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

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

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

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

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

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

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

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

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

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

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Ethical approval: Not required

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

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

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