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Short Communication

Successful treatment and digestive decolonisation of a patient with osteitis caused by a carbapenemase-producing *Klebsiella pneumoniae* isolate harbouring both NDM-1 and OXA-48 enzymes

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ABSTRACT

Objectives: Carbapenem resistance in *Klebsiella pneumoniae* is an increasing problem worldwide and infections caused by this bacterium can be difficult to treat. This study reported the case of a patient from Romania, who was hospitalised in Bulgaria after an accident trauma. He then came to France for treatment of an osteitis caused by a *Klebsiella pneumoniae* carrying both bla_{NDM-1} and bla_{OXA-48}.
Method: The resistome of this extremely drug-resistant bacterium was analysed both with phenotypic (large antibiotic susceptibility testing) and genomic methods (genome sequencing). The genetic environment of the two carbapenemases was studied.
Results: Klebsiella pneumoniae ST307 carrying both a bla_{NDM-1} and bla_{OXA-48} gene was located on two different plasmids: Inc L/M and IncFII. The patient was successfully treated by a combination of intravenous colistin (9 MUI, then 4.5 MUI bd), intravenous fosfomycin (4 g tds) and oral doxycycline (100 mg bd) for 3 months. Faecal microbiota transplantation was successfully conducted for stool carriage.
Conclusion: The ST307 type is becoming endemic in hospital environments and is frequently associated with carbapenem resistance. Treatment of infection caused by multidrug-resistant bacteria is a clinical challenge, and the use of old antibiotics associated with screening and decolonisation of the reservoirs can be an efficient therapeutic alternative.

1. Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) have become, in the last decade, a major global concern, particularly in healthcare settings [1]. Carbapenemases are the most powerful β-lactamases, being able to hydrolyse almost all β-lactams. Of all the carbapenemases, the OXA-48 carbapenemase is currently the one that is the most rapidly spreading in many European countries [1]. In France, the first OXA-48-producing isolate was a *Klebsiella pneumoniae* identified in Paris in 2009 from the sputum of a Tunisian patient [2]. Subsequently, OXA-48-producing Enterobacteriaceae isolates were found in patients transferred from countries around the Mediterranean sea [1], causing large hospital outbreaks in western European countries [1].

Otherwise, isolates containing New Delhi metallo-β-lactamase (NDM-1) have been circulating in India as early as 2006, two years before the first European case was identified [3]. Since 2008, there has been repeated import of NDM-1-positive bacteria from the Indian subcontinent to Europe, in addition to being endemic in the Middle East, Northern Africa, and the Balkans [3]. The first identification of NDM-1 in France was in 2009, corresponding to an imported *Escherichia coli* isolate from India [4]. Two years later, the first reported case of community-acquired NDM-1 was identified in southern France, highlighting the risk of autochthonous acquisition [5].

This study reported the case of a patient who travelled for medical care from Bulgaria to France. This patient had an osteitis caused by a carbapenemase-producing *K. pneumoniae* harbouring

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both bla_{NDM} and bla_{OXA-48} genes. He also had stool carriage of a *K. pneumoniae* that was also carrying the two genes. Genome of this extremely-drug-resistant isolate was sequenced and analysed. The management of the infection included surgical and antibiotic treatment and a faecal microbiota transplantation, which are reported here.

2. Material and methods

2.1. Case report

At the end of 2015, a 43-year-old man was admitted to a hospital in the south of France suffering from septic pseudarthrosis of his left arm and left femur. Three years before, in Romania, the patient had had a car accident that resulted in both left humeral and open left femoral fractures. He underwent a humeral plate osteosynthesis and external fixation of his femur. Fifteen days later, the patient was transferred to a tertiary hospital in Bulgaria for fever and suppuration of the leg wound (Fig. 1). He then underwent a second surgical intervention for debridement and external fixation replacement. Two months before his admission in France, due to an infection of the pin site, a second external fixation replacement was performed. Despite several lines of empirical antibiotic therapy, the infection persisted, and the patient decided to travel to France for medical care. In the current hospital, all osteosynthesis material was removed and bone samples were taken and cultured in the laboratory with standard procedures.

