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Surveillance and Molecular Epidemiology of *Klebsiella pneumoniae* Isolates That Produce Carbapenemases: First Report of OXA-48-Like Enzymes in North America

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A study was designed to characterize nonrepeat isolates of carbapenemase-producing *K. pneumoniae* obtained from the SMART worldwide surveillance program during 2008 and 2009. Characterization was done by PCR and sequencing for bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{OXA}, bla_{KPC}, and plasmid-mediated quinolone resistance and virulence factors (VFs). Genetic relatedness was determined with pulsed-field gel electrophoresis (PFGE) using XbaI and multilocus sequence typing. A total of 110 isolates were included; 47 possess genes that encode *K. pneumoniae* carbapenemases (KPCs), 26 NDMs, 19 VIMs, 13 OXA-48-like, and 5 imipenems (IMPs). We identified 3 different major sequence types (STs) among 65% of the isolates (i.e., ST11 [$n = 11$], ST147 [$n = 23$], and ST258 [$n = 38$]). ST11 and ST147, producing OXA-48-like and NDMs, were found in Argentina, Turkey, Greece, Italy, and India; ST258, producing KPCs, was present in the United States, Israel, Greece, and Puerto Rico. The major STs consisted of up to 4 different pulsotypes, and each pulsotype had a specific geographical distribution. A new ST, named ST903, with bla_{IMP-26}, was identified in the Philippines, while two bla_{OXA-48}-positive isolates were detected in the United States. There were no significant differences in the distribution of the VFs between the isolates; all were positive for *fimH*, *mrkD*, *wabG*, and *ureA*. This is the first report of OXA-48-like enzymes in North America. Our study highlights the importance of surveillance programs using molecular techniques as powerful tools to identify the importance of international sequence types.

The *Enterobacteriaceae*, most notably *Escherichia coli* and *Klebsiella pneumoniae*, are among the most important causes of serious hospital-acquired and community onset bacterial infections in humans (1). Since β-lactam antibiotics are a major drug class used to treat serious community onset or hospital-acquired infections caused by *Enterobacteriaceae*, resistance to these agents will continue to challenge clinical therapeutic choices. Of special concern is the development of resistance to the carbapenems, since these agents are often the last line of effective therapy available for the treatment of infections caused by multiresistant *Enterobacteriaceae* (2).

Most important within the *Enterobacteriaceae* is the increasing recognition of isolates that produce carbapenemases, which cause resistance to the carbapenems. They include the class A (*K. pneumoniae* carbapenemase [KPC] types), the class B (metallo-β-lactamases [MBLs] [i.e., VIM, imipenem {IPM}, and NDM types]), and the class D (e.g., OXA-48-like enzymes) oxacillinases (3).

Multilocus sequence typing (MLST), which uses sequence variation in a number of housekeeping genes to define sequence types (STs), or clones, is an excellent tool for evolutionary studies to show common-ancestry lineages among bacteria (4). It has led to the definition of major sequence types and the recognition of successful widespread international antimicrobial-resistant sequence types, such as *E. coli* ST101, which produces NDMs (5); *E. coli* ST131, which produces various extended-spectrum β-lactamases (ESBLs) (6); and *K. pneumoniae* ST258, which produces KPCs (7).

Several worldwide surveillance studies have shown the emergence of *Enterobacteriaceae* that produce carbapenemases, especially KPCs in the United States and NDMs in the Indian subcontinent (8–10). However, limited information is available about the molecular epidemiology of carbapenamase-producing *Enterobacteriaceae* from different parts of the world. A study was designed to characterize nonrepeat isolates of carbapenemase-producing *K. pneumoniae* obtained from the SMART worldwide surveillance program during 2008 and 2009.

MATERIALS AND METHODS

Bacterial isolates. One hundred and ten nonduplicate isolates of *K. pneumoniae* from intra-abdominal infections collected as part of the SMART surveillance program during 2008 and 2009 were included in this study (11). Isolates were obtained from Europe (20 sites), Asia (27 sites), North America (20 sites), Latin America (12 sites), the South Pacific (6 sites), and the Middle East (2 sites).

