Staphylococcal toxic shock syndrome (TSS) was originally described in menstruating women and linked to TSS toxin 1 (TSST-1)–producing *Staphylococcus aureus*. Using UK national surveillance data, we ascertained clinical, molecular and superantigenic characteristics of TSS cases. Average annual TSS incidence was 0.07/100,000 population. Patients with nonmenstrual TSS were younger than those with menstrual cases but had the same mortality rate. Children <16 years of age accounted for 39% of TSS cases, national surveillance data, we ascertainment clinical, molecular and superantigenic characteristics of TSS cases. Average annual TSS incidence was 0.07/100,000 population. Patients with nonmenstrual TSS were younger than those with menstrual cases but had the same mortality rate. Children <16 years of age accounted for 39% of TSS cases,
Staphylococcal toxic shock syndrome (TSS) is a life-threatening illness characterized by fever, rash, desquamation, organ dysfunction, and shock. In 1980, the use of highly absorbent tampons in the United States triggered an outbreak of menstrual TSS (mTSS) in young women, and TSS incidence peaked at 13.7/100,000 population (1). Changes in tampon manufacture and advice regarding tampon use helped halt the epidemic. TSS is a notifiable illness in the United States; in 2004–2014, average annual incidence varied from 0.03–0.05/100,000 population (2). In the United Kingdom and other countries in Europe, staphylococcal TSS is not a notifiable illness, so the clinical, microbiological, and toxicogenic features of TSS remain poorly described.

TSS is attributed to staphylococcal superantigens that cause massive T-cell activation and cytokine release (3). TSS toxin 1 (TSST-1) is associated with 95% of mTSS cases and 50% of TSS cases caused by nonmenstrual infective foci (nmTSS) (4). Although 24 different staphylococcal superantigens have been described, including staphylococcal enterotoxin (SE) and enterotoxin-like superantigens (5), SE types A, B, and C are implicated in remaining nmTSS cases (3,6), despite the lack of data from Europe.

TSST-1 is encoded by the gene tst, which is carried on mobile genetic elements (MGE) named staphylococcal pathogenicity islands (SaPIs) that lie within the S. aureus chromosome. SaPIs are linked to specific S. aureus genetic families, known as lineages (7). Within human S. aureus strains, tst is carried on SaPI1, SaPI2, and SaP68111 (8,9). Known regulators of tst include the S. aureus accessory gene regulator operon (agr) via the effector molecule RNAIII (10), the staphylococcal respiratory response regulator AB (SrrAB) (10), a glucose catabolite repressor CcpA (11), the staphylococcal accessory regulator A, σb (12) and the SaeRS 2-component system (13).

mTSS strains are reported to belong to a single S. aureus lineage (14,15) corresponding to multilocus sequence type–clonal complex (MLST-CC) 30, a lineage prevalent in the United Kingdom (16). Staphylococcal methicillin resistance is mediated by mecA or mecC genes within the mobile genetic element staphylococcal cassette chromosome mec (SCCmec), of which there are 12 types (17,18). Methicillin-sensitive S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA) strains that are members of CC30 carry tst on SaPI2 (19,20).

In this study, we aimed to characterize the clinical and molecular epidemiology of TSS in England, Wales, and Northern Ireland. We further determined superantigen production by dominant S. aureus strain types.

Methods

Case Identification
Public Health England (PHE) requests the referral of all TSS-associated isolates to the national reference laboratory for characterization, including toxin gene profiling. We identified clinician-diagnosed staphylococcal TSS cases from a database of referred S. aureus isolates from England, Wales, and Northern Ireland during January 2008–December 2012 using the search term “toxic shock syndrome.” Clinical and demographic data from the accompanying isolate referral form (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/2/17-0606-Techapp1.pdf) that had been recorded contemporaneously were scrutinized for accuracy by a clinician (H.S.) before inclusion in the study.

We classified TSS cases in patients ≤16 years of age as pediatric. We classified cases in female patients 12–60 years of age as mTSS if the infection was associated with menstruation or positive vaginal culture for S. aureus. We classified the remaining cases as nmTSS. All cases had an associated S. aureus isolate.

The average annual incidence of TSS was calculated as cases per 100,000 population using Office for National Statistics UK population estimates (http://www.ons.gov.uk/ons/datasets-and-tables/index.html) and was based on data from 2009 and later (due to changes in reporting practice from November 2008 prompted by national guidance on toxin-producing S. aureus). We used total population data for the United Kingdom excluding Scotland as the denominator for all TSS and nmTSS cases; the total female population 12–60 years of age as the denominator for mTSS cases, reflecting the age range of this group; and the number of children <16 years of age as the denominator for pediatric cases. We included data from 2008–2012 in all other analyses.

