First outbreak of multidrug-resistant *Klebsiella pneumoniae* carrying \( \text{bla}_{\text{VIM-1}} \) and \( \text{bla}_{\text{SHV-5}} \) in a French university hospital

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**Objectives:** We studied eight imipenem-resistant isolates of *Klebsiella pneumoniae* involved in an outbreak in a French teaching hospital.

**Methods:** The eight isolates were recovered from clinical specimens or rectal swabs. Antibiotic susceptibilities were determined using standard agar diffusion and dilution methods including synergy tests. PFGE was used to study the relatedness of isolates. Genes encoding \( \beta \)-lactamases were characterized by transfer assays, specific amplification and cloning.

**Results:** The eight isolates were closely related by PFGE analysis and highly related to a *K. pneumoniae* strain from Greece. They were highly resistant to \( \beta \)-lactams, including aztreonam and imipenem (MIC \( \geq 32 \) mg/L), and were positive by the imipenem-EDTA disc synergy test. Isolates were also resistant to aminoglycosides, newer quinolones and sulfamethoxazole, and showed an intermediate level of resistance to tetracycline. VIM-1 and SHV-5 \( \beta \)-lactamases were revealed in all isolates by PCR. The analysis of plasmid contents of *Escherichia coli* DH10B electroporants expressing the VIM-1 \( \beta \)-lactamase or the SHV-5 \( \beta \)-lactamase confirmed that the two enzymes were coded by two different plasmids. The \( \text{bla}_{\text{VIM-1}} \) gene was part of a class 1 integron that also included \( \text{aac6}, \text{dhfrI} \) and \( \text{aadA} \) genes and was similar to those reported from strains isolated in Greece.

**Conclusions:** This study confirms the potential risk of spread of multiresistant bacteria with international transfer of patients.

Keywords: imipenem resistance, class I integrons, liver transplants

**Introduction**

The emergence of acquired metallo-\( \beta \)-lactamases (MBLs) in Gram-negative bacilli is becoming a therapeutic challenge because these \( \beta \)-lactamases possess a broad hydrolysis spectrum that includes virtually all \( \beta \)-lactams, except the monobactam aztreonam. IMP- and VIM-type enzymes are the two major types of MBLs reported. Most acquired MBL genes that have been reported were inserted on mobile elements (especially integron-borne gene cassettes). The first member of the VIM-family determinants, VIM-1, was identified from a clinical isolate of *Pseudomonas aeruginosa* in Verona, Italy.\(^1\) VIM-type \( \beta \)-lactamases have been described in various geographical areas\(^2\) and have been reported in several enterobacterial species but *P. aeruginosa* remains the most important known reservoir of these enzymes.\(^3\) Outbreaks of the VIM-type MBLs have been reported mostly in *P. aeruginosa*, but also recently in *Klebsiella pneumoniae* in Greece.\(^3\)

We report the first outbreak of colonizations and infections due to a *K. pneumoniae* strain producing VIM-1 MBL in a French University Hospital, which followed transfer of a patient from Greece.

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First outbreak of multidrug-resistant Klebsiella pneumoniae

Material and methods

Hospital setting and patients

Imipenem-resistant K. pneumoniae isolates were recovered from patients hospitalized at the Hepatobiliary Surgical Centre of the Paul Brousse Hospital. It is an 81 bed centre including 15 ICU beds, and admits ~500 patients per year, of whom ~15% are referred from outside of France. Since 2002, screening of extended-spectrum β-lactamase (ESBL)-producing strains of Gram-negative bacilli using rectal swabs has been instituted for all patients admitted to the ICU.4

Bacterial strains

One hundred and sixteen imipenem- and aztreonam-resistant isolates of K. pneumoniae were recovered from eight patients (68 strains from clinical specimens and 48 from rectal swabs). As all isolates exhibited the same resistance pattern, the first isolate from each patient was included in the study. Three imipenem-resistant strains of K. pneumoniae isolated in Greece (K1, K5 and K8)3 were used as comparators in the typing methods.

Clonal relation between isolates by PFGE analysis

Genomic DNA, prepared as described previously5 and digested with XbaI (Ozyme, New England Biolabs Inc., Saint Quentin en Yvelines, France) was subjected to PFGE with the CHEF DRIII device (Bio-Rad). The resulting restriction patterns were interpreted as reported by Tenover et al.6

Antibiotic susceptibility and synergy testing

Agar dilution and disc diffusion tests were performed according to the recommendations of the Comité de l’Antibiogramme de la Société Française de Microbiologie (http://www.sfm.asso.fr). Class A extended-spectrum and plasmid-mediated class C β-lactamas were detected using synergy tests7,8 (http://www.sfm.asso.fr). To detect MBL production, a synergy test using imipenem and EDTA-nam (1, 2 or 4 mg/L).

