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Travel-Related Carbapenemase-Producing Gram-Negative Bacteria in Alberta, Canada: the First 3 Years

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We describe here the characteristics of Alberta, Canada, patients with infections or colonizations with carbapenemase-producing Gram-negative bacteria during 2010 to 2013 that were linked to recent travel outside Canada. Antimicrobial susceptibility was determined by broth microdilution, and isolates were characterized using PCR, sequencing, and multilocus sequencing typing. A broth mating study was used to assess the transferability of resistance plasmids, which were subsequently characterized. All the patients ($n = 12$) included in our study had contact with a health care system while abroad. Most of the patients presented with urinary tract infections (UTIs) and were admitted to hospitals within weeks after their return to Alberta. Secondary spread occurred in 1 case, resulting in the death of another patient. The carbapenemase-producing bacteria ($n = 17$) consisted of *Escherichia coli* (sequence type 101 [ST101], ST365, ST405, and ST410) with NDM-1, *Klebsiella pneumoniae* (ST15, ST16, ST147, ST258, ST340, ST512, and ST972) with NDM-1, OXA-181, KPC-2, and KPC-3, *Acinetobacter baumannii* with OXA-23, *Providencia rettgeri* with NDM-1, *Enterobacter cloacae* with KPC-2, and *Citrobacter freundii* with NDM-1. The *bla*$_{NDM-1}$ gene was associated with various narrow- (i.e., IncF) and broad- (i.e., IncA/C and IncL/M) host-range plasmids with different addiction factors. Our results show that NDM-producing *K. pneumoniae*, belonging to a variety of sequence types with different plasmid scaffolds, are regularly imported from India into Alberta. Clinical microbiology laboratories should remain vigilant in detecting bacteria with carbapenemases.

Gram-negative bacteria, most notably *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are among the most important causes of serious hospital-acquired and community-onset bacterial infections in humans, and resistance to antimicrobial agents in these bacteria has become an increasingly relevant problem (1). Of special concern is the development of resistance to the carbapenems, as these agents are often the last line of effective therapy available for the treatment of infections caused by multiresistant Gram-negative bacteria (2). Most important is the recognition of isolates that harbor carbapenemases that cause resistance to the carbapenems. These enzymes include the class A (i.e., KPC types), the class B (or the metallo-β-lactamases) (i.e., VIM, IPM, and NDM types), and the class D oxacillinases (i.e., OXA enzymes) (3).

NDM was first described in *K. pneumoniae* and *E. coli* isolated from a Swedish patient who was previously hospitalized in New Delhi, India (4). Subsequently, bacteria with NDM-1 have been recognized in over 40 countries on every continent, except Antarctica, and these bacteria are considered endemic in the Indian subcontinent (5). NDM-1 has most commonly been reported in *E. coli* and *K. pneumoniae* but has also been found in a variety of other members of the *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas* spp., and *Vibrio cholerae* (6, 7).

KPCs were first reported in the late 1990s from a *K. pneumoniae* isolated in North Carolina, and to date, more than 10 different KPC variants have been described (8). KPC β-lactamases (especially KPC-2 and -3) have been described in various enterobacterial species, especially *Klebsiella* spp. and *E. coli* (9). Several nosocomial outbreaks have been reported from the United States and European, South American, Asian, and Middle Eastern countries (8, 10, 11). KPC-producing bacteria are considered to be endemic in certain parts of the world, such as the northeastern United States, Puerto Rico, Colombia, Greece, Israel, and China, and are important causes of nosocomially acquired infections in some parts of these countries (11).

