EPIDEMIOLOGY AND CONTROL OF THE FIRST REPORTED VANCOMYCIN-RESISTANT *ENTEROCOCCUS* OUTBREAK AT A TERTIARY-CARE HOSPITAL IN BANGKOK, THAILAND

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Abstract. This retrospective study described the first reported vancomycin-resistant enterococci (VRE) outbreak from June 2013 through January 2014 at a tertiary-care hospital in Bangkok, Thailand. After the index case was detected in an 18-bed medical intermediate care unit, a number of interventions was implemented, including targeted active surveillance for VRE, strict contact precautions, enhanced standard precautions, dedicated units for VRE cases, extensive cleaning of the environment and the restricted use of antibiotics. VRE isolates were evaluated by polymerase chain reaction and random amplified polymorphic DNA (RAPD) testing. A prevalence case-control study was conducted. Among 3,699 culture samples from 2,671 patients screened, 74 patients (2.8%) had VRE. The positivity rate declined from 15.1% during week 1 to 8.2% during week 2 and then 1.4% during week 3. By weeks 4-9, the prevalences were 0-2.7%. However, the prevalence rose to 9.4% during week 10 and then subsequently declined. All VRE isolates were *Enterococcus faecium* and had the *vanA* gene. RAPD analysis revealed a single predominant clone. Multivariate analysis showed mechanical ventilation for ≥ 7 days was a predictive factor for VRE colonization [odds ratio (OR) 11.47; 95% confidence interval (CI): 1.75-75.35; p=0.011]. This experience demonstrates VRE can easily spread and result in an outbreak in multiple-bed units. Active surveillance, early infection control interventions and rapid patient cohorting were important tools for control of this outbreak. Patients requiring mechanical ventilator for ≥7 days were at higher risk for VRE acquisition.

Keywords: *Enterococcus*, vancomycin resistance, epidemiology, infection control, Thailand

INTRODUCTION

Vancomycin-resistant enterococci (VRE) are important nosocomial pathogens associated with increased mortality, longer hospital stays and higher cost compared to vancomycin-susceptible enterococci (Lode, 2009). Treatment of VRE infection is challenging due to multi-drug resistance (Torres-Viera and Dembry, 2004). Data from the United States, Europe and some countries in Asia shows a growing incidence of VRE infections (Lee *et al*, 2004; National Nosocomial Infections Surveillance System, 2004; Hidron *et al*, 2008; Lu *et al*, 2012; Meyer *et al*, 2013). In Thailand, VRE isolates comprise 0.81-1.9% of clinical isolates (Nilgate *et al*, 2003; Thongkoom *et al*, 2012). However, there are limited data regarding VRE outbreaks and intervention strategies in resource-limited settings. In this report, we describe the first reported VRE outbreak and molecular epidemiology at our institution, a tertiary-care hospital in Thailand. We determine the risk factors for VRE acquisition and evaluated the impact of the control measures.

MATERIALS AND METHODS

Setting

Ramathibodi Hospital is a 910-bed university hospital with 12,000 admissions per year. It is a tertiary care center with renal and bone marrow transplant units, a burn unit, a medical intensive care unit (ICU), a coronary care unit (CCU), 4 surgical ICUs, a medical intermediate care unit and a surgical intermediate care unit. The total number of ICU, CCU and intermediate care unit beds is 73.

Outbreak

Ramathibodi Hospital initiated targeted active surveillance for VRE coloni-zation in 2012. There were 2 VRE isolates from the urine of patients at single-patient rooms of medical private unit in May and October 2012. Final identification revealed both isolates were *Enterococcus faecalis* carrying of the *vanB* gene. Active surveillance cultures from patients in the adjacent rooms were negative for VRE.

