Antimicrobial Treatment and Containment Measures for an Extremely Drug-Resistant *Klebsiella pneumoniae* ST101 Isolate Carrying pKPN101-IT, a Novel Fully Sequenced *bla*KPC-2 Plasmid

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An extremely drug-resistant *Klebsiella pneumoniae* isolate, sequence type ST101, was isolated from a patient in Italy. We describe antibiotic treatment, measures to clear and contain the infection, and a complete sequence analysis of a novel large plasmid, pKPN101-IT, harboring the *bla*KPC-2 gene and arising from the threatening recombination of different worldwide-distributed backbones.

Multidrug resistance is emerging worldwide among *Enterobacteriaceae* at an alarming rate, causing both nosocomial and community-acquired infections (16). The effective propagation of the Ambler class A KPC-type carbapenemases has become a major public health concern: these enzymes hydrolyze all beta-lactam drugs with the exception of cephamycins and cause frequent nosocomial outbreaks and life-threatening infections (20). Notably, the efficient spreading of the *bla*KPC genes rises from their mobilization on a Tn3-like transposon (8). The sequential appearance of distinct *bla*KPC alleles is suggestive of adaptation in response to antibiotic pressure and highlights the ease with which plasmid-borne variants can spread among *Enterobacteriaceae* (7).

KPC dissemination has also been associated with a highly epidemic international clone of *Klebsiella pneumoniae*, sequence type (ST) 258. Nowadays, ST258 and derivatives (such as ST512 and ST745) are predominant worldwide among KPC-positive *K. pneumoniae* isolates, but additional KPC-producing clones (ST147, ST11, ST307, ST437, ST554, and ST745, among others) have been reported (1, 5, 6, 11).

As part of an epidemiological study (26), in July 2011, a patient was diagnosed with an extremely drug-resistant *K. pneumoniae* strain belonging to a rare sequence type, ST101, which had never been observed in our region. Here we describe the characterization and deep-sequencing analysis of a novel plasmid harboring the *bla*KPC-2 gene that arises from the threatening recombination of different worldwide-distributed backbones.

From November 2010 to June 2011, a 57-year-old man was hospitalized in different neurological and neurorehabilitation clinics within the Florence province, Italy. In April 2011, the patient developed bacterial meningitis, which was empirically treated for 2 weeks with piperacillin-tazobactam (4.5 g/day) and tigecycline (150 mg/day). In May 2011, therapy was shifted to meropenem (3 g/day) and rifampin (600 mg/day) for 10 days. In June 2011, bacteriological screening of bronchoalveolar lavage (BAL) fluid and urine samples revealed the presence of a multidrug-resistant (MDR) *K. pneumoniae* strain, which was treated with a cocktail of rifampin (600 mg/day), tigecycline (100 mg/day), and colistin (480 mg/day) for 3 weeks (Table 1). The patient was subsequently discharged. At the beginning of July 2011, the patient was rehospitalized in the neurosurgery ward of the Padua Teaching Hospital. At admission, the BAL fluid was positive for *K. pneumoniae*. The antibiogram, determined by the Vitek automated system (bioMérieux, Marcy l’Etoile, France) and interpreted according to the EUCAST clinical breakpoints v.2.0 document (9), revealed that the isolate was resistant to all drugs except for colistin (Table 1). Therefore, the patient was administered colistin (480 mg/day), tigecycline (60 mg/day), and rifampin (600 mg/day): after 4 weeks, urine and blood samples were negative for the presence of *Enterobacteriaceae*. Toxicity of both colistin and tigecycline required monitoring of the hepatorenal function during antimicrobial therapy, which demonstrated high creatinine levels (159 μmol/liter). Therefore, as soon as perirectal and bronchial samples from the patient were negative for MDR organisms, therapy was shifted to meropenem (4 g/day) and trimethoprim-sulfamethoxazole (240 and 1,200 mg/day, respectively) and maintained as a precautionary measure for 10 days. By the end of August, surveillance perirectal swabs highlighted that the patient was still colonized with an MDR *K. pneumoniae* isolate susceptible to colistin only (Table 1). Antibiotic treatment with colistin (480 mg/day) and tigecycline (150 mg/day) for 20 days apparently cleared the MDR *K. pneumoniae* colonization. Therefore, antibiotic treatment was switched once more to meropenem (4 g/day) and trimethoprim-sulfamethoxazole (240 and 1,200 mg/day, respectively). However, 10 days later, an MDR *K. pneumoniae* strain was again diagnosed even though no clinical symptoms of infection were reported (Table 1). The MDR *K. pneumoniae* isolates collected from the patient were characterized by means of PCR-mediated detection and sequencing of antibiotic resistance determinants, as previously described (24): the *bla*KPC-2 and *bla*TEM-1 genes were found.

