

Vancomycin-resistant enterococci with reduced daptomycin susceptibility in Singapore: prevalence and associated factors

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SUMMARY

Prevalence of vancomycin-resistant enterococci (VRE) and use of daptomycin are increasing in Asia. To determine the prevalence of daptomycin non-susceptible enterococci (DNSE) and understand factors associated with reduced daptomycin susceptibility in VRE, we conducted a case-control study in a 1600-bed adult tertiary hospital in Singapore. All VRE isolates from inpatients in 2012 were tested for daptomycin susceptibility. Patients with VRE isolates of daptomycin minimum inhibitory concentration (MIC) $\geq 3 \mu\text{g/ml}$ were classified as daptomycin-reduced susceptible VRE (DRS-VRE) and those with daptomycin MIC $< 3 \mu\text{g/ml}$ classified as daptomycin-susceptible VRE (DS-VRE). Medical records were reviewed for clinical and epidemiological data. None of 243 VRE isolates had MIC $> 4 \mu\text{g/ml}$ (DNSE). About half (135, 55%) had reduced susceptibility to daptomycin (MIC 3–4 $\mu\text{g/ml}$). None in the DS-VRE group had prior exposure to daptomycin. After adjusting for age, gender, comorbidity, hospitalization duration, surgical history, indwelling device use, and duration of antibiotic exposure in the prior 3 months, > 1 movement between wards [odds ratio (OR) 0.35, 95% confidence interval (CI) 0.16–0.74, $P = 0.006$] and minocycline resistance (OR 0.45, 95% CI 0.25–0.84, $P = 0.011$) were independently associated with DRS-VRE. Our study suggests that daptomycin exposure, > 1 movement between wards, and resistance to minocycline, were associated with reduced daptomycin susceptibility in VRE.

Key words: DNSE, epidemiology, reduced daptomycin susceptibility, risk factors, VRE.

INTRODUCTION

Vancomycin-resistant enterococci (VRE) have been recognized as a leading cause of hospital-acquired infections (HAI) globally since its emergence in 1986 in the UK and France [1]. VRE infections, with the organism's ability to acquire resistance and its intrinsic resistance to various antibiotics [2] coupled with

the scarcity of reliable antimicrobial therapy for the effective treatment of serious bacteremia [3], present a clinical dilemma for infectious disease physicians. Enterococci are the third most common organism causing nosocomial bloodstream infections, with vancomycin resistance observed in 60% of *Enterococcus faecium* [4].

Daptomycin is one of a limited number of antibiotics with effective bactericidal activity against VRE [5]. Soon after its approval for use by the US Food and Drug Administration in 2003, reports of infections with daptomycin non-susceptible enterococci (DNSE) emerged and cases have been described in patients both with and

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without prior daptomycin exposure [6, 7]. The mechanism for the development of daptomycin non-susceptibility in enterococci has not been well elucidated [8], although there is the suggestion that DNSE colonization or infection increased with increasing daptomycin use [9, 10].

In Singapore, daptomycin was approved for use in 2008 and utilization has increased over the years [11]. Although daptomycin non-susceptible *Staphylococcus aureus* bacteraemia have been reported, little is known about the epidemiology of DNSE in Singapore and Asia. Since the first report of VRE in 1994, VRE rates have increased in Singapore, mirroring the pattern seen in Europe a decade earlier [12]. This study aims to determine the prevalence of DNSE in an Asian country with an increasing VRE prevalence and daptomycin use, and to understand the factors associated with reduced daptomycin susceptibility in VRE.

METHODS

We conducted a case-control study in a 1600-bed adult tertiary hospital in Singapore. The prevalence of clinical VRE infections in the hospital increased from 9·97/100 000 deaths and discharges in 2010 to 77·11/100 000 in 2012. Correspondingly, daptomycin utilization increased by 6·4-fold from an average monthly defined daily dose/1000 patient-days of 0·2 in 2010 to 1·28 in 2012. The hospital had an active VRE screening programme where surveillance cultures were taken from 'high-risk' patients who were on renal dialysis or had a history of prior hospitalization in a foreign or private hospital in the preceding 12 months, on admission to the hospital. In addition, whenever a VRE infection was detected in an inpatient, contact screening of all patients who were in the same ward as the index patient for the duration of the patient's hospital stay prior to isolation was carried out.

Clinical and surveillance samples were plated on selective medium chromID VRE plates (bioMérieux, USA) and incubated aerobically at 35–37 °C for 48 h.

