Recurrent healthcare-associated community-onset infections due to *Klebsiella pneumoniae* producing VIM-1 metallo-β-lactamase

Aggeliki Poulou, Nicholas Spanakis, Spyros Pournaras, Vassiliki Pitiriga, Kyriaki Ranellou, Fani Markou, and Athanassios Tsakris

1Department of Microbiology, Serres General Hospital, Serres, Greece; 2Department of Microbiology, Medical School, University of Athens, Athens, Greece; 3Department of Microbiology, Medical School, University of Thessaly, Larissa, Greece

*Corresponding author. Tel: +30-210-7462011; Fax: +30-210-7462210; E-mail: atsakris@med.uoa.gr

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Objectives: To investigate the extrahospital dissemination of carbapenem-resistant *Klebsiella pneumoniae* isolates and the mechanisms of acquired resistance.

Methods: Patients who were referred to the outpatient department of Serres General Hospital with community-onset infections due to carbapenem-resistant *K. pneumoniae* isolates during August 2007–October 2008 were included in the study. The selected isolates were tested by determination of agar dilution MICs, phenotypic carbapenemase testing and PFGE. PCR and sequencing analyses were employed for identification of *bla* genes and mapping of the integron carrying the metallo-β-lactamase (MBL) gene. The location of the MBL allele was investigated by mating experiments, plasmid analysis and PCR assays.

Results: Twenty-four carbapenem-resistant *K. pneumoniae* isolates causing urinary tract infections were recovered from 12 outpatients. Six of the patients presented with recurrent infections within a period of 1–6 months after the initial extrahospital isolation. All patients reported prior hospitalization within the preceding 4 months, whilst two were infected by carbapenem-resistant *K. pneumoniae* isolates during their previous hospitalization. Imipenem, meropenem and ertapenem MICs ranged from 8 to 64 mg/L, 4 to 32 mg/L and 8 to 128 mg/L, respectively. All studied isolates as well as those obtained from prior hospitalization belonged to a single PFGE clone. They harboured a plasmid-mediated *bla*VIM-1 gene in an integron structure that has been previously described among *K. pneumoniae* isolates causing hospital-acquired infections in Greece.

Conclusions: This is the first study to document the dissemination of an MBL-producing *K. pneumoniae* strain in the community. The successful strain caused recurrent community-onset infections and was most likely acquired during patients’ previous hospitalization.

Keywords: class B carbapenemases, integrons, extrahospital, combined-disc test, EDTA

Introduction

Acquired metallo-β-lactamases (MBLs) are zinc-dependent enzymes that have been of growing concern over the last decade, because of their capacity to readily hydrolyse most of the β-lactam antibiotics, including carbapenems, and their increasing dissemination among Gram-negative pathogens. MBL genes are usually embedded within class 1 integrons with various compositions of gene cassettes, which may be located in the chromosome or the plasmidic DNA. The increase in MBL acquisition in Gram-negative organisms can be attributed to the propagation of IMP- and VIM-type MBLs, signifying a large reservoir of the respective gene cassettes.

During the last few years the incidence of infections caused by MBL-producing Enterobacteriaceae has been significantly increased by means of several intrahospital outbreaks reported mainly in Southern Europe. In Greece, VIM-type MBL-producing *Klebsiella pneumoniae* isolates constitute one of the broadest categories of multidrug-resistant *K. pneumoniae* isolates. Furthermore, relatively large interhospital outbreaks due to the spread of single or multiple VIM-1-producing *K. pneumoniae* clones have been recently investigated and reported in Greek studies. In all cases *bla*VIM-1 was located on plasmids of various sizes (50–150 kb) and was part of the variable region of class 1 integrons that also included gene cassettes conferring resistance to aminoglycosides and trimethoprim.
However, contrary to the well-established dissemination in the community of multidrug-resistant K. pneumoniae strains, such as extended-spectrum β-lactamase (ESBL) producers,9–11 the emergence of community-onset infections caused by MBL-producing K. pneumoniae has not been reported in Greece or other regions worldwide. Moreover, no information exists regarding the epidemiological aspects of these infections outside hospitals.

