Characterization of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and extended-spectrum beta-lactamase-producing *Escherichia coli* in intensive care units in Canada: Results of the Canadian National Intensive Care Unit (CAN-ICU) study (2005–2006)

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BACKGROUND: Methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and vancomycin-resistant enterococci (VRE) are important hospital pathogens in Canada and worldwide.

OBJECTIVES: To genotypically and phenotypically characterize the isolates of MRSA, VRE and ESBL-producing *E coli* collected from patients in Canadian intensive care units (ICUs) in 2005 and 2006.

METHODS: Between September 1, 2005, and June 30, 2006, 19 medical centres participating in the Canadian National Intensive Care Unit (CAN-ICU) study collected 4133 unique patient isolates associated with infections in ICUs. Isolates of MRSA underwent *mec*A polymerase chain reaction (PCR) and Panton-Valentine leukocidin analysis; they were typed using pulsed-field gel electrophoresis. All isolates of *E coli* with ceftriaxone minimum inhibitory concentrations greater than or equal to 1 µg/mL were tested for the presence of an ESBL using the Clinical Laboratory Standards Institute double-disk diffusion method. Subsequently, PCR and sequence analysis were used to identify bla_{SHV}, bla_{TEM} and bla_{CTX-M}. Isolates of VRE were tested for the presence of *van*A and *van*B genes by PCR.

RESULTS: Of the 4133 ICU isolates collected, MRSA accounted for 4.7% (193 of 4133) of all isolates. MRSA represented 21.9% (193 of 880) of all *S aureus* collected during the study; 90.7% were health care-associated MRSA strains and 9.3% were community-associated MRSA strains. Resistance rates for the isolates of MRSA were 91.8% to levofloxacin, 89.9% to clarithromycin, 76.1% to clindamycin and 11.7% to trimethoprim-sulfamethoxazole; no isolates were resistant to vancomycin, linezolid, tigecycline or daptomycin. ESBL-producing *E coli* accounted for 0.4% (18 of 4133) of all isolates and 3.7% (18 of 493) of *E coli* isolates. All 18 ESBL-producing *E coli* were PCR-positive for CTX-M, with $bla_{CTX-M-15}$ occurring in 72% (13 of 18) of isolates. All ESBL-producing *E coli* displayed a multidrug-resistant phenotype (resistant to third-generation cephalosporins and one or more other classes of antimicrobials), with 77.8% of isolates resistant to ciprofloxacin, 55.6% resistant to trimethoprim-sulfamethoxazole, 27.8% resistant to gentamicin and 26.3% resistant to doxycycline; all isolates were susceptible to ertapenem, meropenem and tigecycline. VRE accounted for 0.4% (17 of 4133) of all isolates and 6.7% (17 of 255) of enterococci isolates; 88.2% of VRE had the *van*A genotype. Isolated VRE that were tested were uniformly susceptible to linezolid, tigecycline and daptomycin.

CONCLUSIONS: MRSA isolated in Canadian ICUs in 2005 and 2006 was predominately health care-associated (90.7%), ESBL-producing *E coli* were all CTX-M producers (72% $bla_{CTX-M-15}$) and VRE primarily harboured a *van*A genotype (88.2%). MRSA, ESBL-producing *E coli* and VRE were frequently multidrug resistant.

Key Words: *CAN-ICU; ESBL* E coli*; Intensive care; MRSA; Resistance; VRE*

La caractérisation du staphylocoque doré méthicillinorésistant, de l'entérocoque vancomycinorésistant et de l'*Escherichia coli* producteur de bêta-lactamase à large spectre aux unités de soins intensifs du Canada : Les résultats de l'étude sur les unités de soins intensifs au Canada (2005-2006)

HISTORIQUE : Le staphylocoque doré méthicillinorésistant (SARM), l'*Escherichia coli* producteur de bêta-lactamase à large spectre (BELS) et l'entérocoque vancomycinorésistant (EVR) sont des pathogènes importants dans les hôpitaux du Canada et d'ailleurs dans le monde.

OBJECTIFS : Caractériser le génotype et le phénotype des isolats de SARM, d'EVR et d'*E coli* producteur de BELS prélevés chez des patients hospitalisés dans des unités de soins intensifs (USI) du Canada entre 2005 et 2006.