In May 2013, the Public Health Services of Alberta Health in Canada issued provincial surveillance and management guidelines for carbapenem-resistant Gram-negative bacteria (12). These guidelines were issued directly as a consequence of an outbreak that occurred in April 2012. Health care facilities are required to notify regional public health authorities regarding patients that are colonized or infected by these bacteria, and laboratories are requested to refer isolates to an antimicrobial resistance referral center for additional testing and confirmation of the resistance mechanisms. We undertook a study to characterize imported cases due to Gram-negative bacteria that produce carbapenemases in Alberta, Canada, that occurred over a 3-year period before the Alberta Health guidelines became available. (This work was presented at the 53rd Intersci. Conf. Antimicrob. Agents Chemother., Denver, CO, 10 to 13 September 2013 [13].)

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MATERIALS AND METHODS

Alberta, Canada. The province of Alberta is located in western Canada, has a population of just over 3.7 million people, and is Canada's fourth most populous province. Alberta has ethnic diversity, with the people of Chinese and East Indian origin representing nearly 7% of Alberta's population. Aboriginal Albertans make up approximately 3% of the population. In line with the rest of Canada, the majority of Albertans originated from England, Scotland, Ireland, and Wales, but large numbers also came from other parts of Europe, notably Germany, France, Ukraine, and Scandinavia. Alberta is home to the second highest proportion (2%) of Francophones in western Canada (after Manitoba).

Alberta Health Services (AHS) is the provincial health authority responsible for overseeing the planning and delivery of health support and services to adults and children living in the province of Alberta. AHS has been organized so as to separate acute hospital facilities from smaller hospitals and community services, the latter of which are organized into five zones (North, Edmonton, Central, Calgary, and South). The Calgary zone includes five major acute-care sites, including Foothills Medical Centre, Peter Lougheed Centre, Rockyview General Hospital, South Health Campus, and Alberta Children's Hospital. The Edmonton zone includes eight acute-care sites (hospitals) in the metropolitan area, which include the University of Alberta Hospital (Edmonton), Royal Alexandra Hospital (Edmonton), Gray Nuns Hospital (Edmonton), Misericordia Community Hospital (Edmonton), Sturgeon Community Hospital (St. Albert), Leduc Community Hospital (Leduc), WestView Health Centre (Stony Plain), and Fort Saskatchewan Community Hospital (Fort Saskatchewan).

Patients. The first description of travel-related carbapenemase-producing bacteria was of a case that occurred during April 2010 when *E. coli* with NDM-1 was isolated from a patient with pyelonephritis and prostatitis who returned to Canada after recent hospitalization in India (14). Subsequently, 11 additional patients infected or colonized with carbapenemase-producing bacteria were identified up to the end of April 2013. The majority of cases ($n = 7$) were identified in the Edmonton zone, and 4 occurred in the Calgary zone, while 1 case was identified in the Rural South zone. Some of these cases ($n = 4$) had previously been reported (14–17); however, we identified additional patients ($n = 8$) and performed supplementary characterization (i.e., plasmid analysis and virulence factors) that had not been reported in the original publications.

Screening patients for carbapenemase-producing bacteria. Surveillance cultures (i.e., from rectal, wound, ostomy, and endotracheal suction sources) were performed in clinical microbiology laboratories from the Edmonton and Calgary zones and were implemented to identify all epidemiologically linked patients who may have become colonized. This process was directed by the infection prevention and control departments on an individual basis at each acute-care center in Edmonton and Calgary and formed part of the contact tracing and unit-wide prevalence screening procedures. No screenings of environmental or health care workers were performed.

Screening swabs obtained from patients were placed into a Copan M40 Transystem containing Amies gel transport medium. Stool and endotracheal suction specimens were submitted in sterile containers without transport medium. The Centers for Disease Control and Prevention (CDC) (Atlanta, GA) protocol was used to screen for carbapenemase-producing Gram-negative bacteria (18).

