Original Article

Screening for Vancomycin-resistant Enterococci Using Stools Sent for *Clostridium difficile* Cytotoxin Assay is Effective: Results of a Survey of 300 Patients in a Large Singapore Teaching Hospital

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Abstract

Introduction: To assess the efficacy of screening stools sent for *Clostridium difficile* cytotoxin assay (CDTA) for surveillance of vancomycin-resistant enterococci (VRE). Materials and Methods: From April to May 2005, all stools submitted for CDTA were also cultured for VRE using vancomycin containing culture media. Isolates were identified to species level and vancomycin resistance confirmed, followed by polymerase chain reaction (PCR) for detection of vancomycin resistance genes and DNA fingerprinting. Over 2 consecutive days during that period, stool specimens or rectal swabs were also obtained from all patients in high-risk units (haematology, oncology, renal and intensive care). Fifty-one patients in each group were compared in terms of VRE risk factors previously identified. Results and Discussion: The prevalence of VRE in both groups was similar [3/204 (1.5%) in the CDTA arm and 1/97 (1.0%) in the high-risk arm; *P* = 1.0, Fisher's exact test]. Prevalence of risk factors for VRE colonisation, including age, duration of hospitalisation, exposure to antibiotics, exposure to surgical procedures, presence of malignancy and diabetes mellitus was similar in both groups (*P* > 0.05). Only renal failure (*P* < 0.05) was more common in the high-risk group. All 4 isolates of VRE identified were genetically distinct by variable number tandem repeat (VNTR) typing; 3 were *Enterococcus faecium* (2 with the vanB gene, 1 with vanA) and one *E. faecalis*. Conclusion: Less than 2% of our high-risk patients are VRE carriers. In-hospital VRE screening using stools sent for CDTA is a simple, reasonable surrogate for screening individual high-risk patients as the patient risk profile is similar and the yield comparable in a low-prevalence setting.

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Introduction

Vancomycin-resistant enterococci (VRE) have emerged as a major cause of nosocomial infections worldwide over the past 2 decades. In an effort to prevent the spread of vancomycin resistance, the United States Centers for Disease Control and Prevention have recommended that culture surveys of stools or rectal swabs be performed periodically, with the frequency determined by the population at risk and the hospital units involved.[1] Screening in a low-prevalence setting has been found to be effective in eradicating VRE.[2,3] Ongoing surveillance has also been reported to be beneficial in controlling endemic VRE.[4]

Risk factors for nosocomial colonisation or infection with VRE previously identified include advanced age, prolonged duration of hospitalisation, interhospital transfer, exposure to antibiotics,[5,6] exposure to surgical procedures and presence of an immunocompromised state.[6,7] Positive *Clostridium difficile* cytotoxin assay (CDTA) was also associated with VRE colonisation, suggesting that patients at risk for *C. difficile* infection may serve as reservoirs for VRE, possibly because of common risk factors.[8]

VRE have been identified in Singapore since 1994.[9] The first reported outbreak was of 26 cases in December 2004 in another large tertiary hospital in Singapore.[10] In response

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to that outbreak and a subsequent larger outbreak in that same hospital, baseline studies in high-risk patients in other Singapore hospitals were performed. This two-month, prospective, three-part study was designed to assess the efficacy of screening stools sent for *C. difficile* cytotoxin assay for estimating the hospital prevalence of VRE.

Materials and Methods

The study hospital is a 900-bed tertiary teaching hospital serving southwestern Singapore.

Part 1: CDTA Study

From 1 April 2005 to 31 May 2005, all stools sent to the microbiology laboratory for CDTA by the treating clinicians were also screened for VRE as described below. All stool samples sent for CDTA were screened for VRE, irrespective of the clinical indications for CDTA testing.

Part 2: Point Prevalence Study

The point prevalence study included all patients in high-risk units in our hospital, namely the haematology, oncology, renal and intensive care units. Over a period of 2 consecutive days (12 May 2005 and 13 May 2005), stool specimens or rectal swabs were obtained from consenting patients and screened for VRE.

Part 3: Cohort Study

The objective of the cohort study was to compare the risk profile for VRE colonisation, in patients involved in the CDTA study and the point prevalence study.

A patient in the CDTA group was defined as any of the first 51 patients who had stools sent for CDTA during the study period from 1 April 2005 to 31 May 2005. Patients were identified from the microbiology request records.

