Four Carbapenem-Resistant Gram-Negative Species Carrying Distinct Carbapenemases in a Single Patient

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Carbapenem-resistant Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, and Acinetobacter baumannii were isolated from a single patient, each producing different carbapenemases (NDM-1, KPC-2, IMP, and OXA-23, respectively). The NDM-1-producing E. coli strain was preceded by a clonally related carbapenem-susceptible strain a month earlier, suggesting in vivo acquisition of blaNDM-1.

CASE REPORT

A 46-year-old man was admitted to a hospital in Jiangxi Province in Eastern China with headache, nausea, and vomiting in June 2012. Cerebrospinal fluid (CSF) examination revealed an elevated protein level and pleocytosis, dominated mainly by mononuclear cells. Empirical antituberculous therapy was initiated for a probable diagnosis of tuberculous meningitis. However, progression of hydrocephalus was detected by computed tomography (CT) scan, which required placement of an external ventricular drain to control the CSF pressure. In a repeat CSF specimen, Cryptococcus neoformans was revealed by Indian ink staining and culture, and amphotericin B and flucytosine were initiated for the treatment of cryptococcal meningitis. Serology for HIV and immunological-autoimmune-rheumatological workup failed to reveal an evident underlying immunodeficiency or immune-related disorder. Imipenem (1 g every 12 h [q12h]) was used empirically for 1 month because of fever and leukocytosis. The patient was transferred to Huashan Hospital in Shanghai in September 2012 due to persistent hyperpyrexia and altered mental status.

After approximately 1 week of antituberculous (isoniazid, rifampin, ethambutol, and levofloxacin) and antifungal (fluconazole and flucytosine) treatment, the patient began to improve and his temperature returned to normal levels. Subsequently, extended-spectrum cephalosporin (ceftazidime, amikacin, and ciprofloxacin) and antifungal (itraconazole) were initiated, and carbapenem-resistant A. baumannii, carbapenem-resistant Klebsiella pneumoniae, and carbapenem-resistant Enterobacter aerogenes were isolated from his sputum. After 2 weeks, he developed fever again (40.2°C), and carbapenem-resistant K. pneumoniae was isolated from the CSF. Intravenous tigecycline (50 mg q12h) and cefoperazone-sulbactam (3 g q12h) was initially used for 1 week, and then cefoperazone-sulbactam was replaced by intravenous fosfomycin (8 g q12h) according to the antimicrobial susceptibility testing results and continued for three more weeks. His temperature returned to normal levels in late October. Along with continual isolation of extensively drug-resistant A. baumannii and carbapenem-resistant E. aerogenes from his sputum, a carbapenem-resistant E. coli strain was also identified in the sputum in early November. Although aggressive management was continued, the clinical status of the patient deteriorated. The patient developed a persistently high fever (approximately 39°C), became unconscious, and was eventually transferred to a local hospital for palliative care in the middle of November (Fig. 1). A ventilator was not used during the patient’s hospitalization.

During his 2-month stay at Huashan Hospital, a total of 34 bacterial isolates belonging to four Gram-negative species were identified in various culture specimens from the patient, 13 of which were available for this study, including three isolates each of E. coli (EC1, EC2, EC3), K. pneumoniae (KP1, KP2, KP3), and E. aerogenes (EA1, EA2, EA3) and four isolates of A. baumannii (AB1, AB2, AB3, AB4). All the available strains had been isolated from sputum samples except EC2 and KP1, which were from the urine and the CSF samples, respectively. These isolates were identified by the Vitek2 system (bioMérieux) in the Department of Clinical Microbiology.

Clonal relationships were analyzed using pulsed-field gel electrophoresis (PFGE) (1). Three K. pneumoniae isolates, three E. aerogenes isolates, and four A. baumannii isolates showed identical PFGE types and had the same susceptibility profile, indicating they likely represented the same strains. Almost all isolates were nonsusceptible to imipenem, ertapenem, piperacillin-tazobactam, ceftazidime, amikacin, and ciprofloxacin but remained susceptible to polymyxin B and tigecycline (Table 1). β-Lactamase and 16S rRNA methyltransferase genes were identified by PCR amplification and sequencing (2–4). KP1 belonged to sequence type 11 (ST11) and carried blaKPC-2, blaDHA-1, and rmtB. EA1 carried blalmp-1, blalCTX-M-1, and armA. AB1 belonged to ST208 and harbored blalOXA-23, blalOXA-51, and armA. armA and rmtB are 16S rRNA methyltransferase genes encoding resistance to aminoglycosides. The carbapenem resistance could not be transferred from these three strains to E. coli J53 by conjugation experiments despite repeated attempts.

Of the 3 E. coli isolates collected, EC1 and EC3 were identified from sputum 1 month apart and showed the same sequence type (ST101) and the same PFGE type. On the other hand, EC2 isolated from urine was ST405 and showed a PFGE type different from
All 3 Escherichia coli isolates were resistant to piperacillin-tazobactam, ceftazidime, and ciprofloxacin while susceptible to polymyxin B and tigecycline. Both EC1 and EC3 were resistant to amikacin, and EC3 displayed resistance to carbapenems. The MICs of imipenem and ertapenem for EC3 were 8/16 μg/ml and 64/128 μg/ml, respectively. EC3 carried blaNDM-1, blaDHA-1, armA, and rmtB; EC2 harbored blaCTX-M-14; EC1 only had armA (Table 2). Plasmids of various sizes were observed in EC1 and EC3, none of which were shared. EC3 had two plasmid bands between chromosomal fragments and the 182-kb marker. EC1 had a 182-kb plasmid band and 1 smaller plasmid band. An approximately 180-kb IncA/C-IncFIA plasmid carrying blaNDM-1 could be transferred from EC3 to E. coli J53 by conjugation. The transconjugant carried blaNDM-1 and rmtB and showed resistance to carbapenems, piperacillin-tazobactam, ceftazidime, and amikacin and susceptibility to ciprofloxacin, polymyxin B, and tigecycline (Table 2).

