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2 ***Pseudomonas aeruginosa* ST357 with VEB ESBLs in the UK: relatedness and**
3 **resistance**

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21 ESBLs are uncommon in *Pseudomonas aeruginosa* in the United Kingdom (UK), but may be
22 frequent e.g. in parts of the Middle East [1]. The types of ESBL predominantly found in the
23 species are unusual, with VEB and PER often encountered, rather than the CTX-M, SHV, and
24 TEM variants that dominate in Enterobacteriaceae [1,2]

25 From 2006-16, the Antimicrobial Resistance and Healthcare Associated Infections
26 (AMRHA) Reference Unit confirmed 30 VEB-positive *P. aeruginosa* isolates by PCR. Seven
27 of these were from an outbreak in North West (NW) England, the early stages of which were
28 described previously [2]; the remaining 23 were reference service submissions selected for
29 *bla*_{VEB} PCR tests based on either: (i) a ceftazidime MIC ≥ 256 mg/L reduced to ≤ 32 mg/L by 2
30 mg/L clavulanate; or (ii) being from the same hospital as known positives and being resistant
31 to ceftazidime at ≥ 256 mg/L, irrespective of ceftazidime/clavulanate potentiation.

32 Despite their coming from 20 different hospitals we were struck that all 30 isolates
33 had the VNTR profile 13, 2, 1, 5, 2, 3, 6, 5, x, determined as previously described [3], where
34 'x' is variable. This profile corresponds to sequence type (ST) 357, a recognised 'high-risk *P.*
35 *aeruginosa* clone' for metallo β -lactamases (MBLs) [4]. These findings led us to screen 38
36 further ST357 isolates – referred for typing but not susceptibility testing – identifying an
37 additional 12 *bla*_{VEB} positives and giving a final working collection of 42 VEB-positive ST357
38 isolates from 36 patients at 26 hospitals (2006: one isolate, 2008: one isolate, 2010: four
39 isolates, 2012: seven isolates, 2013: ten isolates, 2014: seven isolates, 2015: eleven isolates,
40 2016: one isolate). Although we receive incomplete information on patients' origins and prior
41 travels, many isolates were likely imports to the UK: among 15 isolates from 13 patients
42 hospitalized in the London area 11 were from private hospitals with international clientele,
43 including many admissions from the Middle East. Seven isolates originated from patients
44 involved in a fire in Bucharest and transferred to different UK hospitals following prior
45 hospitalisation in Romania.

46 Fourteen isolates also had MBLs, as predicted from phenotypes and confirmed by
47 PCR; 13 of these, including seven from the NW outbreak, had *bla*_{VIM}; one had *bla*_{NDM}.
48 Irrespective of MBL co-production, the VEB-positive isolates were extremely resistant to
49 antipseudomonal agents, as determined by BSAC agar dilution with EUCAST breakpoints
50 (<http://www.eucast.org>). Ceftazidime MICs all were ≥ 256 mg/L, carbenicillin ≥ 1024 mg/L,
51 cefepime ≥ 64 mg/L and piperacillin/tazobactam ≥ 32 mg/L, indicating consistent high-level
52 resistance. All but one isolate were resistant to imipenem (41/42; 97.7%) whilst resistance to
53 meropenem was observed in 40/42 (95.3%). Resistance to ciprofloxacin (MICs ≥ 4 mg/L) was
54 universal and that to aminoglycosides was near universal (tobramycin MICs all ≥ 16 mg/L,
55 100% non-susceptible; amikacin MICs 8- ≥ 64 mg/L, 41/42 (97.7%) non-susceptible;
56 gentamicin MICs 4- ≥ 32 mg/L, 41/42 (97.7%) non-susceptible). All remained susceptible to
57 colistin (MICs ≤ 2 mg/L). Where MBLs were absent (28/42 isolates), carbapenem resistance
58 likely depended on loss of porin OprD, which is frequent in *P. aeruginosa*, although this was
59 not examined directly.

60 Despite their frequent ceftazidime/clavulanate synergy, all of the isolates were
61 resistant to ceftolozane/ tazobactam, with MICs >16 mg/L, regardless of MBL co-production;
62 it remains unclear whether this reflects poor penetration of *P. aeruginosa* by tazobactam or
63 tazobactam having little capacity to inactivate VEB enzymes. The behaviour of
64 ceftazidime/avibactam was more interesting. Avibactam evidently penetrates *P. aeruginosa*
65 as it potentiates ceftazidime against AmpC-derepressed strains, and reportedly can inhibit
66 extracted VEB β -lactamases [5]. Nevertheless, and despite avibactam being used at double
67 the concentration of clavulanate, resistance to ceftazidime/avibactam was observed in 95.3%
68 of the isolates (40/42), with MICs above the 8+4 mg/L breakpoint and mostly remained above
69 those of ceftazidime/clavulanate (Table 1).

