

1 ***Escherichia coli* Harboring *mcr-1* and *bla*_{CTX-M} on a Novel IncF Plasmid: First report of**
2 ***mcr-1* in the USA**

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10 **Running Title:** Colistin resistance in the USA

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

34 *E. coli* MRSN 388634 was cultured from the urine of a 49 year-old female who presented to a
35 clinic in Pennsylvania on April 26th 2016 with symptoms indicative of a UTI. The isolate was
36 forwarded to WRNMMC, where susceptibility testing indicated an ESBL phenotype (Table 1).
37 The isolate was included in the first 6 ESBL-producing *E.coli* selected for colistin susceptibility
38 testing, and it was the only isolate to have a MIC of colistin of 4 μ g/ml (all others had MICs \leq
39 0.25 μ /ml). Colistin MIC was confirmed by microbroth dilution and *mcr-1* detected by real-time
40 PCR (6). Whole genome sequencing (WGS) of MRSN 388634 was performed on a PacBio RS II
41 and Miseq benchtop sequencer.

42 *E. coli* MRSN 388634 belonged to ST457, a rare *E.coli* ST first identified in 2008 from a urine
43 culture in the UK (7). It was subsequently identified from a bloodstream culture in Italy, where it
44 was found to harbor the carbapenemase gene *bla*_{KPC-3} and *bla*_{CTX-M-55} (8). MRSN 388634 carried
45 15 antibiotic resistance genes, but no carbapenemases, that were harbored on two plasmids
46 (Table 2).

47 The first plasmid, pMR0516mcr, was 225,707 bp in size and belonged to incompatibility group
48 F18:A-:B1 (9). BLAST analysis indicated that pMR0516mcr represented a novel IncF plasmid.
49 Notably, it shares 89 Kb of homologous sequence with pHNSHP45-2, a *mcr-1* carrying IncHI2
50 plasmid described by Liu and colleagues (1). This shared sequence contains *mcr-1* in association
51 with *ISApII* (1), but in pMR0516mcr it is in a different location and orientation (Figure 1).
52 pMR0516mcr also carried 7 additional antibiotic resistance genes, including the ESBL gene
53 *bla*_{CTX-M-55} (Table 2). The second plasmid, pMR0416ctx, was ~47 Kb in size and was assigned to
54 IncN (Table 2). It carried 7 antibiotic resistance genes including *bla*_{CTX-M-14}. A complete
55 description of both plasmids is under preparation.

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

65 **Nucleotide Accession Numbers.** The Short Read Archive (SRA) file for MRSN 388623 has
66 been deposited at Genbank under Accession number SRP075674.

67 **Acknowledgements.**

68 This study was funded by the U.S. Army Medical Command, the Global Emerging Infections
69 Surveillance and Response System, and the Defence Medical Research and Development
70 Program. All authors declare no conflict of interest.
71 The identification of specific products or scientific instrumentation does not constitute
72 endorsement or implied endorsement on the part of the author, DoD, or any component agency.
73 While we generally excise references to products, companies, manufacturers, organizations, etc.
74 in government produced works, the abstracts produced and other similarly situated research
75 presents a special circumstance when such product inclusions become an integral part of the
76 scientific endeavour.
77 Material has been reviewed by the Walter Reed Army Institute of Research. There is no
78 objection to its presentation. The views expressed in this article are those of the author and do
79 not reflect the official policy of the Department of Army/Navy/Air Force, Department of
80 Defense, or the U.S. Government.

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119 **Table 1. Antibiotic resistance profile of MRSN 388634**

Antibiotic	MIC ($\mu\text{g/ml}$)¹
Amikacin	≤ 8
Amoxicillin/clavulanate	16/8
Ampicillin	>16
Aztreonam	>16
Cefazolin	>16
Cefepime	>16
Ceftazidime	>16
Ceftriaxone	>32
Ciprofloxacin	>2
Colistin	4
Ertapenem	≤ 0.25
Gentamicin	>8
Imipenem	≤ 0.25
Levofloxacin	>4
Meropenem	≤ 0.25
Nitrofurantoin	≤ 16
piperacillin-tazobactam	4/4
Tetracycline	>8
Tobramycin	>8
Trimethoprim/sulfamethoxazole	>2/38

120

121 ¹ MIC's were determined using the BD Phoenix (BD Diagnostics Systems, MD, USA) with
122 panels NMIC/ID 133 except for colistin, which was performed using E-test and manual
123 microbroth dilution, and both gave MICs of colistin = 4 μ g/ml.

