Methicillin-susceptible ST398 Staphylococcus aureus responsible for bloodstream infections: an emerging human-adapted subclone?

VALENTIN-DOMELIER, Anne-Sophie, et al.

Abstract

In the course of an annual 3-month bloodstream infections (BSI) survey conducted during a four-year period in 31 healthcare institutions located in three noncontiguous French regions, we report 18 ST398 Staphylococcus aureus BSI. ST398 BSI incidence showed a seven-fold increase during the study period (0.002 per 1,000 patient days in 2007 vs. 0.014 in 2010). ST398 BSI isolates differed from the pig-borne multiresistant clone: 17/18 BSI isolates were methicillin susceptible and none was of t011, t034 or t108 pig-borne spa-types. ST398 BSI isolates had homogenous resistance patterns (15/18 with only Ery(r)) and prophagic content (all harboured the hlb-converting Sau3int phage). The clustering of BSI and pig-borne isolates by spa-typing and MLVA, the occurrence of Sau3int phage in BSI isolates and the lack of this phage in pig-borne isolates suggest that the emergence of BSI isolates could have arisen from horizontal transfer, at least of the Sau3int phage, in genetically diverse MSSA ST398 isolates. The acquisition of the phage likely plays a role in the increasing ability of the lysogenic ST398 isolates to colonize human. [...]

Reference

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Methicillin-Susceptible ST398 *Staphylococcus aureus* Responsible for Bloodstream Infections: An Emerging Human-Adapted Subclone?

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Introduction

Primarily described in Europe, ST398 methicillin-resistant *S. aureus* (MRSA) is now a worldwide threat associated with livestock, their human contact and food products [1]. Association with animals or animal farming is recognized as major risk factor for infections associated with ST398 MRSA. Skin, soft tissue and invasive infections are mainly associated with isolates of spa-types t011, t034 or t108 [1]. Beside this, there are few recent reports of infections caused by ST398 *S. aureus* in persons lacking livestock-associated (LA) risk factors. Most of these involve MSSA of spa-type t571 [2-4]. Previous characterization of ST398 pig-borne isolates have indicated that most are MRSA strains of *agr* type I, do not contain any genes encoding the major staphylococcal virulence factors, harbour genes involved in the colonization and the early stages of infections such as the *cna* gene and the hyaluronidase gene and appear deficient in the type I restriction system (*kodS-kodR*) [5]. This is a system whose main function is to limit horizontal gene transfer. Unlike the pig-borne strains, ST398 isolates associated with infections in persons lacking LA risk factors are mostly MSSA strains, harbor *cdaC-cadM* prophagic genes shared with USA300 (ST8) virulent clone, and include remnants of SCCme Cul cassette [2,4].

Reporting of *Staphylococcus aureus* bloodstream infections (BSI) is often mandatory. Reduction of hospital-acquired (HA) MRSA BSI rates is a performance target [6]. A longitudinal survey of BSI under way since 2002 in the region Centre of France showed a decreasing incidence rate of *S. aureus* BSI from 2002 to 2006 and an increased incidence of methicillin-sensitive *S. aureus* (MSSA) BSI since 2006 [7]. The emergence of MSSA ST398 BSI cases was described in the same context in 2009 [2].
To assess the incidence of ST398 BSI cases at a national level, we screened for ST398 all *S. aureus* BSI cases diagnosed into three noncontiguous French regions and comprising a total of 9,329 acute-care beds, participated in the study: three located into three noncontiguous French regions and comprising a microbiological study of *S. aureus* involves an annual 3-month survey of all cases of BSI, and a previously reported [7], a BSI surveillance program which have been conducted in 2007, 2008, 2009 and 2010. The survey covered 2,436,583 acute-care patient days (PD). *S. aureus* BSI were defined according to international definitions (CDC). The variables studied included patient age and sex, portal of entry (skin [primitive cutaneous form or superinfection of a skin breach], surgical site, lungs, urine, intravascular device, or digestive tract), BSI origin (community-acquired or nosocomial), death within 7 days of BSI diagnosis, and duration of hospital stay. The data were analyzed with Epi Info version 6 software. Ethical approval of the BSI surveillance program was obtained at the national level from the Réseau Alerte Investigation Surveillance des Infections Nosocomiales (RAISIN).

