Evaluation of Polymyxin B-based and Dual-Carbapenem Combinations against Carbapenem-Resistant Enterobacteriaceae co-harbouring *mcr-1* and *KPC-2*

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Disclosures

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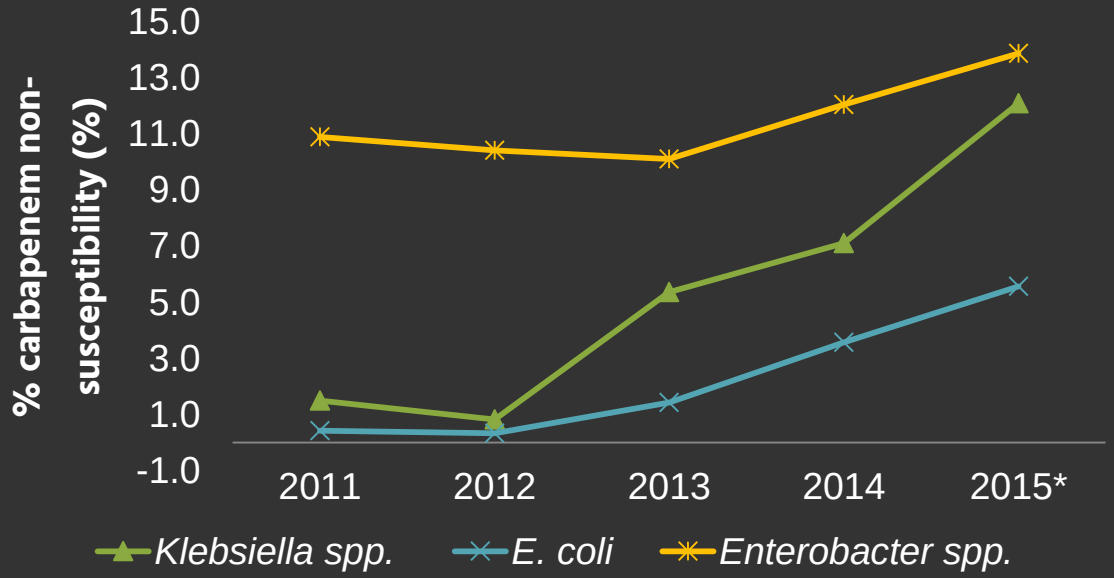
A serious public health threat

CARBAPENEM-RESISTANT ENTEROBACTERIACEAE (CRE)

https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

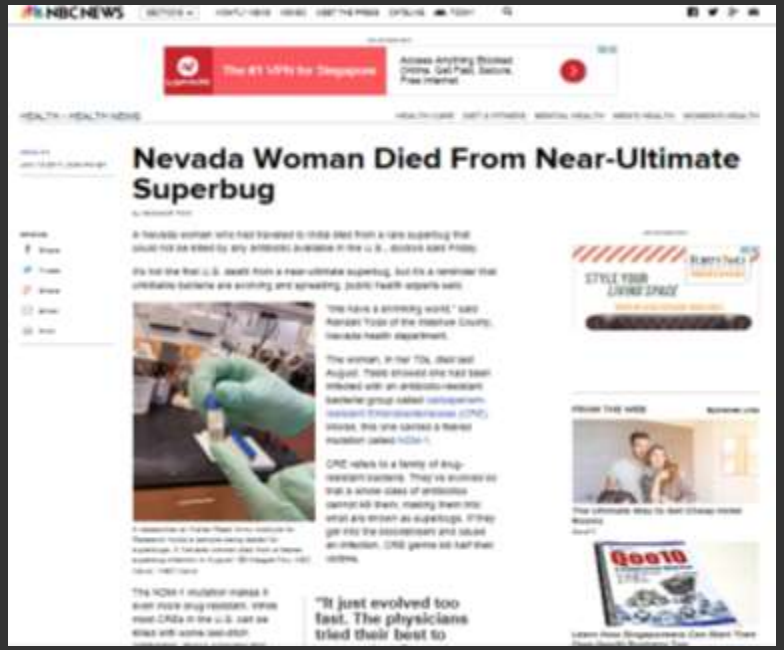
Increasing carbapenem resistance in Singapore



JQ Teo et al. Microorganisms 4 (1). 2016 Feb 16.

