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.

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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 concern 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 MR1_22697_F1 (5′-CAGTATTGTTGACGCGCTATGTA-3′) and MR1_22810_R1 (5′-CCCACACCCAGGACTACCAT-3′) and the hydrolysis probe MR1_22763_Pb1 (FAM-TGGTCTCGG/ ZEN/CTTGTCGGTCTGAGGC-3′IABkFQ) (Integrated DNA Technologies, Coralvile, 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 (Bio-Mérieux, 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 MHCl 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 MHCl: 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 up to 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 coexisting 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 pNHSP45 (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 MHCl 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>
<thead>
<tr>
<th>Patient ID† (age, y)</th>
<th>Underlying conditions</th>
<th>Time from admission to collection of specimen (specimen type)</th>
<th>Antimicrobial drug use &lt;1 mo before isolation</th>
<th>mcr-1–positive species (colistin MIC, μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (55)</td>
<td>Acute myeloid leukemia 4 mo after bone marrow transplant</td>
<td>4 mo (stool sample‡)</td>
<td>Piperacillin/tazobactam, meropenem</td>
<td>Asymptomatic colonization</td>
</tr>
<tr>
<td>2 (68)</td>
<td>Primary sclerosing cholangitis with liver transplant in China in 2004; currently on sirolimus and prednisolone; right hepatectomy in 2008 for right diffuse ischemic bile injury; history of recurrent cholangitis</td>
<td>On admission with sepsis workup for biliary sepsis resulting from biliary anastomotic stricture (blood culture)</td>
<td>None</td>
<td>Recovered</td>
</tr>
<tr>
<td>3 (2)</td>
<td>Autologous bone marrow transplant for stage IV neuroblastoma</td>
<td>14 d (stool sample‡)</td>
<td>Piperacillin/tazobactam</td>
<td>Asymptomatic colonization</td>
</tr>
<tr>
<td>4 (57)</td>
<td>Hepatitis B virus–related hepatocellular carcinoma; recurrent pyogenic cholangitis; recurrent biliary sepsis with portal vein thrombosis; cerebellar stroke in 2013</td>
<td>On admission with sepsis workup for biliary sepsis resulting from biliary stricture and recent transarterial chemoembolization (blood culture)</td>
<td>None</td>
<td>Recovered</td>
</tr>
<tr>
<td>5 (80)</td>
<td>Duke’s B carcinoma of rectum [lower anterior resection in 1996]; carcinoma of thyroid [post-thyroidectomy]; hypertension; diabetes mellitus</td>
<td>On admission with sepsis workup for symptomatic urinary tract infection (mid-stream urine sample)</td>
<td>None</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

†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, C. gattii 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 antibody 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 flucytosine (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).

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.