MAJOR ARTICLE

Population-Based Epidemiological Study of Infections Caused by Carbapenem-Resistant *Pseudomonas aeruginosa* in the Calgary Health Region: Importance of Metallo-β-Lactamase (MBL)–Producing Strains

Kevin B. Laupland,[1,2,3,6,7] Michael D. Parkins,[1,7] Deirdre L. Church,[1,2,5,7] Daniel B. Gregson,[1,2,5,7] Thomas J. Louie,[1,4,7] John M. Conly,[1,2,4,5,6,7] Sameer Elsayed,[2,4,5,7] and Johann D. D. Pitout[2,4,5,7]

Departments of [1]Medicine, [2]Pathology and Laboratory Medicine, [3]Critical Care Medicine, and [4]Microbiology and Infectious Diseases, University of Calgary, [5]Division of Microbiology, Calgary Laboratory Services, [6]Centre for Anti-microbial Resistance, and [7]Calgary Health Region, Calgary, Alberta, Canada

Background. A study was conducted in the Calgary Health Region between May 2002 and April 2004 to define the population-based epidemiological characteristics of infections caused by imipenem-resistant *Pseudomonas aeruginosa* and to explore the clinical outcomes due to metallo-β-lactamase (MBL)–producing and non–MBL-producing strains.

Methods. Detailed clinical information was obtained by chart review, and phenotypic and molecular characterizations were performed using the MBL E-test, polymerase chain reaction with sequencing, and pulsed-field gel electrophoresis.

Results. A total of 228 patients with infections caused by imipenem-resistant *P. aeruginosa* were identified (annual incidence, 10.5 cases/100,000 population), with the highest incidence rate in those ⩾75 years old. MBL-producing strains (98/228) were associated with higher rates of multidrug resistance and bacteremia. Ninety MBL-producing strains also produced VIM-2, 4 produced IMP-7, and 4 were unclassified. A cluster of VIM-2–producing strains was responsible for a nosocomial outbreak during 2003. The case-fatality rate was significantly higher for infections caused by MBL-producing strains than for those caused by non–MBL-producing strains (25% vs. 13%; relative risk, 1.98 [95% confidence interval, 1.00–3.90]; $P = .05$).

Conclusion. MBL-producing *P. aeruginosa* strains were associated with a higher case-fatality rate and invasive disease. Our study highlights the potential importance of molecular laboratory techniques in infection control and patient care.

Carbapenems, including meropenem and imipenem, are recognized as among the most potent agents with activity against *Pseudomonas aeruginosa* [1]. Although recent laboratory-based surveys have found low rates of resistance to these agents in gram-negative organisms, resistance to carbapenems in *P. aeruginosa* is increasing [2–6]. Resistance to carbapenems in *P. aeruginosa* is often due to impermeability that arises via the loss of the OprD porin, the up-regulation of an active efflux pump system present in the cytoplasmic membrane of these organisms, or the production of metallo-β-lactamases (MBLs) that hydrolyze all carbapenems [7–9]. The presence of these mechanisms can potentially lead to treatment failure if carbapenems are used. However, MBL-producing strains are of additional importance from an infection-control perspective, because they may be responsible for horizontal transmission of the resistance gene(s) conferring carbapenem resistance in other strains of *P. aeruginosa* or even in unrelated gram-negative organisms [10–13]. Although patients from several continents with infections caused by MBL-producing *P. aeruginosa* strains have been described, few

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Reprints or correspondence: Dr. Johann D. D. Pitout, Div. of Microbiology, Calgary Laboratory Services, No. 9, 3535 Research Rd. NW, Calgary, Alberta, Canada T2L 2K8 (johann.pitout@cls.ab.ca).

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Table 1. Susceptibility of metallo-β-lactamase (MBL)–producing and non–MBL-producing imipenem-resistant *Pseudomonas aeruginosa* strains to routinely tested antimicrobial agents.

