3. Slavin M, van Hal S, Sorrell TC, *et al*. Invasive infections due to filamentous fungi other than Aspergillus: epidemiology and determinants of mortality. *Clin Microbiol Infect* 2015; 21. 490.e1−10.
4. Ong A, Blyth CC, Bency R, *et al*. Fatal mycotic aneurysms due to *Scedosporium* and *Pseudallecheria* infection. *J Clin Microbiol* 2011; 49: 2067−71.
5. Thomson S, Alibhai K, Winkelaar G, *et al*. Case report of vertebral osteomyelitis and mycotic abdominal aortic aneurysm caused by *Scedosporium apiospermum* in a lung transplant patient with cystic fibrosis. *Transplant Proc* 2015; 47: 204−9.
6. Blasco-Lucas A, Reyes-Juarez JL, Nazarena M, *et al*. Aortic arch mycotic aneurysm due to *Scedosporium Apiospermum* reconstructed with homografts. *Ann Thorac Surg* 2015; 99: 2218−20.
7. Ogawa Y, Sato M, Tashiro M, *et al*. Rapid development of a mycotic aneurysm of the intracranial artery secondary to Scedosporium apiospermum sinusitis. *Med Mycol Case Rep* 2016; 14: 30−2.
8. Rodriguez-Tudela JL, Berenguer J, Guarro J, *et al*. Epidemiology and outcome of Scedosporium prolificans infection, a review of 162 cases. *Med Mycol* 2009; 47: 359−70.
9. Douglas AP, Chen SCA, Slavin MA. Emerging infections caused by non-Aspergillus filamentous fungi. *Clin Microbiol Infect* 2016; 22: 670−80.
10. Gil-Lamaignere C, Winn RM, Simitsopoulou M, *et al*. Interferon gamma and granulocyte-macrophage colony-stimulating factor augment the antifungal activity of human polymorphonuclear leukocytes against Scedosoporium spp.: comparison with Aspergillus spp. *Med Mycol* 2005; 43: 253−60.
11. Goldman C, Akiyama MJ, Torres J, *et al*. *Scedosporium apiospermum* infections and the role of combination antifungal therapy and GM-CSF: a case report and review of the literature. *Med Mycol Case Rep* 2016; 11: 40−3.
12. Abzug MJ, Walsh TJ. Interferon-gamma and colony-stimulating factors as adjuvant therapy for refractory fungal infections in children. *Pediatr Infect Dis J* 2004; 23: 769−73.

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Vancomycin dependent *Enterococcus*: an unusual mutant?

Sir,

Vancomycin resistant enterococci (VRE) are increasingly important healthcare associated pathogens. The incidence of infection and colonisation with these organisms has risen significantly both in Australia[1] and around the world. Interestingly, there have been rare reports describing isolates of *Enterococcus* that both require and are dependent on vancomycin for growth. Herein, we report two such cases, where we isolated this vancomycin-dependent *Enterococcus* (VDE) from rectal surveillance swabs. Both patients in question had received prolonged oral vancomycin therapy for the treatment of recurrent *Clostridioides difficile* infection.

The first patient was a 54-year-old woman with relapsed acute myeloid leukaemia on palliative chemotherapy. She had suffered from multiple recurrences of *C. difficile* colitis and was on a prolonged treatment course of oral vancomycin. She was known to be colonised with *vanB* VRE *faecium*, identified on a rectal swab, predating her vancomycin treatment course. A rectal surveillance swab several weeks after commencement of vancomycin, isolated three phenotypically different colony types on chrom ID VRE (bioMérieux, France). All three isolates were identified by the matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF; Bruker Daltronics, USA) assay as *Enterococcus faecium*. Following standard laboratory procedures, the three colonies were subcultured onto horse blood agar (HBA) with a 30 μg vancomycin disc (Oxoid, UK) and incubated in 5% CO_2 at 35°C for 48 h. One of the *E. faecium* colonies only exhibited growth adjacent to the vancomycin disc (Fig. 1). Further investigation using a vancomycin Etest (bioMérieux) on Mueller-Hinton agar (MHA) confirmed this unusual vancomycin dependence with the density of growth of the organism increasing with higher concentrations of vancomycin on the Etest strip (Fig. 1). Susceptibility testing using the VITEK2 GP card (bioMérieux) was unsuccessful as the instrument terminated due to insufficient growth of the organism. Molecular testing using the Roche LightCycler VRE detection platform (Roche Diagnostics, Germany) detected a *vanB* gene within this VDE isolate.

