Epidemiology and Control of the First Vancomycin-resistant *Enterococcus* Outbreak in a Tertiary-Care Hospital in Bangkok, Thailand

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Presented in part: 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2014), Barcelona, 10–13 May 2014.
Abstract

Background: The aim of this study was to describe the first vancomycin-resistant Enterococci (VRE) outbreak including intervention strategies, molecular epidemiology, and identify risk factors for VRE acquisition in resource-limited setting.

Methods: This is a retrospective study from June 2013 through January 2014. Cases were patients with VRE infection or colonization in medical and surgical units. After the index case was detected in an 18-bed medical intermediate care unit, a bundle of interventions was implemented, including targeted active surveillance for VRE, strict contact precautions, enhanced standard precautions, dedicated units for VRE cases, extensive cleaning of environment, and encourage the restricted use of antibiotics. VRE isolates were characterized by polymerase chain reaction and random amplified polymorphic DNA (RAPD). A prevalence case-control study was conducted to identify predictive factors.

Results: Among 3,699 culture samples from 2,671 patients screened, 74 (2.8%) patients had positive VRE. The positive rate declined from 15.1% in week 1 to 8.2% in week 2, and 1.4% in week 3. During week 4-9, the prevalence was 0-2.7%. However, the prevalence rose to 9.4% in week 10, and subsequently declined. All VRE isolates were Enterococcus faecium and carried the vanA gene. RAPD analysis demonstrated a single predominant clone. Multivariate analysis showed that receipt of mechanical ventilation $\geq$ 7 days was a predictive factor for VRE colonization (OR 11.47, 95% CI 1.75-75.35; p=0.011).

Conclusions: VRE can easily spread and result in outbreak in multiple-bed units. Active surveillance, early infection control intervention, and rapid patient cohorting were important tools in rapid control of the outbreak. Patients requiring mechanical ventilator $\geq$ 7 days were at risk for VRE acquisition.
Keywords: Vancomycin resistance; Enterococcus; Epidemiology; Infection control
Background

Vancomycin-resistant enterococci (VRE) are important nosocomial pathogens that are associated with increased mortality, longer hospital stay, and higher cost in comparison to vancomycin-susceptible enterococci [1]. Effective antimicrobial treatment for VRE infection is limited due to the resistance to many antibacterial agents [2]. Data from United States, Europe, and some countries in Asia showed growing incidence of VRE infection [3-7]. In Thailand, VRE isolates ranged from 0.81-1.9% of clinical isolates [8, 9]. VRE outbreak was reported in many countries in Asia. Evidence of epidemiologic links between inpatients and environment, suggesting cross transmission, assumably from undetected VRE carriers was described in Taiwan [10]. However, there are little experiences and limited data regarding VRE outbreak and intervention strategies in resource-limited settings. In this report, we describe the first VRE outbreak in our hospital, a tertiary-care hospital in Thailand, including molecular epidemiology. We determined risk factors for VRE acquisition, and evaluated the impact of a bundle of control measures.

Methods

Setting

Ramathibodi hospital is a 910-bed university hospital with 12,000 admissions per year. It is a tertiary care center with kidney and bone marrow transplant units; burns unit; 1 medical intensive care unit (ICU), 1 coronary care unit (CCU), 4 surgical ICUs, 1 medical intermediate care unit, and 1 surgical intermediate care unit. Total beds of ICUs, CCU and intermediate care units are 73 beds.
Outbreak

Ramathibodi hospital has initiated targeted active surveillance for VRE colonization since 2012. There were 2 VRE isolates from urine culture of patients at single rooms of medical private unit in May and October 2012. Final identification of both isolates was Enterococcus faecalis carrying of the vanB gene. Active surveillance culture from patients in the adjacent rooms was negative for VRE.

The first hospital VRE outbreak began in early June 2013. The index case was identified from positive VRE in urine culture at an 18-bed medical intermediate care unit, providing an intermediate level of care between medical ICUs and the general medical units. There was no single-patient room for patients requiring contact precautions in this unit. This patient had acute exacerbation of chronic obstructive pulmonary disease, and was admitted to male medical unit on April 14, 2013. He had complicated clinical conditions with pneumonia, pneumothorax, upper gastrointestinal bleeding, and was transferred to medical intermediate care unit on April 16, 2013. Targeted active surveillance for VRE colonization was conducted in 5 closed contact patients in the same cubicle, and two were found positive. Subsequently, VRE screening in all remaining 14 patients in the same unit was performed, which 5 more patients were positive for VRE (figure 1).