Antimicrobial susceptibility testing. MICs of ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), cefoxitin (FOX), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ertapenem (ERT), IPM, amikacin (AMK), ciprofloxacin (CIP), tigecycline (TIG), and colistin (COL) were determined using dehydrated broth microdilution MicroScan panels (Siemens Healthcare Diagnostics, IL) or in-house panels (COL) following 2012 CLSI and manufacturers' guidelines (12). Throughout this study, results were interpreted using 2012 CLSI breakpoints for broth dilution (12). The European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoint was used for COL, and the FDA breakpoint was used for TIG.

Received 14 August 2012 Returned for modification 7 October 2012
Accepted 9 October 2012
Published ahead of print 15 October 2012
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doi:10.1128/AAC.01686-12

β-Lactamase gene identification. KPC, OXA-48-like, VIM, IMP, and NDM-carbapenemases were identified using PCR amplification and sequencing as described previously (10). A microarray assay (Check-MDR CT101; Check-Points, The Netherlands) was also used to detect additional β-lactamases, as described previously (10).

Plasmid-mediated quinolone resistance (PMQR) determinants. The amplification of the *qnrA*, *qnrS*, and *qnrB* genes was undertaken in all isolates with multiplex PCR (13). *aac(6′)-lb* and *qepA* were amplified in separate PCRs using primers and conditions previously described (14, 15). The variant *aac(6′)-lb-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 using a multiplex PCR described by Doi and Arakawa (16).

Virulence factors in *K. pneumoniae*. PCR as described by Brisse et al. was used to determine the presence of virulence genes that have previously been associated with virulence in *K. pneumoniae* (17). They 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 fimbrial 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).

PFGE. The genetic relatedness of the ESBL-producing *K. pneumoniae* isolates was examined by pulsed-field gel electrophoresis (PFGE) following the extraction of genomic DNA and digestion with XbaI using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention, Atlanta, GA (18). The subsequent PFGE analyses were performed on a Chef Mapper apparatus (Bio-Rad Laboratories, Hercules, CA). PFGE banding patterns were analyzed with BioNumerics software (Applied Maths, Kortrijk, Belgium), and the relatedness was calculated by the unweighted-pair group method using average linkages (UPGMA) algorithm, with similarity of bands calculated using the Dice coefficient. Cluster designation was based on the criteria of Tenover et al. (19).

MLST. MLST was performed using seven conserved housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) (20). A detailed protocol of the MLST procedure, including allelic type and ST assignment methods, is available in MLST databases from the Pasteur Institute, Paris, France (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). New alleles and STs were submitted to the MLST website and were approved. The eBURST V3 algorithm (http://eburst.mlst.net/) was used to demonstrate phylogenetic relationships among closely related STs. Those STs that differed from the ancestor at one of the seven MLST alleles were defined as single-locus variants (SLVs), while STs that differed at two of the seven MLST alleles were defined as double-locus variants (DLVs). Clonal complexes (CCs) were defined as groups of STs that are related to each other by SLV level. For the purpose of this study, we included major sequence types only if 10 or more isolates belonged to that specific sequence type.

Statistical methods. Data were analyzed using Stata 11.2 (StataCorp, College Station, TX). Fishers' exact and χ^2 tests were used for comparing categorical variables among two and multiple groups, respectively. *P* values of less than 0.05 were deemed to represent statistical significance.

Nucleotide sequence accession number. The sequence of VIM-33 was submitted to GenBank under accession number JX258134.

RESULTS

Bacterial isolates, β-lactamases, susceptibilities, PMQR, and RNA methylases. A total of 110 *K. pneumoniae* isolates that produce carbapenemases were included in this study: 47 that produce KPCs (i.e., KPC-2 [*n* = 23], KPC-3 [*n* = 18], and KPC-11 [*n* = 6]), 26 that produce NDM-1, 19 that produce VIMs (i.e., VIM-1 [*n* = 16], VIM-5 [*n* = 1], VIM-27 [*n* = 1], and VIM-33 [*n* = 1]), 13 that produce OXA-48-like (i.e., OXA-48 [*n* = 6], OXA-163 [*n* = 2], and OXA-181 [*n* = 5]), and 5 that produce IMP-26 (Table 1). Some of the isolates also produced ESBLs (SHV-2, -5, -12, and -31; CTX-M-3, -14 and -15) and plasmid-mediated AmpC β-lactamases (CMY-2 and DHA-1) (Table 2 shows details).

