AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

LETTER TO THE EDITOR

Detection of *mcr-1* among *Escherichia coli* Clinical Isolates Collected Worldwide as Part of the SENTRY Antimicrobial Surveillance Program in 2014 and 2015

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The initial report of the plasmid-mediated colistin resistance gene *mcr-1* detected in *Escherichia coli* isolates from China (1) and the numerous follow up publications reporting livestock and clinical isolates carrying this gene raise serious concerns about the potential for the dissemination of a mobile gene encoding resistance to one of the few treatment options that have been used for infections caused by multidrug-resistant (MDR) and carbapenem-resistant *Enterobacteriaceae* (CRE) isolates displaying elevated MICs for more widely used and less toxic antimicrobial agents.

In this report, 390 *E. coli* and *Klebsiella pneumoniae* clinical isolates displaying elevated colistin MIC results (≥4 μg/ml) collected during a 2-year period as part of a large global surveillance program were screened for the presence of *mcr-1*. In addition, 314 CRE isolates collected as part of the SENTRY Antimicrobial Surveillance Program during 2015 were screened for *mcr-1*. This report includes an *mcr-1*-harboring *E. coli* isolate from the United States collected in May 2015.

During 2014 and 2015, 21,006 *E. coli* (n = 13,526) and *K. pneumoniae* (n = 7,480) isolates were collected in 183 hospitals located in the Asia-Pacific region (n = 15), Europe (n = 46), Latin America (n = 9), and North America (n = 113) as part of the SENTRY Program. Isolates were tested for susceptibility against colistin and other antimicrobial agents by the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (2). Categorical interpretations from CLSI document M100-S26 (3), the EUCAST website for colistin (http://www.eucast.org/clinical_breakpoints/), or the U.S. Food and Drug Administration (FDA) package inserts for tigecycline and ceftazidime-avibactam were applied. Quality control was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, and the results were within specified ranges as published by the CLSI (3).

A total of 390 (1.9% of overall isolates) *E. coli* (n = 59; 0.4% for this species) and *K. pneumoniae* (n = 331; 4.4% for this species) isolates displayed colistin MICs of ≥4 μg/ml (the EUCAST resistance breakpoint for *Enterobacteriaceae*) and were submitted for a PCR assay targeting *mcr-1*. Amplicons generated were sequenced on both strands; nucleotide and deduced amino acid sequences were analyzed and compared with those available via Internet sources. Among colistin-resistant isolates, 19 (4.9% of colistin-resistant [by EUCAST criteria] isolates and <0.1% overall) were positive for *mcr-1*, including 8 isolates from 2014 and 11 isolates from 2015. These 19 isolates were all *E. coli* (32.2% of colistin-resistant *E. coli* isolates; 0.1% overall for this species) distributed in 10 countries (Table 1): Belgium (1 isolate), Brazil (1 isolate), Germany (5 isolates), Hong Kong (1 isolate), Italy (4 isolates), Malaysia (1 isolate), Poland (1 isolate), Russia (1 isolate), Spain (3 isolates), and the United States (1 isolate). Isolates positive for *mcr-1* were associated with bloodstream infections (8 isolates), skin and skin structure infections (5 isolates), urinary tract infections (3 isolates), respiratory tract infections (2 isolates), and intra-abdominal infections (1 isolate).

The only *mcr-1*-positive *E. coli* isolate detected in the United States in this study was recovered in May 2015, prior to the first report of an *mcr-1*-producing *E. coli* isolate detected in the United States, which was recovered from the urinary tract of a patient hospitalized in Pennsylvania (4). In contrast to the first report, the isolate included in this study was susceptible to several antimicrobial agents, including cefepime (MIC, 2 μg/ml), piperacillin-tazobactam (4 μg/ml), ceftolozane-tazobactam (1 μg/ml), ceftazidime-avibactam (0.25 μg/ml), imipenem (≤0.12 μg/ml), meropenem (≤0.015 μg/ml), doripenem (≤0.06 μg/ml), gentamicin (0.5 μg/ml), amikacin (2 μg/ml), tobramycin (0.5 μg/ml), nitrofurantoin (8 μg/ml), fosfomycin (64 μg/ml), and tigecycline (0.5 μg/ml). This isolate was resistant to ciprofloxacin and levofloxacin (>4 μg/ml for both), trimethoprim-sulfamethoxazole (>4 μg/ml), ceftazidime (>32 μg/ml), aztreonam (>16 μg/ml), and ceftriaxone (>8 μg/ml) and carried two β-lactam resistance genes: $bla_{\text{SHV-5}}$ and $bla_{\text{TEM-1}}$.