**Materials and Methods**

**BSI epidemiological survey method**

**Study population.** Thirty-one healthcare institutions (HCIs) located into three noncontiguous French regions and comprising a total of 9,329 acute-care beds, participated in the study: three university hospitals, – one located in the Centre region (1309 beds), one in the Franche-Comté region (1112 beds) and one in the Bretagne region (1330 beds) –, and 28 HClIs located in the Centre region and comprising six general hospitals (364 to 580 beds), 10 private clinics (68 to 344 beds) and 12 local hospitals (58 to 141 beds). **Study design.** Following methods and a study design previously reported [7], a BSI surveillance program which involves an annual 3-month survey of all cases of BSI, and a microbiological study of *S. aureus* strains isolated from BSI cases have been conducted in 2007, 2008, 2009 and 2010. The survey covered 2,436,583 acute-care patient days (PD). *S. aureus* BSI were defined according to international definitions (CDC). The variables studied included patient age and sex, portal of entry (skin [primitive cutaneous form or superinfection of a skin breach], surgical site, lungs, urine, intravascular device, or digestive tract), BSI origin (community-acquired or nosocomial), death within 7 days of BSI diagnosis, and duration of hospital stay. The data were analyzed with Epi Info version 6 software. Ethical approval of the BSI surveillance program was obtained at the national level from the Réseau Alerte Investigation Surveillance des Infections Nosocomiales (RAISIN).

**Bacteriology**

BSI-associated *S. aureus* isolates were collected during each of the four three-month periods and conserved at -80°C. The isolates were identified as *S. aureus* according to routine methods in each laboratory. All *S. aureus* isolates were then sent to the laboratory of the Réseau des Hygiénistes du Centre. Twelve isolates representative of the major European pig-borne ST398 MSSA and MRSA strains, and three unrelated virulent strains were also studied. **Antimicrobial susceptibility testing.** The disk diffusion method (Bio-Rad, France) was used to test the antibiotic susceptibilities of all isolates. According to the French National Committee recommendations [8], the antibiotics tested were penicillin G, cefoxitin, erythromycin, lincomycin, pristinamycin, tetracycline, kanamycin, tobramycin, gentamicin, rifampin (rifampicin), fusidic acid, fosfomycin, pefloxacin, cotrimoxazole, vancomycin, and teicoplanin. **ST398 PCR.** Detection of ST398 among *S. aureus* isolates first revealed low diversity among antibiotic susceptibility patterns (table 2). The MRSA isolate was found resistant to tetracycline only; the 17 respectively, by PCR amplification using a procedure previously described by Jarraud et al. [10]. spa-types were determined for all isolates as previously described and were assigned through the spa-type database www.ridom.de/spaserver [11]. Phage integrase multiplex PCR. Sequences corresponding to seven prophagic genes were sought by PCR amplification using a procedure previously described by Goerke et al. [12]. **VNTR analysis.** According to François et al. [13], multiplex PCR amplifications with eight primer pairs that target gene regions with variable numbers of tandem repeats were resolved by microcapillary electrophoresis and automatically assessed by cluster analysis to compare ST398 BSI isolates with pig-borne ST398 MSSA and MRSA isolates and reference strains. **PFGE.** PFGE was performed according to the previously described procedure [14]. Apal, EagI and CfoI PFGE patterns were analyzed with the Gelcompar package (Gelcompar software, Applied Maths, France) and interpreted according to international recommendations [14].

**Statistical data**

Chi-square tests and Fisher’s exact test (two-tailed) were used to test associations, and a P value of 0.05 was considered significant.

**Results**

During the study period, 858 *S. aureus* BSI were diagnosed in 566 males and 292 females. BSI were associated with MSSA in 670 cases (78.1%) and MRSA in 188 (21.9%). Among the 858 BSI, 556 were hospital-acquired (HA) (64.8%), resulting in a mean incidence of 0.352 per 1,000 PD (table 1). The HA-BSI incidence increased 7.1% during the study (0.239 in 2007 vs 0.256 in 2010, table 1) due mostly to the 20.4% increase of MSSA BSI incidence (0.167 in 2007 vs 0.201 in 2010) whilst MRSA declined by 23.5% (0.071 in 2007 vs 0.055 in 2010). The portal of entry was known in 667 cases (77.7%). The increase in *S. aureus* HA-BSI incidence was mainly due to MSSA HA-BSI associated with surgical sites (0.035 in 2007 vs 0.043 in 2010) or intravenous devices (0.050 in 2007 vs 0.067 in 2010) (table 1). By contrast, incidence of IVD-associated and surgery-associated MRSA BSI did not vary during the study period. Among 755 patients (88.0%) whose survival outcome was documented in the database, 80 deaths (10.6% mortality) were documented.

