Persistence of NDM-1-producing enterobacteria over a 6-month period in a French community patient.

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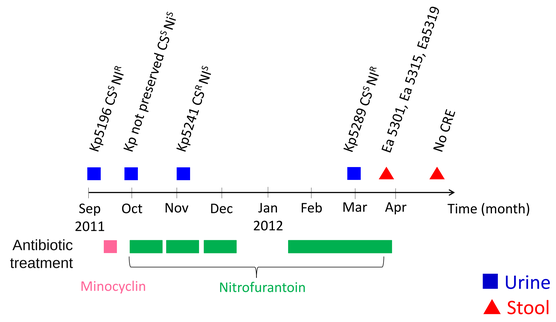
Introduction

On 09/01/2011, Kp5196, a colistin susceptible (CS^S) and nitrofurantoin resistant (NI^R) NDM-1-producing *Klebsiella pneumoniae*, was responsible for a urinary tract infection (UTI) in a French community patient without history of foreign travel (**1**). Two-months later, a second UTI episode was this time due to the CS^R NI^S NDM-1-producing *K. pneumoniae,* Kp5241. Thus, nitrofurantoin therapy was implemented.

The aim of this study was to look for the persistence of NDM-1-producing enterobacteria in the urine and the stools of this patient over a 6-month period. The strains were analyzed for their epidemiological relationship, antibiotic resistance phenotype and conjugative plasmid content.

Case report

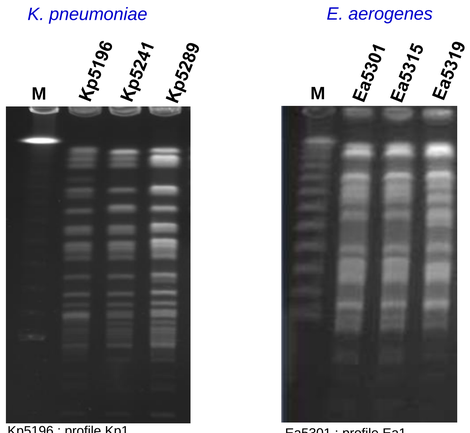
Time distribution of NDM-1-producing enterobacteria



Kp, *K. pneumoniae*, Ea, *Enterobacter aerogenes*
Colistin susceptible (CS^S), colistin resistant (CS^R), nitrofurantoin susceptible (NI^S), nitrofurantoin resistant (NI^R), CRE, carbapenem-resistant enterobacteria

Molecular typing

*Xba*I PFGE patterns



Kp5196 : profile Kp1
Kp5241 : profile Kp2 (91.3% *vs* Kp1)
Kp5289 : profile Kp3 (91.6% *vs* Kp1)

Ea5301 : profile Ea1
Ea5315 : profile Ea1
Ea5319 : Ea2 (84.6% *vs* Ea1)

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Material and Methods

- Antibiotic susceptibility testing: disk diffusion method (http://www.sfm.microbiologie.org).
- Screening of ertapenem-resistant enterobacteria in the fecal samples: CHROMagar KPC medium (CHROMagar).
- Strain identification: API20E system (bioMerieux) and urea-indole medium (bioMerieux).
- Strain typing: PFGE using *Xba*I and the CHEF DRIII apparatus (BioRad).
- *K. pneumoniae* typing: MLST (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).
- Resistance gene analysis: PCR amplification and sequencing.
- Plasmid analysis:
  - Conjugative transfer to *Escherichia coli* C600 (Azide resistant).
  - Plasmid size determination by S1 nuclease-PFGE analysis (**2**).
  - Analysis of resistance gene loss after repeated subcultures without selective pressure.

