In Vitro Selection of *Enterobacter cloacae* with Cefepime, Meropenem, and Ceftazidime-avibactam Generate Diverse Resistance Mechanisms

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Disclosure

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Arixa Pharmaceuticals
Astellas Pharma
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Fox Chase Chemical Diversity Center, Inc.
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The Medicines Co.
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VenatoRX
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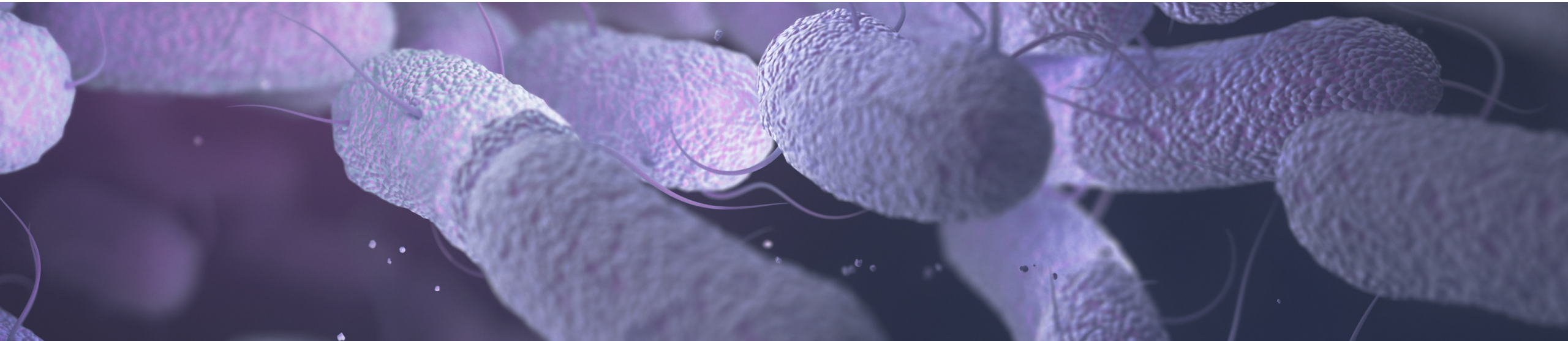
Wockhardt
Yukon Pharmaceuticals
Zai Lab
Zavante Therapeutics

E. cloacae

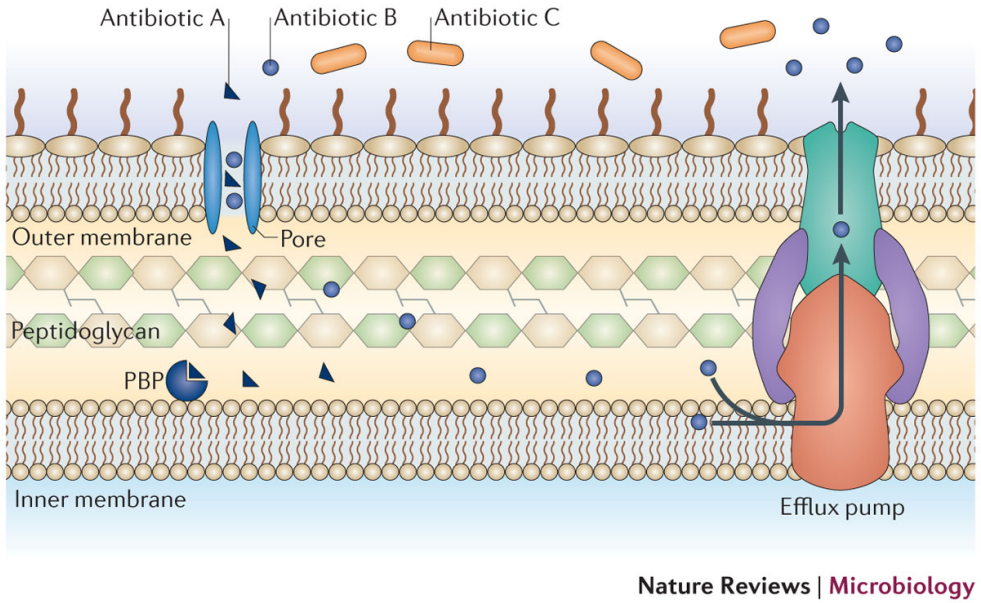
- *Enterobacter cloacae* causes a variety of human infections
- This organism was included in the ESKAPE pathogen list due to its ability to cause serious infections and develop resistance during treatment
- Acquired β-lactamases are not the most common β-lactam resistance mechanism in *E. cloacae*

