First report of the predominance of clonal complex 398 *Staphylococcus aureus* strains in osteomyelitis complicating diabetic foot ulcers: a national French study

E. Senneville[1], M. Brière[2,3], C. Neut[4], N. Messad[2], G. Lina[5], J.-L. Richard[6], A. Sotto[2,3], J.-P. Lavigne[2,7] and The French Study Group on the Diabetic Foot*

1) *Service des Maladies Infectieuses et Tropicales, CH Dron, Tourcoing,* 2) *Institut National de la Santé et de la Recherche Médicale, U1047, Université Montpellier 1, UFR de Médecine, Nîmes Cedex,* 3) *Service des Maladies Infectieuses et Tropicales, CHU Caremeau, Nîmes Cedex,* 4) *Faculté de Pharmacie, Service de Microbiologie Clinique, Université Lille 2, Lille Cedex,* 5) *Faculté de Médecine Laennec, Institut National de la Santé et de la Recherche Médicale, U851, Centre National de Référence des Staphylocoques, Université Lyon 1, Lyon Cedex,* 6) *Maladies de la Nutrition et Diabétologie, CHU Nîmes, Le Grau du Roi and* 7) *Laboratoire de Bactériologie, CHU Caremeau, Nîmes Cedex France*

Abstract

Staphylococcus aureus is the most common pathogen cultured from diabetic foot infection including diabetic foot osteomyelitis. This French multicentre study determined the genetic content of *S. aureus* isolated from 157 consecutive cases admitted to 12 diabetic foot centres between 2008 and 2011. We describe for the first time the emergence of the CC398 methicillin-susceptible *S. aureus* clone, the main clone in diabetic foot osteomyelitis, and its tropism for bone. This clone spreads to humans from an animal source through its intrinsic virulence. This adaptation of *S. aureus* isolates looks to be a worrisome problem and should be carefully monitored.

Keywords: Clonal complex 398, diabetic foot, methicillin-susceptible *Staphylococcus aureus*, osteomyelitis
Original Submission: 21 May 2013; **Revised Submission:** 2 August 2013; **Accepted:** 6 August 2013
Editor: P. Tassios
Article published online: 28 August 2013
Clin Microbiol Infect 2014; **20:** O274–O277
10.1111/1469-0691.12375

Corresponding author: J.-P. Lavigne, Institut National de la Santé et de la Recherche Médicale, U1047, Université de Montpellier 1, UFR de Médecine, 186 Chemin du Carreau de Lanes, 30908 Nîmes cedex 02, France
E-mail: jean.philippe.lavigne@chu-nimes.fr

*Members of the French Study Group on the Diabetic Foot are in Appendix 1.

Foot ulcers are common in diabetic patients, with a prevalence as high as 25% [1]. These ulcers frequently become infected, and spread of infection to soft tissue and bony structures is a major causal factor for lower-limb amputation [2], making early diagnosis and adequate treatment essential. Depending on the setting and severity of infection, prevalence of bone involvement widely ranges from 10 to 60% [3]. Chronic ulcers of the foot are at high risk for infection and secondary spread to underlying bone structures [4]. Consequently, all factors that contribute to delayed wound healing may increase the risk for developing diabetic foot osteomyelitis (DFOM). Although *Staphylococcus aureus* is the most common pathogen cultured from bone in DFOM in western countries [5,6], there is currently no report on the clones of this microorganism in this condition. *Staphylococcus aureus* clonal complex (CC) 398, which is commonly found as a colonizing strain in pig farming, is emerging as a cause of human infections. In recent years, increasing numbers of studies throughout the world have identified CC398 strain as a livestock colonizing strain, but continuously increasing and alarming cases of human infections have been reported [7]. The present study reports for the first time the emergence of CC398 strains in a French cohort of patients with diabetic foot infections (DFI) and its tropism for bone.