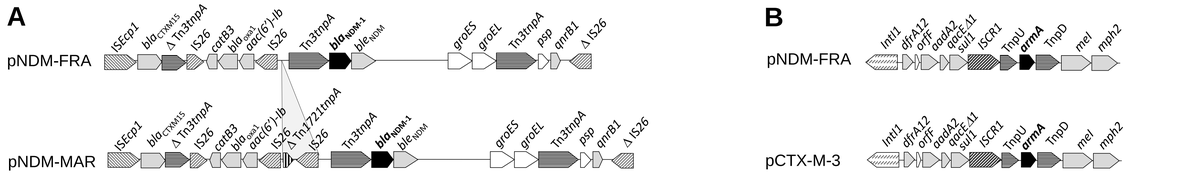
Antibiotic resistance phenotypes and associated genes

Strain	Sample	Isolation date (mo/da/yr)	Sequence type	Pulsotype (*Xba*I)	pNDM-FRA (size, kb)	Resistance genes located on the conjugative pNDM-FRA plasmid [a]									Not transferable resistances[b]
						ß-lactams			Aminoglycosides			SSS + TMP	Q	CHL	
Kp5196	Urine	09/01/2011	ST15	Kp1	270	$bla_{CTX-M-15}$	bla_{oxa1}	bla_{NDM-1}	*aac(6')Ib'*	*aadA2*	*armA*	*sul1*	*dfrA12*	*qnrB1*	*catA1*	CS^S NI^R
Kp5241	Urine	11/05/2011	ST15	Kp2	300	$bla_{CTX-M-15}$	bla_{oxa1}	bla_{NDM-1}	*aac(6')Ib'*	*aadA2*	*armA*	*sul1*	*dfrA12*	*qnrB1*	*catA1*	CS^R NI^S
Kp5289	Urine	03/01/2012	ST15	Kp3	300	$bla_{CTX-M-15}$	bla_{oxa1}	bla_{NDM-1}	*aac(6')Ib'*	*aadA2*	*armA*	*sul1*	*dfrA12*	*qnrB1*	*catA1*	CS^S NI^R
Ea5301/Ea5315	Stool	03/21/2012	ND	Ea1	300	$bla_{CTX-M-15}$	bla_{oxa1}	bla_{NDM-1}	*aac(6')Ib'*	*aadA2*	*armA*	*sul1*	*dfrA12*	*qnrB1*	*catA1*	CS^S NI^R
Ea5319	Stool	03/21/2012	ND	Ea2	Nd	$bla_{CTX-M-15}$	bla_{oxa1}	bla_{NDM-1}	*aac(6')Ib'*	*aadA2*	*armA*	*sul1*	*dfrA12*	*qnrB1*	*catA1*	CS^S NI^R

[a], SSS, sulfamethoxazole; TMP, trimethoprim; CHL, chloramphenicol. [b] Colistin susceptible (CS^S), colistin resistant (CS^R), nitrofurantoin susceptible (NI^S), nitrofurantoin resistant (NI^R). ND, not determined.

pNDM-FRA plasmid analysis

Schematic representation of the pNDM-FRA DNA sequences surrounding the bla_{NDM-1} (A) and *armA* (B) genes and comparison with the sequences of pNDM-MAR (A) and pCTX-M3 (B)



Genes or open reading frames (ORFs) are indicated by boxes and their transcriptional orientations are indicated by arrows. The same label is used to represent homologous genes. The antibiotic/antiseptic resistance genes are indicated in black for bla_{NDM-1} and *armA* genes and in grey for other resistances. bla_{NDM-1}, New Delhi metallo-ß-lactamase; $bla_{CTX-M15}$, class A ß-lactamase; bla_{OXA-1}, class D ß-lactamase; *armA*, 16S rRNA methylase; *aac(6')-Ib'*, aminoglycoside acetyltransferase; *aadA2*, aminoglycoside adenyltransferase; *mel*, macrolide efflux protein; *mph1-2*, macrolide 29-phosphotransferase; *dhfrA12*, dihydrofolate reductase; *sul1,* dihydropteroate synthase; *qacΔE1*, quarternary ammonium compounds; *catB3*, chloramphenicol acetyl transferase; *qnrB1*, quinolone resistance protein; ble_{NDM}, bleomycin resistance protein. The genes other than antibiotic/antiseptic resistance genes are labeled in light white: *groES-groEL*, chaperonin cluster; *psp* operon, transcriptional activator and phage shock proteins. The ORFs encoding for transposases /resolvases or putative transposases are indicated as hatched boxes as follows: IS*Ecp1*, Tn*3tnpA*, IS*26*, Tn*1721*, IS*CR1*, Tnp*U*, TnpD, and *IntI1*, class 1 integrase. Symbols are: Δ, genes that are truncated; Tn, transposon; tnpA, transposase. The shaded triangle symbolizes a deletion.

