The 16S rRNA gene sequencing revealed that the isolate was in fact a K. georgiana, with 99.85% similarity to the sole available sequence from K. georgiana ATCC 51603 (GenBank accession number AF047186.1), while the similarity index was 98.91% when compared with K. cryocrescens ATCC 33435 (GenBank accession number AF310218.1). The isolate was resistant to all β-lactams (ceftazidime MIC, 16 mg/L; cefepime MIC, 32 mg/L; ampicillin/sublactam MIC, ≥256 mg/L; imipenem MIC, 256 mg/L; meropenem MIC, 128 mg/L; and ertapenem MIC, 128 mg/L), but susceptible to other drugs such as amikacin (MIC, 2.0 mg/L), ciprofloxacin (MIC ≤0.125 mg/L), tigecycline (MIC, 0.5 mg/L) and polymyxin B (MIC, 0.25 mg/L). ESBL phenotypic and genotypic tests were negative. Both the MHT and BA-based assay for carbapenemase detection were positive. The presence of the blaφKPC gene was confirmed by PCR and the gene sequencing revealed 100% identity with blaφKPC.2. The plasmid electroporation into E. coli Top10 resulted in transformants that presented positive results using the MHT and the BA-based method, and the presence of the KPC gene was confirmed by PCR. The transformants demonstrated the acquisition of a ~36 kb plasmid and the genetic environment analysis of blaφKPC suggested that the gene was inserted in a Tn801-like transposon. We obtained a partial sequence of 1461 bp of this transposon (130 bp upstream to 459 bp downstream of the KPC gene) demonstrating 99.8% similarity with the sequence of a KPC-2-producing Enterobacter cloacae, in which the KPC gene is inserted in a Tn801-like transposon (GenBank accession number JN048640.1).

High-level resistance to carbapenems was observed in K. georgiana, suggesting the involvement of additional resistance mechanisms. Since porin expression was not investigated, we cannot exclude the possibility that the K. georgiana phenotype results from the interplay between permeability alteration and KPC-2 production.

KPC-producing isolates have been associated with serious infection and high mortality, probably due to the difficulty of treating these infections. This work presents a description of a KPC-2 enzyme in the rare pathogen K. georgiana, highlighting the ability of KPC to spread to unusual pathogens.

Funding
This work was supported by Fundo de Incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre (09-270), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (10/0026-1), Brazil. A. P. Z. and A. L. B. are research fellows from the National Council for Scientific and Technological Development, Ministry of Science and Technology, Brazil.

Transparency declarations
None to declare.

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J Antimicrob Chemother 2012
do:10.1093/jac/dks270
Advance Access publication 10 July 2012

Emergence of resistance to fosfomycin used as adjunct therapy in KPC Klebsiella pneumoniae bacteremia: report of three cases

Drosos E. Karageorgopoulos1, Vivi Miriagou2, Leonidas S. Tzouvelekis3, Kalliopi Spyridopoulou1 and George L. Daikos1*

1First Department of Propaedeutic Medicine, University of Athens, Laikon General Hospital, Athens, Greece; 2Laboratory of Bacteriology, Hellenic Pasteur Institute, Athens, Greece; 3Department of Microbiology, University of Athens, Athens, Greece

*Corresponding author. Tel: +30-2107462636; Fax: +30-2107462635; E-mail: gdaikos@med.uoa.gr

Keywords: Enterobacteriaceae, carbapenemases, antibiotic combinations, bloodstream infections

Sir,

Fosfomycin has recently been proposed as an adjunct to other active agents for the treatment of KPC-producing Klebsiella pneumoniae (KPC-Kp) infections.1,2 We describe here three
critically ill immunocompromised patients in their fourth or fifth decade who received fosfomycin combined with other antimicrobials for the treatment of KPC-Kp bloodstream infections in a hospital in Athens. Before initiation of fosfomycin, all three patients were treated with antibiotic combinations commonly used against KPC-Kp bacteremia; however, their condition deteriorated rapidly. Fosfomycin was added as a last-resort drug despite the lack of relevant clinical experience.