Between 1 January 2008 and 31 December 2010, we prospectively and consecutively screened outpatients attending one of the 12 participating French foot clinics for DFI. The local ethics committee (South Mediterranean III) approved this study. After informed consent was obtained, only patients with a documented DFI were included if they had not received any antibiotic agents in the previous month. Patients were suspected of having osteomyelitis of the foot if they had at least two of the following clinical criteria: (1) a wound with duration ≥2 weeks, located over an underlying bony prominence, with an area >2 cm^2 or a depth >3 mm; (2) a positive probe-to-bone test; and (3) abnormalities consistent with the diagnosis of osteomyelitis either on plain X-rays, radionuclide procedures (three-phase bone scan and/or labelled leucocyte imaging) or magnetic resonance imaging. After wound debridement, samples for bacterial culture were obtained by swabbing the wound base, needle aspiration or tissue biopsy for skin and soft tissue infection (SSTI) and by transcutaneous bone biopsy using a previously described procedure [5]. Only patients with monomicrobial culture for *S. aureus* were included. Genus, species and antibiotic susceptibilities were determined using the Vitek 2 card (BioMérieux, Marcy-l'Etoile, France) and interpreted according to the recommendations of the French Society for Microbiology (http://www.sfm-microbiologie.org). The Alere StaphyType DNA microarray was used according to protocols that have been previously detailed [8]. This technology allowed the identification of the clonal complex

(CC) of strains. The affiliation of isolates was assessed by an automated comparison of hybridization profiles to a collection of previously characterized reference strains [8]. Screening for the presence of prophage ϕSa3 (*scn*, *chp*, *pvl*, *tetM*) in CC398 was obtained by DNA array [9,10]. The presence of each clonal complex in *S. aureus* strains was compared according to the clinical condition (DFOM or SSTI) using Fisher's exact test. Statistical analysis was performed using the S-PLUS 2000 software package (Insightful Corporation, Seattle, WA, USA) and results were considered significant for $p < 0.05$.

During the study period, 157 patients (Table 1) were recruited, in whom *S. aureus* was the sole organism isolated from the bacterial culture of their DFI. Seventy-seven wounds (49%) were associated with osteomyelitis and 80 with SSTI without involving the bone. Among the 77 patients, 81 *S. aureus* isolates were detected; two different *S. aureus* strains (α-haemolytic and β-haemolytic colonies) were isolated from four patients. Prevalence of methicillin-resistant *S. aureus* (MRSA) was identical in cultures from DFOM ($n = 13$, 16%) and from SSTI ($n = 8$, 10%). As shown in Table 2, the *S. aureus* isolates displayed a high clonal diversity. A total of 18 known CCs and 11 singletons were identified. CC398 methicillin-sensitive *S. aureus* (MSSA; $n = 31$) was the most frequently found CC in DFOM with a prevalence of 38.3% and was significantly associated with this condition ($p < 0.001$). Of the MSSA strains, 45.6% belonged to the CC398 clone. On the other hand, CC45 MSSA ($n = 13$, 16.3%) was significantly associated with SSTI (p 0.05). The antibiotic susceptibilities showed that the CC398 strains were susceptible to all tested antibiotics except penicillin G. The strains were isolated in 10 of the 12 foot clinics. No link could be found between each patient harbouring this clone, suggesting a national propagation of this strain in DFOM. No relation with animal farming density or seasonal effect was noted; the clone was distributed throughout the study period. Interestingly strains with different appearances on blood agar media had the same gene content. Regarding the MRSA strains isolated from DFOM, they mainly belonged to three CCs: CC8 MRSA (Lyon Clone, $n = 9$), the main clone present in French hospitals, CC5 MRSA (New Paediatric, $n = 2$) and ST22 MRSA (Barnim Epidemic Strain, UK-EMRSA-15, $n = 1$). CC398 strains were characterized by some distinctive features regarding the distribution of virulence factors: absence of enterotoxins, presence of haemolysins, and genes encoding intracellular adhesion proteins, *cap5*, together with three genes encoding MSCRAMM (*bbp*, *clfA*, *clfB*). All the isolates were found in *agr* group I. Moreover, the CC398 strains belonged to human MSSA clades carrying the prophage ϕSa3 due to the presence of genes encoding human-specific immune evasion cluster (*chp*, $n = 29$/