In vitro loss of resistance genes and concomitant evolution of plasmid size

Kp5196 derivatives	Deletion frequency (%)	Plasmid size (kb)	Antibiotic resistance gene [b]									
			bla			*aac(6')Ib'*	*aadA2*	*armA*	*sul1*	*dfrA12*	*qnrB1*	*catA1*
			CTXM-15	OXA-1	NDM-1							
Kp5196 [a]	-	270	+	+	+	+	+	+	+	+	+	+
Kp5196 (1)	1.0	260	+	+	-	+	+	+	+	+	+	+
Kp5196 (2)	4.2	255	+	+	-	+	+	+	+	+	-	+
Kp5196 (3)	11.5	245	-	+	-	-	-	-	-	-	-	-
Kp5196 (4)	1.0	ND	-	-	-	-	-	-	+	+	-	-

[a] parental strain, [b] Results of a single experiment.

Results

Analysis of strain persistence

On 03/01/2012, the patient was still under nitrofurantoin, and suffered a UTI caused by the CS^S NI^R NDM-1-producing *K. pneumoniae,* Kp5289. Screening of the patient's stools using the CHROMagar KPC medium showed predominance of wild-type *Pseudomonas aeruginosa* and few CS^S NI^R NDM-1-producing enterobacteria. A total of 94 colonies were tested and gave a negative urease test, as well as the 10 strains of *E. aerogenes* collected at random and analyzed by the API20E system. Three of them were further investigated. Antibiotic therapy was then stopped and 1 month later these strains had disappeared from the fecal and urine samples.

All *K. pneumoniae* strains belonged to the same ST15 clone, had a PFGE Dice index >91% and harbored the same plasmid carrying bla_{NDM-1}, $bla_{CTXM-15}$, bla_{OXA-1} *qnrB1*, and *armA.* Three *E. aerogenes* isolated from stools (Ea5301, Ea5315 and Ea5319) carried the same plasmid, including 2 that were identical by PFGE analysis, and the remaining one (Ea5319) that showed a Dice index of 84.6% compared to the 2 other ones.

Plasmid analysis

The conjugative plasmid, pNDM-FRA, belonged to the IncH incompatibility plasmid group. Analysis of the bla_{NDM-1} gene surrounding sequences revealed a very similar organization to that present on the recently described pNDM-MAR plasmid (267 kb, GenBank accession number, JN420336). This incH plasmid was carried by a ST15 NDM-1-producing *K. pneumoniae* collected from Moroccan patients (**3, 4**). In contrast with pNDM-FRA, it did not carry any 16S rRNA methylase gene (**4**). The locus containing the *armA* gene shared homology with that described in a IncL/M plasmid, pCTX-M3 (GenBank accession number, AF550415).

Analysis of in vitro plasmid evolution

After 8 subcultures without selective pressure, 100 clones of Kp5196 were analyzed for the presence of antibiotic resistance genes. Various resistance phenotypes were obtained with frequencies ranging from 1.0 to 11.5%. The strains exhibited different resistance genes and contained plasmids with different sizes.

Conclusions

Our study showed that infection and fecal carriage of NDM-1-producing enterobacteria persisted over a 6-month period and disappeared after removal of the antibiotic selective pressure.

All *K. pneumoniae* strains isolated from this patient were highly related. The in vivo transfer of bla_{NDM-1}-carrying plasmid between *K. pneumoniae* and *E. aerogenes* was demonstrated.

Analysis of the pNDM-FRA plasmid showed extensive similarities with the IncH plasmid, pNDM-MAR, isolated from Moroccan ST15 *K. pneumoniae* strains (**4**). In addition, it contained a locus with the *armA* gene found on other plasmids, e.g. the incL/M plasmid, pCTX-M-3, suggesting either acquisition or deletion of resistance modules. Our in vitro experiments confirmed the frequent occurrence of deletions in pNDM-FRA plasmid. They are likely due to the transposition of genetic elements, since the latter are well known to promote such rearrangements. This plasmid analysis threw some light onto the possible in vivo evolution of this novel type of IncH plasmid.

References

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