linezolid, and tigecycline. Although the clinical significance of these findings is unknown, the decline in drug effectiveness against S. aureus infections represents a looming threat to patient health and highlights the possibility of a return to illness and death rates similar to those before antimicrobial drugs were available.

Acknowledgments
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Address for correspondence: Mohit Kumar, Biotechnology and Bioinformatics, NIIT University, Neemrana, Rajasthan-301705, India; email: kumarmohit@yahoo.com

Colistin-Resistant Enterobacteriaceae Carrying the mcr-1 Gene among Patients in Hong Kong


Authors affiliations: Queen Mary Hospital, Hong Kong, China (S.C.Y. Wong, J.H.K. Chen, V.C.C. Cheng); The University of Hong Kong, Hong Kong (H. Tse, P.-L. Ho, K.-Y. Yuen)

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To the Editor: Colistin belongs to the last line of bactericidal antimicrobial drugs active against multidrug-resistant gram-negative bacteria such as carbapenemase-producing Enterobacteriaceae and carbapenem-resistant Acinetobacter baumannii. Consequently, the discovery of the plasmid-mediated colistin-resistant gene mcr-1 in Escherichia coli (1) raises concerns in the medical community because colistin might be useless in treating infections caused by mcr-1–carrying Enterobacteriaceae.

During December 8, 2015–January 8, 2016, we conducted prospective laboratory surveillance of mcr-1–carrying Enterobacteriaceae and Acinetobacter species in a university-affiliated tertiary hospital serving a population of ≈0.53 million in Hong Kong, China. Clinical specimens were processed by using standard operating procedures for different specimen types (2). All Enterobacteriaceae and Acinetobacter spp. isolates were plated onto MH1 agar, which is Mueller-Hinton agar (BD Diagnostics, Sparks, MD, USA), supplemented with 1 µg/mL colistin sulfate (Sigma-Aldrich, St. Louis, MO, USA) for overnight incubation at 37°C in air. Intrinsically colistin-resistant organisms, including Proteus spp., Providencia spp., Serratia spp., and Morganella morganii, were excluded. E. coli ATCC 25922 was used as a negative control. We screened bacteria that grew on MHC1 for mcr-1 by real-time PCR that used specific primers MCR1_22697_F1 (5′-CACCTATGGACCGTCATAGA-3′) and MCR1_22810_R1 (5′-CCCCAGACCATGATACGCAT-3′) and the hydrolysis probe MCR1_22763_Pb1 (FAM-TGGTCTCGG/ZEN/TTTGGCTCGTGTAGGCG-3′ABkFQ) (Integrated DNA Technologies, Coralville, IA, USA). The complete mcr-1 gene found in PCR-positive isolates was amplified and sequenced by specific primers. The colistin MIC of positive isolates was measured by using Etest strips (BioMérieux, Marcy l’Etoile, France). Susceptibility to other antimicrobial drugs was determined by using the Kirby-Bauer disk diffusion method, according to Clinical and Laboratory Standards Institute guidelines (3). We retrieved clinical details of patients whose sample had mcr-1–carrying Enterobacteriaceae from the hospital clinical management system.
A total of 1,324 Enterobacteriaceae and 103 Acinetobacter spp. isolates were screened on MHC1 agar and isolated from blood, urine, stool, or respiratory samples; wound swab specimens; and other sterile and nonsterile body fluids, tissues, or swab specimens. Of the total 1,427 isolates, 62 (4.3%) grew on MHC1: 26 E. coli, 24 Klebsiella spp., 7 Enterobacter spp., 4 Salmonella spp., and 1 Citrobacter sp. Among these 62 isolates, 1 Enterobacter cloacae and 4 E. coli isolates were mcr-1 positive. All gene sequences were 100% identical to that of mcr-1 in E. coli strain SHP45 (GenBank accession no. KP347127), which was isolated from a pig farm specimen in China (1,3). Of the 5 mcr-1-positive isolates, 2 were from blood cultures from patients with biliary tract infection, 1 from a mid-stream urine specimen from a patient with symptomatic urinary tract infection, and 2 from stool specimens from asymptomatic patients. The range of colistin MICs of the 5 mcr-1-positive isolates was 3–64 µg/mL; all were susceptible to carbapenem. One E. coli isolate (from patient 4) exhibited extended-spectrum β-lactamase activity (Table). Patient 3 resided in mainland China before this admission; patient 2 received a liver transplant in China in 2004. None of the 5 patients had a history of colistin treatment.

Finding the mcr-1 gene in 0.4% of Enterobacteriaceae clinical isolates in Hong Kong is expected because of the high proportion of livestock and meat imported from China, where prevalence of colistin-resistant isolates is the 103 (55) Enterobacteriaceae and 103 Acinetobacter spp. isolates were screened on MHC1 agar and isolated from blood, urine, stool, or respiratory samples; wound swab specimens; and other sterile and nonsterile body fluids, tissues, or swab specimens. Of the total 1,427 isolates, 62 (4.3%) grew on MHC1: 26 E. coli, 24 Klebsiella spp., 7 Enterobacter spp., 4 Salmonella spp., and 1 Citrobacter sp. Among these 62 isolates, 1 Enterobacter cloacae and 4 E. coli isolates were mcr-1 positive. All gene sequences were 100% identical to that of mcr-1 in E. coli strain SHP45 (GenBank accession no. KP347127), which was isolated from a pig farm specimen in China (1,3). Of the 5 mcr-1-positive isolates, 2 were from blood cultures from patients with biliary tract infection, 1 from a mid-stream urine specimen from a patient with symptomatic urinary tract infection, and 2 from stool specimens from asymptomatic patients. The range of colistin MICs of the 5 mcr-1-positive isolates was 3–64 µg/mL; all were susceptible to carbapenem. One E. coli isolate (from patient 4) exhibited extended-spectrum β-lactamase activity (Table). Patient 3 resided in mainland China before this admission; patient 2 received a liver transplant in China in 2004. None of the 5 patients had a history of colistin treatment.