MÉTHODOLOGIE : Entre le 1er septembre 2005 et le 30 juin 2006, 19 centres médicaux participant à l'étude sur les unités de soins intensifs au Canada ont prélevé 4 133 isolats uniques chez des patients, associés à des infections à l'USI. Les isolats de SARM ont subi une réaction en chaîne de la polymérase *mec*A (PCR) et une analyse de la leucocidine de Panton-Valentine et ont été typés par électrophérèse en champ pulsé.

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Tous les isolats d'*E coli* dont la concentration inhibitoire minimale de ceftriaxone était supérieure ou égale à 1 ug/L ont fait l'objet de tests pour déceler la présence de BELS au moyen de la méthode de diffusion à double disque du *Clinical Laboratory Standards Institute*. Par la suite, le PCR et l'analyse de séquence ont permis de repérer le bla_{SHV}, le bla_{TEM} et le bla_{CTXT-M}. Les isolats d'ERV ont fait l'objet de tests pour déceler la présence de gènes *van*A et *van*B par PCR.

RÉSULTATS : Parmi les 4 133 isolats prélevés à l'USI, le SARM représentait 4,7 % (193 sur 4 133) de tous les isolats et 21,9 % (193 sur 880) de tous les cas de staphylocoque doré prélevés pendant l'étude, dont 90,7 % étaient des souches de SARM associées au système de santé et 9,3 %, des souches non nosocomiales. Les isolats de SARM résistaient à un taux de 91,8 % à la lévofloxacine, de 89,9 % à la clarithromycine, de 76,1 % à la clindamycine et de 11,7 % au triméthoprim-sulfaméthoxazole. Aucun isolat n'était résistant à la vancomycine, au linézolide, à la tigécycline ou à la daptomycine. L'*E coli* producteur de BELS représentait 0,4 % (18 sur 4 133) de tous les isolats et 3,7 % (18 sur 493) des isolats d'*E coli*. Les 18 cas d'*E coli* producteurs de BELS étaient PCR-positifs au CTX-M, le $bla_{CTX-M-15}$ se produisant dans 72 % (13 sur 18) des isolats. Tous les cas d'*E coli* producteurs de BELS possédaient un phénotype multirésistant (résistant aux céphalosporines de troisième génération et à au moins une autre classe d'antimicrobiens), 77,8 % des isolats étant résistants à la ciprofloxacine, 55,6 % au triméthroprim-sulfaméthoxazole, 27,8 % à la gentamicine et 26,3 % à la doxycycline. Tous les isolats étaient susceptibles à l'ertapénem, au méropénem et la tigécycline. L'ERV représentait 0,4 % (17 sur 4 133) de tous les isolats et 6,7 % (17 sur 255) des isolats d'entérocoque, tandis que 88,2 % des cas d'ERV étaient dotés du génotype *van*A. Les ERV isolés ayant fait l'objet d'un test possédaient la même susceptibilité au linézolide, à la tigécycline et à la daptomycine.

CONCLUSIONS : Le SRAM isolé dans les USI canadiennes en 2005 et 2006 s'associait surtout au système de santé (90,7 %), l'*E coli* producteur de BELS était toujours producteur de CTX-M (72 % $bla_{CTX-M-15)}$ et l'EVR hébergeait surtout un génotype *van*A (88,2 %). Le SRAM, l'*E coli* producteur de BELS et l'EVR étaient souvent multirésistants.

The increasing prevalence of antimicrobial-resistant bacteria may threaten the ability of physicians to effectively treat infected patients and underscores the need for continued surveillance (1-4). Antimicrobial-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) (community-associated MRSA [CA-MRSA] and health care-associated MRSA [HA-MRSA]), extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* family such as *Escherichia coli*, and vancomycin-resistant enterococci (VRE), are increasing in prevalence in regions of Canada, the United States and globally (5-15). Available therapeutic options for the treatment of these antibiotic-resistant organisms are sometimes limited because these organisms frequently display a multidrug-resistant (MDR) phenotype (4,7,14,15).

The purpose of the present study was to genotypically and phenotypically characterize the isolates of MRSA, VRE and ESBL-producing *E coli* collected from patients in Canadian intensive care units (ICUs) between 2005 and 2006.

