

**Table.** Neutralizing antibody titers against TBEV and JEV among members of the Japan Self-Defense Forces screened in early 2017\*

Patient age, y	Received tick bite in previous 10 y	Antibody titer†	
		TBEV	JEV
42	3 times	80	<20
48	1 time	40	<20

\*JEV, Japanese encephalitis virus; TBEV, tickborne encephalitis virus.

†Neutralizing titer was defined as the reciprocal of the highest dilution of serum.

These 2 unrecognized subclinical TBEV infections were serologically diagnosed, demonstrating that humans who are particularly at risk for tick bites are partly asymptotically infected with TBEV in Hokkaido. Because flaviviruses are known to serologically cross-react with other close flaviviruses (8), we tested serum against JEV, the only other endemic flavivirus in Japan, and successfully excluded its possibility. The antibody titer was lower than that in persons with clinically apparent cases (e.g., >1,600), perhaps because the virus replication was limited among subclinical cases or antibody had decayed since infection.

Our findings echo similar cross-sectional survey results among persons recently bitten by ticks in Xinjiang and Inner Mongolia, China (9). Although the estimated frequency in Japan was as low as 0.7%, this figure should not be regarded as small, considering that >30,000 persons serve in the Northern Army. In addition, frequently bitten persons include not only JSDF members but also dairy farmers, foresters, and hikers. Seroepidemiologic survey with greater sample size and broader scope of study participants are needed to identify persons at high risk for infection and determine the pros and cons of specific countermeasures, including vaccination (10). Such surveys also are needed to measure the virulence of TBEV of the so-called Far-Eastern subtype because the detection of subclinical or mild cases may lead to an overall decrease in its case-fatality risk, which is perceived as high (1).

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## ***bla*<sub>CTX-M-27</sub>-Encoding *Escherichia coli* Sequence Type 131 Lineage C1-M27 Clone in Clinical Isolates, Germany**

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We examined extended-spectrum  $\beta$ -lactamase-producing isolates from livestock, humans, companion animals, food, and the environment during 2009–2016 in Germany for the presence of CTX-M-27 allele within *Escherichia coli* sequence type (ST) 131. *E. coli* ST131 C1-M27 was exclusively present in humans; its incidence increased from 0% in 2009 to 45% in 2016.

During the past 20 years, *Escherichia coli* sequence type (ST) 131 has emerged as a prevalent vehicle for extended-spectrum  $\beta$ -lactamases (ESBL) worldwide. Particularly prevalent are isolates of the clade ST131 C/H30R, which frequently are associated with urinary tract infections and bacteremia (1,2). Although the ESBL production of the predominant subgroup ST131 C2/H30Rx is conferred by the CTX-M-15 allele, the emerging subgroup C1 often is associated with other CTX-M alleles, such as CTX-M-14 and CTX-M-27 (3). An increase in C1/H30R ST131 isolates was initially reported among clinical isolates in Japan; most of those were identified as members of the recently defined clade C1-M27 (90% of C1/H30R) (3). More recently, a dramatic rise from 0% to 65% in the incidence of ST131 C1/H30R *bla*<sub>CTX-M-27</sub> isolates in the fecal carriage of children in France during 2010–2015 was reported (4). In addition, ST131 isolates harboring *bla*<sub>CTX-M-27</sub> have been reported sporadically from other countries (3). We examined ESBL-producing isolates from livestock, humans, companion animals, food, and the environment during 2009–2016 in Germany for the CTX-M-27 allele.

We analyzed a representative subset of 953 sequenced isolates from a collection of 4,386 nonrepetitive ESBL-producing *E. coli*, which were obtained through 2 national research networks investigating the incidence of antimicrobial resistance: ESBL and Fluoroquinolone Resistance in Enterobacteriaceae (RESET) and German Center for Infection Research (DZIF) in Germany (online Technical Appendix 1, <https://wwwnc.cdc.gov/EID/article/23/10/17-0938-Techapp1.xlsx>). In silico multilocus sequence typing (MLST) identified 159 (17%) of the 953 isolates as ST131 (online Technical Appendix 2, <https://wwwnc.cdc.gov/EID/article/23/10/17-0938-Techapp2.pdf>). The most prevalent ESBL genes in the studied isolates were *bla*<sub>CTX-M-15</sub> (73 [46%]), followed by *bla*<sub>CTX-M-27</sub> (24 [15%]), *bla*<sub>CTX-M-1</sub> (18 [11%]), *bla*<sub>CTX-M-14</sub> (15 [9%]), and others (*bla*<sub>CTX-M-3/11/17/24/36/47</sub>) (10 [6%]).