Infection Control Measures

All VRE-positive cases were placed under contact isolation. After additional VRE-positive cases were found in all three cubicles at medical intermediate care unit, this unit was frozen; meaning that the 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 from medical intermediate care unit to
other units within 30 days, were tracked and screened for VRE at the end of the first week of the outbreak. Seven positive cases were identified from 19 patients undergoing surveillance cultures, and 1 clinical culture from all three general medical units on the same floor as medical intermediate care unit (figure 1). Universal contact precautions in all VRE-positive medical units were implemented to all patients regardless of their VRE status. No new admission was allowed into 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 medical ICU, all 6 surgical units, and hemodialysis unit. One out of 8 samples from medical intensive care unit, and 4 out of 34 samples from 2 surgical units were positive for VRE (figure 1). Contact precautions in positive cases and contact patients in the same cubicles were performed at these units.

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 were on contact precautions, while the patients in the non-contact VRE zone were on standard precautions. After the number of patients at medicine units decreased, the medical intermediate care unit and one general medicine unit were dedicated for positive VRE patients, on day 12 of the outbreak. On day 26 of the outbreak, one newly renovated unit was temporarily assigned for relocation of positive cases from the previous two dedicated units, and admission was allowed to those medicine units into contact zone, and non-contact VRE zone. In contact cases, follow up surveillance culture was performed every 3 days until 3 negative VRE culture or 1 positive VRE culture was reached; for positive VRE cases, surveillance was performed every
week until 3 negative VRE culture was reached. Discontinuation of contact precautions was
allowed after 3 consecutively negative VRE cultures.

Dedicated patient care items including stethoscope, blood pressure cuff, disposable food
container and drinking water were individually provided for confirmed positive and VRE-contact
cases. Enhanced environmental cleaning was maintained throughout the outbreak. Hydrogen
peroxide vaporization was used as a part of terminal cleaning. Antimicrobial therapy, particularly
vancomycin, was encouraged to discontinue when 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 culture for VRE from rectal swab was performed in contact cases
every 3 days, and non-contact cases who admitted more than 2 weeks in positive VRE units
every 2 weeks during admission. Environmental culture for VRE was collected from bed rails,
overbed tables, chairs, ventilators, vital signs monitors, infusion pump, drip stands, blood
pressure-cuffs, urinals, bedpans, washbasins, faucets, curtains, light switches and patient lift
medical scales. Screening culture was performed on bile-esculin agar plates supplemented with 6
mg/L vancomycin. Suspected colonies of Enterococcus species were selected and sub-cultured
onto sheep blood agar and tested for vancomycin and teicoplanin resistance by the disc diffusion.
Identification to the species level of the suspected organism was performed using conventional
biochemical test and MALDI-TOF (Bruker, Germany). The MIC testing of vancomycin,
teicoplanin, daptomycin, and linezolid was determined by Trek Sensititre broth microdilution
Detection of van resistance genes was performed using PCR method. Whole-cell DNA was extracted and purified using the Qiaquick PCR purification kit (Qiagen, Chatsworth, CA) according to manufacturer’s instruction. The vanA sequence of forward primer was 5’-CATGAATAGAATAAAAGTTGCAATA-3’ and the reverse primer was 5’-CCCCTTTAACGCTAATACGATCAA-3’ [11]. The vanB sequence of forward primer was 5’-AAGCTATGCAAGAAGCCATG-3’ and the reverse primer was 5’-CCGACAATCAAATCATCCTC-3’ [12]. Epidemiological typing was evaluated by random amplified polymorphic DNA (RAPD), using ERIC1 and ERIC2 primers, as previously described [13].

**Prevalence case-control study**

A prevalence case-control study was conducted among colonized and non-colonized VRE patients. Patients who ever stayed in medical intermediate care unit during June 1-30, 2013, and had at least one VRE surveillance culture were enrolled into the study. A case was defined as any patient with at least one positive VRE from surveillance culture after admission, during the first two weeks of the outbreak. Control patients were patients with negative VRE from surveillance culture. Medical records and laboratory reports were reviewed until the first VRE-positive culture in cases, and until discharge from medical intermediate care unit in controls. Data including age, sex, co-morbidities, antibiotic exposure, invasive devices, specific procedures, and length of hospital stay were collected and compared.