Antimicrobial agent	MBL-producing strains (n = 98)	Non–MBL-producing strains (n = 130)	RR (95% CI)	P
Tobramycin	90 (92)	24 (18)	5.0 (3.4–7.17)	<.0001
Gentamicin	92 (94)	65 (50)	1.9 (1.55–2.23)	<.0001
Ciprofloxacin	93 (95)	33 (25)	3.7 (2.77–5.04)	<.0001
Piperacillin	13 (13)	8 (6)	2.2 (0.93–5.00)	.1
Piperacillin-tazobactam	6 (6)	7 (5)	1.1 (0.39–3.28)	1.0
Ceftazidime	87 (89)	8 (6)	14.4 (7.35–28.33)	<.0001

NOTE. Data are no. (%) of strains, unless otherwise indicated. CI, confidence interval; RR, relative risk.

patients with these infections have been identified in North American medical centers [14, 15], and population-based studies defining the epidemiological characteristics of these infections have not been reported.

Given the importance of carbapenem-resistant *P. aeruginosa* strains as multidrug-resistant pathogens and our recognition of their presence in our region, we undertook a study to investigate the clinical epidemiological characteristics and outcomes of infections caused by these organisms. We conducted a population-based, laboratory surveillance study of all infections caused by imipenem-resistant *P. aeruginosa* in the Calgary Health Region (CHR), to define the incidence rate and demographic risk factors for their acquisition. We also investigated the specific occurrence, clinical features, molecular epidemiological characteristics, and clinical outcomes of infections caused by MBL-producing and non–MBL-producing strains.

PATIENTS, MATERIALS, AND METHODS

Patient population. The CHR provides all publicly funded health care services to the >1 million people residing in Calgary, Airdrie, and numerous adjacent surrounding communities, covering an area of 37,000 km^2 [16]. Acute care is provided principally through 1 pediatric and 3 large adult hospitals (acute care centers 1, 2, and 3). A centralized laboratory (Calgary Laboratory Services [CLS]) performs the routine clinical microbiological services for both the community and hospital sites within the CHR. The base study population consisted of all patients with a first infection caused by an imipenem-resistant *P. aeruginosa* strain identified by the CLS between 1 May 2002 and 31 April 2004.

Study protocol. All patients with infections caused by imipenem-resistant *P. aeruginosa* strains were prospectively identified by the CLS during the surveillance period. An infection was defined as the isolation of *P. aeruginosa* from clinical specimens and the presence of symptoms and signs of infection, whereas positive cultures from routine surveillance swab specimens were strictly excluded. Basic demographic information (age, sex, and community- or hospital-based submission site) and microbiological data (site of infection and results of testing for susceptibility to antimicrobial agents) were obtained for all patients. Detailed clinical information was then collected from all adult patients (≥18 years old) admitted to any of the 3 acute care institutions in the CHR. Patients were deemed to have community-onset disease if the infection was incubating at the time of hospital admission (i.e., a culture performed within 24 h of admission was positive for imipenem-resistant *P. aeruginosa*) or if the first culture that was found to be positive for imipenem-resistant *P. aeruginosa* was obtained within 48 h of admission. The presence of comorbid illnesses was defined using criteria described elsewhere [17]. Chart reviews were conducted using standardized review forms, and results were then entered into a study database and linked with microbiological data by use of Access 2003 (Microsoft). The ethics review boards at the University of Calgary and Calgary Health Region approved this study.

Clinical microbiological and molecular testing. Consecutive, nonduplicate imipenem-resistant *P. aeruginosa* strains (MIC >8 μg/mL) collected at CLS during May 2002–April 2004 were included in this study. Strains were identified to the species level with Vitek AMS (bioMérieux Vitek). Susceptibility to antimicrobial agents was determined using Vitek AMS, microdilution panels (Microscan Gram negative NMIC30; Dade Behring Canada), and disk diffusion techniques that used the NCCLS criteria for broth dilution and disk diffusion (table 1) [18]. The presence of MBLs was evaluated in imipenem-resistant strains by use of both an EDTA screen test and the MBL E-test (AB BioDisk) in accordance with the manufacturer's instructions [19, 20].

