tests from Panama City versus other areas of Panama and might result in a sampling bias. Despite these limitations, the recent Zika outbreak has shown the speed at which vectorborne diseases can spread and highlights the importance of detecting emerging viruses like PTVs.

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References
1. Armien B, Pascale JM, Munoz C, Lee SJ, Choi KL, Avila M, et al. Incidence rate for hantavirus infections without pulmonary syndrome, Panama. Emerg Infect Dis. 2011;17:1936–9. http://dx.doi.org/10.3201/eid1710.101717
2. Fauci AS, Morens DM. Zika virus in the Americas—yet another arbovirus threat. N Engl J Med. 2016;374:601–4. http://dx.doi.org/10.1056/NEJMp1600297
3. Tesh RB, Chaniotis BN, Peralta PH, Johnson KM. Ecology of viruses isolated from Panamanian phlebotomine sandflies. Am J Trop Med Hyg. 1974;23:258–69.
4. Palacios G, Wiley MR, Travassos da Rosa AP, Guzman H, Quiroz E, Sayji N, et al. Characterization of the Punta Toro species complex (genus Phlebovirus, family Bunyaviridae). J Gen Virol. 2015;96:2079–85. http://dx.doi.org/10.1099/vir.0.000170
5. Xu F, Chen H, Travassos da Rosa AP, Tesh RB, Xiao SY. Phylogenetic relationships among sandfly fever group viruses (Phlebovirus: Bunyaviridae) based on the small genome segment. J Gen Virol. 2007;88:2312–9. http://dx.doi.org/10.1099/vir.0.82860-0
6. Liu DY, Tesh RB, Travassos Da Rosa AP, Peters CJ, Yang Z, Guzman H, et al. Phylogenetic relationships among members of the genus Phlebovirus (Bunyaviridae) based on partial M segment sequence analyses. J Gen Virol. 2003;84:465–73. http://dx.doi.org/10.1099/vir.0.18765-0
7. Sánchez-Seco MP, Echevarría JM, Hernández L, Estévez D, Navarro-Mari JM, Tenorio A. Detection and identification of Toscana and other phleboviruses by RT–nested-PCR assays with degenerated primers. J Med Virol. 2003;71:140–9. http://dx.doi.org/10.1002/jmv.10465
8. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4. http://dx.doi.org/10.1093/molbev/msw054
9. Valderrama A, Tavares MG, Andrade Filho JD. Anthropogenic influence on the distribution, abundance and diversity of sandfly species (Diptera: Phlebotominae: Psychodidae), vectors of cutaneous leishmaniasis in Panama. Mem Inst Oswaldo Cruz. 2011;106:1024–31. http://dx.doi.org/10.1590/S0074-02762011000800021

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mcr-1 Colistin Resistance in ESBL-Producing Klebsiella pneumoniae, France

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We report intestinal carriage of an extended-spectrum β-lactamase–producing Klebsiella pneumoniae strain with high-level resistance to colistin (MIC 24 mg/L) in a patient in France who had been hospitalized for fungal meningitis. The strain had the mcr-1 plasmid gene and an inactivated mgrB gene, which are associated with colistin resistance.
Resistance to colistin in gram-negative bacteria stems mainly from structural modifications of bacterial lipopolysaccharide. These modifications include addition of 4-amino-4-deoxy-L-arabinose or phosphoethanolamine caused by chromosomal mutations in genes encoding the 2-component systems PhoPQ and PmrAB, or mutations in the mcr-1 gene, a negative regulator of PhoPQ (1).

The recent discovery of a horizontally transferable plasmid-mediated mcr-1 gene encoding a phosphoethanolamine transferase is a cause for concern, but few mcr-1–positive clinical strains of Klebsiella pneumoniae have been reported so far in Europe (2). Colocalization of carbapenemases or extended-spectrum β-lactamase (ESBL) genes and the mcr-1 gene on the same plasmids is of concern because it might lead to pandrug resistance (1,3). We report mcr-1 colistin resistance in ESBL-producing K. pneumoniae isolated from a patient in France.

The patient was a 38-year-old man who had chronic granulomatous disease that was diagnosed when he was 8 months old. Since then, he has had several minor and major diseases and conditions, including primitive femoral osteitis, hepatic abscesses, disseminated candidiasis, and bacteremia, which required several treatments with antimicrobial drugs. However, the patient was never given colistin.

In April 2016, he was hospitalized for surgical removal of a thyroid abscess. Fungal cultures of the abscess grew Aspergillus fumigatus. Despite antifungal treatment with amphotericin B and flucytosine, fungal meningitis, cerebral abscesses, disseminated candidiasis, and bacteremia, which required several treatments with antimicrobial drugs. However, the patient was never given colistin.

In August 2016, systematic culture of a rectal swab specimen showed an ESBL-producing strain of K. pneumoniae; 2 previous rectal screenings showed negative results. The strain was resistant to colistin (MIC >4 mg/L). Resistance was determined by using a broth microdilution assay (BD Phoenix Instrument; Becton Dickinson, Franklin Lakes, NJ, USA) but not on a high colistin concentration or the presence of vancomycin, which can increase in medium might be related to a high colistin concentration or the presence of vancomycin. Lack of colistin resistance can be confirmed within 2 h by using an in-house rapid polymyxin Nordmann-Poirel test (3,4). The mcr-1 gene can be rapidly detected by real-time polymerase chain reaction (PCR) of DNA extracts obtained from bacterial strains or directly from stool samples (2,5,6).

