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Vaginal *Staphylococcus aureus* Superantigen Profile Shift from 1980 and 1981 to 2003, 2004, and 2005▽

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We determined vaginal *Staphylococcus aureus* superantigens. Staphylococci were quantified from tampons/diaphragms in 2003 to 2005, with counts compared to those determined in 1980 and 1981. In 2003 to 2005, more women were colonized than in 1980 and 1981 (23 versus 12%). Enterotoxins G and I and enterotoxin-like superantigens M and N declined, but enterotoxin-like superantigens K, L, and Q increased.

The composition of the aerobic human vaginal microfloras has been extensively studied (2, 10, 14, 22–24). These floras are dominated by lactobacilli; however, other aerobic bacteria may also be present, including *Staphylococcus aureus*. The bacterial density of *S. aureus* changes dramatically during menstruation, increasing logarithmically in the vagina compared to during nonmenses (31). A major end product of *Lactobacillus* metabolism, lactic acid, is responsible for maintaining the vaginal pH at approximately 4 at times other than menstruation (2, 14). During menstruation, lactobacilli appear to be unable to maintain vaginal pH, and the rise in pH corresponds with rises in *S. aureus* levels (31).

Studies performed in 1980 and 1981, at the time that toxic shock syndrome (TSS) was being described, suggest that 10 to 15% of women were colonized with *S. aureus* vaginally (5, 15, 18, 31), and of women with vaginal *S. aureus*, 25 to 40% of the strains made TSS toxin 1 (TSST-1) (5, 18, 31). In contrast, a recent study suggested that vaginal colonization with *S. aureus* during menstruation is higher than previously determined (38). This small study using fluorescent in situ hybridization analysis, a non-culture-based technique, reported that all women were colonized with *S. aureus* vaginally during menstruation (38). Very recently, however, a large prevalence study was published, which suggested that vaginal colonization with *S. aureus* was approximately 9% (26). One goal of the present study was to use techniques similar to those we employed 25 years ago (31), during the menstrual TSS epidemic, to assess whether or not more Minnesota women were colonized vaginally with *S. aureus* in 2003 to 2005 than in 1980 and 1981. In addition, we examined whether or not there has been a shift in *S. aureus* superantigen (SAg) production, including that of TSST-1, staphylococcal enterotoxins (SEs), and SE-like (SEl) SAgs (16), over the 25-year time period.

The principal cause of vagina-associated TSS is TSST-1, which is produced by some isolates of *S. aureus* (4, 20, 32, 33). This toxin has been reported to be produced by 1 to 5% of vaginal *S. aureus* isolates from healthy women (5, 18, 26, 31). TSST-1 is one member of a large family of exotoxins referred to as SAgs based on their mechanism of T-lymphocyte activation (19). SAgs produced by *S. aureus* include TSST-1, SEs, and SEl SAgs (16, 20). These toxins have the capability of causing massive cytokine release as a result of $CD4^+$ T-lymphocyte and macrophage activation, with consequent production of TSS (20). A recent article suggested that SEG and SEI are associated with small numbers of menstrual TSS cases (11).

A randomly selected group of mucosal *S. aureus* isolates from 1980 and 1981, including isolates from women during menses and nonmenses ($n = 30$), were compared to randomly collected mucosal isolates from 2003 to 2005 ($n = 30$) for SAg gene profile. The SAg gene profiles of all strains were determined by PCR. *S. aureus* cultures were grown overnight in Todd Hewitt broth (Becton Dickinson, Sparks, MD) at 37°C with shaking at 200 rpm. A sample of the grown cultures (1.5 ml) was added to a microcentrifuge tube and spun at 14,000 × *g* for 2 min. The supernatants were aspirated, and DNA was extracted from the pellets according to the DNeasy Tissue Handbook provided by the supplier of the kit (QIAGEN, Valencia, CA). The PCR primers used are listed in Table 1.