Davin-Regli et al., CMR, 2019
Boucher et al., CID, 2009



β-lactam resistance in *E. cloacae*

- Important contributors to β-lactam resistance, alone and in combination, are:
  - Overexpression of the constitutive AmpC
  - Outer membrane mutations decreasing β-lactams permeability
  - Increased efflux
- ESBLs and carbapenemases encoding resistance have been described in this species



Davin-Regli et al., CMR, 2019

Treatment of *E. cloacae* infections

- Due to resistance to many β-lactam agents, cefepime and carbapenems have been used for treatment of *E. cloacae* infections
- The increased use of carbapenems to treat infections caused by *E. cloacae* could have generated higher resistance levels in this species
- *E. cloacae* is the second most common carbapenem-resistant *Enterobacterales* (CRE; data from the SENTRY Program)
  - Most CR-*E. cloacae* isolates do not produce carbapenemases

Davin-Regli et al., CMR, 2019

Ceftazidime-avibactam

- Ceftazidime-avibactam is approved by the United States Food and Drug Administration (US FDA) and by the European Medicine Agency (EMA)
- Avibactam restores the activity of ceftazidime in the presence of Ambler class A (ESBLs and KPC), class C (AmpC), and some class D (OXAs) enzymes

Complicated urinary tract infections, including pyelonephritis

Complicated intrabdominal infections with metronidazole

Hospital acquired pneumonia

Objective

We subjected 6 *E. cloacae* isolates to 10-day serial passage with cefepime, meropenem, and ceftazidime-avibactam to evaluate resistance level and mechanism in the mutant strains

Methods

- Baseline and mutant isolates were susceptibility tested by reference broth microdilution (CLSI; M07, 2018) against cefepime, meropenem, and ceftazidime-avibactam (inhibitor at 4 mg/L)
- Serial passaging was performed in broth microdilution by inoculating the highest growth well from the broth microdilution panels into new panels
- Colonies growing in the highest antimicrobial concentrations were submitted to short-read whole genome sequencing (WGS) on a MiSeq (Illumina, San Diego, California, USA) and analyzed for β-lactam resistance mechanisms

β-lactam resistance genes	
AmpC	MarA
AmpR	MarB
AmpD	MarR
OmpC	RamA
OmpF	RamR
AcrA	CsrA
AcrB	RobA
TolC	SoxS