TABLE 1. Demographic and clinical characteristics of study patients

Characteristics	Patients with CC398 strains $n = 31$	DFOM $n = 77$	SSTI $n = 80$	Total $n = 157$
Age (range), years	66 (38–91)	66 (38–85)	66.5 (33–101)	66.5 (33–101)
Male/female, *n* (%)	22 (71)/9 (29)	58 (75.3)/19 (24.7)	58 (72.5)/22 (27.5)	116 (73.9)/32 (26.1)
Type 1/type 2 diabetes mellitus	4 (12.9)/27 (87.1)	7 (9.1)/70 (90.9)	14 (17.5)/66 (82.5)	21 (13.4)/136 (86.6)
HbA1c (%)	8.5	8.4	8.4	8.4
Cardiovascular disease				
Absence	9 (29.0)	23 (29.9)	16 (20)	39 (24.8)
Coronary heart disease	9 (29.0)	23 (29.9)	31 (38.8)	54 (34.4)
Peripheral arterial disease	18 (58.1)	45 (58.4)	57 (71.3)	102 (65.0)
Arterial hypotension	25 (80.6)	60 (77.9)	65 (81.3)	125 (79.6)
Stroke	1 (3.2)	5 (6.5)	5 (6.3)	10 (6.4)
Nephropathy				
Absence	6 (19.4)	15 (19.5)	29 (36.3)	44 (28.0)
Microalbuminuria	6 (19.4)	11 (14.3)	26 (32.5)	37 (23.6)
Proteinuria	10 (32.3)	28 (36.4)	20 (25)	48 (30.6)
Renal failure	9 (29.0)	23 (29.9)	28 (35)	51 (32.5)
Neuropathy				
Peripheral	31 (100)	77 (100)	75 (93.8)	152 (96.8)
Autonomic	7 (22.6)	12 (15.6)	20 (25)	32 (20.4)
Diabetic retinopathy				
Absence	12 (38.7)	32 (41.6)	31 (38.8)	63 (40.1)
Non-proliferative diabetic retinopathy	13 (41.9)	33 (41.6)	40 (50)	73 (46.5)
Proliferative diabetic retinopathy	6 (19.4)	12 (15.6)	10 (12.5)	22 (14.0)
Lifestyle factors				
Obesity	12 (38.7)	31 (40.3)	32 (40)	63 (40.1)
Smoking	8 (25.8)	19 (24.7)	22 (27.5)	41 (26.1)
Alcoholism	5 (16.1)	12 (15.6)	14 (17.5)	26 (16.5)
Sedentary	25 (8.06)	60 (77.9)	62 (77.5)	122 (77.7)
First wound/Recurrence	8 (25.8)/23 (74.2)	17 (22.1)/60 (77.9)	19 (23.8)/61 (76.2)	36 (22.9)/121 (77.0)
IWGDF-IDSA grade				
2 (Mild)	0 (0)	0 (0)	38 (47.5)	38 (24.2)
3 (Moderate)	24 (77.4)	54 (70.1)	42 (52.5)	96 (61.1)
4 (Severe)	7 (22.6)	23 (29.9)	0 (0)	23 (14.7)

DFOM, diabetic foot osteomyelitis; HbA1c, glycated haemoglobin; IDSA, Infectious Diseases Society of America; IWGDF, International Working Group of the Diabetic Foot; SSTI, skin and soft tissue infection.
Values are median and interquartile ranges (25th–75th percentile) or numbers and percentages.

TABLE 2. Distribution of clonal complexes among *Staphylococcus aureus* strains isolated from diabetic foot osteomyelitis (DFOM) and skin and soft tissue infection (SSTI) in diabetic foot ulcers in a French population

Clonal complexes	DFOM *n* = 81	SSTI *n* = 80	Total *n* = 161	p *DFOM* vs *SSTI*
CC398-MSSA	31 (38.3)	4 (5)	35 (21.7)	<0.001
CC7-MSSA	4 (4.9)	14 (17.5)	18 (11.2)	NS
CC8-MRSA	9 (11.1)	6 (7.5)	15 (9.3)	NS
CC45-MSSA	2 (2.5)	13 (16.3)	15 (9.3)	0.05
CC30-MSSA	5 (6.2)	9 (11.3)	14 (8.7)	NS
None[a]	7 (8.6)	3 (3.8)	10 (6.2)	NS
CC18-MSSA	0 (0)	9 (11.3)	9 (5.6)	NS
CC59-MSSA	6 (7.4)	1 (1.3)	7 (4.3)	NS
CC25/28-MSSA	0 (0)	7 (8.8)	7 (4.3)	NS
CC5-MSSA	4 (4.9)	3 (3.8)	7 (4.3)	NS
CC8-MSSA	3 (3.7)	0 (0)	3 (1.9)	NS
CC5-MRSA	3 (3.7)	0 (0)	3 (1.9)	NS
CC9-MSSA	0 (0)	3 (3.8)	3 (1.9)	NS
CC1-MSSA	0 (0)	3 (3.8)	3 (1.9)	NS
CC15-MSSA	3 (3.7)	0 (0)	3 (1.9)	NS
CC101-MSSA	0 (0)	2 (2.5)	2 (1.2)	NS
CC7-MRSA	0 (0)	2 (2.5)	2 (1.2)	NS
CC97-MSSA	2 (2.5)	0 (0)	2 (1.2)	NS
CC22-MSSA	1 (1.2)	1 (1.3)	2 (1.2)	NS
CC22-MRSA	1 (1.2)	0 (0)	1 (0.6)	NS

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.
[a]None, the clonal complexes of these strains are not known and are absent in databanks. They correspond to different singletons. NS, not significant.

