# Genesis of a KPC-producing Klebsiella pneumoniae after in vivo transfer from an imported Greek strain

**F Barbier[1,2,3], E Ruppé (etienne.ruppe@bch.aphp.fr)[1,3], P Giakkoupi[4], L Wildenberg[1], J C Lucet[5], A Vatopoulos[4], M Wolff[2], A Andremont[1]**

1. Department of Bacteriology, EA3964 and National Reference Centre for Antimicrobial Resistances in Commensal Flora, Bichat-Claude Bernard Hospital (Assistance Publique-Hôpitaux de Paris) and Paris-7 University, Paris, France
2. Medical Intensive Care Unit, Bichat-Claude Bernard Hospital (Assistance Publique-Hôpitaux de Paris) and Paris-7 University, Paris, France
3. These authors contributed equally to this work
4. Department of Microbiology, National School of Public Health, Athens, Greece
5. Infection Control Unit, Bichat-Claude Bernard Hospital (Assistance Publique-Hôpitaux de Paris) and Paris-7 University, Paris, France

**Citation style for this article:**
Citation style for this article: Barbier F, Ruppé E, Giakkoupi P, Wildenberg L, Lucet JC, Vatopoulos A, Wolff M, Andremont A. Genesis of a KPC-producing Klebsiella pneumoniae after in vivo transfer from an imported Greek strain. Euro Surveill. 2010;15(1):pii=19457. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19457

This article has been published on 7 January 2010

**We document here the *in vivo* transfer of *bla*$_{KPC-2}$ between intensive care unit-acquired and a commensal strain of *Klebsiella pneumoniae* in a French patient after his repatriation from Greece. This first report of *in vivo* transfer of a *bla*$_{KPC-2}$ between two *K. pneumoniae* strains raises further concerns about the spread of carbapenem resistance among *Enterobacteriaceae*.***

## Introduction

Carbapenems are the cornerstone of therapy against multidrug-resistant (MDR) enterobacteria, notably those expressing extended-spectrum β-lactamases (ESBL). To date, enterobacterial strains producing Ambler class A *Klebsiella pneumoniae* carbapenemases (KPC) remain very scarce in western European countries and correspond almost exclusively to imported clones from endemic areas, namely, the United States, Israel and Greece [1]. The *bla*KPC genes are located in a set of plasmid-borne Tn*4401*-type transposons [2], with recent evidence of interspecies conjugative transfer [3,4]. Here, we provide the first evidence of *in vivo* transfer of *bla*$_{KPC-2}$ between two *K. pneumoniae* strains from a single patient, one imported from Greece and the other from the commensal flora, leading to the emergence of a new KPC-2-producing strain in France.

## Case report and study

A French man in his 70s who was travelling in Greece was admitted to the intensive care unit (ICU) of a hospital in Athens on 30 April 2009 (day 0) for intestinal bleeding complicated by haemorrhagic shock and multiple organ failure. Several nosocomial infections occurred during his five-week long ICU stay in Athens, including a catheter-related bloodstream infection (BSI, day 25) due to a carbapenem-resistant *K. pneumoniae* strain that was also resistant to fluoroquinolones, co-trimoxazole, and aminoglycosides except gentamicin. This episode resolved after catheter removal and a one-week course of intravenous colistin. Subsequent clinical improvement allowed medical repatriation in France, and the patient was transferred to the ICU of a hospital in Paris (day 42). Intestinal carriage of MDR enterobacteria was routinely screened at admission by plating a rectal swab on ChromID ESBL medium (BioMérieux). One carbapenem-resistant *K. pneumoniae* strain (CHA-1) was isolated, and expressed the same co-resistances as the one involved in the BSI episode (Table). As the patient had never been hospitalised previously, we assume that he acquired the CHA-1 in the ICU in Athens.

The patient was discharged to a general medical ward on day 62. He did not receive carbapenems or other β-lactams after his transfer from Greece. On day 92, a second rectal swab was cultured on ChromID ESBL medium. Overnight growth yielded *K. pneumoniae* for which subsequent antibiotic susceptibility testing showed two distinct phenotypes. Subculturing recovered CHA-1 and another *K. pneumoniae* strain designated as CHA-2 (Table). According to the latest breakpoints published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5], CHA-2 was resistant to ertapenem, intermediate susceptible to doripenem, and susceptible to imipenem and meropenem (Table).

