as well as recognition of relatively frequent detection of MPO mRNA in ALL blasts, which does not correlate consistently with immunophenotyping and cytochemical findings.^{1,6}

Advances in molecular techniques have resulted in better subclassification of acute leukaemias and improved diagnostic certainty.^{2,6} Several sequencing studies have demonstrated a large degree of genomic heterogeneity in MPAL as well as differences between MPAL subtypes (B/myeloid and T/myeloid), although notably only a small number of triphenotypic leukaemia were included in these studies.^{9,10} *MLLT10* (also known as *AF10*) fusions involving multiple partner genes, including *DDX3X*, have been reported in both paediatric and adult T-acute lymphoblastic leukaemia and MPAL.^{9,11–13} Similar to previous reports the fusion detected in our patient retains the octapeptide motif-leucine-zipper (OM-LZ) domain of *MLLT10* at its C-terminus, a critical region required for leukaemogenesis.¹⁴ Although the role of the *DDX3X* RNA helicase in *DDX3X-MLLT10* fusions is less well understood, these fusions have only been observed in males, suggesting that the absence of wild type *DDX3X* may also contribute to leukaemia development.

Our case illustrates the diagnostic, and therefore therapeutic, implications of correctly identifying and subsequently confirming the triphenotypic lineage using multiple modalities. It highlights that in spite of the diagnostic advances in recent years, namely molecular testing and improvements in multiparametric flow cytometry, there remains a need for integration of multiple techniques to confirm complex and poorly differentiated acute leukaemias. Additionally, this case adds to the existing body of literature for triphenotypic acute leukaemia as defined by the most recent diagnostic criteria, for which there are only scarce data available.

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Vancomycin variable *Enterococcus* (VVE), *E. faecium*, harbouring the vanA gene complex

Sir,

In this article we describe the isolation from a blood culture specimen of a vancomycin variable *Enterococcus* (VVE), *Enterococcus faecium*, harbouring the vanA gene complex and a methicillin resistant *Staphylococcus aureus* (MRSA). We highlight the importance of using molecular methods for detecting VVE from sterile sites.

VVE are *E. faecium* harbouring the vanA gene or vanB gene complex but being phenotypically vancomycin susceptible. VVE vanA was first described in 2011.¹ VVE can only be detected by molecular methods and cannot be cultured on selective vancomycin containing media. Different clones of VVE have caused nosocomial outbreaks.² This makes the detection of VVE important in clinical samples in order to ensure relevant antibiotic treatment and in screening samples to mitigate nosocomial spread. VVE detection is challenging; the clone is likely to be unrecognised, which facilitates further spread. VVE outbreaks through transmission in health care settings have been described in previous studies. Hammerum et al. describe a clonal shift in vanA *E. faecium* isolates from clinical samples obtained from patients in Denmark from 2015 to the first quarter (Q1) of 2019. They report in 2019 that VVE ST1421-CT1134 vanA *E. faecium* became the most dominant vanA *E. faecium* clone and has spread throughout Denmark.² In Australia in 2018 the Australian Group of Antimicrobial Resistance (AGAR) reported 10 VVE that harboured the

vanA gene and eight that harboured the *vanB* gene.³ Therefore, accurate and timely detection of VVE is imperative in preventing hospital outbreaks and providing effective antibiotic therapy in those with infections.

In the index case presented in this article the presence of the *vanA* gene within the blood culture specimen of this patient was confirmed by real time polymerase chain reaction (PCR)⁴ and by GeneXpert *vanA vanB* assay (Cepheid, USA). The concurrent presence of the methicillin resistant *Staphylococcus aureus* isolate was confirmed by PCR in detecting the *mecA* and *femA* genes.⁵ Testing of the patient rectal swabs on admission on chromogenic agar (ChromID VRE agar; bioMérieux, Australia), and for several months while hospitalised on screening, did not detect VRE. Phenotypic susceptibility tests were performed on both the VVE and MRSA isolates using Vitek2 XL and Etest (both bioMérieux) and results are shown in Table 1. Both isolates were susceptible to daptomycin, and the patient was treated with daptomycin. Subsequently the VVE was not isolated again from blood cultures or any rectal swabs; however, the MRSA persisted with further positive blood cultures, and the patient was switched to vancomycin after the daptomycin MIC level rose to 4 mg/L on the Vitek2 XL and 2 mg/L by Etest.

VRE resistance in *vanA* bearing enterococci isolates is typically mediated by the group of genes *vanR*, *vanS*, *vanH*, *vanA*, and *vanX*, which are usually carried on the Tn1546 transposon. The expression of these genes leads to replacement of the C-terminal D-Ala residue with D-Lac during cell wall synthesis, thus modifying the vancomycin binding target. The transposon is often located in plasmids, making it easy to transfer among enterococcal strains. The *vanA* transposon is composed of seven genes including *vanR* and *vanS* (which regulate the expression of vancomycin resistance); *vanH*, *vanA*, *vanX*, *vanY* (vancomycin resistance) and *vanZ* (teicoplanin resistance). Whole genome sequencing

analysis (MiSeq; Illumina, USA) of the VVE isolate isolated from this blood culture specimen revealed that this isolate contained *vanR*, *vanS*, *vanH* and *vanA* but did not contain *vanX*, *vanY* and *vanZ*. The isolate was multilocus sequenced and was shown to be ST1424. The genome analysis also revealed partial deletion of the *vanA* gene (83 bp deletion; 92% coverage). This isolate was phenotypically sensitive to vancomycin and teicoplanin. The vancomycin sensitivity may be explained by the partial deletion in the *vanA* gene or the absence of *vanX* and *vanY*, while the teicoplanin sensitivity may be explained by the absence of the *vanZ* gene (Fig. 1).

