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► **To cite this version:**

Myriam Chiaruzzi, Alexia Barbry, Anaëlle Muggeo, Anne Tristan, Isaline Jacquemond, et al.. Vaginal Tampon Colonization by *Staphylococcus aureus* in Healthy Women. *Applied and Environmental Microbiology*, 2020, 86 (18), pp.e01249-20. 10.1128/AEM.01249-20 . hal-02989503v1

**HAL Id: hal-02989503**

**<https://hal.science/hal-02989503v1>**

Submitted on 23 Nov 2020 (v1), last revised 26 Aug 2022 (v2)

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1 Vaginal Tampon Colonization by Staphylococcus aureus in Healthy Women

2  
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12  
13 **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).

14  
15 **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.

16  
17 KEYWORDS Staphylococcus aureus, colonization, healthy women,  
... menstruation,  
18 vagina  
19  
20 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 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).

21  
22 *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.

## 23 24 RESULTS

25 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

25... cefuroxime, n=1 cefpodoxime, n=1 pivmecillinam), cyclines (n=4  
... doxycycline, n=3 vibramycin, n=2 tetracycline, n=1 physiomycline),  
... fosfomycin (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%]).

26  
27 *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}.

28  
29 *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 estroprogestative  
... pills (P=0.9), or sexual intercourse within the last 5 days (P=0.4).

30  
31 *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

31... (24/28 [86%] versus 4/177 [2%],  $P < 0.001$ ). *tst*, *sea*, *egc*, and *seh* were  
... significantly associated with CC30 (24/27 [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).

32

33

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

35 Characteristic Value<sup>a</sup>

36 Median age, yrs (IQR) 32 (12)

37 History of salpingitis 1/727 (0.1)

38 Antibiotics within the last 15 days 39/727 (5)

39 Contraception

40 None 327/723 (45)

41 Estroprogestative pills 243/723 (34)

42 Intrauterine devices 153/737 (21)

43 Sexual intercourse within the last 5 days 212/728 (29)

44 Tampon brands 728 (99)

45 Tampax 309 (42)

46 Nett 199 (27)

47 Carrefour 44 (6)

48 Doulys 34 (5)

49 Natracare 20 (3)

50 OB 17 (2)

51 Labell 15 (2)

52 Casino 12 (2)

53 Auchan 11 (2)

54 U 11 (2)

55 Siempre 10 (1)

56 Others<sup>b</sup> 36 (5)

57 Tampon absorbencies

58 1 droplet 12/583 (2)

59 2 droplets 149/583 (26)

60 3 droplets 237/583 (41)

61 4 droplets 125/583 (21)

62 5 droplets 26/583 (4)

63 6 droplets 34/583 (6)

64 Tampon with applicator 381/614 (62)

65 Tampon carrying time, median (IQR) 4 h 5 min (2 h)

66 Tampon colonized by *S. aureus* 198/737 (27)

67 Detection of one *S. aureus* strain 191/737 (26)

68 Detection of two different *S. aureus* strains 7/737 (1)

69 <sup>a</sup>Data are given as no./total (percent) or no. (percent) unless otherwise  
... noted.

70 <sup>b</sup>Only tampon brands used by  $\geq 10$  participants are reported.

71

72 Among the subset of women with tampons colonized by *S. aureus*, those who  
... declared sexual intercourse within the last 5 days were less frequently

72... 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.

73

#### 74 DISCUSSION

75 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 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

76

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

78 Characteristic

79 Value for healthy women volunteers

80 P

81 *S. aureus* positive

82 (n = 205)

83 *S. aureus* negative

84 (n = 532)

85 Median age, yrs (IQR) 31 (12) 32 (11) 0.1

86 Tampon carrying time, median (IQR) 4 h 0 min

87 (2 h 50 min)

88 4 h 20 min

89 (3 h 0 min)

90 0.3

91 Tampon brands  
92 Tampax 79/309 (26) 230/309 (74) 0.6  
93 Nett 59/197 (28) 138/197 (72)  
94 Otherb 60/216 (28) 156/216 (72)  
95 Tampon with applicator 90/164 (55) 291/450 (65) 0.031  
96 Absorbencies 0.96  
97 1 and 2 droplets 33/157 (21) 128/426 (30)  
98 3 and 4 droplets 106/157 (68) 256/426 (60)  
99 5 and 6 droplets 18/157 (11) 42/426 (10)  
100 Antibiotics within the last 15 days 7/197 (4) 38/529 (7) 0.08  
101 Contraception  
102 None 78/327 (24) 249/327 (76) 0.07  
103 Estroprogestative pills 67/196 (34) 176/527 (33) 0.9  
104 Intrauterine devices 53/198 (28) 102/529 (19) 0.033  
105 Sexual intercourse within the last 5 days 62/194 (32) 150/524 (29) 0.4  
106 aData are given as no./total (percent) unless otherwise noted.  
107 bBrands of tampon other than Tampax and Nett were clustered into one  
... group for the statistical analysis.