PCR amplification and molecular characterization of transferable β-lactamases

PCR with blaTEM, SHV, CTX-M, CMY, VIM, IMP, SPM, ACC, FOX specific primers9 and subsequent sequencing of PCR products were performed.

Transfer of β-lactam resistance

Conjugation experiments were carried out between each test isolate and Escherichia coli K12 J53-2 (met pro Rif)10 in broth medium. Transfer of resistance by electroporation was performed with plasmid DNAs (Qiagen Midi Kit, Coutaboeuf, France) transformed into E. coli DH10B (Invitrogen SARL, Cergy Pontoise, France) by electroporation (Bio-Rad). Transconjugants or electroporants were selected on rifampicine (256 mg/L) and either imipenem (1, 2 or 4 mg/L) or aztreonam (1, 2 or 4 mg/L).

Plasmid DNA analysis

Plasmid DNA was extracted by the alkaline lysis method10. Plasmid DNA was purified from electroporant cells with the Qiagen Plasmid Midi Kit (Qiagen). For fingerprinting analysis, plasmid DNA was digested with EcoRI (Ozyme).

Cloning experiments

DNA fragments obtained from Sau3A partially digested genomic DNA were ligated into the vector pACYC184 digested with BamH1. E. coli DH10 transformants were selected on Mueller–Hinton agar supplemented with 1 and 4 mg/L of imipenem or 2 and 8 mg/L of aztreonam. The inserted DNA fragments were sequenced on both strands.

Nucleotide accession number

The nucleotide sequence of the integron described in this paper has been submitted to EMBL-GenBank under accession no. AJ870988.

Results and discussion

Description of the outbreak and clonal relatedness of the isolates

The outbreak of imipenem-resistant K. pneumoniae occurred in the surgical centre during a 6 month period. The index case (Patient M) was a patient transferred from Greece for fulminant hepatitis and the strain of K. pneumoniae was detected at admission. This patient was carrying the multiresistant strain in his digestive flora and was not infected. The outbreak included the spread of the strain to seven other patients (Table 1). The last patient (K) was detected after discharge during the follow-up.

PFGE analysis identified one major profile with three subtypes (Ia, Ib and Ic) (Figure 1). The Ic subtype (patients V and S) showed four banding differences. Comparison of the PFGE profiles with those three strains isolated in Greece indicated that our epidemic strain was identical with the strain K5 reported in Athens teaching hospitals.3

Antibiotic susceptibility and synergy testing and identification of β-lactamase

Identification performed by using the API20E gave identical results except for isolates recovered from two patients (S and V) that were lysine-decarboxylase negative. These two isolates corresponded to the subtype Ic in PFGE. All isolates were highly resistant to β-lactams (>64 mg/L), including aztreonam, and resistant to imipenem (MICs ≥32 mg/L). All isolates were positive by the EDTA disc synergy test indicating the presence of an MBL.

PCR using the primers VIM-1 up and VIM-1 low yielded a 261 bp amplification product that suggested the presence of a blaVIM gene, which was confirmed by sequencing to encode VIM-1 MBL. The PCR-based screening for ESBL revealed the presence of a SHV-type β-lactamase, identified by sequencing as SHV-5. Luzzaro et al.11 identified both SHV-12 and VIM-4 in two strains of Enterobacter cloacae. Scoulia et al.12 reported the spread of E. coli strains producing both VIM-1 and a CTX-M-type β-lactamase and recently Galani et al.13 identified the presence of the β-lactamases VIM-2 and IBC-1 in a strain of E. coli. Antimicrobrial phenotype to other antibiotics showed resistance to almost all aminoglycosides (MICs for gentamicin of 8 mg/L, newer quinolones (MICs of ciprofloxacin ≥128 mg/L) and the combination of sulfamethoxazole and trimethoprim. Isolates showed an intermediate resistance level to tetracycline with MICs of 16 mg/L.
Transfer of β-lactam resistance and plasmid analysis

Transfer by conjugation was achieved for only one isolate (Patient F) on selective agar containing aztreonam, and the E. coli J53 transconjugant displayed a typical ESBL phenotype. As reported for VIM-1 and VIM-2 β-lactamases, imipenem resistance was not transferred by conjugation suggesting that the corresponding gene is not located on a transferable plasmid. For the other isolates the transfer was obtained by electroporation. E. coli DH10B electroporants selected on imipenem showed an MBL phenotype (e.g. resistant to all β-lactams with the exception of aztreonam) while those selected on aztreonam corresponded to the ESBL phenotype.