Mucosal *S. aureus* isolates from 1980 and 1981 contained the genes for SEl-M and -N and for SEG and SEI significantly more often than those from 2003 to 2005 (Table 2). In contrast, the genes for SEl-K, -L, and -Q SAgs increased in frequency during this same time period. Genes for other SAgs remained relatively constant, including that for TSST-1, the principal cause of vaginal TSS. These data are important for the following reasons. (i) The SEG, SEI, and SEl-M, -N, and -O SAg genes have been reported to be linked together on a pathogenicity island-like DNA element and have been occasionally associated with TSS (11, 12). In the United States this DNA linkage appears to be incomplete, as, for example, only 13 and 7% of isolates, respectively, contained the gene for SEl-O in 1980 and 1981 and in 2003 to 2005, fewer isolates than contained other members of the pathogenicity island. This observation was true also for other members of the pathogenicity island (data not shown) in that each member of the SAg cluster was absent in multiple strains that were positive for other

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TABLE 1. PCR primers for SAgs

Primer name	Primer sequence (5′ to 3′)	Approximate size (bp)
SEA forward	ATTGTTTTGGGGGAGTTTGAAGTT	400
SEA reverse	TACATTGCGTTTTATTGGTTGCTC	
SEB forward	GTATGATGATAATCATGTATCAGCAATA	640
SEB reverse	CGTAAGATAAACTTCAATCTTCACATC	
SEC forward	GAGTCAACCAGACCCTATGCC	610
SEC reverse	CGCCTGGTGCAGGCATC	
SED forward	GAGACTAGCCGCAATCTATCC	650
SED reverse	GCTGCATTTAGTAATGCTGGCTG	
SEE forward	GGTAGCGAGAAAAGCGAAG	450
SEE reverse	GCCTTGCCTGAAGATCTAGCTC	
SEG forward	TGAATGCTCAACCCGATCCTAAAT	580
SEG reverse	CAAACCAAAAACTTGTATTGTTCTTTTCA	
SEH forward	TTCACATCATATGCGAAAGCAGAA	620
SEH reverse	CAGATTTTAAAGTTTTATTGTCTTCA	
SEI forward	CGTATGCTCAAGGTGATATTGGTG	580
SEI reverse	AAAAACTTACAGGCAGTCCATCTCC	
SEl-J forward	TTTAGGATCCCTACAGAACCAAAGG	900
SEl-J reverse	GTTTCCATGGATAGCAAAAATGAAAC	
SEl-K forward	GTGTCTCTAATAATGCCAGCGCTC	650
SEl-K reverse	TTTGGTAGCCCATCATCTCC	
SEl-L forward	CACCAGAATCACACCGCTTA	450
SEl-L reverse	TCCCCTTATCAAAACCGCTAT	
SEl-M forward	TTTTGCTATTCGCAAAATCATATCGCA	800
SEl-M reverse	TCAACTTTCGTCCTTATAAGATATTTCTAC	
SEl-N forward	TGAGATTGTTCTACATAGCTGCAA	720
SEl-N reverse	AATTAGATGAGCTAACTGTTCTATTATCAC	
SEl-O forward	TAGTGTAAACAATGCATATGCAAATG	950
SEl-O reverse	ATTATGTAAATAAATAAACATCAATATGATA	
SEl-P forward	GGAAGCTAAAGCAGAGACAC	660
SEl-P reverse	CCCGTTTCATATGAAGTGCCACC	
SEl-Q forward	GCTTCAAGGAGTTAGTTCTGG	500
SEl-Q reverse	CTCTCTGCTTGACCAGTTCCGGTG	
TSST-1 forward	GAAATTTTTCATCGTAAGCCCTTTGTTG	625
TSST-1 reverse	TTCATCAATATTTATAGGTGGTTTTTCA	

members. (ii) The genes for SEl-K, -L, and -Q are often present on pathogenicity islands, transposons, and bacteriophages, sometimes in association with the gene for TSST-1 (7, 8, 17, 37). The linkage of these genes also has become disrupted, with strains of *S. aureus* often missing one or more SAgs (data not shown).