108  
109 TABLE 3 Characteristics of *S. aureus* strainsa  
110 Clonal  
111 complex agr type Superantigen gene(s)  
112 Presence  
113 of mecA  
114 No. of strains  
115 (n = 205)  
116 CC1 agr3 sea, seb, seh, sek, seq 2  
117 sea, seh, sek, seq 2  
118 seh, sek 1  
119 CC5 agr2 tst, sec, sel, sed, sej, ser, egc Yes 1  
120 tst, sed, sej, ser, egc Yes 1  
121 sea, egc 1  
122 sed, sej, ser, egc Yes 1  
123 sed, sej, ser, egc Yes 1  
124 sed, sej, ser, egc 1  
125 sed, sej, ser, egc 4  
126 egc, sep 1  
127 egc 8  
128 CC6 agr1 sea 3  
129 CC7 agr1 – 6  
130 CC8 agr1 tst 1  
131 sea, sed, sej, ser Yes 2  
132 sea 1  
133 seb 1  
134 sec, sel, egc 1  
135 sed, sej, ser 1  
136 egc 1  
137 – 16  
138 CC9 agr2 egc 4  
139 CC12 agr2 seb, sep 1  
140 CC15 agr2 seb 1

141 – 14  
142 CC20 agr1 seb, egc 1  
143 sec, sel, egc 1  
144 CC22 agr1 tst, sec, sel, egc 1  
145 egc 7  
146 CC25 agr1 egc 2  
147 CC30 agr3 tst, sea, seb, seh, sek, egc 1  
148 tst, sea, seh, egc 3  
149 tst, sea egc 14  
150 tst, egc 6  
151 sea, egc 3  
152 CC45 agr1 sec, sel, egc 14  
153 egc 4  
154 CC45 agr4 egc 1  
155 CC59 agr1 seb, seb, sek, seq 3  
156 CC88 agr3 sec, sel 1  
157 – 1  
158 CC97 agr1 sec, sel 1  
159 – 2  
160 CC101 agr1 – 1  
161 CC121 agr4 egc 3  
162 CC182 agr1 egc 1  
163 CC188 agr1 – 1  
164 CC398 agr1 – 56  
165 aCC, clonal complex; agr, accessory gene regulator; tst, TSST-1 gene; sea  
... to ser, staphylococcal enterotoxin A  
166 to R genes; egc, enterotoxin gene cluster including seg, sei, sem, and  
... sen and/or seo; mecA, methicillin  
167 resistance gene A; -, no detection.  
168  
169 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).  
170  
171 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



171... 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.

172  
173 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,

174  
175 TABLE 4 Characteristics of *S. aureus* strains codetected in tampons

176 Participant no. Clonal complex *agr* type Superantigen gene(s)

177 96 CC25 *agr1* *egc*

178 CC30 *agr3* *tst*, *sea*, *egc*

179 126 CC22 *agr1* *egc*

180 CC398 *agr1* –

181 137 CC15 *agr2* *tst*, *sed*, *sej*, *ser*, *egc*

182 CC25 *agr1* *egc*

183 256 CC15 *agr2* –

184 CC30 *agr3* *tst*, *sea*, *egc*

185 273 CC5 *agr2* *egc*

186 CC398 *agr1* –

187 291 CC22 *agr1* *egc*

188 CC30 *agr3* *tst*, *sea*, *egc*

189 650 CC121 *agr4* *seb*, *egc*

190 CC30 *agr3* *tst*, *sea*, *egc*

191  
192 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.

193  
194 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

194... 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).

195  
196 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.

197  
198 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.

199  
200 MATERIALS AND METHODS

201 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 Civil 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

201... 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 multilocus 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 nonparametric Wilcoxon test for  
... quantitative variables and Fisher's exact test for categorical variables.

202

#### 203 ACKNOWLEDGMENTS

204 Myriam Chiaruzzi was supported by a research grant from the Regional  
... Council of Picardie. This work was supported by the LABEX ECOFECT  
... (ANR–11–LABX–0048) of the University of Lyon within the program  
... Investissements d'Avenir (ANR–11–IDEX–0007), operated by the French  
... National Research Agency (ANR) and the FINOVI foundation. We are grateful  
... to the members of the Staphylococcus National Reference Center,  
... especially François Vandenesch and Frédéric Laurent, as well as Gery  
... Lamblin, and Pierre Adrien Bolze for fruitful comments and technical  
... help. We declare no conflict of interest.

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