Methicillin-susceptible *Staphylococcus aureus* clonal complex 398: high prevalence and geographical heterogeneity in bone and joint infection and nasal carriage

F. Valour[1,2,3,*], J. Tasse[3,*], S. Trouillet-Assant[3], J.-P. Rasigade[1,3], B. Lamy[4], E. Chanard[5], P. Verhoeven[6], J.-W. Decousser[7], H. Marchandin[4], M. Bès[3,8,9], C. Chidiac[2,3], F. Vandenesch[3,8,9], T. Ferry[2,3] and F. Laurent[1,3,8,9] on behalf of the Lyon Bone and Joint Infection study group[†]

1) *Bacteriology Department, Groupement Hospitalier Nord,* 2) *Infectious Diseases Department, Hospices Civils de Lyon,* 3) *International Centre for Research in Infectious Diseases, INSERM U1111, Université Claude Bernard Lyon 1, Lyon,* 4) *Bacteriology Department, Montpellier University Hospitals, Montpellier,* 5) *Bacteriology Department, Novescia, Lyon,* 6) *Bacteriology Department, Saint Etienne University Hospitals, St Etienne,* 7) *Bacteriology Department, Hôpital Henri-Mondor, Assistance Publique – Hôpitaux de Paris, Paris,* 8) *Bacteriology Department, Groupement Hospitalier Est, and* 9) *French National Reference Centre for Staphylococci, Hospices Civils de Lyon, Lyon, France*

Corresponding author: F. Valour, Laboratoire de Bactériologie, Groupement Hospitalier Nord, 103 Grande-Rue de la Croix-Rousse, 69004 Lyon, France
E-mail: florent.valour@chu-lyon.fr

*These authors contributed equally to this work.
[†]Lyon Bone and Joint Infection study group members are given in Appendix 1.

Abstract

The prevalence of clonal complex (CC) 398 methicillin-susceptible *Staphylococcus aureus* (MSSA) was unexpectedly high among bone and joint infections (BJIs) and nasal-colonizing isolates in France, with surprising geographical heterogeneity. With none of the major, most-known staphylococcal virulence genes, MSSA CC398 BJI was associated with lower biological inflammatory syndrome and lower treatment failure rates.

Keywords: Clonal complex 398, bone and joint infection, methicillin-susceptible *Staphylococcus aureus*, molecular epidemiology, nasal carriage, virulence factors
Original Submission: 1 September 2013; **Revised Submission:** 15 December 2013; **Accepted:** 21 January 2014
Editor: P. Tassios
Article published online: 26 January 2014
Clin Microbiol Infect 2014; **20**: O772–O775
10.1111/1469-0691.12567

In addition to being a frequent colonizing organism, *Staphylococcus aureus* is one of the leading causes of human suppurative infections, such as bone and joint infections (BJIs). Although wild isolates are susceptible to methicillin (methicillin-susceptible *S. aureus* (MSSA)), methicillin-resistant *S. aureus* (MRSA) isolates emerged from MSSA initially in hospital settings (so-called hospital-acquired MRSA) and then in the community (community-acquired MRSA) by acquiring the SCC*mec* element harbouring the *mec*A gene, which encodes a specific penicillin-binding protein (PBP2a). In the past 10 years, livestock has been described as a third MRSA reservoir (livestock-associated MRSA), notably because of the worldwide spread of MRSA of sequence type (ST) 398 or related STs clustered in clonal complex (CC) 398 [1,2]. Initially reported as an animal colonizer, MRSA CC398 has been shown to be responsible for various human infections [2]. Whole genome sequence analysis has provided evidence that this MRSA clone probably originated in humans as MSSA, and then jumped to livestock, where it acquired methicillin resistance-associated genes [1]. However, little is known about this MSSA CC398 counterpart. Poorly described in animals, this clone was recently reported in people lacking livestock-associated risk factors as a rare pathogen in various conditions such as bloodstream, respiratory tract and skin and soft tissue infections, and infective endocarditis in several countries, as well as a rare human nasal commensal [3–7]. However, the role of MSSA CC398 in BJIs has not been described.