Seven hundred and eighty-five isolates were available for microbiological analysis (91.5%). PCR targeting *sau1-1*, followed by MLST for positive isolates, demonstrated that 18 (17 MSSA, 1 MRSA) out of these 785 isolates (2.3%) belonged to ST398. Prevalence was the highest among MSSA (17/615, 2.8% vs 1/170, 0.6% for MRSA; p = .072). ST398 BSI incidence showed a marked increase during the study period (0.002 in 2007 vs 0.014 in 2010). Patients with ST398 BSI did not differ from patients with non ST398 BSI by gender nor age. The portal of entry was known in 14 cases (77.8%) (table 2). BSI associated with a surgical site and those related to IVD were the most frequent (4/14, 28.6% for each of the two portals of entry). A digestive portal of entry was found for three BSI cases. Digestive portals of entry were over six times more common among ST398 compared with other cases (3/14 [21.4%] for ST398 vs 23/633 [3.5%]; p = .014). The occurrence of death within 7 days of diagnosis was documented in all cases and two deaths were documented among the 18 cases (11.1%). Examination of patient history did not reveal any exposure to animal husbandry or retail meat products among the 18 ST398 cases.

Microbiological study of the 18 ST398 isolates first revealed low diversity among antibiotic susceptibility patterns (table 2). The MRSA isolate was found resistant to tetracycline only; the 17
MSSA isolates were susceptible to all tested antibiotics (n = 2, 11.8%) or only resistant to erythromycin (n = 15, 88.2%). Significant associations were found, first, between ST398 MSSA isolates and the sole resistance to erythromycin (15/17, 88.2% for ST398 vs 52/598, 8.7% for nonST398 MSSA; p < .0001), and second, between ST398 MRSA and tetracycline resistance (1/1, 100% for ST398 vs 1/169 0.6% for non ST398 MRSA; p = .012).

Spa typing identified seven related spa-types. The two spa-types t571 and t1451, that are infrequently associated with pig-borne isolates, were found with 9 and 4 BSI isolates, respectively. No isolate was assigned to classical pig-borne spa-types (i.e. t011, t034 or t108). None of the 18 ST398 isolates were positive for tst nor luk genes. Using phage integrase multiplex PCR, Sa3int phage was found in all ST398 isolates (figure 1). The prophagic content differed with the pig-borne isolates (figure 1). None of the 12 pig-borne isolates harbored Sa3int phage; one isolate didn’t harbor any phage, three isolates harbored Sa6int phage, 5 isolates harbored Sa2int phage and two isolates harbored both Sa2int and Sa6int phages. MLVA distinguished clearly all the studied ST398 from the three non ST398 reference S. aureus isolates (figure 1). The population of ST398 isolates was divided into four divisions, named I to IV. Division I clustered the t571 MRSA pig-borne isolate and all the t571 MSSA BSI isolates. Division II was divided into two branches: IIA containing all t1451 BSI isolates, and IIB clustering all the t108 MSSA and MRSA pig-borne isolates. Division III was subdivided in IIIA and IIIB. IIIA clustered the MRSA t899 BSI isolate with the MRSA t899 pig-borne isolates, and IIIB clustered the MRSA and MSSA t011 pig-borne isolates. Division IV was subdivided into two branches. IVA comprising comprised two MSSA BSI isolates respectively characterized by spa-types 5635 and 6605, and IVB clustering the pig-borne MRSA t034 isolates. PFGE using ApaI, EagI and Crf9I restriction enzymes revealed 3, 6 and 3 pulsotypes, respectively, mostly clustered into a homogenous division with UPGMA analysis.