**TABLE 1**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Carbenapenem</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IPM</td>
</tr>
<tr>
<td>KP1</td>
<td>KPC-2</td>
<td>11</td>
</tr>
<tr>
<td>EA1</td>
<td>IMP</td>
<td>NA</td>
</tr>
<tr>
<td>AB1</td>
<td>OXA-23</td>
<td>208</td>
</tr>
<tr>
<td>EC3</td>
<td>NDM-1</td>
<td>101</td>
</tr>
</tbody>
</table>

*KP, Klebsiella pneumoniae; EA, Enterobacter aerogenes; AB, Acinetobacter baumannii; EC, Escherichia coli.*

Carbapenems have been widely used for infections caused by multidrug-resistant bacteria. In the meantime, infections by carbapenem-resistant Gram-negative bacteria have become common in health care institutions (5). At our hospital, the incidence of carbapenem-resistant Enterobacteriaceae (CRE) increased rapidly since the first CRE (E. coli) isolate had been recovered from the urine sample of a patient in March 2002. In a recent study, Hu et al. also revealed that blaKPC-2 and blaIMP-positive strains accounted for 84.4% and 5.2% of CRE isolated from our hospital from April 2009 to January 2012, respectively (6). Here, we reported an unusual case with the identification of carbapenem-resistant E. coli, K. pneumoniae, E. aerogenes, and A. baumannii from a single patient, and each species produced different carbapenemases (NDM-1, KPC-2, IMP, and OXA-23).

Mechanisms of acquisition of carbapenem-resistant Gram-negative bacteria include patient-to-patient spread and transfer of carbapenemase genes between different Gram-negative bacterial species within an individual (7). The transfer of genetic elements conferring antibiotic resistance might play an important role in the association between cocolonization and multidrug resistance. Sidjabat et al. identified KPC-3 β-lactamases from 3 species of Enterobacteriaceae recovered from the same patient over a 5-month period (8). In this case, we also observed that carbapenem resistance could be transferred easily from EC3 to E. coli J53.

Most blaNDM-1, blaKPC-2, and blaOXA-23 resistance genes were identified to be located on a plasmid in CRE, whereas blaOXA-23 can be both chromosome and plasmid mediated and has been found almost only in A. baumannii (9). It is well known that certain plasmids can be stably maintained in limited similar bacterial species, while others are able to replicate in a broader host range.

**FIG 1** The timeline of bacterial isolation and antibiotic usage. All the preservation isolates were identified from sputum samples, except an E. coli isolate from the urine on 11 October and a K. pneumoniae isolate from the CSF sample on 12 October.
To our knowledge, this is the first case where four different carbapenem-producing Gram-negative bacterial species in a single patient were identified in four different Gram-negative species recovered from a single patient. Drieux et al. have described the cocarcocolonization of four carbapenem-resistant strains producing different carbapenemases. The unusual transfer of the carbapenem resistance may attribute to the incompatibility of the resistance plasmids. Thus, the unlikelihood of the transfer of the carbapenem resistance may attribute to the incompatibility of the resistance plasmids. Drieux et al. have described the cocarcocolonization of four carbapenem-resistant strains producing different carbapenemases. Drieux et al. have described the cocarcocolonization of four carbapenem-resistant strains producing different carbapenemases.

Different levels of carbapenem MICs were observed among E. coli isolates of the same PFGE type in the present case. In contrast to clonal expansion, horizontal gene transfer via plasmids appears to be the dominant mechanism of the global dissemination of NDM-1. We suspected that the EC3 strain might have emerged by in vivo transfer of blaNDM-1 from an unknown species to the recipient EC1. This hypothesis was supported by successful conjugation experiment. Different plasmid profiles of the two strains with identical PFGE types suggest that restructuring of the plasmids may have occurred during the transfer of blaNDM-1.

In conclusion, our study provides new information about the coexistence of carbapenem-resistant Gram-negative bacilli in clinical settings. This finding poses a major challenge in formulating antimicrobial treatment plans.

ACKNOWLEDGMENTS

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REFERENCES


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TABLE 2 Antimicrobial susceptibilities of 3 clinical isolates of E. coli and one transconjugant of EC3

<table>
<thead>
<tr>
<th>Isolate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isolation date (yr/mo/day)</th>
<th>Source</th>
<th>ST</th>
<th>Resistance gene(s)</th>
<th>MIC (&lt;sub&gt;b&lt;/sub&gt;g/ml)</th>
<th>ETP</th>
<th>CAZ</th>
<th>AMK</th>
<th>CIP</th>
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<tr>
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<td>2012/10/10</td>
<td>Sputum</td>
<td>101</td>
<td>armA</td>
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<td>64</td>
<td>&gt;128</td>
<td>&gt;128</td>
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<tr>
<td>EC2</td>
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<td>Urine</td>
<td>405</td>
<td>blac&lt;sub&gt;TX,M-14&lt;/sub&gt;</td>
<td>0.125</td>
<td>0.25</td>
<td>&gt;128</td>
<td>4</td>
<td>128</td>
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<tr>
<td>EC3</td>
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<td>Sputum</td>
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<td>blasnDM-1, blagmHA-1, armA, rmtB</td>
<td>8</td>
<td>64</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>64</td>
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<tr>
<td>TC of EC3</td>
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<td>NA</td>
<td>blasnDM-1, rmtB</td>
<td>2</td>
<td>4</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>0.125</td>
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