de Aperfeiçoamento de Pessoal de Nível Superior, and, Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro, Brazil. This manuscript represents a portion of a thesis submitted by T.F.R. to the Federal University of Rio de Janeiro, Brazil, as a partial fulfillment of the requirements for her doctorate degree.

Tatiana F. Robaina,[1] Gabriella S. Mendes,[1] Fabrício J. Benati, Giselle A. Pena, Raquel C. Silva, Miguel A.R. Montes, Renata Otero, Gloria F. Castro, Fernando P. Câmara, and Norma Santos

Author affiliation: Federal University of Rio of Janeiro, Rio de Janeiro, Brazil

References

1. Scuda N, Hofmann J, Calvignac-Spencer S, Ruprecht K, Liman P, Kühn J, et al. A novel human polyomavirus closely related to the African green monkey–derived lymphotropic polyomavirus. J Virol. 2011;85:4586–90. http://dx.doi.org/10.1128/JVI.02602-10
2. Babakir-Mina M, Ciccozzi M, Farchi F, Bergallo M, Cavallo R, Adorno G, et al. KI and WU polyomaviruses and CD4+ cell counts in HIV-1–infected patients, Italy. Emerg Infect Dis. 2010;16:1482–5. http://dx.doi.org/10.3201/eid1609.100211
3. Machado DM, Fink MC, Pannuti CS, Succi RC, Machado AA, do Carmo FB, et al. Human polyomaviruses JC and BK in the urine of Brazilian children and adolescents vertically infected by HIV. Mem Inst Oswaldo Cruz. 2011;106:931–5. http://dx.doi.org/10.1590/S0074-02762011000800006
4. Jeffers L, Webster-Cyriaque JY. Viruses and salivary gland disease (SGD): lessons from HIV SGD. Adv Dent Res. 2011;23:79–83. http://dx.doi.org/10.1177/0022034510396882
5. Berger JR, Miller CS, Mootoor Y, Avdiushko SA, Kryscio RJ, Zhu H. JC virus detection in bodily fluids: clues to transmission. Clin Infect Dis. 2006;43:e9–12. http://dx.doi.org/10.1086/504947
6. Sundsfjord A, Spein AR, Lucht E, Flaegstad T, Seternes OM, Traavik T. Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. J Clin Microbiol. 1994;32:1390–4.
7. Bialasiewicz S, Whiley DM, Lambert SB, Nissen MD, Sloots TP. Detection of BK, JC, WU, or KI polyomaviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. J Clin Virol. 2009;45:249–54. http://dx.doi.org/10.1016/j.jcv.2009.05.002
8. Agostini HT, Ryschkewitsch CF, Stoner GL. Genotype profile of human polyomavirus JC excreted in urine of immunocompetent individuals. J Clin Microbiol. 1996;34:159–64.
9. Sharp CP, Norja P, Anthony I, Bell JE, Simmonds P. Reactivation and mutation of newly discovered WU, KI, and Merkel cell carcinoma polyomaviruses in immunosuppressed individuals. J Infect Dis. 2009;199:398–404. http://dx.doi.org/10.1086/596062
10. Jeffers LK, Madden V, Webster-Cyriaque J. BK virus has tropism for human salivary gland cells in vitro: implications for transmission. Virology. 2009;394:183–93. http://dx.doi.org/10.1016/j.virol.2009.07.022

[1]These authors contributed equally to this article.

Address for correspondence: Norma Santos, Departamento de Virologia, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Cidade Universitária, CCS–Bl. I, Ilha do Fundão Rio de Janeiro–RJ, 21.941-972, Brazil; email: nsantos@micro.ufrj.br

Carbapenem-hydrolyzing Oxacillinase-48 and Oxacillinase-181 in Canada, 2011

To the Editor: In 2001, a *Klebsiella pneumoniae* isolate from a patient in Turkey was found to harbor a novel class D carbapenem-hydrolyzing oxacillinase, OXA-48 (1). Although this enzyme hydrolyzes carbapenems at a low level and shows weak activity against expanded-spectrum cephalosporins, it is often associated with other β-lactamases and is multidrug resistant. Reports of *Enterobacteriaceae* harboring OXA-48 have been described across Europe, the Mediterranean area, and the Middle East (2). In addition, OXA-181, which differs from OXA-48 by 4 aa substitutions, has been described in India (2). We describe the emergence of OXA-48 and OXA-181 in Canada.

