

I TEST DI SENSIBILITA' ED I TEST DI SINERGIA PER I MICROORGANISMI MULTI-RESISTENTI

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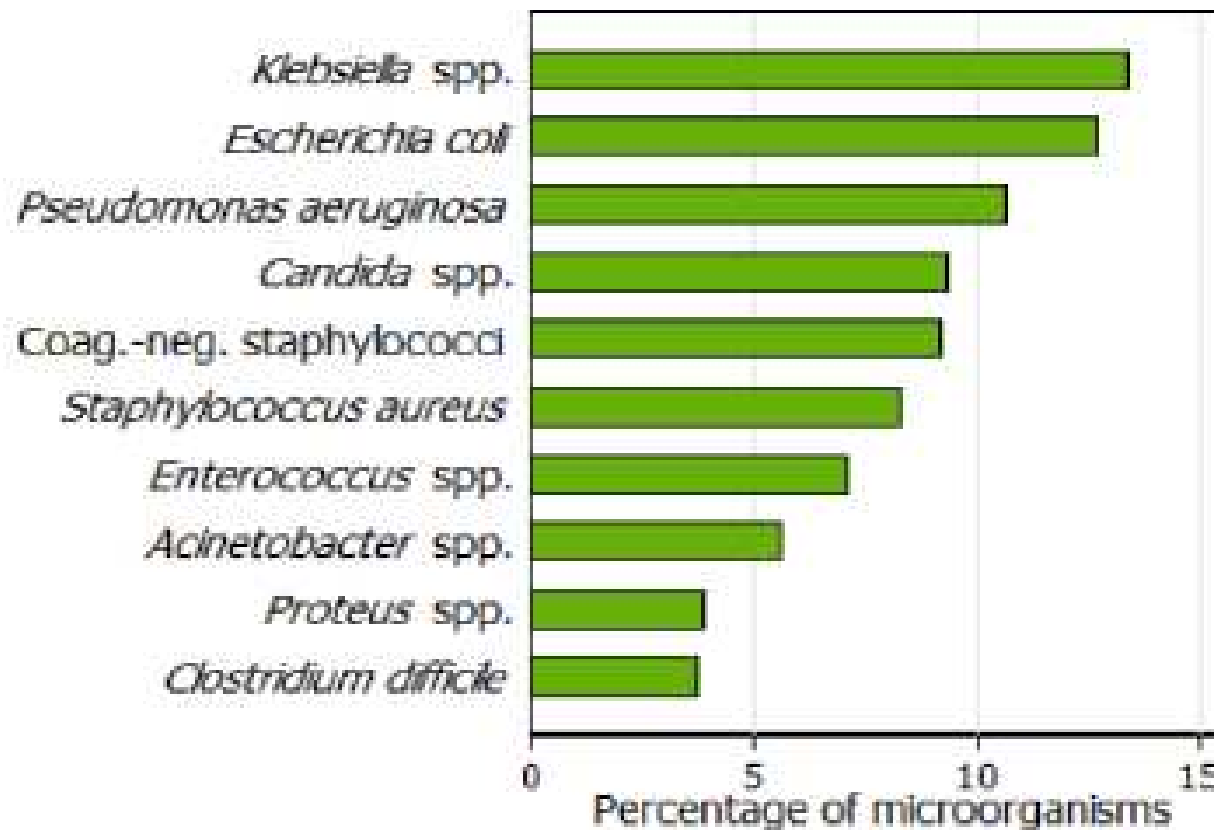
Dipartimento Biotecnologie Mediche



UNIVERSITÀ
DI SIENA

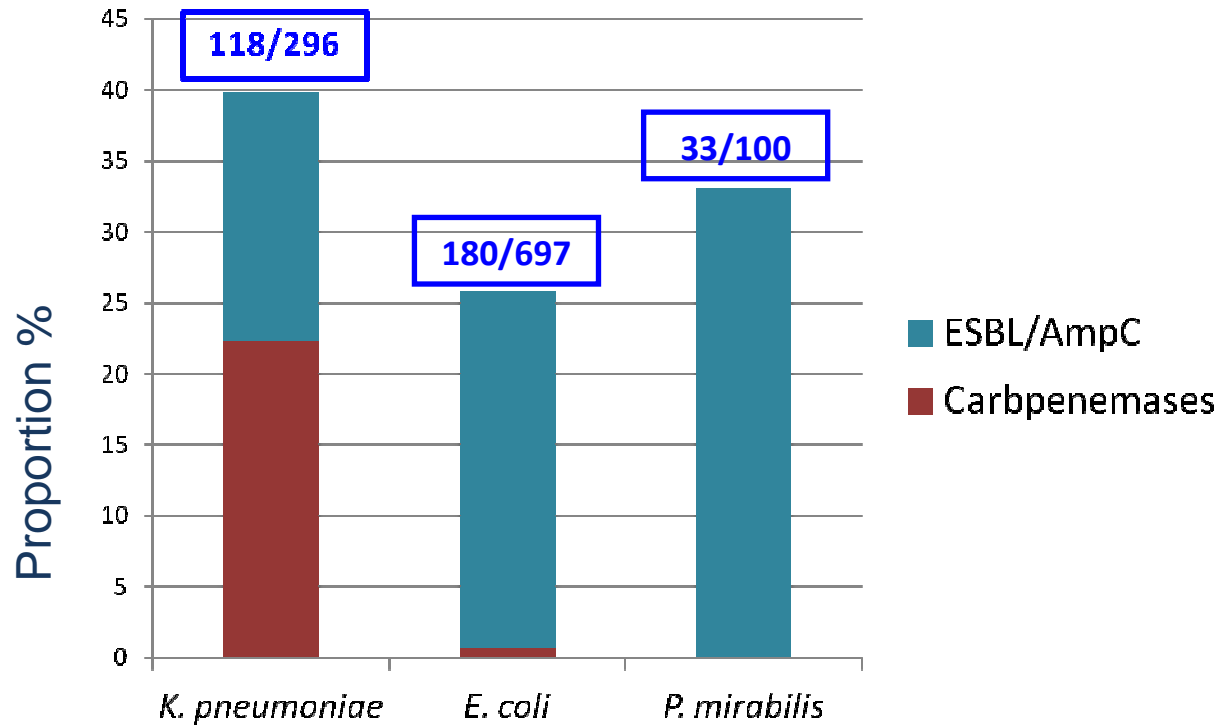
1240

Top ten pathogens causing HAIs, Italy



2013 AMCLI-COSA Surveillance (1/15-Oct) 10/14 centers

Consecutive non-replicate isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, with MIC for 3GC or/and ERT >1



KPC+ *K. pneumoniae*

Antibiotic	MIC mg/L(S/I/R)
Amp/Sulb	>32 R
Pip/Tazo	>128 R
Ceftriaxone	>64 R
Ceftazidime	>64 R
Cefepime	>64 R
Ertapenem	>32 R
Imipenem	>32 R
Meropenem	>32 R
Amikacin	>64 R
Gentamicin	2 S
Tobramycin	>16 R
Ciprofloxacin	>4 R
Tigecycline	1 S
Colistin	0.25 S

Carbapenem-resistant *Klebsiella pneumoniae*, treatment

Conclusions	Study design	n. studies	n. patients	Ref.
Support use of carbapenem-based combination therapy	Retrospective observational	3	269	<i>Qureshi et al. AAC 2012; Daikos et al AAC 2014; Tumbarello et al. CID 2012</i>
Support the use of tigecycline-colistin combination	Prospective observational	1	53	<i>Zarkotou et al. CMI 2011</i>
	Retrospective observational	1	26	<i>Sbrana et al. CID 2013</i>
Support the use of combination vs monotherapy	Retrospective observational; Prospective observational; Case series	6	403	<i>All previous and Hirsch JAC 2010</i>

