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ORIGINAL ARTICLE

Increasing incidence of bloodstream infections due to *Staphylococcus aureus* clonal complex 398 in a French hospital between 2010 and 2017

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Abstract

The epidemiology of *Staphylococcus aureus* is changing and several surveillances worldwide have evidenced an increasing incidence of *S. aureus* bloodstream infections (BSIs). Here, we described the long-term epidemiology of the emergent clonal group CC398 among *S. aureus* isolated from BSIs in our French university hospital between 2010 and 2017. Each patient with at least one blood culture positive with *S. aureus* during the study period was included ($N = 1455$). Cefoxitin susceptibility was determined using the disk diffusion method according to EUCAST recommendations. CC398 isolates were first screened from the whole *S. aureus* collection with a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) typing method confirmed by a CC398-specific PCR. In our hospital, the incidence of hospital- and community-acquired BSIs due to *S. aureus* and MSSA increased in parallel between 2010 and 2017 while that of BSIs with MRSA decreased. The prevalence of CC398 isolates among *S. aureus* from BSIs increased from 3.6 in 2010 to 20.2% in 2017 ($p < 0.05$). CC398-MRSA emerged but remains very sparse. Our data suggested that CC398-MSSA disseminates in the community. We showed here the emergence and the diffusion of CC398-MSSA, a subclone associated with invasive infections, in our hospital. The monitoring of this particular human-adapted *S. aureus* clone is needed and genomic studies will have to identify the determinants of its diffusion.

Keywords Bloodstream infection · Epidemiology · Methicillin-susceptible *Staphylococcus aureus* · *Staphylococcus aureus* CC398

Introduction

Staphylococcus aureus is the second most common cause of bloodstream infection (BSI) [1]. BSIs due to *S. aureus* are associated with short-term mortality rates of 15–30%, long-term excess mortality, and increased healthcare costs [1]. The epidemiology of *S. aureus* appears to be changing and several surveillances worldwide have evidenced an increase in the incidence of BSIs with *S. aureus*. The European Antimicrobial Resistance Surveillance System including 27 countries revealed an increased burden of BSIs with *S. aureus* in the 2000s by 2.3% per year and rising levels of BSIs due to methicillin-susceptible *S. aureus* (MSSA) [2]. However, this trend was not confirmed by other international studies. Regional surveys even showed a substantial decrease of the burden of BSIs with *S. aureus* overtime [3]. Trends of BSIs with *S. aureus* may be influenced by the local prevalence of MSSA and methicillin-resistant *S. aureus* (MRSA) and by the population structure of the bacterial species.

S. aureus sequence type 398 (ST398) belongs to the clonal complex 398 (CC398). CC398 emerged almost concomitantly in livestock and humans via two distinct subpopulations: (i)

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ST398-MRSA appeared in the early 2000s and became a worldwide threat associated with livestock [4, 5], and (ii) ST398-MSSA emerged probably a few years later as a pathogen increasingly responsible for invasive infections in patients without livestock contact [6–8]. Hence, BSIs due to CC398-MSSA increased in incidence since 2007 in France and are associated with a high risk of mortality [9, 10]. We demonstrated in a previous study that CC398-MSSA BSIs are frequently healthcare-associated and affect fragile patients with a history of neurological disease [10].

We determined here the trend of the prevalence of CC398 *S. aureus* among the *S. aureus* isolated from BSIs in a French university hospital between 2010 and 2017.

Material and methods

Setting and study period

We conducted a retrospective cohort study from 2010 to 2017 in the Besançon University Hospital (Eastern France), a 1200-bed teaching hospital with approximately 50,000 admissions and 320,000 patient days annually.

Patients included and BSI classification

Each hospitalized patient with at least one blood culture positive with *S. aureus* during the study period was included. Day care admissions were excluded. We only kept for further analysis the *S. aureus* isolate from the first BSI episode with MSSA and/or the first BSI episode with MRSA for each patient, each year. We classified BSIs with *S. aureus* as either community- or hospital-acquired. A BSI was classified as community-acquired or hospital-acquired when the first positive blood culture was collected ≤ 2 days or > 2 days after admission, respectively. Hospital-acquired BSIs incidence density was calculated using the number of patient days as the denominator. Community-acquired BSIs incidence density was calculated using the number of admissions as the denominator.

Statistical analysis

Trends were explored using a Poisson regression analysis. The comparison of categorical variables was performed using a χ^2 test, Fisher's exact test, or Pearson's χ^2 test as appropriate. A p value less than 0.05 was considered significant. Data were analyzed by using the STATA® software (version 14.1, StataCorp. College Station, TX, USA).

