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Composite SCCmec Element in Single-locus Variant (ST217) of Epidemic MRSA-15 Clone

To the Editor: Since early epidemiologic studies of methicillin-resistant *Staphylococcus aureus* (MRSA) were published, it has been clear that the majority of nosocomial MRSA infections worldwide are caused by isolates derived from a few highly epidemic MRSA (EMRSA) clones. These are thought to have emerged through acquisition of the staphylococcal cassette chromosome *mec* (SCCmec) element by successful methicillin-susceptible *S. aureus* strains, within 5 major lineages or clonal complexes (CCs) including CC22 (1). Although epidemic clones are found worldwide, shifts of the predominant clones over time in which the emerging and usually more antibacterial drug–susceptible clones replace the older ones have been

noted in countries, in small regions within countries, and in single hospitals (2). The reasons and mechanisms of such replacement as well as the epidemiologic dynamics leading to the success of a particular epidemic clone are largely unknown.

In Italy, isolations of classical EMRSA clones such as ST8-MRSA-I, ST247-MRSA-I, and ST239-MRSA-III decreased from the 1990s to the 2000s; during the same period ST228-MRSA-I increased, became established, and turned into the predominant clone in Italy (3). The genesis of other clones, such as ST8-MRSA-IV and ST22-MRSA-IV, which were associated with a tendency towards decreased multidrug resistance, was documented during 2000–2007 (3). Similar to occurrences in other European countries, the gentamicin-susceptible Panton-Valentine leukocidin–negative ST22-MRSA-IV clone, also known as EMRSA-15 (1), is now becoming predominant in Italy, replacing ST228-MRSA-I in hospital settings (4).

As part of another investigation, we recently isolated a MRSA strain from the nasal swab samples of a 5-year-old boy and his parents. The 3 isolates shared the same antibacterial drug resistance pattern (oxacillin and ciprofloxacin resistance) and proved to be identical by pulsed-field gel electrophoresis, SCCmec typing, and *agr* typing. Remarkably, ≈2 months earlier, the child had been admitted to a pediatric hospital for 10 days to be evaluated and treated for behavioral problems. A MRSA isolate, which was identified in a nasal sample obtained and analyzed just before discharge in the absence of clinical symptoms and was not further investigated, showed the same antibacterial drug resistance pattern as the 3 isolates collected later. In the absence of an epidemiologic history of exposure outside the hospital, it seems reasonable to assume that the strain was acquired by the child in the hospital and then transmitted to his parents.

Investigation of the genetic background of the strain isolated from the child's specimen, designated as Lu1, led to its assignment as ST217, a single-locus variant of EMRSA-15 within the same CC, CC22 (<http://saureus.mlst.net>), *agr* group I, and *spa* type t965. At times associated with ST22-MRSA-IV strains, t965 is a single repeat variant of t032, which is the most prevalent *spa* type of EMRSA-15; t965 and closely related *spa* types have been reported mainly in Germany and the United Kingdom (<http://spa.ridom.de>). Strain Lu1 was Pantone-Valentine leukocidin-negative and lacked the ACME (arginine catabolic mobile element) cluster. By using current criteria (5), the SCCmec element was assigned to type IV(2B), which is consistent with the combination of a class B *mec* complex and a *ccrA2B2* (type 2) *ccr* complex. However, an additional *ccr* locus (*ccrC*, type 5) was found in the J3 region between *orfX* and IS431. The SCCmec element was thus identified as a composite type IV(2B&5). Sequencing of the *ccrC*-IS431 segment (3,217 bp, GenBank accession no. HG315670), followed by performing analysis by using BLAST (<http://blast.ncbi.nlm.nih.gov>), displayed variable alignment scores and high-level nucleotide identities to the corresponding regions found downstream of *ccrC* in the SCCmec elements of MRSA reference strains of types III(3A), IV(2B&5), V(5C2&5), and VII(5C1) (5). The highest identity (3,216/3,217 nt) was with JCSC6944 (GenBank accession no. AB505629), an unspecified animal isolate of type V(5C2&5) from Japan, subtype c, belonging to the livestock-associated MRSA clone CC398 (6).

Very few ST217 strains, and none from Italy, are currently found in the MLST database (<http://saureus.mlst.net>), and data on such strains are scant in the literature. In particular, ST217-MRSA-IV was 1 of the dominant MRSA lineages isolated from patients

in a hospital in Switzerland (7) and was detected in food samples of animal origin in Spain (8).

The composite SCCmec organization we detected in strain Lu1, featuring 2 *ccr* complexes (type 2 and 5), is similar to that described in 2 isolates that belong to different genetic lineages: 1 (ST100, CC5), later designated ZH47 (5,9), was identified in a sample from an inpatient in Switzerland (7); and the other (ST59, CC59, community-associated MRSA) from a pediatric patient in Taiwan (10).

