Prevalence of \textit{mcr-1} in the Cecal Contents of Food Animals in the United States

Richard J. Meinersmann,\textsuperscript{a} Scott R. Ladely,\textsuperscript{b} Jodie R. Plumlee,\textsuperscript{a} Kimberly L. Cook,\textsuperscript{a} Eileen Thacker\textsuperscript{a}

\textsuperscript{a}USDA Agricultural Research Service, Athens, Georgia, USA; \textsuperscript{b}USDA Food Safety and Inspection Service, Athens, Georgia, USA

\textbf{ABSTRACT} A survey of 2,003 cecal content samples from chickens, turkeys, cattle, and swine at slaughter facilities in the United States was conducted to estimate the prevalence of the \textit{mcr-1} gene conferring resistance to colistin in \textit{Enterobacteriaceae}. Two cecal samples from swine had \textit{Escherichia coli} with IncI2 plasmids bearing the \textit{mcr-1} gene.

\textbf{KEYWORDS} \textit{Escherichia coli}, antimicrobial resistance, colistin

The discovery of a plasmid-borne gene (\textit{mcr-1}) that confers resistance to polymyxin E (colistin) in \textit{Escherichia coli} (1) has raised fears that the readily transferable gene will limit treatment options, especially if found in multidrug-resistant organisms. Since its first discovery in China, it has since been reported on every continent except for Antarctica and Australia (2). Plasmids with the gene have been found in \textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, \textit{Shigella sonnei}, and \textit{Salmonella enterica}. These pathogens have been isolated from humans, chickens, turkeys, and swine (2). At this time, \textit{mcr-1} has been isolated from four human patients in the United States (3–7), but there has not been published, to our knowledge, any systematic study of prevalence in the United States.

Since food animals are common sources for human infection by these organisms, a study was undertaken to determine the prevalence of the \textit{mcr-1} gene in the United States. The Food Safety and Inspection Service (FSIS) participates in the National Antimicrobial Resistance Monitoring System (NARMS) by collecting samples of cecal contents from slaughtered animals at all federally inspected slaughter plants according to a schedule and methods described by FSIS Directive 10,100.1 (8). For this study, 2,003 cecal samples taken from 21 March to 3 August 2016 that were sent to the FSIS laboratory in Athens, GA, were tested, including 1,077 samples from cattle, 238 samples from chickens, 563 samples from swine, and 125 samples from turkeys. The samples were diluted (10 g into 90 ml) in buffered peptone water (BPW) and incubated overnight at 37°C. At that time, an aliquot of the BPW was collected and diluted 1:10 in BPW with 2 \textmu{g}/ml colistin and incubated overnight at 37°C. The inoculated colistin containing BPW was then tested by PCR using the primers and conditions described by Liu et al. (1). Primers UNI338F and UNI1100R (9) for the 16S rRNA gene were included for a positive PCR control; samples that were negative for the 16S rRNA gene were excluded from the study. All cultures with PCR producing any band of approximately the appropriate size for \textit{mcr-1} were streaked on MacConkey’s agar supplemented with 2 \textmu{g}/ml colistin and incubated again overnight. If a characteristic \textit{Enterobacteriaceae} colony was then observed, it was reisolated on MacConkey’s agar with colistin, and the next day, a colony was used for retesting by PCR. Presumptive positive isolates were identified to the species level using the Vitek 2 system (bioMérieux), and the presence of the \textit{mcr-1} gene was confirmed by sequencing (10, 11).
Susceptibility to colistin was determined by the agar dilution method using Mueller-Hinton agar supplemented with doubling concentrations of colistin ranging from 2 to 32 \( \mu g/ml \). Susceptibilities to a panel of 14 other antimicrobials were determined by the broth microdilution using the Sensititre system (Thermo Fisher Scientific). The antimicrobials included amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole. MICs were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (17) when available, or by using the criteria established by the NARMS (http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM528751.pdf).

Two isolates were found carrying \textit{mcr-1}, both from cecal samples from swine, yielding an overall prevalence in food animals at slaughter of 0.1% and prevalence in swine at slaughter of 0.35%. This is considerably lower than prevalences recently reported for food animals in Europe (12) and Asia (13). Characteristics of the two isolates are listed in Table 1. It can be seen that the two isolates are substantially different from each other, have little virulence potential, and bear little in common with isolates worldwide bearing \textit{mcr-1} other than the observation that the \textit{mcr-1} gene is being carried on an \textit{IncI2} plasmid, as has been reported several times. Figure 1 is a ring map that compares all of the \textit{mcr-1}-bearing \textit{IncI2} plasmids available in GenBank as of 4 August 2016. The plasmids are distinguishable largely by multiple insertion/deletion events. A notable observation is the presence of an \textit{ISA\textit{pl}}\textit{1} family transposase immediately upstream of the \textit{mcr-1} gene in six of the 16 plasmids. Neither of the swine cecal isolates had that transposase, but \textit{pSLy1} (CP015913) had a different transposase (\textit{IS}91\textit{family}) that interrupted the \textit{nikB} gene. This is noteworthy, because the product of \textit{nikB} is involved in conjugation.