Bacterial isolates

All *S. aureus* isolates retrieved from blood cultures were systematically stored at the Centre de Ressources Biologiques Filière Microbiologique, Besançon (CRB-FMB, Biobanque BB-0033-00090). All the isolates were identified as *S. aureus* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Microflex LT (Bruker Daltonik GmbH, Bremen, Germany) with a log score value ≥ 2 according to manufacturer's recommendations. Susceptibility to cefoxitin was determined using the disk diffusion method according to EUCAST recommendations [11]. Data were collected retrospectively from January 2010 to December 2017.

Detection of *S. aureus* clonal complex 398

We screened all *S. aureus* BSI isolates using a MALDI-TOF MS method that detects the CC398 lineage [12]. For each isolate, we analyzed the spectra of two deposits of protein extract. Isolates with at least one spectrum classified as CC398 by MALDI-TOF MS were confirmed by a CC398-specific PCR [13].

Results

The incidences of BSIs due to *S. aureus* and MSSA increase between 2010 and 2017

During the eight-year survey, we identified 1496 incident cases of BSIs with *S. aureus* from 1455 different patients. Five, 31, and 1419 patients had three, two, and one episode of BSI with *S. aureus* at different years of the survey, respectively. One patient had a BSI with MRSA and a BSI with MSSA in the same calendar year. Of these 1496 BSIs with *S. aureus*, 159 were due to MRSA (10.6%) and 1337 to MSSA (89.4%) (Table 1). The proportion of hospital-acquired BSIs with *S. aureus* was 49.5% ($N = 740$). The incidence of hospital- and community-acquired BSIs due to *S. aureus* and MSSA increased in parallel between 2010 and 2017 while that of BSIs with MRSA decreased ($p < 0.05$) (Fig. 1a, b).

The prevalence of *S. aureus* CC398 increases among *S. aureus* from BSIs

Over the study period, 1342 (89.7%) *S. aureus* isolates from BSIs could be collected and tested for CC398. Among them, 12.7% ($N = 170$) belonged to CC398 and 87.3% ($N = 1172$) belonged to other clonal complexes. The prevalence of CC398 isolates among *S. aureus* from BSIs increased from 3.6 in 2010 to 20.2% in 2017 ($p < 0.05$) (Fig. 1c). BSIs due to

Table 1 Distribution of *Staphylococcus aureus* bloodstream infections in the University Hospital of Besançon (France), 2010–2017

	2010 *N* (%)	2011 *N* (%)	2012 *N* (%)	2013 *N* (%)	2014 *N* (%)	2015 *N* (%)	2016 *N* (%)	2017 *N* (%)	Total *N* (%)
BSIs with *S. aureus*	168 (–)	162 (–)	169 (–)	183 (–)	187 (–)	215 (–)	220 (–)	192 (–)	1496 (–)
BSIs with MSSA	138 (82.1)	138 (85.2)	153 (90.5)	166 (90.7)	168 (89.9)	195 (90.7)	197 (89.5)	182 (94.8)	1337 (89.4)
BSIs with MRSA	30 (17.9)	24 (14.8)	16 (9.5)	17 (9.3)	19 (10.1)	20 (9.3)	23 (10.5)	10 (5.2)	159 (10.6)
Hospital-acquired	84 (50.0)	83 (51.2)	90 (53.2)	90 (49.2)	93 (49.7)	100 (46.5)	108 (49.1)	92 (47.9)	740 (49.5)
Community-acquired	84 (50.0)	79 (48.8)	79 (47.8)	93 (50.8)	94 (50.3)	115 (53.5)	112 (50.9)	100 (52.1)	756 (50.5)
S. aureus isolates from BSIs	165 (–)	145 (–)	155 (–)	172 (–)	170 (–)	167 (–)	200 (–)	168 (–)	1342[a] (–)
MSSA	135 (81.8)	121 (83.4)	141 (91.0)	156 (90.7)	165 (97.1)	153 (91.6)	179 (89.5)	159 (94.6)	1209 (90.1)
MRSA	30 (18.2)	24 (16.6)	14 (9.0)	16 (9.3)	5 (2.9)	14 (8.4)	21 (10.5)	9 (5.4)	133 (9.9)
Hospital-acquired	83 (50.3)	74 (51.0)	82 (52.9)	84 (48.8)	84 (49.4)	75 (44.9)	95 (47.5)	83 (49.4)	660 (49.2)
Community-acquired	82 (49.7)	71 (49.0)	73 (47.1)	88 (51.2)	86 (50.6)	92 (55.1)	105 (52.5)	85 (50.6)	682 (50.8)
CC398	6 (3.6)	8 (5.5)	14 (9.0)	22 (12.8)	22 (12.9)	27 (16.2)	37 (18.5)	34 (20.2)	170 (12.7)