DNA template preparation and duplex polymerase chain reaction (PCR) amplification for the simultaneous detection of bla_{IMP} and bla_{VIM} β-lactamase genes were performed on a Thermal Cycler 9600 apparatus (Applied Biosystems) with primers and conditions described elsewhere [20]. Strains that were phenotypically positive for MBLs but were negative for either the bla_{VIM} or the bla_{IMP} genes (4 in total) by PCR were further in-

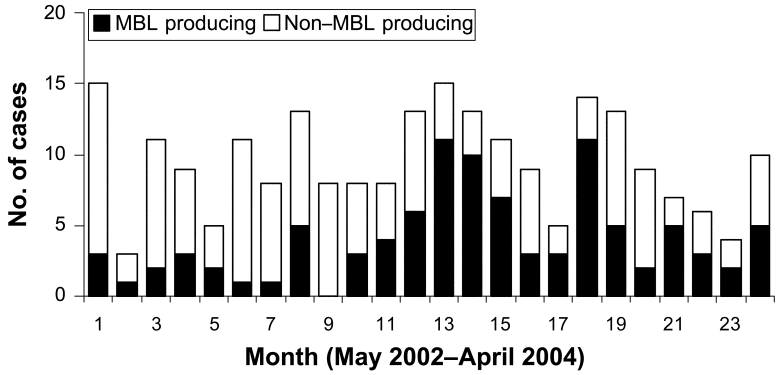

Figure 1. Occurrence of infections caused by metallo-β-lactamase (MBL)–producing and non–MBL-producing imipenem-resistant *Pseudomonas aeruginosa* strains in the Calgary Health Region.

vestigated for the presence of SPM MBLs by use of primers and conditions described elsewhere [21].

Sequencing of the VIM and IMP genes was performed using the class 1 integron primers 5CS (5′-GGCATCCAAGCAGCAAG-3′) and 3CS (5′-AAGCAGACTTGACCTGA-3′) in combination with IMP and VIM primers, respectively. To ensure that the entire MBL allele was obtained, 2 different amplicons per strain were sequenced in an overlapping fashion; a 900-bp fragment was obtained with the 5CS and VIM2004B primers, a 1-kb fragment was obtained with the VIM2004A and 3CS primers, a 1.2-kb fragment was obtained with the 5CS and IMPB primers, and a 1-kb fragment was obtained with the IMPA and 3CS primers. Automated sequencing was performed on the PCR products with the ABI Prism 3100 genetic analyzer (Applied Biosystems) and Sequence Analysis software [20].

All of the MBL-producing strains and 45 of the non–MBL-producing strains were typed with pulsed-field gel electrophoresis (PFGE) after the extraction of genomic DNA and digestion with *Spe*I, as described elsewhere [22]. The subsequent PFGE analyses were performed on a CHEF-MAPPER apparatus (Bio-Rad Laboratories). DNA relatedness was calculated on the basis of the Dice coefficient [23], and strains were considered to be genetically related if the Dice coefficient correlation was $\geq$80%, which corresponds with the possibly related (4–6 bands difference) criteria of Tenover et al. [24].

Analysis. All analyses were performed using Stata (version 8.0; StataCorp). Variables were assessed using histograms obtained before analysis, to identify the underlying distribution. Means $\pm$ SDs were used to describe normally or near normally distributed variables and were compared using Student's *t* test. Medians with interquartile ranges (IQRs) were used to describe nonnormally distributed variables and were compared using the Mann-Whitney *U* test. Differences in proportions were compared using Fisher's exact test. The annual incidence rate (per 100,000 population) of infections caused by imipenem-resistant strains was calculated by placing the number of new cases annually among CHR residents in the numerator and the 2002–2003 population estimates of the CHR on the basis of 2001 census data in the denominator [16]. A CHR resident was defined as having a listed residence within the boundaries of the CHR established as of April 2003 [16]. Age- and sex-specific incidence rates and relative risk (RR) determinations with 95% confidence intervals (CIs) were calculated as described elsewhere [17].