Because recent reports have documented an increase in the number of C1-M27 clade isolates in Japan and France, we investigated *bla*<sub>CTX-M-27</sub>-encoding ST131 isolates in more detail. All ST131 isolates with *bla*<sub>CTX-M-27</sub> were of serogroup O25b and harbored a *fimH30* allele, except for 1 isolate that was of serogroup O16 and carried a *fimH41* allele. Recently, the M27PP1 prophage-like region was defined as a specific marker for C1-M27 clade (3,4). This region was present in 23 of 24 *bla*<sub>CTX-M-27</sub>-harboring isolates. Phylogenomic

analysis revealed that these 23 isolates belong to clade C1/H30R (online Technical Appendix 2 Figure).

We identified contigs with F1:A2:B20 plasmid replicons in sequences from 20 of 24 isolates; the remaining isolates harbored contigs with F1:A6:B20, F1:A2:B20, F1:A2:B-, and F29:A-B10 plasmid incompatibility groups. We sequenced the genome of 1 representative isolate (H105) to completion (GenBank accession numbers: chromosome, CP021454; plasmid, CP021871) and confirmed that the *bla*<sub>CTX-M-27</sub>-encoding contig was indeed part of a plasmid harboring the F1:A2:B20 replicon (5). This plasmid is highly conserved in isolates of ST131 and is probably ancestral to the C1/H30R clade because it is present in all the *bla*<sub>CTX-M-27</sub>-positive ST131 isolates, regardless of whether they harbor antimicrobial resistance genes (5).

Core genome phylogenetic comparisons of all C/H30R ST131 isolates, based on alignment to the closed genome of *E. coli* ST131 lineage C1-M27 isolate H105, revealed an average of 292 single-nucleotide polymorphisms (SNPs). In contrast, isolates within the C1-M27 clade were separated by <100 SNPs. Comparative analyses of 13 isolates reported from Japan showed that these isolates share  $\approx$ 85% of the genome with those from Germany. Isolates from both countries exhibit an average difference of 59 SNPs, indicating clonality and possible evolution from a single common ancestor (online Technical Appendix 2). Metadata of the C1-M27 isolates showed 19 of 24 isolates were obtained in 2015 and 2016, indicating recent emergence.

Our results provide evidence for the recent emergence of ST131 subgroup *fimH30*-O25b, clade C1-M27, harboring *bla*<sub>CTX-M-27</sub>, in Germany and reinforce observations made elsewhere. The data suggest an ongoing shift in CTX-M alleles associated with ST131 infections worldwide that now warrants further attention.

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## Angiostrongylus cantonensis Eosinophilic Meningitis in an Infant, Tennessee, USA

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*Angiostrongylus cantonensis*, the rat lungworm, is the most common infectious cause of eosinophilic meningoencephalitis worldwide. This parasite is endemic to Southeast Asia and the Pacific Islands, and its global distribution is increasing. We report *A. cantonensis* meningoencephalitis in a 12-month-old boy in Tennessee, USA, who had not traveled outside of southwestern Tennessee or northwestern Mississippi.

In 2016, a 12-month-old, fully vaccinated boy was admitted to a hospital in Memphis, Tennessee, USA, for evaluation of 18 days of daily fever, irritability, decreased oral intake, and emesis. His medical history was unremarkable, and he had no known contact with sick persons. He had not traveled outside the area comprising southwestern Tennessee and northwestern Mississippi. He lived in a nonagricultural rural area and was exposed to a vaccinated family dog. Wild rats had been observed in and around the home, and rat droppings had been found in the child's bed. Raccoons were seen on the property; however, contact, either direct or through fomites such as latrines, was not reported. During a 17-day period, 2 evaluations by his primary care physician and 4 emergency department visits resulted in the diagnosis of fever of unknown origin and inpatient admission.

