

CC9 Livestock-Associated *Staphylococcus aureus* Emerges in Bloodstream Infections in French Patients Unconnected With Animal Farming

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We report 4 bloodstream infections associated with CC9 *agr* type II *Staphylococcus aureus* in individuals without animal exposure. We demonstrate, by microarray analysis, the presence of *egc* cluster, *fnbA*, *cap* operon, *lukS*, *set2*, *set12*, *splE*, *splD*, *sak*, *epiD*, and *can*, genomic features associated with a high virulence potential in humans.

Keywords. *Staphylococcus aureus*; CC9; bloodstream infections; France.

Staphylococcus aureus is a leading cause of bloodstream infection (BSI), associated with high levels of morbidity and mortality. Over the past 50 years, *S. aureus* has undergone changes in its genetic makeup, resulting in the emergence of clones that are successfully transmitted and cause disease in hospital and community settings. *Staphylococcus aureus* also colonizes and infects animals, particularly livestock. *Staphylococcus*

aureus belonging to clonal complexes 398 (CC398) and CC9 is associated worldwide with livestock, their human contacts, and food products. To date, human infections with livestock-associated (LA) *S. aureus* isolates have generally occurred in farmers or veterinary surgeons [1].

However, in France [2] and worldwide [3], CC398 strains have recently emerged in patients without animal exposure, in whom they have caused BSI. Likewise, during an annual prospective, longitudinal BSI survey initiated in 2002 in France [4], we identified, in 2011 and 2012, the 4 first cases of BSI due to CC9 *S. aureus*, in patients without animal exposure. We document this emergence by reporting the clinical context and determining the genomic content of these CC9 isolates.

METHODS

BSI Epidemiologic Survey Method

A BSI surveillance program and a microbiologic study of *S. aureus* isolates from BSI cases have been conducted since 2002, in the central region of France (2.5 million inhabitants). The methods, study design, and data for the years 2000–2008 have been reported elsewhere [4].

Microbiologic Methods

BSI-associated *S. aureus* isolates were collected during each survey period and sent to a central laboratory. Antimicrobial drug susceptibility testing was performed by the disk diffusion method (Bio-Rad). The *mecA* and *cfr* genes were detected by polymerase chain reaction (PCR) [4, 5]. DNA macrorestriction and pulsed-field gel electrophoresis (PFGE) were used for typing [4]. PCR targeting *sau1-hsdS1* was used for the detection of CC398 isolates [6]. For multilocus sequence typing (MLST), *S. aureus* isolates were analyzed as previously described [4]. *Spa* types were determined for all isolates as previously described and were assigned through the database www.ridom.de/spaserver [4]. Typing for *agr* was performed and isolates were classified as *agr* types I to IV [4]. PCR was performed to detect virulence genes (*lukS-PV*, *lukF-PV*, *tst*, *eta*, *etb*, and the genes encoding enterotoxins A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, U, and R) [7]. In DNA microarray experiments, isolates were studied with a previously described oligonucleotide microarray [4].

Ethics Statement

The isolates were obtained from clinical samples as part of the annual surveillance studies carried out in accordance with