Hospital and provincial public health laboratories in Canada voluntarily submitted *Enterobacteriaceae* isolates to the National Microbiology Laboratory. Isolates submitted by the laboratories were not susceptible to carbapenems and were to be tested by PCR for carbapenemase genes (KPC, NDM, IMP, VIM, OXA-48, and GES) (3). During April–November 2011, a total of 4 isolates (3 *K. pneumoniae*, 1 *Escherichia coli*) tested positive for the *bla*OXA-48–type gene. Sequencing, using the primers preOXA-48A and -48B (4), revealed that 3 of the isolates (*K. pneumoniae* 11-882 and 11-2720 and *E. coli* 11-1498) possessed the *bla*OXA-48 gene, and the other isolate (*K. pneumoniae* 11-2568) possessed the *bla*OXA-181 gene. We conducted additional β-lactamase PCR and sequencing as described (3) (Table). The Modified Hodge test (using a 10-μg disk of ertapenem and meropenem) showed that all isolates were strongly positive for carbapenemase production.

Table. Patient data and antimicrobial drug susceptibility profiles for isolates of *Klebsiella pneumoniae* and *Escherichia coli* harboring OXA-type carbapenemases, Canada, 2011*

Variable	Bacterial isolate, strain no., OXA-type				Plasmid		
	K. pneumoniae			*E. coli*			
	11-882, OXA-48	11-2568, OXA-181	11-2720, OXA-48	11-1498, OXA-48	p48-11-882	p181-11-2568	*E. coli* DH10B†
Patient data					NA	NA	NA
Recent travel	NK	India	Lebanon	Dubai	NA	NA	NA
Site of bacterial isolation	Urine	Perirectal	Urine	Skin swab	NA	NA	NA
Infection/colonization	NK	Colonization	Infection	Colonization	NA	NA	NA
Hospitalization	NK	General ward	Outpatient	ICU	NA	NA	NA
Bacterial isolate data							
β-lactamase	TEM-1, SHV-11	TEM-1, SHV-11, CTX-M-15, OXA-1	SHV-11, OXA-1	TEM-1, CTX-M-24	TEM-1	Neg	NA
Sequence type	395	147	831	38	NA	NA	NA
Antimicrobial drug susceptibility test, drug‡							
Vitek2,§ MIC μg/mL							
Amikacin	≤2.0	4.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0
Aztreonam	≤1.0	≥64.0	≤1.0	32.0	≤1.0	≤1.0	≤1.0
Cefazolin	32.0	≥64.0	16.0	≥64.0	≥64.0	32.0	≤4.0
Cefepime	≤1.0	≥64.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0
Ceftriaxone	≤1.0	≥64.0	≤1.0	≥64.0	≤1.0	≤1.0	≤1.0
Ciprofloxacin	≥4.0	≥4.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Ertapenem	≥8.0	≥8.0	4.0	4.0	≥8.0	4.0	≤0.5
Gentamicin	≤1.0	≥16.0	≤1.0	≥16.0	≤1.0	≤1.0	≤1.0
Imipenem	4.0	≥16.0	≤1.0	≤1.0	8.0	2.0	≤1.0
Meropenem	1.0	≥16.0	≤0.25	0.5	8.0	≤0.25	≤0.25
Tigecycline¶	1.0	2.0	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
TMP/SXT	≥320.0	≥320.0	≤20.0	≥320.0	≥320	≤20	≤20.0
Etest,§ MIC μg/mL							
Imipenem	1.0	24.0	0.38	0.38	0.5	0.5	0.19
Meropenem	0.5	24.0	0.19	0.38	0.125	0.19	0.032
Ertapenem	2.0	≥32.0	0.5	2.0	0.38	0.75	0.008
Colistin#	0.38	0.5	0.5	0.5	0.125	0.125	ND
Disk diffusion,** zone diameter, mm							
Imipenem	20	8	23	22	24	25	33
Meropenem	20	7	23	22	22	28	35
Ertapenem	16	6	19	16	23	22	35