Multilocus sequence typing (MLST) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) revealed that...
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<thead>
<tr>
<th>Isolate type</th>
<th>Sample type(s)</th>
<th>Place of isolation</th>
<th>Date of isolation (day.mo.yr)</th>
<th>ST</th>
<th>MIC (mg/liter)</th>
<th>Antimicrobial therapy</th>
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<td>21.05.2011 –</td>
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<td>64</td>
<td>/H11350 64 /H11350 16 /H11350 16 2 /H11349 0.5 Colistin (480 mg/day), tigecycline (100 mg/day), and rifampin (600 mg/day) for 20 days</td>
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<td>BAL fluid</td>
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<td>28.06.2011 – 19.07.2011</td>
<td>101</td>
<td>64</td>
<td>/H11350 64 /H11350 16 /H11350 16 2 /H11349 0.5 Colistin (480 mg/day), tigecycline (60 mg/day), and rifampin (600 mg/day) for 30 days</td>
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<td>Meropenem (4 g/day) and trimethoprim-sulfamethoxazole (240 and 1,200 mg/day, respectively) for 15 days</td>
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**Table 1. Properties of the K. pneumoniae strains isolated from the patient and of the pKPN101-IT-transformed and untransformed laboratory E. coli strains.**

**a** CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem; TGC, tigecycline; CST, colistin; NA, not applicable; –, not available.

**b** E. coli TOP10 is a laboratory strain transformed with pKPN101-IT.
all isolates belonged to ST101 (Table 1). This was the first isolation of *K. pneumoniae* ST101 in the Padua Teaching Hospital. An outbreak of this carbapenem-nonsusceptible ST has been very recently reported in Tuscany (18), where the patient was initially hospitalized.

Interestingly, OXA-48- and CTX-M-15-producing ST101 strains have been described in the rest of the world (8,21); in particular, KPC-positive ST101 *K. pneumoniae* strains have been recently reported in Brazil and in the United States (6,27).

Plasmid localization of the *bla*KPC-2 gene was confirmed by electroporation of a plasmid DNA preparation obtained by phenol-chloroform extraction from the ST101 isolate into *Escherichia coli* TOP10 (Invitrogen Ltd., Paisley, United Kingdom) with selection on ampicillin agar plates (100 mg/liter). Plasmid DNA extracted from transformed *E. coli* cells was used for deep sequencing using a 454 FLX sequencing platform (Roche). Plasmid sequencing was performed with the 454 pyrosequencing technology (19) using a shotgun approach. A total of 6,401 reads, with an average length of 419 bp, were obtained, resulting in 25× coverage. Sequences were assembled into 8 contigs using Newbler 2.6 (contig average length, 13,132 bp; N50 contig size, 64,311 bp); the finishing step was accomplished by primer-specific PCR and Sanger sequencing. Annotation was carried out using the RAST server (2).

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**FIG 1** Structural features of the pKPN101-IT plasmid. (A) Schematic map of the pKPN101-IT plasmid. Starting from the outside, arrows indicate coding sequences (CDSs) (CDSs with defined functions are shown in black, and hypothetical CDSs are represented in gray). The black arc placed over the 10,000 tick highlights the presence of the transposon Tn4401a. The GC content has been calculated for bins of 100 nucleotides (nt) and is reported in the inner circular histogram. The average value is 52.7%, ranging from 24% (light gray) to 75% (black). (B) Comparative view of the major features present in the plasmid pKPN101-IT (GenBank accession number JX283456), sequenced in this study, and plasmids pKpQIL-IT (GenBank accession number JN233705) and pKP048 (GenBank accession number FJ628167). Genes involved in drug resistance are shown in black, while gray boxes represent transposon-related genes, resolvase genes, and insertion sequences. Other significant genes are represented as white boxes.