This bacteraemia case with VVE and MRSA raises several important questions, previously discussed by Downing *et al.*⁶

Should vancomycin be avoided in patients with serious VVE infections? A patient colonised with this organism was recently described, and it was shown that the isolate developed resistance to vancomycin *in vivo* during vancomycin therapy.⁷ It has been reported that exposure of VVE strains to vancomycin *in vitro* leads to the development of vancomycin resistance by the constitutive expression of *vanHAXYZ* gene clusters.⁸ The use of vancomycin to treat VVE infections could provide selective pressure that may lead to the development of therapy induced resistance and treatment failure. Therefore, it would be sensible to avoid the administration of vancomycin to patients infected with VVE strains.

Given the introduction of real time PCR in many laboratories, should molecular testing be the standard of care for identifying VVE from all sterile sites as recommended by AGAR (Australian Group Antimicrobial Resistance)? Our laboratory performs *vanA/vanB* PCR testing of any *Enterococcus* isolates obtained from a sterile site. Further surveillance data such as those supplied by AGAR are needed to better understand the prevalence of VVE in clinical specimens. If VVE becomes an established nosocomial pathogen,

Table 1 Susceptibility profiles (MICs mg/L) of *Enterococcus faecium* harbouring the *vanA* gene complex (VVE) and MRSA isolated from the bacteraemia

Isolate	Vitek2 XL						Etest		
	Antibiotics (MICs)								
	PEN	AMP	VAN	TEIC	LZD	DAP	VAN	DAP	TEIC
VVE	≥64	≥32	≤0.5	1	1	NT	1	2	1
MRSA	>0.5	NT	≤0.5	≤0.5	2	1	1	0.5	1

AMP, ampicillin; DAP, daptomycin; LZD, linezolid; MIC, minimum inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; NT, not tested; PEN, penicillin; TEC, teicoplanin; VAN, vancomycin; VVE, vancomycin variable enterococci.
Vitek 2 XL cards used staphylococci card AST-P656, enterococci card AST-P643.

Fig. 1 Schematic diagram of the *vanA* transposon showing partial deletion of the *VanX*, *VanY* and *VanZ* regions.

appropriate routine genotypic testing of *E. faecium* clinical isolates is recommended, particularly those from sterile sites, to determine the presence of vancomycin resistance genes.

Should we worry about the colonisation of VVE since the routine non-molecular VRE screening methods do not reliably identify VVE and did not detect the index case summarised here? A large hospital outbreak due to VVE has been previously described in Ontario⁹ and Denmark,² thus highlighting that VVE is able to spread within the community and health care facilities. Further research is required to determine the sensitivity of various selective agars with and without vancomycin in detecting VVE and whether this strategy in combination with molecular testing is cost-effective.

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Genomic sequencing of hypervirulent *Klebsiella pneumoniae* with novel patterns of virulence and global epidemiological linkage

Sir,

Klebsiella pneumoniae is an increasingly important cause of infections in humans due to its capability to acquire a range of virulence factors and antibiotic resistance genes.^{1,2} Many infections occur in hospitalised patients with impaired host defences usually as a consequence of immunodeficiency or chronic comorbidity.^{1,2}

Hypervirulent *K. pneumoniae* have also emerged, particularly in Asia, and can cause invasive infections in healthy hosts.^{1,2} Clinical disease is typically characterised by primary hepatic abscesses, with complications of endophthalmitis, meningitis, and further metastatic abscesses.^{1,2} Hypervirulent variants often demonstrate a hypermucoid phenotype, as determined by the ‘string test’.^{1,2}

Key genotypic factors associated with increased virulence include: mucoid phenotype genes *rmpA/A2*; iron acquisition systems salmochelin, aerobactin, yersiniabactin, and the genotoxin colibactin. These virulence factors are carried by mobile genetic elements. *Klebsiella pneumoniae* isolates usually harbour a subset, or none, of the genes encoding for these virulence factors. Strains carrying a full hand of virulence factors have rarely been reported to cause prostatic abscesses,^{1,2} and usually only in the context of metastatic disease with additional risk factors such as diabetes mellitus.¹ Men who have sex with men (MSM) have not been reported to be at increased risk.

A previously well, 23-year-old man of Chinese ancestry, presented to a hospital in Melbourne, Australia, with three days of fevers, dysuria, and purulent urethral exudate culminating in urinary retention on the day of presentation. The patient had been in a stable sexually active relationship with a male partner for the previous 6 months. The patient practised only receptive anal intercourse without regular condom use. Three months prior to presentation, he had travelled to Shanghai, China to visit friends and relatives. He reported no sexual contact while overseas.

On presentation, the patient was febrile at 38.6°C, tachycardic at 136 beats/min, tachypnoeic at 24 breaths/min, and had a blood pressure of 132/83 mmHg. Examination noted purulent urethral exudate, lower abdominal pain without peritonism, and a tender prostate. The remainder of the examination was unremarkable.

The peripheral leukocyte count was $9.2 \times 10^9/L$ (normal $4.0–12.0 \times 10^9/L$) and a C reactive protein (CRP) was 69.3 mg/L (normal <5.0 mg/L). Renal function was normal, and liver function testing revealed a mild transaminitis with an