Complex ecological interactions of *Staphylococcus aureus* in tampons during menstruation.

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Table S1. Clinical and biological characteristics of the cases of menstrual toxic shock syndrome included in the study.

Patient	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**
Sample nb	92	93	94	95	96	97	98
Age (year)	18	17	30	21	14	15	17
Tampon brand	Tampax	NR	Tampax	NR	NR	Tampax	Net
Time of carriage	7h	Overnight	Overnight	24h	4h	Overnight	Overnight
Hospitalisation unit	ICU	ICU	ICU	ICU	ICU	Medicine	ICU
TSS CDC criteria	Probable	Probable	Probable	Probable	Confirmed	Probable	Probable
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hypotension	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cutaneous rash	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Desquamation	NR	NR	NR	NR	Yes	NR	Yes
Number of system involvement	3/7	3/7	6/7	4/7	3/7	5/7	4/7
***S. aureus* strain ref.**	ST20141074	ST20140803	ST20160181	ST20151989	ST20151978	ST20160223	ST20161423
***S. aureus* genotype**	CC5 *tst+*	CC30 *tst+*	CC30 *tst+*	CC30 *tst+*	CC30 *tst+*	CC30 *tst+*	CC22 *tst+*

ICU, Intensive Care Unite; NR, Not Recorded. *S. aureus* was genotyped using diagnostic DNA microarrays and identibac *S. aureus* Genotyping® (Alere) [2].

Table S2. Demographic and clinical data.

Factor		***S. aureus* vaginal carriage**	
		Yes	No
Age *(years)*			
	≤ 25	21	25
	26-35	14	26
	> 35	9	13
Tampon brand			
	Tampax®	19	24
	Nett®	10	19
	Other	15	21
Tampon use duration *(hours)*			
	0-4	19	30
	4-8	19	32
	> 8	5	2
Sexual intercourse in the previous 5 days			
	Yes	16	24
	No	27	39
Contraceptive method			
	OP pill	23	37
	IUD	7	7
	None	13	20
Delay between tampon collection and analysis *(days)*			
	0	5	26
	1	17	27
	2	9	7
	3	3	3
	4	3	1
	5	1	0
	6	2	0
	7	1	0
	8	1	0
	9	1	0

OP, oestroprogestative; IUD, intra-uterine device.

Table S3. Comparison of 16S rRNA gene sequencing approaches to the culturomic approach for quantifying *Staphylococcus aureus* abundances

Sample number	**Menstrual toxic shock**[a]	***nuc* PCR**[b]	***tst* PCR**[c]	***S. aureus* abundance**	
				Metabarcoding data (reads)	**Culturomic data (CFU/mL)**
92	1	1	1	9527	10000000
93	1	1	1	5327	10000000
94	1	1	1	9	10000000
95	1	1	1	140	10000000
96	1	1	1	60	10000000
97	1	1	1	1331	10000000
7a	0	1	1	28	< 100
11	0	1	1	1719	10000000
24	0	1	1	17	1000
52	0	1	1	93	10000000
1a	0	1	0	55	100000
10	0	1	0	15	10000
16	0	1	0	5070	10000000
19a	0	1	0	1240	10000000
22	0	1	0	138	1000000
23a	0	1	0	8	< 100
28a	0	1	0	14	< 100
30a	0	1	0	13	100
33	0	1	0	178	100000
36	0	1	0	9	10000
37	0	1	0	6	< 100
38	0	1	0	149	1000000
39	0	1	0	180	100000
42	0	1	0	6	1000000
43a	0	1	0	36	1000
44a	0	1	0	47	< 100
45	0	1	0	131	100000
46	0	1	0	19	< 100
48	0	1	0	75	10000000
49a	0	1	0	53	1000
51	0	1	0	654	10000
53a	0	1	0	81	100
59a	0	1	0	35	1000000