The first hospital VRE outbreak began in early June 2013. The index case was identified from a positive VRE urine culture at an 18-bed medical intermediate care unit, providing intermediate level care between the medical ICU and the general medical units. There were no single-patient rooms for patients requiring contact precautions in this unit. The index patient had an acute exacerbation of chronic obstructive pulmonary disease, and was admitted to the male medical unit on April 14, 2013. He had complicated clinical conditions of pneumonia, a pneumothorax and upper gastrointestinal bleeding and was transferred to the medical intermediate care unit on April 16, 2013. Targeted active surveillance for VRE colonization was conducted in 5 close contact patients in the same cubicle and two were found positive. VRE screening was subsequently conducted among the 14 remaining patients in the same unit, 5 more were also positive for VRE.

Infection control measures

All the VRE-positive cases were placed in contact isolation. After 5 additional VRE-positive cases were found in all three cubicles of the medical intermediate care unit, the unit was frozen, meaning patients were not allowed to move into this unit or relocate to other units. Contact patients, defined as all patients in, or recently moved out of the medical intermediate care unit to other units within the previous 30 days, were tracked and screened for VRE at the end

of the first week of the outbreak. Of the 19 contact patients in three general medical units on the same floor as the medical intermediate unit, 7 were identified to have VRE-positive from surveillance cultures, and 1 was found to have VRE from clinical culture. Universal contact precautions were implemented for all the VRE-positive medical units regardless of VRE status. No new admissions were allowed to these units, except known positive or contact patients.

Eleven days after the first VRE cluster, expanded VRE screening was done in high-risk units, including the medical ICU, all 6 surgical units and the hemodialysis unit. One out of 8 samples from the medical intensive care unit, and 4 out of 34 samples from 2 surgical units were positive for VRE. Contact precautions were put in place for these positive cases and for contact patients in the same cubicles.

For patient cohorting, each unit was divided into 3 zones: confirmed positive VRE zone, VRE-contact zone and non-contact zone. The patients in the confirmed positive VRE and VRE-contact zones had contact precautions implemented, while the patients in the non-contact VRE zone had standard precautions continued. After the number of patients in the medicine units decreased, the medical intermediate care unit and one general medicine unit were dedicated for VRE-positive patients on day 12 of the outbreak. On day 26 of the outbreak, one newly renovated unit was temporarily assigned to relocate the positive cases from the two previously dedicated VRE units, and admissions were allowed into the contact zone, and the non-contact VRE zone of those medicine units. Follow-up surveillance cultures were performed every 3 days among contact cases until 3 cultures were negative for VRE or 1 positive VRE cul-

ture was found. For VRE-positive cases, surveillance was performed every week until 3 cultures were negative for VRE. Contact precautions were discontinued after 3 consecutive cultures were negative for VRE.

Dedicated patient care items were individually provided for confirmed positive and VRE-contact cases. Enhanced environmental cleaning was maintained throughout the outbreak. Hydrogen peroxide vaporization was used for terminal cleaning. Antimicrobial, particularly vancomycin therapy was discontinued as soon as appropriate. Active communication and education with all involved healthcare workers were done by infectious diseases physicians and infection control nurses.

Microbiological and molecular determinations

Targeted active surveillance cultures for VRE from rectal swab specimens were obtained every 3 days from contact cases and every 2 weeks from non-contact cases who had been admitted for more than 2 weeks to VRE-positive units. Environmental samples were also obtained. Screening cultures were performed on bile-esculin agar plates supplemented with 6 mg/l vancomycin. Suspected colonies of *Enterococcus* species were subcultured onto sheep blood agar and tested for vancomycin and teicoplanin resistance by disc diffusion. Species identification was performed using conventional biochemical tests and MALDI-TOF (Bruker-Franzen Analytik, Bremen, Germany). MIC testing for vancomycin, teicoplanin, daptomycin and linezolid was conducted with a Trek Sensititre broth microdilution panel for gram-positive bacteria (Trek Diagnostic System, Cleveland, OH). Detection of *vanA* and *vanB* resistance genes was

performed using a PCR method (Clark *et al*, 1993; Elsayed *et al*, 2001). Whole-cell DNA was extracted and purified using a Qiaquick PCR purification kit (Qiagen, Chatsworth, CA) following the manufacturer's instructions. Epidemiological typing was performed using random amplified polymorphic DNA (RAPD) with ERIC1 and ERIC2 primers, as described previously (Versalovic *et al*, 1991).