To investigate MSSA CC398 in BJIs, we conducted a retrospective study of all patients with monomicrobial or polymicrobial MSSA BJI (i.e. clinical evidence of infection and at least one reliable MSSA-positive bacteriological sample) diagnosed in four French geographical areas between 2009 and 2012 (Fig. 1). Because nasal carriage of staphylococci is associated with a high risk of *S. aureus* infection, a control population of nasal-colonizing isolates was established in two of the participating centres, obtained by nasal sampling of patients admitted for orthopaedic surgery (excluding patients with BJIs) or in intensive-care units. *S. aureus* characterization was initially performed with the automated system Vitek-2 (bioMérieux, Marcy l'Etoile, France).

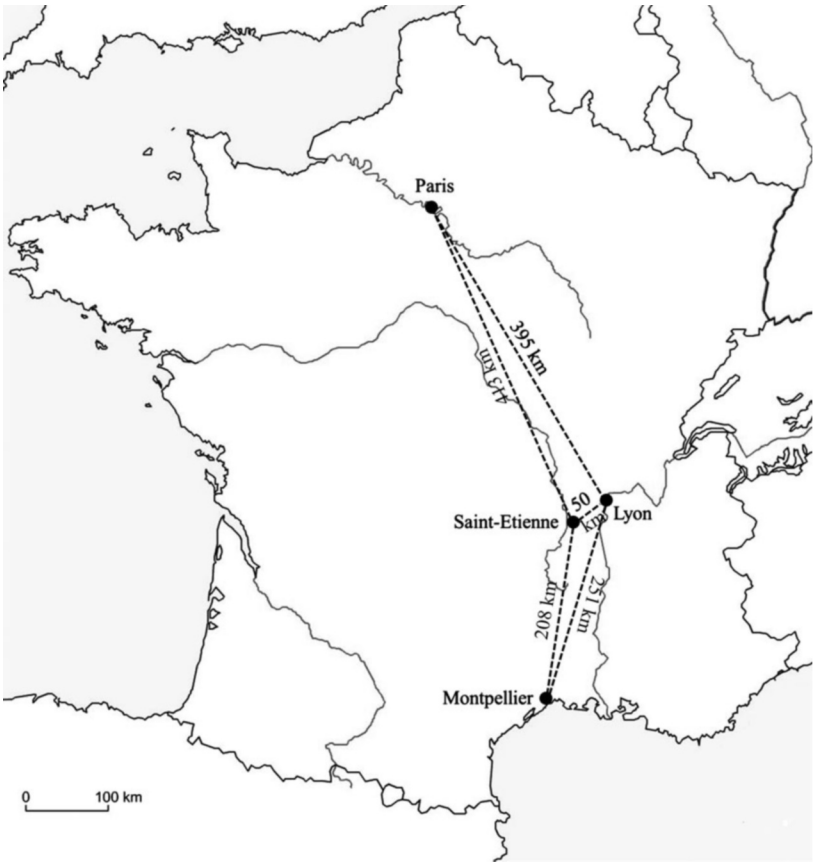

FIG. 1. Map showing the four geographical areas included in the study.

A total of 485 isolates from patients with MSSA BJIs (Lyon, *n* = 173 (Hospices Civils de Lyon, *n* = 75; Novescia, *n* = 98); Montpellier, *n* = 132; Saint-Etienne, *n* = 96; Paris, *n* = 84) were screened by the use of CC398-specific PCR targeting the *sau1–hsdS1* gene, as previously described [8]. Within this MSSA BJI collection, 68 of the 485 isolates (14.0%) belonged to CC398. For comparison, 31 (9.0%) of the 346 nasal-colonizing MSSA isolates (Hospices Civils de Lyon, from 2010 to 2012, *n* = 228; Montpellier, 2012, *n* = 118) were CC398. Because most previous international staphylococcal clonal distribution studies have mainly focused on MRSA, it is difficult to determine whether this clone is emerging or has been neglected thus far. However, several facts argue for emergence. Concerning nasal-colonizing strains, CC398 accounted for only two of 829 (0.2%) MSSA isolates in a Dutch study published in 2008, and two of 52 (3.8%) Spanish MSSA isolates in 2009 [3,4]. With respect to BJIs, Luedicke *et al.* reported a high diversity of staphylococcal genetic backgrounds for the 2005–2006 period in Germany, with a distribution of the major CCs similar to that in our study, but no CC398 [9]. These differences may have been influenced by geographical area, but the hypothesis that this clone emerged and rapidly spread is supported by the fact that only one CC398 strain was identified in 2008 during screening of BJI MSSA strains isolated in our hospital in the period between 2001 and 2008 among 52 isolates (1.9%; p 0.081).