Based on antimicrobial susceptibility data from the outpatient department of Serres General Hospital, a number of community-onset infections were due to carbapenem-resistant, phenotypically MBL-positive K. pneumoniae isolates. This prompted the design of the present prospective study in which we describe the first reported extrahospital propagation of MBL-producing K. pneumoniae isolates causing community-onset infections.

**Methods**

**Patient data and definitions**

Patients who were referred to the outpatient department of Serres General Hospital with community-onset infections due to carbapenem-resistant K. pneumoniae isolates during a 15 month period (August 2007–October 2008) were included in the study. The specific hospital is a 400 bed acute care hospital serving a population of ~200 000 inhabitants. Patients with community-onset infection due to carbapenem-resistant K. pneumoniae isolates were defined as those who were referred from the community to the outpatient department of the hospital for assessment and had symptomatic infection, with clinically significant isolation of imipenem-non-susceptible (MIC > 4 mg/L) and/or ertapenem-non-susceptible (MIC > 2 mg/L) K. pneumoniae, from a specimen taken in the outpatient department. The community-onset infection was defined as healthcare associated if the patient fulfilled any of the previously proposed criteria.12 Recurrent urinary tract infection was defined as repeated infection of the kidneys or bladder, with at least 2 weeks follow-up monitoring after cessation of antimicrobial therapy.13 Demographic characteristics of the patients, medical history in terms of underlying diseases and hospitalization or surgery interventions in the preceding year, permanent urinary catheter usage and previous antibiotic consumption were recorded.

**Identification of bacterial isolates and susceptibility testing**

Identification and initial susceptibility testing were performed using the Microscan system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Identification was confirmed using the API 20NE system (bioMérieux, Marcy l’Étoile, France).

The agar dilution method was applied in order to confirm the preliminary susceptibility to β-lactams (aztreonam, cefepime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin/tazobactam), aminoglycosides (amikacin, gentamicin, tobramycin), colistin, tigecycline and ciprofloxacin. The Escherichia coli ATCC 25922 reference strain was used as a control and results were interpreted according to CLSI criteria.14 Among carbapenem-resistant isolates, phenotypic detection of MBL production was based on the performance of the combined-disc test with imipenem and EDTA15 and the Etest MBL assay (AB Biodisk, Solna, Sweden), while that of KPC carbapenemase production was based on the performance of the combined-disc test with meropenem and boronic acid.16

**PCR amplifications and sequence analysis**

The possible carriage of β-lactamase genes was tested by PCR using specific primers and amplification conditions for carbapenemase (bla_VIM, bla_SPA, bla_OXA), ESBL (blaTEM, blaSHV, blaCTX-M, blaGES/IBC) and plasmidic AmpC genes using consensus primers and amplification conditions.16–20

Integron mapping was performed by using PCR assays combining primers specific for 5′-CS and 3′-CS conserved sequences with primers specific for bla_OXA, daaC, dfrH1, aadA, qac and sul genes.21 PCR products were purified using ExoSAP-IT reagent (USB Corporation, Cleveland, OH, USA) and used as templates for nucleotide sequencing on both strands with an ABI Prism 377 DNA sequencer (Perkin-Elmer, Applied Biosystems, Foster City, CA, USA).

**Conjugation experiments and plasmid analysis**

Mating experiments were performed by using E. coli 26R793 (lac–rif) as the recipient strain. Transconjugants were selected on MacConkey agar plates containing 100 mg/L rifampicin and ertapenem at concentrations ranging from 0.25 to 1 mg/L. bla_VIM-bearing transconjugants were analysed for plasmids by an alkaline lysis procedure. The plasmidic DNA bands were extracted from the agarose gel and used as the template DNA in a PCR for the detection of the bla_VIM gene.

**PFGE analysis**

PFGE of XbaI-digested genomic DNA of the VIM-producing K. pneumoniae isolates was performed using a CHEF-DRII system (Bio-Rad, Hemel Hempstead, UK) and banding patterns were compared visually.22 The carbapenem-resistant K. pneumoniae isolates recovered from all patients including those recovered from recurrent episodes of community-onset infections were analysed. In addition, the isolates were genotypically compared with those that had been previously isolated in the wards of the hospital where the outpatients with community-onset infections were hospitalized. For comparison, three historical K. pneumoniae isolates from our hospital were included as controls.