A patient in the high-risk group was defined as any patient in a high-risk unit who had participated in the point prevalence study. Patients who had stools sent for CDTA testing were excluded from the "high-risk" group.

The first 51 patients from the CDTA group were identified and compared with 51 randomly selected patients from the high-risk group. Patient medical records and computerised databases were reviewed for demographics, underlying disease, length of hospital stay, institutional transfer, procedures performed during the hospitalisation and presence of indwelling devices. We recorded the prevalence of inpatient antimicrobial use in each group by review of data from the inpatient medical records and computerised pharmacy databases.

Microbiology

All stool samples and rectal swabs collected were tested for VRE. Culture specimens were inoculated onto Enterococosel agar (BBL Microbiology Systems, Cockeysville, MD), supplemented with vancomycin 6 μg/mL. Plates were examined after 24 and 48 hours of incubation at 37°C. Isolates from this plate were identified to species level by standard microbiologic techniques, including pyrrolidonyl-aryl-amidase, catalase, bile esculin hydrolysis and growth in 6.5% NaCl.

All enterococci isolates were tested for levels of vancomycin resistance and vancomycin-resistance genotype. Antimicrobial susceptibility testing was determined using the Vitek 2 automated susceptibility panel (BioMerieux, France), and minimum inhibitory concentrations confirmed with E test (AB Biodisk, Solna, Sweden). The presence of specific vancomycin-resistance genotype *VanA* or *VanB* was detected by use of polymerase chain reaction (PCR) assays based on previously described protocols.[11] Isolates were further characterised by variable number tandem repeats (VNTRs) as previously described by Top et al.[12]

Statistical Methods

Data were abstracted into a standardised form, reviewed for errors, and statistically analysed using the Statistical Package for the Social Sciences (SPSS), version 12.0. Categorical variables were analysed by the χ^2 or Fisher's exact test. Student's *t*-test was used to compare the means of continuous variables. *P* values of <0.05 were considered significant. All *P* values were based on two-sided tests.

Results

During the study period, 204 patients had stools sent for CDTA and were included in the first part of the study. For the second part of the study, all patients in the high-risk units agreed to participate in the point prevalence survey and stools or rectal swabs obtained for screening. A total of 97 patients from high-risk units were screened. For the cohort study, 51 patients in each of the above 2 groups were selected and compared in terms of VRE risk factors.

Patients in the CDTA group and high-risk group were comparable in age, gender as well as in ethnicity and average length of hospital stay. The details are listed in Table 1.

In almost all instances, prevalence of risk factors for VRE colonisation was similar in both groups. In univariate analysis, no significant differences existed with regard to length of hospital stay, procedures performed during the hospitalisation and presence of indwelling devices. No differences were found with respect to inpatient antimicrobial use, including vancomycin and cephalosporins.

Renal failure was found to be more common in the high-risk group than in the CDTA group [15 (29%) versus 5 (9.8%); $P = 0.01$]. Diabetes mellitus was also more common

Table 1. Comparison of Patients in the CDTA Group and High-risk Group*

Variable	CDTA patients (n = 51)	High-risk patients (n = 51)	*P* value
Baseline characteristics			
Male, %	43.1	52.9	0.33
Age, y	57.8 ± 20.3	58.4 ± 17.1	0.89
Chinese, %	66.7	52.9	0.23
Clinical characteristics			
Duration of hospitalisation, d	11.3 ± 16.9	10.1 ± 10.0	0.65
Time in intensive care, d	4.6 ± 10.6	3.8 ± 9.1	0.71
Institutional transfer, n (%)	3 (5.9)	3 (5.9)	1.0
Underlying conditions, n (%)			
Diabetes	12 (23.5)	21 (41.2)	0.06
Renal failure	5 (9.8)	15 (29.4)	0.01
Haematologic cancer	7 (13.7)	5 (9.8)	0.54
Other cancer	15 (29.4)	12 (23.5)	0.50
Invasive procedures or devices, n (%)			
Gastrointestinal surgery	3 (5.9)	6 (11.8)	0.49
Central arterial or venous catheter	20 (39.2)	20 (39.2)	1.0
Urinary catheter	18 (35.3)	19 (37.3)	0.84
Nasogastric tube	16 (31.4)	12 (23.5)	0.38
Mechanical ventilation	15 (29.4)	10 (19.6)	0.25
Anti-infective therapy, n (%)			
Aminoglycosides	17 (33.3)	14 (27.5)	0.52
Cephalosporins	28 (54.9)	26 (51.0)	0.69
Fluoroquinolones	18 (35.3)	11 (21.6)	0.12
Metronidazole	11 (21.6)	8 (15.7)	0.45
Vancomycin	14 (27.5)	13 (25.5)	0.82
Multiple antibiotics†	32 (62.3)	25 (49.0)	0.16