Surprisingly, heterogeneity in geographical distribution was observed. The prevalence of CC398 among MSSA BJI isolates was only 3.1% in Saint-Etienne, reached 10.4% in Lyon, and was as high as 19.0% in Paris and 23.5% in Montpellier. The frequency in Montpellier was significantly different from that observed in Saint-Etienne (p 0.002) and Lyon (p 0.009), and the frequency in Paris was different from that in Saint-Etienne (p 0.002) and Lyon (p 0.046). It is of note that the lower prevalence of MSSA CC398 was found in two cities that are close to each other (Lyon and Saint-Etienne; 50 km). Similarly, an important difference was observed in MSSA CC398 rate among nasal-colonizing isolates between Lyon (*n* = 9/228; 3.9%) and Montpellier (*n* = 22/118; 18.6%; p 0.013). This heterogeneity in geographical prevalence raises the question of the driving mechanism. It may lie in greater endemic diffusion in some areas, possibly related to specific routes of transmission, risk factors, and environmental conditions, or the emergence of subpopulations associated with the acquisition of particular genetic traits in other areas.

In an ancillary study, MSSA BJIs identified in our hospital (n = 75) between 2011 and 2012 were extensively characterized with respect to genetic background, virulence and resistance genes, and clinical characteristics. CC assignment on the basis of DNA microarray results (StaphyType; Alere, Jena, Germany) was in agreement with the CC398-specific PCR results [10,11]. As expected for the MSSA collection, a great diversity of CCs was observed (n = 20), among which CC398 was the fourth most common (n = 8; 10.7%), behind CC30 (n = 12; 16.0%), CC5 (n = 10; 13.3%), and CC45 (n = 9; 12.0%). CC398 was associated with three different *spa* types, namely t571 (n = 6), t034 (n = 1), and t002 (n = 1). No significant differences were observed between CC398 and other strains with respect to demographic characteristics and the clinical presentation of BJIs (Table 1). Conversely, MSSA CC398 BJIs were significantly associated with a lower biological inflammatory syndrome (p 0.035) and lower treatment failure rates (0% vs. 37.3%, p 0.032). These results should be interpreted with caution, because of the large number of statistical tests performed and the small number of included cases of each clone. Nevertheless, this low virulence profile is in agreement with DNA microarray analysis revealing no particular resistance gene (except for an isolated resistance to macrolides related to the *ermT* gene), and none of the major, most well-known staphylococcal virulence genes, with the exception of one strain bearing the genes for enterotoxins C and L. This may also explain why this 'unspecialized' pathogen is associated with a large panel of diseases [3–7]. However, all strains harboured the *chp* and *scn* genes on a mobile genetic element belonging to the immune evasion cluster and β-haemolysin-converting bacteriophages and described as being associated with *S. aureus* human host specificity for the ST398 lineage that has been lost in the animal-adapted MRSA CC398 [1,12]. The presence of such mobile genetic elements provides evidence for the capacity of the MSSA CC398 clone to be highly receptive to horizontal gene transfer, which might lead to human and/or virulence adaptation. This hypothesis was recently confirmed by the emergence and spread of a Panton–Valentine leukocidin-positive CC398 subpopulation in Chinese skin and soft tissue infections, reaching 64.3% of CC398 isolates in a recent study [13]. This last report is very worrying; the acquisition of this hypervirulent phenotype by a clone that is well adapted to humans and able to be transmitted throughout the community (as demonstrated by high nasal colonization rates in some areas) may have a major impact on public health, and warrants regular monitoring.