RESULTS

Incidence. During the study period, a total of 1583 *P. aeruginosa* strains were isolated at the CLS, and 228 patients presented with a newly diagnosed infection caused by imipenem-resistant *P. aeruginosa* strains. Of these 228 patients, 195 (86%) were CHR residents, and this gave an overall annual incidence rate of 10.5 cases/100,000 population. Eighty CHR residents were infected with MBL-producing strains, and 115 were infected with non–MBL-producing strains, and this gave annual incidence rates of 3.7 and 5.3 cases/100,000 residents, respectively. Although the overall incidence rate of infection caused by imipenem-resistant strains was stable throughout the study period, significant variation occurred in the number of infections caused by imipenem-resistant strains identified each month (figure 1). The proportion of infections caused by MBL-producing strains was also much higher in the second year of the study, compared with that in the first year of the study (67/116 [58%] vs. 31/112 [28%] infections; $P < .001$). The overall median age of the patients was 70.4 years (IQR, 54.3–81.4 years), and 121 patients (53%) were men. A direct relationship was found between patient age and acquisition of an infection caused by an imipenem-resistant strain (figure 2). There was no significant overall difference in incidence observed between men and women.

Clinical characteristics. Of the 215 adult patients identified in the study, 166 (77%) were admitted to 1 of the 3 acute care centers, 8 (4%) were treated through either CHR emergency

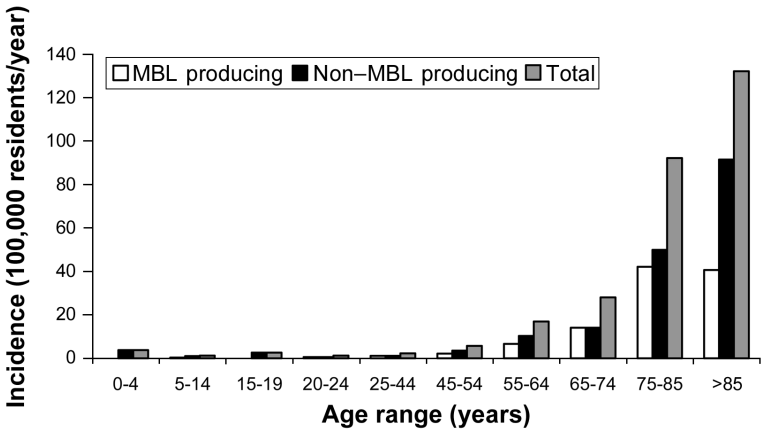

Figure 2. Age-specific annual incidence of infections caused by metallo-β-lactamase (MBL)–producing and non–MBL-producing imipenem-resistant *Pseudomonas aeruginosa* strains in the Calgary Health Region.

departments or hospital-based ambulatory care clinics, and 41 (19%) were treated elsewhere. Records for clinical information were available for review for 173 patients (80%). The subsequent analysis of clinical characteristics and outcome data presented refers to this cohort, unless otherwise stated.

A total of 83% (144/173) of the infections were classified as being nosocomially acquired, which gave an overall incidence rate of ~0.8 cases/1000 discharges. The median time from hospital admission to the development of a nosocomial infection was 20.5 days (IQR, 8–37 days). The nosocomial acquisition rate of imipenem-resistant strains was somewhat higher at acute care center 1, compared with that at the 2 smaller acute care centers (1.1 vs. 0.6 cases/1000 discharges; $P = .0001$), which was attributed to a substantially higher incidence rate of infections caused by MBL-producing strains at that hospital (0.8 vs. 0.2 cases/1000 discharges; RR, 5.0 [95% CI, 2.89–8.97]; $P < .0001$).

Table 2 illustrates various clinical features that were different in patients with MBL-producing strains, compared with those in patients with non–MBL-producing strains. The most common clinical diagnosis in patients with imipenem-resistant strains was urinary tract infection, which was present in 70 patients (40%); pneumonia was present in 53 patients (31%), and soft tissue infection was present in 35 patients (20%) (20 patients presented with nonsurgical infections, and 15 presented with surgical wound–related infections). With the exception of bacteremia without clinical focus (table 2), no significant differences in clinical foci due to MBL-producing and non–MBL-producing strains were observed. The majority (70%) of patients had received at least 1 antimicrobial agent within the 14 days preceding the first infection caused by an imipenem-resistant strain. With the exception of aminoglycosides, quinolones, penicillins, and antifungal agents (table 1), there was no difference observed with respect to exposure to other classes of antimicrobial agents.