**Discussion**

The study confirms the emergence among French patients of BSI due to ST398, a *Staphylococcus aureus* strain formerly found primarily in association with pigs [2]. The incidence suggests a concurrent increase of MSSA BSI and ST398 BSI. In addition, BSI related to surgical site or an IVD were found preponderant among ST398 BSI cases and among increasing HA-MSSA BSI. This suggests that the increasing incidence of ST398 may play a

| Year   | Number of BSI incidence | Sex of the patient | Portal of entry of the BSI | Death within 7 days |
|--------|-------------------------|--------------------|---------------------------|--------------------|
| BSI    | M | F | Skin | Surgical Site | Pulmonary | Urinary | IVD | Digestive | others | Unknown | 2007 | 2008 | 2009 | 2010 | 2007 | 2008 | 2009 | 2010 | 2007 | 2008 | 2009 | 2010 | All | CA | HA |
| CA all | 251 | 0.103 | 162 | 89 | 86 | 27 | 13 | 6 | 33 | 86 | 21 | 27 nk | 54 (83 nk) |
| 2007  | 50 | 0.083 | 28 | 22 | 12 | 6 | 2 | 3 | 12 | 15 | 3 | 2 nk | 3 (2 nk) |
| 2008  | 64 | 0.103 | 42 | 22 | 24 | 7 | 5 | 3 | 25 | 6 (2 nk) |
| 2009  | 66 | 0.105 | 45 | 21 | 23 | 4 | 5 | 3 | 11 | 20 | 3 (10 nk) |
| 2010  | 71 | 0.121 | 47 | 24 | 27 | 10 | 1 | 7 | 26 | 9 (13 nk) |
| HA all | 419 | 0.172 | 280 | 139 | 50 | 82 | 30 | 30 | 128 | 15 | 18 | 66 | 33 (56 nk) |
| 2007  | 101 | 0.167 | 69 | 32 | 14 | 21 | 6 | 10 | 30 | 3 | 3 | 14 | 15 (1 nk) |
| 2008  | 97 | 0.156 | 68 | 29 | 12 | 18 | 7 | 6 | 27 | 3 | 5 | 19 | 11 (1 nk) |
| 2009  | 103 | 0.164 | 73 | 30 | 12 | 18 | 9 | 7 | 32 | 4 | 4 | 17 | 4 (26 nk) |
| 2010  | 118 | 0.201 | 70 | 48 | 12 | 25 | 8 | 7 | 39 | 5 | 6 | 16 | 3 (28 nk) |
| MRSA  | 188 | 0.077 | 124 | 64 | 45 | 18 | 21 | 25 | 30 | 5 | 5 | 39 | 26 (20 nk) |
| CA all | 51 | 0.021 | 36 | 15 | 18 | 7 | 7 | 2 | 17 | 4 (7 nk) |
| 2007  | 9 | 0.015 | 5 | 4 | 1 | 1 | 2 | 5 | 2 (1 nk) |
| 2008  | 15 | 0.024 | 9 | 6 | 5 | 3 | 3 | 4 | 2 |
| 2009  | 13 | 0.021 | 11 | 2 | 5 | 3 | 2 | 1 | 5 (0 nk) |
| 2010  | 14 | 0.024 | 11 | 3 | 7 | 3 | 1 | 3 | 0 (2 nk) |
| HA all | 137 | 0.056 | 88 | 49 | 27 | 18 | 14 | 18 | 30 | 5 | 3 | 22 | 22 (13 nk) |
| 2007  | 43 | 0.071 | 28 | 15 | 6 | 6 | 4 | 4 | 11 | 3 | 2 | 7 | 7 (1 nk) |
| 2008  | 33 | 0.053 | 20 | 13 | 10 | 6 | 4 | 2 | 5 | 1 | 5 | 5 (1 nk) |
| 2009  | 29 | 0.046 | 19 | 10 | 5 | 2 | 3 | 7 | 6 | 1 | 5 | 5 (5 nk) |
| 2010  | 32 | 0.055 | 21 | 11 | 6 | 4 | 3 | 5 | 8 | 1 | 5 | 5 (6 nk) |
| All   | 858 | 0.352 | 566 | 292 | 181 | 100 | 78 | 68 | 158 | 26 | 56 | 191 | 80 (103 nk) |
| CA    | 302 | 0.124 | 198 | 104 | 104 | 34 | 20 | 6 | 35 | 103 | 25 (34 nk) |
| HA    | 556 | 0.228 | 368 | 188 | 77 | 100 | 44 | 48 | 158 | 20 | 21 | 88 | 55 (69 nk) |

1Intra-Venous Devices
2nk not known.

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role within the global trend toward increased incidence of MSSA BSI. More generally, however, the emergence of ST398 human infections may offer insight into the genetics of bacterial adaptation to human infection.