Finally, a particular ability of this clone to cause BJIs remains questionable. On the one hand, the high prevalence of MSSA CC398 in BJIs and the difference between BJI and nasal-colonizing isolates, especially in our institution (nine MSSA CC398/228 nasal-colonizing isolates (3.9%) as compared with 10.4% CC398 BJIs (p 0.028)), suggest a particular tropism of this clone for bone and joints. In Montpellier, the very widespread diffusion of the clone as indicated by the huge prevalence, even in carriage (23.5% of all MSSA), possibly masks the phenomenon, and makes it more difficult to obtain statistically significant data when comparing the prevalence of CC398 in BJIs and carriage. On the other hand, a global rise in the prevalence of MSSA CC398 has been observed in France in various pathogenic conditions, including bloodstream infections [5,7]. Moreover, DNA microarray analysis failed to highlight specific genetic virulence associated with BJIs. In any case, this rise in MSSA CC398 prevalence in BJIs as well as in many other infectious conditions may be attributable to specific unknown virulence factors or specific expression

TABLE 1. Characteristics of the 75 patients with methicillin-susceptible *Staphylococcus aureus* (MSSA) bone and joint infection (BJI) included at the Lyon University Hospitals, and comparison between clonal complex (CC) 398 and other clones

	Total	CC398	Other clones	p
Total patients	75	8	67	
Demographic characteristics				
Sex (male)	47 (62.7)	6 (75.0)	41 (61.2)	0.364
Age (years)	52.7 ± 20.3	49.9 ± 17.0	53.1 ± 20.7	0.377
Charlson's comorbidity index	2.4 ± 2.8	1.8 ± 2.7	2.5 ± 2.8	0.492
BJI characteristics				
Arthritis	26 (34.7)	4 (50)	22 (32.8)	0.278
Prosthesis joint infections	16 (21.3)	1 (12.5)	15 (22.4)	0.455
Osteomyelitis	38 (50.7)	4 (50)	34 (50.7)	0.629
Osteosynthesis device infections	25 (33.3)	4 (50)	21 (31.3)	0.249
Vertebral osteomyelitis	11 (14.7)	0 (0)	11 (16.4)	0.262
Vertebral osteosynthesis device infections	5 (6.7)	0 (0)	5 (7.5)	0.560
Acute BJI	47 (62.7)	6 (75)	41 (61.2)	0.364
Delay from symptoms to diagnosis (days)	52.5 ± 362.9	3.4 ± 3.3	58.4 ± 383.9	0.246
Biological inflammatory syndrome	67 (89.3)	5 (62.5)	62 (92.5)	0.035
CRP (mg/L)	110 ± 113	62 ± 96	116 ± 115	0.193
Leukocyte count (10^9/mL)	10.2 ± 4.2	7.6 ± 2.3	10.5 ± 4.2	0.048
Neutrophil count (10^9/mL)	7.8 ± 4.0	5.3 ± 2.0	8.1 ± 4.1	0.035
Local complication	58 (77.3)	6 (75)	52 (77.6)	0.583
Fistula	38 (50.7)	2 (25)	36 (53.7)	0.122
Abscess	31 (41.3)	5 (62.5)	26 (38.8)	0.182
Bacteraemia	22/33 (66.7)	1/2 (50)	21/31 (67.7)	0.562
Outcome				
Treatment failure	25 (33.3)	0 (0)	25 (37.3)	0.032

CRP, C-reactive protein. Acute BJI: time from the onset of symptoms to diagnosis of ≤4 weeks. Biological inflammatory syndrome: CRP level of >10 mg/L at the time of diagnosis. Treatment failure: (i) persistence of septic symptoms despite appropriate surgical and medical treatment; (ii) relapse owing to the same MSSA after cessation of treatment; or (iii) the need for a new operation for sepsis more than 5 days after the initial operation.
Results are presented as n (%) and mean ± standard deviation. Comparisons were performed by use of a t-test with Welch's correction for continuous variables, and the chi-squared or Fisher exact test for dichotomous variables, as appropriate.

profiles impacting on phenotypic characteristics, such as biofilm formation and/or interactions with bone cells, which remain to be evaluated.

