First reported cases of linezolid-resistant vancomycin-resistant enterococci in South-East Asia: A report of three cases and literature review

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Abstract
Infections with vancomycin-resistant enterococci (VRE) have limited treatment options, with linezolid the mainstay of therapy. The emergence of linezolid-resistant vancomycin-resistant enterococci (LRVRE) further restricts the therapeutic options. LRVRE isolated from clinical specimen have not previously been reported in South-East Asia. Here, we report three clinical cases of LRVRE from a teaching hospital in Malaysia. Three isolates of Enterococcus faecium were isolated from three different patients. These isolates were determined to be resistant to both vancomycin and linezolid by Etest gradient diffusion test and Vitek2 automated antibiotic susceptibility testing, respectively. Retrospective analysis of the microbiological and clinical data of the three patients was undertaken. LRVRE were isolated from from tissue and peritoneal fluid of two patients who underwent complicated abdominal surgery. Another patient with underlying acute lymphoblastic leukaemia developed neutropenia following chemotherapy. LRVRE were isolated from the blood culture. All three patients were not treated with linezolid. The possible risk factors for LRVRE acquisition are administration of multiple broad-spectrum antimicrobials and disruption of the gastrointestinal mucosa. The appearance of LRVRE in increasingly widespread geographic locations may lead to a global health threat. Judicious use of broad-spectrum antimicrobials and infection-control practices are crucial to curb its spread.

Keywords
Linezolid resistance, vancomycin-resistant enterococci, Enterococcus faecium, South-East Asia

Introduction
Vancomycin-resistant enterococci (VRE) is a known pathogen causing hospital-associated infection. Linezolid resistance among isolates of VRE (linezolid-resistant VRE (LRVRE)) has been reported in the USA and Europe since early 2000s. LRVRE in Asia was reported relatively recently – in 2009 in Korea and in 2014 in India.¹,² Linezolid-resistant vancomycin-susceptible enterococci were reported from human clinical specimen in Thailand in 2012.³ To our knowledge at the time writing of this report, LRVRE has not yet been reported in South-East Asia. The three cases of LRVRE reported here may herald the emergence of LRVRE in the region.

Case reports
LRVRE isolated from routine microbiological analysis of clinical samples in a Malaysian teaching hospital were identified. Antibiotic susceptibility testing (AST) was performed using Kirby Bauer disc diffusion methods. Strains with vancomycin or linezolid resistance were further tested with an E-Test and Vitek2 automated susceptibility testing, respectively, for their minimal inhibitory concentration (MIC). All AST and MIC results were interpreted according to Clinical and Laboratory Standard Institute criteria.⁴

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Case 1
Case 1 was a 48-year-old woman with locally advanced endometrioid adenocarcinoma of the ovary who had undergone subtotal hysterectomy and bilateral oophorectomy. She had received multiple courses of chemotherapy for her illness. She presented with recurrence of the malignancy with bladder and bowel involvement. Operative management of anterior pelvic exenteration with colostomy and ileal conduit creation was done. This was complicated with surgical site infection (SSI), leading to anastomotic leak, wound dehiscence and enterocutaneous fistula. She was empirically treated for the complicated SSI with intravenous (i.v.) cefoperazone in combination with metronidazole, and subsequently escalated to piperacillin-tazobactam and meropenem in combination with vancomycin. Initial intraoperative tissue specimen revealed a fully susceptible *Enterococcus faecium*. Subsequently, three revision operations were done for wound debridement, repair of fistula and nephrostomy for urinary diversion. Repeat tissue culture grew *E. faecium* which was resistant to both vancomycin and linezolid. In addition, the isolate was also resistant to all beta lactam antibiotics tested and demonstrated high-level resistance to gentamicin. Automated antibiotic susceptibility testing (AST) by Vitek2 revealed that the isolate was susceptible to tigecycline and intermediate to quinupristin/dalfopristin. The patient was treated with i.v. tigecycline for a week for complicated SSI in combination with operative source control. Repeat tissue cultures after the completion of tigecycline therapy revealed no growth.