METHODS

Bacterial isolates

Study isolates were obtained from the Canadian National Intensive Care Unit (CAN-ICU) study <www.can-r.ca> (16). The CAN-ICU study included 19 medical centres across Canada (western Canada – British Columbia, Alberta, Saskatchewan and Manitoba; central Canada – Ontario and Quebec; and eastern Canada – Maritime provinces). The ICUs selected represented tertiary care medical centres from all regions of the country. Between September 2005 and June 2006, each centre collected a maximum of 300 consecutive, clinically significant isolates obtained from blood, urine, tissue or wound, and respiratory specimens (one pathogen per cultured site per patient) of ICU patients. Surveillance swabs; duplicate isolates; and eye, ear, nose and throat swabs were excluded, as were anaerobic bacteria and fungi. Isolates were shipped to the reference laboratory (Health Sciences Centre, Winnipeg, Manitoba) on Amies charcoal swabs, subcultured onto appropriate media and stocked in skim milk at –80°C until further testing was conducted.

Antimicrobial susceptibility testing

Following two subcultures from frozen stock, the in vitro activities of cefazolin, ceftriaxone, cefepime, ciprofloxacin, clarithromycin, clindamycin, dalbavancin, daptomycin, doxycycline, ertapenem, gentamicin, levofloxacin, linezolid, meropenem, moxifloxacin, piperacillin-tazobactam, tigecycline, trimethoprim-sulfamethoxazole and vancomycin were determined by microbroth dilution in accordance with the 2006 Clinical Laboratory Standards Institute (CLSI) guidelines (M7-A7 and M100-S16) (16). Antimicrobial agents were obtained as laboratory-grade powders from their respective manufacturers. Stock solutions were prepared and dilutions were made as described by the CLSI guidelines (M7-A7). The minimum inhibitory concentrations (MICs) of the antimicrobial agents for the isolates were determined using 96-well custom-designed microtitre plates. These plates contained doubling antimicrobial dilutions in 100 µL/well of cation-adjusted Mueller-Hinton broth and were inoculated to achieve a final concentration of approximately 5×10^5 colony-forming units/mL. They were then incubated in ambient air for 24 h before reading. Colony counts were performed periodically to confirm inocula. Quality control was performed using American Type Culture Collection Quality Control organisms – *Streptococcus pneumoniae* 49619, *S aureus* 29213, *Enterococcus faecalis* 29212, *E coli* 25922 and *Pseudomonas aeruginosa* 27853.

For all antimicrobials tested, MIC interpretive standards were defined according to the CLSI break points (M100-S16). For tigecycline, the following susceptible, intermediate and resistant interpretive breakpoints (Food and Drug Administration) were used – *S aureus*, less than or equal to 0.5 µg/mL (susceptible); *Enterococcus* species, less than or equal to 0.25 µg/mL (susceptible); and *Enterobacteriaceae* family, less than or equal to 2 µg/mL (susceptible), less than or equal to 4 µg/mL (intermediate), and greater than or equal to 8 µg/mL (resistant).

Characterization of MRSA, ESBL-producing *Enterobacteriaceae* family and VRE

MRSA: Potential MRSA isolates were confirmed using the CLSI disk-diffusion method and *mec*A polymerase chain reaction (PCR). All isolates of MRSA were tested for Panton-Valentine leukocidin and typed using pulsed-field gel electrophoresis following the Canadian standardized protocol to assess whether the isolates were CA-MRSA or HA-MRSA (10,11,17,18). Pulsed-field gel electrophoresis fingerprints were analyzed with BioNumerics version 3.5 (Applied Maths, Belgium) using a position tolerance of 1.0 and an optimization of 1.0. Strain relatedness was determined as previously described (19). Fingerprints were compared with the national MRSA fingerprint

database and were grouped into one of 10 Canadian epidemic MRSA strains (CMRSA-1, CMRSA-2, etc) as previously described (11). In the present study, CA-MRSA and HA-MRSA were defined genotypically and not epidemiologically. Any MRSA with a CMRSA-7 (USA400/MW2) or CMRSA-10 (USA300) genotype was labelled as CA-MRSA, while all other genotypes (eg, CMRSA-1 [USA600], CMRSA-2 [USA100] and CMRSA-4 [USA200]) were labelled as HA-MRSA.

ESBL testing: Any *E coli* or *Klebsiella* species with a ceftriaxone MIC of greater than or equal to 1 µg/mL was identified as a potential ESBL producer. ESBL producers were confirmed using the CLSI double-disk diffusion method. PCR and DNA sequence analyses were used to identify bla_{SHV}, bla_{TEM} and $bla_{CTX\text{-}M}$ genes among isolates, as previously described (9,12,13).

