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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References

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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 μg 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<sub>NDM-1</sub>, bla<sub>KPC</sub>, bla<sub>IMP</sub>, bla<sub>VIM</sub>, bla<sub>GES</sub>, bla<sub>OXA-24-like</sub>, bla<sub>NMC</sub>), and β-lactamase-conferring (bla<sub>SHV</sub>, bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>OXA-1</sub>, bla<sub>CMY-2</sub>) 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<sub>IMI-1</sub>, and 1 (0.1% [95% CI 0.003–0.599]) from Bangladesh contained E. aerogenes harboring bla<sub>IMI-2</sub>. Of 101 mollusk samples, 3 (3.0% [95% CI 0.62–8.44]) clam samples imported from Vietnam contained E. cloacae harboring bla<sub>NDM-1</sub>, bla<sub>TEM</sub>, and bla<sub>OXA-1</sub>. All isolates with carbapenemase genes were phenotypically resistant to ampicillin, cefoxitin, and amoxicillin/clavulanic acid; some were multiclass-resistant (Table).

Isolates harboring bla<sub>NDM-1</sub> genes contained no plasmid DNA. However, using electroporation into E. coli, we showed that the bla<sub>IMI-2</sub> gene was plasmid-mediated; the plasmid contained the IncFII(Yp) replicon. The bla<sub>NDM-1</sub> genes were nontransformable into E. coli, although the 2 isolates contained IncHI2, IncFIB, and IncFII replicons. The location of the bla<sub>NDM-1</sub> 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<sub>NDM-1</sub>, bla<sub>OXA-1</sub>, and bla<sub>TEM</sub>; 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<sub>IMI-1</sub> 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<sub>VCC-1</sub> (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<sub>NDM-1</sub> and bla<sub>IMI-type</sub> 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>
<thead>
<tr>
<th>Sample type, resistant species</th>
<th>No. (%) samples [95% CI]</th>
<th>Origin of seafood</th>
<th>Gene</th>
<th>Antibiotic profile</th>
<th>ST†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp, n = 928</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. cloacae</td>
<td>2 (0.2) [0.03–0.78]</td>
<td>Vietnam</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;</td>
<td>AMC-AMP-(AZM)-FOX‡</td>
<td>ST411; ST412</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>1 (0.1) [0.003–0.599]</td>
<td>Bangladesh</td>
<td>bla&lt;sub&gt;IMI-2&lt;/sub&gt;</td>
<td>AMC-AMP-FOX</td>
<td>NA</td>
</tr>
<tr>
<td>Bivalve mollusks, n = 101</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. cloacae</td>
<td>2 (2.0) [0.24–6.97]</td>
<td>Vietnam, clam</td>
<td>bla&lt;sub&gt;NDM-1&lt;/sub&gt;, bla&lt;sub&gt;TEM&lt;/sub&gt;, bla&lt;sub&gt;OXA-1&lt;/sub&gt;</td>
<td>AMC-AMP-FOX-TIO-CRO-CHL-CIP-GEN-STR-FIS-TET-TMP/SXT</td>
<td>ST479</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>3 (3.0) [0.62–8.44]</td>
<td>Vietnam, clam</td>
<td>bla&lt;sub&gt;IMI-1&lt;/sub&gt;</td>
<td>AMC-AMP-(AZM)-FOX§</td>
<td>ST477; ST478; ST373</td>
</tr>
<tr>
<td>Cephalopods, n = 240</td>
<td>0 [0.00–1.53]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Miscellaneous, n = 59</td>
<td>0 [0.00–6.06]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*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.
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→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