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

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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 Tn4401-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 *EcoRI*-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

	<i>K. pneumoniae</i> strain CHA-1	<i>K. pneumoniae</i> strain CHA-2	<i>K. pneumoniae</i> strain BW1	<i>K. pneumoniae</i> strain TcBW1m ^a	<i>E. coli</i> strain J53	<i>E. coli</i> strain TcJ53-1	<i>E. coli</i> 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 <i>bla</i> 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 Drigalski 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 bla_{VIM-1} -negative/ bla_{KPC-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.

FIGURE

XbaI-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.

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.

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*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 *bla*_{KPC} 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>