Conjugation rate studies were performed using streptomycin-resistant \textit{E. coli C600} as the recipient, as described by Liu et al. (1), except that transconjugants were selected on LB plates with colistin (4 mg/liter) and streptomycin (1,000 mg/liter). Since the parent strain for \textit{pSLy1} was also resistant to streptomycin, the plasmid was first transferred to \textit{E. coli J53} by conjugation, as previously described (16), which was pansusceptible except for resistance to sodium azide (200 mg/liter). A transconjugant of \textit{E. coli J53} with resistance to colistin (4 mg/liter) but susceptibility to streptomycin was then mated with \textit{E. coli C600} to determine conjugation efficiency. The results of this conjugation were highly varied (between \( 6.1 \times 10^{-5} \) and \( 3.8 \times 10^{-1} \) transconjugants per recipient). In contrast, in conjugation experiments with \textit{E. coli C600} as the recipient and the second colistin-resistant isolate as the donor, the \textit{pSLy21} plasmid was transferred at a more consistent rate (2.14 \pm 0.55 \times 10^{-4} transconjugants per recipient).

In conclusion, the \textit{mcr-1} gene conferring resistance to colistin was found in the cecal contents from swine in the United States. It is noteworthy that the routine NARMS

### Table 1: Isolate characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>\textit{E. coli}</td>
<td>\textit{E. coli}</td>
</tr>
<tr>
<td>Serotype</td>
<td>O160:H40</td>
<td>O undeterminable:H32</td>
</tr>
<tr>
<td>Sequence type</td>
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<td>ST-132</td>
</tr>
<tr>
<td>Host</td>
<td>Swine</td>
<td>Swine</td>
</tr>
<tr>
<td>State of origin</td>
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<td>Illinois</td>
</tr>
<tr>
<td>Colistin MIC (( \mu g/ml ))</td>
<td>16</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Antimicrobial resistance</td>
<td>Collistin, ampicillin, streptomycin, sulfisoxazole, tetracycline</td>
<td>Colistin</td>
</tr>
<tr>
<td>Plasmid bearing \textit{mcr-1}</td>
<td>\textit{IncI2}</td>
<td>\textit{IncI2}</td>
</tr>
<tr>
<td>Other plasmids</td>
<td>\textit{IncF1B, IncI1, and two unclassified}</td>
<td>None</td>
</tr>
<tr>
<td>GenBank sequences</td>
<td>CP015912, CP015913 (\textit{pSLy1}), CP015914, CP015915, CP015916, CP015917</td>
<td>CP016404, CP016405 (\textit{pSLy21})</td>
</tr>
<tr>
<td>VirulenceFinder results</td>
<td>\textit{astA, lpfA}</td>
<td>\textit{astA, gad}</td>
</tr>
</tbody>
</table>

\*VirulenceFinder at http://cge.cbs.dtu.dk; see reference 14.*
testing of the same samples did not discover colistin-resistant isolates, but the samples were tested for *Salmonella* and not *E. coli*, and NARMS does not routinely analyze for colistin resistance. At this time, there is not enough information to determine the source of the colistin-resistant isolates. To date, there has only been one *mcr-1* gene with a sequence that differs from all the others, and that is only by one base, so the gene itself appears to be phylogenetically young and is rapidly spreading. However, the plasmids on which *mcr-1* is appearing are fairly heterogeneous. Most of the reports

FIG 1 Comparative map of *mcr-1*-bearing IncI2 plasmids. A BRIG map (15) was constructed using GenBank accession number KP347127 as the reference sequence (innermost circle and outermost circle coding sequence [CDS] annotations). The complete circle represents only the length of that reference, which is 64,015 bp. The second (blue) and third (magenta) inner circles represent the *mcr-1*-positive plasmids we found (GenBank accession numbers CP015913 and CP016405, respectively). The remaining red circles represent *mcr-1*-positive IncI2 plasmids found in NCBI as of 3 August 2016. The intensity of the color shading indicates similarity to the reference, which in this illustration shows most of the sequences are highly conserved or totally absent. The *mcr-1* gene is at approximately the 4:30 clock position with a clockwise orientation. A transposase (ISApl1 family in reference sequence) can be seen immediately upstream of the *mcr-1* in 6 of the plasmids.
of mcr-1 genes have been on IncI2 plasmids, but this is the first time the mcr-1-bearing IncI2 plasmid was found in cecal contents from swine.

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REFERENCES