We obtained subcultures of the strain from the patient on Columbia CNA agar containing 10 mg/L of colistin and 15 mg/L of nalidixic acid and 5% sheep blood (CNA; bioMérieux) but not on Thayer-Martin agar medium containing unknown concentrations of vancomycin, colistin, amphotericin B, and trimethoprim (VCA3; bioMérieux). Lack of growth on this medium might be related to a high colistin concentration or the presence of vancomycin, which can potentiate colistin activity (6). Further investigations using the E-test method (Polymyxin B E-test strip; bioMérieux, Marcy l’Etoile, France).

The strain was sent to the French National Reference Center for Antibiotic Resistance in Enterobacteriaceae (Hôpital Gabriel Montpied, Clermont-Ferrand, France), which confirmed phenotypic resistance to colistin and identified the mcr-1 gene by using PCR and previously described primers (2). Whole-genome sequencing showed that the K. pneumoniae strain had genotype ST15 and confirmed the presence of the mcr-1 gene on a 33,303-kb transferable plasmid of incompatibility group IncX4 (online Technical Appendix). This plasmid differed by only 4 mutations from mcr-1.2–encoding plasmid pMCR-1.2.IT (GenBank accession no. KX236309) previously characterized in Italy (4). Conjigation of the plasmid into Escherichia coli K12 conferred colistin resistance (MIC increased from 0.25 mg/L to 4 mg/L) to the E. coli strain.

Other resistance genes were also identified (Table), including the ESBL-encoding gene bla_SHV-106 (online Technical Appendix). None of them were localized with the mcr-1 gene on the IncX4 plasmid. Moreover, insertion of mobile element IS5 in the mgrB gene was detected, which is also associated with colistin resistance (5). No mutations were found in the prmA, pmrB, phoP, and phoQ genes.

There is currently no commercial medium to screen gram-negative bacteria harboring the mcr-1 gene. Nordmann et al. (6) described an in-house SuperPolymyxin medium composed of eosin methylene blue agar, 3.5 mg/L of colistin sulfate, 10 mg/L of daptomycin, and 5 mg/L amphotericin B, which showed excellent sensitivity and specificity. Colistin resistance can be confirmed within 2 h by using an in-house rapid polymyxin Nordmann-Poirel test (7). The mcr-1 gene can be rapidly detected by real-time PCR of DNA extracts obtained from bacterial strains or directly from stool samples (2,5,6).

We obtained subcultures of the strain from the patient on Columbia CNA agar containing 10 mg/L of colistin and 15 mg/L of nalidixic acid and 5% sheep blood (CNA; bioMérieux) but not on Thayer-Martin agar medium containing unknown concentrations of vancomycin, colistin, amphotericin B, and trimethoprim (VCA3; bioMérieux). Lack of growth on this medium might be related to a high colistin concentration or the presence of vancomycin, which can potentiate colistin activity (6). Further investigations using

| Resistance gene | Target antimicrobial drug |
|-----------------|--------------------------|
| mcr-1 and inactivation of mgrB by IS5 insertion | Colistin |
| bla_SHV-106     | β-lactams                |
| aac(3)-IId and aadA16-like | Aminoglycoside |
| aac(6′)-Ib-cr   | Quinolone and aminoglycoside |
| fosA5           | Fosfomycin               |
| sulI and folP   | Sulfonamide              |
| dfrA27          | Trimethoprim             |
| tetD            | Tetracycline             |

*ESBL, extended-spectrum β-lactamase.*
CNA+ medium did not identify intestinal carriage of ESBL-negative but mcr-1–positive enterobacteria in the index case-patient. On the basis of these results, rectal screening of 39 contacts was performed by using an ESBL-screening medium (BLSE agar [MacConkey agar and Drigalski agar]; bioMérieux). All of the tests showed negative results.

The origin of the mcr-1 strain remains unknown. Nosocomial acquisition cannot be ruled out because colistin-resistant strains harboring the mcr-1 gene might have been isolated in the hospital but not identified because this resistance mechanism was initially reported in February 2016. Food might also be incriminated (I); one study identified a 21% mcr-1 prevalence among ESBL-producing E. coli in calves in France (10).

Multiple antimicrobial drug therapy for this patient might have selected for this multidrug-resistant bacteria. The presence of a plasmid containing the mcr-1 and ESBL or other resistance genes in the same strain might be involved in selection of colistin-resistant strains during administration of any ineffective antimicrobial drug (3). Development of efficient tools for rapid detection of mcr-1–harboring strains should be a priority to prevent dissemination of these strains in hospital settings.