A cerebrospinal fluid (CSF) sample taken by lumbar puncture on day 20 of illness showed eosinophil-predominant pleocytosis, mild hypoglycorrhacia, and a mildly elevated protein level (Table). Magnetic resonance imaging of the brain and spine showed scattered areas of restricted diffusion throughout the brain parenchyma, leptomeningeal enhancement, and multifocal nodular enhancement along the ventral portion of multiple spinal levels. Serologic testing was negative for *Toxocara canis/cati*, *Strongyloides stercoralis*, *Ehrlichia chaffeensis*, *Rickettsia rickettsiae*, Epstein-Barr virus, HIV, and *Toxoplasma gondii*; a rapid plasma reagin was also negative. Tuberculin skin testing was negative. Results of CSF PCR for *Streptococcus pneumoniae*, herpes simplex virus, and enteroviruses were negative; CSF cryptococcal antigen testing was also negative. Due to concern for infection with *Baylisascaris procyonis*, the raccoon roundworm, physicians prescribed albendazole and dexamethasone. The patient's temperature returned to normal, and his symptoms resolved. Upon discharge, he was to complete 3 weeks of albendazole and tapering doses of corticosteroids. Attending physicians repeated lumbar punctures on days 28, 41, and 56 (Table).

Physicians sent samples (CSF and serum) taken on day 20 to the Centers for Disease Control and Prevention (Atlanta, GA, USA) to test for *B. procyonis* roundworms and samples taken on day 56 to test for *Angiostrongylus cantonensis*, the rat lungworm. Results were negative for *B. procyonis* but positive for *A. cantonensis*. In addition, serum samples obtained at the time of the initial lumbar puncture were positive for *A. cantonensis* antibodies by investigational whole-worm Western blot.

The first documented human infection with *A. cantonensis* worms occurred in 1944 in Taiwan. Since then, >2,800 cases among humans have been reported; most have been in Southeast Asia and the Pacific islands (*I*; online Technical Appendix, <https://www.cdc.gov/EID/article/23/10/17-0978-Techapp1.pdf>). In the late 1950s, the first report of human *A. cantonensis* infection in the

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## Technical Appendix

### Whole-Genome Sequencing

On the isolates examined in this study (Technical Appendix Table), we conducted whole-genome sequencing using Illumina platforms (either MiSeq or NextSeq), as described previously (1,2). Briefly, genomic DNA was isolated from overnight cultures by using the Purelink Genomic DNA Mini kit (Invitrogen, Darmstadt, Germany). For short-read whole-genome sequencing, an Illumina Nextera XT library (Illumina Netherlands BV, Eindhoven, the Netherlands) was constructed and sequenced. The *bla*<sub>CTX-M-27</sub>-encoding *Escherichia coli* sequence type (ST) 131 isolate H105 a member of the lineage C1/H30R was sequenced for its complete genome using PacBio RSII system (Pacific Biosciences, Menlo Park, CA, USA). GenBank accession numbers for the chromosome and plasmid are CP021454 and CP021871, respectively.

### In Silico Analysis

For genome assembly of Illumina reads we used Spades V.3.6 (3). Annotation was performed with Prokka V1.11 (4) using standard default parameter settings. PacBio data were assembled de novo based on 59,447 PacBio long reads with an average read length of 10,355 bp using RS\_HGAP\_Assembly.3, included in the SMRT Portal version 2.3.0. Illumina short reads were mapped onto the assembled sequences using Burrows–Wheeler Aligner to obtain a highly accurate genome with QV60 final quality. Assembly quality was assessed through QUAST v2.3 (5), and contigs with >500 bp were considered for further analysis. Multilocus sequence typing (MLST) was carried out by mlst-package (<https://github.com/tseemann/mlst>). The *bla*<sub>CTX-M</sub> profiles, fim-type, serotype, and virulence gene were determined by Resfinder, FimTyper,