TABLE 1 Numbers of *K. pneumoniae* isolates that produce different carbapenemases[a]

Carbapenemase	Country of isolation
KPC types (*n* = 47)	
KPC-2 (*n* = 23)	Greece (*n* = 14), USA (*n* = 7), Israel (*n* = 1), Puerto Rico (*n* = 1)
KPC-3 (*n* = 18)	USA (*n* = 9), Israel (*n* = 8), Columbia (*n* = 1)
KPC-11 (*n* = 6)	Greece (*n* = 6)
NDM-1 (*n* = 26)	India (*n* = 26)
OXA-48-like (*n* = 13)	
OXA-48 (*n* = 6)	Turkey (*n* = 3), Argentina (*n* = 1), USA (*n* = 2)
OXA-163 (*n* = 2)	Argentina (*n* = 2)
OXA-181 (*n* = 5)	India (*n* = 5)
VIM types (*n* = 19)	
VIM-1 (*n* = 16)	Greece (*n* = 13), Spain (*n* = 2), Italy (*n* = 1)
VIM-5 (*n* = 1)	Greece (*n* = 1)
VIM-27 (*n* = 1)	Turkey (*n* = 1)
VIM-33 (*n* = 1)	Greece (*n* = 1)
IMP-26 (*n* = 5)	Australia (*n* = 2), Philippines (*n* = 3)

[a] *n*, no. of isolates.

All the isolates included in this study were nonsusceptible (NS) (i.e., either intermediate or resistant) to SAM, FOX, CTX, CRO, ERT, IPM, or CIP, and 97% were NS to TZP and FEP, 95% to AMK, 32% to COL, and 15% to TIG.

Eight (7%) of the *K. pneumoniae* isolates that produced carbapenemases were positive for *aac(6′)-lb-cr*, 23 (21%) for *aac(6′)-lb-cr* with *qnrB*, 1 (1%) for *aac(6′)-lb-cr* with *qnrS*, 5 (5%) for *qnrB*, 4 (4%) for *qnrA*, and 1 (1%) for *qnrS* (Table 3). All the isolates were negative for *qepA*.

Twenty (18%) of the *K. pneumoniae* isolates that produced carbapenemases were positive for *armA*, 2 (2%) for *armA* with *rmtB*, 5 (5%) for *rmtC*, and 2 (2%) for *rmtB* (Table 3).

Virulence factors. All 110 isolates tested positive for *fimH*, *wabG*, and urea; 108 (98%) were positive for *mrkD*, 105 (95%) for *uge*, 11 (10%) for *kfuB*, and 7 (6%) for *alls*. All the isolates were negative for *magA*, *rpmA*, and aerobactin (Table 3).

Pulsed-field gel electrophoresis. PFGE identified 3 major clusters (with more than 10 isolates per cluster) of *K. pneumoniae* among 72 isolates, which were designated clusters A (*n* = 11), B (*n* = 23), and C (*n* = 38). Ten minor clusters (with less than 10 isolates per cluster) were also identified: clusters D (*n* = 2), E (*n* = 2), F (*n* = 2), G (*n* = 4), H (*n* = 2), I (*n* = 2), J (*n* = 3), K (*n* = 2), L (*n* = 4), and M (*n* = 2). The isolates that belonged to clusters A to M had >80% similar PFGE profiles among the isolates of each cluster. Interestingly, isolates that belonged to clusters A, H, I, and L exhibited >60% similarity of PFGE profiles to cluster C, and this suggested that they were related to cluster C. The remaining isolates (*n* = 13) were not clonally related, i.e., exhibited <60% similar PFGE profiles and did not show patterns similar to those from clusters A to M.