The precise characterization of 18 strains of ST398 revealed new characteristics of this emerging S. aureus clone. First and foremost, all the BSI isolates, but none of the porcine isolates, incorporated the Sa3int phage. We also showed that the 18 BSI isolates differed greatly from “classical” pig-borne isolates with respect to Spa-type and antimicrobial susceptibility. The very prevalent pig-borne Spa-types t011, t034 and t108 were not found in ST398 BSI isolates, belonging mainly to the Spa-types t571 and t1451. In addition, all but one of our ST398 BSI strains had completely homogenous resistance patterns (resistant to erythromycin only). This confirms their difference from pig-borne clones, whose heterogeneity of resistance patterns and Spa-types suggests a rapidly evolving group [15].

The ST398 BSI isolates also showed substantial diversity. Only one pair presented strictly similar Spa-type, PFGE and MLVA patterns, and 17 were distributed into multiple distinct divisions designated by Spa-typing and MLVA. Within all of these MLVA divisions, ST398 BSI isolates clustered at varying distances from pig-borne ST398 isolates. The single MRSA BSI isolate was very close from an MRSA t699 pig-borne isolate. The 17 MSSA ST398 BSI isolates, in contrast, in the 17 other cases, clustered at a higher genetic distance not only from pig-borne isolates, but also from other ST398 isolates within the same divisions.

This diversity may be related to the ST398’s apparent shift toward human hosts. Deficiency in SauI type I restriction system, resulting in hypersusceptibility to acquisition of foreign genes, have been described in ST398 clone [16]. This would account for both the rapid acquisition of genetic diversitiy, and, in particular, to the acquisition of virulence genes for a particular host. Our observations regarding the genome content of the ST398 BSI isolates suggest that their emergence could have arisen as a consequence of recent horizontal transfers in diverse ST398 pig-borne isolates.

Horizontal transfer of phage allows bacteria to adapt to specific niches, and may lead to the emergence of new clones. Prophagic content differences have been hypothesized between pig-borne and human-adapted ST398 strains [4,17]. In our study, the Sa3int phage distinguished all ST398 BSI isolates from all porcine isolates tested (figure 1). Pig-borne SR398 isolates were associated with colonization and virulence in humans [12]. This lends weight to the hypothesis that a human-adapted ST398 recently evolved from pig-borne strains, and is able to accept genes conferring the ability to colonize humans, possibly by virtue of the Sa3int phage with which it is associated. This would explain the sudden ability of the emerging ST398 to infect humans in the absence of exposure to livestock or meat.

The nature of the precise gene transfer events that occurred between human and animal isolates is not elucidated. Different hypotheses for the origin of ST398 clones in different species may be formulated. The different ST398 clones could have originated from the chromosomal diversification of a common ancestor. The lateral transfer hypothesis, however, is supported by recent

| Isolate code | Date and site of isolation | Characteristics of the patient | Characteristics of the BSI | Antibiotic resistance | Spa-type | PFGE patterns |
|--------------|---------------------------|--------------------------------|---------------------------|----------------------|----------|---------------|
|              | year | Region¹ | Sex | Age | Origin (Community- or Hospital-acquired) | Portal of entry | Death within 7 days | M | E | Te | Apa | Eag | CrfN |
| 89           | 2010 | C       | M   | 49  | HA Digestive                        | Surgical Site   | +                | +         | 899 | A   | A   | A   |     |     |
| 90           | 2008 | B       | M   | 63  | HA Surgical Site                   |                | +                |           | 571 | B   | B   | C   |     |     |
| 91           | 2010 | B       | M   | 53  | CA not known                       |                | +                |           | 571 | B   | B   | C   |     |     |
| 92           | 2007 | C       | M   | 75  | HA IVD                             |                | +                |           | 5635| B   | C   | C   |     |     |
| 93           | 2009 | C       | M   | 58  | HA IVD                             |                | +                |           | 1451| B   | C   | C   |     |     |
| 94           | 2009 | B       | M   | 59  | CA not known                       |                | +                |           | 571 | B   | C   | C   |     |     |
| 95           | 2009 | B       | M   | 80  | HA Skin                            |                | +                |           | 571 | B   | C   | C   |     |     |
| 96           | 2009 | FC      | M   | 73  | HA Skin                            |                | +                |           | 6605| B   | C   | C   |     |     |
| 97           | 2010 | C       | M   | 84  | HA Surgical Site                   |                | +                |           | 1451| B   | C   | C   |     |     |
| 98           | 2010 | C       | M   | 59  | HA Digestive                       |                | +                |           | 1451| B   | C   | C   |     |     |
| 99           | 2010 | FC      | M   | 74  | HA Pulmonary                       |                | +                |           | 571 | B   | C   | C   |     |     |
| 100          | 2010 | C       | F   | 69  | HA IVD                             |                | +                |           | 9378| B   | C   | C   |     |     |
| 101          | 2008 | C       | M   | 36  | HA IVD                             |                | +                |           | 571 | B   | D   | C   |     |     |
| 102          | 2009 | C       | F   | 69  | HA Surgical Site                   |                | +                |           | 571 | B   | D   | C   |     |     |
| 103          | 2009 | C       | M   | 68  | HA Surgical Site                   |                | +                |           | 571 | B   | D   | C   |     |     |
| 104          | 2010 | B       | M   | 85  | CA not known                       |                | +                |           | 571 | B   | D   | C   |     |     |
| 105          | 2008 | B       | M   | 77  | CA not known                       |                | +                |           | 1451| B   | F   | C   |     |     |
| 106          | 2010 | C       | F   | <1  | HA Digestive                       |                | +                |           | 571 | C   | E   | B   |     |     |