Suspect colonies were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik GmHB, Germany); antimicrobial susceptibility testing was performed for ampicillin, ciprofloxacin, clindamycin, erythromycin, gentamicin (high level), linezolid, minocycline, penicillin, teicoplanin, and vancomycin, using the Vitek 2 System (bioMérieux) [13]. Select strains that were identified with low discrimination were confirmed using an in-house multiplex polymerase chain reaction based on primer sets published by Dutka-Malen *et al.* [14].

All VRE isolates from patients hospitalized from 1 January to 31 December 2012 were tested for daptomycin susceptibility using the Etest (bioMérieux). We defined daptomycin-reduced susceptible VRE (DRS-VRE), by using the criteria of the Clinical and Laboratory Standards Institute [15], as patients with a VRE isolate with daptomycin minimum inhibitory concentration (MIC) $\geq 3 \mu\text{g/ml}$ from screening or clinical cultures. Daptomycin-susceptible VRE (DS-VRE) was defined as patients with VRE isolates with daptomycin MIC $< 3 \mu\text{g/ml}$. For patients with VRE isolated from multiple samples, we only included the first VRE isolate and examined exposures prior to the first isolation of VRE.

We reviewed medical records for clinical and epidemiological data, and recorded patients' demographics, comorbidities, prior healthcare exposures, current hospitalization experiences, antibiotic exposures and durations within the past 3 months, antimicrobial susceptibilities and genotypes of VRE. Additionally, Charlson's comorbidity index [16] was derived using coding algorithms. Clinical infection was determined as part of the hospital infection control unit's routine workflow, after review of patients for signs and symptoms of infection based on guidelines by the Centers for Disease Control and Prevention's National Healthcare Safety Network.

We computed odds ratios (ORs) and 95% confidence intervals (CIs), to compare differences in exposures and covariates between DRS-VRE and DS-VRE. Next, we constructed a multiple logistic regression model to control for confounding and assess for independent factors associated with DRS-VRE colonization or infection. We included variables decided *a priori* as factors associated with reduced daptomycin susceptibility based on prior knowledge from literature review to be associated with DNSE colonization and infection. We used the Hosmer–Lemeshow test to check for goodness of fit for the model. All statistical analyses were performed using Stata v. 12 (Stata Corp., USA).

Ethical approval

This study was approved by the Domain Specific Review Board of the National Healthcare Group, Singapore (DSRB- 2013/00228).

RESULTS

In total, 243 VRE isolates were analysed, including both VanA (133, 55%) and VanB (110, 45%) genotypes. The majority of VREs (213, 88%) were isolated from screening cultures. Of the VRE clinical infections, urine was

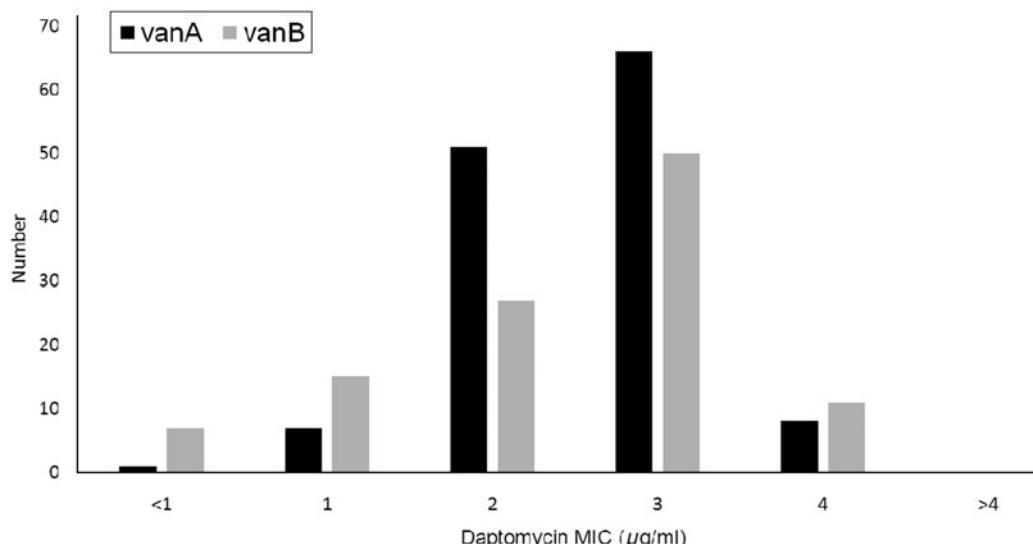


Fig. 1. Daptomycin minimum inhibitory concentration (MIC) levels by vancomycin-resistant enterococcus genotype.

found to be the main source (67%). The daptomycin MICs were distributed similarly between genotypes (Fig. 1).