*NK, not known; NA, not applicable; ICU, intensive care unit; Neg, negative; TMP/SXT, trimethoprim/sulfamethoxazole; ND, not done.
†OXA-48–type plasmids were electroporated into *E. coli* DH10B cells.
‡Additional antimicrobial drugs on panel: ampicillin, ampicillin/sulbactam, nitrofurantoin, piperacillin/tazobactam, tobramycin.
§Test from bioMérieux Canada Inc., St. Laurent, Quebec, Canada.
¶MIC breakpoints for tigecycline were based on the US Food and Drug Administration criteria for *Enterobacteriaceae*: susceptible, ≤2 μg/mL; intermediate, 4 μg/mL; resistant, ≥8 μg/mL.
#MIC breakpoints for colistin followed Clinical Laboratory Standards Institute breakpoint criteria for *Acinetobacter* spp.: susceptible, MIC ≤2 μg/mL; resistant, MIC ≥4 μg/mL.
**Test from Clinical Laboratory Standards Institute (www.clsi.org/source/orders/free/m100-s22.pdf).

The Table shows clinical data and antimicrobial drug susceptibility profiles for the *K. pneumoniae* and *E. coli* harboring OXA-type carbapenemases. Half of the isolates were from men >65 years of age. Three of the case-patients had a history of travel to regions where OXA-48–type carbapenemases are endemic (Lebanon, India, and Dubai), and while still abroad (shortly before being hospitalized in Canada), 2 case-patients had sought medical attention.

Pulsed-field gel electrophoresis of 3 *K. pneumoniae* isolates digested with *Xba*I showed unique fingerprint patterns (data not shown). Multilocus sequence typing (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) of *K. pneumoniae* 11-2568, 11-882, and 11-2720 showed that they belonged to sequence type (ST) 147, ST395, and ST831, respectively. ST395 has been identified in *K. pneumoniae* from Morocco, Amsterdam, and France harboring blaOXA-48 (2). E. coli 11-1498 was shown to belong to sequence type 38, previously described in a nosocomial outbreak and in isolated cases of OXA-48 in France; travel to Morocco and Egypt was linked to those cases (5,6). Phylogenetic grouping of the *E. coli* isolate revealed that it belonged to group D, which is associated with virulent extraintestinal strains (7).

Antimicrobial drug susceptibilities were determined by using Vitek 2 (GN-25) (bioMérieux, Canada Inc., St Laurent, Quebec, Canada) and interpreted by using the 2012 guidelines (M100-S22) of the Clinical Laboratory Standards Institute (www.clsi.org/source/orders/free/m100-s22.pdf) (Table). All but 1 isolate (*K. pneumoniae*

11-2720) was multidrug resistant (defined as resistant to >3 antimicrobial drug classes). All isolates were resistant to ampicillin, cefazolin, and piperacillin/tazobactam. Two isolates were resistant to broad-range cephalosporins, both of which contained the CTX-M–type extended-spectrum β-lactamase. All isolates were also resistant to ertapenem, 2 of 4 were resistant to imipenem, and 1 was resistant to meropenem. Results were confirmed by Etest (bioMérieux, Canada Inc.) and Clinical Laboratory Standards Institute disk diffusion, with the exception of results for *K. pneumoniae* 11-882, which showed susceptibility to meropenem and imipenem by Etest and intermediate susceptibility by disk diffusion (Table). All isolates were susceptible to tigecycline and colistin.

PCR mapping, using previously described primers (8), identified the *bla*OXA-48 gene located on the transposon Tn1999. The *bla*OXA-181 gene was found downstream of IS*Ecp1*, as described (4). We isolated plasmid DNA by using QIAGEN Plasmid Mini Kits (QIAGEN, Mississauga, ON, Canada) and attempted to transfer the plasmid harboring the *bla*OXA-48–type gene, using electroporation into *E. coli* DH10B, as described (3). Plasmid transfer of *bla*OXA-48–type genes was successful only for *K. pneumoniae* 11-882 and 11-2568. PCR revealed the transfer of *bla*TEM-1 and *bla*OXA-48 along with a plasmid of ≈114 Kb (p48-11-882). In addition, *bla*OXA-181 was transferred along with a plasmid of »4 Kb (p181-11-2568) with no additional β-lactamases present.

Replicon typing (9,10), using primers for incompatibility group IncR (IncRrv 5′-GTGTGCTGTGGTTATGCCTCA-3′), showed that p48-11-882 belonged to IncR and to the OXA-48–associated IncL/M. Plasmid p181-11-2568 was negative for all replicons tested.

