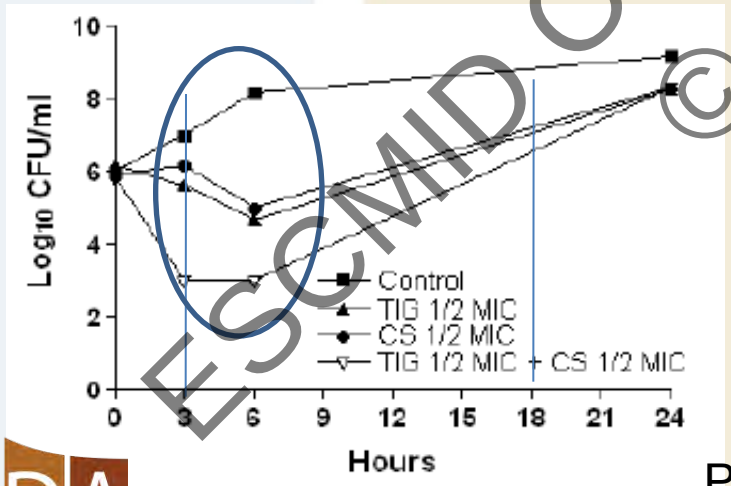
Synergy studies – time kill method

Organism	Agent combination	% isolates with synergy
A. baumannii	Rifampin	88-100%
	Carbapenems	100%
P .aeruginosa	Rifampin	100%
	Carbapenems	
	Azythromcin	100%

Landman D. CMR 2008

Synergy of colistin/tigecycline against A. baumannii

- 24 isolates tested using checkerboard method
  - 2 showed synergy (with FIC=0.5)
  - 1 showed antagonism (FIC=4.25)
  - 2 showed borderline antagonism (2<FIC <4)
  - 19 indeference