Last resort antibiotic

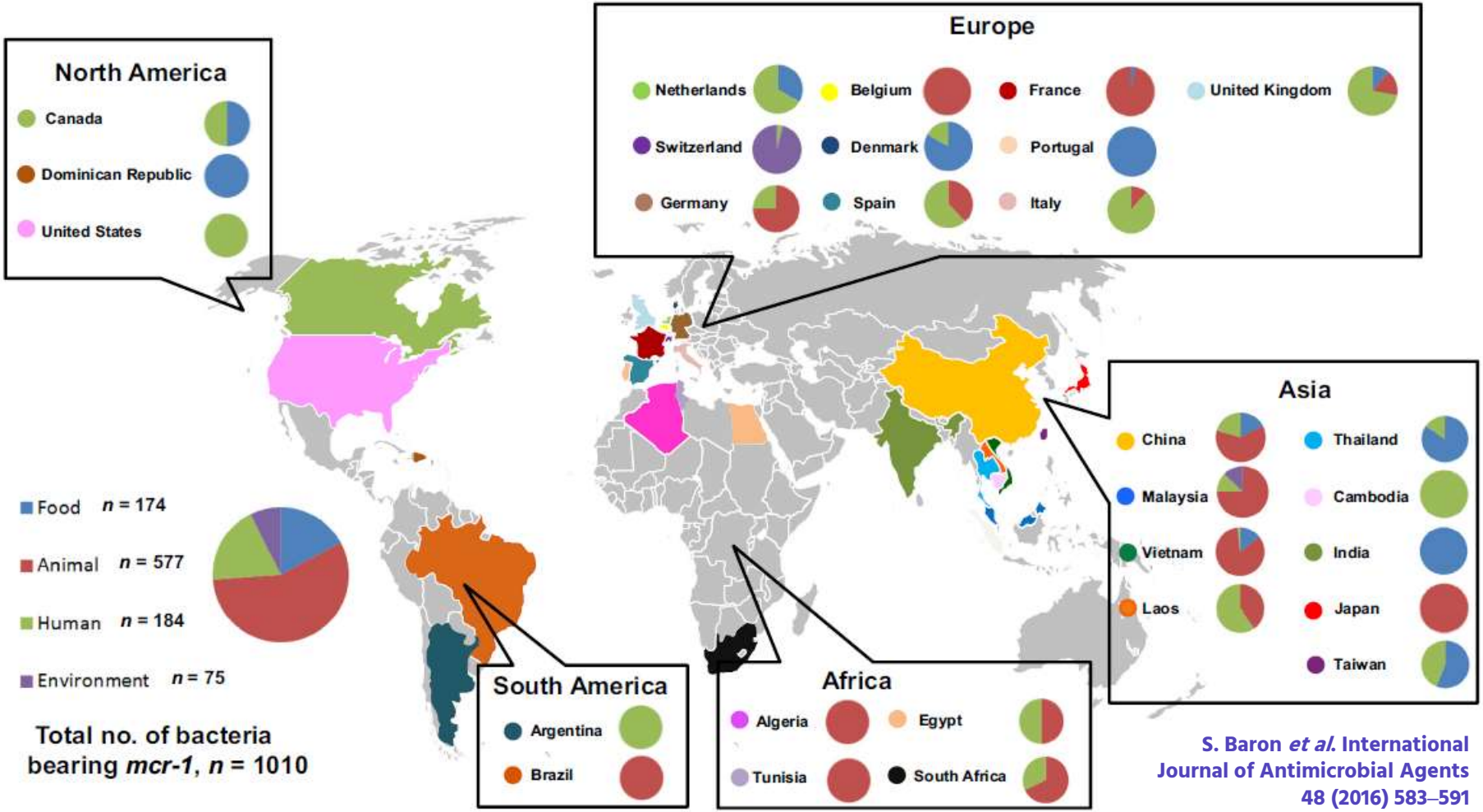
Polymyxin, an old antibiotic, is increasingly being used for multi-drug resistant infections



http://www.nbcnews.com/health/health-news/nevada-woman-died-near-ultimate-superbug-n706641

POLYMYXIN RESISTANCE

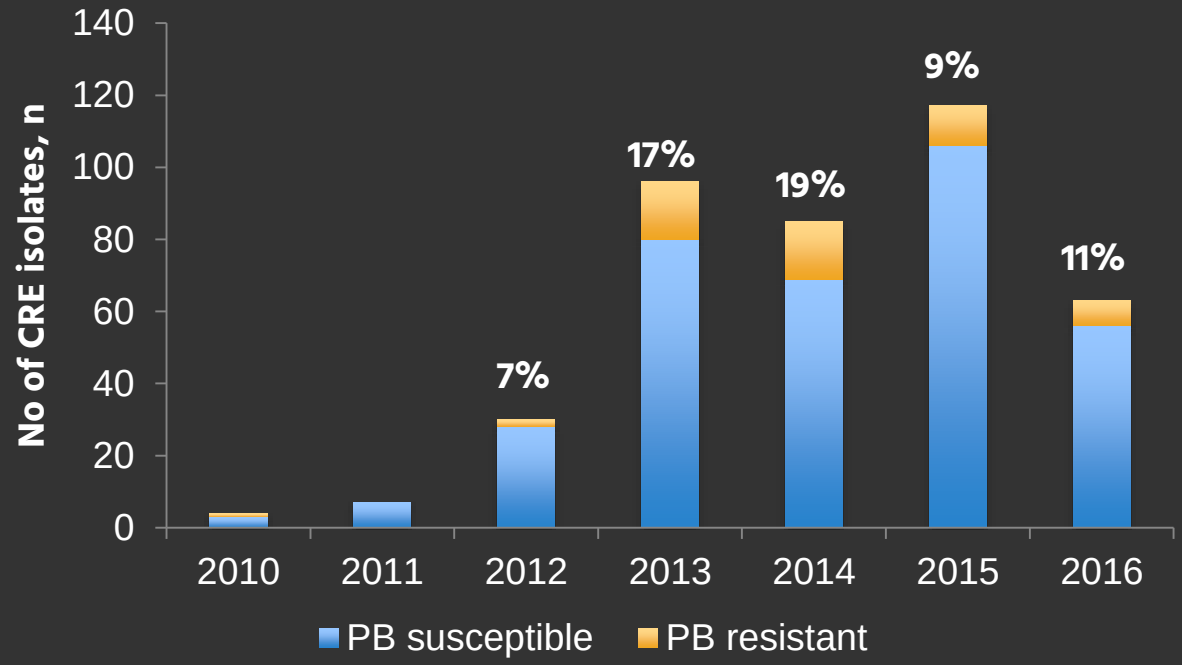
ANOTHER NAIL IN THE ANTIBIOTIC COFFIN…



S. Baron *et al.* International Journal of Antimicrobial Agents 48 (2016) 583–591

Fig. 2. Global distribution of plasmid-mediated *mcr-1* colistin-resistant strains isolated from environments, foods, animals and humans (November 2015 to April 2016).