71a	0	1	0	7	100
75	0	1	0	6	< 100
77	0	1	0	46	100
79a	0	1	0	6	100
82	0	1	0	441	10000000
83a	0	1	0	21	100000
85	0	1	0	21	100000
88a	0	1	0	17	100
89a	0	1	0	37	10000000
1b	0	0	0	8	0
2	0	0	0	4	0
3	0	0	0	6	0
4	0	0	0	14	0
5	0	0	0	18	0
6	0	0	0	7	0
7b	0	0	0	26	0
8	0	0	0	57	0
13	0	0	0	60	0
14	0	0	0	14	0
17	0	0	0	59	0
18	0	0	0	6	0
19b	0	0	0	11	0
20	0	0	0	39	0
23b	0	0	0	7	0
25	0	0	0	11	0
26	0	0	0	15	0
28b	0	0	0	6	0
32	0	0	0	23	0
34	0	0	0	47	0
41	0	0	0	8	0
43b	0	0	0	37	0
44b	0	0	0	26	0
59b	0	0	0	2	0
64	0	0	0	41	0
65	0	0	0	22	0
66	0	0	0	13	0
67	0	0	0	2	0
68	0	0	0	5	0
69	0	0	0	16	0
70	0	0	0	13	0

71b	0	0	0	38	0
72	0	0	0	6	0
73	0	0	0	46	0
74	0	0	0	8	0
76	0	0	0	15	0
78	0	0	0	38	0
79b	0	0	0	6	0
80	0	0	0	11	0
81	0	0	0	7	0
83b	0	0	0	22	0
84	0	0	0	26	0
87	0	0	0	13	0
88b	0	0	0	5	0
89b	0	0	0	78	0

[a] 1= Menstrual toxic shock ; 0 healthy woman.

[b] *nuc* PCR targets thermonuclease gene specific to *S. aureus* (1= positive; 0 =negative).

[c] *tst* PCR target the gene encoding TSST-1 (1= positive; 0 =negative).