2.2. Microbiological procedures

Samples were inoculated on blood agar medium (Biomérieux, Marcy l'Etoile, France) and chocolate polyvitex agar under aerobic atmosphere at 37 °C for 48 h. Additionally, one blood agar plate was inoculated under anaerobic conditions for 10 days at 37 °C. Screening for stool carriage was performed using the ChromeID CARBA SMART medium (Biomérieux, France). Bacterial identification was performed by Matrix Assisted Laser Desorption Ionisation - Time of Flight (MALDI-TOF), as previously described [6]. Antimicrobial susceptibility testing was performed according the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Minimum Inhibitory Concentration (MIC) of tigecycline and minocycline, doxycycline and imipenem were determined using Etest (Biomérieux, France), while colistin MIC was obtained using the UMIC microdilution method (Biocentric, Bandol, France). Real-time PCR of the carbapenemase genes (bla_{OXA-48}, bla_{NDM}, bla_{KPC}) was performed on every strain. Conjugation test was performed in an azide-resistant *E. coli* J53 strain. Transconjugant selection was performed on Luria Bertani agar (Beckton Dickinson, Le Pont de Claix, France) supplemented with 120 μg/mL sodium azide and 4 μg/mL ertapenem. Carbapenemase PCR performed on transconjugants confirmed the presence of the two carbapenemase genes.

2.3. Genome sequencing and analysis

The genome of one multidrug-resistant (MDR) *K. pneumoniae* KP_DC isolated from the articular liquid (GenBank accession no. NJGM00000000) was sequenced by Miseq technology (Illumina Inc, San Diego, CA, USA) with a paired-end strategy. The genome was assembled with A5 software [7], aligned with Mauve [8] to the reference strain ATCC43816 KPRR (GenBank accession number CP009208.1), and annotation was performed using Prokka [9] and Arg-annot [10] for research of antibiotic-resistance genes. Plasmid was found using PlasmidSeeker [11] and then reconstituted by mapping the reads of the genome with the reference sequence found with PlasmidSeeker using CLC Genomics Workbench version 7.5 (Qiagen, Hilden, Germany). The FAB formula of the IncF plasmid was determined using the Center for Genomic Epidemiology platform (https://cge.cbs.dtu.dk/).

3. Results

Both bone biopsies from the humerus and femur were positive for a carbapenemase-producing *K. pneumoniae*. Screening for stool carriage also found a carbapenemase-producing *K. pneumoniae.* All isolates remained susceptible to fosfomycin, nitrofurantoin, tigecycline, minocycline and colistin (Table 1). All these isolates harboured both NDM and OXA-48 genes, which was confirmed by PCR (Table 1). Treatment was started with a combination of intravenous colistin (9 MUI, then 4.5 MUI bd), intravenous fosfomycin (4 g tds) and oral doxycycline (100 mg bd) for 3 months (Fig. 1). No adverse effects were observed during the treatment. The kidney and liver functions, which were normal before treatment, remained unchanged.

Faecal microbiota transplantation (FMT) was performed on day 10 of hospitalisation at the current institution. In brief, as

