Pathogenic Lineage of mcr-Negative Colistin-Resistant Escherichia coli, Japan, 2008–2015

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To the Editor: Colistin is a last-line drug for treatment of multidrug-resistant, gram-negative bacterial infections, including those caused by *Escherichia coli*. We report colistin-resistant *E. coli* isolates from Japan, including a global-spreading pathogenic lineage, serotype O25b:H4, sequence type (ST) 131, and subclone H30-R (O25b:H4-ST131-H30R).

We tested 514 *E. coli* isolates obtained from clinical specimens taken at Sapporo Clinical Laboratory Inc. (Sapporo, Japan) and Sapporo Medical University Hospital in Japan during 2008–2009 (1) and 2015, respectively. Samples were processed according to Clinical and Laboratory Standards Institute guidelines (2). Identification of O25b:H4-ST131, O25b, H4, and ST131 were determined as described previously (1). For identification of the H30Rx subclone of O25b:H4-ST131, H30 was determined by PCR using a specific primer set (3). R was determined according to ciprofloxacin MIC, and X was determined by detecting 2 single-nucleotide polymorphisms, as previously described (4).

Four *E. coli* isolates exceeded the colistin resistance breakpoint (>2 mg/mL) (Table). None of the patients from whom the *E. coli* isolates were derived had a history of colistin treatment. Three of the 4 colistin-resistant isolates belonged to a pandemic lineage, O25b:H4-ST131-H30R, which has been isolated from urinary tract and bloodstream infections (3,4). The frequency of colistin-resistant ST131 *E. coli* isolates among O25b:H4-ST131 was 2.2%. This lineage is fluoroquinolone resistant and is frequently resistant to β-lactams because it possesses CTX-M-type extended-spectrum β-lactamase genes (1,3,4).

The colistin-resistant isolates reported were resistant to fluoroquinolones, and 1 (SME296) was resistant to cephalosporins (due to expression of *blaCTX-M-1*). Another colistin-resistant *E. coli* isolate (SME222) belonged to O18-ST416, which is also known as an extraintestinal pathogenic *E. coli* (5), although this lineage has not previously been reported to exhibit colistin resistance.

The colistin-resistant *E. coli* isolates we identified were sensitive to carbapenems and aminoglycosides, including amikacin, whereas previously it was reported that some *E. coli* ST131 isolates exhibited resistance to carbapenems by possessing carbapenemases, such as NDM-1 and KPC-2; the NDM-1–possessing ST131 isolate also exhibited resistance to amikacin (6,7). Thus, these findings may affect future antimicrobial choices because of the clonal dominance, multidrug resistance, and pathogenicity of the isolates.

Recent studies reported a plasmid-mediated colistin resistance gene, *mcr-1*, in various countries (8). In addition, a novel plasmid-mediated colistin resistance gene, *mcr-2* (76.7% nucleotide identity to *mcr-1*), was found in *E. coli* isolates in Belgium (9). These genes encode a phosphoethanolamine transferase family protein, which modifies the lipid A component of lipopolysaccharide (8,9). The colistin-resistant *E. coli* isolates we identified did not possess *mcr-1* or *mcr-2*, although the MICs for colistin were the same as or higher than that of the transconjugant of a *mcr-1*–harboring plasmid in an *E. coli* ST131 isolate (4 mg/L) reported by Liu et al. (8). Thus, these colistin-resistant isolates may have other colistin resistance mechanisms. For example, modification of lipid A with 4-amino-4-deoxy-arabinose or phosphoethanolamine, caused by chromosomal mutations in *mrgB*, *phoPQ*, and *pmrAB* genes, might occur and could be responsible for the resistance. This polymyxin-resistance mechanism is seen in *Enterobacteriaceae*; however, other novel mechanisms are also conceivable.

In conclusion, we report colistin resistance in a major global-spreading extraintestinal pathogenic *E. coli* strain, O25b:H4-ST131-H30R, in Japan. This strain acquired colistin resistance without carrying a plasmid bearing the *mcr* gene. Clarifying the colistin-resistance mechanisms in these isolates is necessary if we are to forestall the emergence of multidrug (including

Table. Characterization of colistin-resistant *Escherichia coli* isolates, Japan, 2008–2015*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specimen type</th>
<th>Patient age, y/sex</th>
<th>Year</th>
<th>Serotype</th>
<th>ST</th>
<th>MIC, mg/L†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PIP</td>
</tr>
<tr>
<td>SRE34</td>
<td>Urine catheter</td>
<td>UNK/F</td>
<td>2008</td>
<td>O25b:H4</td>
<td>131-</td>
<td>128</td>
</tr>
<tr>
<td>SRE44</td>
<td>Urine catheter</td>
<td>UNK/M</td>
<td>2008</td>
<td>O25b:H4</td>
<td>131-</td>
<td>64</td>
</tr>
<tr>
<td>SME222</td>
<td>Indwelling</td>
<td>76/M</td>
<td>2015</td>
<td>O18</td>
<td>416</td>
<td>2</td>
</tr>
<tr>
<td>SME296</td>
<td>Urine</td>
<td>67/M</td>
<td>2015</td>
<td>O25b:H4</td>
<td>131-</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

*All isolates were phylogenetic group B2. AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CPD, cefpodoxime; FEP, cefepime; CST, colistin; GEN, gentamicin; IPM, imipenem; PIP, piperacillin; PMB, polymyxin B; R, resistant; S, susceptible; ST, sequence type; UNK, unknown.†EUCAST (http://www.eucast.org/) breakpoints were used for resistance determination because the colistin breakpoint for *E. coli* was undetermined by the Clinical and Laboratory Standards Institute. MICs were determined by the agar dilution method unless otherwise stated. Breakpoints: PIP, >16; CAZ, >16; CPD, >1; FEP, >4; PMB, >8; GEN, >4; AMK, >16; CIP, >1; CST, >2.‡Broth microdilution method.
Colistin–resistant O25b:H4-ST131-H30R. The worst-case scenario is the global spread of this isolate, which has acquired resistance to the last-line antimicrobial drug, colistin.

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References


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