[a] Over the study period, 1342 (89.7%) *S. aureus* isolates from BSIs could be collected and tested for CC398

CC398-MRSA appeared in 2015 but the prevalence of MRSA CC398 remained < 2% in 2017 (Fig. 1c). The proportion of community- and hospital-acquired BSIs due to CC398 increased in parallel over the study period ($p < 0.05$).

Discussion

The MALDI-TOF MS rapid detection assay accurately detects *S. aureus* CC398 [12]. Since the negative predictive value of this screening technique is very high (97%), false negatives are unlikely. In addition, we confirmed MALDI-TOF MS-detected CC398 with a highly specific PCR, thus limiting the number of false positives [13, 14]. Overall, we confirmed here that MALDI-TOF MS detects CC398 quickly, unexpansively, and accurately.

The incidence of BSIs with *S. aureus* in hospitalized patients increased over the last decade in our University hospital. This was due to the progression of BSIs with MSSA that largely compensated the decrease of incidence of BSIs with MRSA. Our results confirm the trends in Europe [2, 15]. The decreasing incidence of BSIs with MRSA in our French hospital is consistent with that reported from a nationwide surveillance and arising because of the national infection control program on MRSA [16]. We found that the distributions between hospital- and community-acquired BSIs with *S. aureus* were similar. The increasing proportion of MSSA and CC398 among *S. aureus* responsible for hospital-acquired BSI mirrored that of MSSA and CC398 among *S. aureus* responsible for community-acquired BSI. The prevalence of CC398-MSSA among *S. aureus* responsible for BSIs increased steadily from 2010 to 2017. CC398-MRSA emerged in 2015 but remains very sparse, with only one isolate in 2015 and two isolates in 2016 and 2017. Only one BSI due to MRSA-CC398 was acquired in the hospital (in 2017). Since our goal was to assess the proportion of CC398 isolates among a collection of *S. aureus* isolates, we focused our efforts on the identification of the clone with a screening (using MALDI-TOF MS) followed by a PCR confirmation. Spa typing would have been of low benefit, but a whole-genome sequencing approach could have helped understand the dynamic of the CC398 evolution [17].

Overall, our results confirm the spread of CC398-MSSA previously demonstrated from 2010 to 2014 in our hospital, also reported in other settings in Europe [10, 18–20]. Although we ignore the evolution of the population structure of *S. aureus*, the emergence of CC398 probably participates to the increased incidence of BSIs with MSSA in our hospital.

Unfortunately, *S. aureus* isolates from BSIs were not systematically collected before 2010. Thus, we cannot precisely date the emergence of CC398-MSSA in our hospital. However, we participated to a multicentric study from 2007 to 2010, which typed the MSSA isolates from BSIs from the last quarter of each year. This study retrieved the two first CC398-MSSA isolates in our hospital in 2009 and 2010 [9]. In addition, CC398 was absent from a collection of French MSSA in the beginning of the 2000s [21]. At the same time, other parts of the world, including Europe, the USA, and China witnessed the emergence of CC398-MSSA isolates [7]. Altogether, these data suggest that CC398-MSSA emerged globally as an invasive pathogen in the mid-2000s.

We found that CC398-MSSA spread both in the community and hospital settings, in line with the dissemination of ST398-MSSA in the community reported elsewhere [17, 18, 22, 23]. Prevalence studies should be carried out to test whether healthy population in the community is a reservoir for CC398 *S. aureus*.

It is acknowledged that CC398 evolved from an ancestral clade adapted to humans and a clade adapted to animals.