Carbapenem-resistant *Klebsiella pneumoniae* treatment

MAJOR ARTICLE

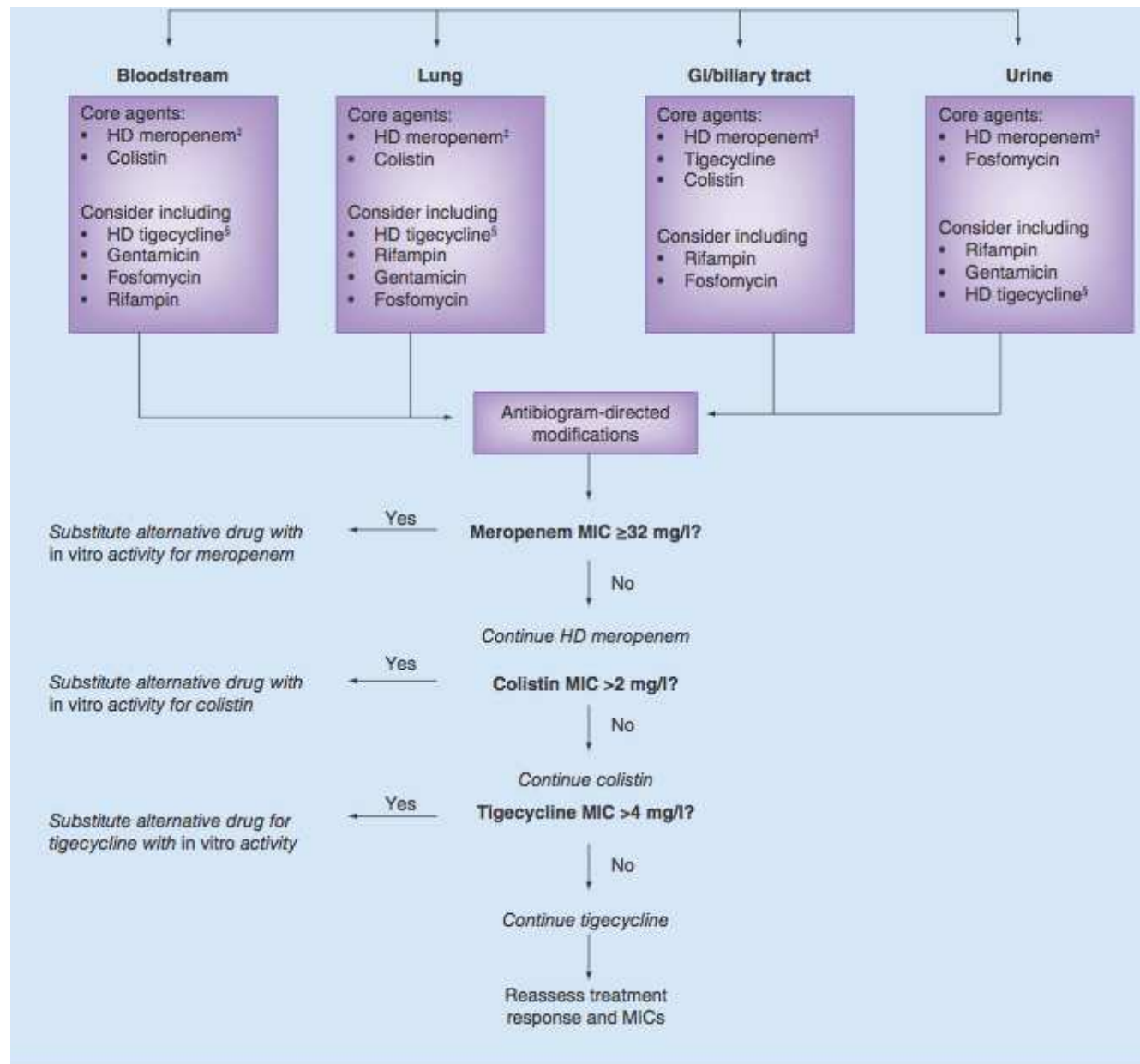
Predictors of Mortality in Bloodstream Infections Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *K. pneumoniae*: Importance of Combination Therapy

Mario Tumbarello,¹ Pierluigi Viale,² Claudio Viscoli,³ Enrico Maria Treccarichi,¹ Fabio Tumietto,² Anna Marchese,⁴ Teresa Spanu,⁵ Simone Ambretti,⁶ Francesca Ginocchio,³ Francesco Cristini,² Angela Raffaella Losito,¹ Sara Tedeschi,² Roberto Cauda,¹ and Matteo Bassetti^{3,7}

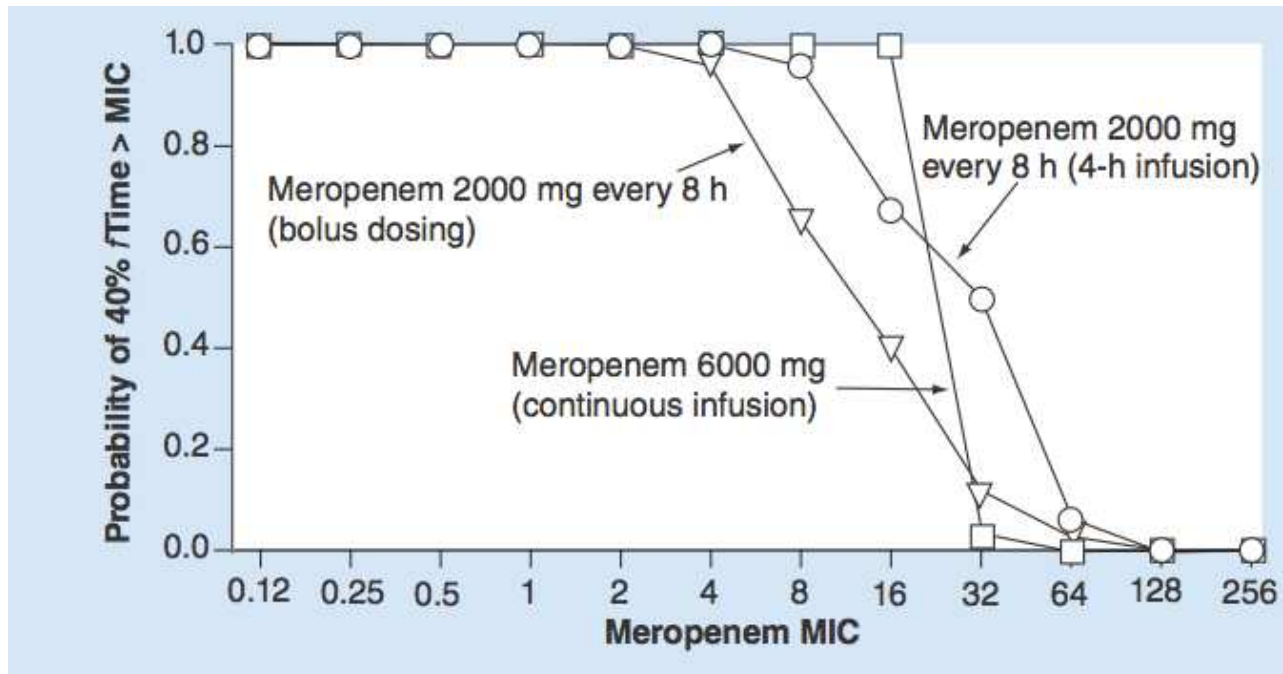
Meropenem+Tigeciclina+Colistina

Meropenem+Tigeciclina+Gentamicina

Therapy Carbapenem-resistant *Klebsiella pneumoniae*



Carbapenem-resistant *Klebsiella pneumoniae*, Italy



Meropenem MIC (mg/L)	Total	No. (%)	
		Nonsurvivors	Survivors
1	1	0	1 (100)
2	4	0	4 (100)
4	10	2 (20)	8 (80)
8	4	1 (25)	3 (75)
≥16	17	6 (35.2)	11 (64.7)
Total	36	9 (25)	27 (75)