Case 2
A 70-year-old man with no medical illness was admitted with sudden-onset epigastric pain. Physical examination revealed a pulsatile mass at the epigastric region which was later diagnosed as a leaking infrarenal abdominal aortic aneurysm. Resection of the aneurysm and insertion of an inlay graft were done. During his hospital stay, he contracted hospital-acquired pneumonia (HAP) caused by carbapenem-resistant Klebsiella pneumoniae. Subsequently, he developed a pulmonary embolism with cardiac arrest, and required mechanical ventilation for cardiorespiratory failure. He was treated empirically with i.v. piperacillin-tazobactam and i.v. cefepime, and was later changed to ertapenem in combination with amikacin based on antibiotic susceptibility results. He responded to antibiotic therapy and managed to be extubated after two weeks. However, the operation was complicated with anastomatic leak of the right common iliac artery graft and small-bowel perforation. A femoral-femoral bypass with ligation of the leakage and end-to-end anastomosis of the small bowel with peritoneal lavage were performed. The peritoneal fluid was taken in the operation theatre and grew *E. faecium* which was resistant to both vancomycin and linezolid. Signs and symptoms of peritonitis were absent, and the patient remained afebrile. Therefore, the operative culture-positive LRVRE was of doubtful significance, and this was not treated.

Case 3
A 20-year-old woman with relapsed Pre-B acute lymphoblastic leukaemia was admitted for a second cycle of chemotherapy. Following that, she developed neutropenia and intermittent fever with a non-productive cough. She was treated empirically with i.v. cefepime for neutropenic sepsis and HAP. She remained febrile and neutropenic for the next four days before developing colicky abdominal pain with diarrhea. The antimicrobial therapy was escalated to i.v. meropenem to include more resistant organisms. At the same time, oral vancomycin and i.v. metronidazole were commenced for the possibility of *Clostridium difficile*–associated diarrhoea. However, *C. difficile* toxin was not detected. Thus, vancomycin and metronidazole were discontinued after five days. The neutropenia subsequently resolved, as did the fever, diarrhea and cough. Two sets of blood cultures taken during the febrile episode grew LRVRE. She was then discharged with the plan for another cycle of chemotherapy a week later; and the blood culture taken before discharge revealed no growth. The source of the LRVRE was likely from the gastrointestinal tract as a result of chemotherapy-related mucosal barrier injury. We regard both isolates as transient bacteremia which resolved following the recovery of the immune system.

The clinical characteristics of the three cases are summarised in Table 1.

### Discussion
Enterococci are commonly isolated from clinical samples involving the gastrointestinal tract as normal gut commensals. They constitute a minor portion of the gut microbiota in comparison to anaerobic commensals. However, selective pressure exists in patients who received broad-spectrum antibiotics, enabling them to dominate the gastrointestinal tract microbiota and greatly enhancing their potential to cause hospital-acquired infections.

*E. faecium* and *Enterococcus faecalis* are the most common clinical isolates. *E. faecium* is typically more multidrug resistant than *E. faecalis*. Linezolid is considered the mainstay of treatment of vancomycin-resistant *E. faecium* infections. Linezolid belongs to the antibiotic class of oxazolidinones, and acts by binding to the 50S ribosome unit at the interface with 30S unit, thereby inhibiting bacterial protein synthesis. Resistance to linezolid in enterococci is mediated by mutations in the 23S ribosomal RNA (rRNA), with the RNA R2576T point mutation being the most frequently reported mutation. The occurrence of LRVRE appears to be infrequent, with a reported incidence of 1.8% of VRE isolates in the linezolid compassionate use program. The overall incidence worldwide is unknown. Linezolid resistance among enterococci regardless of vancomycin susceptibility is also low, ranging from 0.12% to 1.07% from linezolid susceptibility analysis in 32 countries.

Risk factors for VRE colonisation include broad-spectrum antibiotic use, vancomycin use, gastrointestinal procedures, host immune status (immunosuppression, neutropenia), prolonged hospital stay and admission to the intensive care unit or oncology unit. These risk factors are evident in all three cases presented here demonstrating that the presence of antibiotic-selective pressure with compromised host immunity and gastrointestinal mucosal barrier, combined with a prolonged unresolved underlying condition, set the stage for the emergence of multidrug-resistant enterococci. However, risk factors for acquisition of LRVRE are yet to be defined.
Table 1. Clinical characteristics of cases with LRVRE.