* Values are expressed as the mean ± SD unless otherwise noted

† Defined as having 2 or more antibiotics administered during period of hospitalisation

in the high-risk group, but this was not statistically significant [21 (41%) versus 12 (24%); $P = 0.06$]. Renal failure was positively associated with diabetes in this sample ($P = 0.001$). The prevalence of risk factors for VRE colonisation is further detailed in Table 1.

VRE were isolated from 3 of 204 patients in the CDTA group, giving a rate of 1.5% (95% CI, 0.5 to 4.2). In the high-risk group, 1 of 97 patients was positive for VRE, giving a rate of 1.0% (95% CI, 0.2 to 5.6). The prevalence of VRE in both groups was similar ($P = 1.0$, Fisher's exact test).

Species identification demonstrated that 3 isolates were *Enterococcus faecium* and 1 *E. faecalis*. Of the 3 *E. faecium* isolates, 2 demonstrated the VanB genotype and 1 was VanA. All 4 isolates of VRE identified were genetically distinct by VNTR typing.

Discussion

Enterococci resistant to vancomycin were first reported in England in 1988.[13] Enterococci are the fourth most common pathogens associated with nosocomial infection in intensive care units,[14] with VRE accounting for 28.5% of all enterococcal isolates.[15] Preventing the spread of VRE, for which very limited bactericidal therapy is available, should be a high priority in all hospitals. Current screening strategies that have been shown to be effective include screening of high-risk patients, as well as surveillance programmes that encompass all patients.[3,4,7]

Less than 2% of our high-risk patients are VRE carriers. All 4 isolates of VRE identified were genetically distinct, suggesting that there is very limited local transmission of VRE at our hospital. However, resistant strains or possibly mobile genetic elements coding for resistance genes are being introduced periodically from other institutions.[16]

In a review of the relationship between VRE and *C. difficile*, Gerding[17] first postulated the relationship between infection with these organisms. Safdar and Maki,[7] in a large systematic review, also showed the commonality of risk factors in patients infected with antimicrobial-resistant bacteria including methicillin-resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase producing gram-negative bacilli, VRE and *C. difficile*. These risk factors are similar to those explored in our study, including type and extent of antimicrobial usage, advanced age, extended length of stay and underlying disease.

Our analysis shows that the patient risk profile, with the exception of renal failure, is similar in patients screened for *C. difficile* and patients at high risk for VRE infection or colonisation. The prevalence of VRE was also similar in both groups. Hence, VRE screening using stools sent for CDTA gives a comparable yield to screening using actively collected stool specimens and rectal swabs in a low prevalence setting.

A previous study by Ray et al[18] found that a *C. difficile*-focused surveillance strategy identified 67% of all colonised patients. When combined with the screening of high-risk patients, 87% of all colonised patients were identified. In another study, Katz et al[19] found that a *C. difficile*-focused surveillance programme detected 73% of what an intensive surveillance programme (involving patients on high-risk units and selected low-risk units, those on wards with VRE-colonised patients, and individuals admitted from other facilities with VRE) found, with substantial cost savings. In our study, 75% (3 of 4) of detected VRE-colonised patients were identified through screening stools sent for CDTA.

Testing stools sent for CDTA eliminates the need for a separate specimen collection, which may even increase the risk of transmission from colonised patients. It also involves considerable savings in terms of manpower and administrative costs. It is also less invasive and might be less of a problem in neutropenic patients for whom rectal

swabs are sometimes a concern. Culturing stools sent for CDTA for VRE is a strategy that is easily contained and managed within a busy clinical laboratory and might be considered in a low-resource setting. While unable to identify all VRE cases, screening stools sent for CDTA gives an accurate reflection of the point prevalence of VRE, as shown in our study. We would like to propose in-hospital screening using stools sent for CDTA as a simple, reasonable surrogate for screening individual high-risk patients in a low-prevalence setting such as ours.[20] The low prevalence (1.0% of high-risk patients) and genetically unique isolates found in this study show a lack of endemicity of VRE in our hospital. In hospitals where VRE are not endemic, screening, accompanied by infection control measures, is crucial to prevent endemicity of VRE.

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