Occurrence of bla<sub>KPC-2</sub>, bla<sub>CTX-M</sub>, and mcr-1 in Enterobacteriaceae from Well Water in Rural China

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ABSTRACT We report on the coexistence of mcr-1 and bla<sub>CTX-M</sub> in multidrug-resistant, extended-spectrum β-lactamase-producing Escherichia coli belonging to the sequence type 10 complex isolated from well water in rural China. Raoultella ornithinolytica with bla<sub>KPC-2</sub> was also detected in well water from the same area. This study shows that genes coding for resistance to last-resort antibiotics are present in wells in rural China, indicating a potential source of antibiotic resistance.

KEYWORDS ESBL, antibiotic resistance, mcr-1

Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing members of the family Enterobacteriaceae such as Escherichia coli have spread worldwide both as commensal bacteria and as causative agents of infection in humans and animals (1–3). Carbapenems and polymyxins are regarded as last-resort antibiotics for the treatment of severe infections in humans caused by multidrug-resistant members of the family Enterobacteriaceae (4, 5). However, this paradigm was challenged when the plasmid-mediated colistin resistance gene mcr-1 was reported in members of the family Enterobacteriaceae isolated from humans and food animals (6). E. coli with both mcr-1 and the EBSL-encoding gene bla<sub>CTX-M</sub> (7–9) and the carbapenemase-encoding gene bla<sub>NDM</sub> (10, 11) have been isolated from both humans and animals, indicating that resistance to these last-resort antibiotics is emerging. The coexistence of mcr-1 and bla<sub>CTX-M</sub> in E. coli has also been reported in surface waters such as rivers and ponds in Switzerland and Malaysia (9, 12).

In rural areas of China, well water is the primary source of irrigation water and drinking water for humans and farm animals. In this study, 71 wells were sampled in 12 rural villages of county A (n = 43) and 12 villages in county B (n = 28) in the Shandong province of China during July and September 2015 (Fig. 1). There was a long history of pig breeding in county A and a long history of chicken manure application for intensive vegetable cultivation in county B. About 1,000 ml of well water was collected in sterile bottles and filtered through sterile membrane filters with a pore size of 0.45 μm (Millipore, Billerica, MA). The filters were aseptically put into ESwab tubes (Copan, Brescia, Italy). Each sample was subsequently cultured on chromID ESBL, chromID CARBA, and chromID OXA-48 agar plates (bioMérieux, Marcy l’Etoile, France) for 18 to 24 h at 37°C to identify presumptive ESBL- and/or carbapenemase-producing members of the family Enterobacteriaceae. Identification to the species level was obtained by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Antimicro-
bial susceptibility profiles were determined by the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2016) and the EUCAST criteria (http://www.eucast.org/clinical_breakpoints/). The \textit{mcr-1}, \textit{bla}_{CTX-M}, \textit{bla}_{NDM}, \textit{bla}_{KPC}, \textit{bla}_{VIM}, and \textit{bla}_{OXA-48} genes were screened for by PCR (6,13, 14). PCR products were verified by sequencing (BGI Company, Beijing, China), and amplicons with at least 99% similarity to the \textit{mcr-1} amplicon were considered \textit{mcr-1}. Multilocus sequence typing (MLST) was performed according to the scheme at http://mlst.warwick.ac.uk/mlst/mlst/dbs/Ecoli.

Ten ESBL-producing \textit{E. coli} isolates were detected in a total of 71 samples, all of which came from county A (10/43, 23.3%) (Table 1). The \textit{mcr-1} gene was identified in two isolates from two different samples (ECcz1 and ECdw2) that also carried the \textit{bla}_{CTX-M-14} and \textit{bla}_{CTX-M-65} genes, respectively. Antibiotic susceptibility testing revealed that both strains exhibited multidrug-resistant phenotypes (resistance to more than two antibiotic classes), but both were susceptible to meropenem, amikacin, and

\begin{figure}
\centering
\includegraphics[width=\textwidth]{genotypes.png}
\caption{The genotypes of \textit{E. coli} and \textit{R. ornitholytica} isolates from agricultural counties A and B. DEM, digital elevation model.}
\end{figure}
TABLE 1 Characteristics of ESBL-producing *E. coli* with *mcr-1* and carbapenemase-producing *R. ornithinolytica* isolated from well water

