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Detection and Characterization of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* Isolates in Canada: Results from the Canadian Nosocomial Infection Surveillance Program, 1995–2006▽†

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We describe the epidemiology of heterogeneously resistant *Staphylococcus aureus* (hVISA) identified in Canadian hospitals between 1995 and 2006. hVISA isolates were confirmed by the population analysis profiling-area under the curve method. Only 25 hVISA isolates (1.3% of all isolates) were detected. hVISA isolates were more likely to have been health care associated (odds ratio [OR], 5.1; 95% confidence interval [CI], 1.9 to 14.2) and to have been recovered from patients hospitalized in central Canada (OR, 3.0; 95% CI, 1.2 to 7.4). There has been no evidence of vancomycin "MIC creep" in Canadian strains of methicillin (meticillin)-resistant *S. aureus*, and hVISA strains are currently uncommon.

Strains of *Staphylococcus aureus* with heterogeneous resistance to vancomycin (hVISA) have MICs considered to be in the susceptible range (≤2.0 μg/ml) but contain a subset of the bacterial population that expresses the resistance phenotype (7). This heteroresistance may represent a preliminary step toward the development of vancomycin-intermediate *S. aureus* (VISA) (6). Infections due to hVISA have been associated with treatment failure and prolonged or persistent bacteremia in some studies (2, 3, 16) but not in others (9, 11, 19). Although hVISA strains have been identified in many parts of the world, relatively little is known about their prevalence or epidemiology (3, 7, 15, 17). The most appropriate method for the laboratory identification of hVISA is also uncertain. Currently, the "gold standard" for hVISA detection is the population analysis profiling-area under the curve (PAP-AUC) method, but this method is laborious and is not routinely used in clinical microbiology laboratories (30). The purpose of this study was to describe the epidemiology of hVISA recovered from hospitalized patients in Canada. The performance of the Etest macromethod (EAS 003; AB Biodisk, Solna, Sweden) and the glycopeptide resistance detection (GRD) Etest strips (AB Biodisk) was also evaluated.

Prospective surveillance for methicillin (meticillin)-resistant *S. aureus* (MRSA) in hospitalized patients has been conducted by the Canadian Nosocomial Infection Surveillance Program (CNISP) since 1995, involving 48 sentinel hospitals across the country working in collaboration with the Centre for Communicable Diseases and Infection Control and the National Microbiology Laboratory, both of the Public Health Agency of Canada (24, 25). Most (94%) of the hospitals are tertiary-care teaching hospitals. Surveillance for MRSA was laboratory based, and only hospitalized patients newly identified as colonized or infected with MRSA were included. The presence of infection caused by MRSA was determined using standard surveillance definitions (8). To maintain site-specific confidentiality, the hospitals were grouped into three geographic regions: western Canada (16 hospitals from the provinces of British Columbia, Alberta, Saskatchewan, and Manitoba), central Canada (24 hospitals from Ontario and Quebec), and eastern Canada (8 hospitals from New Brunswick, Nova Scotia, and Newfoundland and Labrador).

MRSA isolates obtained from 1995 to 2006 were included in this study. Only one isolate, generally the first, from each patient was included in the study. Vancomycin susceptibility testing was done by broth microdilution (4). A total of 475 isolates (all 271 with a vancomycin MIC of 2.0 μg/ml and a geographically representative subset of 204 isolates with a vancomycin MIC of 1.0 μg/ml) were selected for additional testing to identify hVISA. Isolates were screened for the presence of the hVISA phenotype by using the Etest macromethod and GRD Etest strips in accordance with the manufacturer's instructions. All isolates identified by the Etest macromethod or GRD Etest strips as potential hVISA isolates ($n = 57$) and 18

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TABLE 1. *In vitro* susceptibility to vancomycin and detection of hVISA in MRSA isolates obtained in surveillance done by the CNISP, 1995 to 2006

Yr	No. (%) of isolates[a] with vancomycin MIC (μg/ml) of:			No. screened for hVISA phenotype	No. (%) of hVISA strains
	≤0.5	1.0	2.0		
1995	94 (47)	101 (50)	7 (8)	19	1 (5.3)
1996	53 (19)	222 (76)	16 (5)	28	2 (7.1)
1997	80 (13)	502 (84)	16 (3)	30	4 (13.3)
1998	303 (40)	453 (59)	6 (1)	20	2 (10.0)
1999	263 (27)	684 (69)	44 (4)	58	5 (8.6)
2000	174 (50)	166 (47)	11 (3)	25	1 (4.0)
2001	187 (49)	197 (51)	4 (1)	18	1 (5.6)
2002	32 (8)	324 (82)	40 (10)	61	2 (3.3)
2003	15 (4)	376 (86)	45 (10)	66	2 (3.0)
2004	78 (13)	524 (85)	12 (2)	33	4 (12.1)
2005	342 (58)	239 (41)	6 (1)	27	0
2006	329 (42)	388 (50)	64 (8)	90	1 (1.1)
Total	1,951 (31)	4,192 (65)	271 (4)	475	25 (5.3)