The second patient was a 68-year-old man with a history of paroxysmal nocturnal haemoglobinuria and mesothelioma who was admitted to the intensive care unit (ICU) with septic shock due to pneumonia. He had a history of recurrent episodes of debilitating *C. difficile* colitis and had been treated on multiple occasions with short courses and subsequently a prolonged weaning regimen of oral vancomycin. Previous surveillance swabs had isolated both *vanB* and *vanA* VRE *faecium*. Similar to the first patient, ICU admission VRE rectal surveillance swabs identified two phenotypically different colonies of *E. faecium* which demonstrated growth most apparent around the vancomycin disc on subculture on HBA. One of the VDE isolates also demonstrated 'partial teicoplanin dependence' (i.e., no growth in the absence of teicoplanin, with growth in the presence of low concentrations of teicoplanin and inhibition at higher teicoplanin concentrations) (Fig. 2).

Since first described in 1993, there have been a very small number of VDE cases worldwide. The first case described by Fraimow *et al.* was a vancomycin-dependent *E. faecalis* isolated in the urine of a patient on a course of intravenous vancomycin for >100 days.[2] Subsequent case series have described the detection of vancomycin-dependence in *E. faecalis*, *E. faecium* and *E. avium* from a variety of clinical specimens.[2−10] These included both stool or rectal surveillance cultures and more invasive specimens such as blood, urine and intra-abdominal fluid. Dever *et al.* describe a similar case to ours, where they isolated a vancomycin-dependent *E. faecium* in stool of a patient 10 days after treatment with oral vancomycin for *C. difficile* infection.[3] Likewise, both our patients were also found to be carriers of VDE with no clinically significant disease.

The development of resistance to glycopeptides in enterococci is thought to be due to acquisition of genetic elements carrying *vanA* and *vanB* that result in the production of peptidoglycan cell wall precursors that confer resistance to vancomycin. Normal bacterial cell walls are composed of the dipeptidoglycan d-alanyl d-alanine ('d-ala d-ala'). When exposed to vancomycin, organisms with acquired resistance are able to synthesise an alternative d-alanyl d-lactate ('d-ala d-lac') cell wall precursor that binds to vancomycin with significantly lower affinity. In the absence of vancomycin, VRE still retain their ability to make the original d-ala d-ala. Conversely, due to potential amino acid substitutions or deletions in the *ddl* gene, which encodes d-ala d-ala ligases, vancomycin-dependent organisms are unable to produce d-ala d-ala, and are only able to produce d-ala d-lac cell wall precursors.[2,4,5,8] Therefore their growth is dependent on the continued presence of vancomycin. These VDE strains can spontaneously revert to vancomycin-independent growth either by restoration of d-

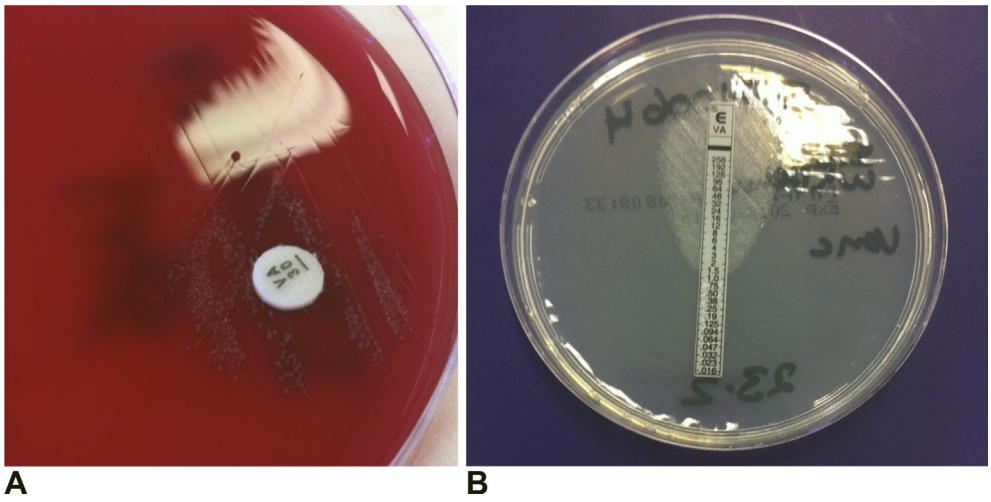

Fig. 1 (A) Vancomycin dependence with growth most pronounced surrounding vancomycin disc on horse blood agar (HBA) incubated in 5% CO_2 at 35°C for 48 h. (B) Vancomycin dependence with organism growth density increasing at higher concentrations of vancomycin in Etest strip on Mueller-Hinton agar (MHA).

ala d-ala ligase activity[11] (due to further *ddl* gene mutations) or by constitutive expression of d-ala d-lac (as a result of mutations in the vicinity of the autophosphorylation site of the $VanS_B$ sensor). Vancomycin-independent revertants are usually apparent when strains are subcultured to media without vancomycin.