Outcome. The median length of hospitalization was 45 days (IQR, 20–87.5 days), and 62 patients required admission to an intensive care unit (ICU) for a median stay of 11 days (IQR, 4.5–23.5 days). A total of 19% (33/173) of patients died, which gave a crude case-fatality rate for infections caused by MBL-producing strains that was significantly higher than that caused by non–MBL-producing strains (23/93 [25%] vs. 10/80 [13%] deaths; RR, 1.98 [95% CI, 1.00–3.90]; $P = .05$).

Microbiological assessment. Results of susceptibility testing for routinely tested antimicrobial agents are shown in table 1. Resistance to ≥ 2 different classes of antimicrobial agents was significantly more common in MBL-producing strains, compared with that in non–MBL-producing strains (95/98 [97%] vs.77/130 [59%] strains; RR, 1.64 [95% CI, 1.41–1.90]; $P < .0001$). The different classes of antibiotics were defined as follows: penicillins, cephalosporins, aminoglycosides, and quinolones. The majority (87/98; 89%) of MBL-producing strains were resistant to ≥ 3 classes of antibiotics (in addition to imipenem), whereas resistance to antibiotics was uncommon (9/130; 7%) in non–MBL-producing strains ($P < .0001$). It was interesting to note that 13 (13%) of 98 MBL-producing strains and 8 (6%) of 130 non–MBL-producing strains were resistant to piperacillin. Meropenem resistance was present in 81 (68%) of 119 non–MBL-producing strains. Additional susceptibility testing was selectively performed on some MBL-producing strains, and resistance was found to aztreonam in 9 (15%) of 61 strains, to colistin in 7 (11%) of 66 strains, and to amikacin in 81 (80%) of 101 strains.

Of the 228 *P. aeruginosa* strains isolated during the study period, 90 (39%) were positive for bla_{VIM} genes, and 4 (2%) were positive for bla_{IMP} genes. The remaining 134 strains (59%) were negative for both VIM and IMP genes. The 4 strains that

Table 2. Clinical characteristics of patients with infections caused by metallo-β-lactamase (MBL)–producing and non–MBL-producing imipenem-resistant *Pseudomonas aeruginosa* strains in the Calgary Health Region.

Clinical characteristic	MBL-producing strains (n = 93)	Non–MBL-producing strains (n = 80)	RR (95% CI)	P
Median age (IQR)	67.2 (54.6–78.7)	73.9 (57.9–82.2)	…	.04
Male sex	59 (63)	39 (49)	1.3 (0.99–1.71)	.06
Nosocomial acquisition	81 (87)	63 (79)	1.1 (0.97–1.27)	.16
⩾1 hospital admission in previous year	33 (35)	41 (51)	0.7 (0.49–0.98)	.05
Nursing home resident	14 (15)	14 (18)	0.9 (0.44–1.69)	.40
Respiratory disease	28 (30)	37 (46)	0.7 (0.44–0.96)	.04
Transplant recipient	11 (12)	2 (3)	4.7 (1.08–20.72)	.02
Cancer	34 (37)	20 (25)	1.54 (0.96–2.45)	.07
Heart disease	46 (49)	58 (73)	0.68 (0.53–0.87)	<.01
Bacteremia without clinical focus	12 (13)	3 (4)	3.4 (1.01–11.76)	.06
Recent[a] aminoglycosides	12 (13)	3 (4)	3.4 (1.01–11.76)	.05
Recent[a] penicillins	32 (34)	15 (19)	1.83 (1.07–3.14)	.03
Recent[a] antifungals	14 (15)	2 (3)	6.0 (1.41–25.70)	<.01
Recent[a] quinolones	36 (39)	15 (19)	2.1 (1.22–3.48)	.005

NOTE. Data are no. (%) of strains, unless otherwise indicated. CI, confidence interval; IQR, interquartile range; RR, relative risk.