[a] Antimicrobial susceptibility testing was done by the by broth microdilution method (4).

other randomly selected isolates were tested by the PAP-AUC method as previously described (30) (see Table S1 in the supplemental material). Isolates were defined as hVISA if the vancomycin MIC was ≤2.0 μg/ml and the PAP-AUC ratio was >0.90 compared to the Mu3 reference strain. Reference strains of VISA (Mu50, ATCC 700699), hVISA (Mu3, ATCC 700698), MRSA (ATCC 43300), and methicillin- and vancomycin-susceptible *S. aureus* (ATCC 29213) were included as control organisms.

Isolates were typed by pulsed-field gel electrophoresis (PFGE) following SmaI digestion of genomic DNA (18, 25) and analyzed with BioNumerics software, version 5.1 (Applied Maths, Austin, TX). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was done by PCR as previously described (21).

Patients with hVISA were compared to those without hVISA. Differences in categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared using the Wilcoxon rank sum test. Multivariate logistic regression analysis was done to evaluate the statistical significance of descriptive variables. Variables were selected for inclusion in the regression model if at least five of the patients had the characteristic and the variable was associated with hVISA with a P value of ≤0.10 in the univariate analysis.

A total of 6,397 unique patient isolates of MRSA underwent susceptibility testing. No vancomycin-resistant *S. aureus* or VISA isolates were detected. The frequency distribution of vancomycin MICs for each study year is displayed in Table 1. The modal MIC in each year remained 1.0 μg/ml, and there was no increase in the proportion of isolates with higher vancomycin MICs. Of the 475 MRSA strains selected for hVISA screening, 57 were identified as possible hVISA strains by either of the Etest methods. A total of 25 MRSA strains were confirmed as hVISA by PAP-AUC (Table 1). Of the 271 isolates with a vancomycin MIC of 2.0 μg/ml, 22 (8.1%) had the hVISA phenotype, whereas only 3 (1.5%) of the 204 MRSA isolates with a vancomycin MIC of 1.0 μg/ml were hVISA strains ($P < 0.001$).

A summary of the epidemiological and microbiological features associated with the hVISA and non-hVISA isolates is presented in Table 2. In the multivariate analysis, hVISA strains were more likely to have been recovered from patients hospitalized in central Canada (adjusted odds ratio [OR], 3.0; 95% confidence interval [CI], 1.2 to 7.4; $P = 0.02$) and to have been health care associated (adjusted OR, 5.1; 95% CI, 1.8 to 14.2; $P = 0.002$). Among the hVISA isolates, the most common PFGE profile was CMRSA-1 (resembling USA600), which was found more often in the hVISA strains (56%) than in the non-hVISA strains (21%) examined ($P < 0.001$). There were no hVISA strains identified from among the MRSA strains associated with community acquisition, CMRSA-10 (USA300) and CMRSA-7 (USA400).

The Etest macromethod and GRD Etest strips identified 12 and 57 possible hVISA strains, respectively. GRD Etest strips correctly identified all 25 strains subsequently confirmed as hVISA by PAP-AUC (sensitivity, 100%), but there were 32 strains identified as hVISA that were not confirmed by PAP-AUC (specificity, 36%). In contrast, the Etest macromethod identified only 11 of the hVISA strains (sensitivity, 44%), with one false-positive test result (specificity, 98%). Most (84%) of the errors obtained with the Etest methods occurred with CMRSA-1 (USA600) strains.

In previous studies, considerable variability in the prevalence of hVISA has been reported, with rates ranging from less than 1% to as high as 50% (3, 7, 9, 11–13, 15, 17, 23, 27). In this study, only a small number of the strains tested were found to be hVISA, and no VISA strains were identified. Among the strains with a vancomycin MIC of 2.0 μg/ml, the hVISA rate was 8.1%, whereas only 1.5% of the strains with a vancomycin MIC of 1.0 μg/ml were hVISA. Using these rates and assuming that none of the strains with lower vancomycin MICs would be hVISA, it can estimated that the overall prevalence of hVISA strains among MRSA isolates recovered from hospitalized patients in Canada would have been approximately 1.3%. These results are similar to those reported in an analysis of 14 studies from around the world that identified only 2.2% of MRSA strains as hVISA (15). "Vancomycin creep" is a term that has been used to describe a gradual increase in vancomycin MICs over time (23, 26, 29). However, this has not been observed universally (1, 19) and we did not find a significant increase in the modal vancomycin MIC or in the proportion of isolates with MICs of ≥2.0 μg/ml in Canadian MRSA strains over a span of 12 years.

