The chimeric nature of the virus strain was confirmed by RT-PCR with primers spanning possible recombination sites and analysis of overlapping reads from next-generation sequencing.

Annotation of the sequence of SeCoV/GER/L00930/2012 performed on the basis of SeCoV/Italy/213306/2009 identified a similar putative coding sequence with a TGEV backbone and a spike coding sequence similar to that for PEDV (online Technical Appendix panel B). Downstream of the spike protein–coding open reading frame (ORF), an additional hypothetical ORF was identified in both SeCoV sequences. The coded amino acid sequences (27 aa in the virus from Germany and 30 aa in the virus from Italy) resembled an N- and C-terminally truncated TGEV nonstructural protein 3a. The difference of 3 aa between the 2 strains is the result of a 10-bp deletion at the 3′-end of the hypothetical ORF, which shifted the stop 3 codons to the 5′-end (online Technical Appendix Figure, panel B) in SeCoV/GER/L00930/2012. This deletion is apparently located within the potential 3′ recombination site (online Technical Appendix Figure, panel B).

It is tempting to speculate that SeCoV/Italy/213306/2009 is a precursor of SeCoV/GER/L00930/2012, and that other members of this novel genotype are still undetected. These viruses might be targets of secondary mutation and recombination events. Therefore, more chimeric CoVs should be identified to determine the potential origin of the recombination event.

In conclusion, we detected an enteric CoV that resembled the TGEV/PEDV chimeric virus reported by Boniotti et al. (1). Although these findings support the notion that CoV genomes are subject to mutations and recombination events, problems in disease diagnosis can be foreseen. In countries where porcine epidemic diarrhea, transmissible gastroenteritis, or both of these diseases are reportable, correct diagnosis and reporting might be difficult. Thus, diagnosticians should be aware of possible recombinants of swine CoVs. Diagnostic problems can be prevented by use of a double-check strategy with techniques specific for different genome regions. Apart from diagnostic obstacles, the effect of virus recombinations in terms of virulence and organ tropism is unknown and needs further investigations.

References

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To the Editor: Colistin is an old-generation antimicrobial agent; however, because it is one of the few agents that remain effective against multidrug-resistant gram-negative bacteria (e.g., carbapenem-resistant Pseudomonas aeruginosa and Enterobacteriaceae), its clinical usefulness is being increasingly recognized (1). Previous reports have described the mechanisms of colistin resistance (2) as being chromosomally mediated and not associated with horizontal gene transfer. However, from 2011 through 2014, a plasmid-encoded colistin-resistance gene, mcr-1, was identified in colistin-resistant Escherichia coli isolated in

Figure. Electron micrograph of a new chimeric swine enteric coronavirus (SeCoV/GER/L00930/2012), Germany, 2012. Scale bar indicates 100 nm.
China, particularly from animals. Specifically, mcr-1–positive isolates were found in 21% of healthy swine at slaughter, 15% of marketed pork and chicken meat, and 1% of hospitalized human patients (3). A study of E. coli isolated from healthy cattle, swine, and chickens in Japan during 2000–2014 found only 2 (0.02%) of 9,308 isolates positive for mcr-1 (4). We report the rates at which mcr-1 was detected in our stored collection of E. coli isolates from diseased swine (swine with diarrhea or edema disease), hereafter referred to as swine-pathogenic E. coli.

We recently analyzed swine-pathogenic E. coli strains isolated from diseased swine throughout Japan during 1991–2014 (5). We analyzed all swine disease-associated E. coli strains isolated from the 23 Livestock Hygiene Service Centers in Japan (including prefectures that covered 75% of total swine production in Japan in 2014) and sent to the National Institute of Animal Health for diagnostic purposes during 1991–2014. Among the 967 strains examined, 684 (71%) belonged to E. coli serogroup O139, O149, O116, or OSB9.

In the study reported here, we investigated these 684 strains for susceptibility to colistin and for mcr-1 carriage. The strains from the 4 predominant serogroups (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/7/16-0234-Techapp1.pdf) can be considered representative of swine-pathogenic E. coli strains isolated from farm animals, but not food products, in Japan. MICs were determined by using the agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (6). The presence of mcr-1 was detected by PCR (3).

Among the 684 strains examined, colistin MICs exhibited a bimodal distribution of 0.25–128 µg/mL and peaked at 0.5 and 16 µg/mL (online Technical Appendix Figure). According to the European Committee on Antimicrobial Susceptibility Testing criterion (7), in which isolates with an MIC of ≥4 µg/mL are considered colistin resistant, 309 (45%) of the 684 strains were classified as colistin resistant. The gene mcr-1 was detected in 90 (13%) strains, and the MICs for these mcr-1–positive strains ranged from 8 to 128 µg/mL (online Technical Appendix Figure). Among the 309 colistin-resistant strains, mcr-1–positive and mcr-1–negative isolates had the same 50% and 90% MICs, 16 and 32 µg/mL, respectively. These results indicate that a high proportion of swine-pathogenic E. coli in Japan are resistant to colistin, that mcr-1 has already been widely disseminated among these strains, and that the level of colistin resistance mediated by mcr-1 is similar to that mediated by mcr-1–independent mechanisms.