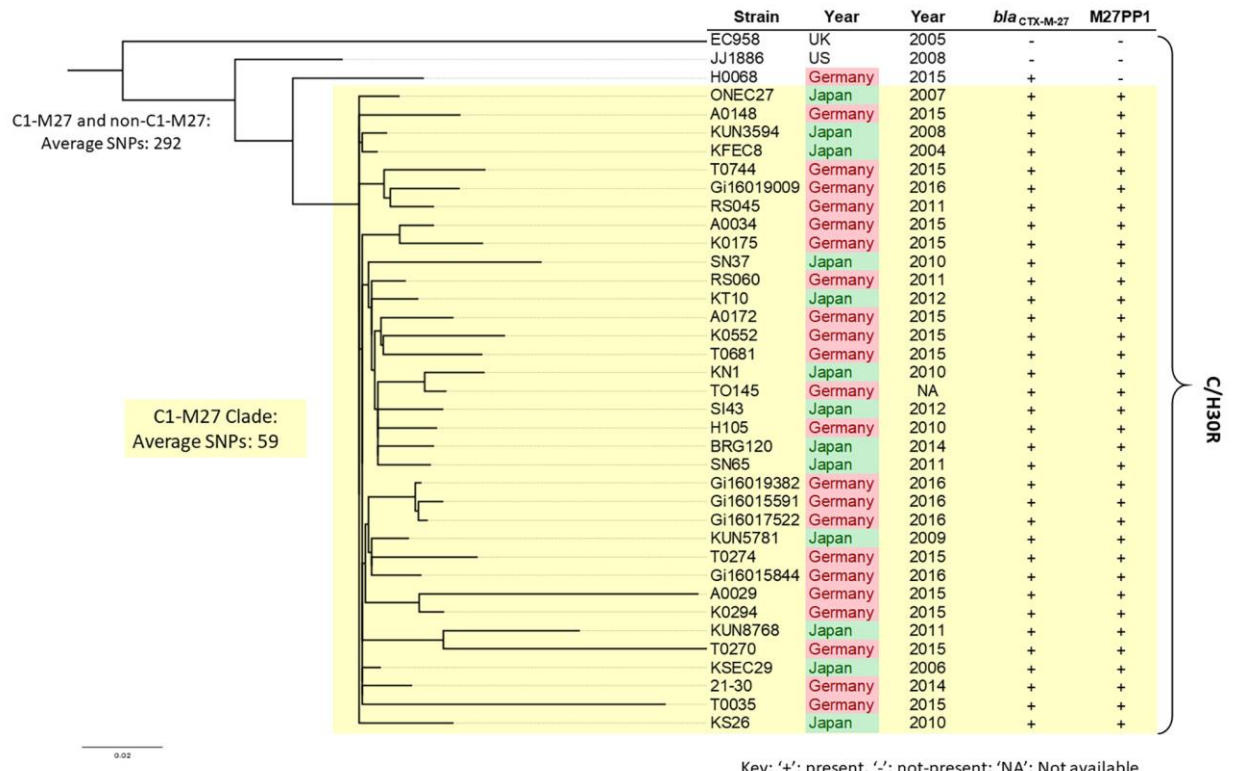


SeroTypeFinder, and VirulenceFinder, respectively (6–9). Plasmid incompatibility groups and plasmid MLST was performed using PlasmidFinder and pMLST (10). The presence of the M27PP1 region was determined by LS-BSR (11). For core genome analysis, draft genomes of the 24 isolates from Germany were compared with isolates from Japan (n = 13) using Harvest Suite (version 1.2) (12) with a default parameter *E. coli* EC958 and *E. coli* H105 were used as reference genomes for between- and within-clade comparisons (Technical Appendix Figure). Genome sequencing data for the *bla*<sub>CTX-M-27</sub>-encoding isolates are deposited in European Nucleotide Archive under accession no. PRJEB21697.

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**Technical Appendix Figure.** Core genome based phylogenomic analysis of *Escherichia coli* sequence type (ST) 131 C1-M27 isolates from Germany and Japan (13). The tree is rooted to EC958. An average of 59 single-nucleotide polymorphisms were identified in core genome of C1-M27 clade, whereas an average of 292 single-nucleotide polymorphisms was recognized between C1-M27 and non-C1-M27 clade. Scale bar indicates nucleotide substitutions per site.