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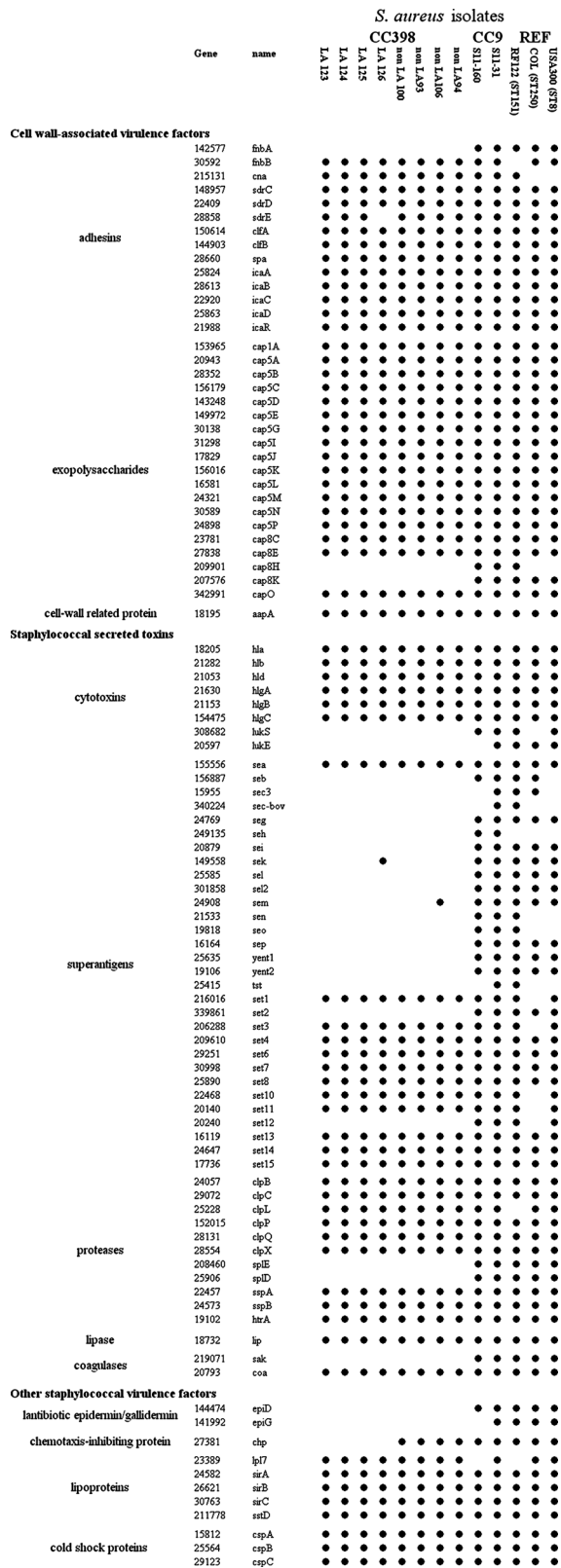


Figure 1. Microarray data for virulence factors, obtained with the 2 CC9 isolates, 8 CC398 isolates, and 3 reference strains (RF122, COL, and USA300). Abbreviation: *S. aureus*, *Staphylococcus aureus*.

French healthcare recommendations. Ethics approval for these surveillance studies was obtained at the national level from the Réseau Alerte Investigation Surveillance des Infections Nosocomiales.

RESULTS

During the 2007–2012 period, 723 cases of *S. aureus* BSI were diagnosed in our network and 685 (94.7%) *S. aureus* isolates were available for analysis. *Sma*I PFGE patterns were obtained for all but 27 isolates (3.9%), which were assigned to CC398 by MLST. During the 2011 and 2012 survey periods, 4 isolates presented a similar PFGE pattern that had never been observed before, and were thus subjected to MLST; all were assigned to CC9.

The 4 CC9-associated BSI cases were diagnosed at different hospitals, some distance apart and with no epidemiologic link. Two cases were genitourinary-associated BSI: a case of urinary-associated BSI in an 85-year-old man and a case associated with orchitis in a 58-year-old man with diabetes mellitus. A third BSI with no recognized portal of entry was identified in an 87-year-old man. The remaining case was a central venous catheter-associated infection in a 69-year-old man. An examination of patient histories and epidemiologic investigation revealed an absence of exposure to animal for all these patients.

The CC9 isolates were all of *agr* type II. Three of the 4 CC9 isolates had the same resistance pattern (only Ery^R), whereas the fourth was susceptible to all antibiotics. *mecA* and *cfr* were not detected. *Spa* typing identified 3 related *spa* types: t587 (2 isolates), t1939 (1 isolate), a *spa* type assigned to a CC9 pig-borne isolate [8], and t8666 (1 isolate). The 4 isolates had sequences corresponding to the genes encoding the enterotoxins G, I, M, N, O, and U, known as the *egc* cluster. By contrast, they had no sequences corresponding to *lukS-PV* and *lukF-PV*, encoding Pantan-Valentine leukocidin; *tst*, encoding toxic shock syndrome toxin 1; the *eta* and *etb* genes, encoding exfoliatins A and B, respectively; or the genes encoding enterotoxins A, B, C, D, E, H, J, K, L, P, Q, and R.