None of the isolates was determined to be daptomycin non-susceptible based on the Clinical Laboratory Standards Institute's antimicrobial susceptibility testing standards of MIC $>4 \mu\text{g}/\text{ml}$ [15]. However, about half of the patients (135, 55%) had reduced susceptibility to daptomycin with MIC 3–4 $\mu\text{g}/\text{ml}$. Two-thirds (20/30) of clinical isolates had reduced daptomycin susceptibility compared to 54% (115/213) of screening isolates (OR 1.70, 95% CI 0.72–4.27, $P = 0.191$).

There was no difference in age and gender between the DRS-VRE and DS-VRE groups (Table 1). Both groups had similar prior healthcare exposures, with more than 70% having been hospitalized in the preceding year. Of the 135 DRS-VRE patients, more than two-thirds (67.4%) had a Charlson comorbidity score of ≥ 3 and 76.3% were immunosuppressed. The mean duration of hospital stay for all VRE patients was 26 days. Of note, DRS-VRE isolates (14.8%) were more likely to be from clinical infections than DS-VRE isolates (9.3%). Interestingly, patients who had >1 movement between wards during the hospitalization episode of VRE isolation (OR 0.57, 95% CI 0.33–0.98, $P = 0.040$) were less likely to have DRS-VRE. Compared to DRS-VRE, DS-VRE patients were more likely to have experienced >1 movement between beds and to have been exposed to indwelling devices during hospitalization.

None of the DS-VRE patients in the study cohort had been exposed to daptomycin in the preceding 3 months, compared to three of the DRS-VRE patients. An antimicrobial exposure in the prior 3 months was not significantly different between the two groups, with exposure to penicillin being the longest. Duration of exposure to cephalosporin (OR 0.94, 95% CI 0.89–1.00, $P = 0.044$) was observed to be protective against DRS-VRE. Of note, VRE isolates that were resistant to minocycline were half as likely to have reduced daptomycin susceptibility as those who were not (OR 0.49, 95% CI 0.28–0.85, $P = 0.012$). In both DRS-VRE and DS-VRE, $>90\%$ of VRE isolates were resistant to erythromycin.

There was no difference in VRE genotypes, hospitalization exposure in the prior 1 year, number of movements between beds during hospitalization, number of types of antibiotics exposed to, exposure to surgical interventions, and admission to the intensive care unit, between DRS-VRE and DS-VRE patients. Four (20%) out of the 20 DRS-VRE infected patients died during the hospitalization, compared to one (10%) out of 10 DS-VRE patients [OR 2.00, 95% CI 0.26–15.62, $P = 0.640$ (Fisher's exact test)].

In multivariate analyses, the two independent factors associated with DRS-VRE isolation were >1 movement between wards (OR 0.35, 95% CI 0.16–0.74, $P = 0.006$) and resistance to minocycline (OR 0.45, 95% CI 0.25–0.84, $P = 0.011$), after adjusting for age, gender, Charlson's comorbidity score ≥ 3 , duration of

Table 1. Univariate and multivariate analyses of risk factors associated with daptomycin-reduced susceptible vancomycin-resistant enterococci (DRS-VRE)

