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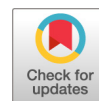
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Vaginal Tampon Colonization by *Staphylococcus aureus* in Healthy Women

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ABSTRACT Tampons recovered from a cohort of 737 healthy women (median age, 32 years) were analyzed for the presence of *Staphylococcus aureus*. A total of 198 tampons (27%) were colonized by *S. aureus*, 28 (4%) by a strain producing toxic shock syndrome toxin 1 (TSST-1). *S. aureus* was detected more frequently in tampons that did not require an applicator for their insertion (74/233 [32%] versus 90/381 [24%]; odds ratio [OR] = 1.51 [95% confidence interval, 1.04 to 2.17]) and in women who used an intrauterine device for contraception (53/155 [34%] versus 145/572 [27%]; OR = 1.53 [95% confidence interval, 1.05 to 2.24]). The *S. aureus* strains isolated from tampons belonged to 22 different clonal complexes (CCs). The most prevalent CC was CC398 *agr1* ($n = 57$ [27%]), a clone that does not produce superantigenic toxins, followed by CC30 *agr3* ($n = 27$, 13%), producing TSST-1 (24/27 [89%]), the principal clone of *S. aureus* involved in menstrual toxic shock syndrome (MTSS).

IMPORTANCE Menstrual toxic shock syndrome (MTSS) is an uncommon severe acute disease that occurs in healthy menstruating women colonized by TSST-1-producing *S. aureus* who use intravaginal protection, such as tampons and menstrual cups. The catamenial product collected by the protection serves as a growth medium for *S. aureus* and allows TSST-1 production. Previous studies evaluated the prevalence of genital colonization by *S. aureus* by vaginal swabbing, but they did not examine tampon colonization. This study demonstrated a high prevalence of tampon colonization by *S. aureus* and the presence of the CC30 TSST-1 *S. aureus* clone responsible for MTSS in tampons from healthy women. The results support the vaginal carriage of this lineage in healthy women. In addition, the higher prevalence of *S. aureus* within tampons that do not require an applicator indicates a crucial role for handwashing before tampon handling to decrease the risk of tampon contamination.

KEYWORDS *Staphylococcus aureus*, colonization, healthy women, menstruation, vagina

Menstrual toxic shock syndrome (MTSS) is an uncommon severe acute disease characterized by fever, hypotension, and multiorgan failure (1). It occurs in healthy menstruating women colonized by *Staphylococcus aureus* that produces the toxic shock syndrome toxin 1 (TSST-1) who have no protective antibodies against this toxin and use intravaginal protection, such as tampons or menstrual cups (1–3). The catamenial products collected by the intravaginal protection serve as a growth medium, allowing *S. aureus* proliferation and TSST-1 production (4). TSST-1 then gains

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access to the bloodstream and induces systemic illness (5). Vaginal carriage of *S. aureus* with a TSST-1-producing isolate represents the main risk factor for the development of MTSS in susceptible women (3).

S. aureus is an opportunistic pathogen that expresses a variety of virulence factors. Among them, TSST-1 and staphylococcal enterotoxins (SEs) belong to the family of superantigens (6). These toxins are able to corrupt the immune response by triggering aberrant T-cell activation with massive cytokine release, which is responsible for the shock observed in both MTSS and non-MTSS (1, 2, 6). The majority of human isolates of *S. aureus* are capable of producing at least one of these superantigens (6). However, only a portion of *S. aureus* isolates carry the TSST-1 gene (*tst*). *tst* is carried by mobile genetic islands, staphylococcal pathogenicity islands, inserted in the *S. aureus* chromosome (7). *tst* has been found in at least eight different *S. aureus* genetic lineages, the clonal complexes (CCs): CC1, CC5, CC8, CC12, CC22, CC30, CC45, and CC59 (8–13).

Interestingly, since the description of MTSS, most of the clinical isolates recovered from MTSS have been of the CC30 *S. aureus* lineage, initially described as bacteriophage lytic group I (8, 14). The origin of the epidemiological link between the TSST-1-producing CC30 *S. aureus* lineage and MTSS remains to be determined, but it may reflect the prevalence of vaginal carriage in healthy women who use tampons.

The aim of the present study was to determine, from tampons recovered from healthy women, the rate of tampon colonization by *S. aureus*, the factors contributing to *S. aureus* tampon colonization, and the characterization of the genetic lineages and toxin gene contents of the *S. aureus* isolates.