VRE: Potential VRE isolates were confirmed using the CLSI vancomycin disk-diffusion testing; they underwent *van*A and *van*B PCR, as well as DNA fingerprinting to assess genetic similarity, as previously described (14,20).

RESULTS

Patient demographics and specimen types

A total of 4133 organisms from 2580 patients (or 1.6 isolates per patient) were collected from ICUs across Canada in 2005 and 2006; they were taken during any time of the patient's ICU admission. 59.3% (2451 of 4133) of isolates were collected from male patients, while 40.7% (1682 of 4133) were collected from female patients. Patient age breakdown was 17 years of age or younger, 13.7%; between 18 and 64 years of age, 46.7%; and 65 years of age or older, 39.6%. 54.8% of organisms were obtained from respiratory sites – 17.7% from blood, 13.9% from wounds and intravenous sites, and 13.6% from urine.

Most common organisms isolated from ICUs

Table 1 describes the 20 most common organisms isolated from ICUs across Canada in 2005 and 2006. The most common Gram-positive cocci included methicillin-susceptible *S aureus* (MSSA), coagulase-negative staphylococci/*Staphylococcus epidermidis*, *Enterococcus* species, *S pneumoniae* and MRSA, which represented 39.5% of all pathogens. The most common Gram-negative bacilli included *E coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia* and *Serratia marcescens*, which made up 45% of all pathogens in ICUs. All MRSA, VRE and ESBL *E coli* underwent genotypic characterization and antimicrobial susceptibility studies.

TABLE 1
The 20 most common organisms isolated from patients in 19 intensive care units across Canada between 2005 and 2006 (n=4133)

Ranking	Organism	Isolates, n (%)
1	*Staphylococcus aureus* – MSSA	687 (16.6)
2	*Escherichia coli*	493 (11.9)
3	*Pseudomonas aeruginosa*	419 (10.1)
4	*Haemophilus influenzae*	329 (8.0)
5	CNS – *Staphylococcus epidermidis*	273 (6.6)
6	*Enterococcus* species	255 (6.2)
7	*Streptococcus pneumoniae*	244 (5.9)
8	*Klebsiella pneumoniae*	224 (5.5)
9	*S aureus* – MRSA	193 (4.7)
10	*Enterobacter cloacae*	164 (4.0)
11	*Stenotrophomonas maltophilia*	108 (2.7)
12	*Serratia marcescens*	100 (2.4)
13	*Moraxella catarrhalis*	78 (1.9)
14	*Klebsiella oxytoca*	77 (1.8)
15	*Streptococcus pyogenes*	49 (1.2)
16	*Enterobacter aerogenes*	47 (1.1)
17	*Citrobacter freundii*	39 (0.9)
18	*Streptococcus agalactiae*	39 (0.9)
19	*Proteus mirabilis*	38 (0.9)
20	*Acinetobacter baumannii*	28 (0.7)
	Other*	249 (6.0)

CNS Coagulase-negative staphylococci; MRSA Methicillin-resistant S aureus*; MSSA Methicillin-susceptible* S aureus. **Other* – Acinetobacter *species,* Burkholderia *species,* Bacillus *species,* Citrobacter *species,* Corynebacterium *species,* Enterobacter *species,* Haemophilus *species,* Micrococcus *species,* Morganella *species,* Neisseria *species,* Pseudomonas *species,* Salmonella *species,* Serratia *species,* Staphylococcus *species and* Streptococcus *species*