TABLE 2 Sequence types of *Klebsiella pneumoniae* that produce carbapenemases

Sequence type	Carbapenemase	ESBL or AmpC	Country of isolation
ST11 ($n = 11$)			
Pulsotype 11A ($n = 3$)	OXA-48	CTX-M-15	Turkey, Argentinia
Pulsotype 11B ($n = 3$)	NDM-1	CTX-M-15, DHA-1	India
Pulsotype 11C ($n = 2$)	OXA-163		Argentina
Pulsotype 11D ($n = 3$)	NDM-1	CTX-M-15	India
ST147 ($n = 23$)			
Pulsotype 147A ($n = 9$)	NDM-1	CTX-M-15, CMY-2	India
Pulsotype 147B ($n = 2$)	OXA-181	CTX-M-15	India
Pulsotype 147C ($n = 12$)	VIM-1, −27, 33	SHV-12, -31, DHA-1	Greece, Italy
ST258 ($n = 38$)			
Pulsotype 258A ($n = 7$)	KPC-3	SHV-12, CTX-M-14	USA, Israel
Pulsotype 258B ($n = 6$)	KPC-3		USA, Israel
Pulsotype 258C ($n = 20$)	KPC-2, −11	SHV-12	Greece, Israel
Pulsotype 258D ($n = 5$)	KPC-2	SHV-12	USA, Puerto Rico
ST1 ($n = 2$)	VIM-1		Spain
ST14 ($n = 2$)	NDM-1	CTX-M-15, CMY-2	India
ST15 ($n = 2$)	OXA-48, −181	CTX-M-15	USA, India
ST17 ($n = 4$)	KPC-2, VIM-1, OXA-48		Greece, Turkey
ST340 ($n = 2$)	NDM-1	SHV-2, CTX-M-15	India
ST418 ($n = 2$)	KPC-2		USA
ST43 ($n = 3$)	OXA-181, VIM-5	SHV-2, CTX-M-15, CTX-M-3	India, Turkey
ST478 ($n = 2$)	IMP-26	CTX-M-15	Australia
ST512 ($n = 4$)	KPC-3		Israel, Columbia
ST626 ($n = 2$)	IMP-26		Phillipines
Other STs ($n = 13$)[a]	NDM-1, VIM-1, OXA-48, KPC-2, −3, and IMP-26	CTX-M-15, SHV-5, DHA-1, CMY-2,	India, Greece, USA, Philippines

[a] Including ST101, ST20, ST231, ST271, ST278, ST29, ST336, ST34, ST391, ST437, ST572, ST676, and ST903; most of them ($n = 9$) were NDM-1 producers, with CTX-M-15 from India.

MLST. MLST identified the "major" clusters as follows: cluster A, ST11; B, ST147; and C, ST258. The "minor" clusters belonged to the following sequence types: cluster D, ST1; E, ST14; F, ST15; G, ST17; H, ST340; I, ST418; J, ST43; K, ST478; L, ST512; and M, ST626. ST11, ST340, ST418, and ST512 are SLVs of ST258 and explained the similarity of PFGE patterns between these sequence types. The remaining 13 isolates were identified as ST101, ST20, ST231, ST271, ST278, ST29, ST336, ST34, ST391, ST437, ST572, ST676, and ST903 (Table 2). ST903, isolated in the Philippines, was described for the first time in this study with a novel allele (i.e., *phoE151*). ST903, with bla_{IMP-26}, was positive for *armA*, *rmtB*, and *qnrB*. The eBURST analysis of ST903 showed that it is not associated with any clonal complex.

ST11 and ST258 each consisted of 4 different pulsotypes, and ST147 consisted of 3 pulsotypes (i.e., there were >90% similar PFGE profiles among the isolates of each pulsotype). The geographical distribution and the presence of ESBL and AmpC β-lactamases of the different major sequence types ST11, ST147, and ST258, with their respective pulsotypes, are shown in Table 2. It was interesting that the isolates within each pulsotype produced similar carbapenemases and ESBLs and had the same geographical distribution (e.g., isolates that belonged to pulsotype 147A produced NDM-1 with CTX-M-15 and were present in India, while isolates that belonged to pulsotype 147D produced VIMs with SHV-12 and were mostly present in Greece).