Antimicrobial susceptibilities. Antimicrobial susceptibility was determined with MicroScan NEG MIC 38 panels (Siemens, Burlington, ON). The MICs of the following drugs were determined: amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefoxitin (FOX), ceftriaxone (CRO), ceftazidime (CAZ), aztreonam, (ATM), imipenem (IPM), meropenem (MER), ertapenem (ERT), amikacin (AMK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), and tigecycline (TIM). Additional susceptibility tests for colistin (CST) were performed using Etest methodology according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France). Throughout this study, results were interpreted using the 2012 CLSI criteria for broth dilution (19). The European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints (i.e., for *K. pneumoniae* and *E. coli*) were used for CST and the FDA breakpoint was used for TIM.

β-Lactamase identification. The presence of carbapenemase was determined with the modified Hodge test (19) and the Mastdiscs ID inhibitor combination disks (20) (Mast Group, Ltd., Merseyside, United Kingdom). PCR amplification and sequencing for bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{CMY}, bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, $bla_{OXA-48-like}$, $bla_{OXA23-like}$, $bla_{OXA24-like}$, $bla_{OXA51-like}$, and $bla_{OXA58-like}$ were carried out on the isolates with a GeneAmp 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) using PCR conditions and primers as previously described (6, 14, 21–24). The presence of IS*ABA125* and ble_{MBL} among bla_{NDM}-positive bacteria was detected using PCR conditions and primers as previously described (6), while the different Tn*4401* isoforms (i.e., a, b, or c) were identified among the bla_{KPC} genes according to the method of Cuzon and colleagues (25).

Plasmid-mediated quinolone resistance determinants. The amplification of the *qnrA*, *qnrS*, and *qnrB* genes was undertaken with a multiplex PCR (26). *AAC(6′)-Ib* and *qepA* were amplified in a separate PCR using primers and conditions as previously described (27, 28). The variant *aac(6′)-Ib-cr* was further identified by digestion with *BstF5*I (New England BioLabs, Ipswich, MA).

16S rRNA methylation. The amplification of genes encoding 16 RNA methylases was determined as described previously (29, 30).

Multilocus sequencing typing. Multilocus sequencing typing (MLST) on the *K. pneumoniae* was performed using seven conserved housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) as previously described (31). MLST was performed on the *E. coli* using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*). A detailed protocol of the MLST procedure, including allelic type and sequence type (ST) assignment methods, is available at the MLST Databases at the Environmental Research Institute (ERI), University College Cork website (http://mlst.ucc.ie/mlst/dbs/Ecoli). MLST was performed on the *A. baumannii* using seven conserved housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*). A detailed protocol of the MLST procedure, including allelic type and sequence type (ST) assignment methods, is available at the Pasteur MLST databases (http://www.pasteur.fr/recherche/genopole/PF8/mlst/primers_Abaumannii.html).

Plasmid analysis. Plasmid sizes on the *Enterobacteriaceae* were determined as previously described (32) and assigned to plasmid incompatibility groups by PCR-based replicon typing (33, 34). Plasmid addiction systems were determined using PCR as described before (35). Conjugation experiments were performed by mating-out assays with nutrient agar containing meropenem (MER) (1 μg/ml) and using *E. coli* J53 (azide, 100 μg/ml) as the recipient.

Virulence factors for *E. coli* and *K. pneumoniae*. We assessed the presence of extraintestinal pathogenic *E. coli* (ExPEC)-associated virulence genes and the housekeeping gene *uidA* (β-glucuronidase) for *E. coli* by multiplex PCR (36). These included the following: F10 *papA* (P fimbriae subunit variant), *papACEFG* (genes of the P fimbriae operon), *sfa/foc* (S or F1C fimbriae), *focG* (F1C fimbriae adhesin), *iha* (adhesion siderophore), *fimH* (type 1 fimbriae), *tsh* (temperature-sensitive hemagglutinin), *hra* (heat-resistant agglutinin), *afa/dra* (Dr-binding adhesins), *hlyD* (α-hemolysin), *sat* (secreted autotransporter toxin), *pic*, (serine protease), *vat* (vacuolating toxin), *astA* (enteroaggregative *E. coli* toxin), *cnF1* (cytotoxic necrotizing factor), *iroN* (salmochelin [siderophore] receptor), *fyuA* (yersiniabactin [siderophore] receptor), *ireA* (siderophore receptor), *iutA* (aerobactin [siderophore] receptor), *kpsM* II (group 2 capsule), K1, K2, and K5 (group 2 capsule variants), *kpsM* III (group 3 capsule), *usp* (uropathogenic-specific protein), *traT* (serum resistance-associated), *ompT* (outer membrane protease T), *iss* (increased serum survival), H7 *fliC* (flagellin variant), and *malX* (pathogenicity island marker). Isolates were defined as ExPEC if positive for ≥2 of *papA* and/or *papC* (P fimbriae