13% OF CRE ISOLATES WERE PB-RESISTANT



J. Teo *et al.* 27th ECCMID (23rd Apr 2017) Poster P0711

Study objective

- To evaluate the *in vitro* activity of multiple antibiotics in combination with polymyxin B against *mcr-1*-harbouring CRE through time-kill studies

METHODS

Study Isolates

- CRE prospective surveillance in Singapore General Hospital
- Retrospective screening for *mcr-1* via WGS and/or PCR for the period 2013 - 2015

Susceptibility Testing (MIC)

- Microbroth dilution panels (Trek Diagnostics, West Sussex, UK)

Study Antibiotics

Antibiotic	Simulated Dosing Regimens	Concentration (mg/L)
Polymyxin B	30,000IU/kg/day	2
Ertapenem	1g every 24h	15
Aztreonam	8g every 24h (infused over 24h)	24
Cefepime	2g every 8h (infused over 4h)	50
Piperacillin/ tazobactam	4.5g every 6h (infused over 4h)	35/7
Meropenem	2g every 8h (infused over 3h)	20
Doripenem	2g every 8h (infused over 4h)	26
Imipenem	1g every 6h (infused over 1h)	12.5
Levofloxacin	750mg every 24h	8
Rifampicin	600mg every 12h	4
Tigecycline	100mg every 12h	2

Polymyxin B Combinations Time-kill Studies

Antibiotic	Simulated Dosing Regimens	Concentration (mg/L)
Polymyxin B	30,000IU/kg/day	2
Ertapenem	1g every 24h	15
Aztreonam	8g every 24h (infused over 24h)	24
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Levofloxacin	750mg every 24h	8
Rifampicin	600mg every 12h	4
Tigecycline	100mg every 12h	2

Dual Carbapenems Time-kill Studies

Antibiotic	Simulated Dosing Regimens	Concentration (mg/L)
Polymyxin B	30,000IU/kg/day	2
Ertapenem	1g every 24h	15
Aztreonam	8g every 24h (infused over 24h)	24
Cefepime	2g every 8h (infused over 4h)	50
Piperacillin/ tazobactam	4.5g every 6h (infused over 4h)	35/7
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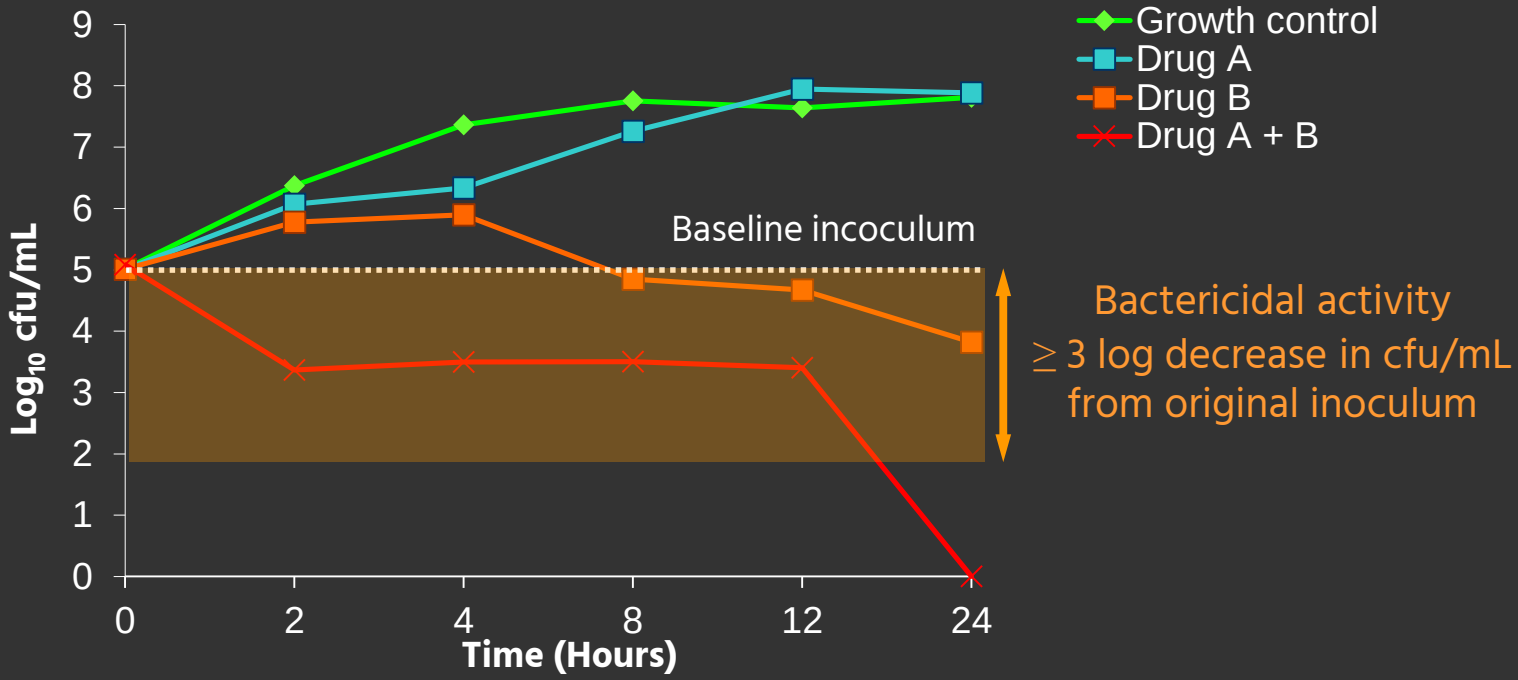
Study Endpoint