31 and *scn*, *n* = 30/31) and absence of *pvl* and *tetM* (associated with livestock isolates).

The present French multicentre study highlighted for the first time the emergence and spread of CC398 clone in DFI and its tropism for bone. For many years, this clone has been described in various livestock and most studies reported infection with MRSA. As an example, swine have been identified as a major reservoir for CC398 MRSA isolates [11]. Various clinical infections with livestock-associated CC398 MRSA have been reported around the world [11–13]. Recently, the emergence of an animal-independent CC398 MSSA clone has been documented in several countries. However, Price *et al.* [9] reported that the human CC398s were the original CC398 clone from which the livestock-associated clones emerged. Our strains had a human origin, indicated by the presence of the human innate immunomodulatory genes carried by ϕSa3 prophage that plays crucial roles in human niche adaptation [9,10]. The limited surveillance of MSSA has precluded an accurate assessment of the global spread of CC398 [10]. Hence, this emergence was described in community households in northern Manhattan [12]. Since that case, some reports have described CC398 MSSA not as a colonizer but as an infectious agent including cases of necrotizing pneumonia and invasive bloodstream infections in young healthy individuals [14,15]. The concern that these strains may represent a more virulent CC398 subtype is further supported by the higher prevalence of these strains in bloodstream infections of patients who had no documented exposure to livestock [14]. In France this emergence was also described in bloodstream infections [16]. In addition to the intrinsic virulence exhibited by CC398 MSSA in previous studies, the potential to acquire other virulence factors such as Panton–Valentine leukocidin [17,18], as well as resistance to multiple classes of antimicrobial drugs [9] warrants reinforced surveillance. Another animal clone, *S. aureus* CC97, was detected in our isolates. This clone was shown to have a better adaptation to animals than to humans and has been associated with bovine mastitis [19] with little impact on humans [20]. In the present study, the two CC97 *S. aureus* strains were isolated in DFOM as previously observed [21], suggesting a bone tropism of these clones of animal origin. The adaptation of animal *S. aureus* isolates to humans is a worrisome problem and should be carefully monitored.

Acknowledgements

The authors thank all the participants of the Foot Clinics for help in recruiting patients and all the patients for agreeing to be included in this study. We thank Laure Vidal-Navarro, INSERM U1047 for her technical assistance and Anne Blanc-Potard, Julien Chamard, Céline Groul-Viaud, the Research Clinical Technicians of the study, Nîmes University Hospital for their help in data processing. This manuscript will be presented at the 32th RICAI (November 2012), Paris, France.

Funding

This work was supported by grants from the Hospital Project of Clinical Research 2007 (2007-A00678-45), the French Society of Diabetes (ALFEDIAM grant 2008), a Pfizer Grant, the Languedoc-Roussillon Region (Chercheur d'avenir Grant 2009) and the National Institute of Health and Medical Research (INSERM). NM is a PhD student supported by Infectiopôle Sud Foundation, Marseille (Prof. D. Raoult).

Transparency Declaration

The authors declare no conflicts of interest.

Appendix 1 Participants of the French Study Group on Diabetic Foot

University Hospital, Nîmes: Nicole Bouziges, Nathalie Jourdan, Sophie Schuldiner; University Hospital, Montpellier: Antoine Avignon,

Christian Carrière, Ariane Sultan; University Hospital, Nice: Evelyne Bernard, Bertrand Canivet, Alexandre Fredenrich, Alice Gaudart, Luce Landraud, Emmanuel Lemichez; University Hospital, Toulouse: Maryse Archambaud, Eric Bonnet, Jacques Martini; University Hospital, Bordeaux: Michel Dupon, Henri Gin, Jeanne Maugein; AP-HP La Pitié-Salpétrière, Paris: Agnès Heurtier, Jérôme Robert; University Hospital, Reims: Christophe De Champs, Dominique Malgrange; University Hospital, Clermont-Ferrand: Richard Bonnet, Olivier Lesens; General Hospital, Narbonne: Elodie Gleize, Jean–François Thuan, Nathalie Vigier; University Hospital, Nantes: David Boutoille, Jocelyne Caillon; AP-HP Lariboisière, Paris: Pierre-Jean Guillausseau, Marie Laloi-Michelin, Laurent Raskine