TABLE 2. SAg gene profile of vaginal *S. aureus* isolates

SAg gene	1980–1981 (n = 30)		2003–2005 (n = 30)		P value[a]
	No. positive	% Positive	No. positive	% Positive	
TSST-1	9	30	12	40	0.59
SEA	8	27	8	27	1.00
SEB	7	23	3	10	0.30
SEC	11	37	9	30	0.78
SED	1	5	4	13	0.35
SEE	6	30	6	30	1.00
SEG	26	87	9	30	**≪0.001**
SEH	3	10	3	10	1.00
SEI	28	93	10	33	**≪0.001**
SEl-J	7	23	8	27	1.00
SEl-K	7	23	23	77	**≪0.001**
SEl-L	7	23	29	97	**≪0.001**
SEl-M	26	87	7	23	**≪0.001**
SEl-N	23	77	5	17	**≪0.001**
SEl-O	4	13	2	7	0.67
SEl-P	2	7	0	0	0.49
SEl-Q	3	10	18	60	**≪0.001**

[a] Data were analyzed by Fisher's exact test (two sided). P values of less than 0.05 were considered statistically significant and are in boldface.

The significance of this shift in SAg profile is incompletely understood. However, it is tempting to speculate that a number of unrecognized subclinical or clinical conditions will be associated with the shift. For example, desquamative inflammatory vaginitis and vulvodynia have been described as conditions or collections of conditions of unknown cause but with appearances consistent with bacterial infection (1, 21, 36). Desquamative inflammatory vaginitis, for example, responds to clindamycin and is characterized by increased vaginal discharge, vaginal wall erythema, epithelial cell exfoliation, and replacement of lactobacilli with gram-positive cocci. Clindamycin has the interesting property of inhibiting staphylococcal SAg production independent of effects on bacterial growth (30). Future studies should examine the role of SAgs in such illnesses.

Through the use of antibodies, we were able to demonstrate that the SAgs TSST-1 and SEA to SEC (the only ones tested) were produced in detectable amounts in all strains where the respective SAg genes were present (data not shown).

Both tampons and contraceptive diaphragms have been associated with TSS since the initial TSS descriptions in 1978 to 1981 (6, 9, 34, 35). The relative risk of TSS development in association with tampon and contraceptive diaphragm use is 1/100,000 to 3.5/100,000 (9, 33). Numerous studies have attempted to determine the reason that tampons are associated

with TSS. Studies have determined that TSST-1 production depends on a neutral pH (29). Normally, the pH of the vagina changes to neutral during menstruation, but at times other than menstruation, the pH is highly acidic (2, 10, 14, 23–25). In addition, toxin production occurs at 37°C, in the presence of at least 2% oxygen balanced with 7% CO_2 and protein (13, 29). These studies led investigators to suggest that the tampon association with TSS may depend on introduction of oxygen vaginally, since the vagina is anaerobic in the absence of tampons (13, 29, 39, 40). In addition, studies suggest that certain surfactants added to tampons, such as pluronic L92, increase TSST-1 production by *S. aureus* (28). Despite the reduction of tampon absorbency since 1984, cases of menstrual TSS continue to occur today (33). There have been only limited studies to explain the association of TSS with contraceptive diaphragm use. In one in vitro study, contraceptive diaphragms were observed to inhibit production of TSST-1 (27). The inhibitory effect was independent of the presence of seminal fluid. In 1983, Baehler et al. (3) suggested that the use of contraceptive diaphragms by women over 24-h time periods caused *S. aureus* numbers to increase dramatically.

We also determined vaginal colonization with *S. aureus* through vaginal swabs taken at times other than menses and from women using tampons in 1980 and 1981 compared with women using tampons and contraceptive diaphragms (nonmenses) in 2003 to 2005 to assess whether there has been a change in vaginal colonization. Healthy women, aged 18 to 40 years ($n = 180$), were recruited during 2005 to provide tampons after 4 to 6 h of use on day 2 of menstruation for quantitative determination of *S. aureus*. Day 2 of menstruation was predictive of maximum numbers of *S. aureus* vaginally. An additional 82 healthy women, aged 18 to 40 years, who used contraceptive diaphragms (Ortho-McNeil Pharmaceutical, Inc., Raritan, NJ) with the enclosed spermicide nonoxynol 9 were studied in 2003 for quantifying *S. aureus* at times other than menstruation. Each woman inserted a diaphragm, which was left for 2 min and then removed and analyzed. A second diaphragm was then inserted, used for 7 to 8 h, and then removed for analysis of changes in *S. aureus*. For comparison to the tampon and contraceptive diaphragm studies, data from a previous study, which characterized the vaginal microflora and *S. aureus* in healthy women during 1980 and 1981 (31), were evaluated for *S. aureus*. These organisms were isolated from tampons used during menstruation ($n = 95$) or from vaginal swabs during nonmenstruation ($n = 205$). In all instances, volunteer subjects were treated in accordance with Institutional Review Board approval.