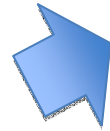
64% vs 58%

Roberts et al. JAC 2009
Tumbarello et al. CID 2012

Carbapenem based regimen

Meropenem^{HD}

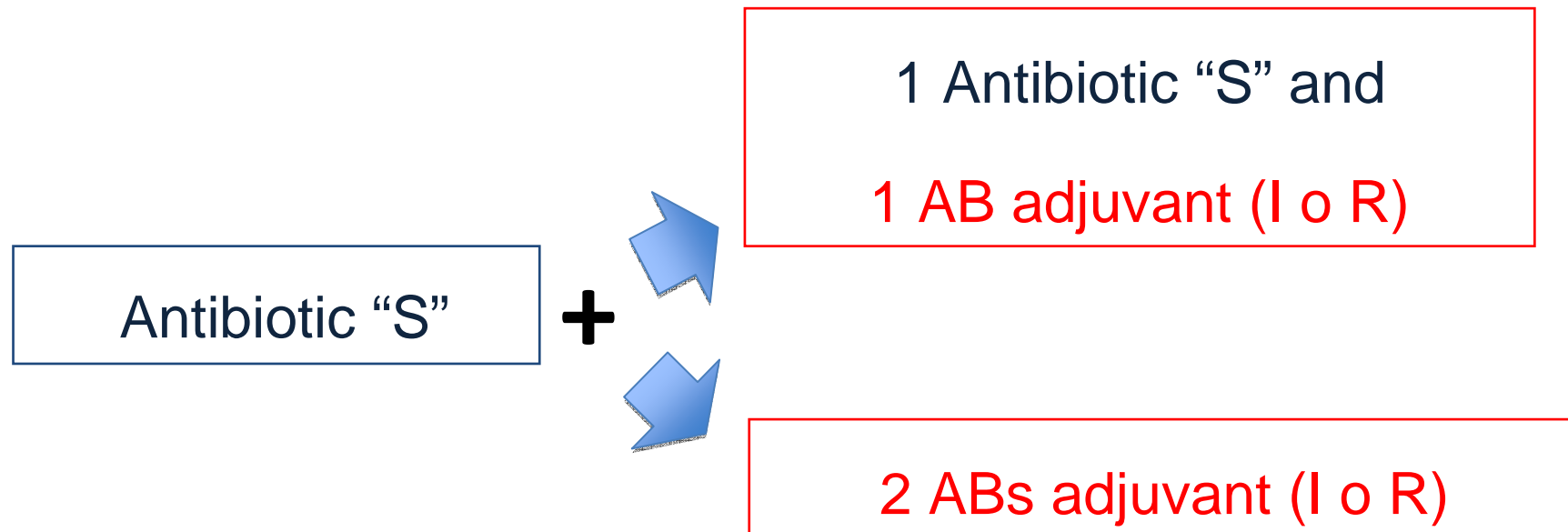
+



2 Antibiotics "S"

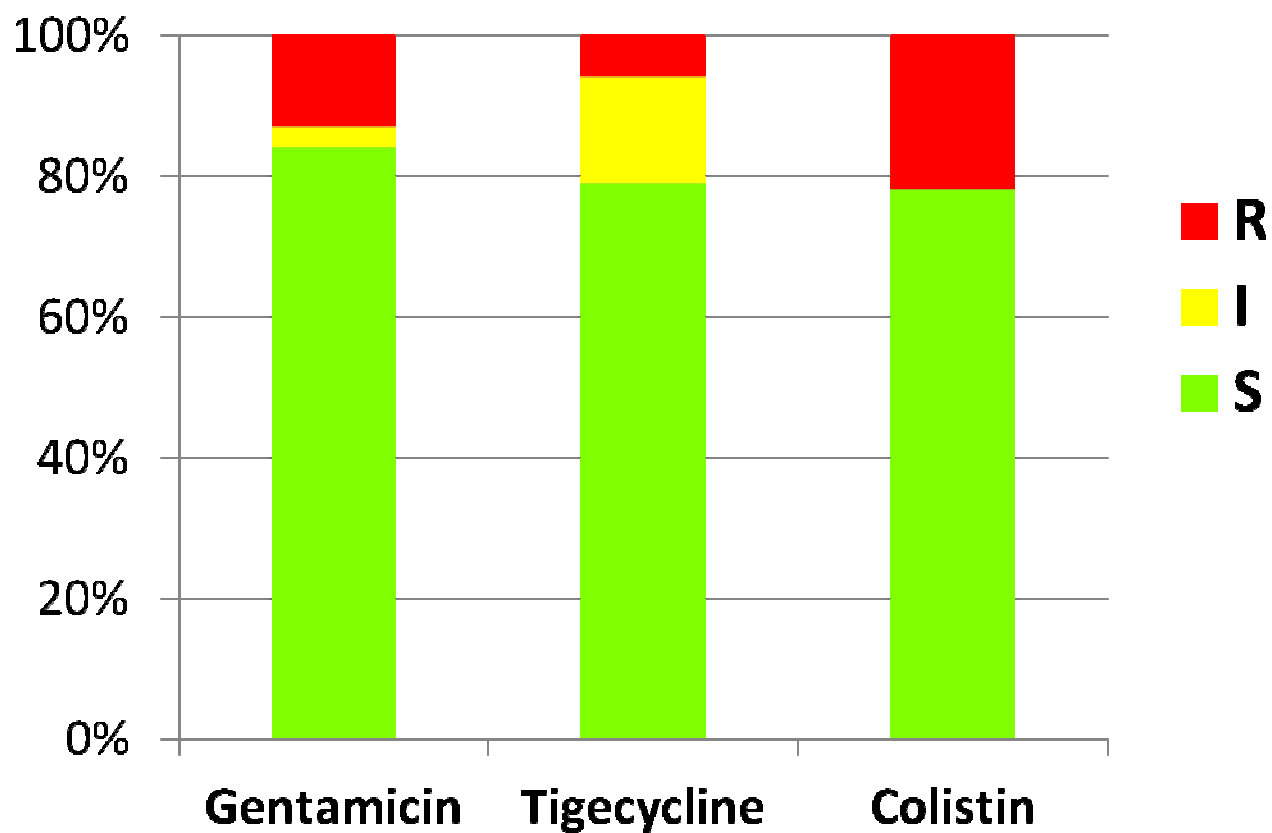
Antibiotic "S" and
AB adjuvant (I or R)

Carbapenem sparing regimen



Backbone agents

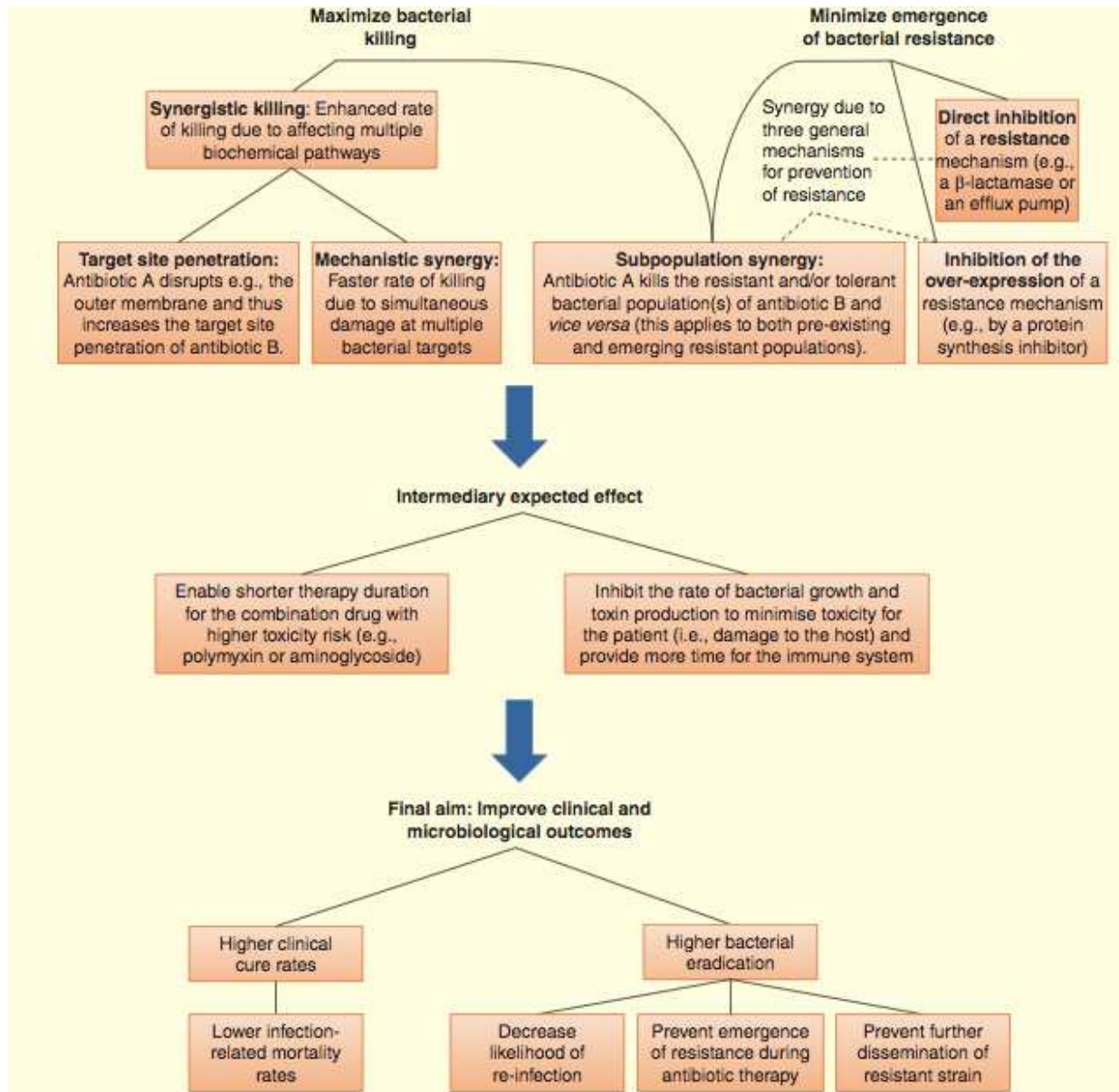
Sensibilità delle KPC-Kp (Sorveglianza nazionale Italiana 2011, n=204 isolati)