Transformants were resistant to ampicillin, cefazolin, and piperacillin/tazobactam but susceptible to third- and fourth-generation cephalosporins. This finding is not surprising because OXA-48 and OXA-181 show weak activity against expanded-spectrum cephalosporins (1,4). Resistance to third- and fourth-generation cephalosporins in clinical isolates is likely caused by a combination of additional mechanisms, such as porin mutations, or additional β-lactamases, such as CTX-M–type, extended-spectrum β-lactamases (Table).

We report the emergence of OXA-48 and OXA-181 in North America. The emergence of carbapenem-resistant *Enterobacteriaceae* in Canada is of concern because they are difficult to detect in the laboratory, and treatment options are lacking. The history of travel by the patients in our study to regions where carbapenem-resistant *Enterobacteriaceae* are endemic highlights the necessity for understanding the potential risk factors associated with acquiring these multidrug-resistant pathogens.

Acknowledgments

We acknowledge Karen Pike and Jayshree Somani for contributions in gathering patient data

Laura F. Mataseje, David A. Boyd, Linda Hoang, Miguel Imperial, Brigitte Lefebvre, Mark Miller, Susan M. Poutanen, Diane Roscoe, Barbara M. Willey, and Michael R. Mulvey

Author affiliations: National Microbiology Laboratory, Winnipeg, Manitoba, Canada (L.F. Mataseje, D.A. Boyd, M.R. Mulvey); British Columbia Center for Disease Control, Vancouver, British Columbia, Canada (L. Hoang, M. Imperial); Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, Quebec, Canada (B. Lefebvre); SMBD–Jewish General Hospital, Montreal, Quebec, Canada (M. Miller); University of Toronto, Toronto, Ontario, Canada (S.M. Poutanen); University Health Network/Mount Sinai Hospital, Toronto (S.M. Poutanen, B.M. Willey); and Vancouver General Hospital, Vancouver (D. Roscoe)

DOI: http://dx.doi.org/10.3201/eid1901.120706

References

1. Poirel L, Heritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004;48:15–22. http://dx.doi.org/10.1128/AAC.48.1.15-22.2004
2. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother. 2012;67:1597–606. http://dx.doi.org/10.1093/jac/dks121
3. Mataseje LF, Bryce E, Roscoe D, Boyd DA, Embree J, Gravel D, et al. Carbapenem-resistant gram-negative bacilli in Canada 2009–10: results from the Canadian Nosocomial Infection Surveillance program (CNISP). J Antimicrob Chemother. 2012;67:1359–67. http://dx.doi.org/10.1093/jac/dks046
4. Potron A, Nordmann P, Lafeuille E, Al Maskari Z, Al Rashdi F, Poirel L. Characterization of OXA-181, a carbapenem-hydrolyzing class D b-lactamase from *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2011;55:4896–9. http://dx.doi.org/10.1128/AAC.00481-11
5. Crémet L, Bourigault C, Lepelletier D, Guillouzouic A, Juvin ME, Reynaud A, et al. Nosocomial outbreak of carbapenem-resistant *Enterobacter cloacae* highlighting the interspecies transferability of the blaOXA-48 gene in the gut flora. J Antimicrob Chemother. 2012;67:1041–3. http://dx.doi.org/10.1093/jac/dkr547
6. Poirel L, Bernabeu S, Fortineau N, Podglajen I, Lawrence C, Nordmann P. Emergence of OXA-48–producing *Escherichia coli* clone ST38 in France. Antimicrob Agents Chemother. 2011;55:4937–8. http://dx.doi.org/10.1128/AAC.00413-11
7. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66:4555–8. http://dx.doi.org/10.1128/AEM.66.10.4555-4558.2000
8. Giani T, Conte V, Di Pilato V, Aschbacher R, Weber C, Larcher C, et al. *Escherichia coli* from Italy producing OXA-48 carbapenemase encoded by a novel Tn1999 transposon derivative. Antimicrob Agents Chemother. 2012;56:2211–3. http://dx.doi.org/10.1128/AAC.00035-12
9. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005;63: 219–28. http://dx.doi.org/10.1016/j.mimet.2005.03.018

10. Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother. 2012;56:559–62. http://dx.doi.org/10.1128/AAC.05289-11

Address for correspondence: Michael R. Mulvey, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB R3E 3R2, Canada; email: michael.mulvey@phac-aspc.gc.ca