In the 2005 tampon study, *S. aureus* densities per tampon ($n = 37$) averaged 8.5×10^9 CFU (range, 10^4 to 10^{11} CFU). One tampon was excluded from CFU/tampon determination since this participant had swarming *Proteus* vaginally, which prevented accurate plate counts. The total *S. aureus* CFU/tampon for the remaining 36 tampons were as follows: 7 had 10^{10} to 10^{11} CFU/tampon, 13 had 10^9 to 10^{10} CFU/tampon, 2 had 10^8 to 10^9 CFU/tampon, 1 had 10^7 to 10^8 CFU/tampon, 2 had 10^6 to 10^7 CFU/tampon, 1 had 10^5 to 10^6 CFU/tampon, and 1 had 10^4 to 10^5 CFU/tampon. Although quantification of *S. aureus* (CFU/tampon) was not done for all tampons in the 1980 and 1981 tampon study, the *S. aureus* counts on tampons from day 2 of menses were greater than 10^7/ml (determined by the dilution of tampons in medium and plating onto blood agar). Our experience is that tampons worn for 6 h have on average 5 ml of menses, and assuming that the average volume was the same in 1980 and 1981, the women in the 1980 and 1981 study had more than 5×10^7 CFU of *S. aureus*/tampon.

TABLE 3. Percentages of women positive for *S. aureus* vaginally during both menstruation and nonmenstruation

Yr of isolation	Total no. of study subjects	Subjects with *S. aureus*		Subjects with TSST-1+ *S. aureus*	
		No.	%	No.	%
1980–1981	300	36	12.0	12	4.0
2003–2005	262	60	22.9	11	4.2

Numbers of *S. aureus* isolated vaginally from contraceptive diaphragms in 2003, whether after 2 to 3 min or after 7 to 8 h of wear time, averaged approximately 10^4/diaphragm. This is in contrast to data from a previous study, which suggested staphylococci increase vaginally with contraceptive diaphragm use (3). The reason for the difference in the two studies is unclear but may be related to differences in diaphragm use time.

The overall percentages of vaginal colonization with *S. aureus* in women during menstruation and nonmenstruation using tampons and diaphragms/vaginal swabs in 1980 and 1981, compared to 2003 to 2005, were determined (Table 3). In 1980 and 1981, 36/300 women were colonized vaginally with *S. aureus* (12%). In 2003 to 2005, 60/262 women were colonized vaginally with *S. aureus* (23%). These results indicated that the percentage of women who were *S. aureus* positive in 2003 to 2005 was statistically higher than that in 1980 and 1981 ($P = 0.0005$). However, there was no statistical difference between the percentages of *S. aureus* isolates producing TSST-1 in 1980 and 1981 compared to 2003 to 2005 ($P = 0.54$).

These findings are significant for two reasons. First, the observation that *S. aureus* counts are very high vaginally during menses suggests that surgical procedures that involve vaginal mucosal surface contact should be kept to a minimum at this time or that prospective patients should be prophylactically treated with antibiotics to reduce *S. aureus* numbers. Second, the higher percentage of women colonized in 2003 to 2005 than in 1980 and 1981 is consistent with a shift in *S. aureus* strains, possibly, as suggested above, due to shifts in SAg production.

One final observation was made in this study. Women with *S. aureus* vaginally were also highly likely to be cocolonized with group B streptococci (data not shown).

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