**Statistical analysis**
In the case-control study, data analysis was performed using Stata program version 10.0 software (Stata Corp, College Station, TX). Patients were categorized into two groups on the basis of VRE surveillance culture results. Mean (±SD), median (interquartile range, IQR), and frequencies (percentage) were used to represent the patients’ characteristics in each group. Chi square or Fisher’s exact tests were used to compare categorical variables where appropriate. Student’s t-test and Mann-Whitney U tests were used to compare the means and medians of continuous variables. Binary logistic regression analyses were conducted for multivariate analysis to determine the factors associated with positive VRE. Factors with the p-value of less than 0.1 from univariate analysis were included in the multivariate analysis model, except factors which were correlated. Odd ratios (OR) and their 95% confidence interval (CI) were estimated. A p-value of less than 0.05 was considered to be statistically significant.

Ethics

Ethical approval for this study was received from the institutional reviewed board.

Results

Outbreak description

After the index case of VRE detected in urine, targeted active surveillance revealed 11 more patients from this unit that were colonized with VRE. The rate of positive VRE in patients under screening was highest at 15.1% in week 1, decreased to 8.2%, and 1.4% in week 2, and 3, respectively. During week 3-6, the newly positive VRE culture was found in 5 outpatients who were recently discharged from positive VRE units prior to the detection of index case. There was no new positive case in inpatients during week 4-6 (17 days). Cross-sectional VRE screening
was done continuously in patients who were admitted more than 2 weeks at the previous positive VRE units. No cases were detected by the surveillance. A new VRE-positive patient was detected from urine culture in medical intensive care unit in week 8. In week 10, positive rate increased to 9.4%, which was declined to 5.6% and 4.3% in week 11 and 12, respectively. From week 13-23, the positive rate were at 0-2.6% (0-2 cases/week). In week 24, the positive rate increased to 4.7%. However, the positive rate was declined to 0-1.4% on week 27-33 (0-1 case/week) (Figure 2). We observed higher compliance of healthcare workers with contact precautions, and environmental cleaning after moving to dedicated units for VRE than before moving to dedicated units.

Nineteen positive patients died during the study period. However, none was attributed to VRE infection. One positive patient in week 1 subsequently had VRE-associated urinary tract infection, which was diagnosed in week 3.

**Microbiology and molecular study results**

Of 3,699 perianal and rectal samples from 2,671 patients, 209 VRE were isolated from 74 patients and all were identified as *E. faecium*. All isolates showed resistance to vancomycin (MIC $\geq 32 \mu g/ml$) and teicoplanin (MIC $\geq 32 \mu g/ml$), while remained susceptible to daptomycin (MIC $\leq 4 \mu g/ml$) and linezolid (MIC $\leq 2 \mu g/ml$) according to the Clinical Laboratories Standards Institute (CLSI) breakpoints [14]. The PCR results revealed that all of them carried the *vanA* gene. Molecular typing by RAPD of 32 randomly selected isolates throughout the study period revealed the same RAPD patterns, which were different from VRE isolates from another hospital in Thailand. No isolates were identified from environmental culture.
Prevalence Case-control study results

During the study period, 18 cases and 30 controls were enrolled. The mean (±SD) age was 73.25 (±20.72) years in cases, and 61.53 (±16.23) years in control patients (P = .025). In univariate analysis, age ≥75 years, length of stay ≥3 weeks, receipt of Piperacillin/Tazobactam > 3 days, mechanical ventilator ≥7 days, urinary catheter ≥7 days, and nasogastric tube ≥7 days were more frequent in cases (P < .05) (Table 1). Multivariate analysis identified that patients who were on mechanical ventilation ≥7 days was a predictive factor for VRE colonization (OR 11.47, 95% CI 1.75-75.35; P = .011).

Discussion

It is important to eradicate this outbreak for several reasons. VRE outbreak is emerging first time in our hospital. VRE infection is associated with increased recurrence, mortality, and costs, compare to the susceptible strain infection [15]. Moreover, previous study showed that early interventions resulting in successful control of VRE outbreak [16]. All isolates in this outbreak were vanA Enterococcus faecium with a single predominant clone which indicates an epidemic spread caused by a single strain of VRE, not an endemic setting with circulation of multiple different strains [17].