Prevalence case-control study

We conducted a prevalence case-control study of colonized and non-colonized VRE patients. Patients who had ever stayed in the medical intermediate care unit during June 1-30, 2013 and who had at least one VRE surveillance culture were enrolled in the study. A case was defined as any patient with at least one culture positive for VRE after admission during the first two weeks of the outbreak. Control patients were patients with negative culture for VRE. Medical records and laboratory reports were reviewed until the study endpoints: the first VRE-positive culture for cases and discharge from the medical intermediate care unit for controls.

Statistical analysis

Data analysis was conducted using Stata software version 10.0 (Stata Corp, College Station, TX). Patients were divided into two groups based on the VRE surveillance culture results. The chi-square and Fisher's exact tests were used to compare categorical variables where appropriate. The Student's *t*-test and Mann-Whitney U test were used to compare the means and medians of continuous variables. Binary logistic regression analyses were conducted for multivariate analysis to determine factors associated with positive VRE culture. Factors with a *p*-value <0.1 on univariate analysis were included in the multivariate analysis model, except factors which were correlated. Odds ratios (OR) and their 95% confidence intervals (CI) were estimated. A *p*-value <0.05 was considered statistically significant.

Ethical approval for this study was obtained from the institutional review board.

RESULTS

Outbreak description

After the index case of VRE was detected in the urine, targeted active surveillance revealed 11 more patients from this unit that were colonized with VRE. The VRE positivity rate among screened patients was highest at 15.1% during week 1, decreased to 8.2% in week 2 and then 1.4% by week 3. During weeks 3-6, 5 new patients who were recently discharged home from VRE-positive units were found to have VRE. No new cases of VRE were found for 17 days during weeks 4-6. VRE screening was done continuously on patients admitted for more than 2 weeks in the units with VRE and none were detected. VRE was detected in a urine culture from a patient in the medical intensive care unit during week 8. During week 10, the positivity rate increased to 9.4% and then declined to 5.6% and 4.3% during weeks 11 and 12, respectively. During weeks 13-23, the positivity rates were 0-2.6% (0-2 cases/week). During week 24, the positivity rate increased to 4.7% and then declined to 0-1.4% during weeks 27-33 (0-1 cases/week) (Fig 1).

Nineteen positive patients died during the study period, but none of the deaths were attributed to VRE infection. One VRE-positive patient during week 1 subsequently developed VRE-associated urinary tract infection during week 3.
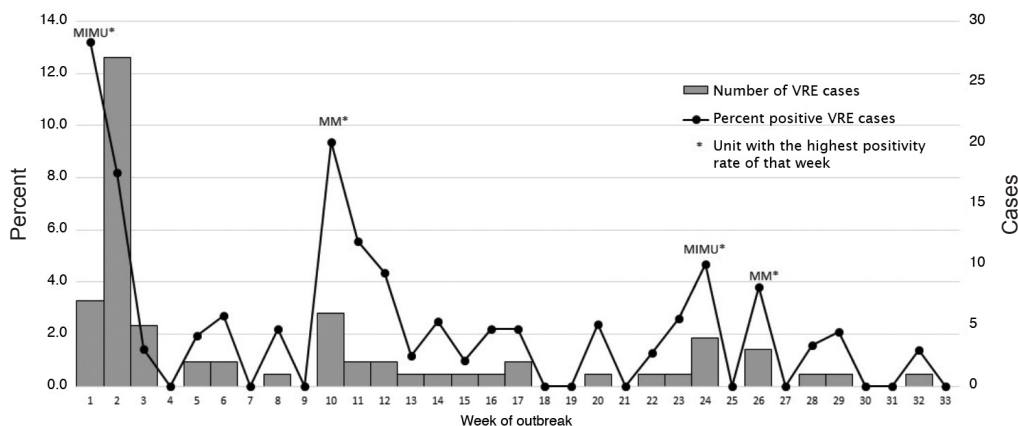
Fig 1–Timeline of positive VRE cases and interventions during June 2013-January 2014.
VRE, vancomycin-resistant *Enterococcus*; MIMU, medical intermediate care unit; MM male medical unit.