RESULTS

Cohort description. Of the 746 female volunteers who participated, 9 reported being hospitalized in the past for complications occurring during the use of periodic tampons or menstrual cups that could potentially correspond to MTSS. In the absence of additional information about the clinical episode, they were considered ineligible for the study. The 737 remaining women included in this study are described in Table 1. Only one volunteer had a history of salpingitis. Antibiotic treatment within the 15 days prior to sampling included beta-lactam antibiotics ($n = 13$ amoxicillin, $n = 4$ amoxicillin-clavulanic acid, $n = 1$ cefuroxime, $n = 1$ cefpodoxime, $n = 1$ pivmecillinam), cyclines ($n = 4$ doxycycline, $n = 3$ vibramycin, $n = 2$ tetracycline, $n = 1$ physiomycline), fosfomicin ($n = 4$), fluoroquinolones ($n = 1$ ciprofloxacin, $n = 1$ ofloxacin, $n = 1$ norfloxacin), azithromycin ($n = 1$), spiramycin plus metronidazole ($n = 1$), and mupirocin ($n = 1$). A total of 728 participants noted the tampon brand. Tampax was the most commonly used tampon brand, followed by Nett, tampons marketed by the supermarket Carrefour, and Doulys from the supermarket Leclerc (Table 1). Other brands represented less than 5% of the collected tampons. Tampon absorbencies were predominantly between 2 and 4 droplets (515/734 [70%]).

***S. aureus* detection in tampons.** *S. aureus* was detected in 198 tampons (27% [Table 1]). Factors that impacted *S. aureus* tampon colonization are summarized in Table 2. The rate of *S. aureus* colonization was increased in women who used a tampon without an applicator (74/233 [32%]) versus women who used tampons with an applicator (90/381 [24%]; odds ratio (OR) = 1.51 [95% confidence interval, 1.04 to 2.17]; $P = 0.031$) and in women who use intrauterine devices (IUDs) for contraception (53/155 [34%] versus 145/572 [27%]; OR = 1.53 [95% confidence interval, 1.05 to 2.24]; $P = 0.033$). Women with IUDs were significantly older than the other women (median age, 34 years (interquartile range [IQR], 29 to 40 years) versus 31 years (IQR, 25 to 37 years); $P < 0.001$).

S. aureus detection from tampons was not significantly impacted by the women's age ($P = 0.2$), tampon carrying time ($P = 0.3$), tampon brand ($P = 0.6$), tampon absorbency ($P = 0.96$), contraception ($P = 0.07$), antibiotic treatment within the last 15 days ($P = 0.08$), contraception with estrogenic pills ($P = 0.9$), or sexual intercourse within the last 5 days ($P = 0.4$).

TABLE 1 Clinical characteristics of the healthy female volunteers ($n = 737$; 2011 to 2017)

Characteristic	Value ^a
Median age, yrs (IQR)	32 (12)
History of salpingitis	1/727 (0.1)
Antibiotics within the last 15 days	39/727 (5)
Contraception	
None	327/723 (45)
Estroprogestative pills	243/723 (34)
Intrauterine devices	153/737 (21)
Sexual intercourse within the last 5 days	212/728 (29)
Tampon brands	728 (99)
Tampax	309 (42)
Nett	199 (27)
Carrefour	44 (6)
Doulys	34 (5)
Natracare	20 (3)
OB	17 (2)
Labell	15 (2)
Casino	12 (2)
Auchan	11 (2)
U	11 (2)
Siempre	10 (1)
Others ^b	36 (5)
Tampon absorbencies	
1 droplet	12/583 (2)
2 droplets	149/583 (26)
3 droplets	237/583 (41)
4 droplets	125/583 (21)
5 droplets	26/583 (4)
6 droplets	34/583 (6)
Tampon with applicator	381/614 (62)
Tampon carrying time, median (IQR)	4 h 5 min (2 h)
Tampon colonized by <i>S. aureus</i>	198/737 (27)
Detection of one <i>S. aureus</i> strain	191/737 (26)
Detection of two different <i>S. aureus</i> strains	7/737 (1)

^aData are given as no./total (percent) or no. (percent) unless otherwise noted.

^bOnly tampon brands used by ≥ 10 participants are reported.

