Development of cefiderocol resistance during treatment in *Klebsiella pneumoniae*

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Case report

ICU Admission

67 year old man repatriated from Iraq

- SARS-CoV-2 acute respiratory failure

Rectal swab

- *E. faecium vanA*
- *E. coli* and *K. pneumoniae* OXA-48

Day 14

Ventilation-acquired pneumonia

<u>Bronchoalveolar lavage:</u>

- *P. aeruginosa*
- *K. pneumoniae*
- *S. maltophilia*
- *E. anopheles*

Initiation of cefepime + levofloxacin

Day 21

<u>Tracheal aspiration:</u>

- Carbapenem-resistant *K. pneumoniae* (**KP1**)
- *P. aeruginosa*
- *E. anopheles*

KP1 « susceptibility »

Resistant to

- Almost everything tested

Susceptible to

- Cefiderocol
- Colistin
- Fosfomycin
- Tigecycline and eravacycline

Phenotypic assays :

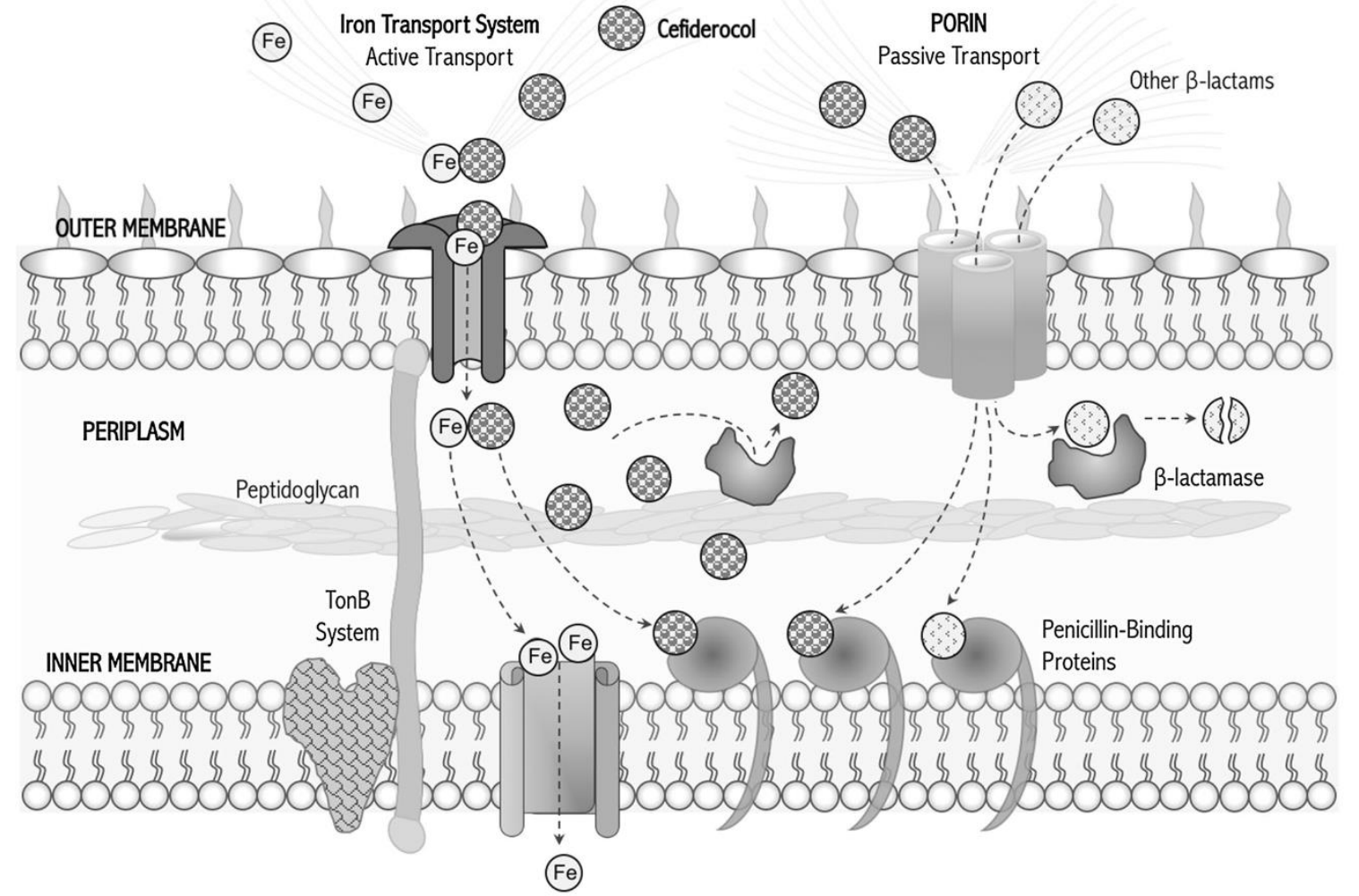
ESBL and carbapenemase producer

MIC (mg/L)	KP1	EUCAST interpretation
Aminoglycosides		R
Quinolones		R
Eravacycline	0.5	No breakpoint
Tigecycline	< 0.5	No breakpoint
Colistin	< 0.5	S
Fosfomycin	< 16	S
Trimethoprim-sulfamethoxazole	> 8	R
Beta-lactams		
Cefiderocol	2	S
Ceftazidime/avibactam	> 16	R
Ceftolozane/tazobactam	> 8	R
Imipenem	8	R
Imipenem/relebactam	8	R
Meropenem	> 16	R
Meropenem/vaborbactam	> 16	R

Treatment changed to cefiderocol (monotherapy on KP1) + ciprofloxacin (Coverage of *P. aeruginosa* and *E. anopheles*)

Cefiderocol

- New cephalosporin combined with a siderophore-like structure
- Binds free iron
- Use porins and siderophore receptors to enter the bacterial cell



Main siderophore receptors in *K. pneumoniae* : ***cirA*** and ***fiu***

Recommended for the treatment of infections caused by multidrug-resistant Gram-negative bacilli
(ESCMID and IDSA Guidelines)

Zhanel et al. – Drugs – 2019

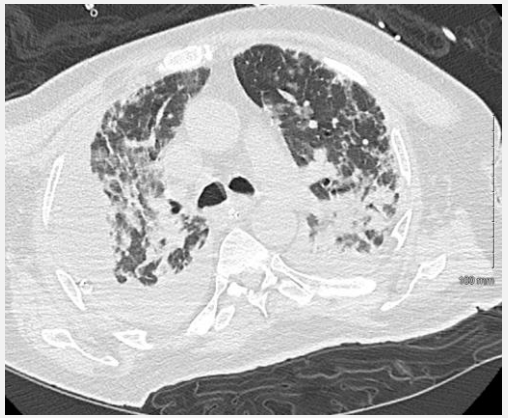