Plasmid analysis showed that all isolates harboured three plasmids of estimated sizes of 100, 130 and >150 kb. The analysis of the plasmid contents of E. coli DH10B electroporants expressing the MBL or those expressing the ESBL in comparison with parental strains indicated that the two β-lactamases were encoded by two

Table 1. Characteristics of patients colonized and/or infected with imipenem-resistant K. pneumoniae

<table>
<thead>
<tr>
<th>Patients</th>
<th>Date of admission</th>
<th>Clinical events</th>
<th>First isolate: date and origin</th>
<th>Ward of suspected acquisition</th>
<th>PFGE profile</th>
<th>ATM</th>
<th>ATM + CLA</th>
<th>ATM + TZB</th>
<th>IMP</th>
<th>IMP + CLA</th>
<th>IMP + EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>12.02.03</td>
<td>Liver transplantation</td>
<td>12.02.03 Rectal swab</td>
<td>ICU (index case transferred from Greece)</td>
<td>Ia</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>32</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>02.02.04</td>
<td>Liver transplantation</td>
<td>02.15.04 Blood culture</td>
<td>Surgical ward</td>
<td>Ia</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>1.5</td>
</tr>
<tr>
<td>L1</td>
<td>09.17.03</td>
<td>Liver transplantation</td>
<td>03.11.04 Tracheal fluid</td>
<td>Surgical ward</td>
<td>Ia</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>1.5</td>
</tr>
<tr>
<td>G</td>
<td>02.12.04</td>
<td>Liver transplantation</td>
<td>04.01.04 Urine culture</td>
<td>ICU</td>
<td>Ia</td>
<td>&gt;128</td>
<td>128</td>
<td>&gt;128</td>
<td>32</td>
<td>32</td>
<td>1.5</td>
</tr>
<tr>
<td>V</td>
<td>04.18.04</td>
<td>Liver cirrhosis</td>
<td>05.19.04 Urine culture</td>
<td>ICU</td>
<td>Ic</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>32</td>
<td>32</td>
<td>1.5</td>
</tr>
<tr>
<td>S</td>
<td>01.06.04</td>
<td>Liver transplantation</td>
<td>05.28.04 Blood culture</td>
<td>ICU</td>
<td>Ic</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>32</td>
<td>32</td>
<td>1.5</td>
</tr>
<tr>
<td>L2</td>
<td>03.08.04</td>
<td>Liver transplantation</td>
<td>06.17.04 Rectal swab</td>
<td>ICU</td>
<td>Ia</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>1.5</td>
</tr>
<tr>
<td>K</td>
<td>01.12.04</td>
<td>Liver transplantation</td>
<td>08.06.04 Rectal swab</td>
<td>Surgical ward</td>
<td>Ia</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;32</td>
<td>32</td>
<td>1.5</td>
</tr>
</tbody>
</table>

ATM, aztreonam; IPM, imipenem; CLA, clavulanic acid at 2 mg/L; TZB, tazobactam at 4 mg/L.

\(^a\)MICs of IMP + EDTA determined by the Etest procedure.

\(^b\)Patient detected in August during the follow up.

Figure 1. PFGE. Lanes 1–3, PFGE profiles of non-related strains of K. pneumoniae; lane 4, strain K1 from Greece; lane 5, strain K5 from Greece; lane 6, strain K8 from Greece; lane 7, isolate M; lane 8, isolate F; lane 9, isolate L1; lane 10, isolate G; lane 11, isolate V; lane 12, isolate S; lane 13, isolate L2; lanes 14, isolate K.
different plasmids. PCR using primers specific for VIM and SHV genes confirmed on each electroporant confirmed these results. The $bla_{VIM}$ gene was present on the larger plasmid (>150 kb) and restriction patterns generated with EcoRI fingerprinting confirmed that VIM-1-encoding plasmids were identical for all isolates.

**Analysis of the class I integron**

Cloning of genomic DNA fragments allowed the isolation of two types of clones, one able to grow on imipenem (1 or 2 mg/L) and the other able to grow on aztreonam (2 or 4 mg/L). The respective phenotypes were consistent with an insert coding for the VIM enzyme in the first clone and an ESBL for the second clone. These results were confirmed by amplification.

Nucleotide sequence analysis of an ~4.5 kb clone carrying the MBL determinant revealed that the $bla_{VIM}$ is part of a class I integron. DNA sequencing showed a gene cassette array that included the $bla_{VIM}$ determinant, a 6′N-aminoglycoside acetyltransferase $aac(6′)-Ib$ gene cassette, the $dhfr$I determinant and another aminoglycoside modifying gene, the adenyltransferase $aadA1$. This structure was preceded by an integrase I gene. Similar cassettes were first reported in *P. aeruginosa*1 and more recently in *E. coli*.3,14

In conclusion, we report the first outbreak of *K. pneumoniae* strains producing both VIM-1 and SHV-5 in France. The strain was imported from Greece. Genetic analysis confirmed that the epidemic strain is closely related to one of the epidemic strains isolated in Athens teaching hospitals and that the $bla_{VIM-1}$ gene is part of a class I integron similar to those reported in isolates of Enterobacteriaceae in Greece.3,14

This study highlights the potential risk of spread of multiresistant bacteria with international transfer of patients and confirms the necessity of systematic screening of these patients at admission.

**Acknowledgements**

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**Transparency declarations**

No declarations were made by the authors of this paper.

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**References**