***S. aureus* characteristics.** Based on colony morphology, only one *S. aureus* strain was isolated per tampon for 191 tampons, whereas two different *S. aureus* strains were coisolated from 7 tampons (Table 1). The 205 *S. aureus* strains were genotyped by DNA microarrays. CC, *agr* type, *mecA*, and the superantigen gene contents of all strains are described in Table 3. The *S. aureus* strains belonged to 22 different CCs. The most prevalent CC was CC398 ($n = 56$ [27%]), followed by CC30 ($n = 27$ [13%]), CC8 ($n = 24$ [12%]), CC5 and CC45 ($n = 19$ [9%]), and CC15 ($n = 15$ [7%]). The other CCs represented less than 5% of the CCs. One hundred twenty-eight strains were *agr1* (62%), 39 *agr2* (19%), 34 *agr3* (17%), and 4 *agr4* (2%). Only six strains were *mecA* positive, four belonged to CC5 *agr2*, and two belonged to CC8 *agr2*. A total of 108/205 (53%) vaginal strains contained at least one gene encoding one of the superantigens. Twenty-eight strains (14%) were *tst* positive: 24 were CC30 (86%), 2 were CC5 (7%), 1 was CC8 (4%), and 1 was CC22 (4%). Concerning the other superantigen genes, *sea* was detected in 32 strains (16%), *seb* in 10 strains (5%), *sec* and *sel* in 20 strains (10%), *sed*, *sej*, and *ser* in 12 strains (6%), the enterotoxin gene cluster *egc* (containing *seg*, *sei*, *sem*, and *sen* and/or *seo*) in 87 strains (42%), *seh* in 9 strains (4%), and *sek* and *seq* in 7 strains (3%). *tst* was significantly associated with the detection of *sea* (18/28 [64%] versus 14/177 [8%], $P < 0.001$), *egc* (27/28 [96%] versus 60/177 [34%], $P < 0.001$) and *seh* (4/28 [14%] versus 5/177 [3%], $P = 0.022$). *tst* was associated with *agr3* (24/28 [86%] versus 4/177 [2%], $P < 0.001$). *tst*, *sea*, *egc*, and *seh* were significantly associated with CC30 (24/27

TABLE 2 Factors that influence *S. aureus* tampon colonization

Characteristic	Value for healthy women volunteers ^a		P
	<i>S. aureus</i> positive (n = 205)	<i>S. aureus</i> negative (n = 532)	
Median age, yrs (IQR)	31 (12)	32 (11)	0.1
Tampon carrying time, median (IQR)	4 h 0 min (2 h 50 min)	4 h 20 min (3 h 0 min)	0.3
Tampon brands			
Tampax	79/309 (26)	230/309 (74)	0.6
Nett	59/197 (28)	138/197 (72)	
Other ^b	60/216 (28)	156/216 (72)	
Tampon with applicator	90/164 (55)	291/450 (65)	0.031
Absorbencies			0.96
1 and 2 droplets	33/157 (21)	128/426 (30)	
3 and 4 droplets	106/157 (68)	256/426 (60)	
5 and 6 droplets	18/157 (11)	42/426 (10)	
Antibiotics within the last 15 days	7/197 (4)	38/529 (7)	0.08
Contraception			
None	78/327 (24)	249/327 (76)	0.07
Esteroprogestative pills	67/196 (34)	176/527 (33)	0.9
Intrauterine devices	53/198 (28)	102/529 (19)	0.033
Sexual intercourse within the last 5 days	62/194 (32)	150/524 (29)	0.4

^aData are given as no./total (percent) unless otherwise noted.

^bBrands of tampon other than Tampax and Nett were clustered into one group for the statistical analysis.

[89%] versus 3/178 [2%]; 21/27 [78%] versus 12/178 [7%], $P < 0.001$; 27/27 [100%] versus 61/178 [34%], $P < 0.001$; 4/27 [15%] versus 5/178 [3%], $P = 0.02$). Of the seven tampons with two strains, four were colonized with *tst*-positive CC30. In six tampons, including those four, the *agr* types of the strains codetected in the tampons were different (Table 4).

Among the subset of women with tampons colonized by *S. aureus*, those who declared sexual intercourse within the last 5 days were less frequently *tst* positive than the others (3/62 [4%] versus 22/132 [17%], $P = 0.022$; OR = 0.25 [95% confidence interval, 0.07 to 0.88]). No other characteristics listed in Tables 1 and 2 had an impact on *S. aureus* *tst* positivity.

