evolutionarily distinct from other AIVs (*5*). This H5N5 strain is a contemporary reassortant virus related to North American and Eurasian strains.

The positive animals we identified originated from a single location on the Antarctic Peninsula, which suggests recent introduction of this AIV H5N5 in the colonies sampled. Antarctica is refuge for most penguin colonies, including the near-threatened emperor penguins. Previous reports suggested that AIV could have caused Adélie penguin chick death (*3*). Four positive samples (including the sequenced virus) were obtained from juvenile chinstrap penguins that were weak, depressed, and possibly ill (i.e., they had ruffled feathers, lethargy, and impaired movement). Thus, additional studies are warranted to assess the health and conservation status of resident bird species and potential pathologic effects of AIV.

These data provide novel insights on the ecology of AIV in Antarctica. Our findings also highlight the need for increased surveillance to understand virus diversity on this continent and its potential contribution to the genetic constellation of AIV in the Americas.

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Pathogenic Lineage of *mcr*-Negative Colistin-Resistant *Escherichia coli*, Japan, 2008–2015

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To the Editor: Colistin is a last-line drug for treatment of multidrug-resistant, gram-negative bacterial infections, including those caused by *Escherichia coli*. We report colistin-resistant *E. coli* isolates from Japan, including a global-spreading pathogenic lineage, serotype O25b:H4, sequence type (ST) 131, and subclone *H*30-R (O25b:H4-ST131-*H*30R).

We tested 514 *E. coli* isolates obtained from clinical specimens taken at Sapporo Clinical Laboratory Inc. (Sapporo, Japan) and Sapporo Medical University Hospital in Japan during 2008–2009 (*1*) and 2015, respectively. Samples were processed according to Clinical and Laboratory Standards Institute guidelines (*2*). Identification of O25b:H4-ST131, O25b, H4, and ST131 were determined as described previously (*1*). For identification of the *H*30Rx subclone of O25b:H4-ST131, *H*30 was determined by PCR using a specific primer set (*3*), R was determined according to ciprofloxacin MIC, and x was determined by detecting 2 single-nucleotide polymorphisms, as previously described (*4*).

Four *E. coli* isolates exceeded the colistin resistance breakpoint (>2 mg/mL) (Table). None of the patients from whom the *E. coli* isolates were derived had a history of colistin treatment. Three of the 4 colistin-resistant isolates belonged to a pandemic lineage, O25b:H4-ST131-*H*30R, which has been isolated from urinary tract and bloodstream infections (*3*,*4*). The frequency of colistin-resistant ST131 *E. coli* isolates among O25b:H4-ST131 was 2.2%. This lineage is fluoroquinolone resistant and is frequently resistant to β-lactams because it possesses CTX-M–type extended-spectrum β-lactamase genes (*1*,*3*,*4*).

The colistin-resistant isolates reported were resistant to fluoroquinolones, and 1 (SME296) was resistant to cephalosporins (due to expression of $bla_{\text{CTX-M-14}}$). Another colistin-resistant *E. coli* isolate (SME222) belonged to O18-ST416, which is also known as an extraintestinal pathogenic *E. coli* (*5*), although this lineage has not previously been reported to exhibit colistin resistance.

The colistin-resistant *E. coli* isolates we identified were sensitive to carbapenems and aminoglycosides, including amikacin, whereas previously it was reported that some *E. coli* ST131 isolates exhibited resistance to carbapenems by possessing carbapenemases, such as NDM-1 and KPC-2; the NDM-1–possessing ST131 isolate also exhibited resistance to amikacin (*6*,*7*). Thus, these findings may affect future antimicrobial choices because of the clonal dominance, multidrug resistance, and pathogenicity of the isolates.

Recent studies reported a plasmid-mediated colistin resistance gene, *mcr-1*, in various countries (*8*). In addition, a novel plasmid-mediated colistin resistance gene, *mcr-2* (76.7% nucleotide identity to *mcr-1*), was found in *E. coli* isolates in Belgium (*9*). These genes encode a phosphoethanolamine transferase family protein, which modifies the lipid A component of lipopolysaccharide (*8*,*9*). The colistin-resistant *E. coli* isolates we identified did not possess *mcr-1*or *mcr-2*, although the MICs for colistin were the same as or higher than that of the transconjugant of a *mcr-1*–harboring plasmid in an *E. coli* ST131 isolate (4 mg/L) reported by Liu et al. (*8*). Thus, these colistin-resistant isolates may have other colistin resistance mechanisms. For example, modification of lipid A with 4-amino-4-deoxy-L-arabinose or phosphoethanolamine, caused by chromosomal mutations in *mgrB*, *phoPQ*, and *pmrAB* genes, might occur and could be responsible for the resistance. This polymyxin-resistance mechanism is seen in *Enterobacteriaceae*; however, other novel mechanisms are also conceivable.

