Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF Plasmid: First report of mcr-1 in the USA

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Running Title: Colistin resistance in the USA

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The recent discovery of a plasmid-borne colistin resistance gene, mcr-1, heralds the emergence of truly pan-drug resistant bacteria (1). The gene has been found primarily in *Escherichia coli*, but has also been identified in other members of the *Enterobacteriaceae* from human, animal, food and environmental samples on every continent (2-5). In response to this threat, starting in May 2016, all extended-spectrum β-lactamase (ESBL)-producing *E. coli* clinical isolates submitted to the clinical microbiology laboratory at the Walter Reed National Military Medical Center (WRNMMC) were tested for resistance to colistin by E-test. Herein we report the presence of mcr-1 in an *E. coli* cultured from a patient with a urinary tract infection (UTI) in the United States.

*E. coli* MRSN 388634 was cultured from the urine of a 49 year-old female who presented to a clinic in Pennsylvania on April 26th 2016 with symptoms indicative of a UTI. The isolate was forwarded to WRNMMC, where susceptibility testing indicated an ESBL phenotype (Table 1). The isolate was included in the first 6 ESBL-producing *E. coli* selected for colistin susceptibility testing, and it was the only isolate to have a MIC of colistin of 4µg/ml (all others had MICs ≤ 0.25 µ/ml). Colistin MIC was confirmed by microbroth dilution and mcr-1 detected by real-time PCR (6). Whole genome sequencing (WGS) of MRSN 388634 was performed on a PacBio RS II and Miseq benchtop sequencer.

*E. coli* MRSN 388634 belonged to ST457, a rare *E. coli* ST first identified in 2008 from a urine culture in the UK (7). It was subsequently identified from a bloodstream culture in Italy, where it was found to harbor the carbapenemase gene *bla*KPC-3 and *bla*CTX-M-55 (8). MRSN 388634 carried 15 antibiotic resistance genes, but no carbapenemases, that were harbored on two plasmids (Table 2).
The first plasmid, pMR0516mcr, was 225,707 bp in size and belonged to incompatibility group F18:A-:B1 (9). BLAST analysis indicated that pMR0516mcr represented a novel IncF plasmid. Notably, it shares 89 Kb of homologous sequence with pHNSHP45-2, a mcr-1 carrying IncHI2 plasmid described by Liu and colleagues (1). This shared sequence contains mcr-1 in association with IS_AplI (1), but in pMR0516mcr it is in a different location and orientation (Figure 1).

pMR0516mcr also carried 7 additional antibiotic resistance genes, including the ESBL gene \textit{bla}_{CTX-M-55} (Table 2). The second plasmid, pMR0416ctx, was ~47 Kb in size and was assigned to IncN (Table 2). It carried 7 antibiotic resistance genes including \textit{bla}_{CTX-M-14}. A complete description of both plasmids is under preparation.

To the best of our knowledge, this is the first report of \textit{mcr-1} in the USA. The epidemiology of MRSN 388634 is noteworthy; the isolate was submitted from a clinic in Pennsylvania, and the patient reported no travel history within the prior 5 months. To date a further 20 ESBL-producing \textit{E. coli} from patients at the WRNMMC have tested negative for \textit{mcr-1}, and are colistin sensitive. However, as testing has only been ongoing for 3 weeks, it remains unclear what the true prevalence of \textit{mcr-1} is in the population. The association between \textit{mcr-1} and IncF plasmids is concerning as these plasmids are vehicles for the dissemination of antibiotic resistance and virulence genes among the \textit{Enterobacteriaceae} (9). Continued surveillance to determine the true frequency for this gene in the USA is critical.

\textbf{Nucleotide Accession Numbers.} The Short Read Archive (SRA) file for MRSN 388623 has been deposited at Genbank under Accession number SRP075674.
Acknowledgements.

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Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation. The views expressed in this article are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or the U.S. Government.


   Upton M. 2008. Major uropathogenic *Escherichia coli* strain isolated in the northwest of 
   England identified by multilocus sequence typing. Journal of clinical microbiology 
   46:1076-1080.

   F, Pantosti A, Rossolini GM, Cerquetti M. 2014. Emergence of *Escherichia coli* 
   ST131 sub-clone H30 producing VIM-1 and KPC-3 carbapenemases, Italy. The Journal 
   of antimicrobial chemotherapy 69:2293-2296.

   of *IncF* plasmids carrying virulence and resistance determinants. The Journal of 
   antimicrobial chemotherapy 65:2518-2529.

    Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using 
    PlasmidFinder and plasmid multilocus sequence typing. Antimicrobial agents and 
    chemotherapy 58:3895-3903.
Table 1. Antibiotic resistance profile of MRSN 388634

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≤8</td>
</tr>
<tr>
<td>Amoxacillin/clavulanate</td>
<td>16/8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Colistin</td>
<td>4</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>≤16</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>4/4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>&gt;2/38</td>
</tr>
</tbody>
</table>
MIC's were determined using the BD Phoenix (BD Diagnostics Systems, MD, USA) with panels NMIC/ID 133 except for colistin, which was performed using E-test and manual microbroth dilution, and both gave MICs of colistin = 4µg/ml.
<table>
<thead>
<tr>
<th>Name</th>
<th>Size (Kb)</th>
<th>Inc</th>
<th>Copy#</th>
<th>Antibiotic resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMR0416ctx</td>
<td>47</td>
<td>N</td>
<td>1</td>
<td>aac(3)-IVa, aph(4)-Ia, blaCTX-M-14, fosA3, mph(A), floR, sul2</td>
</tr>
</tbody>
</table>

1 Plasmid Incompatibility (Inc) group, as determined by Plasmid Finder version 1.2 (10).

2 Average copy number per cell, normalized to the chromosomal read coverage.
Figure 1. Comparison of the homologous region containing mcr-1 shared by pMR0516mcr and pHNSHP45-2. Open arrows represent coding sequences (Green arrows, mcr-1; white arrows, ISap1; purple arrows, metabolic function; blue arrows, plasmid replication and maintenance; grey arrows, hypothetical and unclassified) and indicate direction of transcription. Arrow size is proportional to the gene length. The grey and blue areas between pMR0516mcr and pHNSHP45-2 indicate nucleotide identity >99.9% by BLASTN.
Erratum for McGann et al., *Escherichia coli* Harboring *mcr-1* and *bla*<sub>CTX-M</sub> on a Novel IncF Plasmid: First Report of *mcr-1* in the United States

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Volume 60, no. 7, p. 4420–4421, 2016. Page 4420, right column, line 15: the correct reference for the sequence and description of plasmid pHNSHP45-2 is not reference 1 but is as follows.