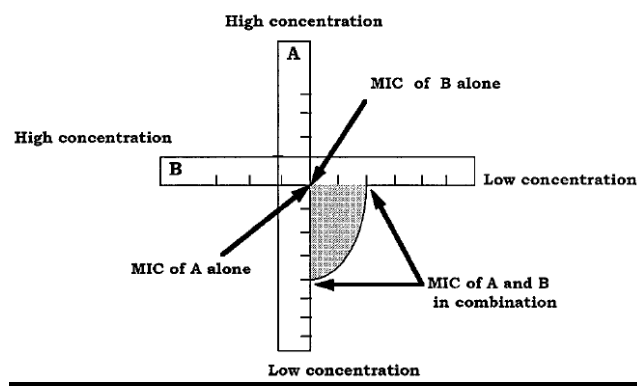
Adjuvant antibiotics

- Colistin
- Tigecycline
- Gentamicin
- Rifampin
- Fosfomycin

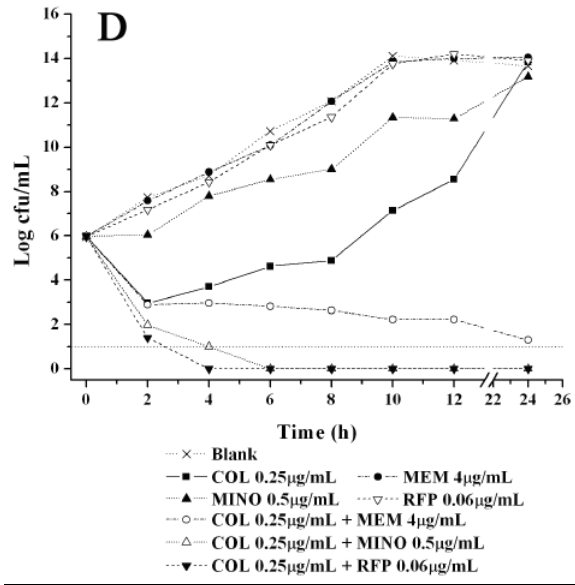
Why to use adjuvant antibiotics? Microbiologic base



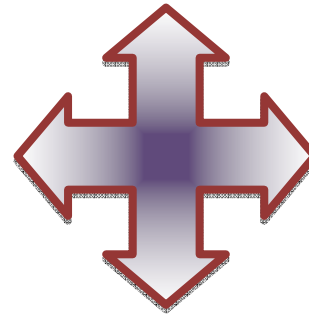
Etest methods



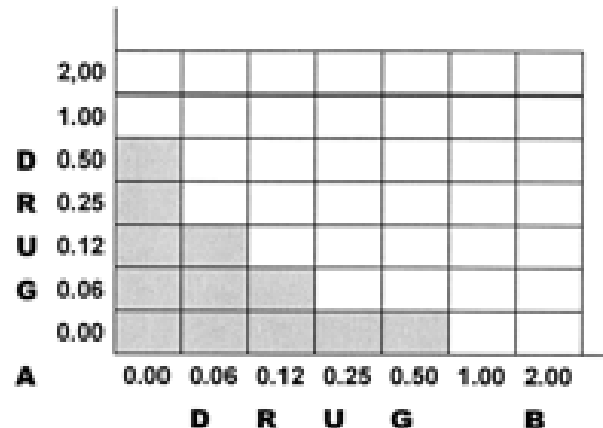
Time-kill assays



Double-disk



Checkerboard assay



Interpretation Checkerboard

Rohner *et al* – AAC 1989 ≤ 0.5 sinergico
 $> 0.5 - \leq 0.75$ additivo
 $> 0.75 - \leq 4$ indifferente
 > 4 antagonista

Bonapace *et al* – DMID 2002 ≤ 0.5 sinergico
 $> 0.5 - \leq 4$ indifferente
 > 4 antagonista

Cai *et al* – J Antibiot 2007 ≤ 0.5 sinergico
 $> 0.5 - \leq 1$ parz. sinergico
 $> 1 - \leq 4$ indifferente
 > 4 antagonista

Zusman *et al* – AAC 2013 ≤ 0.5 sinergico
 $> 0.5 - \leq 1$ additivo
 $> 1 - \leq 4$ indifferente
 > 4 antagonista

Sviluppo di un checkerboard modificato per il test della sinergia

COL ₁₆	COL ₈	COL ₄	COL ₂	COL ₁	COL _{0,5}	COL _{0,25}	RIF ₁₂₈	RIF ₆₄	RIF ₃₂	RIF ₁₆	MEM ₃₂	RIF ₂₄	TIGE _{10,5}
MEM ₁₂₈	MEM ₆₄	MEM ₃₂	MEM ₁₆	MEM ₈	IMI ₁₂₈	IMI ₆₄	IMI ₃₂	IMI ₁₆	IMI ₈	IMI ₄	MEM ₃₂	RIF ₂₄	TIGE _{10,5}
TIGE ₈	TIGE ₄	TIGE ₂	TIGE ₁	TIGE _{0,5}	TIGE _{0,25}	TIGE _{0,125}	MEM ₃₂	RIF ₂₄	IMI ₃₂	IMI ₃₂	MEM ₃₂	RIF ₂₄	TIGE _{10,5}
GENTA ₁₆	GENTA ₈	GENTA ₄	GENTA ₂	GENTA ₁	GENTA _{0,5}	GENTA _{0,25}	TIGE _{10,5}	TIGE _{10,5}	TIGE _{10,25}	TIGE _{10,125}	MEM ₃₂	RIF ₂₄	TIGE _{10,5}
MEM ₃₂	MEM ₃₂	MEM ₃₂	MEM ₃₂	COL ₂₄	TIGE _{10,5}	TIGE _{10,25}	TIGE _{10,5}	MEM ₃₂	MEM ₃₂	IMI ₃₂	IMI ₃₂	RIF ₂₄	TIGE _{10,5}
TIGE _{10,5}	TIGE _{10,25}	GENTA ₂₄	GENTA _{10,5}	RIF ₂₄	COL ₂₄	COL ₂₄	GENTA ₂₄	COL ₂₄	COL ₂₄	TIGE _{10,25}	TIGE _{10,25}	GENTA ₂₄	GENTA _{10,5}
MEM ₂₄	MEM ₂₄	MEM ₂₄	MEM ₂₄	COL ₂₂	TIGE _{10,5}	TIGE _{10,25}	TIGE _{10,5}	MEM ₂₄	MEM ₂₄	IMI ₂₄	IMI ₂₄	RIF ₂₄	TIGE _{10,5}
TIGE _{10,5}	TIGE _{10,25}	GENTA ₂₂	GENTA _{10,5}	RIF ₂₄	COL ₂₂	COL ₂₂	GENTA ₂₂	COL ₂₂	COL ₂₂	TIGE _{10,25}	TIGE _{10,25}	GENTA ₂₂	GENTA _{10,5}
MEM ₂₂	MEM ₂₂	MEM ₂₂	MEM ₂₂	COL ₂₀	TIGE _{10,5}	TIGE _{10,25}	TIGE _{10,5}	MEM ₂₂	MEM ₂₂	IMI ₂₂	IMI ₂₂	RIF ₂₄	TIGE _{10,5}
TIGE _{10,5}	TIGE _{10,25}	GENTA ₂₀	GENTA _{10,5}	RIF ₂₄	COL ₂₀	COL ₂₀	GENTA ₂₀	COL ₂₀	COL ₂₀	TIGE _{10,25}	TIGE _{10,25}	GENTA ₂₀	GENTA _{10,5}
MEM ₂₀	MEM ₂₀	MEM ₂₀	MEM ₂₀	COL ₁₈	TIGE _{10,5}	TIGE _{10,25}	TIGE _{10,5}	MEM ₂₀	MEM ₂₀	IMI ₂₀	IMI ₂₀	RIF ₂₄	TIGE _{10,5}
TIGE _{10,5}	TIGE _{10,25}	GENTA ₁₈	GENTA _{10,5}	RIF ₂₄	COL ₁₈	COL ₁₈	GENTA ₁₈	COL ₁₈	COL ₁₈	TIGE _{10,25}	TIGE _{10,25}	GENTA ₁₈	GENTA _{10,5}