DISCUSSION

In addition to the use of intravaginal protection, vaginal colonization with TSST-1-producing organisms combined with the absence of protective antibodies against TSST-1 represents the major risk factor for the development of MTSS in susceptible women (3). Due to the low incidence of the disease (1/100,000), factors that are associated with an increased risk of MTSS are mainly investigated individually in healthy women (3, 15–20). Using this strategy, we examined the factors that concur with vaginal colonization by *S. aureus* in tampon users.

Few published studies have examined the factors that influence vaginal colonization by *S. aureus*. In these studies, the vaginal sampling was done by swabbing (16–20), whereas we recently showed that using the vaginal tampon for sampling maximizes the detection of vaginal colonization, especially during menstruation (15). In addition, with regard to MTSS, determination of tampon colonization by *S. aureus* appears to be more relevant than vaginal swabs because TSST-1 is preferentially produced by *S. aureus* in the tampon (21, 22).

In this study, we examined factors that contribute to tampon colonization by *S. aureus* in women, with special attention on TSST-1-producing isolates. Only tampons from women without a history of MTSS were included in the study to exclude

TABLE 3 Characteristics of *S. aureus* strains^a

Clonal complex	<i>agr</i> type	Superantigen gene(s)	Presence of <i>mecA</i>	No. of strains (n = 205)
CC1	<i>agr3</i>	<i>sea, seb, seh, sek, seq</i>		2
		<i>sea, seh, sek, seq</i>		2
		<i>seh, sek</i>		1
CC5	<i>agr2</i>	<i>tst, sec, sel, sed, sej, ser, egc</i>	Yes	1
		<i>tst, sed, sej, ser, egc</i>	Yes	1
		<i>sea, egc</i>		1
		<i>sed, sej, ser, egc</i>	Yes	1
		<i>sed, sej, ser, egc</i>	Yes	1
		<i>sed, sej, ser, egc</i>		1
		<i>sed, sej, ser, egc</i>		4
		<i>egc, sep</i>		1
		<i>egc</i>		8
CC6	<i>agr1</i>	<i>sea</i>		3
CC7	<i>agr1</i>	—		6
CC8	<i>agr1</i>	<i>tst</i>		1
		<i>sea, sed, sej, ser</i>	Yes	2
		<i>sea</i>		1
		<i>seb</i>		1
		<i>sec, sel, egc</i>		1
		<i>sed, sej, ser</i>		1
		<i>egc</i>		1
		—		16
CC9	<i>agr2</i>	<i>egc</i>		4
CC12	<i>agr2</i>	<i>seb, sep</i>		1
CC15	<i>agr2</i>	<i>seb</i>		1
		—		14
CC20	<i>agr1</i>	<i>seb, egc</i>		1
		<i>sec, sel, egc</i>		1
CC22	<i>agr1</i>	<i>tst, sec, sel, egc</i>		1
		<i>egc</i>		7
CC25	<i>agr1</i>	<i>egc</i>		2
CC30	<i>agr3</i>	<i>tst, sea, seb, seh, sek, egc</i>		1
		<i>tst, sea, seh, egc</i>		3
		<i>tst, sea, egc</i>		14
		<i>tst, egc</i>		6
		<i>sea, egc</i>		3
CC45	<i>agr1</i>	<i>sec, sel, egc</i>		14
		<i>egc</i>		4
CC45	<i>agr4</i>	<i>egc</i>		1
CC59	<i>agr1</i>	<i>seb, seb, sek, seq</i>		3
CC88	<i>agr3</i>	<i>sec, sel</i>		1
		—		1
CC97	<i>agr1</i>	<i>sec, sel</i>		1
		—		2
CC101	<i>agr1</i>	—		1
CC121	<i>agr4</i>	<i>egc</i>		3
CC182	<i>agr1</i>	<i>egc</i>		1
CC188	<i>agr1</i>	—		1
CC398	<i>agr1</i>	—		56

^aCC, clonal complex; *agr*, accessory gene regulator; *tst*, TSST-1 gene; *sea* to *ser*, staphylococcal enterotoxin A to R genes; *egc*, enterotoxin gene cluster including *seg*, *sei*, *sem*, and *sen* and/or *seo*; *mecA*, methicillin resistance gene A; —, no detection.