**Results**

A total of 157 K. pneumoniae isolates were collected from patients attending the outpatient clinics during a 15 month period. Twenty-four of these isolates exhibited reduced susceptibility to both imipenem and ertapenem and were considered as carbapenem-resistant isolates. These 24 isolates were recovered from urine specimens of 12 patients who presented at the outpatient department of the hospital with symptoms and signs of urinary tract infection.

Susceptibility testing of the 24 isolates showed that MICs of imipenem ranged from 8 to 64 mg/L, while MICs of meropenem were 4–32 mg/L and MICs of ertapenem were 8–128 mg/L. All isolates exhibited resistance to amikacin (MICs 32–64 mg/L), tobramycin (MICs 16–32 mg/L), cefepime, cefetazidime and ciprofloxacin (MICs > 32 mg/L) and piperacillin/tazobactam (MICs 128–256 mg/L), while all were susceptible to aztreonam (MICs 0.5–2 mg/L), gentamicin (MICs 2–4 mg/L) and colistin (MICs 0.5–2 mg/L). All but one of the isolates were susceptible to tigecycline (MICs 0.5–16 mg/L).

The characteristics of patients with community-onset infections are presented in Table 1. The mean age of the 12 patients was 73 years (range 54–83 years) and all except one were male. According to the recorded medical history, all the patients had a
history of previous hospitalization or surgery intervention during the preceding 4 months and therefore their infections were considered to be healthcare-associated community-onset infections. For all 12 patients, the mean time period between prior hospitalization and attendance at the outpatient department with a community-onset urinary tract infection was 45.4 days (range 21–112 days). In 9 of the 12 patients with previous hospital admission, the first community-onset infection was documented within a period of 2 months after hospital discharge, whereas in the remaining 3 patients the first community-onset urinary tract infection was documented within 3–4 months after hospital discharge. It is of note that clinical records revealed that 2 of the 12 patients were infected with carbapenem-resistant K. pneumoniae isolates during their previous hospitalization.

Recurrent community-onset infections due to carbapenem-resistant K. pneumoniae were detected in six outpatients. These patients developed one to three recurrent community-onset infections during the study period. Patients who exhibited recurrent infections usually suffered from papillary transitional carcinoma or had renal lithiasis (Table 1). Among these patients, three developed the recurrent episodes of infection >2 weeks and within 1 month after treatment of the original extrahospital urinary tract infection, whereas in the remaining three the recurrent episodes occurred within a 4–6 month period after the original extrahospital infection.

During previous hospitalization or hospital discharge patients of the study had received several antimicrobials that included trimethoprim/sulfamethoxazole, ciprofloxacin and intravenously administered drugs (amikacin, aztreonam, ticarcillin/clavulanic acid, piperacillin/tazobactam). After the community-onset isolation of the carbapenem-resistant K. pneumoniae isolates, aetiological therapy included the administration of aztreonam alone or with either colistin or gentamicin, resulting in successful outcomes in six outpatients. The eradication of the carbapenem-resistant K. pneumoniae causing recurrent infections was accomplished after the administration of gentamicin alone or with aztreonam.

PFGE results showed that all 12 MBL-producing isolates causing the initial community-onset infections as well as the 12 MBL-producing isolates recovered from recurrent infections belonged to a single clonal type. In the same pulsotype belonged also the two carbapenem-resistant K. pneumoniae isolates that caused hospital-acquired infections during previous hospitalizations of outpatients (Figure 1). However, the three historical K. pneumoniae isolates belonged to different pulsotypes.