Case report

Day 41

Clinical worsening:

- Multiple pulmonary abscesses
- Empyema

Persistance of *K. pneumoniae* (**KP2**), *P. aeruginosa* and *E. anopheles*



Day 53

Cefiderocol resistant *K. pneumoniae* (**KP3**)

Cefiderocol discontinuation

Initiation of ceftazidime/avibactam, aztreonam, fosfomycin, nebulized colistin

Day 82

Elimination of *K. pneumoniae* and *P. aeruginosa* in respiratory samples

Deceased of respiratory failure

Emergence of *K. pneumoniae* resistant to cefiderocol (KP3) after 32 days of treatment

Investigation on cefiderocol resistance : prospective MICs

	KP1 (D21)	KP2 (D41)	KP3 (D53)
Days of cefiderocol treatment	0	20	32
Cefiderocol MIC (mg/L)	2	2	> 8

Method used : Sensititre EUMDROXF (ThermoFisher)

EUCAST Breakpoint : S ≤ 2 mg/L

EUCAST Warning (January 2022): Several lots retraction due to **false susceptibility** to cefiderocol

"If you have bought any of these lots, then please ignore results for cefiderocol"

Retrospective MICs

		KP1	KP2	KP3
Days of cefiderocol treatment		0	18	32
Cefiderocol MIC (mg/L)	Sensititre EUMDROXF (ThermoFisher)	2	2	> 8
	UMIC (Biocentric)	0.25	8	> 32
	ComASP (Liofilchem)	0.25	4	> 128

EUCAST Breakpoint : S ≤ 2 mg/L

Whole Genome Sequencing on KP1, KP2 and KP3

- Nanopore sequencing (MinION Mk1b, R9.4.1 flow-cell)
  - Bioinformatics: trycycler assembly, medaka polishing, kleborate, ResFinder [1]
  - Genome comparison of KP2 and KP3 to KP1

	KP1	KP2	KP3
Sequence Type	ST16		
Acquired resistance genes	aac(6')-Ib', aadA, rmtB, ermB, arr-3, sul1, dfrA1, OXA-9, TEM-1D, CTX-M-15, **NDM-5**		
Increased NDM-5 copy number ? [2]	No		
Mutations in siderophore receptors	-	***cirA*** I194fs (stop codon 219)	***cirA*** stop codon Y359* ***fiu*** A33fs (stop codon 45)
	-	*fhuA* R87Q	*fhuA* R87Q