 Sector 1, MICs of single drugs

 Sector 2, test dual combinations (Σ FIC indexes)

 Sector 3, three-drug combinations

Collezione isolati BSI collaborazione T. Spanu e M. Tumbarello



late 2011-13

95 *K. pneumoniae* KPC
isolates
ST512
ST258

Layout del Miniboard

	1	2	3	4	5	6	7	8	9	10	11	12
A	COL 16	COL 8	COL 4	COL 2	COL 1	COL 0,5	COL 0,25	RIF 128	RIF 64	RIF 32	RIF 16	MEM RIF TIGE0,5
B	MEM 128	MEM 64	MEM 32	MEM 16	MEM 8	IMI 128	IMI 64	IMI 32	IMI 16	IMI 8	IMI 4	TIGE RIF GENTA
C	TIGE 8	TIGE 4	TIGE 2	TIGE 1	TIGE 0,5	TIGE 0,25	TIGE 0,125	MEM COL RIFA	RIF TIGE0,5 COL	IMI COL TIGE0,5	IMI COL RIFA	MEM COL TIGE0,5
D	GENTA 16	GENTA 8	GENTA 4	GENTA 2	GENTA 1	GENTA 0,5	GENTA 0,25	TIGE RIFA	TIGE0,5 RIFA	TIGE0,25 RIFA	TIGE0,125 RIFA	MEM TIGE0,5 GENTA
E	MEM TIGE1	MEM TIGE0,25	MEM GENTA2	MEM GENTA1	COL RIF	TIGE COL	TIGE0,25 COL	TIGE0,5 GENTA	MEM COL	MEM COL	IMI TIGE0,25	IMI GENTA
F	MEM TIGE1	MEM TIGE0,25	MEM GENTA2	MEM GENTA1	COL RIF	TIGE COL	TIGE0,25 COL	TIGE0,5 GENTA	MEM COL	MEM COL	IMI TIGE0,25	IMI GENTA
G	MEM TIGE1	MEM TIGE0,25	MEM GENTA2	MEM GENTA1	COL RIF	TIGE COL	TIGE0,25 COL	TIGE0,5 GENTA	MEM COL	MEM COL	IMI TIGE0,25	IMI GENTA
H	MEM TIGE1	MEM TIGE0,25	MEM GENTA2	MEM GENTA1	COL0,5 RIF	TIGE COL0,5	TIGE0,25 COL0,5	TIGE0,5 GENTA0,5	MEM COL	MEM COL	IMI TIGE0,25	IMI GENTA

Settore 1, MICs dei singoli farmaci

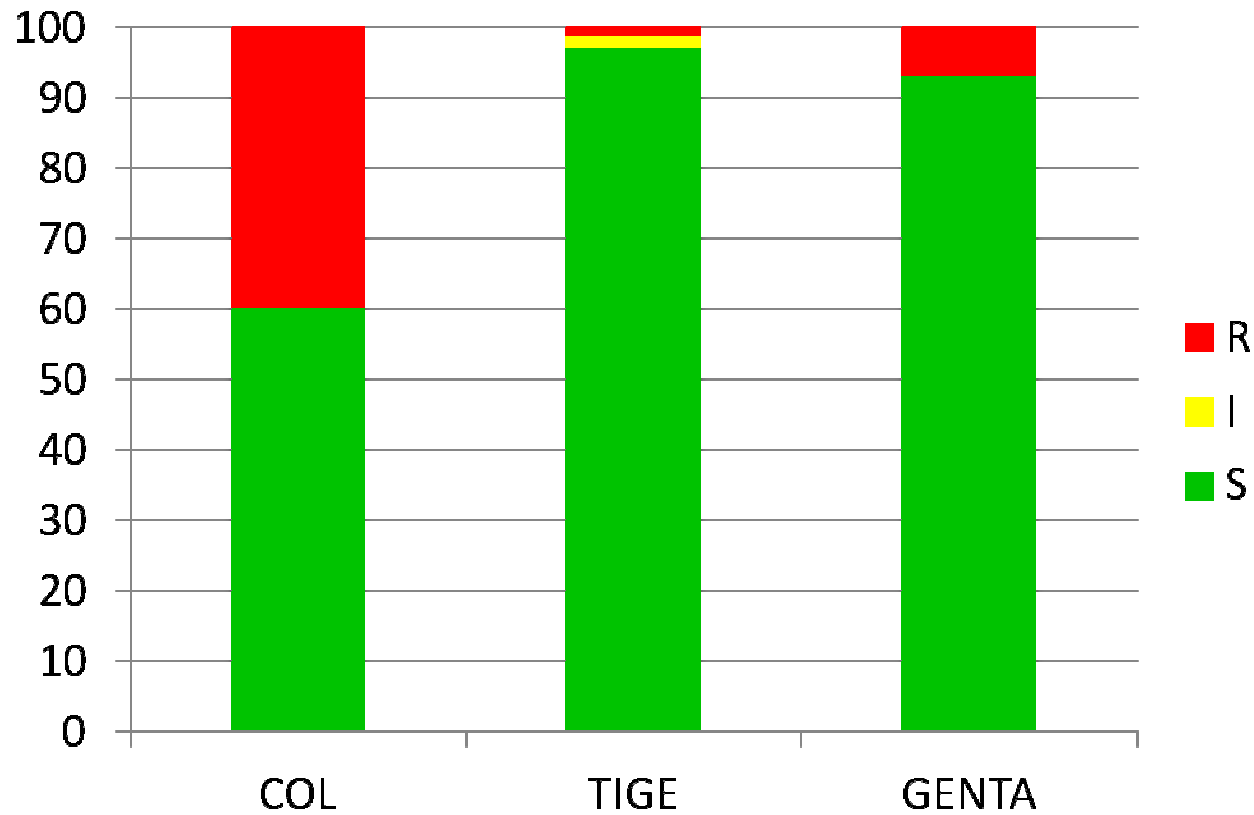
Patent application # FI2013A000203

Sviluppo di un checkerboard modificato per il test della sinergia tra antibiotici contro i CRE

- ◆ Una sola piastra

- ◆ Determinazione della MIC di singoli agenti
 - MER
 - IMI
 - TIG
 - GEN
 - COL
 - RIF

Susceptibility testing (collection): 95 isolates



Retained susceptibility (gentamicin, colistin, tigecyclin)

	Number	Percentage %
Susceptible to all	51	54
Susceptible to 2 drug	42	44
Susceptible to 1 drug	1	1
Resistant to all	1	1