Overall, colistin-resistant *mcr-1*-positive isolates were carbapenem susceptible (all imipenem and meropenem MIC results were ≤0.5 and ≤0.06 μg/ml, respectively). The susceptibility rates for other β-lactam agents (by CLSI criteria) for *mcr-1*-positive isolates were 89.5% for ceftazidime, aztreonam, and cefepime, 78.9% for ceftriaxone, and 73.7% for piperacillin-tazobactam. Ceftazidime-avibactam was active against all isolates tested (100.0% susceptible), and 94.7% of the isolates were susceptible to ceftolozane-tazobactam. Among other antimicrobial classes, the susceptibility rates for these isolates were 10.5% for trimethoprim-sulfamethoxazole and tetracycline, 21.0% for levofloxacin, 89.5% for nitrofurantoin, and 100.0% for amikacin, fosfomycin, and tigecycline.

All CRE isolates collected during 2015 yielded negative results for *mcr-1*. These isolates displayed colistin MICs ranging from

Accepted manuscript posted online 11 July 2016

Citation Castanheira M, Griffin MA, Deshpande LM, Mendes RE, Jones RN, Flamm RK. 2016. Detection of *mcr-1* among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates collected worldwide as part of the SENTRY Antimicrobial Surveillance Program in 2014 and 2015. Antimicrob Agents Chemother 60:5623–5624. doi:10.1128/AAC.01267-16.

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TABLE 1 Occurrence of *mcr-1* among *E. coli* and *K. pneumoniae* clinical isolates collected worldwide during 2014 and 2015 as part of the SENTRY Antimicrobial Surveillance Program[a]

Organism(s) and location where *mcr-1*-positive isolate(s) was observed	No. of *mcr-1*-positive isolates/ no. of colistin-resistant isolates (%)	No. of *mcr-1*-positive isolates with colistin MICs (μg/ml) of:		
		4	8	>8
All colistin-resistant organisms	19/390 (4.9)	10	8	1
E. coli	19/59 (32.2)	10	8	1
Belgium (Antwerp)	1/1	1		
Brazil (Florianopolis)	1/1	1		
Germany (Bonn and Kiel)	5/10	2	3	
Hong Kong	1/1	1		
Italy (Florence, Milan, and Rome)	4/9	3	1	
Malaysia (Kelantan)	1/1		1	
Poland (Warsaw)	1/2	1		
Russia (Yekaterinburg)	1/2	1		
Spain (Madrid)	3/3		2	1
USA (New York City)	1/15		1	

[a] All *K. pneumoniae* isolates were negative for *mcr-1*.

≤0.06 to >8 μg/ml (some overlapping with the MICs for the colistin-resistant subset).

The prevalence of *mcr-1* among *E. coli* isolates was elevated (>30.0% of colistin-resistant isolates), but very low rates were observed for the overall population surveyed by the SENTRY Program (<0.1%). All *K. pneumoniae* isolates yielded negative results for the presence of *mcr-1*. The isolates carrying *mcr-1* were susceptible to various antimicrobial classes; however, the prospect of a mobile gene encoding resistance to colistin evolving among isolates resistant to most clinically available antimicrobial agents is threatening for therapy of serious infection.

This report provides an overview of the occurrence of these isolates in a large collection of global isolates and expands the list of geographies where *mcr-1*-positive isolates have been detected. The potential dissemination of *mcr-1* among *Enterobacteriaceae* isolates highlights the importance of active surveillance of emerging resistance genes, including *mcr-1*, and development of novel antimicrobial agents active against MDR Gram-negative isolates and CRE.

We are performing additional studies to further characterize these isolates, including genetic context analysis of *mcr-1*.

ACKNOWLEDGMENTS

We thank the SENTRY Antimicrobial Surveillance Program participants for their commitment providing bacterial isolates to this program.

JMI Laboratories, Inc., has received research and educational grants in 2014 and 2015 from Achaogen, Actavis, Actelion, Allergan, American Proficiency Institute (API), AmpliPhi, Anacor, Astellas, AstraZeneca, Basilea, Bayer, BD, Cardeas, Cellceutix, CEM-102 Pharmaceuticals, Cempra, Cerexa, Cidara, Cormedix, Cubist, Debiopharm, Dipexium, Dong Wha, Durata, Enteris, Exela, Forest Research Institute, Furiex, Genentech, GSK, Helperby, ICPD, Janssen, Lannett, Longitude, Medpace, Meiji Seika Kasha, Melinta, Merck, Motif, Nabriva, Novartis, Paratek, Pfizer, Pocared, PTC Therapeutics, Rempex, Roche, Salvat, Scynexis, Seachaid, Shionogi, Tetraphase, The Medicines Co., Theravance, ThermoFisher, VenatoRX, Vertex, Wockhardt, Zavante, and some other corporations. Some JMI employees are advisors/consultants for Allergan, Astellas, Cubist, Pfizer, Cempra, and Theravance. With regard to speakers' bureaus and stock options, there are no conflicts of interest to declare.

FUNDING INFORMATION

This study received no specific funding.

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