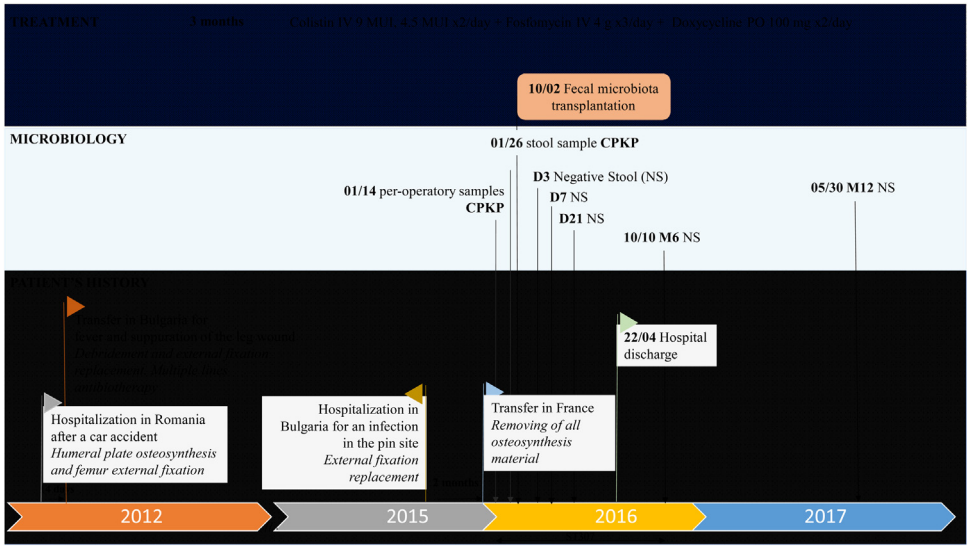

Fig. 1. Timeline of osteoarticular infection in the patient.

Table 1
Antibiotic susceptibility testing, resistance genes and multilocus sequence typing results of the five strains of *Klebsiella pneumoniae* isolated in the per-operatory samples and in stool.

Isolate number	Strain 1		Strain 2		Strain 3 (genome)		Strain 4		Strain 5	
Date of isolation	14/01/2016		14/01/2016		14/01/2016		14/01/2016		26/01/2016	
Nature of sample	Humeral bone biopsy 1		Humeral bone biopsy 2		Humeral bone biopsy 3		Femoral bone biopsy		Stool	
Antibiotic	Ø (mm)	S/I/R	Ø (mm)	S/I/R	Ø (mm)	S/I/R	Ø (mm)	S/I/R	Ø (mm)	S/I/R
Amoxicillin	0	R	0	R	0	R	0	R	0	R
Amoxicillin/clavulanic acid	0	R	0	R	0	R	0	R	0	R
Piperacillin/tazobactam	0	R	0	R	0	R	0	R	0	R
Ceftriaxone	0	R	0	R	0	R	0	R	0	R
Cefepime	9.9	R	9.9	R	9.9	R	10.4	R	10.8	R
Ertapenem	13.3	R	12.3	R	12.3	R	13.2	R	13.6	R
Imipenem	18	I	17.4	I	18	I	18.8	I	17.9	I
Gentamicin	13.6	R	0	R	0	R	13.8	R	0	R
Amikacin	0	R	0	R	0	R	12.7	R	0	R
Ciprofloxacin	10.3	R	16	R	15.9	R	0	R	16.5	R
Trimethoprim/sulfamethoxazole	0	R	0	R	28.1	R	0	R	0	R
Fosfomycin	21.8	S	19.2	S	20.2	S	20.9	S	22.2	S
Nitrofurantoin	18.8	S	17.8	S	17.5	S	18.1	S	19.3	S
MIC antibiotic (μg/mL)										
Colistin (microdilution method)	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S
Imipenem (Etest)	8	I	8	I	6	I	1.5	S	8	I
Minocycline (Etest)	2	S	1.5	S	1.5	S	2	S	1	S
Tigecycline (Etest)	1.5	S	1	S	1	S	1.5	S	1	S
Doxycycline (Etest)	16	–	2	–	2	–	16	–	2	–
Resistome										
Carbapenems	*blaNDM-1* *blaOXA-48*		*blaNDM-1* *blaOXA-48*		*blaNDM-1* *blaOXA-48*		*blaNDM-1* *blaOXA-48*		*blaNDM-1* *blaOXA-48*	
Other ß-lactams	ND		ND		$bla_{CTX\text{-}M\text{-}15}$ $bla_{CTX\text{-}M\text{-}132}$ $bla_{SHV\text{-}28}$ *ampH*		ND		ND	
Aminoglycosides	ND		ND		*rmtC* *strB*		ND		ND	
Fluoroquinolone	ND		ND		*oqxA/ oqxB*		ND		ND	
Sulfamides	ND		ND		*Sul1*		ND		ND	
Fosfomycin	ND		ND		*fosA*		ND		ND	
Sequence type[a]	307		307		307		307		307	

[a] According to the http://bigsdb.pasteur.fr/klebsiella/klebsiella.html database.

previously described [12], an anonymous, fully screened, stool donor was used for FMT. The patient was administered a bowel lavage followed by four doses of oral gentamicin (100 mg) and colistin (2.5 MIU) over 24 h prior to FMT. Following that, 50 g of donor stool were homogenised and diluted in 0.9% NaCl, and 400 mL were administered by nasogastric tube. No adverse events were observed. The patient was placed under contact precautions until three consecutive weekly-collected stool samples were negative for carbapenemase-producing isolate. Control stool samples were still negative 12 months later. The patient recovered with bone consolidation and wound healing after a 12-month follow-up.