70 From our experience, potentiation of ceftazidime by clavulanate coupled with
71 resistance to ceftolozane/tazobactam (MIC >16 mg/L) have good specificity as indicators of

72 VEB ESBLs in *P. aeruginosa*, but sensitivity is poor, with ceftazidime/clavulanate MICs for
73 11.9% of producers identified here remaining above our top concentration of 32+2 mg/L.

74 To date, the genome sequences of three ST357 *P. aeruginosa* isolates have been
75 published, including one with a VEB-1 enzyme from a bloodstream infection in India. More
76 generally, ST357 is well known as a 'high-risk clone' also for VIM and IMP MBLs [4]. These
77 carbapenemases, like VEB enzymes, are integron-borne, and ST357 may have a particular
78 ability to host these elements, We did not examine *bla*_{VEB} gene type or location in this study:
79 previous characterization of isolates from the NW outbreak mostly found *bla*_{VEB-1}, but with
80 *bla*_{VEB-9} in one representative [2]. The *bla*_{VEB} integron was chromosomally located in these
81 outbreak isolates but has also been recorded on plasmids from *P. aeruginosa* in other studies.

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83 Although VEB-positive *P. aeruginosa* remain uncommon in the UK, the present data
84 show that they are repeatedly being introduced, commonly belong to an internationally
85 successful lineage, and are extremely drug resistant - often remaining susceptible only to
86 colistin. At a time when international emphasis is on carbapenemase producers, they should
87 not be underestimated.

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92 **Transparency declaration**

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104 Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, Food
105 Standards Agency, GlaxoSmithKline Services Ltd, Henry Stewart Talks, IHMA Ltd, Kalidex
106 Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme Corp, Meiji Seika Pharmo
107 Co., Ltd, Mobidiag, Momentum Biosciences Ltd, Nordic Pharma Ltd, Norgine
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112 **References**

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114 [1] Al-Agamy MH, Jeannot K, El-Mahdy TS, Samaha HA, Shibl AM, Plésiat P *et al.* Diversity of
115 molecular mechanisms conferring carbapenem resistance to *Pseudomonas aeruginosa*
116 isolates from Saudi Arabia. *Can J Infect Dis Med Microbiol* 2016;2016: 4379686.

117

118 [2] Woodford N, Zhang J, Kaufmann ME, Yarde S, Tomas M, Faris C *et al.* Detection of
119 *Pseudomonas aeruginosa* isolates producing VEB-type extended-spectrum beta-lactamases
120 in the United Kingdom. *J Antimicrob Chemother* 2008;62: 1265-8.

121

122 [3] Turton JF, Turton SE, Yearwood L, Yarde S, Kaufmann ME, Pitt TL. Evaluation of a nine-
123 locus variable-number tandem-repeat scheme for typing of *Pseudomonas aeruginosa*. *Clin*
124 *Microbiol Infect* 2010;16: 1111-6.

125

- 126 [4] Wright LL, Turton JF, Livermore DM, Hopkins KL, Woodford N. Dominance of international
127 'high-risk clones' among metallo- β -lactamase-producing *Pseudomonas aeruginosa* in the UK.
128 J Antimicrob Chemother 2015;70: 103-10.
129
- 130 [5] Lahiri SD, Alm RA. Identification of novel VEB β -Lactamase enzymes and their impact on
131 avibactam inhibition. Antimicrob Agents Chemother 2016;60: 3183-6.

132 **Table 1.** Relationship of ceftazidime/clavulanate and ceftazidime/avibactam MICs for *P.*
 133 *aeruginosa* with *bla*_{VEB}

Ceftazidime/clavulanate (MIC mg/L)*	n	Ceftazidime/avibactam (MIC mg/L)	n	MBL enzymes (n)
4	1	2	1	
		16	4	
8	9	32	3	
		>32	2	
		16	3	VIM (1)
16	14	32	5	
		>32	6	VIM (2)
		8	1	
32	13	16	2	VIM (1)
		32	5	VIM (3)
		>32	5	VIM (3)
>32	5	32	1	VIM(1)
		>32	4	NDM(1), VIM (2)

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135 *MICs of unprotected ceftazidime were ≥ 256 mg/L in all cases

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