MRSA

Of the 193 MRSA (21.9% of all *S aureus*) specimens isolated from ICUs, 175 (90.7%) were HA-MRSA genotypes, while 18 (9.3%) were CA-MRSA genotypes. HA-MRSA-associated genotypes included CMRSA-2 (USA100), 67.6%; CMRSA-6, 15.4%; CMRSA-1 (USA600), 5.9%; CMRSA-8, 4.4%; CMRSA-4 (USA200), 3.7%; CMRSA-3, 0.8%; and CMRSA-9, 0.7%. HA-MRSA genotypes occurred with similar frequencies in all regions of Canada (Table 2). The 18 CA-MRSA specimens were isolated from seven different cities in Canada; however, 15 of 18 (83.3%) were isolated from western Canada. Blood was the most common site from which CA-MRSA was isolated (50%, nine of 18), followed by respiratory (38.9%, seven of 18) and wounds (5.6%, one of 18). The average age of patients with CA-MRSA was 43.2 years (range one to 75 years); CA-MRSA was more frequently isolated from male patients (72.2%, 13 of 18) than female patients. Also, 17 of 18 (94.4%) CA-MRSA isolates were positive for the PVL gene, and 61.1% (11 of 18) of isolates belonged to the CMRSA-10 (USA300) genotype. The CMRSA-7 (USA400) genotype occurred in 38.9% (seven of 18) of isolates. All CMRSA-7 strains were reported from either Saskatchewan or Manitoba, while CMRSA-10 strains were primarily identified in British Columbia (eight of 11). The antimicrobial susceptibilities of the CA-MRSA and HA-MRSA isolates are shown in Table 3. CA-MRSA isolates were more susceptible than HA-MRSA isolates to fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin), gentamicin and all beta-lactams, demonstrating lower MIC for 50% and 90% of isolates to cefazolin, cefepime, ceftriaxone, piperacillin-tazobactam and meropenem. Resistance rates were also lower for CA-MRSA isolates than HA-MRSA isolates for clarithromycin (66.6% versus 92.2%), clindamycin (27.8% versus 81.0%) and trimethoprim-sulfamethoxazole (0.0% versus 12.8%). No isolates of CA-MRSA or HA-MRSA were resistant to vancomycin, linezolid, tigecycline or daptomycin. Dalbavancin was active against all MRSA isolates, with MIC for 50% of isolates and MIC for 90% of isolates with values of 0.06 µg/mL. Of all MRSA, 88.8% were MDR (defined as resistant to three or more of the following: cefazolin and

TABLE 2
Characteristics of 18 community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients in Canadian intensive care units in 2005 and 2006

Isolate number	City (direction)	Specimen source	Age (years)	Sex	PVL	Genotype
58173	Winnipeg (W)	Respiratory	1	F	+	CMRSA-7 (USA400)
61592	Vancouver (W)	Blood	39	M	+	CMRSA-10 (USA300)
62697	Saskatoon (W)	Blood	5	M	+	CMRSA-7 (USA400)
62825	Regina (W)	Blood	25	F	+	CMRSA-7 (USA400)
62996	Victoria (W)	Blood	21	F	+	CMRSA-10 (USA300)
63307	Winnipeg (W)	Respiratory	58	M	+	CMRSA-7 (USA400)
64195	Halifax (E)	Blood	75	F	+	CMRSA-10 (USA300)
64914	Saskatoon (W)	Respiratory	39	M	+	CMRSA-7 (USA400)
65226	Victoria (W)	Respiratory	56	M	+	CMRSA-10 (USA300)
65667	Victoria (W)	Blood	48	M	+	CMRSA-10 (USA300)
66065	Saskatoon (W)	Respiratory	67	M	+	CMRSA-7 (USA400)
66122	Halifax (E)	Blood	69	F	+	CMRSA-10 (USA300)
66303	Vancouver (W)	Respiratory	38	M	+	CMRSA-10 (USA300)
67442	Victoria (W)	Respiratory	36	M	+	CMRSA-10 (USA300)
67878	Victoria (W)	Wound	40	M	+	CMRSA-10 (USA300)
67884	Victoria (W)	Blood	39	M	+	CMRSA-10 (USA300)
68300	Saskatoon (W)	Blood	49	M	–	CMRSA-7 (USA400)
68584	Sydney (E)	Wound	73	M	+	CMRSA-10 (USA300)

– Negative; + Positive; CMRSA Canadian MRSA; E Eastern Canada (Maritime provinces); F Female; M Male; PVL Panton-Valentine leukocidin; W Western Canada (British Columbia, Alberta, Saskatchewan and Manitoba)

TABLE 3
Antimicrobial susceptibilities of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and health care-associated MRSA (HA-MRSA) isolated from patients in Canadian intensive care units in 2005 and 2006