The genome of the *K. pneumoniae* KP_DC isolate was assembled into 71 contigs with lengths ranging from 919 to 733 430 bp and a GC content of 57.4%; 92.3% was found to be genomic DNA but 7.4% of the contig did not map with the reference strain *K. pneumoniae* MGH78578 (NC_009648.1). In silico multilocus sequence typing showed that this strain belonged to the ST307 type. Multilocus sequence typing of the four other isolates with no available genome showed that they also belonged to ST307. The IncFII and IncL/M plasmids were found using PlasmidSeeker [11]. Conjugation tests were positive for the two carbapenemase genes as well as for $bla_{CTX\text{-}M\text{-}15}$ and $bla_{SHV\text{-}28}$ genes.

The IncL/M conjugative plasmid that harboured the $bla_{OXA\text{-}48}$ gene was 61 682 bp in length, with an average G + C content of 51.02% (p2G1140) (Fig. 2a). Comparison with the reference plasmid pOXA-48 (accession number JN626286) found 98% identity and 98% query coverage. The genetic environment of the $bla_{OXA\text{-}48}$ gene included a *Tn1999*-like transposon inserted within a *tir* gene, flanked on both sides by a direct repeat sequence of 9 bp (CGTTCAGCA). In the 3′-5′ direction from the $bla_{OXA\text{-}48}$ gene, the usual *mucB-mucA-pemK-pemI* gene pattern was found. The *Tn1999*-like transposon was flanked on either side by two imperfect insertion sequences (IS): two copies of IS10A on the left and both IS10A and IS1 on the right (Fig. 2a).

The conjugative IncFII plasmid (p1G1140) was an IncFII Y4:A-:B36 carrying a $bla_{NDM\text{-}1}$ gene (Fig. 2b). This plasmid shared 99% cover and 99% identity with the reference plasmid (pRJF866) for a 110 787 bp length, with an average G + C content of 54.72%. The genetic environment of $bla_{NDM\text{-}1}$ gene was made by two IS5 genes with several insertion sequences on each extremity. This transposon also carried a dihydropteroate synthase (Sul1) involved in sulfonamide resistance, a 16S rRNA methyltransferase (RmtC) responsible for aminoglycoside resistance, and a ble_{MBL} gene leading to bleomycin resistance (Table 1).

4. Discussion

Prior surgery and extended hospital stays in countries with high levels of antimicrobial resistance, as well as the presence of wounds, are recognised as risk factors for MDR organism acquisition [1,13]. Antibiotic selection pressure may be an additional factor that influences colonisation with these organisms. In a recent case-control study, prior use of piperacillin-tazobactam, a carbapenem, a quinolone, or metronidazole was