¹E Franche-Comté, C Centre and W Bretagne region
²Intra-Venous Devices
³Antibiotic resistance to methicillin M, erythromycin E or tetracycline Te.
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genomic data demonstrating that the different clones present highly similar core genome, but may be distinguished by highly distinct prophagic elements [17]. The different clones could have originated from horizontal transfer between an isolate with human origin and an isolate with animal origin. This hypothesis is supported by human to animal host switch described already within S. aureus species, with avian adaptation of ST5 human clone [18]. Further work should be done to better understand how the different ST398 clones emerged and spread.

This human-adapted ST398 subclone is increasingly identified in our hospitals [2,19]. It appears to be highly receptive for horizontal gene transfer. Environmental proximity being a dominant factor in determining horizontal transfer, further acquisition of additional genetic elements harboring virulence and antibiotic resistance determinants could arise. For instance, rare cases of PVL-producing ST398 isolates have been reported, mostly from humans lacking livestock exposure [1,20,21]. Here, we identified one isolate of spa-type t899, which may result from recombination with strain belonging to the clone ST9. In S. aureus, variation of spa-type has been described previously as a result of replacement of a large part of chromosome by DNA isolates from other lineages. For instance, the epidemic ST239 has emerged after such a genetic event [22]. Presence of exogenous spa gene with type t899 has been reported previously in ST398 [5] suggesting that recombination would occur frequently in this genetic background. Further investigations are needed to determine if this phenomenon could drive the evolution of ST398 clones.

Much is known over pig-borne MRSA ST398 transmission between animals and humans and between humans [1]. Occupational exposure to pig results in frequent acquisition of pig-borne ST398 MSSA and MRSA isolates and 3 reference strains (COL, MSSA476 and MW2). On the basis of their MLVA fingerprints, the isolates segregated into different clusters. Results of antibiotic susceptibility tests (resistance to Oxa (methicillin), Te (tetracycline), E (erythromycin), K (kanamycin), To (tobramycin)), spa-typing, as well as determination of prophage content of Sa1int to Sa7int genes assessed by Phage integrase multiplex PCRs, are also indicated. The BSI isolates appear underlined with grey colour in the figure.

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Figure 1. Phage content and MLVA dendrogram obtained with the 18 strains of ST398 BSI isolates and 12 strains of ST398 representing the diversity of the pig-borne clone. The dendrogram was calculated using the MLVA data obtained for18 ST398 BSI isolates, 12 pig-borne ST398 MSSA and MRSA isolates and 3 reference strains (COL, MSSA476 and MW2). On the basis of their MLVA fingerprints, the isolates segregated into different clusters. Results of antibiotic susceptibility tests (resistance to Oxa (methicillin), Te (tetracycline), E (erythromycin), K (kanamycin), To (tobramycin)), spa-typing, as well as determination of prophage content of Sa1int to Sa7int genes assessed by Phage integrase multiplex PCRs, are also indicated. The BSI isolates appear underlined with grey colour in the figure.

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