**Case 1**
A liver transplant recipient was referred to our hospital for acute cholangitis, multiple liver abscesses and KPC-Kp bacteremia. Therapy with colistin (120 000 IU/kg/day in two divided doses) plus meropenem (2 g three times daily) cleared the bacteremia and relieved symptoms. On the 25th day, the patient developed sepsis while on this treatment. Blood culture yielded KPC-Kp (isolate 1) that was susceptible to colistin, gentamicin, tigecycline and fosfomycin (MICs 0.125, ≤4, 32 and 32 mg/L, respectively). The meropenem MIC was 4 mg/L. The two largest abscesses were drained and meropenem was replaced by intravenous fosfomycin (4 g four times daily) while the patient continued the same dose of colistin. The patient’s symptoms were initially controlled but 4 days later symptoms recurred and blood culture yielded KPC-Kp exhibiting resistance to fosfomycin, colistin and meropenem (MICs >1024, 4 and >32 mg/L, respectively) (isolate 1 after fosfomycin treatment (isolate 1F)). The patient died of sepsis on the 56th day of hospitalization.

**Case 2**
A patient with acute myelogenous leukaemia developed profound neutropenia (absolute neutrophil count <100/μL) and new onset of fever after chemotherapy. The patient was started empirically on meropenem (2 g three times daily) and gentamicin (240 mg once daily). Blood culture grew KPC-Kp (isolate 2) that was resistant to all antimicrobials tested, except for colistin, gentamicin, tigecycline and fosfomycin (MICs 0.125, ≤4, 0.75 and 32 mg/L, respectively); the MIC of meropenem was 8 mg/L. The patient was severely septic and colistin (150 000 IU/kg/day in two divided doses) was added to the above antimicrobial regimen. Five days later, the symptoms had not improved and blood culture remained positive for KPC-Kp. Meropenem was then replaced by fosfomycin (8 g three times daily). The patient’s condition partially improved and bacteremia was cleared. Eleven days after initiation of fosfomycin and while the patient was on colistin, gentamicin and fosfomycin, fever (>38°C) and bacteremia with KPC-Kp reappeared. The new isolate exhibited high-level resistance to fosfomycin and meropenem (MICs >1024 and >32 mg/L, respectively) while remaining susceptible to colistin, gentamicin and tigecycline (isolate 2F). The patient died 8 days later with polymicrobial bacteremia caused by KPC-Kp, Enterococcus faecium and Candida albicans.

**Case 3**
A kidney transplant recipient was transferred to the intensive care unit (ICU) after cardio-respiratory arrest. On the 15th day in the ICU, the patient developed bacteremia with KPC-Kp (isolate 3). At that time, the patient was being mechanically ventilated and receiving continuous renal replacement therapy. The isolate was susceptible only to fosfomycin (MIC 12 mg/L) and gentamicin (MIC ≤4 mg/L). The MICs of colistin, tigecycline and meropenem were 4, 3 and 8 mg/L, respectively. Treatment with gentamicin (160 mg once daily), meropenem (1 g twice daily) and fosfomycin (3 g three times daily) was initiated. After transient relief of symptoms and control of bacteremia, fever and bacteremia recurred and the patient died of septic shock 11 days later. The KPC-Kp blood isolate exhibited several-fold higher MICs to fosfomycin (128 mg/L), gentamicin (8 mg/L) and meropenem (>32 mg/L) (isolate 3F).

For each of the case-patients, the fosfomycin-resistant KPC-Kp isolates recovered after fosfomycin treatment were most likely mutants of the pre-treatment isolates, as indicated by PFGE typing of XbaI-digested genomic DNA. Also, all PFGE patterns were similar (≥90%) to those of the KPC producers of the sequence type 258 (ST258). Fosfomycin is known for its ability to readily select resistant mutants. Yet, in vitro studies have indicated that its combination with colistin, gentamicin or meropenem prevents the emergence of fosfomycin-resistant K. pneumoniae variants. The three cases we report cast doubts regarding the capability of combination regimens to avert the in vivo development of resistance to fosfomycin in KPC-Kp. It must be pointed out, however, that the parental isolates were not fully susceptible to all the partner drugs, which may partly explain the rapid occurrence of fosfomycin resistance.