Given the similar PFGE pattern obtained, microarray analysis was carried out for 2 of the 4 CC9 isolates. Microarray data were compared with those for 3 reference strains (USA300/CC8, COL/ST250, and RF122/ST151) and 8 previously characterized CC398 isolates [9]. Like the reference strains studied (Figure 1), but unlike CC398 isolates, the BSI-CC9 isolates had sequences corresponding to genes encoding many major staphylococcal virulence factors, some associated with various pathogenicity islands and prophages: FnbA adhesin, capsule operon, leukocidin S, enterotoxins A, B, C3, G, H, I, K, L, M, N, O, and P, superantigens SET2 and SET12, proteases SplE and SplD, and staphylokinase. They also contained the

lantibiotic epidermin/gallidermin gene *epiD* typically harbored by virulent isolates and had a complete type I restriction-modification system (*hsdS-hsdR*), which plays a key role in the limitation of horizontal gene transfer. Nevertheless, the CC9 isolates had 2 major characteristics in common with CC398 isolates: they harbored the *cna* gene encoding the collagen-adhesin associated with colonizing strains and involved in the pathogenesis of osteomyelitis and infectious arthritis, and the gene encoding the chemotaxis inhibitory protein CHIPS, which protects *S. aureus* from human innate immunity [10].

DISCUSSION

In an LA environment, CC9 methicillin-resistant *S. aureus* (MRSA) strains easily colonize and infect humans [1, 11]. But so far, in an animal-free environment, CC9 is a minor lineage associated with scarce nasal carriage [12, 13] and bloodstream infections in human [14, 15]. In a context of increasing incidence of *S. aureus* BSI [16], and following the recent emergence of a new CC398 methicillin-susceptible *S. aureus* (MSSA) lineage, non-LA CC398, causing severe infections in patients without exposure to animals [2, 3], we report the emergence of a second *S. aureus* MSSA lineage, non-LA CC9, responsible for severe human BSI in an animal-free environment.

CC9 LA isolates are of *agr* type II and have an *egc* cluster, but are genetically diverse, as shown by their *spa* types (mostly t899, t1430, and t337), antibiotic susceptibility patterns and, for MRSA, their SCC*mec* elements [17]. Our CC9 isolates were also of *agr* type II and harbored the *egc* cluster, but they remained susceptible to most currently used antibiotics. More remarkably, our non-LA CC9 isolates had several characteristics in common with the successful emerging non-LA CC398 strains. First, 3 of the 4 CC9 isolates had the same resistance pattern (only Ery^R MSSA). Second, they had the human-specific *chp* gene, a gene of phage origin typically harbored by virulent *S. aureus* responsible for severe human infections, and recently identified as a marker of a beta-converting prophage carrying an immune evasion cluster associated with non-LA CC398 isolates [2, 3]. The concomitant presence of this prophage in non-LA CC398 isolates and in non-LA CC9 isolates raises questions about the contribution of horizontal transfer to the virulence of these isolates, which were initially identified as strict animal pathogens.

The simultaneous emergence of invasive infections due to CC398 and CC9 in humans without exposure to animals suggests a rapid epidemiologic change in these *S. aureus* lineages originally clearly associated with livestock. CC9 isolates have been isolated from food items in the Netherlands and Germany [18, 19], so the potential role of food products manufactured from livestock and the route of transmission to patients without animal exposure should be investigated in more detail.

Unlike CC398 isolates that lack several clinically important *S. aureus*-associated virulence factors [20], the BSI CC9 isolates studied here were similar to virulent *S. aureus* strains, with many virulence genes. Concordant with previous observations [14,15], this strongly suggests that non-LA CC9 isolates have a considerable virulence potential, even greater than that of non-LA CC398 strains, given the contents of their respective genomes. In addition, a CC9 isolate bearing the multidrug resistance gene *cfr* has been recently described [5], suggesting that this clone can easily acquire genetic resistance determinants by horizontal transfer.

Our data highlight the benefits of an active surveillance strategy for the early detection of new clones responsible for invasive infections in humans that are adapted to both their host and the hospital setting. In addition, given the specific features of the genomic content of the non-LA CC9 isolates described here, these findings indicate that there is a need for active surveys to study and control the spread of this CC9 clone in humans.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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