Factors leading to the development of colonisation or infection with VDE are unclear. Tambyah *et al.* found in a small case control series that the use of third generation cephalosporins was the most important risk factor in the development of VDE.[10] A possible explanation is that selection pressure from broad spectrum antibiotics promotes VRE colonisation, which in turn when exposed to vancomycin, may lead to emergence of these dependent strains. Notably, both our patients had become VRE colonised from multiple hospital admissions and antibiotic exposures and then had also subsequently received prolonged oral vancomycin courses. Another significant risk factor identified by the authors in the study was renal impairment, which was postulated to magnify vancomycin exposure due to reduced clearance of the antibiotic.

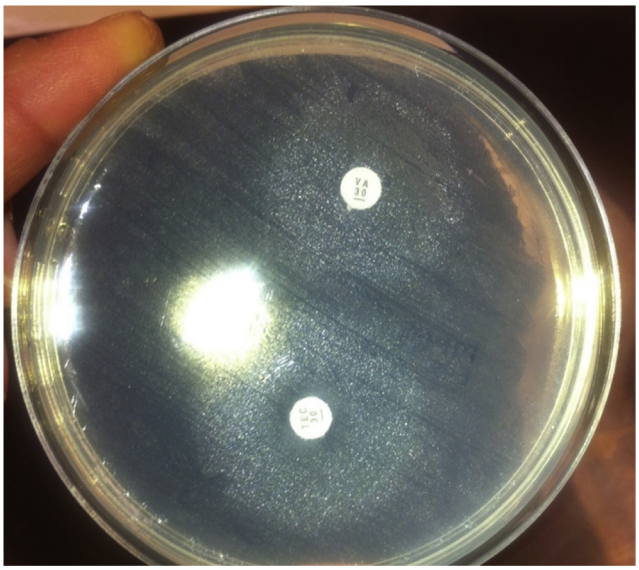

Fig. 2 Isolate exhibiting vancomycin dependence (growth around vancomycin disc) (top) and partial teicoplanin dependence (growth in the presence of low concentrations of teicoplanin with inhibition of growth at higher teicoplanin concentrations immediately around the disc and no growth in the absence of teicoplanin) (bottom).

It has been proposed that VDE can be transmitted like other nosocomial infections via contaminated hands and environmental sources. Kirkpatrick *et al.* described an outbreak of VDE *faecium* in patients in a bone marrow transplant unit receiving protracted prophylactic treatment with vancomycin.[8] All isolates implicated in the outbreak had similar profiles when analysed using pulsed-field gel electrophoresis (PGFE), suggesting likely nosocomial transmission. Implementation of strict infection control measures and judicious prescribing of vancomycin prophylaxis ensured that no further cases were detected or transmitted within the unit.

The identification of VDE poses a challenge in the microbiology laboratory as organisms may fail to grow on both primary cultures (e.g., blood cultures) as well as routine subculture of clinical specimens.[9] Isolation is only successful if specimens are cultured on media containing vancomycin or by placing a vancomycin disc on culture plates. Susceptibility testing is difficult as it requires the addition of either vancomycin or d-ala d-ala as a growth supplement.[12] In both our clinical cases, the vancomycin-dependent isolates were only detected because of our laboratory protocol of subculturing all organisms isolated on chrom ID VRE onto non-selective agar with vancomycin discs. Our first patient also demonstrated the difficulty of using automated systems such as the VITEK2 for identification and susceptibility testing of these organisms due to insufficient growth in the absence of vancomycin.

Many questions remain to be answered about the clinical relevance and the utility of screening for this organism. As routine surveillance for VDE is not widespread in clinical laboratories, the true prevalence of the organism is currently unknown. Performing additional testing for detection of VDE by using media containing vancomycin or media with vancomycin discs in clinical specimens of all patients on vancomycin is thought to be neither cost nor labour effective at present. A reasonable approach may be to screen for the organism in a subset of patients with known previous VRE who

have relevant risk factors such as exposure to broad spectrum antibiotics and prolonged courses of vancomycin. Additional testing for detection may also be warranted in clinical specimens from patients with risk factors who have continuing evidence of infection with an *Enterococcus* that cannot be identified using automated systems such as the VITEK2. To determine the true burden of this organism, further large studies looking at the prevalence of VDE in surveillance rectal swabs of patients 'pre-colonised' with VRE are warranted. The clinical relevance and relative virulence of this organism is also currently unknown due to the lack of clinical data.