Moreover, fosfomycin-resistant variants exhibited higher β-lactam MICs than their parental isolates (Table 1). To reproduce this phenomenon in vitro, suspensions (10^5 cfu/mL) of isolates 1, 2 and 3 were grown in Mueller–Hinton agar containing glucose-6-phosphate (25 mg/L) and fosfomycin (100 mg/L). Stable, one-step fosfomycin-resistant mutants (MICs >1024 mg/L) were obtained at frequencies of 1×10^{-6} to 5×10^{-6}. Similar to the clinical isolates derived after fosfomycin treatment, the in vitro mutants exhibited elevated MICs of β-lactams (data not shown). Activity of non-β-lactams was not affected. Attempts to elucidate the mechanism(s) involved in the reduction of β-lactam activity were not successful. Spectrophotometric determination of total β-lactamase and carbapenemase activities of

<table>
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<th>Antibiotic</th>
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<th>2</th>
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<th>3</th>
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<tr>
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Table 1. Activity of selected antibiotics [Etest MICs (mg/L)] against KPC-producing K. pneumoniae isolates
cell extracts and SDS–PAGE of outer membrane proteins did not reveal differences between the clinical isolates and the respective fosfomycin-resistant mutants selected either in vivo or in vitro. The concomitant increase in β-lactam resistance (a fact that, to our knowledge, is reported here for the first time among fosfomycin-resistant enterobacteria) should be investigated further. It must be noted, however, that the study concerned isolates of a single lineage (ST258). Therefore, the above observations may reflect a clonal characteristic.

Although the addition of fosfomycin as adjunct therapy to other active agents resulted in relief of symptoms and control of bacteraemia in patients with KPC-Kp infections, this effect was transient and was followed by clinical and microbiological relapse due to rapid selection of resistant variants. Additional data are required to determine the benefit from the administration of fosfomycin, if any, in the treatment of infections caused by KPC-Kp.

Acknowledgements
We thank Maria Grammatikou for technical assistance.

Funding
This work was partially supported by a grant from the Hellenic Center for Disease Control and Prevention (KEELPNO).

Transparency declarations
None to declare.

References

Pharmacokinetic interaction between maraviroc and etravirine in HIV-infected patients receiving regimens containing both drugs and no ritonavir-boosted protease inhibitor

Minh Patrick Lê1*, Caroline Solas2, Rodolphe Garraffo3, Marie-Claude Gagnieu4, Patrice Muret5, Patrick Yeni6, Catherine Dhiver7, Christine Katlama8, Isabelle Poizot-Martin9, Jacques Durant10 and Gilles Peytavin1

1 APHP, Bichat-Claude Bernard Hospital, Clinical Pharmacokinetic Department, EA4409 Paris 7 University, Paris, France; 2 APHM La Timone Hospital, Pharmacokinetics and Toxicology, Aix-Marseille University, CR02 UMR911, Marseille, France; 3 CHU Pasteur, Pharmacology, Nice, France; 4 Hospices Civils de Lyon Edouard-Herriot Hospital, Pharmacology, Lyon, France; 5 CHU Besancon, Clinical Pharmacology and Toxicology, Besancon, France; 6 APHP, Bichat-Claude Bernard Hospital, Infectious Diseases, Paris, France; 7 CHU La Conception, Infectious Diseases, Marseille, France; 8 APHP, Pitié-Salpêtrière Hospital, Infectious Diseases, Paris, France; 9 CHU Sainte-Marguerite, Immuno-Hematology CISIH, Marseille, France; 10 CHU L’Archet, Infectious Diseases, Nice, France

*Corresponding author. Tel: +33-1-40-25-84-54; Fax: +33-1-42-63-58-25; E-mail: i.e.minhpatrick@gmail.com

Keywords: HIV, drug interactions, PK, HIV pharmacology

Sir,

Drug–drug interactions (DDIs) between antiretroviral drugs (ARVs) are often complex and difficult to anticipate. The approval of ARVs, such as maraviroc and etravirine, for possible use in patients living with HIV is likely to be important for maintaining ARV efficacy and avoiding toxicity.

As stipulated in international regulatory guidelines, specific DDI studies showing the effects of enzymatic or efflux transporter inhibitors and inducers have been conducted in healthy subjects, but few data are available on maraviroc and etravirine in HIV-infected patients. Maraviroc, a new CCR5 antagonist, is a CYP3A4 substrate demonstrating a favourable pharmacokinetic distribution profile. Etravirine, a non-nucleoside reverse transcriptase inhibitor, is a CYP3A4 inducer and designed to have a high genetic barrier to the development of resistance. Regarding the respective biotransformation pathways of both ARVs, it is as yet unclear which maraviroc dose (300 or 600 mg twice daily) should be chosen in combination with etravirine without any PI/r. The objective of the present study was to determine the...