Previous 2 VRE cases in single-patient rooms of medical private unit did not result in outbreak, whereas this outbreak started and rapidly disseminated within the 18-bed medical intermediate care unit. The spread was possibly enhanced by limited space between beds and no available single-patient room, resulted in ineffective contact precautions. As stated in National Health Service, United Kingdom recommendation (NHS Estates, 2005), most activities carried out at
the bedside can be accommodated within the minimum clear space of 3,600 mm x 3,700 mm around each bed. However, the bed space at our unit is 1,900 mm x 3,000 mm, and was occupied by mechanical ventilator and storage shelf, which could contribute to cross contamination. We did not find VRE isolate from our environmental sampling, however environmental sources could not be excluded, as sampling was performed selectively on possible potential sources, and routine environmental cleaning might affect the yield of culture. Gowns, gloves, stethoscope, and healthcare workers’ hands should still be considered as potential sources [18, 19]. Other potential reservoir for VRE are colonized patients, and proximity to un-isolated positive patients is a risk factor for VRE acquisition [20, 21]. Frequent contacts between staffs, medical students, and nurses to patients may facilitate bacterial spreading. Previous study demonstrated that exposure to the nurse giving care to the positive case is associated with VRE acquisition.[21] Frequent movement of patients between medicine units could also contribute to further outbreak expansion among medicine units. No patient was moved from medicine to surgical units; hence, the possible contributing factor was hospital staff carriers who worked in both medicine and surgical units.

It was difficult to control the outbreak in the unit with high occupancy rate and limited space. However, effective patient cohorting is a key to successful control of the outbreak [16, 22, 23]. For this reason, we prioritized patient cohort by confining VRE patients, potential staff carriers and environmental reservoirs in certain area, reducing number of beds to increase space between beds, and decrease nursing workloads, and finally dedicated specific units for positive patients. These measures helped intensify compliance with isolation guideline, and led to initial successful control of this VRE outbreak.
There was a small number of staff in the outbreak control team and requirement to do multiple tasks against time. Therefore, prompt communication within the team is also important in terms of daily situation updating, and policy implementation. We used technology including electronic mails, chat application on mobile phone, and online file storage service to update the laboratory results and individual unit situation in real time. This strategy helped shortening face-to-face meeting and allowed more time for onsite problem solving.

Emergence of new case after first successful control may result from decreased compliance to contact precautions over time and unavailability of long-term dedicated ward for VRE patients due to administrative problem. Although the number of new VRE cases was subsided by infection control interventions, failure to totally eradicate VRE has been reported [24]. Maintenance of low prevalence of VRE colonization after an outbreak would result in a lower incidence of VRE infection [25]. Therefore, it is necessary to reinforce infection control and have continuous assessment.

Our prevalence case-control study identified that mechanical ventilation of ≥ 7 days is a significant predictive factor for VRE colonization. Previous study showed that long-term receipt of mechanical ventilation was one of the risk factors of VRE bloodstream infections in pediatric patients [26]. This characteristic likely represents dependent patients who require more frequent cares, which may pose the exposure to VRE from contact with healthcare workers and contaminated environment. We did not find other factors associated with VRE colonization, such as nasogastric tubes, central venous catheters, and antibiotic use like in previous study[27], possibly due to our small sample size.
There were several limitations in this study. Multiple interventions were implemented simultaneously that made it difficult to evaluate the effect of individual intervention. However, it was an unavoidable circumstance in outbreak management. We performed RAPD for the molecular typing in our study. Although, Multilocus sequence typing (MLST) is unambiguous, highly reproducible and easy for international comparison of isolates, its main drawback is high cost. While RAPD is less discriminative for typing, it has been used for epidemiologic typing in outbreak investigations [28]. It is rapid, easy to use and economical, which is more practical in our resource-limited setting. We did not perform RAPD on all 209 VRE isolates, owing to our resource limitation. However, we randomly selected 32 VRE isolates to undergo RAPD; 19 isolates (70%) at the peak of outbreak on second week, and sporadically sampled afterward during the study period. RAPD pattern from 32 isolates are similar, and different from the RAPD from other hospital isolates, suggesting of 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 resource-limited setting, and low rate of VRE infection.

Conclusion

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

Competing interests
The authors declare no competing interests.