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Complete plasmid sequencing identified a novel backbone, named pKPN101-IT, that was 107,748 bp in size, encompassing 118 open reading frames (ORFs) with an average GC content of 52.7% (Fig. 1A). Replicon typing analysis, based on the plasmid MLST website (http://pubmlst.org/plasmid/), classified the pKPN101-IT plasmid as an IncFII(k)-type backbone. As recently discussed (29), IncF-like plasmids often provide remarkable virulence and antimicrobial resistance determinants and have a broad host range, covering the entire Enterobacteriaceae family. The newly identified pKPN101-IT plasmid thus has characteristics similar to those of backbones involved in the worldwide dissemination of other resistance determinants, such as blaCTX-M-15 and the recently reported blaNDM-1 (3, 4).

The region embedding blaKPC-2 showed the typical structure of the Tn4401a transposon, with a 100-bp deletion upstream of the blaKPC-2 gene with respect to Tn4401b (12). The transposon included resolvase, transposase, ISKpn7, and ISKpn6 structures, the canonical left and right inverted repeats (IRL and IRR, respectively), and the target site duplication sequences. The pKPN101-IT backbone contained replication, stable inheritance, maintenance, and partitioning modules similar to those reported for other plasmids. BLAST analysis showed important homology to previously characterized KPC-encoding plasmids, including pKpQIL-IT (identified in Italy in a Klebsiella pneumoniae ST258 isolate in 2011 [10]; 71% sequence identity), pKP048 (isolated in China from a K. pneumoniae sample in 2006 [14]; 65% sequence identity) (Fig. 1B), and two recently characterized plasmids embedding NDM-1, pGUE-NDM (9% sequence identity), and pNDM-HK (12% sequence identity) (3, 13) which share the IncFII-type backbone and the region conferring resistance to macrolides, respectively.

Moreover, a complete transfer operon (locus tra-trb) was embedded in pKPN101-IT, suggesting the possibility of wide dissemination. Four complete copies of the IS26 element, each bracketed by IRR and IRL repeats, were detected and encompassed two resistance modules: one containing the 2’-phosphotransferase Mph2, conferring resistance to macrolides, and the second harboring a mercury resistance protein cluster. The Mer operon allows the reduction of toxic Hg(II) to volatile Hg(0), thus facilitating bacterial survival in the environment. The IS26 composite transposons were supposed to have been generated from subsequent plasmid rearrangements and recombination events. In addition, the gene encoding the 16S rRNA methylase ArmA, conferring high-level resistance to all aminoglycosides, was present and coupled with an ISEc29 transposase. Of note, enterobacterial isolates coproducing KPC-2 and ArmA have been reported in China and in Eastern Europe (30, 31).

The overall analysis of pKPN101-IT highlighted how this backbone is a jigsaw of functional and drug resistance modules originating from several recombination events. Patient transfer from Florence to the Padua Teaching Hospital may have caused a new hospital outbreak of the KPC-encoding K. pneumoniae ST101 strain. However, current high awareness of the carbapenem-resistant K. pneumoniae threat has led to prompt adherence to containment procedures. First of all, routine microbiological surveillance performed on all new patients upon admission and weekly on patients in relevant units allowed early identification of the KPC-positive isolate; an ultrarapid real-time screening for KPC from perirectal swabs was set up for this purpose (25). Second, stringent infection and prevention control measures, including contact precautions, such as KPC-positive patient confinement in dedicated rooms, access restrictions to affected areas, instruction in hand hygiene, and increased frequency of environmental cleaning, were implemented. To date, no further ST101 isolates or enterobacterial isolates harboring pKPN101-IT have been detected in our hospital.

Eradication of the MDR strain is an important goal which has
to be achieved by administration of the proper antibiotic therapy. To date, tigecycline has been successfully used in combination with colistin for the treatment of pandrug-resistant *K. pneumoniae* (15, 23). Unfortunately, antimicrobial administration is not always a synonym of complete eradication of the resistant isolate (22, 28). Interestingly, in our case, multiple-antimicrobial administration did not select strains resistant to these drugs, pointing out that, for severe infections, it may be appropriate to combine the few agents that remain active to maximize the chance of efficacy and to minimize selection of further resistance (17).

In conclusion, a new KPC-encoding plasmid has been identified in *K. pneumoniae* ST101; full analysis of the plasmid sequence highlighted (i) its possibility to easily spread among species of the Enterobacteriaceae family and (ii) the extremely high level of association of multiple antibiotic resistance determinants.

**Nucleotide sequence accession number.** The sequence for the novel plasmid pKPN101-IT is available in GenBank under the accession number JX283456.

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**REFERENCES**


