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Effectivity of a double-carbapenem regimen for the treatment of a KPC-producing *Klebsiella pneumoniae* infection in an immunocompromised patient.

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Background

In recent years the number of infections due to carbapenemase-producing *Enterobacteriaceae*, especially *Klebsiella pneumoniae,* has been extensively reported. Within the carbapenemases family one of the most clinically significant carbapenemases are the KPC-type enzymes, which are mediated by plasmids and have disseminated worldwide becoming a public health concern. Carbapenemase-producing *K. pneumoniae* (CPKP) are associated with a high mortality rate (35-56%) and optimal regimens are not yet well defined. CPKP frequently co-expressess additional resistance determinants that confer resistance to several antibiotic families other than betalactams. This situation represents an important threat to public health since therapeutic options against these infections are limited. Combined therapy with more than one antibiotic is recommended nowadays according to retrospective studies. Recently, double-carbapenem regimen has been suggested and used as alternative to treat extensively drug-resistant CPKP. However, it exists limited evidence of the effectiveness of simultaneous administration of two carbapenems to treat CPKP infections among immunocompromised patients.

Case History

A 36-year-old Spanish woman with acute myeloid leukemia diagnosed four months ago (myeloid sarcoma form: retroperitoneal, ovarian and uterine location), was admitted in August 2015 to receive an allo-HSCT (unrelated donor), after conditioning chemotherapy regimen. Previously, she has received anti-infective prophylaxis with fluconazole, acyclovir and nebulized amphotericin and pentamidine; and immunosuppressive therapy (IST) with tacrolimus. In this admission, she presented a neutropenic colitis (absolute neutrophil count, ANC, was 0 cells/mmc) the same day that progenitor cell infusion was performed.
The patient was treated with piperacillin-tazobactam empirically and teicoplanin due to a previous *Enterococcus faecium* bloodstream infection. Bacterial cultures were negative at the time and she did not received any more antibiotics for the moment. On post allo-HSCT day 6, she remained febrile and showed dysuria. Blood cultures done on that day remained negative, whereas in the urine culture grew a carbapenemase-producing *K. pneumoniae* (CPKP) (>10.000 cfu per ml) which was resistant to betalactams and susceptible to gentamycin and colistin (table 1 and 2). Despite the susceptibility to amikacin and tygecicline was not evaluated initially at the urine isolation, piperacillin-tazobactam was empirically replaced by these antibiotics. Twenty-four hours later, the patient persisted febrile with chills and hypotension (no vasopressor and inotropic support was needed). ANC remained at 0 cells/mmc, creatinine was 0.54mg/dL and C-reactive protein was 13.9mg/dL. At this moment, in the blood culture grew the same CPKP. It also grew in a rectal screening but not at central venous catheter cultures.

Clinical progression

This patient was considered to be at a high risk of potential antibiotic-induced nephrotoxicity (hemodynamic instability, neutropenia, early after allo-HSCT and use of other nephrotoxic drugs); so colistin was avoided. Due to a lack of alternative regimens, and on the basis of synergy studies and on previous succesful experiences by other authors, double-carbapenem regimen with ertapenem and meropenem was started. Carbapenem dosage and posology was chosen in consonance with previous experiences based on optimizing PK/PD features: ertapenem (1 g every 24h) was followed one hour later by high dose of meropenem (2 g every 8h, in 3h extended infusion). This regimen was given during 14 days, in the absence of adverse effects. The patient became afebrile in less than 48h and urine cultures and blood cultures underwent negative. The patient achieved clinical and microbiologic success and was discharged 2 weeks later.

Microbiological methods

Antimicrobial susceptibility

- **Disc difussion method**: ampicillin , amoxicillin+ clavulanate, piperacillin+tazobactam, cephalothin, cefuroxime, cefoxitin, ceftazidime, cefotaxime, cefepime, aztreonam, imipenem, meropenem, ertapenem, ciprofloxacin, colistin, cotrimoxazole, gentamycin and amikacin.
- **Etest method**: ertapenem, meropenem, ciprofloxacin, fosfomycin, gentamycin, colistin, tygecicline.

- Interpretation of MICs and zone diameters were done following EUCAST recommendations.

In vitro synergy testing

Direct overlay or fixed ratio method (DOM) (Manno et al., Eur J Clin Microbiol Infect Dis 2003)

Cross or 90° angle method (CM) (White et al., Antimicrob Agents Chemother 1996)

The fractional inhibitory concentration (FIC) was calculated to evaluate the effect of the combination in each Etest synergy method

Time kill assay (TKA) (NCCLS, 1999)

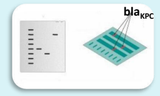
- **Synergy** was defined as a ≥2 log10 decrease in colony count after 24 h by the combination compared to that of the most active single agent alone.
- **Indifference** was defined as a <2 log10 increase or decrease in colony count at 24 h by the combination compared to that of the most active single agent alone.
- **Antagonism** was defined as a ≥2 log10 increase in colony count after 24 h by the combination compared to the most active single agent alone (Pillai et al., 2005).