<table>
<thead>
<tr>
<th>Strain</th>
<th>Deptha (m)</th>
<th>MIC (mg/liter)b</th>
<th>ESBL/ carbapenemase type</th>
<th>mcr-1d</th>
<th>MLST</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECaz1</td>
<td>5.0</td>
<td>1 16 32 &gt;8 8 0.06 4 4</td>
<td>&gt;16 &gt;256 32 4</td>
<td>CTX-M-55</td>
<td>NA *</td>
</tr>
<tr>
<td>ECaj4</td>
<td>4.5</td>
<td>2 16 32 &gt;8 4 0.06 4 4</td>
<td>&gt;16 &gt;256 32 4</td>
<td>CTX-M-55</td>
<td>NA *</td>
</tr>
<tr>
<td>ECaw1</td>
<td>4.5</td>
<td>1 16 256 &gt;8 1 0.06 2 2</td>
<td>&gt;16 128 &gt;256 &gt;32</td>
<td>CTX-M-65</td>
<td>ST2526</td>
</tr>
<tr>
<td>ECbw1</td>
<td>20.0</td>
<td>1 16 32 &gt;8 32 0.06 2 4</td>
<td>&gt;16 128 256 &gt;32 4</td>
<td>CTX-M-55</td>
<td>ST2973</td>
</tr>
<tr>
<td>Ebj3</td>
<td>5.0</td>
<td>1 16 32 &gt;8 8 0.03 &gt;32 &gt;128 &gt;256 &gt;16 &gt;16 256 &gt;32</td>
<td>CTX-M-65</td>
<td>ST1642</td>
<td></td>
</tr>
<tr>
<td>ECCR1</td>
<td>7.0</td>
<td>16 16 8 &gt;8 8 0.06 &gt;32 4</td>
<td>&gt;16 &gt;16 &gt;256 &gt;32 4</td>
<td>STX-14</td>
<td>ST48</td>
</tr>
<tr>
<td>EcDrv1</td>
<td>8.0</td>
<td>16 16 32 &gt;8 16 0.06 &gt;32 8</td>
<td>&gt;16 16 64 256 &gt;32</td>
<td>CTX-M-65</td>
<td>ST162</td>
</tr>
<tr>
<td>EcDrv2</td>
<td>30.0</td>
<td>1 16 8 &gt;8 1 0.03 2 2 128 0.25 2</td>
<td>&gt;16 &gt;16 256 &gt;32</td>
<td>CTX-M-27</td>
<td>ST162</td>
</tr>
<tr>
<td>Ecej1</td>
<td>5.0</td>
<td>2 8 &gt;2 &gt;8 2 0.06 2 4</td>
<td>64 0.5 8 &gt;16 &gt;256 32 4</td>
<td>CTX-M-65</td>
<td>ST2309</td>
</tr>
<tr>
<td>EcW1v</td>
<td>20.0</td>
<td>2 4 64 &gt;8 2 0.5 2 4</td>
<td>128 0.5 1 0.5 &gt;256 &gt;256 &gt;32</td>
<td>CTX-M-55</td>
<td>ST4762</td>
</tr>
<tr>
<td>ROj3</td>
<td>6.0</td>
<td>1 8 32 1 16 16 2 4</td>
<td>128 0.5 0.5 16 &gt;256 32 8</td>
<td>KPC-2</td>
<td>NA *</td>
</tr>
</tbody>
</table>

*aDepth of the well from which the water sample was taken.

bMICs were determined by agar dilution methods. Resistance is indicated by boldface type. Abbreviations: CL, colistin; AMC, amoxicillin-clavulanate; TZP, piperacillin-tazobactam; CTX, cefotaxime; CAZ, ceftazidime; MEM, meropenem; GEN, gentamicin; AMK, amikacin; TE, tetracycline; TGC, tigecycline; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; FOF, fosfomycin; F, nitrofurantoin; FFC, florfenicol. Antimicrobial susceptibility profiles were determined by the agar dilution method recommended by the EUCAST (http://www.eucast.org/mic_distributions_and_ecoffs/), except for tetracycline and florfenicol, for which the Clinical and Laboratory Standards Institute criteria (CLSI 2016) were applied.