	CA-MRSA (n=18)						HA-MRSA (n=175)					
	MIC (µg/mL)			Isolates (%)			MIC (µg/mL)			Isolates (%)		
Antibiotic	MIC_{50}	MIC_{90}	Range	S	I	R	MIC_{50}	MIC_{90}	Range	S	I	R
Ciprofloxacin	16.0	16.0	0.25–16.0	38.9	–	61.1	32.0	32.0	0.25–>16.0	5.1	–	94.9
Clarithromycin	>16.0	>16.0	≤0.25–>16.0	33.4	–	66.6	>16.0	>16.0	≤0.25–>16.0	7.8	–	92.2
Clindamycin	0.25	>8.0	≤0.25–>8.0	72.2	–	27.8	>8.0	>8.0	≤0.25–>8.0	19.0	–	81.0
Dalbavancin	0.06	0.06	≤0.03–0.06	–	–	–	0.06	0.06	≤0.03–0.12	–	–	–
Daptomycin	0.25	0.25	0.12–0.25	100.0	–	0.0	0.12	0.25	0.12–0.5	100.0	–	0.0
Gentamicin	≤0.25	1.0	≤0.25–32	94.4	–	5.6	0.5	1.0	≤0.25–>64.0	83.7	–	16.3
Levofloxacin	4.0	8.0	0.12–8.0	38.9	–	61.1	>32.0	>32.0	0.12–>32.0	5.1	–	94.9
Linezolid	2.0	2.0	1.0–2.0	100.0	–	0.0	2.0	2.0	1.0–4.0	100.0	–	0.0
Moxifloxacin	1.0	2.0	≤0.06–4.0	38.9	11.1	50.0	8.0	>16.0	≤0.06–>16	5.0	–	95.0
Tigecycline	0.12	0.25	0.12–0.25	100.0	–	0.0	0.12	0.5	0.06–0.5	100.0	–	0.0
SXT	0.12	0.12	0.12–0.5	100.0	–	0.0	≤0.12	16.0	≤0.12–>32.0	87.2	–	12.8
Vancomycin	1.0	1.0	0.5–1.0	100.0	–	0.0	1.0	1.0	≤0.25–1.0	100.0	–	0.0

I Intermediate; MIC Minimum inhibitory concentration; MIC_{50} MIC for 50% of isolates; MIC_{90} MIC for 90% of isolates; R Resistant; S Susceptible; SXT Trimethoprim-sulfamethoxazole

piperacillin-tazobactam, ciprofloxacin, clarithromycin, clindamycin, linezolid, vancomycin and tigecycline), compared with only 2.3% of MSSA.

ESBL-producing *E coli*

ESBL-producing *E coli* accounted for 3.7% (18 of 493) of all *E coli* isolates collected. Although ESBL-producing *E coli* were obtained from a variety of Canadian cities, 88.9% (16 of 18) were obtained from central and eastern Canada, whereas only 11.1% (two of 18) were obtained from western Canada (Table 4). The mean age of patients with ESBL-producing *E coli* was 54.1 years (range 25 to 85.5 years), with 10 of 18 (61.1%) isolates from female patients and eight of 18 (44.4%) isolates from male patients. The most common sources of ESBL-producing *E coli* included the urinary tract (50.0%, nine of 18) followed by blood (27.8%, five of 18), and respiratory and wound sites, both at 11.1% (two of 18). The most common genotype of the ESBL-producing *E coli* was CTX-M-15 at 72.2% (13 of 18); followed by CTX-M-2 at 11.1% (two of 18); and CTX-M-1 at CTX-M-9 and CTX-M-14 each at 5.6% (one of 18 each) (Table 4). The *TEM-1* gene (not an ESBL) was identified in 61.1% (11 of 18) of isolates. ESBL-producing *E coli* frequently displayed an MDR phenotype, with 77.8% of isolates demonstrating concomitant resistance to ciprofloxacin, 55.6% demonstrating resistance to trimethoprim-sulfamethoxazole, 27.8% demonstrating resistance to gentamicin and 26.3%

TABLE 4
Characteristics of 18 extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolates from patients in Canadian intensive care units in 2005 and 2006