In conclusion, we report colistin resistance in a major global-spreading extraintestinal pathogenic *E. coli* strain, O25b:H4-ST131-*H*30R, in Japan. This strain acquired colistin resistance without carrying a plasmid bearing the *mcr* gene. Clarifying the colistin-resistance mechanisms in these isolates is necessary if we are to forestall the emergence of multidrug (including

Table. Characterization of colistin-resistant *Escherichia coli* isolates, Japan, 2008–2105*

Strain	Specimen type	Patient age, y/sex	Year	Serotype	ST	MIC, mg/L†									
						PIP	CAZ	CPD	FEP	IPM	GEN	AMK	CIP	CST	PMB
SRE34	Urine catheter	UNK/F	2008	O25b:H4	131-H30Rx	128 (R)	1 (S)	1 (S)	0.06 (S)	0.12 (S)	0.5 (S)	2 (S)	32 (R)	16 (R), 16 (R)‡	8, 8‡
SRE44	Urine catheter	UNK/M	2008	O25b:H4	131-H30Rx	64 (R)	2 (S)	1 (S)	0.13 (S)	0.25 (S)	0.5 (S)	1 (S)	64 (R)	16 (R), 16 (R)‡	16, 8‡
SME222	Indwelling pericardial drain	76/M	2015	O18	416	2 (S)	0.5 (S)	0.5 (S)	<0.03 (S)	0.13 (S)	0.5 (S)	2 (S)	0.03 (S)	4 (R), 4 (R)‡	8, 4‡
SME296	Urine	67/M	2015	O25b:H4	131-H30R	>128 (R)	32 (R)	>128 (R)	16 (R)	0.13 (S)	0.5 (S)	2 (S)	64 (R)	4 (R), 8 (R)‡	1, 4‡

*All isolates were phylogentic group B2. AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CPD, cefpodoxime; FEP, cefepime; CST, colistin; GEN, gentamicin; IPM, imipenem; PIP, piperacillin; PMB, polymyxin B; R, resistant; S, susceptible; ST, sequence type; UNK, unknown.
†EUCAST (http://www.eucast.org/) breakpoints were used for resistance determination because the colistin breakpoint for *E. coli* was undetermined by the Clinical and Laboratory Standards Institute. MICs were determined by the agar dilution method unless otherwise stated. Breakpoints: PIP, >16; CAZ, >16; CPD, >1; FEP, >4; IPM, >8; GEN, >4; AMK, >16; CIP, >1; CST, >2.
‡Broth microdilution method.

colistin)–resistant O25b:H4-ST131-*H*30R. The worst-case scenario is the global spread of this isolate, which has acquired resistance to the last-line antimicrobial drug, colistin.

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Dual Emergence of Usutu Virus in Common Blackbirds, Eastern France, 2015

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To the Editor: Usutu virus (USUV) is a mosquito-borne flavivirus amplified in an enzootic cycle involving passeriform and strigiform birds as reservoir hosts and *Culex* mosquitos as vectors (*1*). Although originating from Africa, USUV has been introduced at least twice into central and western Europe, leading to substantial bird fatalities in central Europe (particularly in Austria, Hungary, Italy, Germany, and Switzerland) since 1996 (*2*). Its zoonotic potential has been recently highlighted in Italy in immunosuppressed patients who sought treatment for encephalitis (*3*).

Even though every country bordering France, apart from Luxembourg, has reported USUV in mosquitoes or wild birds recently, USUV outbreaks had not been reported in France, and only indirect evidence indicated circulation of USUV-like viruses in Eurasian magpies (*Pica pica*) in southeastern France (*4*). In 2015, the French event-based surveillance network SAGIR (*5*) reported increased fatalities of common blackbirds (*Turdus merula*) in 2 departments in eastern France, Haut-Rhin near the German border and Rhône (Figure). Five birds, 2 in Haut-Rhin and 3 in Rhône, were subjected to molecular detection for flaviviruses. During necropsy, their brains, hearts, livers, and kidneys (from 2 birds only) were sampled for RNA extraction and virus isolation. Tissues were homogenized in DMEM with ceramic beads (Qbiogen) and FastPrep ribolyzer (ThermoSavant). Total RNA was extracted with RNeasy kit (Qiagen) and flavivirus genomic RNA was amplified by conventional reverse transcription PCR with all of the tissues from 2 birds in Haut-Rhin that were found dead on August 5–10, 2015, and from 1 bird sampled on September 23 in Rhône (*6*). USUV was systematically identified in blackbird tissues