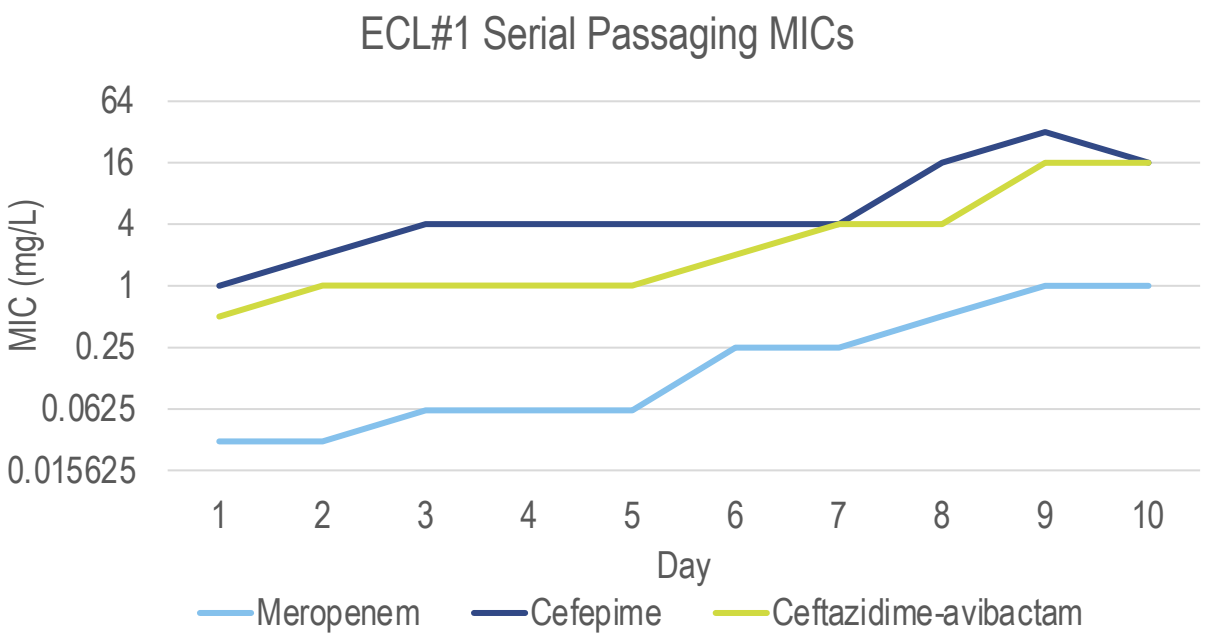
CLSI M7Ed10, 2018

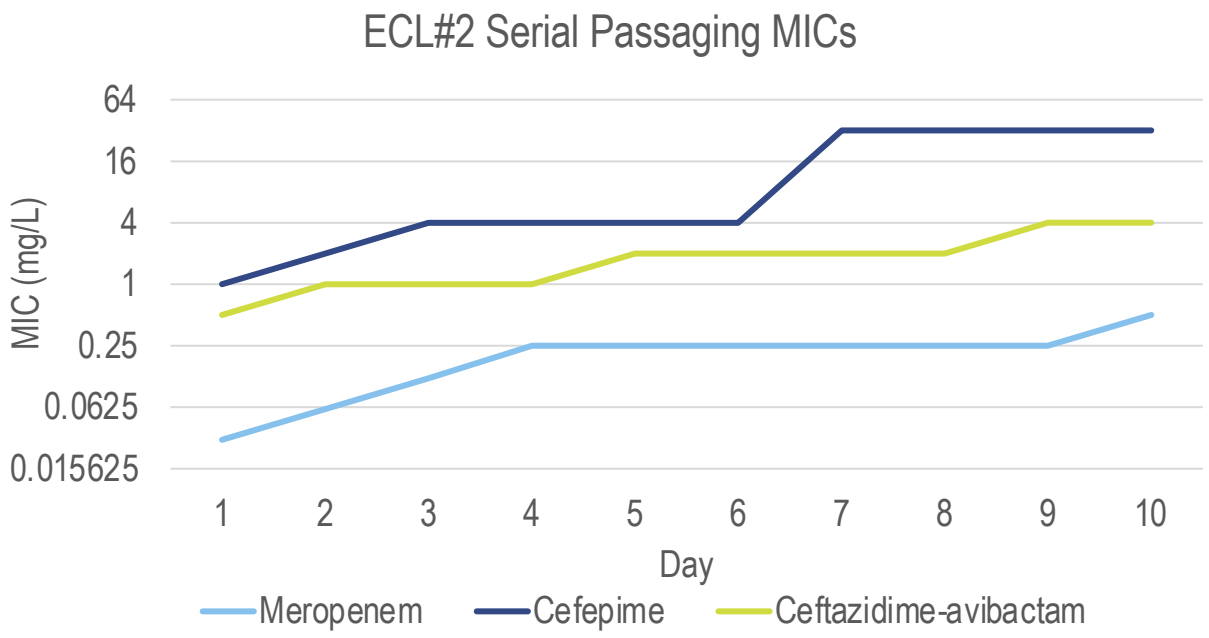
Methods

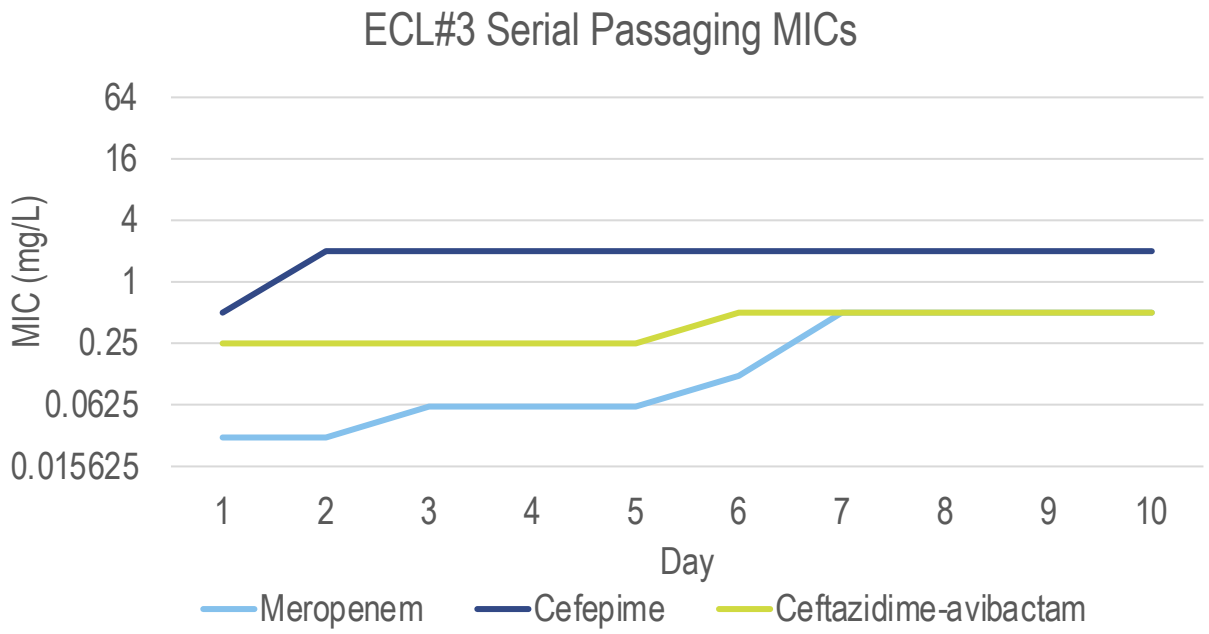
- Final mutants displaying >2-fold changes from the baseline and baseline isolates were sequenced using a long-read technology in a MinIOn (Nanopore, Oxford, UK)
- FASTQ files generated using short and long reads were combined and used for single nucleotide polymorphism (SNP) analysis
- SNPs determined by MAUVE independently and mapped using BWA
- Variant call format (VCF) file had minimum read depth of 4X, >30 map quality, >50 average base quality, no significant strand bias, and >75% of mutations within reads to support the presence of any given alteration
- Indels and uncovered regions were identified using nucDiff (https://omictools.com/nucdiff-tool)