ST11 produced NDM-1, OXA-48-like, and CTX-M-15; was present in India, Argentina, and Turkey; and was often positive for *aac(6′)-Ib-cr* (Table 3). ST147 mostly produced VIMs, NDM-1, and CTX-M-15; was present in India, Greece, and Italy; and was often positive for *aac(6′)-Ib-cr* with *qnrB* and *armA* (Table 3). ST258 produced KPC and SHV-12; was present in Greece, the United States, Israel, and Puerto Rico (1 isolate); and was not associated with PMQR determinants or 16S RNA methylases (Table 3). There were no significant differences in the presence of virulence factors between ST11, ST147, ST258, and the other sequence types (Table 3). Over half of ST258 isolates (53%) were NS to COL, while 35% of ST147 isolates were NS to TIG.

Some interesting geographical distributions were also noted among the minor sequence types. ST1 with VIM-1 was present in Spain; ST14 with KPC-2, VIM-1, and OXA-48 in Greece and Turkey; ST43 with OXA-181 and VIM-5 in India and Turkey; ST478 with IMP-26 in Australia; and ST512 with KPC-2 in Israel and Columbia (Table 2).

DISCUSSION

The pandemics caused by CTX-M-, KPC-, and NDM-producing *Enterobacteriaceae* between 2000 and 2010 highlighted the desperate need for global antimicrobial resistance surveillance systems, especially in resource-limited countries, where novel resistance mechanisms are more likely to emerge (21). These surveillance systems would help to determine the scope of antimicrobial resistance in resource-limited countries, while molecular characterization would ensure the early recognition of

TABLE 3 Characteristics of *K. pneumoniae* sequence types ST11, ST147, and ST258 and other STs that produce carbapenemases

Characteristic	No. (%) with characteristic:				*P* value
	ST11 ($n = 11$)	ST147 ($n = 23$)	ST258 ($n = 38$)	Other STs ($n = 38$)	
Carbapenemase					<0.001
NDM	6 (55)	9 (39)	0	11 (29)	
KPC	0	0	38 (100)	9 (24)	
OXA-48-like	5 (45)	2 (9)	0	6 (16)	
VIM	0	12 (52)	0	7 (18)	
IMP	0	0	0	5 (13)	
ESBL					<0.001
CTX-M-15	5 (45)	11 (48)	0	16 (42)	
CTX-M-14	0	0	3 (8)	0	
Other CTX-Ms	0	0	0	1 (3)	
SHV-12	0	4 (17)	26 (68)	2 (5)	
Other SHVs	0	1 (4)	0	5 (13)	
AmpC					0.233
DHA-1	1 (1)	1 (4)	0	1 (3)	
CMY-2	0	2 (9)	0	1 (3)	
Country of origin					<0.001
India	6 (55)	11 (48)	0	14 (37)	
Philippines	0	0	0	3 (8)	
Australia	0	0	0	2 (5)	
USA	0	0	13 (34)	5 (13)	
Puerto Rico	0	0	1 (3)	0	
Columbia	0	0	0	1 (3)	
Argentina	3 (27)	0	0	0	
Spain	0	0	0	2 (5)	
Greece	0	11 (48)	18 (47)	6 (16)	
Italy	0	1 (4)	0	0	
Turkey	2 (18)	0	0	2 (5)	
Israel	0	0	6 (16)	3 (8)	
Antimicrobial susceptibility					<0.001
Col NS	1 (1)	3 (13)	20 (53)	9 (24)	
Tig NS	2 (18)	8 (35)	2 (5)	5 (13)	
PMQR determinants					0.016
aac(6′)-lb-cr	5 (45)	0	0	3 (8)	
aac(6′)-lb-cr with *qnrB*	1 (1)	11 (48)	0	11 (29)	
aac(6′)-lb-cr with *qnrS*	0	0	0	1 (3)	
qnrB	2 (18)	0	0	3 (8)	
qnrA	0	1 (4)	0	3 (8)	
qnrS	0	0	0	1 (3)	
16S RNA methylases					0.014
armA	3 (27)	7 (30)	0	10 (26)	
armA with *rmtB*	0	0	0	2 (5)	
rmtC	0	4 (17)	0	1 (3)	
rmtB	0	0	1 (3)	1 (3)	
Virulence factors					
uge	9 (82)	23 (100)	38 (100)	37 (97)	0.022
wabG	11 (100)	23 (100)	38 (100)	38 (100)	
urea	11 (100)	23 (100)	38 (100)	38 (100)	
magA	0	0	0	0	
mrkD	11 (100)	23 (100)	38 (100)	36 (95)	0.468
alls	0	2 (9)	0	5 (13)	0.084
kfuBC	0	0	0	11 (29)	<0.001
rpmA	0	0	0	0	
fimH	11 (100)	23 (100)	38 (100)	38 (100)	
Aerobactin	0	0	0	0	