The meropenem epidemiological cutoff for *E. coli* is \( \leq 0.125 \) mg/liter. According to EUCAST, a MIC of \( >0.125 \) mg/liter is used to detect suspected carbapenemase-producing isolates (http://www.eucast.org/mic_distributions_and_ecoffs/).

Symbols: +, present; -, absent.

*dNA, not available.

tigcycline (Table 1). No carbapenemase genes were detected in the *E. coli* isolates. MLST showed that isolate EcDrv2 belonged to sequence type 10 (ST10) and isolate EcCz1 belonged to ST48 (Table 1). ST48 is part of the ST10 clonal complex and differs from ST10 by only a single nucleotide.

No ESBL-producing *E. coli* strains were detected in well water samples from county B. However, there are some essential differences between counties A and B that could have influenced the occurrence of resistant bacteria and ESBL-producing *E. coli*. The well depth in county B (40 to 80 m) was greater than that in county A (4.5 to 30 m), suggesting that depth and temperature can influence the growth or dissemination of resistant bacteria. Shallower wells are also more likely to be influenced by anthropogenic activity. There is also a higher intensity of livestock farming in county A, particularly pig farms, which could have increased the risk of contaminating the shallower wells. Furthermore, in county B, a larger part of the well water is used for irrigation; thus, the wells have a shorter hydraulic retention time, which might influence the dissemination of resistant bacteria.

The two *E. coli* strains that harbored *mcr-1* and *bla*<sub>CTX-M</sub> genes in our study belonged to the ST10 complex, which is the most common ST of *E. coli* detected in human feces in southeastern China (15). The CTX-M ESBL has been reported as the dominant ESBL family in China, with *bla*<sub>CTX-M-14</sub> being the major variant detected in community onset infections in county hospitals across China (16). *bla*<sub>CTX-M-14</sub> is also frequently detected in farm animals, including pigs, and in pets in China. Although *bla*<sub>CTX-M-65</sub> does occur in both humans and pets, it appears to be more common in pigs and broiler chickens (17–20). The other *bla*<sub>CTX-M-65</sub> genes identified in this study (Table 1) are also frequently detected in humans and animals, especially *bla*<sub>CTX-M-55</sub> among clinical isolates. These findings demonstrate the wide spread of *bla*<sub>CTX-M</sub> among *E. coli* isolates from humans, animals, and the environment in China.

Multidrug-resistant *E. coli* with carbapenemase genes and *mcr-1* has previously been reported in clinical samples and constitutes a health risk (10, 21). While none of the *E. coli* isolates carrying *mcr-1* in this study were carbapenemase producers, an isolate of *Raoellella ornithinolytica* carrying the carbapenemase gene *bla*<sub>KPC-2</sub> was also detected in a well water sample. *R. ornithinolytica* is a member of the family *Enterobacteriaceae*, and *bla*<sub>KPC-2</sub>-carrying strains of this species have previously been reported in clinical samples in China (22). Although the isolate was not detected in the same wells as the
E. coli isolates carrying mcr-1, its presence in the same area and its close relatedness to E. coli indicate a potential risk of co-occurrence and transfer of resistance genes. In rural areas, well water is essential in daily life for activities such as tooth brushing and bathing and as drinking water for humans and pigs. Such activities could potentially increase the risk of dissemination of antibiotic-resistant bacteria from the environment to humans and animals.

As only two mcr-1-positive isolates were detected and well water was the only environmental matrix investigated in this study, generalization of our findings should be done with caution. A limitation of this study is that phenotypic screening for ESBL- and/or carbapenemase-producing members of the family Enterobacteriaceae was performed and thus mcr-1-carrying isolates without either phenotype would have remained undetected. From a One Health perspective, further studies involving more samples from humans, animals, and different environmental matrices are urgently needed to study the dissemination dynamics of mcr-1 and other resistance genes.

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We have no conflicts of interest to declare.

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