Strains CHA-1 and CHA-2 were PCR-tested for all major β-lactamase-encoding genes, with subsequent sequencing of the PCR products. Both CHA-1 and CHA-2 carried *bla*$_{KPC-2}$ and *bla*$_{TEM-1}$. In addition, the Ambler class B carbapenemase-encoding gene *bla*$_{VIM-1}$ was detected in strain CHA-1 (Table).

We suspected that the strain CHA-2 had emerged by an *in vivo* co-transfer of $bla_{KPC-2}$/$bla_{TEM-1}$ from the ICU-acquired strain CHA-1 to a recipient wild-type commensal strain of *K. pneumoniae*. This hypothesis was supported by several facts: Firstly, conjugation assays in mixed broth cultures using the rifampicin-resistant Escherichia coli J53 strain as recipient and either CHA-1 or CHA-2 as donors resulted in $bla_{KPC-2}$/$bla_{TEM-1}$-positive J53 transconjugants (conjugation frequency: $10^{-7}$ to $10^{-8}$), suggesting co-transfer of a plasmid carrying both genes. After extraction using the CompactPrep Plasmid Midi Kit (Qiagen), plasmids from both transconjugants yielded identical *Eco*RI-digestion patterns, arguing that CHA-1 and CHA-2 strains harboured the same $bla_{KPC-2}$/$bla_{TEM-1}$-carrying plasmid. $bla_{VIM-1}$ could not be transferred from CHA-1, as already experienced elsewhere [6]. Secondly, the swab from day 92 was re-plated on Drigalski agar. Twenty-five suspected *K. pneumoniae* were isolated, and those that did not grow on subcultures on ChromID ESBL medium were identified and tested for β-lactam susceptibility. Sixteen wild-type isolates of *K. pneumoniae* were thus collected and all yielded identical patterns in an enterobacterial repetitive intergenic consensus (ERIC)-PCR, suggesting that they were duplicates of a single wild-type *K. pneumoniae* strain, designated as BW1 (Table). Pulsed-field gel electrophoresis (PFGE) patterns of strains CHA-1, CHA-2 and BW1 were then compared to those of all KPC-2-producing pulsotypes of *K. pneumoniae* isolated to date in Greece (Figure).

The result indicated that (i) strain CHA-1 belonged to a KPC-2/VIM-1-coproducing pulsotype that is currently spreading in Greek hospitals (pulsotype C) in parallel with the pulsotype A that is the predominant KPC-2-producing pulsotype in Greece [6,7], (ii) CHA-2 did not match with any of the described Greek pulsotypes and (iii) KPC-2-producing strain CHA-2 and

**Table**

Antibiotic resistance phenotypes and acquired *bla* gene contents of enterobacterial strains described in this study

