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**Plasmid-encoded OXA-48 carbapenemase in *Escherichia coli* from Israel**

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**Keywords:** imipenem resistance, oxacillinases, Tn1999, plasmids, class D β-lactamases

Sir,

Carbapenem resistance among Enterobacteriaceae in Israel emerged in 2004 and was observed mainly in *Klebsiella pneumoniae*, but also in *Enterobacter* species and *Escherichia coli*. Since its emergence, carbapenem resistance in these species, both in clinical and colonizing isolates, has been rendered by the production of plasmid-mediated *K. pneumoniae* carbapenemase (KPC).

In late 2007, a woman in her early thirties previously diagnosed with acute lymphoblastic leukaemia, was admitted to the hematology ward in Tel Aviv Sourasky Medical Center. The patient had arrived in Israel from Jordan in order to undergo chemotherapy and, later, bone marrow transplantation. During the first month after transplantation the patient was intermittently febrile and was treated with various antimicrobials, including piperacillin/tazobactam, amikacin, ciprofloxacin, vancomycin, imipenem and voriczam. Two months after admission, while being treated with imipenem and voriconazole, the patient suffered from fever, dyspnoea and renal failure, and was transferred to the intensive care unit. During that period a carbapenem-resistant *E. coli* strain (*E. coli* 1736) was isolated from a Hickman catheter, leading to removal of the catheter and further treatment with ceftazidime and colistin. Treatment with these antibacterial agents cleared the OXA-48-producing *E. coli*, yet unfortunately the patient died 3 months later from a systemic Pseudomonas infection.

*E. coli* 1736 was multidrug resistant, showing resistance to penicillins, piperacillin/tazobactam, aminoglycosides, quinolones and carbapenems, but susceptibility to all cephalosporins, aminopenicillins, tigecycline and colistin (Table 1). Analytical isoelectric focusing (IEF) performed on crude enzyme preparations revealed the presence of two β-lactamases with pIs of 5.4 and 7.2 (data not shown). PCR screening for the presence of β-lactamases in *E. coli* 1736 indicated the presence of *bla* _TEM-1_ and *bla* _OXA-48_ genes corresponding to the pIs of the β-lactamases visualized by IEF. Plasmid analysis of *E. coli* 1736 revealed four plasmids, three of around ≤50 kb in size and a larger plasmid of around 100 kb. Plasmid DNA was purified and transformed into *E. coli* DH10B. Transformant colonies that were screened positive for *bla* _OXA-48_ by PCR harboured the 50 kb plasmid. Southern analysis of plasmid DNA derived from these transformants using a *bla* _OXA-48_-labelled probe demonstrated the presence of *bla* _OXA-48_ on the acquired 50 kb plasmid. Acquisition of this plasmid increased the MICs of imipenem, meropenem and ertapenem without conferring full resistance (Table 1).

PCR mapping of the genetic environment surrounding *bla* _OXA-48_ was performed in collaboration with the laboratory of Professor P. Nordmann (Hôpital de Bicêtre, Paris, France). *bla* _OXA-48_ was found to be located inside Tn1999, similar to the structure described for other enteric strains, such as the *E. coli* strain from Turkey and the *K. pneumoniae* strain from Lebanon.2

*E. coli* 1736 was not resistant to all β-lactam antibiotics as reported for other OXA-48-producing strains, yet it presented a high level of resistance to the commonly used carbapenems (MICs ≥16 mg/L), higher than usually seen in KPC-producing *E. coli* strains isolated in our hospital.3 These high carbapenem MICs suggested the presence of additional resistance mechanisms together with OXA-48 carbapenemase. Outer membrane protein (OMP) produced by *E. coli* 1736 was determined by PCR and sequencing of *ompA*, *ompC* and *ompF* genes. Further OMP analysis was performed by protein extraction and separation on Tris–Tricine gels using SDS-PAGE followed by mass spectrometry (performed in the Biological Mass Spectrometry Facility at the Weizmann Institute of Science). Both methods indicated the absence of at least one major porin, OmpC.

Until the isolation of *E. coli* 1736, carbapenem resistance in *E. coli* in our country was exclusively attributed to the Ambler class A carbapenemase KPC.3 This is the first identified Enterobacteriaceae isolate in our country possessing a carbapenem-hydrolysing oxacillinase. To seek the possible origin of this strain we performed multilocus sequence typing (MLST; http://www.pasteur.fr/recherche/genopole/PGP/MLST/EColi.html), which genotyped the strain as sequence type (ST) 2, an *E. coli* ST that has never been recorded previously in our

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K. pneumoniae was first reported in 2004 in or the plasmid was carried prior to hospitalization. OXA-48 was not detected in Enterobacteriaceae prior to and after this single report. Thus, although the patient was hospital-

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**Table 1.** Antibiotic susceptibility of *E. coli* 1736, *E. coli* transformant and *E. coli* recipient strain DH10B

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC for strain (mg/L)</th>
<th><em>E. coli</em> 1736</th>
<th><em>E. coli</em> transformant</th>
<th><em>E. coli</em> DH10B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.25</td>
<td>0.19</td>
<td>0.095</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.38</td>
<td>0.19</td>
<td>0.095</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&lt;4</td>
<td></td>
</tr>
<tr>
<td>Piperacillin/ tazobactam</td>
<td>64</td>
<td>64</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>8</td>
<td>1.5</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;4</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td></td>
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<tr>
<td>Levofloxacin</td>
<td>&gt;8</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
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<tr>
<td>Imipenem</td>
<td>16</td>
<td>1</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>16</td>
<td>0.5</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>Erlopetin</td>
<td>&gt;32</td>
<td>1</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Doripenem</td>
<td>4</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>0.38</td>
<td>0.047</td>
<td>0.047</td>
<td></td>
</tr>
</tbody>
</table>

*Susceptibility testing of all antibiotics was performed using Vitek-2; carbapenem MICs were additionally tested by agar dilution; MICs of imipenem, meropenem and erlopetin below 0.5 μg/mL and MICs of ceftriaxone, ceftazidime, tigecycline and colistin were determined by Etest.*

Since the emergence of carbapenem-resistant *K. pneumoniae* in Israel during 2006, an active surveillance programme was performed in high-risk patients newly admitted to our institution, and at the national level in long-term care facilities. *bla*OXA-48 was not detected in Enterobacteriaceae prior to and after this single report. Thus, although the patient was hospitalized in our hospital ~2 months prior to the isolation of the OXA-48-producing *E. coli* strain, it is most likely that the strain or the plasmid was carried prior to hospitalization.

Class D OXA-48 carbapenemase among Enterobacteriaceae was first reported in 2004 in *K. pneumoniae* from Turkey and is continuously spreading in the Mediterranean area, as well as in other countries in Europe. Recent reports in the Middle East region stress the urgent need for regional collaboration to confront the spread of resistance.

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We would like to thank the laboratory of Professor P. Nordmann, Hospital de Bicétre, Paris, France, for confirming the results on Tn1999.2.

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**Transparency declarations**

None to declare.

**References**


**Molecular characterization of high-level fluoroquinolone resistance in a clinical isolate of *Haemophilus parainfluenzae***

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**Keywords:** prostatitis, quinolones, QRDRs, quinolone resistance-determining regions

Sir, *Haemophilus parainfluenzae* is commonly implicated as a causative organism in respiratory tract infections. *H. parainfluenzae* has also been frequently associated with the genitourinary tract,