[a] Within the 2 weeks before the development of an infection caused by an imipenem-resistant *P. aeruginosa* strain.

were phenotypically positive for MBLs but negative by PCR for either bla_{VIM} or bla_{IMP} genes were also negative for SPM MBLs. For the purpose of this study, these strains were considered to produce MBLs. PCR with 5C, 3C, and the different combinations of VIM and IMP primers amplified various amplicons ranging in size from 900 bp to 1.2 kb. Sequence analysis of the products obtained with the VIM primers revealed 100% identity with the sequence of the bla_{VIM-2} allele [25], and analysis of the products obtained with the IMP primers revealed 100% identity with the sequence of the bla_{IMP-7} allele [14].

Molecular epidemiological assessment. PFGE revealed 2 closely related restriction patterns (clusters MBL-A [82 strains] and MBL-AR [related to A; 6 strains]) among the 90 VIM-2–producing strains. The remaining 2 VIM-2–producing strains were not related to these clusters. The majority (63/90 [70%]) of these strains were isolated from patients admitted to acute care center 1, 4 (4%) were isolated from patients admitted to acute care center 2, 16 (18%) were isolated from patients admitted to acute care center 3, and 7 (8%) were isolated from patients managed outside the acute care centers. Approximately 90% (56/63) of strains from acute care center 1 were involved in the nosocomial outbreak that occurred because of contaminated faucets at the ICU and the bone marrow transplant unit during April–November 2003 [26]. It was interesting to note that 4 of 6 strains from cluster MBL-AR were isolated from acute care center 3, but these strains were not involved in a nosocomial outbreak. It was established that 20 (74%) of 27 patients infected with cluster MBL-A at acute care centers 2 and 3 and outside the acute care centers did have contact with or were transferred from acute care center 1.

The 4 IMP-7–producing strains belonged to a different cluster (MBL-B) and were related to the strains described in the nosocomial outbreak during the 1990s in our region [14]. The PFGE patterns of the 4 strains that were phenotypically positive but negative by PCR for MBL were as follows: 1 strain belonged to cluster MBL-A, 1 strain belonged to cluster MBL-B, and 2 strains were not related to any of the clusters or to each other. PFGE of the non–MBL-producing strains (only 45 were available for PFGE) showed the following patterns: 10 were closely related (cluster C), 3 belonged to cluster MBL-A (VIM-2 producing), and 32 were not related to any of these clusters or to each other. The majority of strains from cluster C (6/10 [60%]) were from patients admitted to acute care center 2.

DISCUSSION

This study has demonstrated that imipenem-resistant *P. aeruginosa* strains—and, in particular, MBL-producing strains—are common causes of infection within our region. Although the overall occurrence of these infections was stable during the 2-year study period, the relative occurrence of infections caused by MBL-producing strains increased during the second year. We previously recognized and reported a nosocomial outbreak of infections caused by MBL-producing strains that occurred because of contaminated faucets and sinks at the ICU and the bone marrow transplant unit at acute center 1 during 2003 [26]. This explains the increased incidence rate of infections caused by MBL-producing strains during the second year of the present study, but it was unexpected that the incidence rate of infections caused by non–MBL-producing strains decreased as a proportion of the total number of cases (figure 1). One possible explanation could be that the use of carbapenems was restricted during the outbreak, and the removal of selection pressure associated with porin and/or efflux mutants might

have been responsible for the decrease in infections caused by non–MBL-producing strains.

A novel aspect of the present study was the identification of demographic factors associated with the development of infections caused by imipenem-resistant strains in the general population. We observed a significant age-related increase in the incidence rate of infection and a slightly higher but nonsignificant risk in men. This most likely reflects, at least in part, the presence of comorbid disease (figure 2). In the subset of patients admitted to hospitals, those with infections caused by MBL-producing strains were different than patients with non–MBL-producing strains with respect to preceding antibiotic exposure, presence and type of comorbid illness, and invasiveness of infection (table 2). Harris et al. conducted case-control studies to evaluate the risk factors for acquisition of nosocomial infections caused by imipenem-resistant *P. aeruginosa* strains [27, 28]. Exposure to a number of different antibiotics (imipenem, vancomycin, piperacillin-tazobactam, and aminoglycosides), length of hospitalization, and ICU admission were independent risk factors for acquisition of infection caused by imipenem-resistant strains in these cohort studies. Hirakata et al. conducted a case-control study that included 69 patients infected with IMP-producing *P. aeruginosa* strains and found that increased length of hospitalization, antineoplastic therapy, corticosteroids, and indwelling catheters were risk factors for acquisition of these organisms [29]. It should be noted that, with the exception of the population-based demographic risk factor analysis, our study was not designed to identify risk factors for acquisition of imipenem-resistant *P. aeruginosa* strains—or, more specifically, MBL-producing strains—per se.