TABLE 1 The clinical features of patients infected or colonized with travel-related carbapenemase-producing bacteria

Patient no.	Isolate(s)	Age (yr)	Gender[a]	Travel date (mo/yr)[b]	Country visited	Admission on return	Secondary spread	Clinical entity	Reference or source
1	*E. coli* MH01	32	M	4/2010	India (hospitalized in medical ward due to diabetic crisis)	Yes (medical ward for UTI management)	No	UTI[c]	14
2	*K. pneumoniae* KpCG01	52	F	2/2011	India (hospitalized in surgical ward for minor surgical procedure)	No	No	UTI	16
3	*K. pneumoniae* NT11-19	21	M	5/2011	India (hospitalized in surgical ward due to gunshot wound)	Yes (surgical ward for treatment of cellulitis)	No	Cellulitis (wound leg)	This study
4	*C. freundii* NT11-22	87	M	6/2011	India (hospitalized in medical ward due to hypertensive crisis)	Yes (medical ward for hypertensive control)	No	Colonized (rectal)	This study
5	*K. pneumoniae* KpCG02	82	M	11/2011	Greece (hospitalized in medical ward with urinary obstruction)	No	No	UTI	17
6	*K. pneumoniae* KpCG03	61	M	11/2012	India (hospitalized in urology ward for placement of urinary catheter)	Yes (urology ward for UTI management)	No	UTI	This study
7	*E. coli* RN12-40	52	M	6/2012	India (hospitalized in ICU ward due to trauma and blood transfusion)	Yes (surgical ward for management of trauma)	No	Colonized (rectal)	This study
8	*E. coli* RN12-50, *K. pneumoniae* RN12-59	91	F	7/2012	India (hospitalized in medical ward due to congestive heart failure)	Yes (medical ward for management of UTI)	No	UTI	This study
9	*E. coli* EC01, *K. pneumoniae* KP01, *A. baumannii* AB01, *K. pneumoniae* KP02	62	F	3/2013	India (hospitalized in surgical ward and ICU due to trauma)	Yes (surgical ward treatment of cellulitis)	Yes	Cellulitis (wound leg), colonized (rectal)	(15)
10	*P. rettgeri* UR53778, *K. pneumoniae* KPY18268	83	F	3/2013	India (hospitalized in medical ward due to UTI)	Yes (medical ward for management of UTI)	No	UTI	This study
11	*K. pneumoniae* M2800	38	M	4/2012	Israel (hospitalized in surgical ward due to trauma)	Yes (surgical ward for management of trauma)	No	Colonized (rectal)	This study
12	*E. cloacae* S629	27	M	1/2011	Ecuador (hospitalized in ICU and surgical ward due to trauma)	Yes (surgical ward for management of trauma)	No	Cellulitis (wound hip)	This study

[a] M, male; F, female.
[b] Return date to Alberta. All patients received antibiotics while in foreign hospitals.
[c] UTI, urinary tract infection.

major subunit and assembly), *sfa/foc*DE (S and F1C fimbriae), *afa/dra*BC (Dr-binding adhesins), and *kpsM* II (group 2 capsule).