RESULTS

Test Isolates

	EC249	EC250	ENT702
Species	*E. coli*	*E. coli*	*E. aerogenes*
MLST	ST2006	ST2006	N.A.
Isolation site	Rectal swab	Rectal swab	Urine
Carbapenemase	KPC-2	KPC-2	KPC-2
Other beta-lactamases	TEM-1A	TEM-1A, AmpC1	TEM-1B, CTX-M-15
Other resistance determinants	*aadA1, aadA2, mph*(A), *cmlA1, sul3, dfrA12*	*aadA2, mph*(A), *cmlA1, sul3, dfrA12*	*strA, strB, aac(6')Ib-cr, aac(3)-IIa, qnrB66, mph*(A), *catB3, sul2, tet*(A), *dfrA14*
Porin loss	No	No	Yes

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Other beta-lactamases	TEM-1A	TEM-1A, AmpC1	TEM-1B, CTX-M-15
Other resistance determinants	*aadA1, aadA2, mph*(A), *cmlA1, sul3, dfrA12*	*aadA2, mph*(A), *cmlA1, sul3, dfrA12*	*strA, strB, aac(6')Ib-cr, aac(3)-IIa, qnrB66, mph*(A), *catB3, sul2, tet*(A), *dfrA14*
Porin loss	No	No	Yes
	Highly similar → possibly clonal		

Susceptibilities

MIC, mg/L	EC249	EC250	ENT702
Polymyxin B	**4**	**4**	**8**
Aztreonam	≥128	≥128	≥128
Cefepime	≥128	64	≥128
Piperacillin/Tazobactam	≥256/4	≥256/4	≥256/4
Doripenem	8	8	8
Imipenem	≥32	≥32	≥32
Meropenem	≥32	≥32	≥32
Ertapenem	≥32	≥32	≥32
Levofloxacin	≥64	≥64	1
Tigecycline	≤0.25	≤0.25	1
Rifampicin	≥64	≥64	≥64

Susceptibilities

MIC, mg/L	EC249	EC250	ENT702
Polymyxin B	**4**	**4**	**8**
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Imipenem	≥32	≥32	≥32
Meropenem	≥32	≥32	≥32
Ertapenem	≥32	≥32	≥32
Levofloxacin	≥64	≥64	**1**
Tigecycline	≤0.25	≤0.25	1
Rifampicin	≥64	≥64	≥64