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References
1. Baron S, Hadjadj L, Rolain J-M, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents. 2016;48:583–91. http://dx.doi.org/10.1016/j.ijantimicag.2016.06.023
2. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16:161–8. http://dx.doi.org/10.1016/S1473-3099(15)00424-7
3. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. J Antimicrob Chemother. 2016;71:2066–70. http://dx.doi.org/10.1093/jac/dkw274
4. Di Pilato V, Arena F, Tascini C, Cannatelli A, Henriec De Angelis L, Fortunato S, et al. mcr-1.2, a new mcr variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing Klebsiella pneumoniae strain of sequence type 512. Antimicrob Agents Chemother. 2016;60:5612–5. http://dx.doi.org/10.1128/AAC.01075-16
5. Cannatelli A, D’Andrea MM, Giani T, Di Pilato V, Arena F, Ambretti S, et al. In vivo emergence of colistin resistance in Klebsiella pneumoniae producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP mgrB regulator. Antimicrob Agents Chemother. 2013;57:5521–6. http://dx.doi.org/10.1128/AAC.01480-13
6. Nordmann P, Jajou L, Poirel L. A universal culture medium for screening polymyxin-resistant gram-negative isolates. J Clin Microbiol. 2016;54:1395–9. http://dx.doi.org/10.1128/JCM.00446-16
7. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in Enterobacteriaceae. Emerg Infect Dis. 2016;22:1038–43. http://dx.doi.org/10.3201/eid2206.151840
8. Nijhuis RH, Veldman KT, Scheflaut J, Van Essen-Zandbergen A, Wessels E, Claas EC, et al. Detection of the plasmid-mediated colistin-resistance gene mcr-1 in clinical isolates and stool specimens obtained from hospitalized patients using a newly developed real-time PCR assay. J Antimicrob Chemother. 2016;71:2344–6. http://dx.doi.org/10.1093/jac/dkw192
9. Bontron S, Poirel L, Nordmann P. Real-time PCR for detection of plasmid-mediated polymyxin resistance (mcr-1) from cultured bacteria and stools. J Antimicrob Chemother. 2016;71:2318–20. http://dx.doi.org/10.1093/jac/dkw139
10. Haenni M, Météayer V, Gay E, Madec J-Y. Increasing trends in mcr-1 prevalence among ESBL-producing E. coli in French calves despite decreasing exposure to colistin. Antimicrob Agents Chemother. 2016;60:6433–4. http://dx.doi.org/10.1128/AAC.01147-16

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Chromosomal 16S Ribosomal RNA Methyltransferase RmtE1 in Escherichia coli Sequence Type 448

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We identified rmtE1, an uncommon 16S ribosomal methyltransferase gene, in an aminoglycoside- and cephalosporin-resistant Escherichia coli sequence type 448 clinical strain co-harboring blaKPC-2. Long-read sequencing revealed insertion of a 101,257-bp fragment carrying both resistance genes to the chromosome. Our findings underscore E. coli sequence type 448 as a potential high-risk multidrug-resistant clone.

RmtE (RmtE1 and its variant RmtE2) is an uncommon plasmid-mediated 16S rRNA methyltransferase (16S RMtase) found in gram-negative bacteria; only 4 strains have been reported to produce RmtE, all Escherichia coli, including 1 from the University of Pittsburgh Medical
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Technical Appendix

Sequencing of the Klebsiella pneumoniae Strain Genome and Plasmid Carrying the mcr-1 Gene

We used a whole-genome sequencing method (Illumina, San Diego, CA, USA) with 50-bp paired and 60× coverage. Gaps in the plasmid carrying the mcr-1 gene were filled by using PCR and Sanger sequencing.

Characteristics of the SHV-106 Plasmid

Whole-genome sequencing identified a 57-kb plasmid that belonged to incompatibility group IncR and carried the bla\textsubscript{SHV-106} gene. Genomic data were confirmed by extraction of plasmids according to the method of Kado and Liu (1) and hybridization with SHV and IncR probes.

Antimicrobial Drug Susceptibilities of the K. pneumoniae Strain

Antimicrobial drug susceptibilities were determined by using the BD Phoenix Instrument (Becton Dickinson, Franklin Lakes, NJ, USA). The strain showed susceptibility to amoxicillin/clavulanate (MIC 8/2 mg/L), piperacillin/tazobactam (≤4/4 mg/L), temocillin (8 mg/L), cefoxitin (≤4 mg/L), cefepime (≤1 mg/L), aztreonam (≤1 mg/L), ertapenem (≤0.25 mg/L), imipenem (≤0.25 mg/L), meropenem (≤0.125 mg/L), amikacin (≤4 mg/L), tigecycline (1 mg/L), and fosfomycin (32 mg/L); intermediate susceptibility to ticarcillin/clavulanate (16/2 mg/L) and ceftazidime (2 mg/L); and resistance to ampicillin (>8 mg/L), piperacillin (>64 mg/L), ceftriaxone (4 mg/L), tobramycin (>4 mg/L), gentamicin (>4 mg/L), nalidixic acid (>16 mg/L), ciprofloxacin (>1 mg/L), levofloxacin (>2 mg/L), norfloxacin (>2 mg/L), colistin (>4 mg/L), and trimethoprim/sulfamethoxazole (>4/76 mg/L).

Reference

1. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol. 1981;145:1365–73. PubMed