Factor	DRS-VRE (N = 135)	DS-VRE (N = 108)	OR (95% CI)	P	aOR (95% CI)	P
Demographics						
Age, years, mean (s.d.)	73.0 (14.3)	71.5 (14.2)	1.01 (0.99–1.03)	0.416	1.01 (0.99–1.03)	0.447
Female gender	72 (53.3)	50 (46.3)	1.33 (0.80–2.20)	0.276	1.27 (0.70–2.32)	0.418
Comorbidities						
Charlson score (≥ 3)	91 (67.4)	63 (58.3)	1.48 (0.87–2.50)	0.145	1.19 (0.66–2.14)	0.552
Immunosuppressive state*	103 (76.3)	74 (68.5)	1.48 (0.84–2.61)	0.177		
Dementia	26 (19.3)	18 (16.7)	1.19 (0.62–1.25)	0.602		
Liver disease	9 (6.7)	12 (11.1)	0.57 (0.23–1.41)	0.225		
Exposure to healthcare settings and environments						
Nursing-home resident	18 (17.5)	14 (17.1)	1.03 (0.48–2.22)	0.943		
Hospitalization in past 1 year	99 (73.3)	78 (72.2)	1.06 (0.60–1.87)	0.847		
Current hospitalization						
Duration of stay, days, mean (s.d.)	26.2 (42.0)	26.4 (27.6)	1.00 (0.99–1.01)	0.975	1.00 (0.99–1.02)	0.471
Movements between wards, mean (s.d.)	1.5 (2.4)	1.7 (2.5)	0.96 (0.86–1.07)	0.442		
>1 movement between wards	38 (28.1)	44 (40.7)	0.57 (0.33–0.98)	0.040	0.35 (0.16–0.74)	0.006
Movements between beds, mean (s.d.)	2.0 (3.1)	2.4 (3.1)	0.96 (0.88–1.04)	0.277		
>1 movement between beds	53 (39.3)	53 (49.1)	0.67 (0.40–1.12)	0.126		
Indwelling device use†	10 (7.4)	10 (9.3)	0.78 (0.31–1.96)	0.602	0.74 (0.12–4.58)	0.743
Had surgery	70 (51.9)	51 (47.2)	1.20 (0.73–2.00)	0.473	1.61 (0.88–2.94)	0.131
ICU admission	16 (11.9)	13 (12.0)	0.98 (0.45–2.14)	0.965	2.21 (0.43–11.29)	0.336
Antibiotic exposure in past 3 months						
Exposure to any antibiotics	132 (97.8)	102 (94.4)	2.59 (0.63–10.60)	0.186		
Types of antibiotics exposed, mean (s.d.)	3.1 (1.5)	3.1 (1.8)	1.00 (0.88–1.13)	0.983		
Aminoglycoside, days, mean (s.d.)	2.2 (8.5)	1.3 (2.5)	1.03 (0.97–1.10)	0.350	1.06 (0.97–1.16)	0.208
Carbapenems, days, mean (s.d.)	4.2 (8.1)	4.0 (7.2)	1.00 (0.98–1.04)	0.863	1.02 (0.98–1.06)	0.425
Cephalosporin, days, mean (s.d.)	1.8 (3.5)	3.2 (6.6)	0.94 (0.89–1.00)	0.044	0.95 (0.89–1.02)	0.157
Daptomycin, days, mean (s.d.)	0.1 (0.1)	0.0 (0.0)	1.79 (0.50–6.35)	0.371	1.86 (0.46–7.53)	0.382
Fluoroquinolone, days, mean (s.d.)	2.8 (6.5)	4.5 (14.1)	0.98 (0.96–1.01)	0.234	0.98 (0.96–1.01)	0.270
Penicillin, days, mean (s.d.)	11.2 (11.8)	9.6 (8.6)	1.02 (0.99–1.04)	0.221	1.02 (0.99–1.05)	0.137
Polymyxin B, days, mean (s.d.)	0.3 (2.9)	0.8 (4.5)	0.96 (0.88–1.04)	0.290	0.95 (0.86–1.04)	0.290
Tetracycline, days, mean (s.d.)	0.1 (0.7)	0.1 (0.8)	0.97 (0.69–1.37)	0.875	0.92 (0.59–1.43)	0.711
Vancomycin, days, mean (s.d.)	3.7 (7.7)	3.8 (6.7)	1.00 (0.96–1.03)	0.865	0.98 (0.94–1.04)	0.575
Antibiotic resistance pattern of VRE						
Resistant to teicoplanin	73 (54.1)	61 (56.5)	0.91 (0.55–1.51)	0.708		
Resistant to minocycline	31 (23.0)	41 (38.0)	0.49 (0.28–0.85)	0.012	0.45 (0.25–0.84)	0.011
Resistant to gentamicin	63 (47.0)	44 (40.7)	1.29 (0.77–2.15)	0.329		
Resistant to erythromycin	126 (93.3)	106 (98.1)	0.26 (0.06–1.25)	0.093		
Others						
VanA VRE genotype	74 (54.8)	59 (54.6)	0.99 (0.60–1.65)	0.977		
Clinical infection	20 (14.8)	10 (9.3)	1.70 (0.76–3.81)	0.195		

OR, Odds ratio; aOR, adjusted odds ratio; CI, confidence interval.

Values are n (%) unless otherwise indicated.

The percentage is of patients for whom data were available, i.e. excluding the missing values.

* Includes one or more of the following: diabetes with and without chronic complication, leukaemia, lymphoma, any malignancy without metastases, metastatic solid tumour, connective tissue disease, HIV, renal disease.