The PCR methods described by Brisse et al. were used to determine the presence of virulence genes that have previously been associated with virulence in *K. pneumoniae* (37). These included the following: *uge* (encoding UDP galacturonate 4-epimerase), *wabG* (involved in the biosynthesis of the outer core lipopolysaccharide), *ureA* (related to the urease operon), *magA* (mucoviscosity-associated gene A), *mrkD* (type 3 fimbriae adhesion), *allS* (activator of the allantoin regulon), *kfuBC* (iron-uptake system), *rpmA* (regulator of mucoid phenotype), and *fimH* (fimbrial gene encoding type 1 fimbrial adhesion).

RESULTS

Patients and bacteria. During the 3-year period (April 2010 until April 2013), 17 carbapenemase-producing bacteria were isolated from 12 patients with a history of recent travel outside Canada. The details of the patients (including the clinical presentation and country visited) are shown in Table 1. India ($n = 9$) was by far the most common country visited, followed by Greece ($n = 1$), Israel ($n = 1$), and Ecuador ($n = 1$). The majority of patients presented with urinary tract infections (UTIs). All of them had contact with the health care system while traveling abroad (i.e., were admitted to hospitals), and 10 were admitted to a local hospital within 7 days from their return to Alberta. Secondary spread occurred in 1 case, causing the death of another patient in the same unit (15).

The carbapenemase-producing bacteria ($n = 17$) consisted of *K. pneumoniae* ($n = 9$), *E. coli* ($n = 4$), and 1 each of *Enterobacter cloacae*, *Citrobacter freundii*, *Providencia rettgeri*, and *A. baumannii* (Table 1). All of the isolates were nonsusceptible (NS) (i.e., intermediate or resistant) to AMC, TZP, FOX, CRO, CAZ, ATM, IPM, MER, ERT, CIP, and SXT; 14 (82%) were NS to GEN, 15 (88%) to TOB, 11 (65%) to AMK, and 3 (18%) to TIM. All of the isolates remained susceptible to CST.

Active surveillance cultures. The clinical microbiology laboratories in Edmonton and Calgary during the 3-year period received 557 screening specimens from 227 patients with a positive rate of 1.1%. This was directed by the infection prevention and control departments on an individual basis for each acute-care center in Edmonton and Calgary and formed part of the process of contact tracing and unit-wide prevalence screening.

β-Lactamases. The majority ($n = 10$) of the *Enterobacteriaceae* tested positive with the modified Hodge test using MEM. Mast-discs ID inhibitor combination disks indicated that 12 isolates produced metallo-β-lactamase (MBL),while 4 produced class A carbapenemases. *A. baumannii* tested positive with an MBL Etest and the modified Hodge test using IPM as the substrate. PCR results showed that the *Enterobacteriaceae* isolates were positive for bla_{KPC}, bla_{NDM}, bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{CMY}, and $bla_{OXA-48-like}$, and sequencing identified bla_{TEM} as TEM-1, bla_{KPC}