Isolate number	City (direction)	Age (years)	Sex	Specimen source	*CTX/SHV* gene	*TEM* gene	MIC, µg/mL (MIC interpretation)							
							CPM	CTR	CIP	GEN	MER	PTZ	TGC	SXT
59096	Winnipeg (W)	62	M	Blood	CTX-M-15	Negative	8.0	64.0	>16.0	0.5	0.12	128.0	1.0	>32.0
61567	Vancouver (W)	28	M	Wound	CTX-M-15	Negative	32.0	256.0	>16.0	0.5	0.12	64.0	0.25	>32.0
62175	Toronto (C)	73	M	Wound	CTX-M-14	*TEM-1*	4.0	32.0	>16.0	0.5	0.12	2.0	0.25	0.06
62188	Toronto (C)	39	F	Blood	CTX-M-15	Negative	16.0	128.0	>16.0	32.0	0.12	1.0	0.5	0.12
62199	Toronto (C)	54	M	Blood	CTX-M-15	*TEM-1*	16.0	256.0	>16.0	0.5	0.12	8.0	1.0	0.06
62597	Montreal (C)	76	F	Blood	CTX-M-9	*TEM-1*	1.0	8.0	0.06	4.0	0.12	1.0	0.25	>32.0
63106	London (C)	63	M	Resp	CTX-M-15	*TEM-1*	16.0	256.0	>16.0	16.0	≤0.12	8.0	1.0	>32.0
64197	Halifax (E)	70	F	Urine	CTX-M-15	*TEM-1*	≤0.12	2.0	8.0	>16.0	0.12	4.0	0.5	0.06
64532	Dartmouth (E)	25	F	Urine	CTX-M-15	Negative	4.0	>16.0	0.5	16.0	0.12	1.0	1.0	>32.0
64539	Dartmouth (E)	52	F	Urine	CTX-M-2	*TEM-1*	2.0	32.0	1.0	≤0.25	≤0.12	≤1.0	0.5	>32.0
64547	Dartmouth (E)	26	F	Urine	CTX-M-15	Negative	8.0	64.0	>16.0	≤0.25	≤0.12	2.0	0.5	4.0
64611	Halifax (E)	81	F	Urine	CTX-M-15	*TEM-1*	2.0	64.0	>16.0	≤0.25	≤0.12	2.0	1.0	>32.0
64704	Toronto (C)	53	F	Urine	CTX-M-15	Negative	4.0	64.0	16.0	≤0.25	≤0.12	≤1.0	0.25	>32.0
64712	Toronto (C)	85	M	Urine	CTX-M-1	*TEM-1*	16.0	32.0	>16.0	0.5	≤0.12	≤1.0	0.25	>32.0
64771	Toronto (C)	83	F	Blood	CTX-M-15	*TEM-1*	>256.0	>256.0	>16.0	16.0	≤0.12	8.0	0.25	>32.0
66075	Halifax (E)	77	F	Urine	CTX-M-2	Negative	8.0	64.0	0.5	8.0	≤0.12	2.0	0.5	0.06
66956	Montreal (C)	69	M	Urine	CTX-M-15	*TEM-1*	64.0	>256.0	>16.0	0.5	≤0.12	2.0	0.25	0.06
66980	Montreal (C)	69	M	Resp	CTX-M-15	*TEM-1*	64.0	>256.0	>16.0	0.5	≤0.12	2.0	0.25	0.06

C Central Canada (Ontario and Quebec); CIP Ciprofloxacin; CPM Cefepime; CTR Ceftriaxone; E Eastern Canada (Maritime provinces); F Female; GEN Gentamicin; M Male; MER Meropenem; MIC Minimum inhibitory concentration; PTZ Piperacillin-tazobactam; Resp Respiratory; SXT Trimethoprim-sulfamethoxazole; TGC Tigecycline; W Western Canada (British Columbia, Alberta, Saskatchewan and Manitoba)

demonstrating resistance to doxycycline (data not shown). No isolates were resistant to meropenem, ertapenem (data not shown) or tigecycline (Table 4).

VRE

Of the 17 VRE isolated, 52.9% (nine of 17) occurred in eastern Canada, 41.2% (seven of 17) in western Canada and 5.9% (one of 17) in central Canada (Table 5); 76.5% (13 of 17) of isolates were *Enterococcus faecium*, while 23.5% (four of 17) were *Enterococcus faecalis*. The mean patient age was 62.1 years (range 26 to 83 years), and 64.7% (11 of 17) of isolates were from female patients. The source was primarily wound (52.9%, nine of 17), followed by both blood and urine at 17.6% (three of 17) each, and respiratory sites at 5.9% (one of 17). The most common genotype was *van*A (88.2%, 15 of 17). All isolates were resistant to ciprofloxacin. No isolates were resistant to linezolid, daptomycin or tigecycline.

DISCUSSION

The CAN-ICU study was the first national prospective surveillance study assessing antimicrobial resistance in Canadian ICUs. It determined that more than one-half of all infections in the ICUs were respiratory in origin, irrespective of patient age and sex (16). Bloodstream, wound or intravenous origin, and urinary tract infections were less common than respiratory infections in ICU patients, as has been previously documented (21).