† Central venous pressure line, endotracheal tube, peripherally inserted central catheter, urinary catheterization.

hospital stay, history of surgery, presence of indwelling devices, and duration of antibiotic exposure (to aminoglycoside, carbapenem, cephalosporin, daptomycin, fluoroquinolone, penicillin, polymyxin B, tetracycline, vancomycin) in the prior 3 months.

DISCUSSION

Limited data is available on the risk factors associated with DNSE. To the best of our knowledge, this is the first study in Asia to assess the epidemiology of DNSE

and factors associated with reduced daptomycin susceptibility in VRE. Daptomycin non-susceptibility ($\text{MIC} > 4$) was not observed in our patient cohort, although 55% had reduced susceptibility to daptomycin ($\text{MIC } 3\text{--}4 \mu\text{g/ml}$). We observed similar daptomycin resistance patterns in genotypes VanA and VanB. A surveillance study on VRE in Taiwan during the same period also did not report any daptomycin non-susceptibility [17]. The importance of reduced susceptibility to daptomycin has been under-recognized. The presence of mutations associated with daptomycin resistance have been recently observed in VRE isolates with daptomycin MIC $3\text{--}4 \mu\text{g/ml}$, raising concerns about the effectiveness of daptomycin therapy in patients with such VRE infections [18]. Enterococcal isolates with daptomycin MIC $3\text{--}4 \mu\text{g/ml}$ have been observed to harbour mutations in *liaFSR*, a three-component regulatory system controlling cell-envelope stress response, that have been linked with the emergence of daptomycin resistance in enterococci [19]. Another competing pathway associated with *ycyFGHIJ* mutations have been reported to play an important role in the successful treatment of DRS-VRE infections and prevention of development into daptomycin non-susceptibility ($\text{MIC} > 4 \mu\text{g/ml}$) [20].

In our study, we observed a relatively strong association between the duration of daptomycin exposure and daptomycin-reduced susceptibility ($\text{OR } 1.86$, 95% CI $0.46\text{--}7.53$), although statistical significance could not be reached due to the limited number of exposed patients. Another study conducted at the Detroit Medical Center similarly could not conclude that prior daptomycin exposure was a predictor for DNSE, due to the rare occurrence of prior daptomycin exposure [21]. Our findings support the observations of an earlier study at the University of Iowa Hospitals and Clinics which suggested the emergence of daptomycin non-susceptibility under the antimicrobial pressure of prior daptomycin exposure [9]. In that study, the number of patients with DNSE colonization or infection increased rapidly with the surge in daptomycin use.

Although immunosuppression and multiple comorbid conditions were reported to be associated with DNSE [9, 21], they were not observed to be associated with DRS-VRE in our study cohort. Our findings suggest that an immunosuppressive state might not be necessary for the development of daptomycin-reduced susceptibility. We also did not observe an increased risk of DRS-VRE in patients with indwelling devices, although exposure to such

devices was previously reported to be an independent risk factor for DNSE [4].

Interestingly, we observed that VRE isolates that were resistant to minocycline were negatively associated with reduced daptomycin susceptibility. Our observation corroborates the finding of a recent study which reported minocycline resistance in VRE isolates that were susceptible to daptomycin from infected urinary stents in renal transplant patients [22]. Further studies are needed to better understand the mechanisms for antimicrobial resistance.

In our study, patients who had moved between wards were less likely to have DRS-VRE than those who had not ($\text{OR } 0.35$, 95% CI $0.16\text{--}0.74$). This was not previously reported by studies on DNSE and more studies are warranted before any conclusions can be made.

Our study may have been limited by the small number of patients with prior daptomycin exposure. Nonetheless, we identified some interesting factors associated with reduced daptomycin susceptibility in VRE in an Asian country with increasing VRE prevalence and daptomycin use. Our findings suggest an association between daptomycin exposure and reduced daptomycin susceptibility.

CONCLUSIONS

DNSE was not observed in our study population; however, 55% had reduced daptomycin susceptibility with $\text{MIC } 3\text{--}4 \mu\text{g/ml}$. Such reductions in susceptibility to daptomycin have important clinical implications as mutations associated with daptomycin resistance have been observed. Our study suggests that daptomycin exposure, movement between wards, and resistance to minocycline were associated with reduced daptomycin susceptibility in VRE. Active surveillance for reduced susceptibility and non-susceptibility to daptomycin in VRE colonized or infected patients with prior daptomycin therapy is crucial, as the use of daptomycin increases against the backdrop of the VRE surge in Asia.

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DECLARATION OF INTEREST

None.

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