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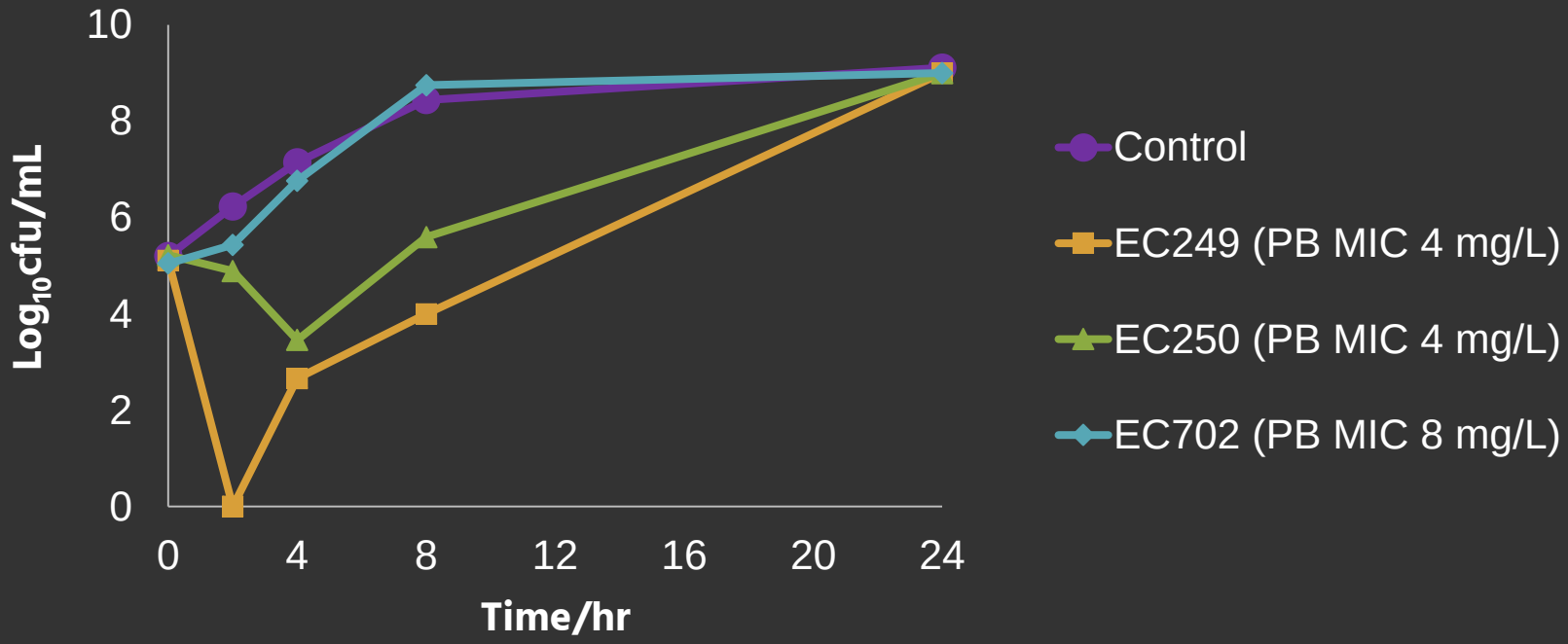
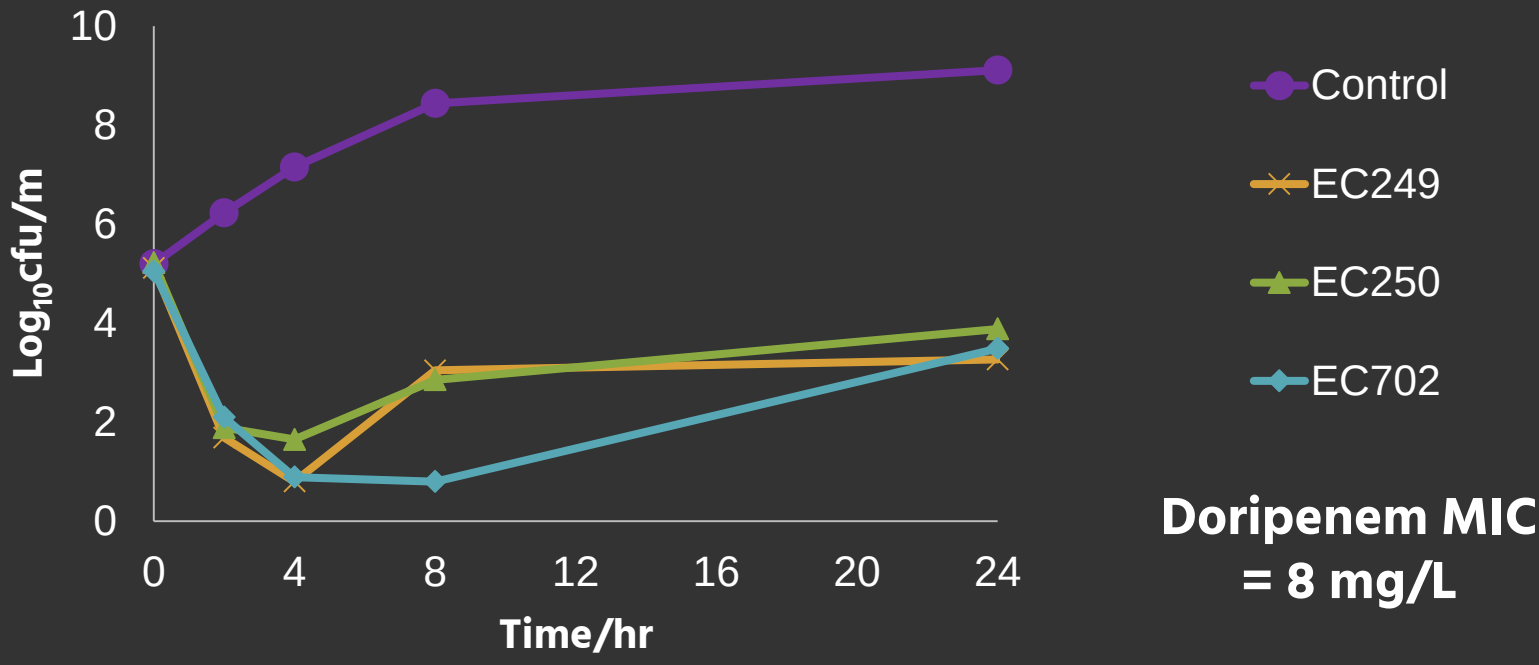
Doripenem	**8**	**8**	**8**
Imipenem	≥32	≥32	≥32
Meropenem	≥32	≥32	≥32
Ertapenem	≥32	≥32	≥32
Levofloxacin	≥64	≥64	**1**
Tigecycline	**≤0.25**	**≤0.25**	**1**
Rifampicin	≥64	≥64	≥64