References

1. Mayfield JA, Reiber GE, Sanders LJ, Janisse D, Pogach LM. Preventive foot care in people with diabetes. *Diabetes Care* 1998; 21: 2161–2177.
2. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005; 336: 1719–1724.
3. Lipsky BA, Peters EJG, Senneville E *et al.* Expert opinion on the management of infections in the diabetic foot. *Diabetes Metab Res Rev* 2012; 28: 163–178.
4. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA. Risk factors for foot infections in individuals with diabetes. *Diabetes Care* 2006; 29: 1288–1293.
5. Senneville E, Melliez H, Beltrand E *et al.* Culture of percutaneous bone biopsy specimens for diagnosis foot osteomyelitis: concordance with ulcer swab cultures. *Clin Infect Dis* 2006; 42: 57–62.
6. Lavery LA, Sariaya M, Ashry H, Harkless LB. Microbiology of osteomyelitis in diabetic foot infections. *J Foot Ankle Surg* 1995; 34: 61–64.
7. Schmidt T, Zündorf J, Grüger T *et al.* Phenotyping of *Staphylococcus aureus* reveals a new virulent ST398 lineage. *Clin Microbiol Infect* 2013; 19: 279–285.
8. Monecke S, Jatzwauk L, Weber S, Slickers P, Ehricht R. DNA Microarray based genotyping of MRSA strains from Eastern Saxony. *Clin Microbiol Infect* 2008; 14: 534–545.
9. Price LB, Stegger M, Hasman H *et al. Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio* 2012; 3: e00305–11.
10. Uhlemann AC, Porcella SF, Trivedi S *et al.* Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *MBio* 2012; 3: e00027-12.
11. Smith TC, Male MJ, Harper AL *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. *PLoS ONE* 2009; 4: e4258.
12. Bhat M, Dumortier C, Taylor BS *et al. Staphylococcus aureus* ST398, New York City and Dominican Republic. *Emerg Infect Dis* 2009; 15: 285–287.
13. Jimenez JN, Velez LA, Mediavilla JR *et al.* Livestock-associated methicillin-susceptible *Staphylococcus aureus* ST398 infection in woman. Colombia. *Emerg Infect Dis* 2011; 17: 1970–1971.
14. van Belkum A, Melles DC, Peeters JK *et al.* Dutch Working Party on Surveillance and Research of MRSA-SOM. Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and humans. *Emerg Infect Dis* 2008; 14: 479–483.
15. Rasigade JP, Laurent F, Hubert P, Vandenesch F, Etienne J. Lethal necrotizing pneumonia caused by an ST398 *Staphylococcus aureus* strain. *Emerg Infect Dis* 2010; 16: 1330.
16. van der Mee-Marquet N, François P, Domelier-Valentin AS *et al.* Bloodstream Infection Study Group of Réseau des Hygiénistes du Centre (RHC). Emergence of unusual bloodstream infections associated with pig-borne-like *Staphylococcus aureus* ST398 in France. *Clin Infect Dis* 2011; 52: 152–153.
17. Davies PR, Wagstrom EA, Bender JB. Lethal necrotizing pneumonia caused by an ST398 *Staphylococcus aureus* strain. *Emerg Infect Dis* 2011; 17: 1152–1153.
18. 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.
19. Rabello RF, Moreira BM, Lopes RM, Teixeira LM, Riley LW, Castro AC. Multilocus sequence typing of *Staphylococcus aureus* isolates recovered from cows with mastitis in Brazilian dairy herds. *J Med Microbiol* 2007; 56: 1505–1511.
20. Schuenck RP, Nouér SA, Winter Cde O *et al.* Polyclonal presence of non-multiresistant methicillin-resistant *Staphylococcus aureus* isolates carrying SCC*mec* IV in health care-associated infections in a hospital in Rio de Janeiro, Brazil. *Diagn Microbiol Infect Dis* 2009; 64: 434–441.
21. Lattar SM, Tuchscherr LP, Centrón D *et al.* Molecular fingerprinting of *Staphylococcus aureus* isolated from patients with osteomyelitis in Argentina and clonal distribution of the *cap5(8)* genes and of other selected virulence genes. *Eur J Clin Microbiol Infect Dis* 2012; 31: 2559–2566.