} 46%

Carbapenem based regimen

+

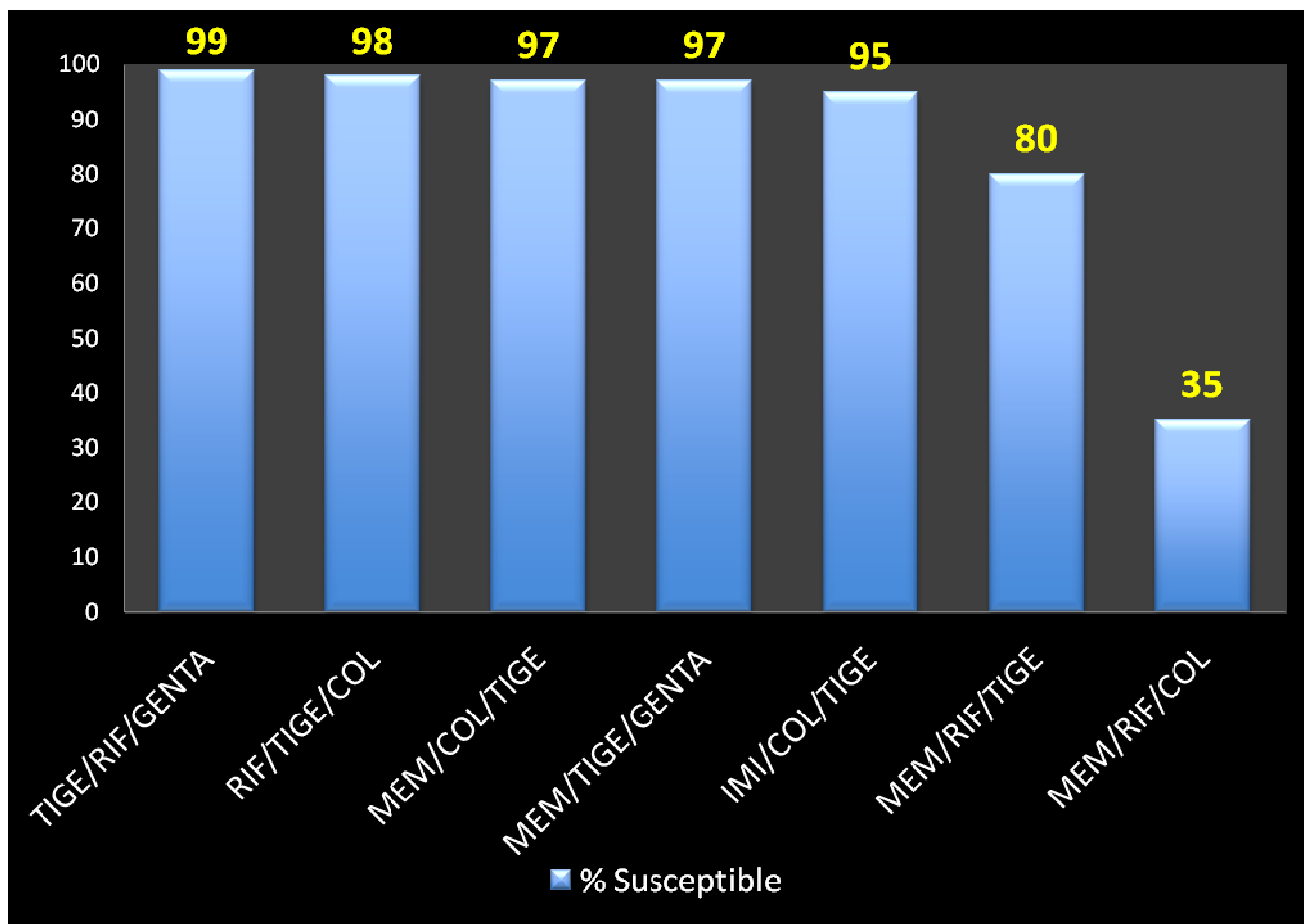
Carbapenem sparing regimen

Layout del Miniboard

	1	2	3	4	5	6	7	8	9	10	11	12				
A	COL 16	COL	COL	COL	COL	COL	COL 0,25	RIF 128	RIF 64	RIF 32	RIF 16	MEM TIGE0,5				
B	MEM 128	M	- MER/COL/RIF - MER/COL/TIG - MER/TIG/GEN - MER/TIG/RIF - IMI/COL/RIF - IMI/COL/TIG - RIF/COL/TIG - RIF/TIG/GEN				IMI 64	IMI 32	IMI 16	IMI 8	IMI 4	TIGE1	RIF GENTA			
C	TIGE 8	T					MEM COL RIFA	TIGE 0,125	MEM COL RIFA	RIF TIGE0,5	IMI COL	IMI COL	RIFA TIGE0,5	MEM COL TIGE0,5		
D	GENTA 16	GE					GENTA 0,25	TIGE 0,25	TIGE 0,5	TIGE 0,5	TIGE 0,25	TIGE 0,25	TIGE 0,125	MEM TIGE0,5	GENTA	
E	MEM TIGE1	M					TIGE 0,25	TIGE 0,5	MEM COL	MEM COL	IMI TIGE0,25	IMI TIGE0,25	MEM TIGE0,25	MEM TIGE0,25	MEM TIGE0,25	MEM TIGE0,25
F	MEM TIGE1	M					TIGE 0,25	TIGE 0,5	MEM COL	MEM COL	IMI TIGE0,25	IMI TIGE0,25	MEM TIGE0,25	MEM TIGE0,25	MEM TIGE0,25	MEM TIGE0,25
G	MEM 0	MEM					MEM	MEM	COL	TIGE	TIGE0,25	TIGE0,5	MEM	MEM	IMI	IMI
H	Conferma della sinergia e screening per l'antagonismo															

▬ Sector 3, combinazione a 3

Association (3 drugs)



Meropenem+Tigeciclina+Colistina

Meropenem+Tigeciclina+Gentamicina

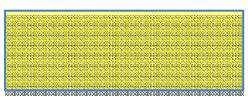
Layout del Miniboard

	1	2	3	4	5	6	7	8	9	10	11	12							
A	COL	COL	COL	COL	COL	COL	COL	RIF	RIF	RIF	RIF	MEM							
B	MEM	- MER/TIG - MER/GEN - MER/COL			MI	- COL/RIF - COL/TIG - TIG/GEN			- TIG/RIF - IMI/TIG - IMI/GEN			RIF							
C	TIGE				TIGE							TIGE	TIGE	TIGE	TIGE	TIGE	TIGE	TIGE	TIGE
D	GENTA				GENTA							GENTA	GENTA	GENTA	GENTA	GENTA	GENTA	GENTA	GENTA
E	MEM	MEM	MEM	MEM	COL	TIGE	TIGE,25	TIGE,5	MEM	MEM	IMI	IMI							
F	TIGE	TIGE,25	GENTA	GENTA	RIF	COL	COL	GENTA	COL	COL	TIGE,25	GENTA							
G	MEM	MEM	MEM	MEM	COL	TIGE	TIGE,25	TIGE,5	MEM	MEM	IMI	IMI							
H	TIGE	TIGE,25	GENTA	GENTA	RIF	COL	COL	GENTA	COL	COL	TIGE,25	GENTA							

Settore 2, test di combinazioni a 2 (ΣFIC)

Lettura del Miniboard

	1	2	3	4	5	6	7	8	9	10	11	12
A	COL 16	COL 8	COL 4	COL 2	COL 1	COL 0,5	COL 0,25	RIF 128	RIF 64	RIF 32	RIF 16	MEM RIF TIGE0,5
B	MEM 128	MEM 64	MEM 32	MEM 16	MEM 8	IMI 128	IMI 64	IMI 32	IMI 16	IMI 8	IMI 4	TIGE1 RIF24 GENTA1
C	TIGE 8	TIGE 4	TIGE 2	TIGE 1	TIGE 0,5	TIGE 0,25	TIGE 0,125	MEM3 COL1 RIFA24	RIF4 TIGE0,5 COL2	IMI3 COL2 TIGE0,5	IMI3 COL1 RIFA24	MEM3 COL2 TIGE0,5
D	GENTA 16	GENTA 8	GENTA 4	GENTA 2	GENTA 1	GENTA 0,5	GENTA 0,25	TIGE1 RIFA24	TIGE0,5 RIFA24	TIGE0,25 RIFA24	TIGE0,125 RIFA24	MEM3 TIGE0,5 GENTA1
E	MEM3 TIGE1	MEM3 TIGE0,25	MEM3 GENTA2	MEM3 GENTA1	COL4 RIF24	TIGE1 COL4	TIGE0,25 COL4	TIGE0,5 GENTA24	MEM3 COL4	MEM3 COL2	IMI3 TIGE0,25	IMI3 GENTA1
F	MEM4 TIGE1	MEM4 TIGE0,25	MEM4 GENTA2	MEM4 GENTA1	COL2 RIF24	TIGE1 COL2	TIGE0,25 COL2	TIGE0,5 GENTA2	MEM4 COL4	MEM4 COL2	IMI4 TIGE0,25	IMI4 GENTA1
G	MEM2 TIGE1	MEM2 TIGE0,25	MEM2 GENTA2	MEM2 GENTA1	COL1 RIF24	TIGE1 COL1	TIGE0,25 COL1	TIGE0,5 GENTA1	MEM2 COL4	MEM2 COL2	IMI2 TIGE0,25	IMI2 GENTA1
H	MEM1 TIGE1	MEM1 TIGE0,25	MEM1 GENTA2	MEM1 GENTA1	COL0,5 RIF24	TIGE1 COL0,5	TIGE0,25 COL0,5	TIGE0,5 GENTA0,5	MEM1 COL4	MEM1 COL2	IMI1 TIGE0,25	IMI1 GENTA1