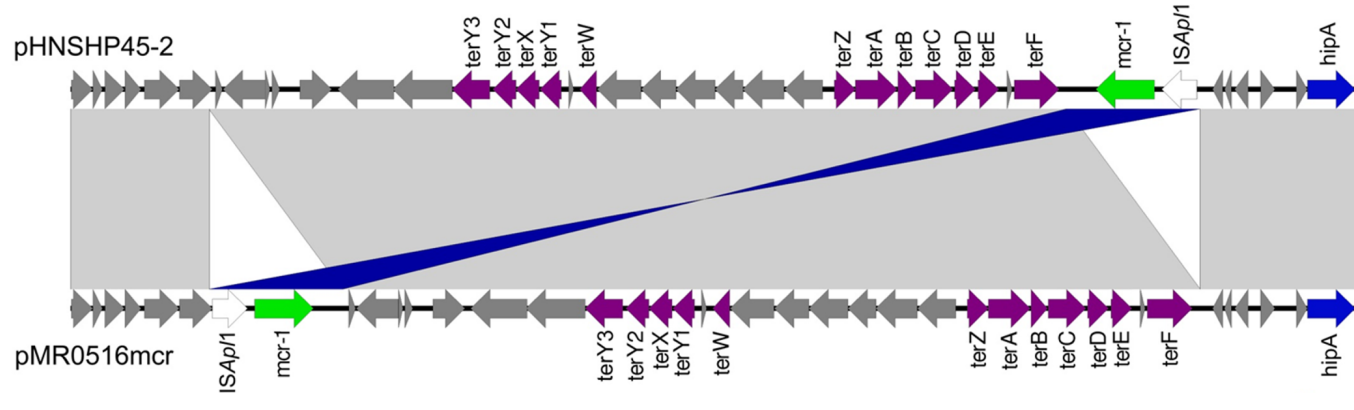
124 **Table 2 Characteristics of plasmids in *E.coli* MRSN 388634**

Name	Size (Kb)	Inc ¹	Copy# ²	Antibiotic resistance genes
pMR0516mcr	225.7	F18:A-:B+	2	<i>strA</i> , <i>strB</i> , <i>bla</i> _{CTX-M-55} , <i>bla</i> _{TEM-1B} , <i>mcr-1</i> , <i>sul2</i> , <i>tet(A)</i> , <i>dfrA14</i>
pMR0416ctx	47	N	1	<i>aac(3)-IVa</i> , <i>aph(4)-Ia</i> , <i>bla</i> _{CTX-M-14} , <i>fosA3</i> , <i>mph(A)</i> , <i>floR</i> , <i>sul2</i>

125

126 ¹ Plasmid Incompatibility (Inc) group, as determined by Plasmid Finder version 1.2 (10).127 ² Average copy number per cell, normalized to the chromosomal read coverage.

128 **Figure 1.** Comparison of the homologous region containing *mcr-1* shared by pMR0516mcr and
129 pHNSHP45-2. Open arrows represent coding sequences (Green arrows, *mcr-1*; white arrows,
130 *ISapII*; purple arrows, metabolic function; blue arrows, plasmid replication and maintenance;
131 grey arrows, hypothetical and unclassified) and indicate direction of transcription. Arrow size is
132 proportional to the gene length. The grey and blue areas between pMR0516mcr and pHNSHP45-
133 2 indicate nucleotide identity >99.9% by BLASTN.



ERRATUM

Erratum for McGann et al., *Escherichia coli* Harboring *mcr-1* and *bla*_{CTX-M} 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.

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Citation McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE. 2016. Erratum for McGann et al., *Escherichia coli* harboring *mcr-1* and *bla*_{CTX-M} on a novel IncF plasmid: first report of *mcr-1* in the United States. *Antimicrob Agents Chemother* 60:5107. doi:10.1128/AAC.01353-16.
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