Molecular Characterization of Isolates
We made MLST-CC assignments on the basis of sequencing the staphylococcal protein A (spa) gene repeat region (21) and referencing spa server (http://spa.ridom.de/mlst.shtml) and MLST (http://saureus.mlst.net) databases. We performed SCCmec detection, typing, and toxin gene profiling (sea-e, seg-j, tst, and pvl only) by multiplex PCR (22,23).
Antimicrobial Susceptibility Testing
For isolates from 2008–2011 (n = 148; online Technical Appendix Table 1), we determined antimicrobial MICs by agar dilution (24) and interpreted them in accordance with European Committee on Antimicrobial Susceptibility Testing guidelines (http://www.eucast.org). We did not determine antimicrobial susceptibilities for isolates from 2012.

TSST-1 Production
Based on molecular epidemiologic findings, we assessed TSST-1 production in all tst-positive CC30 MSSA isolates from the TSS cohort (n = 81), including TSS isolates associated with bacteremia, skin and soft tissue infections (SSTI), and deep infections. We also assessed TSST-1 production in randomly selected tst-positive CC30 MRSA isolates from non-TSS patients (n = 39, including carriage, bacteremia, and SSTI isolates) that had been submitted to the reference laboratory during the study period (online Technical Appendix Table 1). We quantified TSST-1 in cell-free broth-culture supernatants by Western blot comparison with purified TSST-1 protein standards (online Technical Appendix).

T-Cell Proliferation
We obtained normal-donor peripheral blood mononuclear cells (PBMC) from an approved subcollection of the Imperial College NHS Trust Tissue Bank (ICHTB reference R12023) from anonymized consenting healthy donors. We incubated PBMC (1 × 10^6 cells/mL) with cell-free RPMI bacterial supernatants (1:1,000 dilution) prepared from tst-positive CC30 MSSA isolates from the TSS cohort (n = 77; 4 of the isolates did not grow in RPMI) and the randomly selected tst-positive CC30 MRSA isolates (n = 39) that were investigated for TSST-1 production. We cultured the PBMC in RPMI medium (Invitrogen, Hemel Hempstead, UK) supplemented with 10% fetal calf serum at 37°C for 48 h in triplicate (25). We measured proliferation after incorporating 1.0 μCi/well of [3H] thymidine and allowing an additional 16 h incubation.

DNA Sequencing and Analysis
We extracted whole genomic DNA from randomly selected tst-positive CC30 MSSA isolates from the TSS cohort (n = 4) and tst-positive CC30 MRSA isolates (n = 5) (online Technical Appendix Table 1) (26). We prepared libraries using the Nextera-XT DNA Sample Prep Kit (Illumina, Cambridge, UK) and subjected them to MiSeq sequencing (Illumina), generating 150 bp reads. We deposited data in the GenBank short read archive (accession no. SRP082305). We mapped reads to MLST-CC matched reference genomes MRSA252 (GenBank accession no. NC_002952.2 (27) or MN8 (accession no. NZ_CM000952) using SMALT (http://www.sanger.ac.uk/resources/software/smalt/) and determined single-nucleotide polymorphisms (SNPs) by SAMtools and bcftools (28). We performed de novo assemblies using Velvet (https://www.ebi.ac.uk/~zerbino/velvet/) and annotated them using Prokka (http://www.sanger.ac.uk/science/tools/artemis/) to visualize the mapping of sequence reads to the reference strain and manually confirm all polymorphisms. For targeted ecpA sequencing, we amplified and sequenced DNA using forward primer 1: 5′-CACAGTGTCCGCTTGTGTA-3′ and reverse primer 1: 5′-TAAGCCGATCCCTACTGCAC-3′.

Statistical Analysis
We analyzed data with GraphPad Prism 6.0 (GraphPad Software, La Jolla, California, USA). We tested categorical variables using Fisher exact test or χ² test. We summarized non-parametric data by medians and interquartile ranges (IQR) and compared 2 groups by Mann-Whitney U test. We summarized parametric data by means and SDs and analyzed 2 groups by t-test (2-tailed); we considered p<0.05 significant.