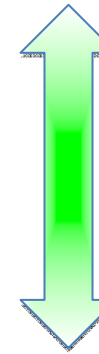
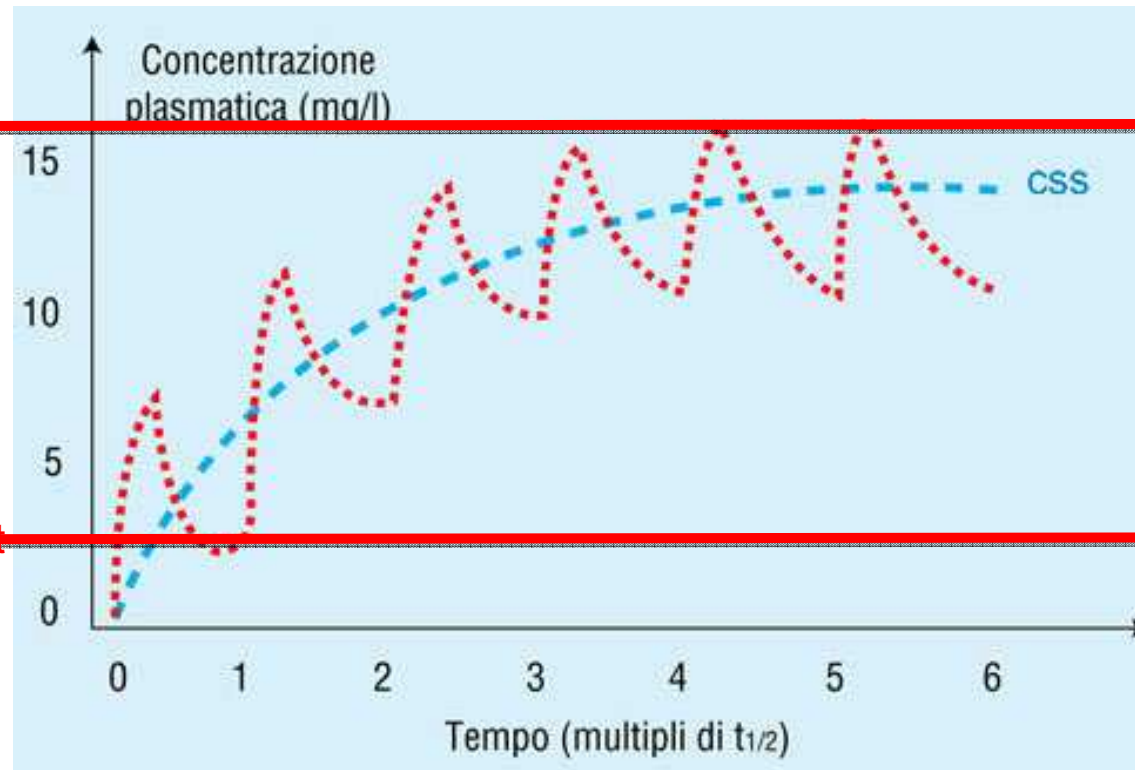
Crescita Batterica

Σ FIC=0.18 → SINERGICO !!

Concentrazioni testate in associazione

C non
raggiungibili

C sotto breakpoint
"S"