Authors’ contributions

DC, PR and SW designed the study. PS and NC carried out PCR and RAPD studies. PT and PS collected data, and analyzed case-control study. SS, MC and PC participated in data acquisition, tracking and follow-up of cases. DC drafted the manuscript. KT critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Sathapatayavongs B, and Wattanasri S for being management consultants, Linasmita P for providing VRE isolates to compare with our hospital isolates, infection control nurses, staff of microbiology laboratory, and inpatient staff for their great cooperation. We also thank the support of hospital administration.
REFERENCES


19. Hayden MK, Blom DW, Lyle EA, Moore CG, Weinstein RA: Risk of hand or glove contamination after contact with patients colonized with vancomycin-resistant enterococcus or the colonized patients' environment. *Infection control and hospital
epidemiology: the official journal of the Society of Hospital Epidemiologists of America


Figure 1 Spot map of 40 positive VRE cases during the first 4 weeks of VRE outbreak
One dot represented one patients. MIMU Medical intermediate care unit, Med Medical unit, Surg Surgical unit, Immunocomp Immunocompromised patient unit

Figure 2 Timeline of positive VRE cases and interventions during June 2013-January 2014
pos positive, VRE Vancomycin-resistant Enterococcus, MIMU Medical intermediate care unit, MM Male medical unit, FM Female medical unit, HPV Hydrogen peroxide vaporization
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case (n=18)</th>
<th>Control (n=30)</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 75 years</td>
<td>10 (56)</td>
<td>7 (23)</td>
<td>4.10 (0.99-17.39)</td>
<td>0.032</td>
</tr>
<tr>
<td>Male sex, n</td>
<td>7 (39)</td>
<td>19 (63)</td>
<td>0.37 (0.09-1.43)</td>
<td>0.140</td>
</tr>
<tr>
<td>Length of stay ≥ 3 weeks</td>
<td>16 (89)</td>
<td>17 (57)</td>
<td>6.12 (1.07-62.09)</td>
<td>0.026</td>
</tr>
<tr>
<td>Underlying</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>6 (33)</td>
<td>8 (27)</td>
<td>1.38 (0.31-5.80)</td>
<td>0.750</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>3 (17)</td>
<td>2 (7)</td>
<td>2.80 (0.28-36.17)</td>
<td>0.350</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (28)</td>
<td>13 (43)</td>
<td>0.50 (0.11-2.04)</td>
<td>0.360</td>
</tr>
<tr>
<td>Antibiotic receiving &gt; 3 days, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4 (22)</td>
<td>3 (10)</td>
<td>2.57 (0.37-19.67)</td>
<td>0.400</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>14 (78)</td>
<td>9 (30)</td>
<td>8.12 (1.80-41.89)</td>
<td>0.002</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>10 (56)</td>
<td>10 (33)</td>
<td>2.50 (0.64-9.82)</td>
<td>0.147</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1 (6)</td>
<td>2 (7)</td>
<td>0.82 (0.01-17.03)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Third or fourth generation cephalosporins</td>
<td>10 (56)</td>
<td>8 (27)</td>
<td>3.44 (0.85-14.08)</td>
<td>0.07</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>5 (28)</td>
<td>4 (13)</td>
<td>2.50 (0.44-14.63)</td>
<td>0.270</td>
</tr>
<tr>
<td>Mechanical ventilation ≥ 7 days</td>
<td>16 (89)</td>
<td>8 (27)</td>
<td>22.00 (3.60-220.89)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Central venous catheter ≥ 7 days</td>
<td>2 (11)</td>
<td>3 (10)</td>
<td>1.13 (0.09-10.93)</td>
<td>&gt;0.99</td>
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<tr>
<td>Urinary catheter ≥ 7 days</td>
<td>14 (78)</td>
<td>9 (30)</td>
<td>8.17 (1.80-41.89)</td>
<td>0.002</td>
</tr>
<tr>
<td>Nasogastric tube ≥ 7 days</td>
<td>17 (94)</td>
<td>13 (43)</td>
<td>22.23 (2.63-982.19)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
Abbreviations: CI confidence interval, SD standard deviation.

Data are no. (%) of patients. \(^a\) Calculated by univariate analysis. \(^b\) Adjusted for matched case patients and control patients.
Spot map of positive VRE cases during the first 4 weeks of the outbreak (n=40)

- week 1
- week 2
- week 3
- week 4