Time-killing of one of the strains which showed synergy by checkerboard

Principe L. Annals of Clin Micro antimicrob 2009

Synergy of colistin/rifampin against A. baumannii

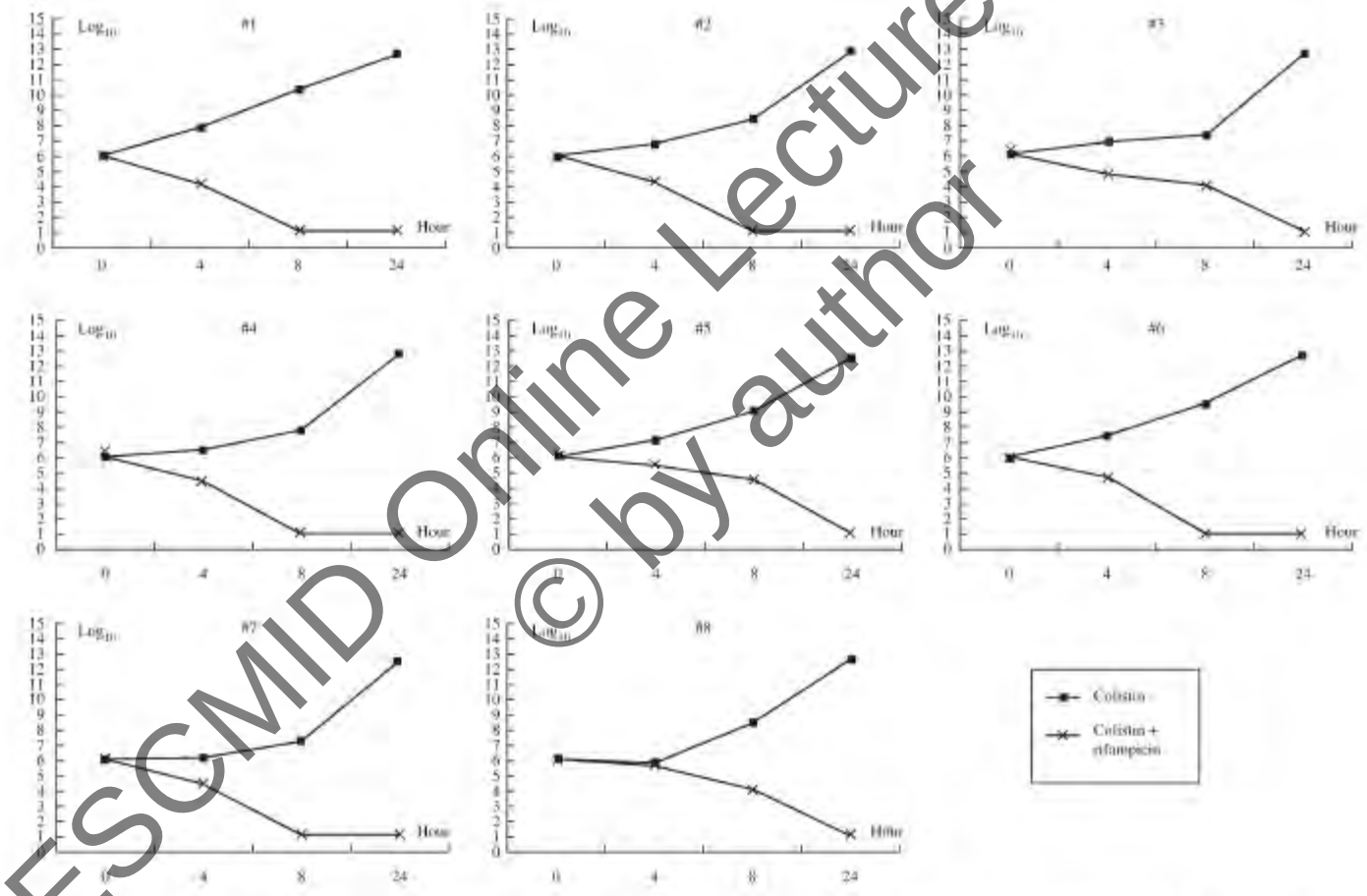


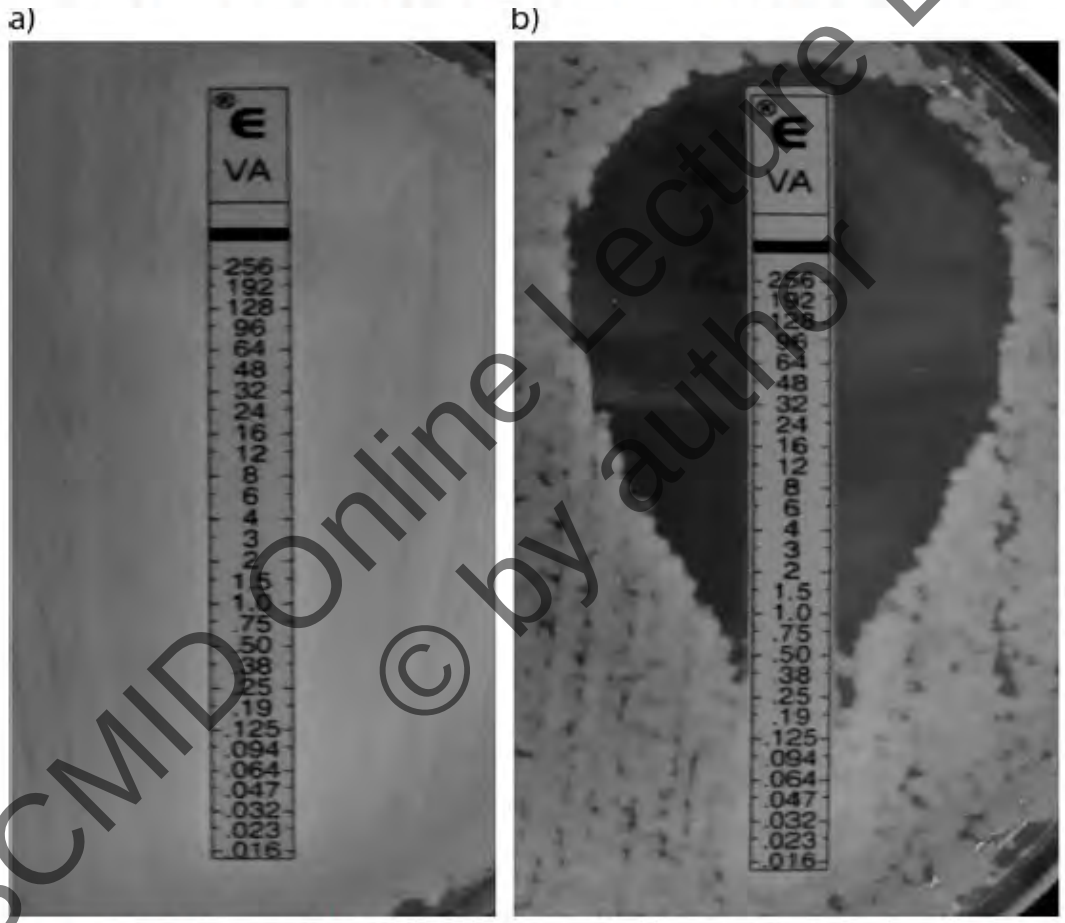
Figure 2. Time–kill curves showing synergistic effect of colistin/rifampicin combination compared with colistin alone against carbapenem-resistant *A. baumannii* strains; viable bacterial counts were reduced by at least $\geq 2 \log_{10}$ cfu/mL compared with colistin alone.