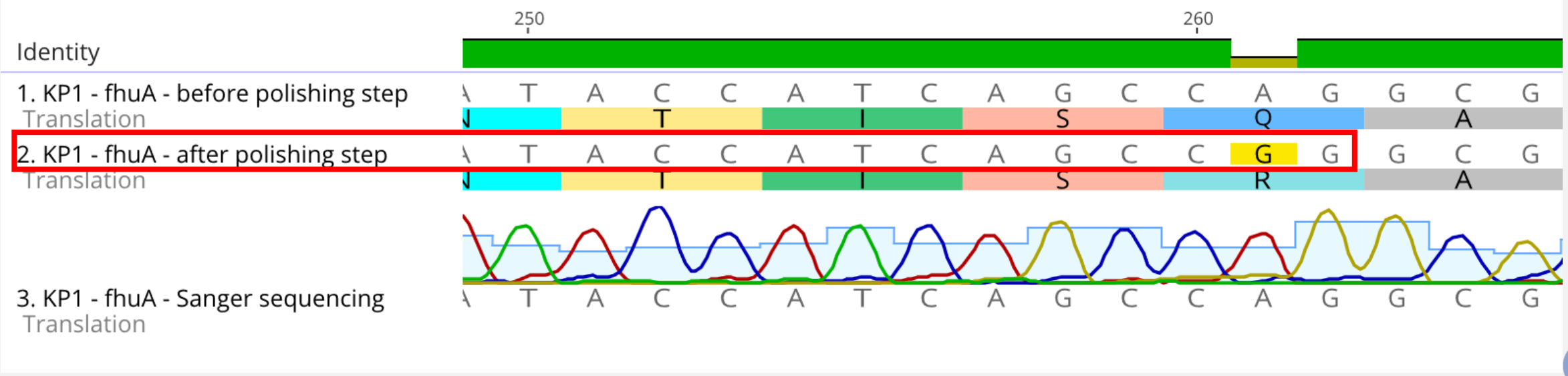
[1] *Wick et al. – Genome Biology – 2021 ; Lam et al. - Nat Commun – 2021 ; Bortolaia et al. – JAC - 2020*

[2] *Simner et al. – CID – 2021*

Whole genome sequencing: where is the *fhuA* mutation ?

Quality control

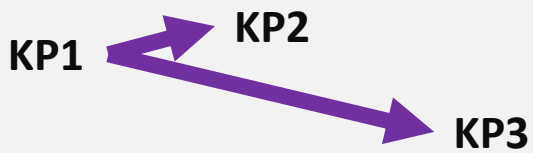
- All suspected mutations verified by Sanger sequencing, raw read mapping and a second WGS run
- *fhuA* :
  - Error introduced during polishing step on KP1
  - Probably due to low coverage in first sequencing
  - Exclusion of *fhuA* mutation



Discussion

- First description of inactivation of two iron transporters
- Mutations in iron transporters (*cirA* and *fiu*) are described *in vitro* [1]
  - Emergence of cefiderocol resistance during treatment due to *cirA* mutations was already observed [2]
  - First description of *fiu* inactivation during cefiderocol treatment

- Strains evolution during treatment
  - Expectation : **KP1** → **KP2** → **KP3**
  - Reality ? :



[1] *Ito et al. – AAC – 2017*
[2] *Klein et al. – CID – 2022 ; Moon et al. – IJAA – 2022 ; Lan et al. – Microbiol Spectr – 2022 ; Jousset et al. – JAC - 2023*

Perspectives

- Evaluate the impact of each mutation (both *cirA* and *fiu*) on cefiderocol resistance

- Homologous recombination in reference *K. pneumoniae* strain
  - Association needed to confer cefiderocol resistance ?
  - Effects of mutations with/without NDM ?

Remaining issues surrounding the use of cefiderocol

- MICs : few reliable cefiderocol AST tests
  - BMD remains gold-standard for MIC, but not adapted to every laboratory
  - Reading can be tricky (EUCAST Guidance document)
  - Elevated MICs in NDM producing bacteria [1]

- Cefiderocol monotherapy as recommended [2]
  - Pulmonary abcesses = high inoculum + low cefiderocol concentrations ?
  - Inoculum effect described *in vitro* [3]

[1] *Bonnin et al. – CMI – 2022*
[2] *ESCMID Guidelines – 2022 ; IDSA Guidelines – 2022*
[3] *Hobson et al. – CMI – 2021*

Thank you