Characterization of Peste des Petits Ruminants Virus, Eritrea, 2002–2011

To the Editor: Peste des petits rumniants (PPR) is a highly contagious viral disease causing high rates of mortality among domestic and wild small ruminants in Africa and Asia. The disease causes economic losses in the countries where it is present. Peste des petits ruminants virus (PPRV) is a negative-sense, single-stranded RNA virus of the genus *Morbillivirus*, in the family *Paramyxoviridae*. Analysis of a small sequence of the PPRV nucleoprotein (NP) gene permits classification of the strains of the unique serotype of circulating PPRV into 4 genetically distinct lineages (*1*–*3*). The geographic distribution of lineages I and II is restricted mainly to western and central Africa and that of lineage III mainly to eastern Africa. Lineage IV is more widely distributed in Southeast Asia, the Arabian Peninsula, and the Middle East. Lineage IV is also currently circulating across northern and central Africa (*1*).

Although PPR is endemic in Eritrea, there are no data on the molecular characterization of the circulating viruses. As part of a program of cooperation between the National Animal and Plant Health Laboratory of Asmara and the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" in Teramo, we analyzed 41 sheep and goat tissue samples that were collected by the Eritrean veterinary service during several outbreaks of PPR. Samples were collected in the following villages: 5 samples in Gahitelay (Northern Red Sea region) in 2003; 4 in Gulee and Weki (Maekel region), 3 in Hukum (Anseba region), and 6 in Torat and Keih Adi (Debub region) in 2005; and 6 in May Harish (Debub region) in 2011. For 17 samples, the region was not recorded; instead, they were identified as "Eritrea," followed by the year of collection. Of the 41 samples, 22 were from goats and 6 from sheep; the source was not recorded for the other 13 samples. Nineteen samples were collected from lymph nodes, 10 from spleen, 9 from lung, and 1 each from tonsil, liver, and trachea. Samples were analyzed in the biosafety level 3 laboratory at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise.

Homogenates were prepared by using a mortar and sterile quartz to grind the tissue samples. The homogenates were then diluted (10% wt/vol) in phosphate-buffered saline, and tissue debris was removed by low-speed centrifugation. For lineage determination, total RNA was extracted by using the High Pure Viral Nucleic Acid Kit (Roche Diagnostic, Mannheim, Germany), RNA was amplified by reverse transcription PCR using the primer set NP3/NP4 (*4*) and the QIAGEN One-Step RT-PCR Kit (QIAGEN, Hilden, Germany). After agarose gel electrophoresis, 34 samples showed specific amplification of the 351-nt fragment of NP gene. PCR products were then purified by using the QIAquick PCR Purification Kit (QIAGEN) and sequenced by using the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Nucleotide sequences were obtained for 24 (59%) samples. The geographic distribution of animals in Eritrea with tissue samples from which we obtained viral sequences is shown in the online Technical Appendix (wwwnc.cdc.gov/EID/pdfs/12-1072-Techapp.pdf). Sequence editing, assembly, and alignment were performed by using BioEdit version 7.0.5.3 (*5*). BLAST (www.ncbi.nlm.nih.gov) was used to find homologous hits in the sequence databases. Phylogenetic analysis (neighbor-joining) with bootstrap (1,000 replicates) was performed using MEGA4 (*6*). We performed phylogenetic analysis of a 255-nt sequence of PPRV NP gene, using a selection of reference strains: 7 new sequences from this study (GenBank accession nos. JX398126, JX398127, JX398128, JX398129, JX398130, JX398131, JX398132) and 34 sequences representing the 4 lineages of PPRV for which sequences are available (Figure). Phylogenetic analysis was repeated by using the distance (neighbor-joining) method in PHYLIP version 3.67 (http://evolution.gs.washington.edu/phylip.html). The results of the phylogenetic analyses showed that the PPRV we isolated from ruminants in Eritrea belongs to the Afro-Asian lineage 4 and could be further distinguished into 2 clusters.

The first cluster consisted of 5 PPRV strains (Eritrea_2002, Eritrea_Gahitelay_2003, Eritrea_Hukum_2005, Eritrea_Torat_2005, and Eritrea_2011), which are related to PPRV strains from Sudan (AlAzaza_BNSUD00) and Cameroon (Cameroon_97). Thus, the data suggest that these 5 isolates from Eritrea are related to viruses that originated in Sudan and Cameroon and spread across central Africa, probably by free ranging wild or susceptible domestic animals.

The second cluster consisted of 2 PPRV strains (Eritrea_Gulee_2005 and Eritrea_May Harish_2011); this