In 2004, colistin-resistant E. coli already represented 77% of the isolates, and the positivity rates varied from year to year (26%–82%) (Figure). First detection of mcr-1–positive strains was in 2007, and the proportion of mcr-1 positivity has risen, especially since 2009 (Figure). During 2013–2014, approximately half of the strains isolated were mcr-1 positive (Figure), and most colistin-resistant strains isolated during these 2 years carried mcr-1 (85% and 62% in 2013 and 2014, respectively). Of note, the rates of mcr-1–positive strains among the 4 serogroups isolated from 2010 through 2014 did not differ significantly (χ² test): 22 (20%) of 110 in O139, 38 (38%) of 100 in O149, 19 (26%) of 73 in O116, and 6 (32%) of 19 in OSB9. This finding suggests that the sharp rise in the proportion of mcr-1–positive strains has been driven by plasmid-mediated horizontal gene transfer, not by the expansion of a specific clone.

In Japan, rates of isolation of colistin-resistant and mcr-1–positive E. coli strains from healthy animals are low, 1.00% and 0.02% of 9,308 strains examined, respectively (4). These low rates may be the result of the prudent use of colistin in Japan. During 2000–2007 in Japan, colistin use in swine did not increase significantly (8). However, our data show that mcr-1 has recently been disseminated among swine-pathogenic E. coli in Japan, which might be associated with the use of colistin to treat disease in swine. Although mcr-1–positive bacteria have not yet been isolated from humans in Japan (4), the sharp increase in swine-pathogenic E. coli in animal strains implies a risk for transmission of mcr-1 from these strains to human-pathogenic bacteria, a serious concern for human medicine. More active surveillance of mcr-1–positive colistin-resistant bacteria in human and animal environments is needed.

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To the Editor: Yellow fever is disease caused by a flavivirus that is transmitted to humans and nonhuman primates through the bites of infected mosquitoes. In 2013, an estimated 130,000 persons in Africa experienced fever with jaundice or hemorrhage associated with yellow fever; ≈78,000 of these infections were fatal (1).

Recently, an outbreak of yellow fever was reported in Angola (2). This serious viral disease affects persons living in and visiting tropical regions of Africa and Central and South America (3). No case of yellow fever had been confirmed in China until this year (3). With the increased population movement between Africa and China, the risk for yellow fever in China is increasing.

In March 2016, a 34-year-old man who had recently returned to China from Angola sought medical treatment at the Shanghai Public Health Clinical Center in Shanghai, China. He reported a 4-day history of malaise, myalgia, weakness, nausea, vomiting, and fever reaching 38.8°C. The patient had been treated with several antimicrobial drugs when he was in Angola, but symptoms did not resolve. He had no history of immunodeficiency or immune-inhibitory drug use. No endocrine, metabolic, or autoimmune abnormalities were found.

Nine years earlier, the patient had undergone cardiac valve replacement for rheumatoid heart disease and was currently receiving warfarin therapy. Because his treating physicians were concerned about the potential effect of yellow fever vaccine on the patient’s international normalized ratio (ratio of reference to measured prothrombin times), the patient traveled to Africa for work without receiving vaccination for yellow fever.

Physical examination revealed a temperature of 37°C. Neither rash nor jaundice were evident. Blood examination revealed a low leukocyte count (1.66 × 10^9 cells/L), a low platelet count (43 × 10^9 cells/L), a low absolute lymphocyte count (0.87 × 10^9 cells/L), and direct bilirubin was 7.4 μmol/L (0.5–17 μmol/L). The patient had normal levels of circulating CD3+ cells (540/mL [690–2,540/mL]), CD8+ cells (97/mL [190–1,140/mL]), and normal levels of CD4+ T-cells.

C-reactive protein level was 4.31 mg/L (reference range 0.0–3.0 mg/L), lactate dehydrogenase was 1,086 U/L (109–245 U/L), alanine aminotransferase was 882 U/L (7–40 U/L), total bilirubin was 13.5 μmol/L (0–17 μmol/L), and direct bilirubin was 7.4 μmol/L (0–5.4 μmol/L). The patient had normal levels of thyroid-stimulating hormone, and no DNA, nuclear, or thyroglobulin antibodies were detected.

Test results for HIV, malaria, and dengue virus infection were negative. Serum and urine samples were positive for yellow fever virus and negative for dengue and...