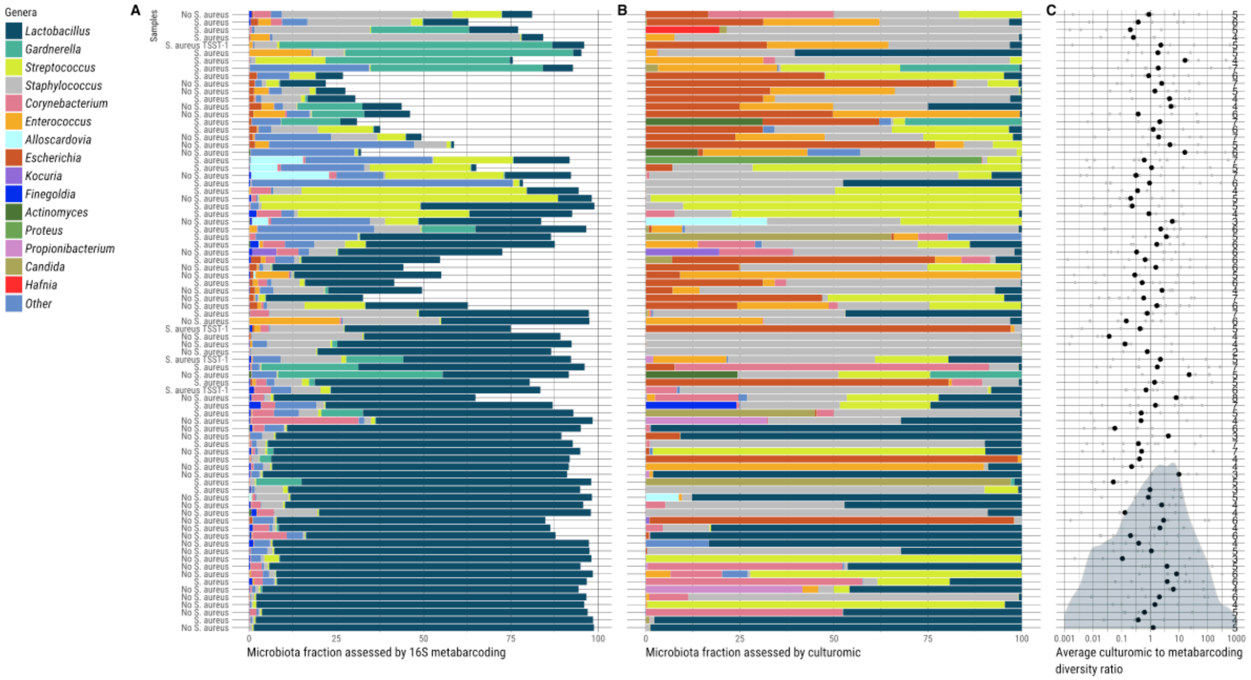


Figure S1. Analysis of the bacterial genera colonizing tampons in healthy women during menstruation. (A) 16S rRNA gene sequences analyses. (B) Culturomic analyses. Each line corresponds to a tampon (n = 81). Tampons were clustered according to their genera composition in (A), and the same order retained in (B) and (C). For each sample, the carriage status of *S. aureus* is indicated (No *S. aureus*, *S. aureus,* or *S. aureus* TSST-1). The 15 most abundant and common genera between the two methods are represented. To simplify the figure, the Other group defines the set of taxa common to both techniques but were not part of the 15 most abundant. In (A), remaining white indicates the percentage of taxa that are not retrieved in both methods. The relative proportion of each genus is represented for each sample analysed by metabarcoding (PM) and culturomic (PC) and the PM/PC ratio calculated ("Other" excluded). Ratios close to 1 exhibited good correspondence between both methods. (C) The PM/PC ratios for each common taxa is represented as grey dot and the average PM/PC ratio as black dot. The average of all finites and non-null ratios were not significantly different from 1 (Student's test, p=0.21). Numbers shown are number of common taxa used to calculate the average PM/PC ratio.