Polymyxin Time-kill Curves

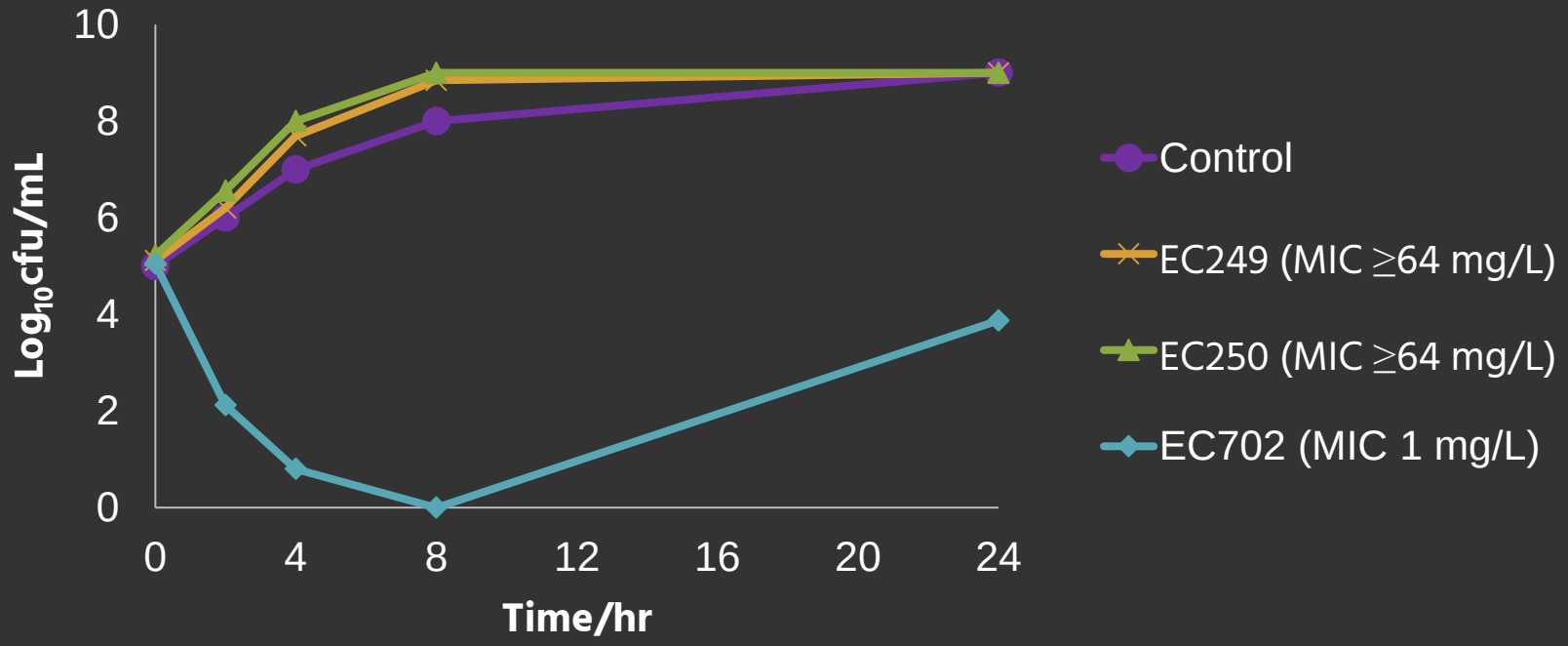


Doripenem Time-kill Curves

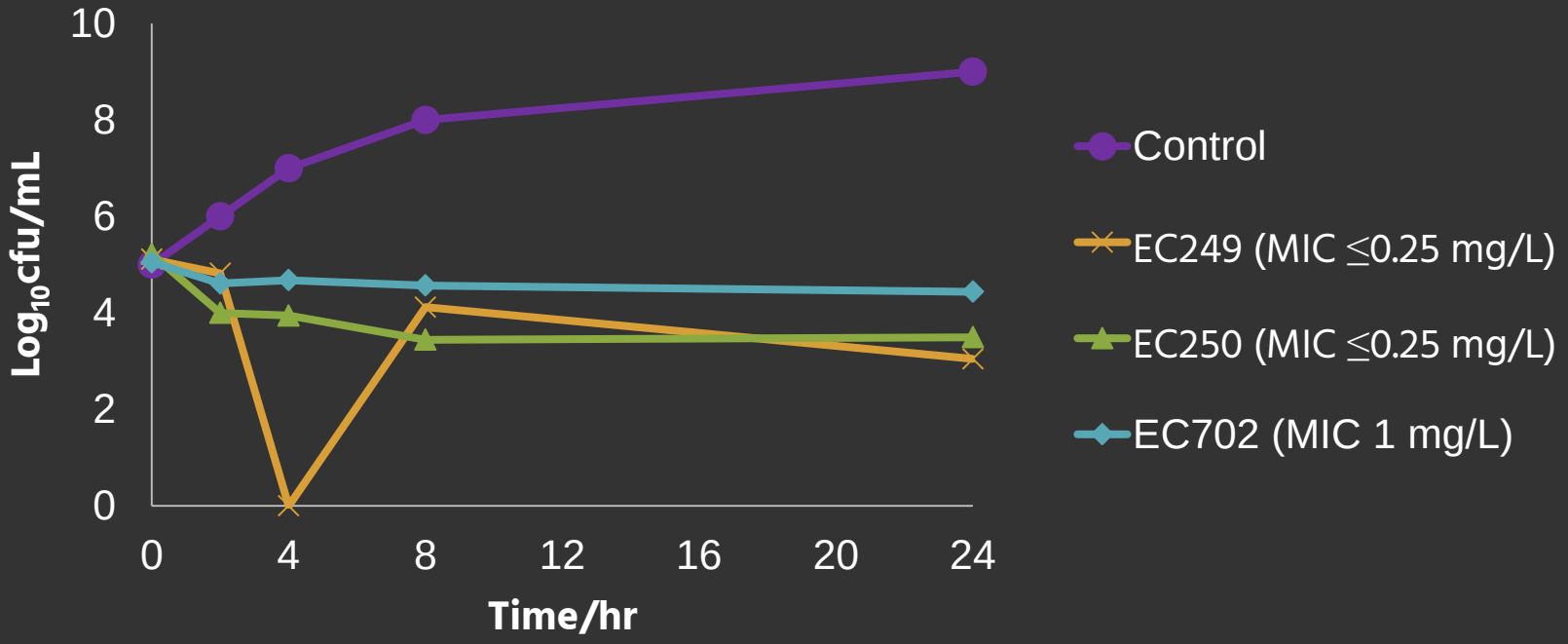


Doripenem MIC = 8 mg/L

Levofloxacin Time-kill Curves

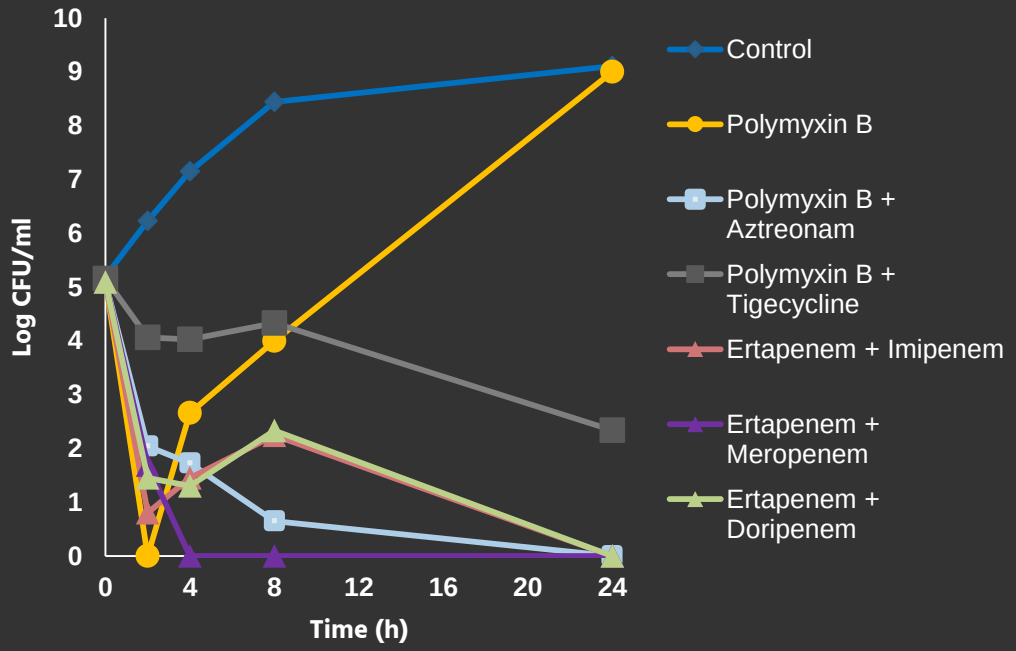


Tigecycline time-kill curves



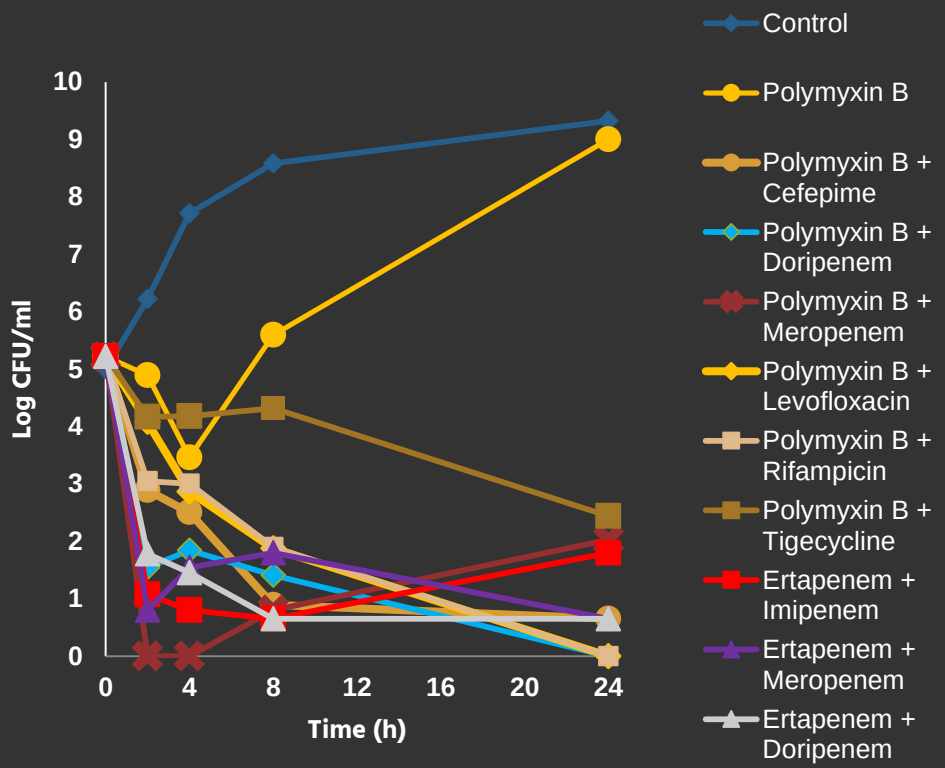
EC249 Combination Time-kill

	Antibiotic	EC249 Log CFU/mL at 24h
Polymyxin	Aztreonam	**0.00**
	Cefepime	4.13
	PT4	9.00
	Doripenem	4.32
	Imipenem	4.05
	Meropenem	2.89
	Rifampicin	2.78
	Tigecycline	**2.34**
	Levofloxacin	5.04
Ertapenem	Doripenem	**0.00**
	Imipenem	**0.00**
	Meropenem	**0.00**