The CAN-ICU study documented that MSSA and MRSA are important pathogens causing respiratory tract infections, bacteremia, and wound or intravenous infections in ICUs in Canada. MRSA accounted for 21.9% of all *S aureus*, and 9.3% of all MRSA causing infections in the ICU were CA-MRSA genotypes. This has not been previously documented in Canada and shows the infiltration of CA-MRSA into Canadian ICUs. All 18 of the CA-MRSA isolates were either USA400 (CMRSA-7) or USA300 (CMRSA-10) genotypes. These two genotypes are the two primary CA-MRSA genotypes reported across North America (11,18,22-24). It appears that infections in Canadian ICUs caused by CA-MRSA are primarily occurring in western Canada, with USA400 (CMRSA-7) the predominant genotype in Saskatchewan or Manitoba, and USA300 (CMRSA-10) the predominant genotype in British Columbia (Table 2).

The present study is the first to document that ESBL-producing *E coli* are becoming more common than ESBL-producing *Klebsiella* species in Canadian ICUs (3.7% of *E coli* were ESBL-producing *E coli*, and 1.8% of *Klebsiella* species were ESBL-producing *Klebsiella* species [data not shown]). The 18 ESBL-producing *E coli* (89.5%) were primarily obtained in central and eastern Canada from urine, blood, the respiratory tract and wounds. All 18 ESBL-producing *E coli* that were isolated displayed an MDR phenotype, with 77.8% demonstrating concomitant resistance to fluoroquinolones and 55.6% demonstrating resistance to trimethoprim-sulfamethoxazole. The study showed that CTX-M with $bla_{\text{CTX-M-15}}$ was the predominant genotype (72%) of ESBL-producing *E coli* in Canada. Other studies (8,9,12,13) assessing ESBL-producing *E coli* have shown that the CTX-M genotype is spreading rapidly in both community and hospital settings. Pitout et al (13) investigated the molecular epidemiology of ESBL-producing *E coli* collected between 2000 and 2005 in the Calgary Health Region in Alberta. These investigators reported

that 64% (354 of 552) of ESBL-producing *E coli* were PCR-positive for bla_{CTX-M} genes, with CTX-M-14 (59.6%) and CTX-M-15 (36.2%) reported most commonly. This study highlights the rapid spread of MDR ESBL CTX-M-15 *E coli* in Canadian ICUs. This genotype is likely spreading rapidly due to the extensive use of third-generation cephalosporins and fluoroquinolones.

The CAN-ICU study showed that VRE represented 6.7% of all enterococci tested, with the *van*A genotype (mostly *E faecium*) making up 88.2% of VRE. This relatively low level of VRE across Canada has been previously documented and likely reflects the active surveillance programs in Canadian hospitals (14). Such programs have been reported to prevent VRE colonization and bacteremia (25). Previous data (5,14) have suggested that the *E faecium* carrying *van*A is the predominant genotype in North America.

Resistance rates of MRSA were high with fluoroquinolones and macrolides, such as clarithromycin and clindamycin (range 76.1% to 91.8%); they were lower, at 11.7%, with trimethoprim-sulfamethoxazole. Thus, trimethoprim-sulfamethoxazole still represents a reasonable empirical treatment for mild to moderate infections caused by CA-MRSA or HA-MRSA. It should be noted that the study found a significant difference between the susceptibilities of CA-MRSA and HA-MRSA. Like others, we report that CA-MRSA was more susceptible to beta-lactams, trimethoprim-sulfamethoxazole, macrolides, clindamycin and fluoroquinolones than HA-MRSA (11). All CA-MRSA and HA-MRSA isolates were susceptible to vancomycin, linezolid, tigecycline and daptomycin. Likewise, all VRE in the study proved to be susceptible to linezolid, tigecycline and daptomycin. MDR ESBL-producing *E coli* isolates were all susceptible to the carbapenems, ertapenem, meropenem and tigecycline. Because nosocomial infections in the ICU are frequently MDR (frequently associated with prior antimicrobial use [6]), some have suggested that involvement of an infectious diseases specialist may help to improve, cure and minimize further resistance development (2).

CONCLUSIONS

MRSA isolated in Canadian ICUs in 2005 and 2006 was predominately HA-MRSA (90.7%), ESBL-producing *E coli* were all CTX-M producers (72% $bla_{CTX-M-15}$) and VRE primarily harboured a *van*A genotype (88.2%). MRSA, ESBL-producing *E coli* and VRE were frequently MDR.

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