Detection of *Pseudomonas aeruginosa* isolates producing VEB-type extended-spectrum β-lactamases in the United Kingdom

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Objectives: The aim of this study was to investigate the presence of VEB enzymes among *Pseudomonas* spp. referred to the UK’s national reference laboratory and with phenotypic evidence of extended-spectrum β-lactamase (ESBL) production.

Methods: Antibiograms were analysed for *Pseudomonas* spp. referred from November 2003 to November 2007. Isolates with /C21 4-fold ceftazidime/clavulanate synergy were screened for *bla*VEB alleles. Genes encoding metallo-β-lactamases (*bla*MBL) were sought in isolates with positive imipenem/EDTA synergy tests. Selected PCR products were sequenced. PFGE of *Spe*I-digested genomic DNA was used to compare isolates.

Results: Forty-nine (3.7%) of 1338 *Pseudomonas* spp. were considered potential ESBL producers; 40 were recovered for molecular testing. *bla*VEB alleles were detected in 32 *Pseudomonas aeruginosa* isolates, comprising diverse PFGE types, from 12 UK hospitals and 1 in India. One UK centre referred 15 isolates with VEB-1 enzyme; these were serotype O15, representing a single PFGE-defined strain that also produced VIM-10 metallo-carbapenemase. This strain was resistant to all β-lactams, aminoglycosides and ciprofloxacin, remaining susceptible only to colistin (MICs ≤1 mg/L). Two other *P. aeruginosa* isolates co-producing both VEB and VIM enzymes were received from two other UK hospitals; one isolate represented inter-hospital spread of the O15 strain and the second was distinct.

Conclusions: VEB enzymes have not been reported previously in the UK, but were produced by 80% of *Pseudomonas* spp. with phenotypic evidence of ESBL production. They co-existed with VIM carbapenemases in two strains, with one responsible for a major hospital outbreak. The incidence of ESBLs may be underestimated in *Pseudomonas* because ESBL phenotypes can be masked by other β-lactam resistance mechanisms.

Keywords: ESBL, VIM metallo-carbapenemase, international clone, hospital outbreak

Introduction

The rise to dominance of CTX-M-type extended-spectrum β-lactamas (ESBLs) among members of the Enterobacteriaceae, particularly *Escherichia coli*, is one of the most dramatic shifts in resistance epidemiology of the early 21st century,1,2 supplementing the problems already caused by TEM- and SHV-derived ESBLs. In addition, a variety of ‘minor’ class A ESBLs have been described,3 with the VEB enzymes representing an increasingly seen group. VEB-1 enzyme was first characterized in an *E. coli* isolate from Vietnam in 1996, with four further sequence variants since detected in widely scattered countries,

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in members of the Enterobacteriaceae or in non-fermenting genera, particularly *Pseudomonas* spp.\(^1\)

Cephalosporin/clavulanate ESBL tests are widely agreed to have, in general, poor sensitivity for *Pseudomonas* spp. However, VEB enzymes are well inhibited by clavulanate, and significant reductions in ceftazidime MICs have been reported for VEB ESBL-producing *Pseudomonas* spp.\(^3,4\) For logistic reasons, the HPA’s Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) determines the susceptibilities of *Pseudomonas* spp. in parallel with Enterobacteriaceae by agar dilution, including on cephalosporin/clavulanate plates. We noted that some *Pseudomonas* isolates showed ceftazidime/clavulanate synergy in these tests and investigated the presence of bla_{VEB} genes among these.

**Materials and methods**

**Bacterial isolates**

Antibiograms were reviewed for all isolates of *Pseudomonas* spp. referred to ARMRL from November 2003 to November 2007. These had been determined by agar dilution methodology. MICs were interpreted using harmonized European Committee for Antimicrobial Susceptibility Testing (EUCAST)/BSAC (v.6) breakpoints. Isolates were suspected to be ESBL producers if they showed a ≥4-fold reduction in ceftazidime MIC in the presence of 4 mg/L clavulanate. These isolates were recovered from storage, either at −70 °C or at room temperature, for molecular investigation.

**Molecular investigations**

Isolates were screened by PCR for bla_{VEB} alleles with primers VEBcas-F and VEBcas-B.\(^5\) In addition, bla_{MBL} alleles (encoding enzymes of the IMP, VIM, SPM, GIM and SIM groups) were sought by multiplex PCR, as described previously,\(^6\) in isolates that also showed positive imipenem/EDTA synergy tests. Selected PCR products were sequenced using dye-terminator chemistry on a CEQ8000 Genetic Analyser (Beckman Coulter, High Wycombe, UK). Sequences were compared and aligned with reference sequences using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and CLUSTAL W (http://www.ebi.ac.uk/clustalw/).

PFGE of SphI-digested genomic DNA was employed to examine the relatedness of selected isolates, with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) used to determine relatedness between isolates.

**Results and discussion**

**MIC analysis and bla_{VEB} screening**

Ceftazidime and ceftazidime/clavulanic acid MICs were reviewed for 1338 *Pseudomonas* spp. isolates referred during the 4 year period. Almost half (630, 47%) were resistant to ceftazidime (MIC > 8 mg/L), which emphasizes the bias towards resistance among isolates submitted to the reference laboratory. For comparison, the BSAC bacteraemia surveillance programme detected ceftazidime resistance in only 1.5% of the *Pseudomonas* spp. isolates tested during 2006 (http://www.bsacsurv.org/mrsweb/bacteraemia).