| | *K. pneumoniae* strain CHA-1 | *K. pneumoniae* strain CHA-2 | *K. pneumoniae* strain BW1 | *K. pneumoniae* strain TcBW1m[a] | *E. coli* strain J53 | *E. coli* strain TcJ53-1 | *E. coli* strain TcJ53-2 |
|---|---|---|---|---|---|---|---|
| Origin | Acquired in Athens ICU | Commensal flora | Commensal flora (putative precursor of strain CHA-2) | Conjugation assay (donor: CHA-1 / recipient: BW1m) | Collection | Conjugation assay (donor: CHA-1 / recipient: J53) | Conjugation assay (donor: CHA-2 / recipient: J53) |
| Date of isolation since hospital admission | Day 42 and day 92 | Day 92 | Day 92 | NA | NA | NA | NA |
| Acquired *bla* genes | $bla_{VIM-1b}$, $bla_{KPC-2}$, $bla_{TEM-1}$ | $bla_{KPC-2}$, $bla_{TEM-1}$ | None | $bla_{KPC-2}$, $bla_{TEM-1}$ | None | $bla_{KPC-2}$, $bla_{TEM-1}$ | $bla_{KPC-2}$, $bla_{TEM-1}$ |
| MIC values, mg/L[c] | | | | | | | |
| Amoxicillin | >256 | >256 | >256 | >256 | 2 | >256 | >256 |
| Amoxicillin + CLA[d] | >256 | 32 | 1.5 | 32 | 2 | 24 | 24 |
| Piperacillin | >256 | >256 | 6 | >256 | 0.75 | 256 | 256 |
| Piperacillin + TZP[e] | >256 | 32 | 1 | 64 | 0.75 | 32 | 48 |
| Cefotaxime | >32 | 2 | 0.047 | 2 | 0.023 | 4 | 4 |
| Ceftazidime | >256 | 2 | 0.094 | 2 | 0.032 | 4 | 4 |
| Aztreonam | >256 | 8 | 0.032 | 6 | 0.016 | 4 | 4 |
| Ertapenem | >32 | 2 | 0.006 | 2 | 0.006 | 0.75 | 0.75 |
| Meropenem | >32 | 2 | 0.012 | 0.75 | 0.006 | 0.25 | 0.38 |
| Doripenem | >32 | 1.5 | 0.016 | 0.75 | 0.006 | 0.25 | 0.25 |
| Imipenem | 32 | 2 | 0.125 | 2 | 0.19 | 0.5 | 0.75 |
| Tobramycin | 16 | 0.25 | 0.25 | 0.19 | 0.064 | 0.064 | 0.064 |
| Amikacin | 16 | 1 | 1 | 1 | 0.38 | 0.25 | 0.25 |
| Gentamicin | 1.5 | 0.5 | 0.5 | 0.5 | 0.094 | 0.094 | 0.094 |
| Ciprofloxacin | >32 | 0.032 | 0.032 | 0.032 | 0.047 | 0.047 | 0.047 |
| Cotrimoxazole | >32 | 0.064 | 0.064 | 0.064 | 0.004 | 0.004 | 0.004 |
| Tigecycline | 0.5 | 1 | 1 | 1 | 0.5 | 0.5 | 0.5 |
| Colistin | 0.125 | 0.125 | 0.125 | 0.125 | 0.19 | 0.125 | 0.19 |

*bla*: beta lactamase; ICU: intensive care unit; NA: not applicable.

[a] Obtained by conjugation assays using a rifampin-resistant mutant of BW1 selected on Szybalski gradients (BW1m, MIC of rifampin > 250 mg/L) as recipient and CHA-1 as donor, and subsequent isolation on Drigaslki agar supplemented with cefotaxime (1mg/L) plus rifampin (250mg/L).

[b] The co-expression of VIM-1 and KPC-2 contributes to explain the higher MICs of β-lactams in strain CHA-1 when compared to the blaVIM-1-egative/blaKPC-2-positive strain CHA-2;

[c] MIC: minimal inhibitory concentrations, as defined by E-test

[d] CLA: clavulanic acid (2 mg/L)

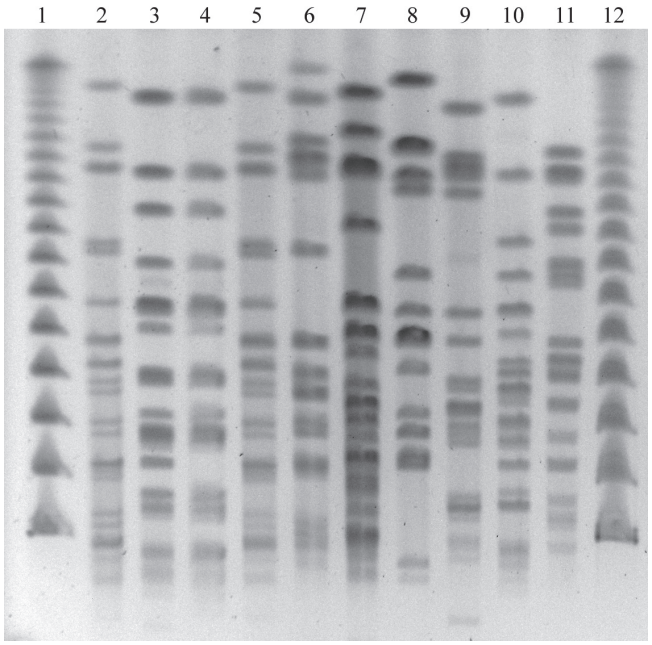
[e] TZP: tazobactam (4 mg/L)