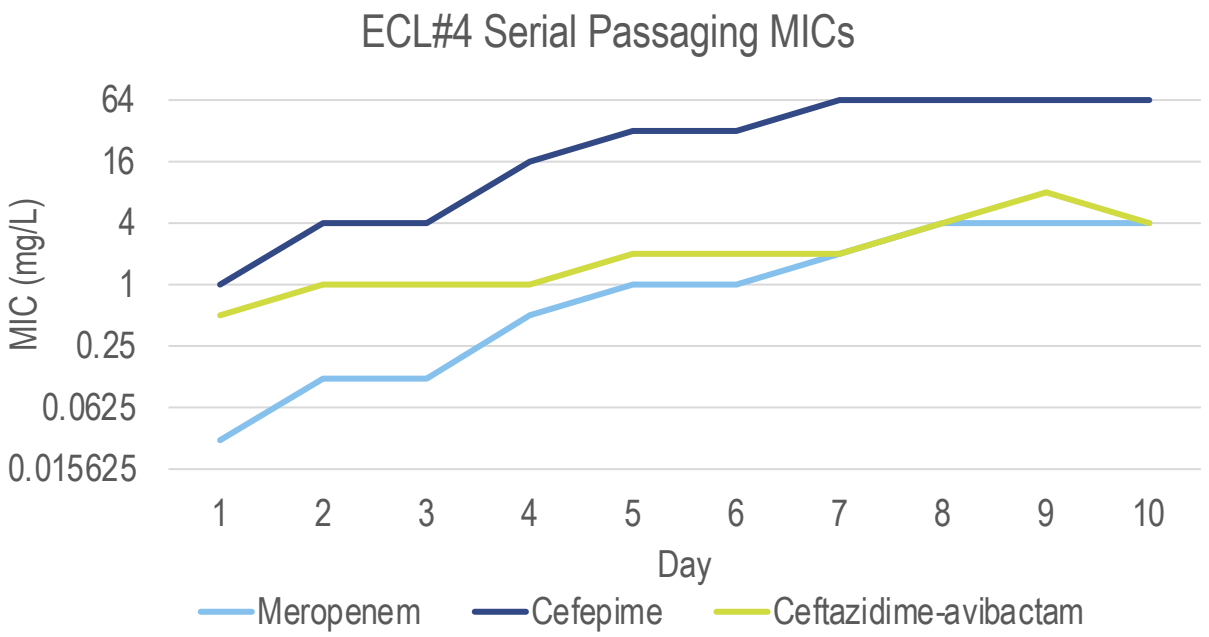
Results

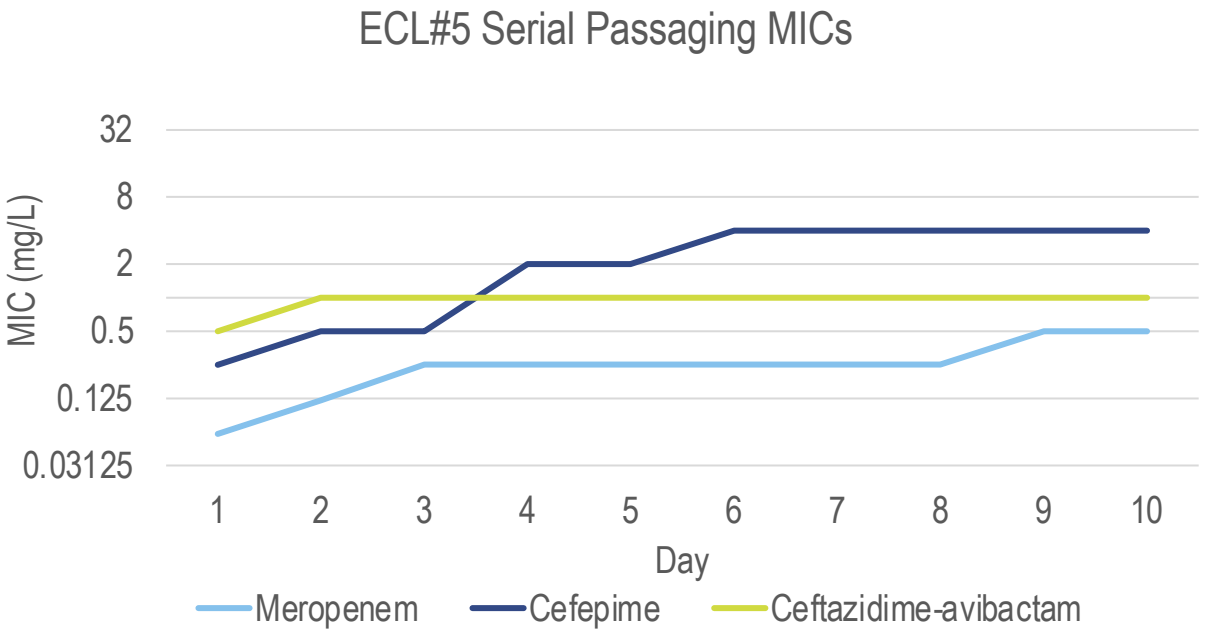
ECL Isolate	MIC (mg/L) Meropenem	Ceftazidime-avibactam	Cefepime	Resistance genes
#1	0.03	0.5	0.5	*act-18*, *aph(6)-Ia*
#2	0.03	0.25	0.25	*act-17*, *fosA*
#3	0.03	0.25	0.5	*act-41*-like, *aadA2*, *sul1*
#4	0.06	0.5	0.5	*act-15*-like, *aph(6)-Ia*
#5	0.06	0.5	0.25	*act-12*-like, *fosA*
#6	0.03	0.25	0.25	*cmh-3*-like, *aph(6)-Ia*, *aph(6)-Id*