novel resistance mechanisms, including the worldwide emergence of certain successful international clones or sequence types (21). Studies that combine surveillance with molecular characterization would also help with the development of rapid techniques to detect novel or emerging resistance mechanisms in travelers returning from areas of endemicity. This would ensure the appropriate implementation of infection control measures and help to curb the worldwide spread of antibiotic resistance.

The SMART program is one of the few worldwide surveillance systems that has a wide representation of microbiology laboratories among resource-limited countries. The program started in 2002 to monitor antimicrobial resistance trends among isolates from intra-abdominal-infection isolates obtained from Asia, Latin America, the South Pacific, and the Middle East. The molecular characterization of the SMART isolates started in 2008. We believe this surveillance program provides a unique opportunity to study the significance and importance of certain sequence types with their respective pulsotypes among *K. pneumoniae* that produce carbapenemases.

Infections with KPC-, VIM-, OXA-48-like-, and NDM-producing *Enterobacteriaceae* have most often been associated with visiting and being hospitalized in areas of endemicity, such as the United States, Greece, and Israel for KPCs; Greece for VIMs; Turkey for OXA-48-like; and the Indian subcontinent for NDMs (21). The results from our study support this notion (Table 1); KPC-producing isolates were mostly found in Greece, the United States, and Israel; NDM-1 producers in India; VIM producers in Greece; and IMP producers in Australia and the Philippines, while *K. pneumoniae* with OXA-48-like β-lactamases were present in India (OXA-181), Turkey (OXA-48), Argentina (OXA-163), and, surprisingly, the United States. To our knowledge, this is the first description of OXA-48-like β-lactamases from the North American continent. The 2 isolates from the United States were obtained during 2009; produced OXA-48 and CTX-M-15; belonged to ST15 and ST336, respectively; and were positive for *aac(6′)-lb-cr* with *qnrB*.

KPC-producing bacteria are endemic in certain parts of the world, and intercontinental dissemination of ST258 has contributed to the worldwide spread of KPC-producing *K. pneumoniae* (7). Of the 47 KPC-producing isolates included in our study, 38 (81%) belonged to ST258, which consisted of 4 different pulsotypes; pulsotypes 258A and 258B with KPC-3 were found in the United States and Israel, pulsotype 258C with KPC-2 and -11 was limited to Greece, while pulsotype 258D with KPC-2 was present in the United States and Puerto Rico (Table 2). A highly epidemic clone of KPC-3-producing *K. pneumoniae* emerged in Israel during 2006, causing several nosocomial outbreaks with high mortality among patients, and was most likely introduced into Israel via travelers from the United States during the early to middle part of 2000 to 2010 (22). The fact that pulsotypes 258A and 258B with KPC-3 were present in the United States and Israel (but absent in KPC isolates from Greece) suggests that there is still intercountry transfer of patients colonized or infected with KPC-3-producing *K. pneumoniae*. In 2008, 32 patients presented with infections due to KPC-producing *K. pneumoniae* in a Columbian hospital; the index case was a medical tourist who traveled from Israel to undergo a liver transplantation (23). We identified ST512, an SLV of ST258, in isolates from Israel and Columbia (Table 2), again supporting the notion that KPC-producing *K. pneumoniae* can travel with travelers and can easily be introduced into a country or region.