hVISA strains have been associated with significant infections, including bloodstream infections, endocarditis, and pneumonia (10, 13, 16, 23). In the present study, hVISA was associated with MRSA infection in only five (20%) patients. A substantial percentage of the patients (80%) were found to be colonized with hVISA, likely because active surveillance for MRSA is commonly done in most Canadian hospitals (20). hVISA strains were found in all parts of the country but were more likely to have been identified in patients hospitalized in the provinces of Ontario and Quebec. This may be because hVISA strains were also more likely to have been associated with the CMRSA-1 (USA600) genotype, which was prevalent in central Canada from 1995

TABLE 2. Epidemiologic and microbiological characteristics associated with hVISA and non-hVISA strains

Characteristic	No. (%) of strains		OR (95% CI) (univariate)	*P* value	OR (95% CI) (multivariate)	*P* value
	hVISA	Non-hVISA[a]				
Mean age (yr)	60.4	60.2		0.97		
Sex						
Male	16 (64)	238 (57)	1.34 (0.58–3.11)	0.54		
Female	9 (36)	180 (43)				
Geographic region[b]						
Western Canada	5 (20)	199 (48)	0.28 (0.10–0.75)			
Central Canada	18 (72)	176 (42)	3.54 (1.45–8.65)	0.003	2.96 (1.18–7.43)	0.02
Eastern Canada	2 (8)	43 (10)	0.75 (0.17–3.32)			
MRSA acquisition						
Health care associated	20 (80)	233 (56)	5.60 (2.16–15.23)	<0.001	5.09 (1.83–14.16)	0.002
Community associated	5 (20)	185 (44)				
MRSA infection						
Yes	5 (20)	198 (47)	0.28 (0.11–0.73)	0.008		
No	20 (80)	220 (52)				
Site of MRSA infection[c]						
Bloodstream	1 (4)	32 (8)	0.50 (0.07–3.83)	0.50		
Surgical site	1 (4)	43 (10)	0.36 (0.05–2.75)	0.31		
Skin/soft tissue	1 (4)	79 (19)	0.17 (0.02–1.34)	0.06		
Respiratory	0	36 (9)		0.13		
Urine	1 (4)	14 (3)	1.20 (0.15–9.52)	0.59		
Other	1 (4)	4 (1)	4.31 (0.63–30.30)	0.25		
PFGE genotype						
CMRSA-1 (USA600)	14 (56)	94 (21)	4.57 (2.01–10.42)	<0.001		
CMRSA-2 (USA100/800)	2 (8)	119 (26)	0.32 (0.09–1.07)	0.05		
CMRSA-10 (USA300)	0	52 (12)		0.07		
Other	9 (36)	185 (41)		0.61		
SCC*mec* type						
I	6 (24)	6 (1)		<0.001		
II	16 (64)	220 (49)		0.14		
III	2 (8)	53 (12)		0.57		
IV	1 (4)	146 (32)		0.003		
Other	0	25				

[a] There were a total of 450 patients with non-hVISA isolates; clinical data were available for 418 patients.
[b] Provinces in western Canada: British Columbia, Alberta, Saskatchewan, and Manitoba. Provinces in central Canada: Ontario and Quebec. Provinces in eastern Canada: New Brunswick, Nova Scotia, and Newfoundland and Labrador.
[c] Some patients had MRSA isolated from multiple sites.

to 2001 (25); clonal spread of hVISA strains has previously been reported (5, 7, 12).

Epidemiological studies of hVISA have been hindered by the lack of a simple and accurate method to identify these strains. Two Etest methods, the Etest macromethod and GRD Etest strips, for the detection of hVISA/VISA isolates have been developed and evaluated with variable results (14, 28, 31, 32). In our evaluation, the Etest macromethod had a low sensitivity (44%) but excellent specificity (98%). In contrast, GRD Etest strips identified all of the hVISA strains (100% sensitivity) but lacked specificity (36%). The high sensitivity of GRD Etest strips may make it a useful screening procedure for detection of VISA or hVISA, but confirmatory testing would be required.

This study presents the results of Canadian national surveillance for MRSA with reduced susceptibility to vancomycin using a standardized PAP-AUC method to identify hVISA. Only a subset of the isolates obtained was screened for the hVISA phenotype, and a small number of hVISA strains with vancomycin MICs of $\leq$1.0 μg/ml may have been missed. As the hVISA phenotype is recognized as "unstable" (22), we may have missed some cases, leading to an underestimate of the prevalence. Our study was unable to collect outcome data or information regarding vancomycin utilization or exposure. Finally, most of the hospitals participating in the surveillance were tertiary-care teaching hospitals in Canada and the results may not be representative of other health care facilities.

In conclusion, VISA and hVISA strains appear to be relatively uncommon in Canada at this time. Improved laboratory methods for rapid and accurate identification of hVISA need to be developed.

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