wild-type strain BW1 displayed strictly identical XbaI-fingerprints, except for one band of approximately 100 kb also observed in CHA-1 that may correspond to the $bla_{KPC-2}$/$bla_{TEM-1}$-carrying plasmid. These data supported the role of BW1, the dominant wild-type *K. pneumoniae* strain within the digestive flora, as the $bla_{KPC-2}$-negative precursor of CHA-2. Lastly, we confirmed that the $bla_{KPC-2}$/$bla_{TEM-1}$-carrying plasmid was transferable from CHA-1 to a rifampicin-resistant BW1 strain obtained on a Szybalski gradient (Table). Some limitations are yet to be considered since we cannot strictly exclude that CHA-2 could have been acquired in Greece and could have been missed in the swab taken on day 42 at admission in France. Likewise, we cannot exclude that acquisition of CHA-2 could have occurred in France although reports on KPC-producing strains remain scarce to date.

This report raises further concerns about the diffusion of carbapenem resistance among enterobacteria. Indeed, that imported strains from endemic areas are able to spread *bla*KPC genes – even in the absence of β-lactam selective pressure, as in this patient – is worrisome, most notably for western European countries where the incidence of KPC-producing pathogens is still low.

## Figure

*Xba*I-PFGE of *K. pneumoniae* strains CHA-1, CHA-2, BW1 and KPC-producing clones disseminated in Greek hospitals




**Lanes 1 & 12:** Lambda Ladder (New England Biolabs)
**Lane 2:** strain CHA-1 $bla_{VIM-1}$ + $bla_{KPC-2}$
**Lane 3:** strain CHA-2 $bla_{KPC-2}$
**Lane 4:** strain BW1 wild type
**Lane 5:** strain 1780 $bla_{VIM-1}$ + $bla_{KPC-2}$ Greek pulsotype C
**Lane 6:** strain 1797 $bla_{VIM-1}$ + $bla_{KPC-2}$ Greek pulsotype G
**Lane 7:** strain 1504 $bla_{KPC-2}$ Greek pulsotype A
**Lane 8:** strain 1370 $bla_{KPC-2}$ Greek pulsotype B
**Lane 9:** strain 1433 $bla_{KPC-2}$ Greek pulsotype D
**Lane 10:** strain 1516 $bla_{KPC-2}$ Greek pulsotype E
**Lane 11:** strain 1643 $bla_{KPC-2}$ Greek pulsotype F

* CHA-2 and BW1 pulsotypes only differ by a ~100-kb band deemed to match the $bla_{KPC-2}$-carrying plasmid (also harboured by strains CHA-1 and 1780) [6].

PFGE: pulsed-field gel electrophoresis.


## Acknowledgements

This work was supported in part by the National Reference Centre for Antimicrobial Resistances in Commensal Flora.


*Erratum: This sentence was replaced on 14 January 2010

## References


1. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9(4):228-36.
2. Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. Genetic structures at the origin of acquisition of the beta-lactamase blaKPC gene. Antimicrob Agents Chemother. 2008;52(4):1257-63.
3. Cai JC, Zhou HW, Zhang R, Chen GX. Emergence of Serratia marcescens, Klebsiella pneumoniae, and Escherichia coli isolates possessing the plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC-2 in intensive care units of a Chinese hospital. Antimicrob Agents Chemother. 2008;52(6):2014-8.
4. Rasheed JK, Biddle JW, Anderson KF, Washer L, Chenoweth C, Perrin J, et al. Detection of the Klebsiella pneumoniae carbapenemase type 2 Carbapenem-hydrolyzing enzyme in clinical isolates of Citrobacter freundii and K. oxytoca carrying a common plasmid. J Clin Microbiol. 2008;46(6):2066-9.
5. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 1.0 December 2009. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_breakpoints_v1.0_20091221.pdf
6. Giakkoupi P, Pappa O, Polemis M, Vatopoulos AC, Miriagou V, Zioga A, et al. Emerging Klebsiella pneumoniae isolates coproducing KPC-2 and VIM-1 carbapenemases. Antimicrob Agents Chemother. 2009;53(9):4048-50.
7. Giakoupi P, Maltezou H, Polemis M, Pappa O, Saroglou G, Vatopoulos A, et al. KPC-2-producing Klebsiella pneumoniae infections in Greek hospitals are mainly due to a hyperepidemic clone. Euro Surveill. 2009;14(21):pii=19218. Available from: http://www.eurosurveillance.org