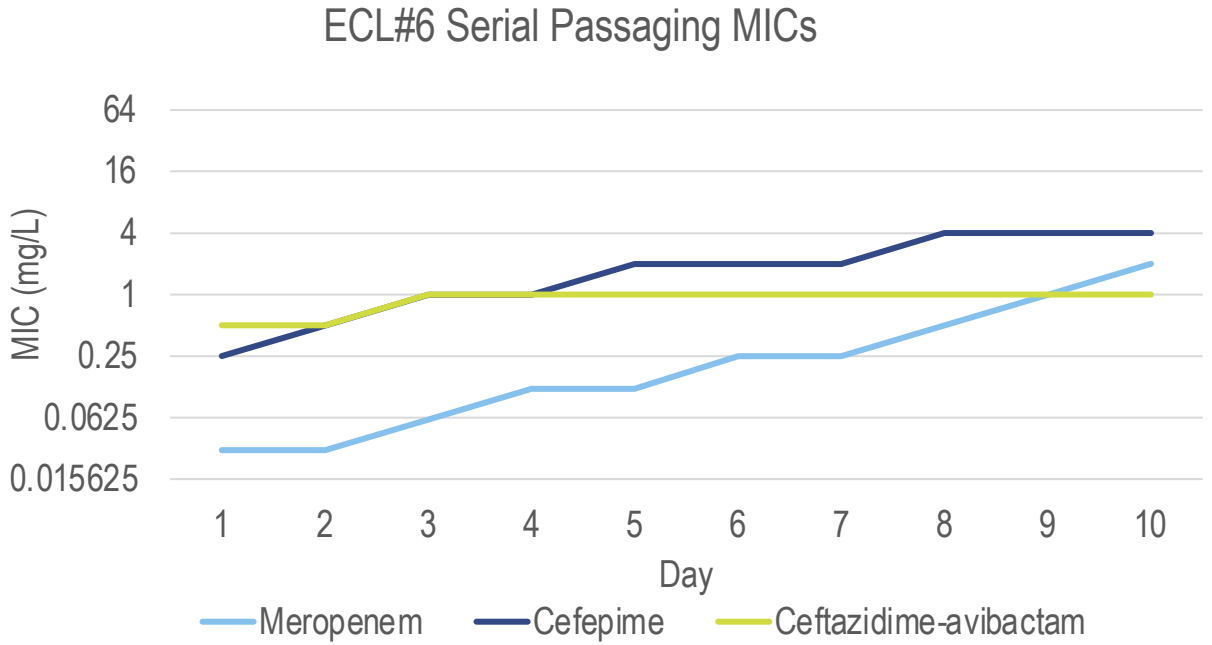

ECL#1 Serial Passaging MICs
MIC (mg/L)
64
16
4
1
0.25
0.0625
0.015625
1 2 3 4 5 6 7 8 9 10
Day
Meropenem
Cefepime
Ceftazidime-avibactam


ECL#2 Serial Passaging MICs
MIC (mg/L)
64
16
4
1
0.25
0.0625
0.015625
1 2 3 4 5 6 7 8 9 10
Day
Meropenem
Cefepime
Ceftazidime-avibactam


ECL#3 Serial Passaging MICs
MIC (mg/L)
64
16
4
1
0.25
0.0625
0.015625
1 2 3 4 5 6 7 8 9 10
Day
Meropenem
Cefepime
Ceftazidime-avibactam


ECL#4 Serial Passaging MICs
MIC (mg/L)
64
16
4
1
0.25
0.0625
0.015625
1 2 3 4 5 6 7 8 9 10
Day
Meropenem
Cefepime
Ceftazidime-avibactam


ECL#5 Serial Passaging MICs
MIC (mg/L)
32
8
2
0.5
0.125
0.03125
1 2 3 4 5 6 7 8 9 10
Day
Meropenem
Cefepime
Ceftazidime-avibactam