Microbiology and molecular study results

Of 3,699 perianal and rectal samples obtained from 2,671 patients, 209 samples were positive for VRE in 74 patients; all were identified as *E. faecium*. All the isolates showed resistance to vancomycin and teicoplanin, and all the isolates were susceptible to daptomycin and linezolid using the Clinical and Laboratory Standards Institute cutoff levels (CLSI, 2013). The PCR results showed all the isolates had the *vanA* gene. Molecular typing by RAPD in 32 randomly selected isolates during the study period showed the same RAPD patterns, which were different from the VRE isolates from another hospital in Thailand. None of the environmental culture results were positive for VRE.

Prevalence case-control study results

Eighteen cases and 30 controls were included in the study. The mean (±SD) age of the cases was 73 (±20) years, and of the controls was 62 (±16) years ($p = 0.025$). On univariate analysis, age ≥75 years, length of stay ≥3 weeks, receipt of piperacillin/tazobactam >3 days, being on a mechanical ventilator ≥7 days, having urinary catheter in place for ≥7 days, and having a nasogastric tube in place for ≥7 days were significantly associated with VRE acquisition (p <0.05) (Table 1). On multivariate analysis, patients on mechanical ventilation for ≥7 days was significantly associated with VRE acquisition (OR 11.47; 95%CI: 1.75-75.35; p =0.011).

DISCUSSION

It was important to eradicate this VRE outbreak emerging for the first time in our hospital. VRE infection is associated with increased likelihood of recurrence, increased mortality, and greater costs than susceptible strain infections (Salgado and Farr, 2003). Previous studies have shown early interventions result in a greater chance of successful control of a VRE outbreak (Lucet *et al*, 2007). All isolates in this outbreak were the *vanA Enterococcus faecium* with a single predominant clone, indicating epidemic spread caused by a single strain of VRE, not an endemic setting with circulation of multiple different strains (Hayden, 2000).

Table 1
Baseline characteristics of cases and controls in the study.

Characteristics	Case n=18 (%)	Control n=30 (%)	OR (95% CI)[a]	p-value[b]
Age ≥75 years	10 (56)	7 (23)	4.10 (0.99-17.39)	0.032
Male sex	7 (39)	19 (63)	0.37 (0.09-1.43)	0.140
Length of stay ≥ 3 weeks	16 (89)	17 (57)	6.12 (1.07-62.09)	0.026
Underlying				
Cancer	6 (33)	8 (27)	1.38 (0.31-5.80)	0.750
Chronic kidney disease	3 (17)	2 (7)	2.80 (0.28-36.17)	0.350
Diabetes mellitus	5 (28)	13 (43)	0.50 (0.11-2.04)	0.360
Antibiotic received for > 3 days				
Vancomycin	4 (22)	3 (10)	2.57 (0.37-19.67)	0.400
Piperacillin/Tazobactam	14 (78)	9 (30)	8.12 (1.80-41.89)	0.002
Carbapenem	10 (56)	10 (33)	2.50 (0.64-9.82)	0.147
Tigecycline	1 (6)	2 (7)	0.82 (0.01-17.03)	>0.99
Third or fourth generation cephalosporin	10 (56)	8 (27)	3.44 (0.85-14.08)	0.07
Hemodialysis	5 (28)	4 (13)	2.50 (0.44-14.63)	0.270
Mechanical ventilation for ≥ 7 days	16 (89)	8 (27)	22.00 (3.60-220.89)	0.0001
Central venous catheter for ≥ 7 days	2 (11)	3 (10)	1.13 (0.09-10.93)	>0.99
Urinary catheter for ≥ 7 days	14 (78)	9 (30)	8.17 (1.80-41.89)	0.002
Nasogastric tube ≥ 7 days	17 (94)	13 (43)	22.23 (2.63-982.19)	0.0005

[a]Calculated by univariate analysis; [b]Adjusted for matched cases and controls.