Acknowledgements

We thank S. Flammier, H. Meugnier and A. Sapin for technical assistance.

Funding

This work was supported by the French Ministry of Health, the French Ministry of Education, and the Institut National de la Santé et de la Recherche Médicale (INSERM). The funders played no role in the study design, data collection and analysis, preparation of the manuscript, or decision to publish.

Transparency Declaration

The authors declare no conflicts of interest.

References

1. Price LB, Stegger M, Hasman H *et al. Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio* 2012; 3: e00305–e00311.
2. Verkade E, Kluytmans J. Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. *Infect Genet Evol* 2013; doi: 10.1016/j.meegid.2013.02.013. [Epub ahead of print].
3. Lozano C, Gomez-Sanz E, Benito D, Aspiroz C, Zarazaga M, Torres C. *Staphylococcus aureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains. *Int J Med Microbiol* 2011; 301: 500–505.
4. van Belkum A, Melles DC, Peeters JK *et al.* Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and humans. *Emerg Infect Dis* 2008; 14: 479–483.
5. Chroboczek T, Boisset S, Rasigade JP *et al.* Clonal complex 398 methicillin-susceptible *Staphylococcus aureus*: a frequent unspecialized human pathogen with specific phenotypic and genotypic characteristics. *PLoS ONE* 2013; 8: e68462.
6. Valentin-Domelier AS, Girard M, Bertrand X *et al.* Methicillin-susceptible ST398 *Staphylococcus aureus* responsible for bloodstream infections: an emerging human-adapted subclone? *PLoS ONE* 2011; 6: e28369.
7. Tristan A, Rasigade JP, Ruizendaal E *et al.* Rise of CC398 lineage of *Staphylococcus aureus* among infective endocarditis isolates revealed by two consecutive population-based studies in France. *PLoS ONE* 2012; 7: e51172.
8. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying sau1–hsdS1. *J Clin Microbiol* 2011; 49: 732–734.
9. Luedicke C, Slickers P, Ehricht R, Monecke S. Molecular fingerprinting of *Staphylococcus aureus* from bone and joint infections. *Eur J Clin Microbiol Infect Dis* 2010; 29: 457–463.
10. Mellmann A, Weniger T, Berssenbrugge C, *et al.* Characterization of clonal relatedness among the natural population of *Staphylococcus aureus* strains by using spa sequence typing and the BURP (based upon repeat patterns) algorithm. *J Clin Microbiol* 2008; 46: 2805–2808.
11. Monecke S, Slickers P, Ehricht R. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. *FEMS Immunol Med Microbiol* 2008; 53: 237–251.
12. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. *J Bacteriol* 2006; 188: 1310–1315.
13. Zhao C, Liu Y, Zhao M *et al.* Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. *PLoS ONE* 2012; 7: e38577.

Appendix 1. Lyon Bone and Joint Infection Study Group

Physicians: F. Ader, F. Biron, A. Boibieux, A. Bouaziz, E. Braun, C. Chidiac, F. Daoud, T. Ferry, J. Karsenty, J. Lippman, P. Miailhes, T. Perpoint, D. Peyramond, M.-P. Vallat, and F. Valour. Surgeons: C. Barrey, M.-H. Fessy, O. Guyen, C. Lienhart, S. Lustig, P. Neyret, F. Trouillet, and G. Vaz. Microbiologists: F. Laurent, J.-P. Rasigade, and F. Vandenesch. Nuclear medicine: E. Deshayes, F. Giammarile, M. Janier, and I. Morelec. Pharmacokinetic/pharmacodynamic specialists: M.-C. Gagnieu, S. Goutelle, and M. Tod. Clinical research assistant: M. Martinez.