ECL#6 Serial Passaging MICs
MIC (mg/L)
64
16
4
1
0.25
0.0625
0.015625
1 2 3 4 5 6 7 8 9 10
Day
Meropenem
Cefepime
Ceftazidime-avibactam

	Fold change for Meropenem MIC results and *E. cloacae* isolates								
Day	#1	#2	#3	#4	#5	#6	Median	Mode	Geo Mean
1	1	1	1	1	1	1	1	1	1.0
2	1	1	1	4	2	1	1	1	1.4
3	2	2	2	4	4	2	2	2	2.5
4	2	4	2	16	4	4	4	4	4.0
5	2	4	2	32	4	4	4	4	4.5
6	8	4	4	32	4	8	6	4	7.1
7	8	4	16	64	4	8	8	8	10.1
8	16	4	16	128	4	16	16	16	14.3
9	32	4	16	128	8	32	24	32	20.2
10	32	8	16	128	8	64	24	8	25.4

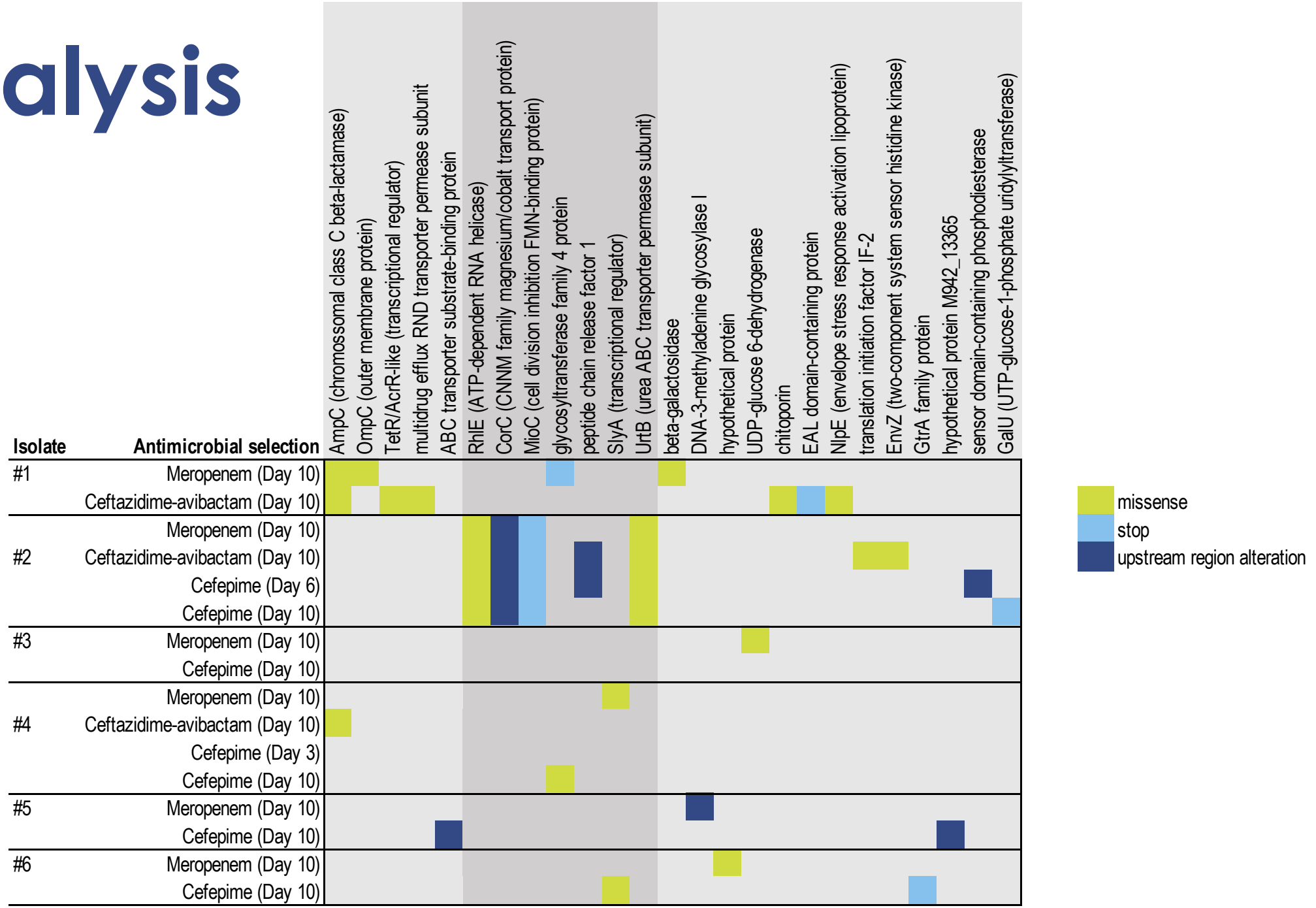
Day	Fold change for Ceftazidime-avibactam MIC results and *E. cloacae* isolates								
	#1	#2	#3	#4	#5	#6	Median	Mode	Geo Mean
1	1	1	1	1	1	1	1	1	1.0
2	2	2	1	2	2	1	2	2	1.6
3	2	2	1	2	2	2	2	2	1.8
4	2	2	1	2	2	2	2	2	1.8
5	2	4	1	4	2	2	2	2	2.2
6	4	4	2	4	2	2	3	4	2.8
7	8	4	2	4	2	2	3	2	3.2
8	8	4	2	8	2	2	3	2	3.6
9	32	8	2	16	2	2	5	2	5.7
10	32	8	2	8	2	2	5	2	5.0