overrepresentation of the TSST1-producing *S. aureus* strains (1). Using this procedure, we found in the large cohort of 737 women aged 14 to 52 years that 27% of them carried tampons colonized by *S. aureus*. This is consistent with our previous observation that 26% of used tampons from healthy women are colonized by *S. aureus* (15). Community profiling has shown that healthy vaginal microbial communities are usually dominated by bacteria from the gut and perineum (15). Interestingly, the percentage of tampons colonized by *S. aureus* was 50% higher for tampons that do not require an applicator. This finding is in agreement with a previous observation (16). As the rate of hand colonization by *S. aureus* is approximately 27% (23), we suspect that the observed

TABLE 4 Characteristics of *S. aureus* strains codetected in tampons

Participant no.	Clonal complex	<i>agr</i> type	Superantigen gene(s)
96	CC25	<i>agr1</i>	<i>egc</i>
	CC30	<i>agr3</i>	<i>tst, sea, egc</i>
126	CC22	<i>agr1</i>	<i>egc</i>
	CC398	<i>agr1</i>	—
137	CC15	<i>agr2</i>	<i>tst, sed, sej, ser, egc</i>
	CC25	<i>agr1</i>	<i>egc</i>
256	CC15	<i>agr2</i>	—
	CC30	<i>agr3</i>	<i>tst, sea, egc</i>
273	CC5	<i>agr2</i>	<i>egc</i>
	CC398	<i>agr1</i>	—
291	CC22	<i>agr1</i>	<i>egc</i>
	CC30	<i>agr3</i>	<i>tst, sea, egc</i>
650	CC121	<i>agr4</i>	<i>seb, egc</i>
	CC30	<i>agr3</i>	<i>tst, sea, egc</i>

difference is related to manual contamination of the tampon during handling for its insertion in the absence of an applicator. Unfortunately, the current design of the study did not include an investigation of *S. aureus* colonization at other body sites, such as the nose, hand, and perineum, to confirm our hypothesis. However, our observation supports the importance of handwashing before and after tampon handling to decrease the risk of tampon contamination and the advantage of an applicator for hygiene during tampon handling. The other factors associated with a higher rate of tampon colonization by *S. aureus* were IUDs for contraception. IUDs were associated with a 50% increase in *S. aureus* detection, as previously suggested in a small survey (16). The reason for this greater vaginal colonization remains unclear. Our hypothesis is that the IUD itself or its string in the vagina could favor *S. aureus* colonization. Supporting this hypothesis, *S. aureus* is known to be a cause of IUD infection, sometimes with non-MTSS as a complication (24–26). However, IUDs are used as contraception in older women, whereas MTSS predominantly occurs in young women (3).

No other significant relationships with tampon colonization by *S. aureus* were observed in our survey. A history of genital herpes simplex virus infection and socioeconomic status have previously been identified as potential risk factors for vaginal colonization by *S. aureus* but were not investigated in this study (16–18).

Over half of the *S. aureus* isolates were shown to carry at least one enterotoxin gene or *tst* detected by our DNA array. The predominant clone, CC398 *agr1*, representing 27% of the isolates, did not carry superantigen genes. None of our CC398 isolates were methicillin-resistant *S. aureus* (MRSA), suggesting that these isolates belonged to the human CC398 lineage and not to the livestock-associated CC398 methicillin-resistant clones (27, 28). This clone is increasingly detected in Europe and identified as highly transmissible among humans (27–29). The predominance of this clone in humans without superantigen genes suggests that superantigens detected by our DNA array are not mandatory for human colonization.

Interestingly, the second most prevalent CC detected in tampons was the CC30 *agr3* clone. This CC is epidemiologically associated with MTSS (8, 14). CC30 *agr3* was predominantly *tst* positive (89%). However, the CC30 *agr3* clone is not restricted to vaginal colonization and is also found in nasal colonization (28).

We also detected other CCs already described to harbor *tst*: CC1, CC5, CC8, CC12, CC22, CC45, and CC59 (8–13). However, *tst* was detected in only a few isolates of these non-CC30 CCs (7% CC5 and 4% CC8 and CC22). Furthermore, these CCs represented only 14% of the *tst*-positive strains. Regardless of the genetic background of the strain, *tst* was significantly associated with *sea*, *egc*, and *seh*, as described previously (12,

29–32). Our observation confirms that CC30 is the more prevalent genetic background of *tst*-positive vaginal *S. aureus* isolates, as observed in MTSS (3, 8–14, 32). The epidemiological link between the TSST-1 CC30 *S. aureus* lineage and MTSS seems to be related to the prevalence of the clone in the vagina.