TABLE 2 Molecular characteristics of bacteria with carbapenemases

Isolate	MLST	β-Lactamases	16S RNA methylase(s)	PMQR[a] determinants	Plasmids carrying bla_{NDM-1} or bla_{KPC} genes			IS*Aba125*	ble_{MBL}	Tn*4401* isoform
					Size (kb)	Replicon typing	Addiction system(s)[b]			
K. pneumoniae KpCG01	ST340	NDM-1, SHV-12, CTX-M-15, TEM-1	*armA*, *rmtC*	*aac(6′)-Ib-cr*	150	FIIk	*ccdA/B*	+	+	ND[c]
K. pneumoniae NT11-19	ST147	NDM-1, CTX-M-15, TEM-1, OXA-30	*rmtF*	*aac(6′)-Ib-cr*, *qnrB*	120	L/M	*pemK*, *vagC/D*	+	−	ND
K. pneumoniae KpCG02	ST258	KPC-2, SHV-12, TEM-1	Neg[d]	Neg	120	FIIk	*vagC/D*	ND	ND	*a*
K. pneumoniae KpCG03	ST147	KPC-2, TEM-1	*armA*	*aac(6′)-Ib-cr*, *qnrB*	110	FIIk	*vagC/D*	ND	ND	*b*
K. pneumoniae RN12-59	ST15	NDM-1, CTX-M-15, CMY-16, TEM-1	*rmtC*, *rmfF*	*qnrS*	150	A/C	*pemK*, *srnB/C*	+	+	ND
K. pneumoniae KP01	ST972	NDM-1, CTX-M-15, OXA-181, TEM-1	*rmtF*	*aac(6′)-Ib-cr*, *qnrS*	150	NT[e]	*vagC/D*	+	+	ND
K. pneumoniae KP02	ST16	NDM-1, CTX-M-15, TEM-1	*rmtF*	*aac(6′)-Ib-cr*, *qnrB*	120	FIIk	*pemK*	+	−	ND
K. pneumoniae KPY18268	ST147	NDM-1, CTX-M-15, OXA-181, TEM-1	*armA*, *rmtF*	*qnrB*	180	A/C	*vagC/D*	+	+	ND
K. pneumoniae M2800	ST512	KPC-3, TEM-1	*armA*	Neg	110	FIIk	*vagC/D*	ND	ND	*a*
E. coli MH01	ST101	NDM-1, CTX-M-15, TEM-1	*armA*, *rmtC*	Neg	80	A/C	*pemK*, *ccdA/B*, *vagC/D*	+	+	ND
E. coli RN12-40	ST365	NDM-1, CTX-M-15, TEM-1	*rmtC*, *rmtF*	Neg	180	A/C	*pemK*, *relE*, *Hok/Sok*	+	+	ND
E. coli RN12-50	ST405	NDM-1, CTX-M-15, CMY-16, TEM-1	*rmtC*, *rmtF*	*qnrS*	150	A/C	*pemK*, *srnB/C*	+	+	ND
E. coli EC01	ST410	NDM-1, CTX-M-15, TEM-1	*rmtF*	*aac(6′)-Ib-cr*	80	A/C	*pemK*, *vagC/D*, *Hok/Sok*	+	−	ND
C. freundii NT11-22	ND	NDM-1, CTX-M-15, OXA-30, TEM-1	*rmtB*, *rmtC*	*aac(6′)-Ib-cr*, *qnrB*	180	A/C	*vagC/D*	+	+	ND
P. rettgeri UR53778	ND	NDM-1, TEM-1	*armA*, *rmtF*	*qnrB*	90	T	Neg	+	+	ND
E. cloacae S629	ND	KPC-2, TEM-1	Neg	*qnrA*	120	L/M	*ccdA/B*	ND	ND	*b*
A. baumannii AB01	ST10	OXA-23, OXA-51-like	*armA*	*aac(6′)-Ib-cr*	ND	ND	ND	ND	ND	ND

[a] PMQR, plasmid-mediated quinolone resistance determinants.
[b] Addiction systems, *pemKI*, plasmid emergency maintenance; *ccdA/B*, coupled cell division locus; *relE*, relaxed control of stable RNA synthesis; *parD/E* and *vagC/D*, virulence-associated proteins; *Hok/Sok*, *pndA/C*, and *srnB/C*, plasmid antisense RNA-regulated systems.
[c] ND, not done.
[d] Neg, negative.
[e] NT, untypeable.

as KPC-2 or KPC-3, bla_{NDM} as NDM-1, bla_{CTX-M} as CTX-M-15, bla_{SHV} as SHV-12, bla_{CMY} as CMY-16, and $bla_{48\text{-like}}$ as OXA-181 (refer to Table 2 for details). The *A. baumannii* isolate was positive for $bla_{OXA\text{-}23\text{-like}}$ and $bla_{OXA\text{-}51}$, and sequencing identified $bla_{OXA\text{-}23\text{-like}}$ as OXA-23 (Table 2).