	Fold change for Cefepime MIC results and *E. cloacae* isolates								
Day	#1	#2	#3	#4	#5	#6	Median	Mode	Geo Mean
1	1	1	1	1	1	1	1	1	1.0
2	4	2	4	8	2	2	3	2	3.2
3	8	4	4	8	2	4	4	4	4.5
4	8	4	4	32	8	4	6	4	7.1
5	8	4	4	64	8	8	8	8	9.0
6	8	4	4	64	16	8	8	8	10.1
7	8	32	4	128	16	8	12	8	16.0
8	32	32	4	128	16	16	24	32	22.6
9	64	32	4	128	16	16	24	16	25.4
10	32	32	4	128	16	16	24	32	22.6

MIC Fold change for *E. cloacae* isolates

	Meropenem			**Ceftazidime-avibactam**			**Cefepime**		
Day	Median	Mode	Geo Mean	Median	Mode	Geo Mean	Median	Mode	Geo Mean
2	1	1	1.4	2	2	1.6	3	2	3.2
3	2	2	2.5	2	2	1.8	4	4	4.5
4	4	4	4.0	2	2	1.8	6	4	7.1
5	4	4	4.5	2	2	2.2	8	8	9.0
6	6	4	7.1	3	4	2.8	8	8	10.1
7	8	8	10.1	3	2	3.2	12	8	16.0
8	16	16	14.3	3	2	3.6	24	32	22.6
9	24	32	20.2	5	2	5.7	24	16	25.4
10	24	8	25.4	5	2	5.0	24	32	22.6

SNP analysis



Conclusions

- Meropenem (range 8 to 128-fold; median 24) and cefepime (4 to 128-fold; median 24) mutants had higher MIC values compared to ceftazidime-avibactam (range 2 to 32; media 5)
- Two isolates had multiple alterations in each of the sequenced mutants
- Mutations in the genes encoding AmpC, OmpC, and efflux regulators were observed in ceftazidime-avibactam and meropenem, meropenem and ceftazidime-avibactam, and cefepime mutants
- 3 of the 6 isolates had mutations in various genes that have not been described in relation to antimicrobial resistance and have roles in cell division, transcription regulation, RNA folding, and efflux

Conclusions

- This study suggests that exposure to cefepime and meropenem could generate isolates with elevated MIC values for these agents in 6 genetically distinct *E. cloacae* clinical isolates
- These high MICs were not observed with ceftazidime-avibactam
- Therapies that prevent the emergence of resistance could reduce the burden of antimicrobial resistance and should be part of stewardship efforts to control this problem

Acknowledgements

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