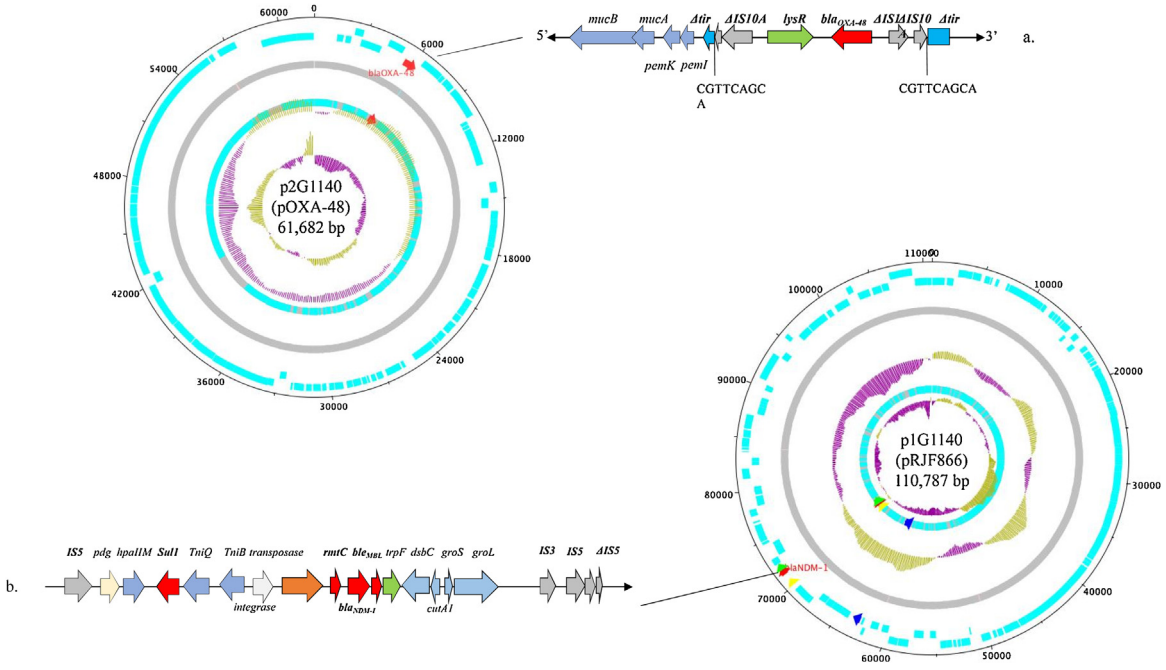

Fig. 2. Representation of plasmids carrying carbapenemase genes compared with plasmids of reference.
(a) Genetic environment of the plasmid-mediated bla_{oxa-48} gene and comparison with the reference plasmid pOXA-48 (accession number JN626286).
(b) Genetic environment of the plasmid-mediated bla_{NDM-1} gene and comparison with the reference plasmid pRJF866 (accession number KF732966).

significantly associated with infections caused by carbapenemase-producing enterobacteria [14]. However, the cumulative number of prior antibiotic exposures appears to be more critical than the use of a specific class of antibiotics [13]. This highlights the importance of screening the digestive colonisation by MDR organisms directly upon admission to hospitals for high-risk patients, especially in patients who have received healthcare in endemic countries or epidemic facilities [13].

Old antibiotics (i.e., colistin, fosfomycin, tetracyclines, mecillinam, temocillin, thiamphenicol, and pristinamycin) have been increasingly reused in the last few years, with rising numbers of clinical studies evaluating their efficacy for the treatment of MDR bacterial infections, and pharmacokinetic/pharmacodynamic studies reassessing their optimal dosing [15]. But despite the evidence that these old antibiotics are still effective, mostly available as generics, they are not universally marketed [16]. The current patient was successfully treated with fosfomycin, despite the presence of a fosfomycin-resistance gene *fosA* in the genome of the sequenced isolate. The *fosA* gene is widely distributed in the *K. pneumoniae* species and conferred a fosfomycin MIC to approximatively 24 mg/L, allowing its use in clinical practice (MIC cut-off according to EUCAST guidelines = 32 mg/L).

It has been shown that stool carriage of CPE could be extended for as long as 40 months [17]. Faecal microbiota transplantation has been proposed as an efficient way of reducing the duration of colonisation by CPE, as it has emerged as therapy for MDR bacterial decolonisation [12]. It was successfully used in the current case, since faecal samples were still negative 1 year later.

The ST307 appeared in literature in 2013 from strains isolated from clinical samples between 2007 and 2010 in Texas but recent analyses showed that this clone emerged in the mid-1990s and spread worldwide [18]. This clone has been frequently associated with ESBL and carbapenemase genes [18] and is becoming prevalent in hospital environments. Double carbapenemases-producing bacteria have been increasingly reported in the world [19]. This acquisition of more than one carbapenemase seems to increase the MIC of imipenem [19], limiting its use even in synergistic association with another antibiotic. However, the presence of a double carbapenemase can have no impact on imipenem MIC [19], as it depends on the expression level of the carbapenemases. This is of great concern and must be detected by microbiological screening.

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Competing interest

None.

Ethical approval

Not required.

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