Strain Lu1 (ST217, a single-locus variant of EMRSA-15) might have evolved from the ST22-MRSA-IV clone, which has recently been identified in hospitals in Italy (3,4). Its SCCmec organization may result from recombination events in which a type IV(2B) element acquired the *ccrC*-containing region downstream of *orfX* from SCCmec elements that normally contain it (5). However, a genetic exchange involving MRSA strains of animal origin cannot be excluded, considering the virtually identical sequence of the *ccrC*-IS431 segment shared by strains Lu1 and JCSC6944, the latter being a CC398 LA-MRSA (6), and the isolation of ST217 strains from food samples of animal origin (8).

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***Bartonella quintana* in Body Lice from Scalp Hair of Homeless Persons, France**

To the Editor: *Bartonella quintana* is a body louse-borne human pathogen that can cause trench fever, bacillary angiomatosis, endocarditis, chronic bacteremia, and chronic lymphadenopathy (1). Recently, *B. quintana* DNA was detected in lice collected from the heads of poor and homeless persons from the United States, Nepal, Senegal, Ethiopia, and the Democratic Republic of the Congo and in nits in France (2,3). The head louse, *Pediculus humanus capitis*, and the body louse, *Pediculus humanus humanus*, are obligatory ectoparasites that feed exclusively on human blood (4). Outside of their habitats, the 2 ecotypes are morphologically indistinguishable (1). Sequence variation in the PHUM540560 gene discriminates between head and body lice by determining the genotype of the lice

(5). While surveying for trench fever among homeless persons in shelters in Marseille, France during October 2012–March 2013, we investigated the presence of *B. quintana* DNA in nits, larvae, and adult lice collected from mono-infested and dually infested persons and determined the genotypes of the specimens.

The persons included in this study received long-lasting insecticide-treated underwear; lice were collected by removing them from clothing, including underwear, pants, and shirts. Because body lice reside in the clothing of infested persons except when feeding, they are sometimes called clothing lice.

A total of 989 specimens were tested, including 149 (83 from clothing and 66 from hair) first-instar larvae hatched in the laboratory from eggs collected from 7 dually infested persons, and 840 adult body lice collected from the clothing of 80 mono-infested patients. We included DNA isolated from 3 nits collected from the hair of a mono-infested person who had previously been confirmed as positive for *B. quintana* (6) (Table).

Total DNA was extracted by using an EZ1 automated extractor (QIAGEN, Courtaboeuf, France) and subjected twice to real-time PCR specific for *B. quintana*. The first PCR targeted the 16S-23S intergenic spacer region. Positive samples were confirmed by using a second real-time PCR targeting the *yopP* gene (6). Samples that tested positive for *B. quintana* DNA were analyzed by multiplex real-time PCR that targeted the PHUM540560 gene (5). We used head and body lice that had known genotypes positive

controls. Negative controls were included in each assay.

Of the hatched larvae, 5 (6%) of the 83 recovered from clothing and 7 (11%) of 66 from the hair (Table) of 4 of the 7 dually infested persons were positive for *B. quintana* DNA (online Technical Appendix Table 1 wwwnc.cdc.gov/EID/article/20/5/13-1242-Techapp1.pdf). Of the 840 adult body lice, 174 (21%) collected from 42 (53%) of 80 of the mono-infested persons contained *B. quintana* DNA (Table, online Technical Appendix 2). The multiplex real-time PCR that targeted the PHUM540560 gene clearly identified all nits, larvae, and adult lice as belonging to the body lice lineage. Negative controls remained negative in all PCR-based experiments.

For 2 decades, *B. quintana* DNA has been regularly detected in lice collected from the heads of persons living in poverty, but it had not been detected in head lice that infest schoolchildren (7,8). All of the lice collected during this study that tested positive for *B. quintana* from homeless persons were body lice, including some that were recovered from hair. This observation supports our assertion that body lice are not confined to the body. The 3 eggs that were removed from the hair of a mono-infested homeless person whose samples tested positive for *B. quintana* were also body lice. During the clinical examination, no adult head lice or adult body lice were found on that person, confirming that the patient had been heavily infested with body lice in the past, not head lice. The nits were most likely laid by body lice that migrated toward the patient's head. When a member of

Table. Distribution of *Bartonella quintana* DNA in nits, larvae, and adult body lice collected from hair and clothing of homeless persons in shelters, Marseille, France, October 2012–March 2013*

Location	No. persons		No. (%) lice positive for <i>B. quintana</i> DNA	Reference
	Dually infested, n = 7	Monoinfested, n = 80		
Hair				
Nits	0	3	3 (100)	(6)
Hatched larvae	66	0	7 (10.60)	This study
Clothing				
Hatched larvae	83	0	5 (6.00)	This study
Adults	0	840	174 (20.70)	This study

*All lice were identified as body lice. Study participants were provided with long-lasting insecticide-treated underwear, and killed body lice were collected from the clothing of infested persons.