Song JY. JAC 2007

Potent Synergy and Sustained Bactericidal Activity of a Vancomycin-Colistin Combination versus Multidrug-Resistant Strains of *Acinetobacter baumannii*▽†

N. C. Gordon,[1] K. Png,[2] and D. W. Wareham[1,3]*

Without colistin



Colistin supplemented media

FIG. 1. MICs of vancomycin for *A. baumannii* AB14 (OXA-23 clone 1 isolate) after overnight incubation on unsupplemented Iso-Sensitest agar (a) and Iso-Sensitest agar supplemented with 0.5 μg/ml colistin (b).

AAC 2010

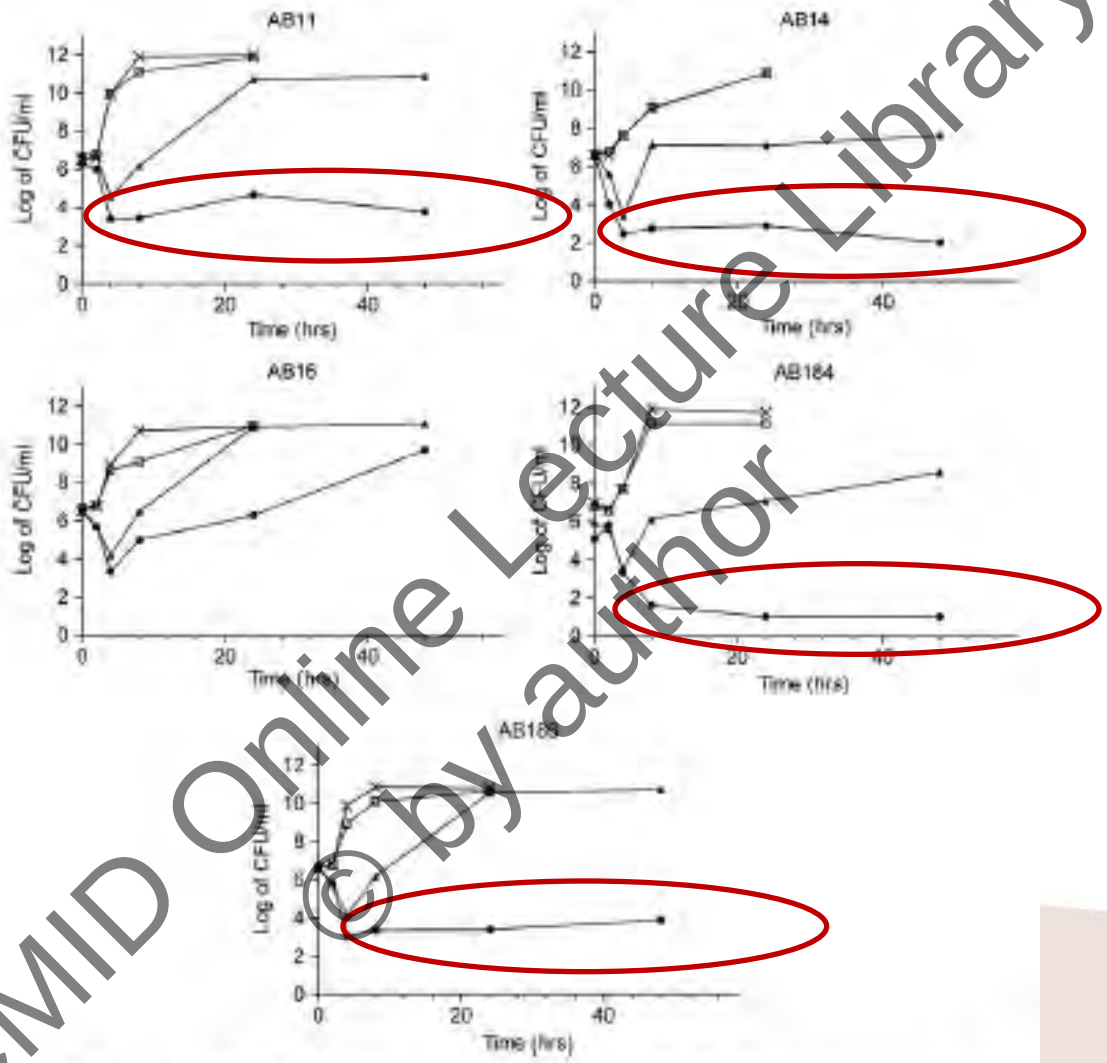
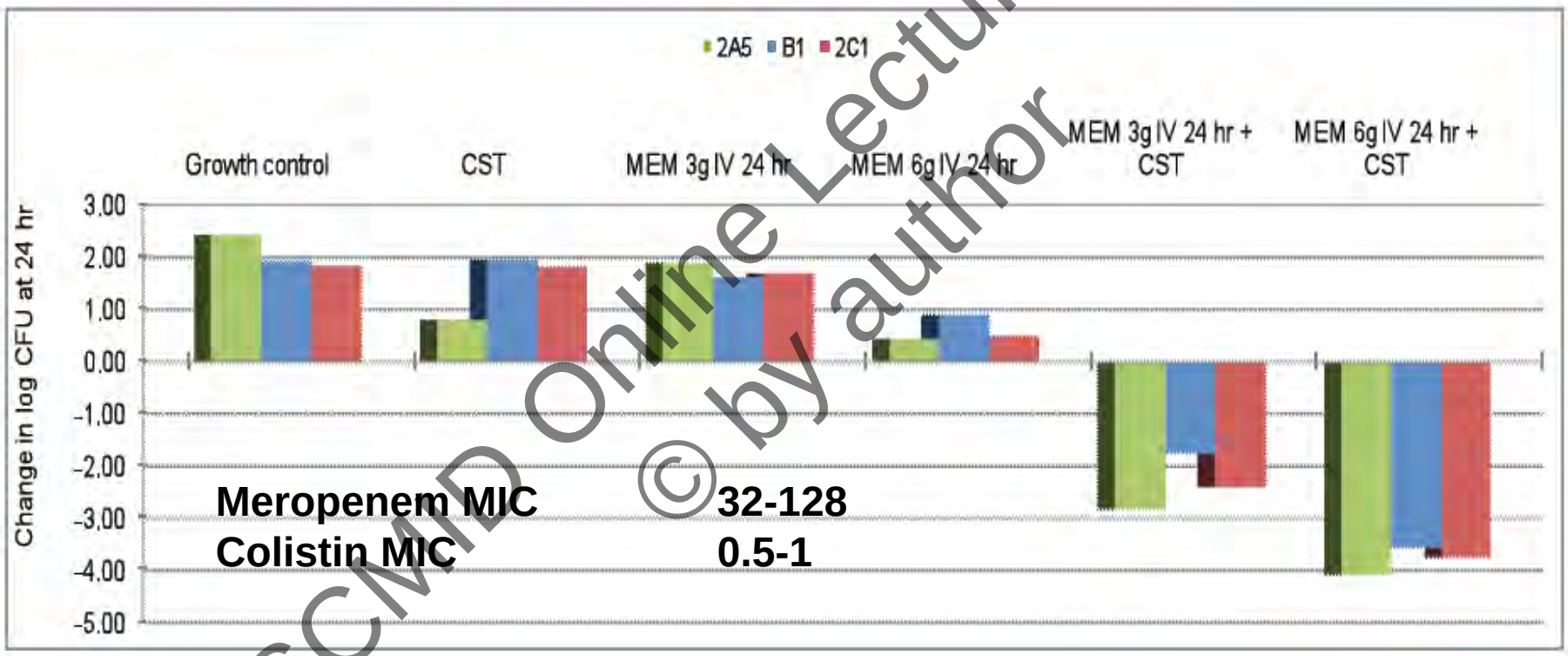