Molecular characterization. MLST identified the *E. coli* isolates as ST101, ST365, ST405, and ST410, while the *K. pneumoniae* isolates belonged to ST15, ST16, ST147, ST258, ST340, ST512, and ST972 (Table 2). MLST identified the *A. baumannii* isolate as ST10. The presence of the different plasmid-mediated quinolone resistance determinants, 16S rRNA methylases, IS*ABA125*, ble_{MBL} (among NDM-producers), and the different *Tn4401* isoforms (among KPC producers) is shown in Table 2.

Plasmid studies. The *Enterobacteriaceae* contained several plasmids ranging from 20 kb to 250 kb in size. The mating-out assays with *E. coli* J53 produced transconjugants with plasmids that ranged from 80 kb to 180 kb in size (Table 2). PCR for plasmid incompatibility groups identified various scaffolds with the bla_{NDM-1} genes that belonged to IncA/C, IncL/M, IncF, and untypeable, while the bla_{KPC}s belonged to IncF and IncL/M (Table 2). The IncF incompatibility group contained the FIIk replicon. The addiction factors associated with the different plasmids are shown in Table 2.

Virulence factors. Among the *K. pneumoniae*, ST147, ST258, ST340, ST512, and ST972 were positive for *mrkD*, *fimH*, *uge*, *wabG*, and *ureA*, while ST15 and ST16 were positive for *kfuBC mrkD*, *fimH*, *uge*, *wabG*, and *ureA*. Among the *E. coli*, ST101 and ST365 were positive for *fimH*, *hra*, *fyuA*, and *uidA* and ST410 was positive for *fimH*, *hra*, *iutA*, *traT*, and *uidA*, while ST405 was positive for *fimH*, *kpsIII*, *PAI*, *iutA*, *fyuA*, *ompT*, *kii*, *PapC*, *traT*, and *uidA*.

DISCUSSION

The easy access of air and ground transportation is making it possible for people to travel to different countries and continents in a matter of hours or days, either as tourists (medical, business, study, or recreational), immigrants, refugees, asylum seekers, or migrant workers. Overseas travel, as a risk factor for the acquisition of infections due to antimicrobial resistant organisms, has recently been described for infections due to CTX-M-producing *E. coli*, as well as for various carbapenemase-producing Gram-negative bacteria, including the NDMs and KPCs (38). We describe the characteristics of Alberta patients with carbapenemase-producing Gram-negative bacteria over a 3-year period, which were linked to recent overseas travel outside Canada. Our results show that NDM-producing *K. pneumoniae* strains, belonging to a

variety of sequence types with different plasmid scaffolds, are regularly imported from India into Alberta, Canada. All of the patients included in our study had contact with the health care system while abroad; most patients presented with UTIs and were admitted to hospitals within weeks after their return to Alberta (Table 1). Fortunately, secondary spread occurred only on a single occasion; however, this had devastating consequences, resulting in the death of a different patient due to infection with OXA-23-producing *A. baumannii* (15). During the 3-year period, we also isolated VIM- and GES-producing *P. aeruginosa* (12 cases) and SME-producing *Serratia marcescens* (4 cases) isolates that were not related to recent foreign hospitalization. VIM-producing *P. aeruginosa* had been previously responsible for nosocomial outbreaks during 2003 to 2004 in the Calgary region (39).

A similar study had previously been published from Finland (40) that documented 25 patients with infection due to carbapenemase-producing *Enterobacteriaceae* over a 4-year period (2008 to 2011); 18 patients had a history of recent travel outside Finland, with Greece being the country most commonly visited. Since *K. pneumoniae* with KPCs and VIMs are endemic in Greece (41), it was not surprising that ST258 and ST147 *K. pneumoniae* with KPCs and VIMs were responsible for 7/18 (39%) of the imported cases in that study (40). NDM-producing bacteria were rarely encountered in the Finnish study.