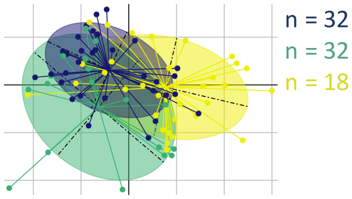
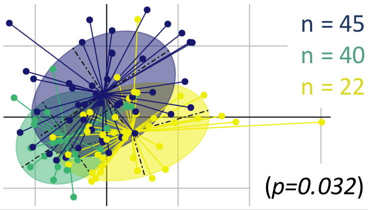
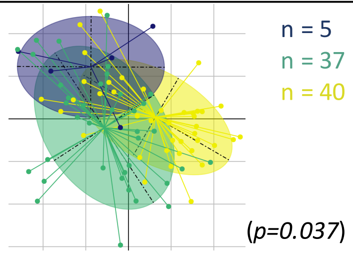
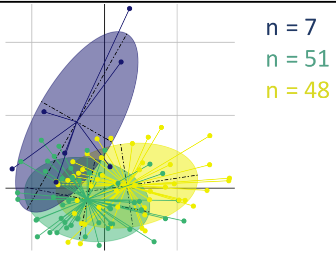
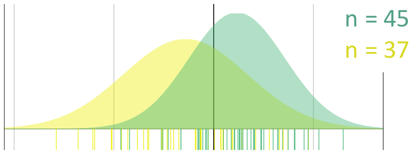
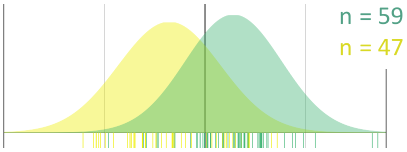
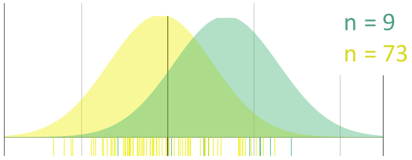
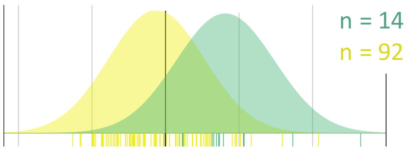
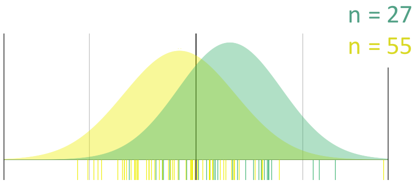
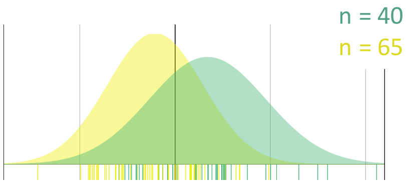
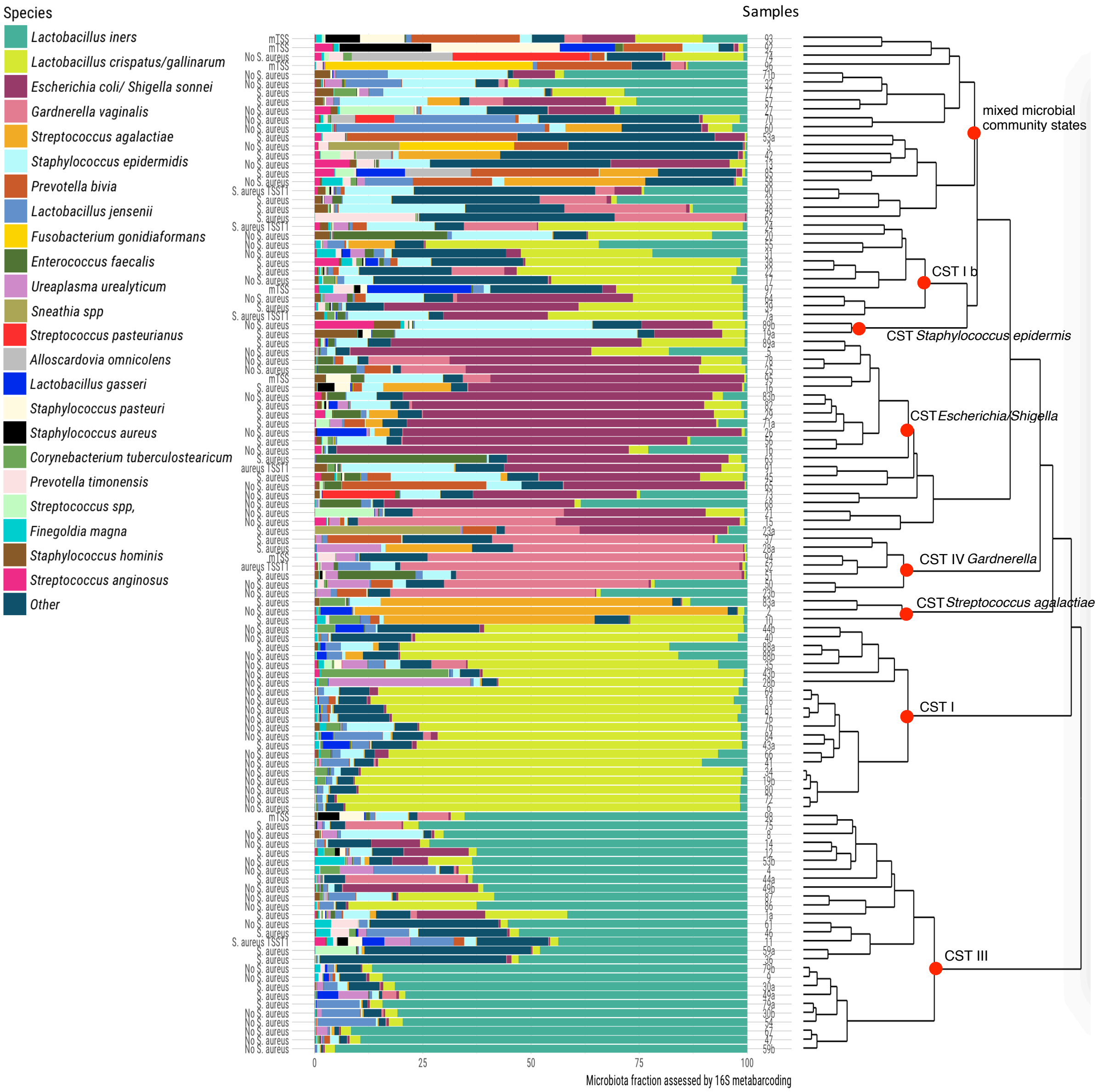
	Culturomic (n = 82)	Metabarcoding (n = 107)
Age of women 🟪 ≤ 25 years 🟩 [25 – 35 years] 🟨 >35 years	 n = 32 n = 32 n = 18	 n = 45 n = 40 n = 22 (*p=0.032*)
Tampon use duration 🟪 >8 hours 🟩]4 – 8 hours] 🟨 [0 – 4 hours]	 n = 5 n = 37 n = 40 (*p=0.037*)	 n = 7 n = 51 n = 48
Contraceptive pill 🟩 Yes 🟨 No	 n = 45 n = 37	 n = 59 n = 47
Contraceptive IUD 🟩 Yes 🟨 No	 n = 9 n = 73	 n = 14 n = 92
Sexual intercourse in the previous 5 days 🟩 Yes 🟨 No	 n = 27 n = 55	 n = 40 n = 65