Clavulanic acid reduced the ceftazidime MICs for 49 (3.7%) isolates by ≥4-fold, and these were considered to be potential ESBL producers; all were highly resistant to ceftazidime (MIC ≥ 64 mg/L). Forty of these potential ESBL producers were recovered for molecular testing, and a bla_{VEB} allele was detected in 32 (80%) of these, referred from 12 UK hospitals and from 1 in India. All of the VEB ESBL-producing isolates were confirmed to be *P. aeruginosa*.

**Recognition of a hospital outbreak: co-production of VEB ESBL and VIM metallo-carbapenemase**

Of the 32 *P. aeruginosa* isolates with VEB ESBLs, 15 were referred from one UK centre (hospital A). These isolates had been recovered from patients on an intensive care unit and were referred in 2003 (1 isolate), 2006 (3 isolates) and 2007 (11 isolates). All belonged to serotype O15 and represented a single PFGE-defined strain (Figure 1). These isolates were multiresistant to carbapenems and other β-lactams, aminoglycosides and ciprofloxacin, but remained susceptible to colistin (Table 1). They showed marked synergy between imipenem and EDTA (Table 1) and were positive for a bla_{VIM} allele in addition to bla_{VEB}. Sequencing of the PCR products from a representative of this strain identified bla_{VEB-1a} and bla_{VIM-10}. The bla_{VEB-1a} allele, which was originally associated with India,\(^7\) encodes classic VEB-1 ESBL, but has an amino acid substitution in the signal peptide.\(^8\) The VIM-10 metallo-carbapenemase is a variant that was originally identified in the UK (GenBank no. AY524989).

A review of records identified the likely ‘index case’ in hospital A’s outbreak. This patient had been transferred to the UK in May 2000 from a hospital in India. A *P. aeruginosa* isolate from this patient was subsequently recovered from storage and referred for testing. It also belonged to serotype O15, was indistinguishable from the outbreak strain by PFGE (Figure 1) and was positive for bla_{VIM}. However, it lacked bla_{VIM} and was susceptible to carbapenems (Table 1).

**Isolates from other hospitals**

Two serotype O15 *P. aeruginosa* isolates from other UK hospitals clustered by PFGE with the strain from hospital A (Figure 1). One of these, from hospital B, co-produced VEB and VIM enzymes and was linked with transfer of a patient from hospital A; the other, from hospital C, produced VEB enzyme only. The latter patient had been repatriated to the UK from Thailand in 2007 and had no known links with hospital A or B. Only one other *P. aeruginosa* isolate co-producing both VEB and VIM β-lactamases was detected (from hospital D). It represented a distinct strain (Figure 1), and a second isolate of this strain (from the same patient) produced only VEB ESBL. The remaining isolates, from eight UK laboratories and one in India, had bla_{VEB} alone, and there was no evidence of strain spread between patients or centres.

In summary, although VEB ESBLs have not been reported previously in the UK, they proved to be the predominant ESBLs among *Pseudomonas* spp., which showed clear phenotypic evidence of ESBL production, present in 80% (32/40) of such isolates. However, bla_{VEB} alleles were not detected in eight potential ESBL-producing *Pseudomonas* isolates, six of which showed ≥16-fold reduction of ceftazidime MICs by 4 mg/L clavulanate. Further work is required to define the ESBL genes responsible for these phenotypes.
We have demonstrated that VEB ESBLs are widespread in the UK, with producers referred from 12 different bacteriology laboratories. Worryingly, VEB ESBLs were found to co-exist with VIM metallo-carbapenemases in two strains of 

*P. aeruginosa*.

In this case, molecular data supported a scenario, in which (i) an O15 strain of *P. aeruginosa* producing VEB-1 ESBL was imported from India in 2000 to hospital A where (ii) it acquired VIM-10 metallo-carbapenemase from an unknown source and then spread to cause the outbreak. Our investigation further suggested that this serotype O15, VEB-positive *P. aeruginosa* strain may be widespread in the Far East, as a second representative (VIM-negative) was imported into the UK in 2007 from Thailand.

Although molecular screening is applied to all ceftazidime-resistant isolates, the incidence of VEB and other ESBLs will be underestimated in *Pseudomonas* spp., because ESBL phenotypes can be masked by the genus’ other β-lactam resistance mechanisms. However, such screening is an unrealistic proposition even in reference laboratories. Fortunately, the presence of ESBLs in *Pseudomonas* spp. rarely causes specific therapeutic dilemmas, and the genus seems not to be a major reservoir of ESBL genes for members of the Enterobacteriaceae, where CTX-M-type enzymes dominate.

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**Transparency declarations**

N. W. and D. M. L. have received research grants and speaking invites from various pharmaceutical companies. D. M. L. has a diversified share portfolio, including holdings in pharmaceutical companies.

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**Table 1. Susceptibilities of variants of the serotype O15 *P. aeruginosa* strain causing a hospital outbreak**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>VEB + VIM (n = 15)</th>
<th>VEB only (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>&gt;32</td>
<td>4</td>
</tr>
<tr>
<td>Imipenem/EDTA</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;32</td>
<td>1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>233</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ceftazidime/clavulnate</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>70</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>35</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Amikacin</td>
<td>53</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Colistin</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Geometric mean MICs for this variant.*
companies. None of these poses a conflict of interest with this work. Other authors: none to declare.

References