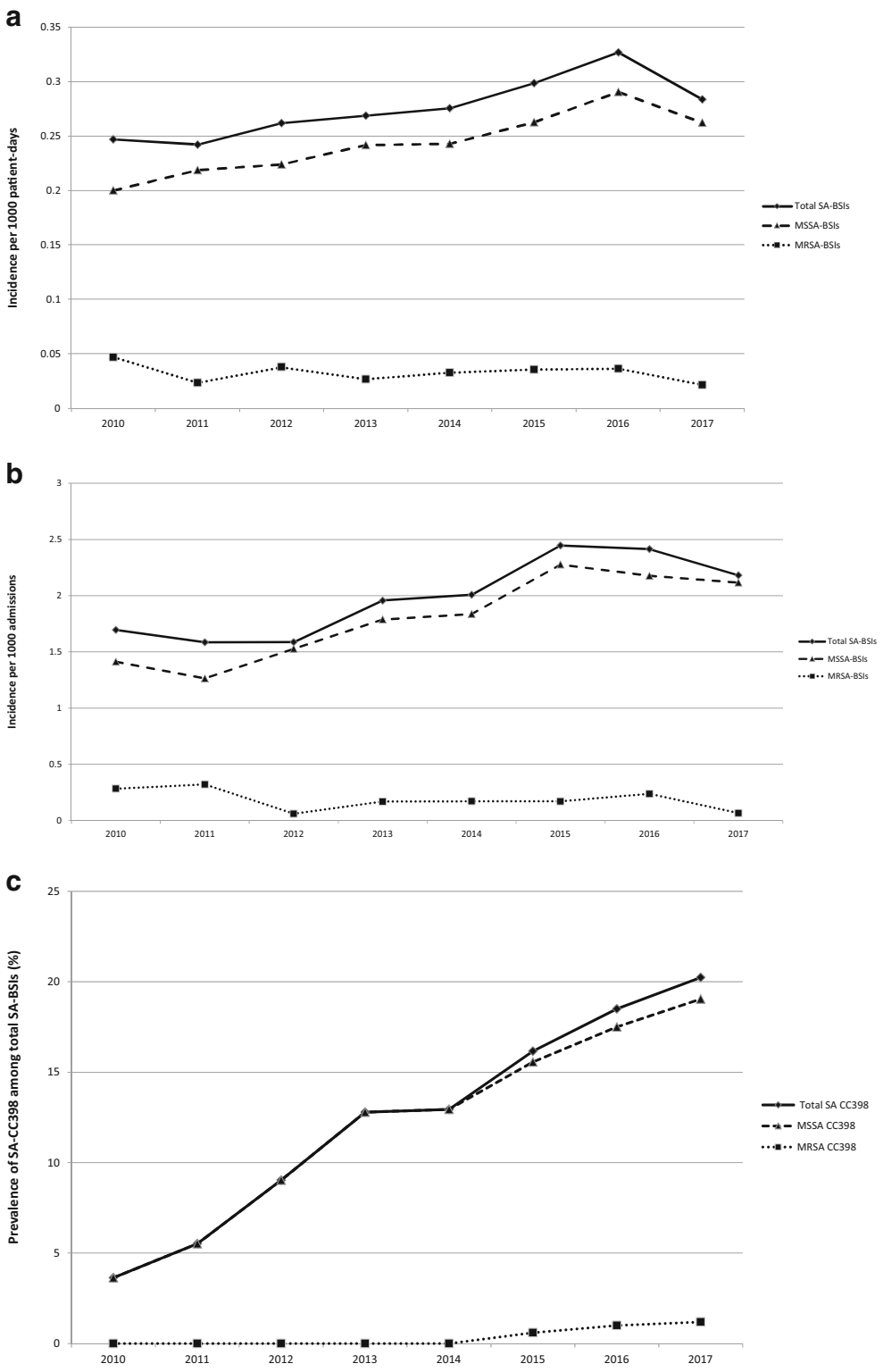

Fig. 1 Trends of *Staphylococcus aureus* bloodstream infections in a French university hospital over an 8-year period (2010–2017). **a** Incidence of hospital-acquired *S. aureus* BSIs ($N = 740$). **b** Incidence of community-acquired *S. aureus* BSIs ($N = 756$). **c** Prevalence of SA-BSIs ST398 among total SA-BSIs

CC398 keeps evolving with the emergence of new subpopulations [24]. The emergence and the spread of CC398 could rely on an increased capacity of adhesion to human cells, a better fitness and transmissibility than other *S. aureus* genotypes, a higher environmental colonization, or its particular ability to acquire resistance and virulence genes [7, 19, 23–25]. The analysis of whole-genome sequences should provide a better understanding of the biological features that could have helped the spread of the clone CC398 [17].

Conclusion

Our local data confirm the emergence and the dissemination in a hospital of CC398-MSSA, a clone associated with invasive infections. However, CC398-MSSA also disseminates in the community. The monitoring of this particular human-adapted *S. aureus* clone is needed and genomic studies will have to identify the determinants of its diffusion.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Informed consent Not applicable.

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