Figure S2. Effect of various factors on the diversity of the vaginal microbiota of healthy women during menstruation analysed by metabarcoding and culturomic approaches. Between-class analysis was performed at the genus level. Colours distinguish between samples belonging to factor groups (age class, carrying time, contraception). The more separation between the colour groups, the more the tested factor impacts the composition of the community. Statistical differences (when any) are indicated (Monte-Carlo test ; $p < 0.05$).



Species
Lactobacillus iners
Lactobacillus crispatus/gallinarum
Escherichia coli/ Shigella sonnei
Gardnerella vaginalis
Streptococcus agalactiae
Staphylococcus epidermidis
Prevotella bivia
Lactobacillus jensenii
Fusobacterium gonidiaformans
Enterococcus faecalis
Ureaplasma urealyticum
Sneathia spp
Streptococcus pasteurianus
Alloscardovia omnicolens
Lactobacillus gasseri
Staphylococcus pasteuri
Staphylococcus aureus
Corynebacterium tuberculostearicum
Prevotella timonensis
Streptococcus spp,
Finegoldia magna
Staphylococcus hominis
Streptococcus anginosus
Other
Samples
mTSS
No S. aureus
S. aureus
S. aureus TSST1
0
25
50
75
100
Microbiota fraction assessed by 16S metabarcoding
mixed microbial community states
CST I b
CST Staphylococcus epidermis
CST Escherichia/Shigella
CST IV Gardnerella
CST Streptococcus agalactiae
CST I
CST III

Figure S3. Analysis of bacterial species colonizing tampons in healthy women and mTSS cases during menstruation by *rrs* gene sequence analyses. Each line represents a tampon and its relative composition in bacterial species. For each sample, the carriage status of *S. aureus* is indicated (no *S. aureus*, *S. aureus*, *S. aureus tst*+), and mTSS specifically indicates mTSS cases carrying S. aureus TSST-1. Some tampons came from the same woman and are indicated by the same sample number (see column samples), "a" meaning that the tampon was colonized by S. aureus and "b" meaning that it was not. The tree was generated using the complete link algorithm and the Euclidean distance~~s~~ between the different tampons' microbial communities. The Other group defines the set of taxa that were not part of the 23 most abundant. Community state type (CST) shown based either on previously literature (Ravel et al 2011) CSTI and CSTIb are dominated by *L. crispatus*, CSTIII by *L. iners*, CSTIV by *G. vaginalis*, or as new CTS fellowed by the community dominating OTUs' affiliation name.