FIG. 2. Time-kill curves showing the effects of unsupplemented Iso-Sensitest broth (control) and broth supplemented with either colistin (1 μg/ml), vancomycin (20 μg/ml), or both for epidemic strains AB11, AB14, AB16, AB184, and AB186 by viable colony counts. Symbols: ×, control; □, vancomycin; ▲, colistin; ●, vancomycin plus colistin.

Gordon AAC 2010

Colistin-Meropenem PK/PD model
Meropenem highly resistant A. baumannii

Figure 2. Changes in Colony Count over 24 hours



Srisupha-Olarn W. ICAAC 2010

In vitro tests for combination

- Are not available in clinical practice
- Difficult to perform, time consuming
- Agreement between methods variable
- Mostly suggest that colisitin combination with carbapenems or rifampin are warrented
- Clinical correlates and relevance ?

Colistin monotherapy vs. combination therapy: evidence from microbiological, animal and clinical studies

N. Petrosillo[1], E. Ioannidou[2] and M. E. Falagas[2,3]

Controls (33), IMI, sulbactam, TOB, RIF, COL[b] were used in monotherapy and in combination therapy (four mice per group). COL was combined only with RIF	RIF + COL (5.59 ± 1.17)	RIF + COL had the same effectiveness as RIF alone regarding lung bacterial counts. For strains of *A. baumanii* that are highly resistant to IMI, a combination of RIF with IMI, TOB or COL may be useful, if resistance to RIF is moderate	Colisitn= Colisitn +Rifampin
Controls (20), RIF (20), COL[a] (22), RIF + COL (20)	Median survival: 4 days. Mortality rate after 6 days: 7/10	Survival with COL (alone or in combination with RIF) was significantly higher vs. controls. Mortality rates on the sixth day of follow-up showed the enhancement of the effect of COL by the addition of RIF. Statistically significant decreases in numbers of bacteria were found in blood and liver of the combined group vs. controls	Colisitn< Colisitn +Rifampin
Controls (20), tachyplesin (20), COL[b] (20), IMI (20), tachyplesir and IMI (20), IMI + COL (20)	Overall deaths: < COL + IMI (3/20), tachyplesin + IMI (2/20)	Combination treatment groups had significantly lower mortality and bacteraemia than monotherapy treatment groups. Tachyplesin III and COL treatments (alone or in combination) resulted in marked decreases ($p < 0.05$) of endotoxin, TNF-α, and IL-6 plasma levels as compared to those of controls	Colisitn< Colisitn +Imipenem
Controls (15), RIF (15), COL[b] + RIF (15)	Mortality at 72 h: COL + RIF (4/15)	Combination of COL and RIF resulted in a significant reduction in bacterial count as compared with COL monotherapy, even though no significant difference was found in positive blood cultures and mortality rates between the two groups	Colisitn=< Colisitn +Rifampin

CMI 2008

Efficacy of monotherapy and combined antibiotic therapy for carbapenem-resistant *Acinetobacter baumannii* pneumonia in an immunosuppressed mouse model