IMP-7–producing *P. aeruginosa* strains were responsible for a nosocomial outbreak involving 24 patients in our region during the 1990s [14]. The IMP-7–producing strains typically were sensitive to piperacillin but resistant to quinolones, aminoglycosides, and ceftazidime and were identical to the strains detected in the present study (table 1). Therefore, it was unexpected to find that >90% of the MBL-producing strains detected in the present study were producing VIM-2. The IMP- and VIM-producing groups are not genetically related to each other (<30% amino acid identity between the groups). It is most likely that strains producing VIM-2 replaced those producing IMP-7 between the 1990s and 2002, and this is supported by the PFGE data. The simultaneous occurrence of 2 different groups of MBLs in a centralized health care region such as the CHR is rare and has been previously described only in Brazil [30]. Although production of VIM-type enzymes in *P. aeruginosa* strains have been reported in Europe, Asia, and South America [31], they have been reported only rarely in North America, including a report of 4 patients in Chicago with infections caused by VIM-2–producing strains [32] and a report of a VIM-7–producing strain from a patient who underwent liver transplantation in Texas [15]. To our knowledge, this is the first report from Canada of VIM-producing *P. aeruginosa* strains.

Our study has also described aspects of the molecular epidemiological characteristics of infections caused by carbapenem-resistant strains in a large, well-defined geographical region. Molecular typing showed that clonally related VIM-2–producing strains (cluster MBL-A) were responsible for a nosocomial outbreak in the ICU and bone marrow transplant unit of acute care center 1 during 2003. Environmental strains isolated from faucets in these units also belonged to cluster MBL-A (T.J.L. and J.D.D.P., unpublished data). Molecular typing further illustrated the ease with which VIM-2–producing strains accompanied patients who were transferred to other acute care centers. However, VIM-2–producing strains did not cause a nosocomial outbreak outside acute care center 1, and this underlines the importance of environmental reservoirs as a cause of nosocomial outbreaks of infections caused by *P. aeruginosa.* The molecular typing also showed that 3 different clusters were present at the different acute care centers within our region (cluster MBL-A at acute care center 1, cluster C at acute care center 2, and cluster MBL-AR at acute care center 3).

Our study has illustrated that MBL-producing strains are an important cause of imipenem resistance in *P. aeruginosa* and strongly supports the notion that clinical microbiological laboratories must be able to distinguish MBL-producing strains from those with other mechanisms responsible for carbapenem resistance. MBL-producing strains were significantly more resistant to various antimicrobial agents than were non–MBL-producing strains (table 1), and this resistance was most likely due to the association of MBL gene cassettes with class I integrons (as has been shown in the present study). The identification of MBL production in *P. aeruginosa* strains also has clinical implications, because such strains were more likely to cause invasive disease and were associated with a higher hospital case-fatality rate, compared with other imipenem-resistant strains. These findings are consistent with the findings of a study by Hirakata et al. [29]. Therefore, the detection and molecular characterization of MBL-producing *P. aeruginosa* strains is important for the purposes of infection prevention and control and, potentially, for defining risk for development of severe disease and adverse outcome.

We hypothesize that strains producing VIM-type enzymes may be associated with increased virulence. Indirect evidence in support of this argument includes the apparently higher case-fatality rate in infections caused by VIM-2–producing strains in the present study. Interestingly, the IMP-7–producing strains that caused the nosocomial outbreak in our region during 2003 appeared to be much less invasive when judged on the basis of the absence of bacteremic illness in the patients [14]. Our findings suggest that the evolution, maintenance, and dissem-

ination of MBL genes in *P. aeruginosa* populations in larger geographic health care regions is a dynamic process that requires ongoing study.

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