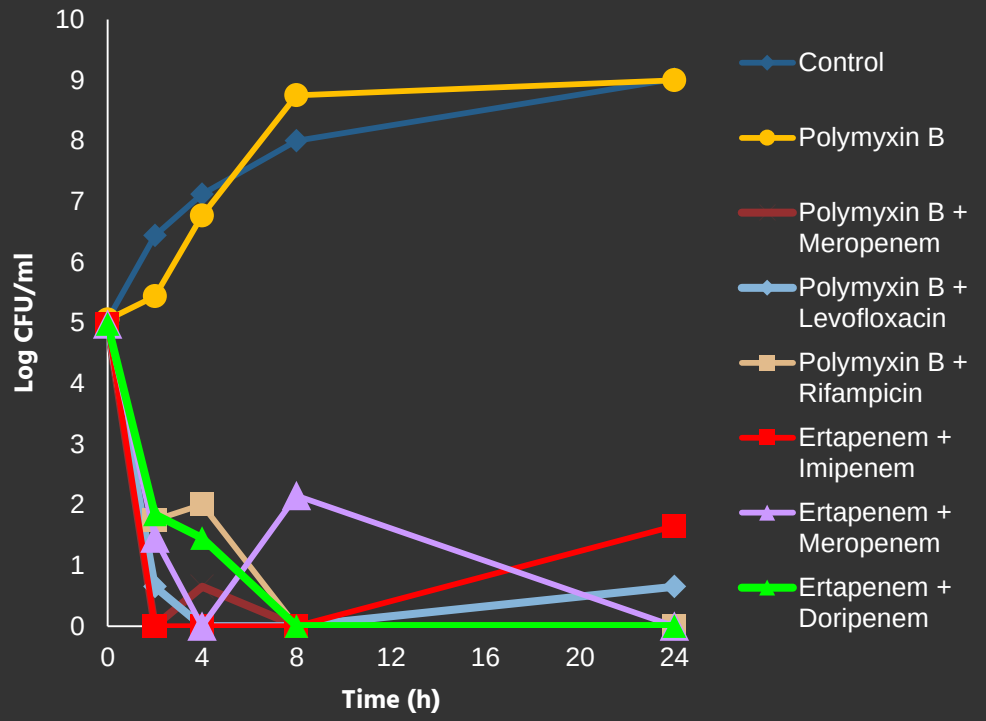
EC250 Combination Time-kill

	Antibiotic	EC250 Log CFU/mL at 24h
Polymyxin	Aztreonam	4.81
	Cefepime	**0.65**
	PT4	9.00
	Doripenem	**0.00**
	Imipenem	5.50
	Meropenem	**2.03**
	Rifampicin	**0.00**
	Tigecycline	**2.44**
	Levofloxacin	**0.00**
Ertapenem	Doripenem	**0.65**
	Imipenem	**1.80**
	Meropenem	**0.65**



EC720 Combination Time-kill

	Antibiotic	EC720 Log CFU/mL at 24h
Polymyxin	Aztreonam	9.00
	Cefepime	3.12
	PT4	9.00
	Doripenem	9.00
	Imipenem	9.00
	Meropenem	**0.00**
	Rifampicin	**0.00**
	Tigecycline	4.76
	Levofloxacin	**0.65**
Ertapenem	Doripenem	**0.00**
	Imipenem	**1.65**
	Meropenem	**0.00**



Combination Time-kill

Antibiotic	Log CFU/mL at 24h		
	EC249	EC250	ENT702
Polymyxin B + Aztreonam	**0.00**	4.81	9.00
Polymyxin B + Cefepime	4.13	**0.65**	3.12
Polymyxin B + Piperacillin/Tazobactam	9.00	9.00	9.00
Polymyxin B + Doripenem	4.32	**0.00**	9.00
Polymyxin B + Imipenem	4.05	5.50	9.00
Polymyxin B + Meropenem	2.89	**2.03**	**0.00**
Polymyxin B + Rifampicin	2.78	**0.00**	**0.00**
Polymyxin B + Tigecycline	**2.34**	**2.44**	4.76
Polymyxin B + Levofloxacin	5.04	**0.00**	**0.65**
Ertapenem + Doripenem	**0.00**	**0.65**	**0.00**
Ertapenem + Imipenem	**0.00**	**1.80**	**1.65**
Ertapenem + Meropenem	**0.00**	**0.65**	**0.00**

Combination Time-kill

Antibiotic	Log CFU/mL at 24h		
	EC249	EC250	ENT702
Polymyxin B + Aztreonam	**0.00**	4.81	9.00
Polymyxin B + Cefepime	4.13	**0.65**	3.12
Polymyxin B + Piperacillin/Tazobactam	9.00	9.00	9.00
Polymyxin B + Doripenem	4.32	**0.00**	9.00
Polymyxin B + Imipenem	Different isolates → different bactericidal combinations		
Polymyxin B + Meropenem			
Polymyxin B + Rifampicin	2.78	**0.00**	**0.00**
Polymyxin B + Tigecycline	**2.34**	**2.44**	4.76
Polymyxin B + Levofloxacin	5.04	**0.00**	**0.65**
Ertapenem + Doripenem	**0.00**	**0.65**	**0.00**
Ertapenem + Imipenem	**0.00**	**1.80**	**1.65**
Ertapenem + Meropenem	**0.00**	**0.65**	**0.00**

Combination Time-kill

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Polymyxin B + Levofloxacin	5.04	**0.00**	**0.65**
Ertapenem + Doripenem	**0.00**	**0.65**	**0.00**
Ertapenem + Imipenem	**0.00**	**1.80**	**1.65**
Ertapenem + Meropenem	**0.00**	**0.65**	**0.00**

EC249 & EC250 similar but different bactericidal combinations

Combination Time-kill

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Polymyxin B + Levofloxacin	5.04	**0.00**	**0.65**
Ertapenem + Doripenem			
Ertapenem + Imipenem			
Ertapenem + Meropenem			

Partnering polymyxin B with antibiotics with lowest MICs may not be the most effective

Combination Time-kill

Antibiotic	Log CFU/mL at 24h		
	EC249	EC250	ENT702
Polymyxin B + Aztreonam	**0.00**	4.81	9.00
Polymyxin B + Cefepime	4.13	**0.65**	3.12
Polymyxin B + Piperacillin/Tazobactam	9.00	9.00	9.00
Polymyxin B + Doripenem	4.32	**0.00**	9.00
Polymyxin B + Imipenem	Dual-carbapenem combinations were bactericidal for all 3 isolates		
Polymyxin B + Meropenem			
Polymyxin B + Rifampicin	2.78	**0.00**	**0.00**
Polymyxin B + Tigecycline	**2.34**	**2.44**	4.76
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Ertapenem + Imipenem	**0.00**	**1.80**	**1.65**
Ertapenem + Meropenem	**0.00**	**0.65**	**0.00**

Conclusions

- Bactericidal combinations are highly strain-specific
- Using individual antibiotics' MIC to select antibiotic combinations is not useful
- Dual carbapenem combinations appear to be the most effective against the tested *mcr-1*+ *KPC* isolates

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All members of Singapore General Hospital Anti-infective Research Lab

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