Joon Young Song, Hee Jin Cheong*, Jacob Lee, Ah Kyeong Sung, Woo Joo Kim

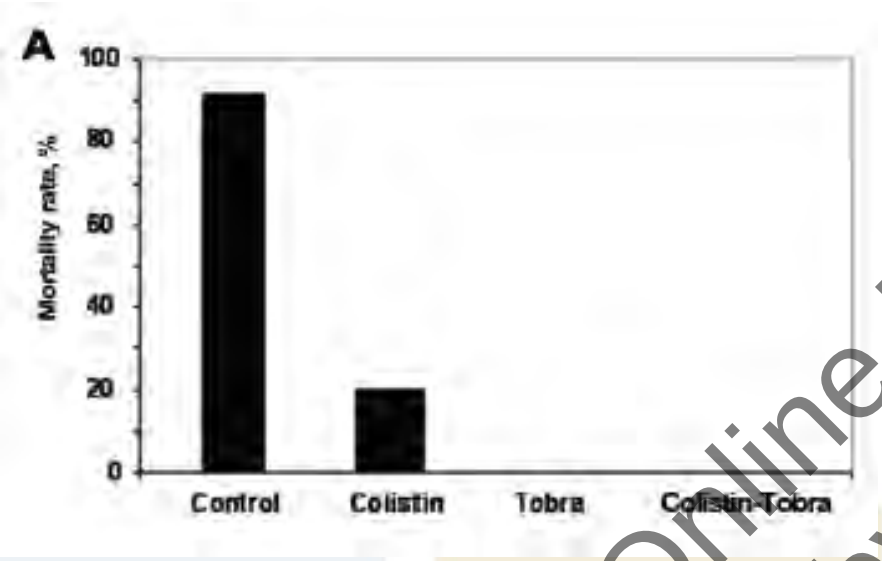
OXA 51 producing A. baumannii: imipenem MIC 64; Colistin MIC <0.5

Treatment	Log10 CFU at 24h	Log10 CFU at 48h
Colistin	9.31	6.35
Imipenem	8.46	6.47
Colistin+Imipenem	9.89	7.15

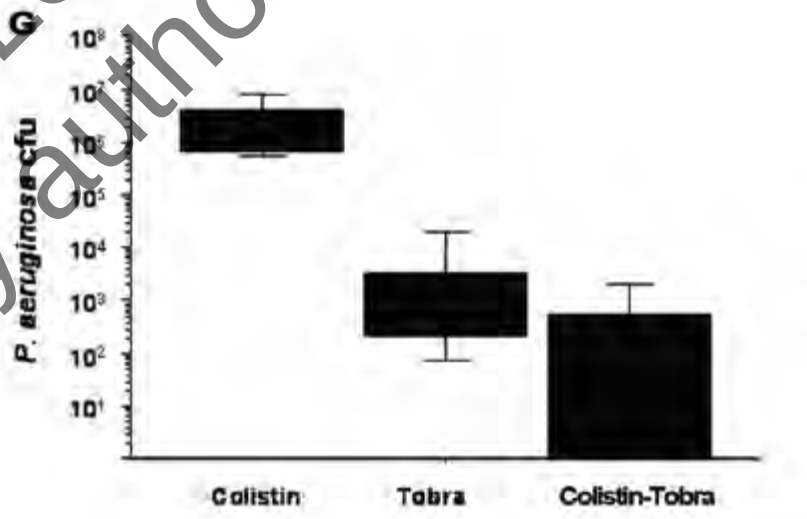
No benefit by combination

Int J Antimicrob Agent 2009

Rat lung infection model
Colistin-Tobramycin combination



Mortality



Lung bacterial counts

Herrmann G. JID 2010

Colistin –Rifampin as effective as Colisitn alone in murine pneumonia model

TABLE 3. *In vivo* results for the experimental pneumonia model

Treatment group[g]	*n*	% Survival	Log_{10} CFU/g of lung (mean ± SD)	% with sterile blood culture
CON	15	0	10.6 ± 0.27	0
IPM	14	28.6[a,f]	7.87 ± 3.43[b,c,d]	30.8[a,b,c,d,e]
RIF	14	71.4[a]	3.05 ± 1.91[a]	78.6[a]
SUL	15	40[a,f]	7.23 ± 4.41[c,d]	33.3[a,b,c]
CST	15	40[a,f]	6.82 ± 3.4[a,c,d]	73.3[a]
RIF + IPM	15	60[a]	2.07 ± 1.82[a]	100[a]
RIF + SUL	15	46.7[a]	2.41 ± 1.37[a]	93.3[a]
RIF + CST	16	43.8[a]	3.4 ± 3.07[a]	93.8[a]
IPM + SUL	14	85.7[a,d,e]	4.22 ± 2.72[a]	50[a,c,d,e]