Finding the mcr-1 gene in 0.4% of Enterobacteriaceae clinical isolates in Hong Kong is expected because of the high proportion of livestock and meat imported from China, where prevalence of colistin-resistant isolates is the 25.4% and 28.0% in pigs and retail chicken meat, respectively (5,6). Our findings highlight several issues. We noted a wide range (3–64 µg/mL) of colistin MICs in the mcr-1-carrying Enterobacteriaceae; the E. cloacae isolate exhibited the highest MIC. This wide variation in MICs has been noted previously (1,7). Whether the variation results from the differential expression of the mcr-1 gene or from potentially unidentified colistin resistance mechanisms co-existing in some isolates is unknown (8).

Our discovery of the mcr-1 gene in an E. cloacae isolate adds diversity to the Enterobacteriaceae species known to be mcr-1 carriers (e.g., E. coli, Klebsiella pneumoniae, and Salmonella sp.) (1,9). An in vitro study showed transfer of mcr-1-carrying pHNSHP45 (i.e., polymyxin-resistant plasmid) to Pseudomonas aeruginosa (1). Consequently, surveillance for the mcr-1 gene should include all clinically relevant Enterobacteriaceae species, and screening for other gram-negative organisms (e.g., P. aeruginosa) infecting humans should be considered.

We show a potential workflow for screening mcr-1 isolates by sequential use of MHC1 agar and real-time PCR. Clinical and Laboratory Standards Institute guidelines have no recommended colistin breakpoints for Enterobacteriaceae (3); however, the European Committee on Antimicrobial Susceptibility Testing recommends a breakpoint of ≥4 µg/mL to define colistin resistance in Enterobacteriaceae (10). Given that some mcr-1-positive isolates may have a colistin MIC of 2 µg/mL (1,7), which is lower than the

<table>
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<tr>
<th>Table. Clinical details of 5 patients infected with mcr-1-carrying Enterobacteriaceae, Hong Kong*</th>
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<tbody>
<tr>
<td>Patient ID† (age, y)</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>1 (55)</td>
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<tr>
<td>2 (68)</td>
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<td>3 (2)</td>
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<td>4 (57)</td>
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<td>5 (80)</td>
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*ID, identifier; ESBL, extended-spectrum β-lactamase.
†Patient 4 was male; others were female.
‡Routine surveillance of stool samples for multidrug-resistant organisms according to infection control protocol (4).
Among immunocompetent persons, we designed MHC1 with a colistin concentration of only 1 µg/mL to minimize false-negative results. However, some colistin-susceptible organisms might grow on MHC1 (<5% in our study), resulting in the low PCR-positive rate for mcr-1 among isolates.

Exact epidemiology of the mcr-1 gene is unknown, indicating a need to conduct accurate surveillance of the gene’s prevalence in humans. Additional mechanisms unique to the mcr-1 gene may contribute to colistin resistance, suggested by the wide variation in colistin MICs among mcr-1–carrying Enterobacteriaceae.

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References

Cryptococcus gattii
Meningitis Complicated by Listeria monocytogenes Infection

Robert G. Deiss, Michael Bolaris, Angel Wang, Scott G. Filler

Author affiliations: Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA (R.G. Deiss); Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda (R.G. Deiss): Naval Medical Center of San Diego, San Diego, California, USA (R.G. Deiss); Harbor-UCLA Medical Center, Los Angeles, California, USA (M. Bolaris, A. Wang, S.G. Filler); David Geffen School of Medicine at UCLA, Los Angeles (S.G. Filler)

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To the Editor: Among immunocompetent persons with cryptococcal disease, infection with a second organism is rare; all reported cases have involved concomitant mycobacterial infections (1). Immunosuppression is not a necessary precondition for infection with Cryptococcus gattii (2), and among immunocompetent persons, C. gattii infection confers high mortality rates: up to 24% according to a large case series (3). In addition, cryptococcomas are frequently observed in patients with C. gattii, as opposed to C. neoformans, infection, commonly necessitating longer courses of treatment. We report a fatal case of C. gattii and Listeria monocytogenes co-infection in an immunocompetent woman with cryptococcomas.

The patient was a previously healthy 23-year-old Hispanic woman who was hospitalized in 2009 after weeks of headache and recent-onset diplopia. Lumbar puncture revealed elevated opening pressure of 52 cm H₂O; elevated leukocytes (1,030 cells/µL: 31% neutrophils, 55% lymphocytes, 14% monocytes); elevated protein concentration (117 g/L); and decreased glucose concentration (30 mg/dL). Cerebrospinal fluid (CSF) cryptococcal antigen (CrAg) titer was 1:64, and culture grew C. gattii. HIV antibod test result was negative. Magnetic resonance imaging of the brain demonstrated scattered enhancing round lesions within the cerebrum and cerebellum, consistent with cryptococcomas. The patient was prescribed intravenous amphotericin B (1 mg/kg/d) and intravenous fluycytosine (2 g/6 h) (Table); after 5 days of therapy, culture of a repeat lumbar puncture sample was negative. The patient was then given intravenous liposomal amphotericin at 7 mg/kg, and after a 14-day induction period she was discharged with instructions to take fluconazole orally (400 mg 2×/d) and to continue amphotericin B infusions (3×/wk) (Table).

Address for correspondence: Kwok-Yung Yuen, Carol Yu Centre for Infection, The University of Hong Kong, Queen Mary Hospital, Pokfulam Rd, Pokfulam, Hong Kong, China; email: kyyuen@hku.hk