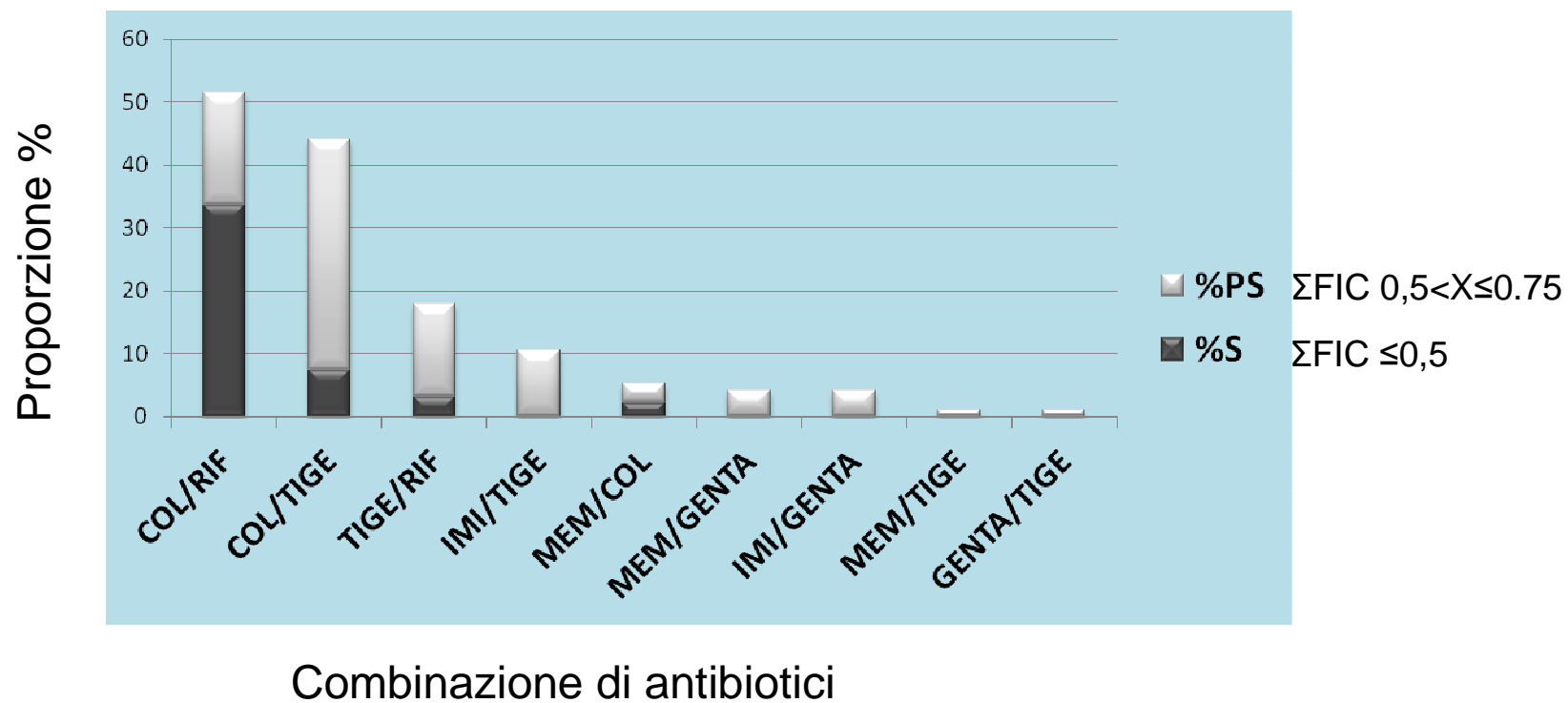


Not evaluable using MINIBOARD

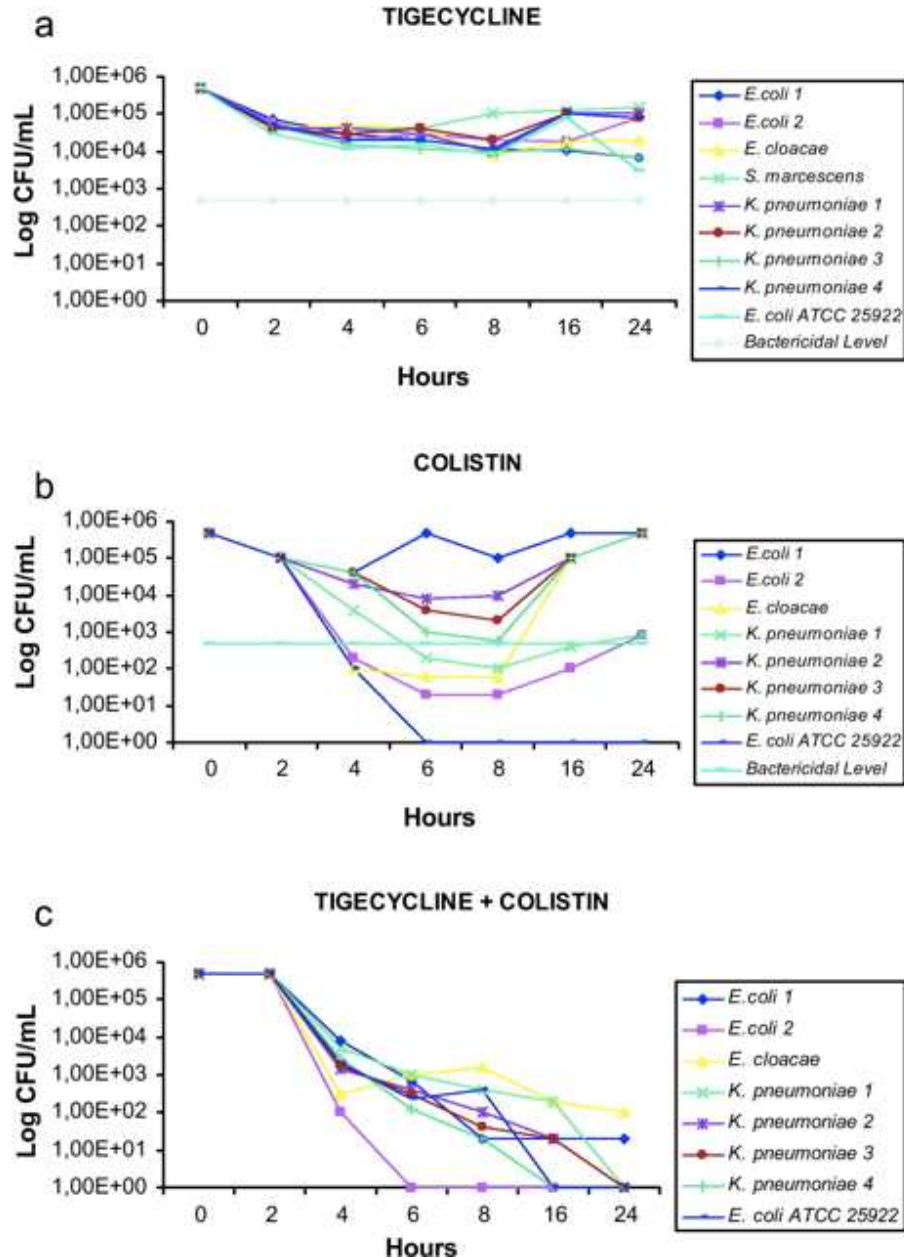
Combination	Synergy not evaluable	Possible synergy not evaluable
COL-RIF	32	3
COL-TIGE	85	31
TIGE-RIF	10	1
MEM-TIGE	80	10
MEM-GENTA	89	86
TIGE-GENTA	95	94
MEM-COL	59	55
IMI-TIGE	80	10
IMI-GENTA	89	86

TOT. EVALUABLE
SYNERGY 236;
POT. SYNERGY 380

Proporzione dei sinergismi osservata con le varie combinazioni tra antibiotici



Time-kill assay TIGE-COL

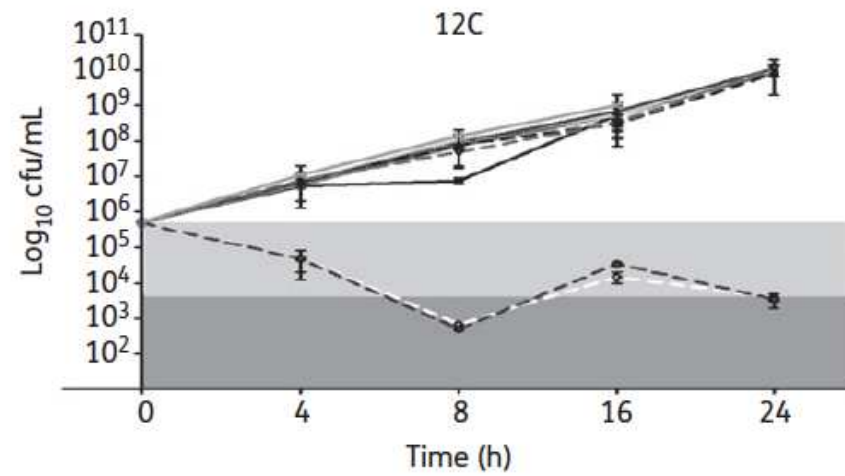
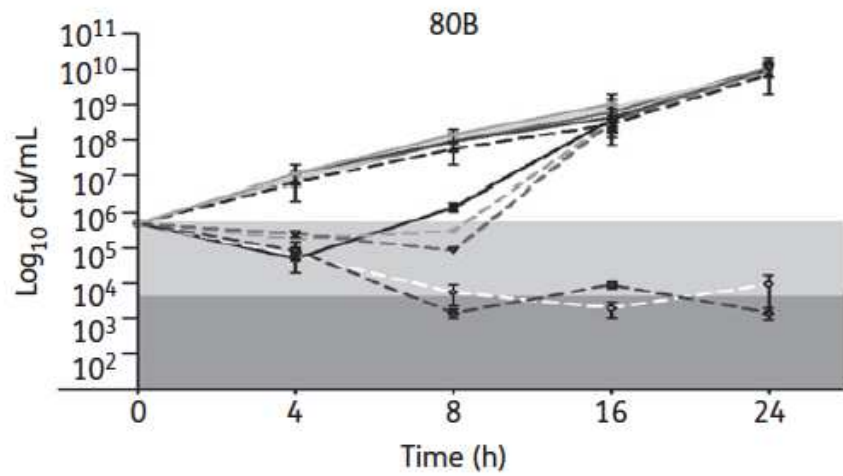


Treatment for infection	n (%)	Infection mortality n (%)
Combination schemes	20 (57.1)	0
Tigecycline combined with		
Colistin	9 (26.5)	0
Gentamicin	3 (8.8)	0
Colistin + carbapenem	2 (5.9)	0
Carbapenem	1 (2.9)	0
Colistin + gentamicin	1 (2.9)	0
Amikacin	1 (2.9)	0
Colistin + gentamicin	2 (5.8)	0
Carbapenem + gentamicin	1 (2.9)	0
Monotherapy	15 (42.9)	7 (46.7)
Colistin	7 (20)	4 (66.7)
Tigecycline	5 (14.7)	2 (40)
Gentamicin	2 (5.9)	0
Carbapenem	1 (2.9)	1 (100)
Total	35	7 (20)

	Non survivors	Survivors
Tigecycline + colistin	7 (13.4)	16 (21.9)
Tigecycline + gentamicin	6 (11.5)	6 (8.2)
Other 2-drug combinations ^e	10 (19.2)	11 (15.1)

Pournaras *et al* – IJAA 2013
 Zarkotu *et al* – CMI 2011
 Tumbarello *et al* – CID 2012

Time-kill assay COL-RIF



■ MEM □ TGC ◆ RIF ▲ CST ● CST/MEM ◆ CST/RIF
 ▼ MEM/CST/TGC ▲ CST/TGC ● CST/RIF/TGC ✕ Untreated culture

Conclusioni

- Crescente richiesta di test di sinergia al laboratorio di Microbiologia Clinica
- Mancanza di test standardizzati per la valutazione delle sinergie nella *routine* (*unmet lab need*)
- Il MINIBOARD può aumentare significativamente le nostre conoscenze sulle sinergie
- Le associazioni a tre farmaci risultano attive *in vitro* nella maggioranza dei casi
- I test di sinergia supportano l'uso di di associazioni che includano tigeciclina/colistina
- Scarsi dati su correlazione diretta tra outcome e sinergia *in vitro* ma evidenze microbiologiche sulla loro utilità

Further Steps

- Studio retrospettivo osservazionale sulla predittività clinica dei test di sinergia (collaborazione M. Tumbarello e T. Spanu)
- Studio validazione inter-laboratorio del MINIBOARD (collaborazione R. Rigoli e C. Scarparo)

GRAZIE dell' ATTENZIONE