Alberta has a substantial population of expatriates from the Indian subcontinent who regularly visit friends and family in India. Some of patients described in this study (4/12) were of East Indian decent. The Indian subcontinent is a region where NDM-producing bacteria are endemic (5), and this would explain why NDM-producing bacteria were so prevalent in our study. The molecular characteristics of the NDM-producing isolates [i.e., the presence of CTX-M-15, *aac*(6′)*-Ib-cr*, and several RNA methylases, including the recent described *rmtF*, IS*ABA125*, ble_{MBL}, and IncF or IncA/C plasmids] are very similar to the features previously described in other NDM-producing *Enterobacteriaceae* that have been linked to travel in the Indian subcontinent (6, 30). We described various narrow- (i.e., IncF) and broad- (i.e., IncA/C) host-range plasmids with different addiction factors associated with bla_{NDM-1}. This supports data from other studies that indicated that the current spread of NDMs is not related to a single type of plasmid or dominant sequence types.

MLST has identified certain epidemic high-risk clones, such as *E. coli* ST405, *K. pneumoniae* ST147, and *K. pneumoniae* ST258, among our collection of travel-related bacteria with carbapenemases (Table 2). The intercontinental dissemination of ST258 had contributed to the worldwide spread of KPC-producing *K. pneumoniae* (11), while *K. pneumoniae* ST147 is an emerging sequence type that had been associated with VIMs (42–44), KPCs (41), and NDMs (16). We compared the presence of virulence factors among the different *K. pneumoniae* sequence types, but failed to identify certain factors that might have been responsible for the international success of ST258 and ST147.

E. coli ST405 with various types of CTX-Ms has a worldwide distribution (45) and has recently been described with NDM-4 isolated in Denmark from a patient that had previously been hospitalized in Vietnam (46). It was interesting to note that *E. coli* ST405 had a significantly higher number of virulence factors than ST101, ST365, and ST410. The exact role of virulence factors in extraintestinal *E. coli* is unknown, and it is unlikely that one set of factors determines virulence among these isolates (47). However, we previously determined that factors such as *sat*, *iutA*, *malX*, *usp*, *iha*, *hra*, and *ompT* could possibly play important roles in the dissemination and ecological success of ST405 (48).

The European Centre for Disease Prevention and Control has developed a communication platform tool that is dedicated to antimicrobial resistance (AMR) in health care-associated infections (HAI) and is referred to as the Epidemic Intelligence Information System (EPIS) AMR-HAI. EPIS AMR-HAI allows experts of national risk assessment bodies within the European Union to rapidly and securely exchange information related to microorganisms with emerging antimicrobial resistance that might have a potential impact in the European Union (38). We recommend that similar communication platforms be established in different provinces and states within the North American continent to ensure the timely communication of emerging antimicrobial resistance mechanisms among public health workers, infection control practitioners, and clinical microbiologists.

In order to prevent the introduction and spread of multiresistant bacteria by returning travelers into the health care systems of their respective home countries, it is essential to isolate them on admission and to rapidly identify patients colonized or infected by these bacteria (15, 38, 49). Screening case contacts appears to be an essential surveillance component for detecting asymptomatic carriers or patients infected or colonized with carbapenemase-producing bacteria. Patients colonized or infected with carbapenemase-producing bacteria should be placed on contact precautions. The added value of active screening of such patients on hospital admission depends on the frequency of carriers among incoming patients, and the use of routine screening remains a controversial issue due to several reasons, including costs.

We believe that the presence of carbapenemases among Gram-negative bacteria is an infection control emergency and that the detection of these bacteria in clinical laboratories is a critical step required for appropriate patient management and infection prevention and control efforts. We recommend preemptive contact isolation and careful attention to routine infection control measures (i.e., hand hygiene, cleaning of shared equipment, and effective environmental cleaning) for patients with a recent history (i.e., 6 months) of foreign hospitalization in areas where bacteria that produce carbapenemases are endemic.

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