Molecular epidemiology

MLST

- PCR specific primers
- Sequencing
- Allelic profiles verified at http://bigsdb.web.pasteur.fr/klebsiella

bla$_{KPC}$ localization



- PCR-based replicon typing
- Southern-blot hybridisation

Antimicrobial resistance markers

Screening for β-lactamases, extended-spectrum-β-lactamases and genes encoding resistance to quinolones and aminoglycosides (*acc(6')-Ib, qnrA, qnrB and qnrS*) by PCR.

bla$_{KPC}$ genetic environment

- PCR mapping
- Sequencing

Microbiological results

Antimicrobial susceptibility

Table1. Etest Method

Antimicrobial agent	MIC (µg/ml)	Interpretation
Ertapenem	≥32	R
Meropenem	≥32	R
Ciprofloxacin	≥32	R
Fosfomycin	≥128	R
Gentamycin	2	S
Colistin	0.0064	S
Tygecicline	1.5	I

Table2. Disc difussion method

Antimicrobial agent	Zone diameter	Interpretation	Antimicrobial agent	Zone diameter	Interpretation
Ampicillin	9	R	Aztreonam	9	R
Amox+clav	9	R	Imipenem	15	R
Pip+ Tz	9	R	Meropenem	16	R
Cephalothin	9	R	Ertapenem	12	R
Cefuroxime	9	R	Ciprofloxacin	9	R
Cefoxitin	9	R	Colistin	21	S
Ceftazidime	9	R	Cotrimoxazol	9	R
Cefotaxime	9	R	Amikacin	9	R
Cefepime	9	R	Gentamycin	19	S

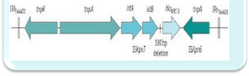
MLST and antimicrobial resistance markers

Belong to sequence type **ST258**

- **KPC-3**
- TEM-1
- SHV-11
- ACC(6')-Ib-cr

bla$_{KPC}$ location and genetic environment

bla$_{KPC}$ is located on *Tn4401*a in an IncFIIk plasmid of 290 Kb



In vitro synergy testing

Antibiotic combination	Direct overlay method ΣFIC	Cross method ΣFIC	Time kill assay (log10 change/24h)
IMI+MER	0.02 SYN	0.9 IND	ND
IMI+ERT	0.05 SYN	0.5 SYN	ND
MER+ERT	0.05 SYN	0.3 SYN	-6 SYN*
ERTA+TIGE	1 IND	ND	ND
MERO+TIGE	1.3 IND	ND	ND
MER+CIP	1 IND	ND	ND
ERTA+CIP	1.2 IND	ND	ND
IMI+CIP	2 IND	ND	ND

- **ΣFIC** = FIC antibiotic A+ FIC antibiotic B.
- **Synergy** (SYN) was defined as ΣFIC ≤0.5, **indifference** (IND) as ΣFIC <0.5–4, and **antagonism** (ANT) as >4 (Pillai et al., 2005).
- **ND**: Not done.
- *Combining 0.5×MIC ERT + 0.5×MIC MEM

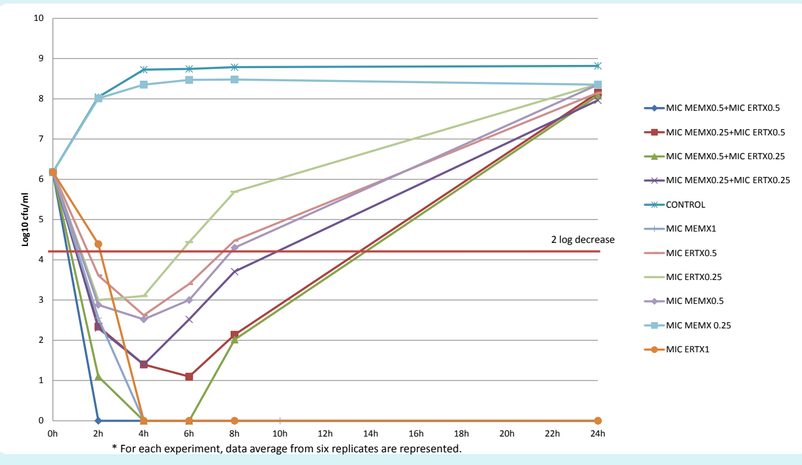
Time kill assay

- Ertapenem MIC by broth dilution was 256 µg/ml
- Meropenem MIC by broth dilution was 128 µg/ml

- For the synergy study by the time kill assay, the following ertapenem (ERT) and meropenem (MEM) combinations were evaluated:
- 1×MIC MEM, 1×MIC ERT, 0.5×MIC MEM, 0.5×MIC ERT, 0.25×MIC MEM, 0.25×MIC ERT, 0.5×MIC ERT + 0.5×MIC MEM, 0.25×MIC ERT + 0.5×MIC MEM, 0.5× MIC ERT + 0.25×MIC MEM, 0.25×MIC ERT + 0.25×MIC MEM.

- Both ertapenem and meropenem monotherapy (0.5x MIC and 0.25x MIC), as well as combinations 0.25×MIC ERT + 0.5×MIC MEM, 0.5×MIC ERT + 0.25×MIC MEM, 0.25×MIC ERT + 0.25×MIC MEM showed an initial reduction followed by significant regrowth at 24h.

- The combination ertapenem plus meropenem showed a **bactericidal effect** at 24h at a concentration of **0.5×MIC ERT + 0.5×MIC MEM.**



* For each experiment, data average from six replicates are represented.

Conclusions

Carbapenemase-producing *K. pneumoniae* bloodstream infections generally have a poor outcome, with a high mortality in immunocompromised patients and high treatment failure rates. We describe a case of a neutropenic patient who developed a carbapenemase-producing *K. pneumoniae* bloodstream infections immediately after allo-HCST. Taking into account the *in vitro* synergy effect of two carbapenems combination, the patient was treated simultaneously with ertapenem and meropenem, achieving clinical and microbiologic success. To our knowledge, this is the first reported case of the successful use of a double-carbapenem regimen in a neutropenic patient. The double-carbapenem regimen could be an effective strategy with little toxicity, especially useful when only nephrotoxic drugs are available. The treatment of these infections should be individuality tailored, a process in which the collaboration with the Microbiology Laboratory is essential to evaluate therapeutic alternatives.