K. pneumoniae isolates that produce VIMs were first reported during 2002 from patients admitted to intensive care units (ICUs) of three teaching hospitals located in Athens, Greece (24). *K. pneumoniae* with VIM-1 is endemic in certain hospitals in Greece, and nosocomial infections caused by these bacteria constitute a major public health problem for the Mediterranean country (25). VIM-27, a single-point variant of VIM-1, has been described in *K. pneumoniae* ST147 recovered from hospitals in Greece (26). Of the 19 VIM-producing isolates included in our study, 12 (63%) belonged to ST147, which consisted of 3 different pulsotypes: pulsotypes 147A with NDM-1 and 147B with OXA-181 were found in India, while pulsotype 147C with VIM-1, -27, and -33 was limited to Greece and Italy (Table 2). VIM-33, first described in this study, is a point mutant variant of VIM-1 and was present in Greece. Interestingly, the 2 VIM-1-producing isolates from Spain belonged to a different sequence type, namely, ST1.

The majority of the patients from North America, Europe, and Australia infected with NDM-producing bacteria were previously hospitalized in the Indian subcontinent (27). All the NDM-1-producing *K. pneumoniae* isolates ($n = 26$) from this study were isolated from sites in India; interestingly, 9 (35%) belonged to ST147 and 6 (23%) to ST11 (i.e., over 50% of NDM-producing *K. pneumoniae* isolates from India belonged to either ST11 or ST147). It is therefore possible that clonal spread of NDM-1 among *K. pneumoniae* isolates in India could be more significant than previously believed.

We identified 3 different major sequence types, namely, ST11, ST147, and ST258, among the majority of isolates (65%) identified during 2008 and 2009. ST11 is a truly international sequence type and has been associated with NDMs (28, 29), KPC (30–32), and CTX-M-15 (33). Our results show that this sequence type also has the ability to produce OXA-48-like enzymes from isolates found in Argentina and Turkey (Table 3). ST147 has only recently been described in Greece (26) and has been associated with VIMs and KPCs in that country (34). This sequence type is also associated with NDMs from Canada (35) and the United Kingdom (28) and VIMs from Scandinavia (36). ST147 in our study was associated with VIMs, NDMs, and OXA-48-like enzymes from *K. pneumoniae* isolates in India, Greece, and Italy (Table 3). These sequence types, especially ST147 and ST258, pose important new public health threats in countries such as the United States, Israel, Greece, and India. Our results also show that these major sequence types are possibly being imported into countries such as Puerto Rico, Columbia, Italy, and Argentina.

A study by Johnson and colleagues investigating a different successful international sequence type, *E. coli* ST131, showed that the unique combination of antimicrobial resistance and certain virulence factors most likely give ST131 a competitive advantage over other isolates of *E. coli*, promoting its clonal expansion and dominance over less virulent and/or more susceptible *E. coli* clones (37). We investigated the susceptibilities and virulence factors of ST11, ST147, and ST258 and compared the results to those for other sequence types identified in this study (Table 3). ST258 was significantly more resistant to colistin, but we failed to identify certain virulence factors that might have been responsible for the international success of ST258 (Table 3).

ST11, ST147, and ST258 are major drug-resistant pathogens among *K. pneumoniae* strains that produce carbapenemases in

different parts of the world. We urgently need well-designed epidemiological and molecular studies to understand the dynamics of transmission, risk factors, and reservoirs for *K. pneumoniae* ST11, ST147, and ST258. This will provide insight into the emergence and spread of these multiresistant sequence types that will hopefully lead to information essential for preventing infections and the spread of these bacteria.

ACKNOWLEDGMENTS

J.D.D.P. and K.B.L. previously received research funds from Merck, Pfizer, and Astra Zeneca.

This work was supported by a research grant from Calgary Laboratory Services (number 73-6350).

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