To complicate matters further, optimal treatment regimens for significant VDE infections is unknown. Cessation of vancomycin alone may not be sufficient to cure infections due to the rapid emergence of vancomycin-independent revertant mutants.[11] The efficacy of antimicrobials such as linezolid and daptomycin are yet to be determined in a clinically significant manner due to the low prevalence of invasive infections. In patients colonised with the organism, just like in VRE, it is important to adhere to strict infection control measures to prevent nosocomial spread.[8]

Though relatively rare, it is important to be aware of this phenomenon of vancomycin-dependence in patients at risk as well as the challenges associated with identification and susceptibility testing of the organism. The emergence of this pathogen also emphasises the importance of antimicrobial stewardship policies in preventing the evolution of increasingly resistant enterococci.

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1. Coombs GW, Pearson JC, Christiansen K, *et al*. Australian group on antimicrobial resistance enterococcus surveillance programme annual report, 2010. *Commun Dis Intell Q Rep* 2013; 37: E199–209.
2. Fraimow HS, Jungkind DL, Lander DW, *et al*. Urinary tract infection with an Enterococcus faecalis isolate that requires vancomycin for growth. *Ann Intern Med* 1994; 121: 22–6.
3. Dever LL, Smith SM, Handwerger S, *et al*. Vancomycin-dependent Enterococcus faecium isolated from stool following oral vancomycin therapy. *J Clin Microbiol* 1995; 33: 2770–3.
4. Green M, Shlaes JH, Barbadora K, *et al*. Bacteremia due to vancomycin-dependent Enterococcus faecium. *Clin Infect Dis* 1995; 20: 712–4.
5. Rosato A, Pierre J, Billot-Klein D, *et al*. Inducible and constitutive expression of resistance to glycopeptides and vancomycin dependence in glycopeptide-resistant Enterococcus avium. *Antimicrob Agents Chemother* 1995; 39: 830–3.
6. Farrag N, Eltringham I, Liddy H. Vancomycin-dependent Enterococcus faecalis. *Lancet* 1996; 348: 1581–2.
7. Stewart B, Hall L, Duke B, *et al*. Vancomycin-dependent enterococci: curious phenomenon or serious threat? *J Antimicrob Chemother* 1997; 40: 734–5.
8. Kirkpatrick BD, Harrington SM, Smith D, *et al*. An outbreak of vancomycin-dependent Enterococcus faecium in a bone marrow transplant unit. *Clin Infect Dis* 1999; 29: 1268–73.
9. Yowler CJ, Blinkhorn RJ, Fratianne RB. Vancomycin-dependent enterococcal strains: case report and review. *J Trauma* 2000; 48: 783–5.
10. Tambyah PA, Marx JA, Maki DG. Nosocomial infection with vancomycin-dependent enterococci. *Emerg Infect Dis* 2004; 10: 1277–81.
11. Van Bambeke F, Chauvel M, Reynolds PE, *et al*. Vancomycin-dependent Enterococcus faecalis clinical isolates and revertant mutants. *Antimicrob Agents Chemother* 1999; 43: 41–7.
12. Sng LH, Cornish N, Knapp CC, *et al*. Antimicrobial susceptibility testing of a clinical isolate of vancomycin-dependent enterococcus using D-alanine-D-alanine as a growth supplement. *Am J Clin Pathol* 1998; 109: 399–403.

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Identification of *Fusobacterium nucleatum* in formalin-fixed, paraffin-embedded placental tissues by 16S rRNA sequencing in a case of extremely preterm birth secondary to amniotic fluid infection

Sir,

According to the World Health Organization, an estimated 15 million babies are born preterm (before 37 completed weeks of gestation) each year, with preterm birth rates increasing in almost all countries that collect reliable data.[1] Globally, complications resulting from preterm birth are the leading cause of death in children under 5 years of age, and those who do survive often have serious long-term health problems and disabilities.[2,3]

One of the most common aetiologies of preterm birth is amniotic fluid infection, caused by bacteria ascending from the vagina into the uterus and infecting the amniotic fluid.[4] Despite the high prevalence of preterm birth secondary to amniotic fluid infection, our understanding of the types and roles of bacteria involved is remarkably limited because in many cases, the causative microorganisms are not known.[5,6]

In order to elucidate the identity of the pathogenic organisms, one needs to perform microbiology studies on a suitable clinical specimen. However, tissues that might contain the causative organisms, such as tissue sample from the neonate or amniotic fluid sample from the mother, are not routinely available for testing in the clinical setting. In contrast, formalin-fixed, paraffin-embedded (FFPE) placental tissues are usually readily accessible for laboratory analysis since pathological examination of placentas has become standard of care in preterm birth cases, and therefore these placentas are routinely sent to the pathology department.

Here, we report the feasibility of performing 16S rRNA sequencing on FFPE placental tissues in bacterial identification in a case of extremely preterm birth secondary to amniotic fluid infection.

A 31-year-old, gravida 3, para 0 woman was admitted at the hospital at 20 4/7 weeks gestational age for fevers and chills. Previous obstetrical history was unavailable. On admission, she had elevated temperature and white cell