

the prevention of *mcr-1* dissemination is needed, particularly to prevent the proliferation of an organism harboring a plasmid with *mcr-1* and a carbapenemase (10).

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Carbapenem-Resistant *Enterobacter* spp. in Retail Seafood Imported from Southeast Asia to Canada

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To the Editor: Carbapenems, antimicrobial drugs of last resort, are recommended only for severe community- and healthcare-associated multidrug-resistant bacterial infections. In Canada, carbapenem-resistant infection rates in hospitals remained low (≤ 0.25 cases/1,000 patient admissions) over 5 years' (2009–2014) surveillance (1). Carbapenemase-producing bacteria have rarely been detected in the food chain in industrialized countries. However, carbapenemase genes were detected in bacteria isolated from produce in Switzerland (2) and seafood in Canada (3); implicated food items originated from Southeast Asia. We conducted targeted sampling to assess, using selective media, the occurrence of carbapenem-resistant *Enterobacteriaceae* in imported seafood products sold in Canada.

For testing, we selected 1,328 retail seafood samples: 928 were imported fresh and frozen raw shrimp collected during 2011–2015 by CIPARS (the Canadian Integrated Program for Antimicrobial Resistance Surveillance), and 400 comprised an assortment of imported niche-market fresh and frozen raw seafood collected specifically for this study during January–April 2015. Product information and origin country were recorded for each sample. We used chromID CARBA agar (bioMérieux, St. Laurent, QC, Canada) to select putative colonies. To determine carbapenemase production on nonsusceptible (zone of inhibition < 25 mm) isolates, we used disk diffusion susceptibility to ertapenem and meropenem (10 μ g each) and the Carba NP test as previously described (4). Isolates were identified to species using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics Ltd, Milton, ON, Canada) and tested for susceptibility using the Sensititre Complete Automated System with the Sensititre NARMS Gram Negative Plate (CMV3AGNF)

(Trek Diagnostic Systems, Oakwood Village, OH, USA). We used single and multiplex PCR to screen isolates for the major carbapenemase-conferring (bla_{NDMP} , bla_{KPC} , bla_{IMP} , bla_{VIM} , bla_{GES} , bla_{OXA-48} -like, bla_{NMC}) and β -lactamase-conferring (bla_{SHV} , bla_{TEM} , bla_{CTX-M} , bla_{OXA-1} , bla_{CMY-2}) genes (5). We performed pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (Illumina Inc., San Diego, CA, USA) on isolates requiring further comparative testing (6). In silico multilocus sequence typing and replicon typing were conducted using the assembled sequence data (SPAdes 3.5.0 [St. Petersburg genome assembler], <http://spades.bioinf.spbau.ru/release3.5.0/manual.html>) and services of the Center for Genomic Epidemiology (<http://www.genomicepidemiology.org>). The transferability of resistance genes was determined by transformation experiments using electrocompetent *Escherichia coli* DH10B cells.

Using selective media methodology, we detected carbapenem-resistant *Enterobacteriaceae* in 8 (0.6% [95% CI 0.26–1.18]) of the 1,328 seafood samples; all 8 were from Southeast Asia (Table). Of the 928 shrimp samples collected as part of CIPARS sampling, 2 (0.2% [95% CI 0.03–0.78]) imported from Vietnam contained *Enterobacter cloacae* harboring bla_{IMI-1} , and 1 (0.1% [95% CI 0.003–0.599]) from Bangladesh contained *E. aerogenes* harboring bla_{IMI-2} . Of 101 mollusk samples, 3 (3.0% [95% CI 0.62–8.44]) clam samples imported from Vietnam contained *E. cloacae* harboring bla_{IMI-1} , and 2 (2.0% [95% CI 0.24–6.97]) clam samples from Vietnam contained *E. cloacae* harboring bla_{NDM-1} , bla_{TEM} , and bla_{OXA-1} . All isolates with carbapenemase genes were phenotypically resistant to ampicillin, cefoxitin, and amoxicillin/clavulanic acid; some were multiclass-resistant (Table).

Isolates harboring bla_{IMI-1} genes contained no plasmid DNA. However, using electroporation into *E. coli*, we showed that the bla_{IMI-2} gene was plasmid-mediated; the plasmid contained the IncFII(Yp) replicon. The bla_{NDM-1} genes were nontransformable into *E. coli*, although the 2 isolates contained IncHI2, IncFIB, and IncFII replicons. The location

of the bla_{NDM-1} gene may therefore be chromosomal or plasmidic. Six different sequence types (STs) of *E. cloacae* were shown by multilocus sequence typing. PFGE results showed that the 2 *E. cloacae* ST479 isolates were indistinguishable, whereas the other isolates were distinct. The *E. cloacae* ST479 isolates harbored bla_{NDM-1} , bla_{OXA-1} , and bla_{TEM} ; were phenotypically resistant to 12 tested antimicrobials drugs; and were from clam samples collected at different retail outlets on different dates. Comparison of ST373 fingerprints with the National Microbiology Laboratory PFGE database containing >170 *E. cloacae* of human origin showed that a human-sourced *E. cloacae* ST373 isolate harboring bla_{IMI-1} shared >75% similarity with a clam-sourced *E. cloacae* isolate. In addition to the carbapenem-resistant *Enterobacteriaceae* findings described here, our findings also show that 1 sample, from a black tiger shrimp (*Penaeus monodon*) originating from India, contained a non-O1, non-O139 *Vibrio cholerae* with a novel class A carbapenemase gene named bla_{VCC-1} (GenBank accession no. KT818596); this isolate has been described elsewhere (6).