Interestingly, *tst* was not the more prevalent superantigen gene detected in tampon isolates. *egc*, a cluster including five different superantigen genes (*seg*, *sei*, *sem*, *sen*, and *seo*), and *sea* were more frequently detected than *tst* (42% and 16%, respectively). In contrast, superantigen genes *seb*, *sec* and *sel*, and *sed*, *sej*, and *ser* were detected less frequently than *tst* (5%, 10%, and 6%, respectively). All of these toxins have been involved in non-MTSS (33, 34). The specific involvement of TSST-1 in MTSS is not explained by the simple prevalence of *tst* among vaginal isolates. The current model involves a specific interaction of TSST-1 with the vaginal mucosa and TSST-1 passage, not efficient with other superantigen toxins (35).

Among women carrying tampons colonized by *S. aureus*, we observed a dramatic decrease in *tst*-positive strains in women who declared sexual intercourse within the last 5 days, with no obvious explanation. The importance of this observation should be moderated by the fact that we did not observe a specific correlation between vaginal colonization by TSST-1-producing strains and any information about the women's health and tampon characteristics or use.

In conclusion, the results from this study confirm the high rate of tampon colonization by *S. aureus* and support the notion that the origin of the epidemiological link between the TSST-1 CC30 *S. aureus* lineage and MTSS is explained by the prevalence of vaginal carriage of this lineage in healthy women. Furthermore, the increased rate of *S. aureus* colonization of tampons that are handled without an applicator supports the importance of handwashing before and after tampon handling to decrease the risk of tampon contamination.

MATERIALS AND METHODS

Ethical review of the study. This study was reviewed and approved by the Ethics Committee (CPP Sud Est IV, Centre Léon Bérard, Lyon, France, no. L16-176). Written consent was obtained from all participants or from the parents/guardians of participants under 18 years of age.

Subjects. Menstruating volunteers were recruited from March 2014 to June 2017 by the National Reference Center for Staphylococci (NRCS) through the gynecology departments of Hospices Civils de Lyon and a national call on social networks and advertising via media. The only inclusion criterion was the use of tampons during menses, regardless of brand, and absence of a potential history of MTSS. Among the volunteers, 76 corresponded to healthy women who participated in a study analyzing the tampon microbiota (15). When multiple tampons were sent to the laboratory by the same woman, only the first tampon received was considered for this study.

Sample collection. For the national tampon collection by autosampling, women were asked to place the tampon in a sterile bag and to send it to the NRCS via express shipping (2-day shipping during business days). Each tampon was accompanied by an information form about the woman's health (i.e., age, history of salpingitis, history of hospitalization for complications occurring during the use of periodic tampons or menstrual cups that could correspond with MTSS, antibiotic use within the last 15 days, contraception methods, and sexual intercourse within the last 5 days) and about the tampon (brand, use of applicator, carrying time).

Menstrual fluid extraction and microbiological analysis. Menstrual fluid was extracted from the tampon by suspending the tampon in 15 ml of sterile distilled water and then pressing it as described previously (15). Fifty microliters of menstrual fluid was spread on a chromogenic plate selective for *S. aureus* (chromID *S. aureus*; bioMérieux, Marcy l'Étoile, France) and incubated at 35°C for 18 to 24 h under aerobic conditions. Suspicious colonies (pink to light pink) were identified by matrix-associated laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (15). All *S. aureus* strains were genotyped using identibac *S. aureus* genotyping (Alere) DNA microarrays as described previously (36), with special regard for accessory gene regulator (*agr*) type, the presence of methicillin resistance gene A (*mecA*), and genes encoding superantigens (*tst* and enterotoxin genes *sea* to *ser*). Isolates were assigned to CCs by comparing the hybridization profiles with those previously characterized by multi-locus sequence typing reference strains (12, 36).

Statistical analysis. The data were analyzed using SPSS Statistics version 21. The results were reported as median and interquartile range (IQR) for nonnormally distributed quantitative variables and as numbers and relative frequencies for categorical variables. Groups were compared using the non-parametric Wilcoxon test for quantitative variables and Fisher's exact test for categorical variables.

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We declare no conflict of interest.

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