All carbapenem-resistant isolates in this study were phenotypically negative for KPC production but positive for MBL production using both the combined-disc test with EDTA and the Etest MBL assay. In accordance with the phenotypic results, PCR and sequencing analysis identified a blaVIM allele in all phenotypically MBL-positive isolates. PCR testing for other bla genes, including various carbapenemases and ESBL and plasmidic AmpC genes, was negative in all cases. PCR mapping exhibited carriage of the MBL gene in a class 1 integron similar to the structure described previously in Greek K. pneumoniae isolates causing hospital-acquired infections.7 More specifically, the variable region included cassettes containing blaVIM, aacA, dhfrI and aada genes. The sequencing of the overlapping PCR amplicons revealed that the class 1 integron contained the intI1 gene with a strong P1 promoter, followed directly by an inactivated (no GGG insertion) P2 promoter and an attI1 site. Subsequently was the blaVIM gene and downstream were aacA7, dhfrI and aadaI alleles prior to the conserved qacEAl/sulI elements.

Conjugation experiments were performed in 12 representative isolates and demonstrated that the MBL determinant could be easily transferred along with resistance to aminoglycosides. Plasmid analysis showed that all transconjugants contained an apparently identical single 110 kb plasmid. PCR on a gel-extracted plasmidic DNA band was positive for the blaVIM

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Table 1. Characteristics of outpatients infected with blaVIM-producing K. pneumoniae isolates

<table>
<thead>
<tr>
<th>Outpatient</th>
<th>Age/sex</th>
<th>No of community-onset UTIs</th>
<th>Days from previous hospitalization</th>
<th>Reason for previous hospitalization</th>
<th>Underlying disease/predisposing factor</th>
<th>Previous receipt of antibiotics&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83/M</td>
<td>1</td>
<td>34</td>
<td>papillary transitional carcinoma</td>
<td>PIICA</td>
<td>ATM, CIP</td>
</tr>
<tr>
<td>2</td>
<td>80/M</td>
<td>1</td>
<td>39</td>
<td>papillary transitional carcinoma</td>
<td>PIICA</td>
<td>CIP, TIM</td>
</tr>
<tr>
<td>3</td>
<td>73/M</td>
<td>1</td>
<td>25</td>
<td>prostatectomy, renal lithiasis</td>
<td>permanent urine catheter</td>
<td>TIM</td>
</tr>
<tr>
<td>4</td>
<td>71/M</td>
<td>2</td>
<td>21</td>
<td>prostatectomy</td>
<td>diabetes melitus</td>
<td>AMK, T2P</td>
</tr>
<tr>
<td>5</td>
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<td>1</td>
<td>82</td>
<td>prostatectomy</td>
<td></td>
<td>AMK, ATM, CIP</td>
</tr>
<tr>
<td>6</td>
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<td>2</td>
<td>40</td>
<td>prostatectomy</td>
<td></td>
<td>AMK, CIP</td>
</tr>
<tr>
<td>7</td>
<td>54/M</td>
<td>2</td>
<td>24</td>
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<td></td>
<td>SXT, T2P</td>
</tr>
<tr>
<td>8</td>
<td>75/M</td>
<td>1</td>
<td>22</td>
<td>prostatectomy</td>
<td></td>
<td>AMK, T2P</td>
</tr>
<tr>
<td>9</td>
<td>79/F</td>
<td>4</td>
<td>37</td>
<td>papillary transitional carcinoma</td>
<td>PIICA, permanent urine catheter</td>
<td>T2P</td>
</tr>
<tr>
<td>10</td>
<td>75/M</td>
<td>1</td>
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<td>prostatectomy</td>
<td>permanent urine catheter</td>
<td>TIM</td>
</tr>
<tr>
<td>11</td>
<td>68/M</td>
<td>4</td>
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<td>renal lithiasis</td>
<td></td>
<td>CIP</td>
</tr>
<tr>
<td>12</td>
<td>75/M</td>
<td>4</td>
<td>76</td>
<td>papillary transitional carcinoma</td>
<td></td>
<td>CIP</td>
</tr>
</tbody>
</table>

<sup>a</sup>During the hospitalization and/or hospital discharge.

M, male; F, female; UTIs, urinary tract infections; PIICA, previous intravesical instillation of chemotherapeutic agents; AMK, amikacin; ATM, aztreonam; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; TIM, ticarcillin/clavulanic acid; T2P, piperacillin/tazobactam.
gene in all transconjugants, indicating that the gene resided in this transferable plasmid.