Seafood, such as shrimp and clams, are raised in aquatic environments with a known potential for water-source contamination (7,8). We found multiple retail seafood samples containing *Enterobacter* spp. harboring bla_{NDM-1} and $bla_{IMI-type}$ genes. This finding suggests that, for humans, the source of carbapenemase-producing *Enterobacter* spp. may not be limited to exposure during travel; contaminated food products may also be a source of exposure (9). The identification, in imported clams, of *E. cloacae* with the same ST and similar DNA fingerprint pattern as an isolate from a human raises concerns of a possible association; however, more work is required before a linkage and direction of transfer can be inferred. Our findings highlight the need for antimicrobial resistance surveillance systems to consider the use of selective media methodology to increase sensitivity for the detection of rare or emerging resistance genes.

Table. Carbapenem-resistant *Enterobacter* species detected in retail seafood products imported from Southeast Asia to Canada*

Sample type, resistant species	No. (%) samples [95% CI]	Origin of seafood	Gene	Antibiogram profile	ST†
Shrimp, n = 928					
<i>E. cloacae</i>	2 (0.2) [0.03–0.78]	Vietnam	bla_{IMI-1}	AMC-AMP-(AZM)-FOX‡	ST411; ST412
<i>E. aerogenes</i>	1 (0.1) [0.003–0.599]	Bangladesh	bla_{IMI-2}	AMC-AMP-FOX	NA
Bivalve mollusks, n = 101					
<i>E. cloacae</i>	2 (2.0) [0.24–6.97]	Vietnam, clam	bla_{NDM-1} , bla_{TEM} , bla_{OXA-1}	AMC-AMP-FOX-TIO-CRO- CHL-CIP-GEN-STR-FIS- TET-TMP/SXT	ST479
<i>E. cloacae</i>	3 (3.0) [0.62–8.44]	Vietnam, clam	bla_{IMI-1}	AMC-AMP-(AZM)-FOX§	ST477; ST478; ST373
Cephalopods, n = 240	0 [0.00–1.53]	NA	NA	NA	NA
Miscellaneous, n = 59	0 [0.00–6.06]	NA	NA	NA	NA

*AMC, amoxicillin/clavulanic acid; AMP, ampicillin; AZM, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; FIS, sulfisoxazole; CRO, ceftriaxone; FOX, cefoxitin; GEN, gentamicin; NA, not applicable (no scheme found); ST, sequence type; STR, streptomycin; TET, tetracycline; TIO, ceftiofur; TMP/SXT, trimethoprim/sulfamethoxazole.

†Determined by multilocus sequence typing.

‡ST412 resistant to AZM.

§ST477 and ST373 resistant to AZM.

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Fluoroquinolone-Resistant *Mycoplasma genitalium*, Southwestern France

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To the Editor: *Mycoplasma genitalium* is a sexually transmitted bacterium involved in nongonococcal urethritis in men and associated with cervicitis and pelvic inflammatory disease in women. Azithromycin regimens have been commonly used as a first-line treatment of these conditions, but a recent increase in *M. genitalium* with azithromycin resistance has been described worldwide; in 2012, resistance in the organism was detected in France at a prevalence of 14% (1). In case of azithromycin failure, moxifloxacin is a second-line treatment; however, moxifloxacin treatment failures have also been reported and are associated with mutations in ParC or GyrA (2).

Prevalence of *M. genitalium* infection was ≈4% in 2013–2014 at Bordeaux University Hospital (Bordeaux, France). To evaluate the prevalence of fluoroquinolone resistance in *M. genitalium* in southwestern France, we examined the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes in *M. genitalium*-positive specimens obtained during 2013–2014. We retrospectively collected (from the Department of Bacteriology, Bordeaux University Hospital) 369 *M. genitalium*-positive urogenital specimens and DNA extracts from 344 patients. The *gyrA* and *parC* QRDRs were amplified and sequenced as described (3,4). We also assayed macrolide resistance-associated mutations using real-time PCR and melting curve analysis (1). To determine resistant genotypes A2058G or A2059G, we sequenced PCR products. Nucleotide positions in the 23S rRNA and amino acid positions in GyrA and ParC were identified according to *Escherichia coli* numbering.

From the 344 *M. genitalium*-positive patients, 200 specimens underwent complete analysis for the *gyrA* and *parC* genes, specimens from 221 patients were investigated for macrolide resistance, and specimens from 168 patients were examined for 23S rRNA, *gyrA*, and *parC* genes. Unsuccessful amplifications could be attributed to low bacterial loads of *M. genitalium* or to the degradation of frozen DNA during storage. Strains from 12/200 patients (6%; 95% CI 3.47%–10.19%) had QRDR mutations, with rates of 6.4% (6/93) for 2013 and 5.6% (6/107) for 2014. This prevalence is in accordance with the 4.5% moxifloxacin resistance described in the United Kingdom in 2011 (3) but lower than prevalences found in small numbers of strains in Japan and Australia during 2006–2014, which ranged from 10% to 47% (4–8).

Strains from 11 patients (patient nos. 6, 8, 12, 20, 23, 28–31, 46, 47) harbored alterations in the ParC QRDR (Table) at positions 80 (Ser→Asn or Ile) or 84 (Asp-84→Tyr or Asn). These mutations have been previously described for *M. genitalium* (4,6–8). In addition, 1 new amino acid alteration, Asn-96→Ser (strain from patient 20), was found in ParC. We detected a GyrA modification with the Ala-93→Thr transition in a strain from 1 patient (patient 3). These 2 amino acid changes were not