Pachon-Ibanez ME. AAC 2010

In Vivo Efficacy of Glycopeptide-Colistin Combination Therapies in a *Galleria mellonella* Model of *Acinetobacter baumannii* Infection▽

M. Hornsey[1] and D. W. Wareham[1,2]*

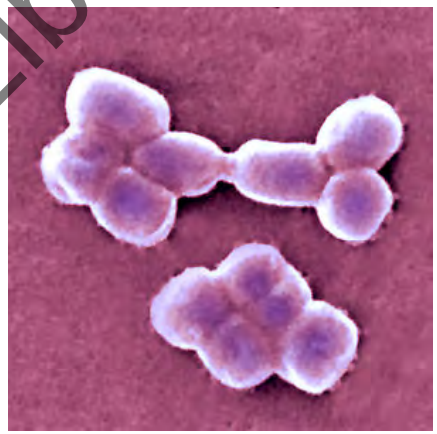


FIG. 2. Survival curves for *A. baumannii* ATCC 19606 (A) and AB210 (B) following treatment with colistin (COL), gentamicin (GENT), teicoplanin (TEIC), or PBS alone. Curves represent a single experiment performed using 16 insects.

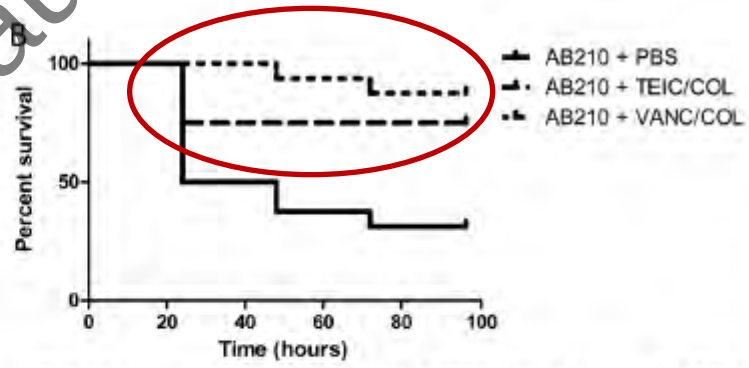


FIG. 3. Survival of *A. baumannii* ATCC 19606 (A) and AB210 (B) following treatment with colistin (COL) in combination with teicoplanin (TEIC) or vancomycin (VANC). For ATCC 19606, $P < 0.001$ for TEIC-COL and VANC-COL versus PBS; for AB210, $P < 0.001$ for VANC-COL and $P < 0.05$ for TEIC-COL versus PBS. Curves represent a single experiment performed using 16 insects.

AAC 2011

Retrospective data from Greek hospitals on carbapenamases producers

Regimen	N	Success	Failues
Colistin Monotherapy	64	35 (55%)	29 (45%)
Combination *	82	66 (81%)	15 (19%)

Inclusion of carbapenem in combination therapy (when MIC is low)

	Carbapenem	N	Success	Failure
≥2 active drugs	no	52	38 (73%)	14 (27%)
≥2 active drugs	yes	30	28 (93%)	2 (7%)

Akova M et al. CMI in press

Colistin-carbapenem combination therapy

- Data from two retrospective cohorts
- Carbapenem-resistant bacteria

	Location, bacteria	Outcome	Colistin monotherapy	Combination therapy
Falagas 2010 [1]	Greece, mostly *A. baumannii*	Failure	2/20 (10%)	14/84 (16.7%)
		Mortality	Not significant	
Qureshi 2012 [2]	US, KPC-producing *K. pneumoniae*	Mortality	4/7 (57%)	1/5 (20%)
Total			**27**	**89**

1 Falagas et al. Int J Antimicrob Agents 2010;
2 [2] Qureshi et al. Antimicrob Agents Chemother 2012

Colisitn Combinations

- Multiple evidence that combination therapy is superior to colistin montherapy
  - Strongest support for carbapenems
- Effectiveness of colistin combinations may vary
  - Between species
  - Between clones within species
  - Within clones – related to mechanisms of resistance
- Diagnostic tests to guide combinations and correlations with clinical outcomes are required

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