**Discussion**

In the last decade, there has been growing concern about the intrahospital and interhospital dissemination of MBL-producing Gram-negative bacteria, leading to outbreaks difficult to control. Particularly, *K. pneumoniae* is a nosocomial pathogen that is frequently implicated in hospital outbreaks due to MBL producers. However, the extrahospital propagation of this multidrug-resistant pathogen has not been previously detected. The present study demonstrates the first emergence of extrahospital infections due to MBL-producing *K. pneumoniae* in the community setting.

Our study cases consisted almost exclusively of males of advanced age, who, according to their medical history, had been admitted to the hospital with genitourinary pathology in the preceding 4 months. Therefore, all infections affecting these specific patients were characterized as healthcare-associated community-onset infections. Actually, considering that the mean time interval between the patients’ hospital discharge and the initial isolation of the VIM-producing *K. pneumoniae* was \(~45\) days, it becomes clear that the transmission of the VIM-producing *K. pneumoniae* clone occurred during their hospitalization. This presumption was further supported by typing results, which revealed that all *K. pneumoniae* isolates causing community-onset infections as well as those causing infection during the previous hospitalization of two patients were genetically indistinguishable. It is also of note that these pathogens commonly caused recurrent urinary tract infections in the community setting. These repeated episodes of urinary tract infection were defined as recurrent episodes (re-infection) because in all cases the interval from treatment of the original infection was >2 weeks and in some cases a negative urine culture was obtained after treatment. The successful cure of these episodes required the administration of gentamicin alone or in combination with aztreonam. The presence of renal lithiasis and the papillary transitional carcinoma of the urinary bladder were usually detected as adverse factors to antimicrobial effectiveness.

In our outpatient community, we have previously documented community-onset infections due to MBL-producing Gram-negative species. More specifically, transmission of acquired MBL-producing *Proteus mirabilis* and *Pseudomonas aeruginosa* isolates in the extrahospital setting of the same geographical area, causing healthcare-associated community-onset infections, has been described. It seems that in this particular hospital setting a high incidence of infections due to different MBL-producing bacterial species has emerged, facilitating in this way their extended dissemination in the community. However, recurrent community-onset *P. mirabilis* infections were not detected, in contrast to the present survey where VIM-1-producing *K. pneumoniae* isolates usually caused recurrent infections. Furthermore, outpatients having no previous exposure to healthcare facilities were not detected in the present report, whereas no evidence of bacteraemia was present in any of the patients, in contrast to the study that described community-onset MBL-producing *P. aeruginosa* infections. Additionally, the time duration of the recurrent episodes in MBL-producing *P. aeruginosa* infections was longer than that of *K. pneumoniae* infections, apparently due to the characteristic ability of *P. aeruginosa* to form biofilms and cause chronic infections.

Although an MBL-producing *K. pneumoniae* strain causing community-onset infections is described herein for the first time.
time, ESBL-producing K. pneumoniae have been widely identified as community pathogens causing urinary tract infections.9–11,26 These studies have investigated the incidence and molecular epidemiology of community infections due to ESBL-producing K. pneumoniae, illustrating their significant public health implications. Moreover, it should be mentioned that the findings of our study coincide with previous observations showing that previous hospitalization and antibiotic treatment as well as male gender over the age of 60 years are risk factors for community-onset urinary tract ESBL infections.27

In conclusion, the acquired knowledge of the present report is that patients during their hospital discharge might carry MBL producers in the intestinal compartment and this colonization can be associated with a high risk of developing community-onset MBL infection. It is also becoming apparent that the community spread of MBL-producing K. pneumoniae is a complex multifactorial problem of high public health significance. This is supported by studies showing that patients with community-onset ESBL infections and their household contacts clearly represent a bacterial reservoir that increases dispersal of resistance in healthy people.28 Urgent implementation of specific interventions at hospital and community levels are certainly required, considering that the potential control measures for such issues have a limited time window for effective action.

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Transparency declarations
None to declare.

References