| Clinical presentation                                                                 | Possible risk factors                                                                 | Clinical interpretation                                           | Phenotypic characteristics       | MIC level                  |
|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------------------------|----------------------------------|-----------------------------|
| 48-year-old female with locally invasive endometrioid ovarian carcinoma              | 1. Complicated abdominal surgery                                                      | Specimen: Tissue Significant (treated and responded)             | VanA phenotype                  | Vancomycin: $\geq 32 \mu g/mL$ | Linezolid: $\geq 8 \mu g/mL$ |
|                                                                                      | 2. Vancomycin used                                                                     |                                                                   | Vancomycin: Resistant           |                             |                             |
|                                                                                      | 3. Broad-spectrum antimicrobical used                                                 |                                                                   | Teicoplanin: Resistant          |                             |                             |
|                                                                                      | 4. Admission to HDU                                                                   |                                                                   |                                  |                             |                             |
| 70-year-old male leaking abdominal aortic aneurysm                                     | 1. Complicated abdominal surgery                                                      | Specimen: Peritoneal fluid Doubtful significance (not treated)  | VanA phenotype                  | Vancomycin: $\geq 32 \mu g/mL$ | Linezolid: $\geq 8 \mu g/mL$ |
|                                                                                      | 2. Multiple broad-spectrum antimicrobical used                                       |                                                                   | Vancomycin: Resistant           |                             |                             |
|                                                                                      | 3. Admission to ICU and HDU                                                           |                                                                   | Teicoplanin: Resistant          |                             |                             |
| 20-year-old lady with relapsed Pre-B acute lymphoblastic leukaemia                   | 1. Chemotherapy induced neutropenia and mucositis                                    | Specimen: Blood Possible transient bacteremia (not treated)      | VanA phenotype                  | Vancomycin: $\geq 32 \mu g/mL$ | Linezolid: $\geq 8 \mu g/mL$ |
|                                                                                      | 2. Multiple broad-spectrum antimicrobical used                                       |                                                                   | Vancomycin: Resistant           |                             |                             |
|                                                                                      | 3. Vancomycin used                                                                   |                                                                   | Teicoplanin: Resistant          |                             |                             |

LRVRE: linezolid-resistant vancomycin-resistant enterococci; MIC: minimal inhibitory concentration; HDU: high dependency unit; ICU: intensive care unit.

Reported risk factors include immunodeficiency, haematological malignancy, solid-organ transplant and prior use of antibiotics such as linezolid, piperacillin-tazobactam or cefepime. The risk factors for acquisition of linezolid-resistant enterococci appears to be similar; regardless of vancomycin susceptibility.

Isolation of enterococci from intra-abdominal infections is common, usually isolated as a part of mixed microbiota. The clinical significance of enterococci isolated in pure growth is controversial. However, intra-abdominal infections with enterococci alone can occur in the presence of substances that promote abscess formation. In cases 1 and 2, the presence of ileal conduits, nephrostomy tube and endovascular graft, respectively, may have promoted adherence, biofilm formation and subsequently the persistence of enterococcal colonisation and infection. Immuno compromised and severely ill patients with intra-abdominal infection from whom enterococci are isolated should be treated with anti-enterococcal antibiotics. Nosocomially acquired enterococcal bloodstream infections are not uncommon. They can be associated with genitourinary, hepatobiliary and vascular catheter-related infections. They are also frequently associated with gastrointestinal translocation. Patients who are debilitated and those who have received broad-spectrum antibiotics are prone to enterococcal bacteraemia. In the third case, chemotherapy and neutropenia may have resulted in the disruption of the gastrointestinal mucosal barrier. Use of broad-spectrum antibiotics and vancomycin probably led to the colonisation by VRE. A combination of these risk factors may have paved the way for VRE bacteraemia. Although we report three cases of LRVRE at our centre, they occurred at different times and locations, and this was not considered to be an outbreak. Nonetheless, the emergence of multidrug-resistant organisms highlights the importance of stringent infection-control measures.

The therapeutic options of LRVRE are limited. In the first two cases presented here, the use of tigecycline may be favoured. This is consistent with the Food and Drug Administration (FDA) indication for the treatment of complicated intra-abdominal infection. Although not FDA approved for the treatment of E. faecium bacteraemia in case 3, recent literature supports the use of high-dose daptomycin, with reduced mortality and improved microbiologic clearance. For improved efficacy, a combination of high-dose daptomycin with β-lactams such as ampicillin, ceftaroline and ertapenem may benefit from the see-saw effect, leading to increased susceptibility to β-lactam antibiotics.

Conclusion

The emergence of LRVRE is an alarming problem. Even more worrying is its spread to geographical regions where this resistance was not previously reported. Infections with LRVRE may become more common in the future, as there is an increase in patient populations with compromised immune systems, and more complicated abdominal surgical procedures are being performed. Further studies may be warranted to define the risk factors for the acquisition of LRVRE. Judicious use of broad-spectrum antibiotics and strict enforcement of infection-control practices are crucial to curb its spread.

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Authors’ contributions

W.C.C. wrote the first draft of the article, and Z.A.R. reviewed and edited the manuscript and approved the final version of the manuscript.

Availability of data and materials

Data sharing is not applicable to this article, as no data sets were generated or analysed during the current study.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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