Two previous cases of VRE in single private rooms did not result in an outbreak, but this outbreak started and rapidly disseminated in this 18-bed medical intermediate care unit. The spread was possibly enhanced by limited space between beds and no available single-patient rooms, resulted in ineffective contact precautions. The National Health Service in the United Kingdom recommend bedside activities require a minimum clear space of 3,600 mm x 3,700 mm around each bed (NHS Estates, 2005). However, the clear space in our unit is 1,900 mm x 3,000 mm, and this is occupied by a mechanical ventilator and a storage shelf, which can contribute to cross contamination. We did not find VRE in any of the environmental samples, however environmental sources cannot be excluded, since sampling was performed selectively on possible potential sources, and routine environmental cleaning might have affected the results. Gowns, gloves, stethoscopes, and healthcare workers' hands should still be considered as potential sources of VRE (Hayden *et al*, 2008). Other potential reservoir for VRE acquisition include colonized patients and proximity to un-isolated positive patients (Boyce *et al*, 1994). Frequent contact of patients by staff, medical students and nurses may facilitate bacterial spread. A previous study found exposure to nurses giving care to the positive case was associated with VRE acquisition (Boyce *et al*, 1994). Frequent movement of patients between medicine units can contribute to further spread among medicine units. No

patients were moved from the medical to surgical units, meaning spread to surgical unit could have occurred via hospital staff.

It was difficult to control the outbreak because of the high occupancy rate and the limited space. Patient cohorting is key to successful control of an outbreak (Jochimsen *et al*, 1999; Lucet *et al*, 2007). We confined VRE-positive patients, potential hospital staff carriers and environmental reservoirs to certain areas, reducing the number of beds to increase the space between beds, decreased the nurse workload and dedicated specific units for positive patients. These measures helped improve compliance with isolation guidelines, and initial control of this VRE outbreak.

There were few staff in the outbreak area keeping them busy doing multiple tasks. Communication within the team is important for daily situation updating, and policy implementation. We used technology, such as emails, group messages by smartphone and online file storage to obtain laboratory results and individual unit situation. This strategy helped shortening the length of face-to-face meetings allowing more time for onsite problem solving.

Emergence of the new cases after first successful control may have resulted from decreased compliance with contact isolation precautions and the lack of available long-term dedicated unit for VRE patients due to administrative problems. Although the number of new VRE cases declined with infection control interventions, failure to totally eradicate VRE has been reported (Lai *et al*, 1998). Maintenance of a low prevalence of VRE colonization after an outbreak can reduce the incidence of VRE infection (Calfee *et al*, 2003). Therefore, it is necessary to reinforce infection control measures and conduct continuous reassessments.

In our study, mechanical ventilation for ≥7 days was significantly associated with VRE acquisition. A previous study found long-term mechanical ventilation is associated with VRE bloodstream infections in pediatric patients (Haas *et al*, 2010). This likely reflects dependent patients who require more frequent care, increasing exposure to VRE from contact with healthcare workers and contaminated environment.

There were several limitations in this study. Multiple interventions were implemented simultaneously making it difficult to determine the effect of any single intervention. However, this is unavoidable during outbreak management. We did not perform the RAPD test on all 209 VRE isolates, due to limited resources. Instead, we randomly selected 32 VRE isolates to evaluate with RAPD: 19 isolates (70%) during the peak of the outbreak in the second week and the other isolates sampled randomly throughout the outbreak. The RAPD patterns for the 32 isolates were similar, but different from the RAPD results from other hospital isolates, suggesting a monoclonal outbreak. We have not yet analyzed the cost-effectiveness of the multifaceted control measures. Further studies are needed to determine cost-effectiveness, particularly in a resource-limited setting, and a low rate of VRE infection.

VRE can easily spread causing an outbreak, particularly in multiple-bed units. However, it was possible to control the outbreak with targeted active surveillance, early infection control interventions, rapid communication within the infection control team, and cohorting VRE cases in dedicated units. Mechanical ventilation ≥7 days was a significant predictive factor for VRE acquisition.

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