Results
Incidence of TSS
During January 2008–December 2012, a total of 195 TSS case isolates were referred to PHE. We excluded 15 cases from the study (duplicate isolates from the same case, 4 cases; isolates submitted for quality control testing, 2 cases; isolates from cases incorrectly recorded as TSS, 9 cases), leaving 180 microbiologically confirmed TSS cases with isolates. Because of missing clinical data, we were unable to classify 3 isolates as mTSS or nmTSS and could not ascertain the sex of 1 patient with nmTSS.

We considered the apparent rise in cases during 2008–2009 an artifact of increased clinical awareness of severe toxigenic S. aureus disease from late 2008, prompted by national guidance on toxin-producing S. aureus (Figure 1). Beginning in 2009, mTSS referrals declined annually, whereas nmTSS cases remained stable. By 2012, cases of nmTSS outnumbered mTSS. Overall, most cases were nonmenstrual (107, 59.4%). Average annual incidence per 100,000 population was 0.07 (95% CI 0.05–0.10) for all cases, 0.09 (95% CI 0.06–0.14) for menstrual cases, and 0.04 (95% CI 0.02–0.06) for nonmenstrual cases.

Clinical Characteristics of TSS Patients
Despite an overall preponderance of female case-patients, we found no gender difference among nmTSS cases (Table 1). The median age of the cohort was 19 years; patients with nmTSS were younger than those with mTSS (median 15.0 vs. 21.5 years; p = 0.01).
Of the TSS cases studied, 39% (71/180) occurred in children ≤16 years of age; one sixth of all TSS case-patients were <1 year of age (Figure 2). The median age of pediatric TSS case-patients was 4 years, with an average annual incidence of 0.14/100,000 children (95% CI 0.08–022). However, among children <1 year of age, the average annual incidence increased to 0.45/100,000 (95% CI 0.26–0.79). Most pediatric nmTSS cases were related to burns (26.8%, 15/56) or SSTIs (25%, 14/56).

Five percent of all patients with TSS had died at the time of referral of the isolate. We found no difference in fatality rate between mTSS and nmTSS cases and no association with age (online Technical Appendix Table 2). The infective focus in nmTSS cases was SSTI (n = 41), primary bacteremia (n = 15), burns (n = 15), deep abscess (n = 13), respiratory tract (n = 10), bone and joint (n = 4), unknown (n = 6), and other sites (n = 3). We found no association between site of infection and S. aureus lineage (online Technical Appendix Figure 1).

Molecular Characteristics of TSS Isolates
Among 180 TSS S. aureus isolates, we identified 88 spa types associated with 15 different MLST-CCs (online Technical Appendix Table 3). The leading cause of both mTSS and nmTSS was CC30 MSSA, accounting for >50% of infections (Figure 3), although we found a stronger association of CC30 with mTSS than with nmTSS (72.9% vs. 36.4%; p<0.0001; online Technical Appendix Table 3). CC30 MSSA was also the leading cause of TSS among pediatric cases (31/71). We identified only 7 MRSA TSS isolates (online Technical Appendix Table 4).

TSS isolates carried 3 superantigen genes on average (online Technical Appendix Table 5). The most common superantigen gene among both mTSS and nmTSS isolates was tst (Table 2; online Technical Appendix Figure 2), with the exception of the other 2 prevalent superantigen genes, seg and sei, that are carried on an enterotoxin gene cluster (egc) along with selm/n/o/u in most S. aureus isolates (5). The tst gene was associated with mTSS (Table 2) and strongly associated with the CC30 lineage of S. aureus (online Technical Appendix Table 6). The superantigen gene sea combined with tst was also linked to mTSS (Table 2), whereas sea alone was associated with CC30 (online Technical Appendix Tables 5, 6); sec was linked to nmTSS (Table 2) and CC45 (online Technical Appendix Table 5). Ten nmTSS cases were associated with isolates that lacked any superantigen gene tested; 7 were CC15, highlighting severe disease attributable to this lineage that was unexplained by the presence of major superantigens (online Technical Appendix Tables 5, 6).

Antimicrobial Susceptibility of TSS Isolates
Most isolates were MSSA (mecA negative). The rate of resistance to erythromycin was 9.2%; to ciprofloxacin, 8.5%; to tetracycline, 3.5%; and to teicoplanin, 1.4%. For 7 mecA-positive MRSA-TSS isolates, the resistance rate to ciprofloxacin was 57.1%; to erythromycin, 42.6%; and to clindamycin, 14.3%.

As MRSA-related TSS is rarely reported we examined these cases in more detail. All 7 MRSA cases were nonmenstrual, affecting mainly male patients; 3 were associated with SSTIs. The median patient age was 34 (IQR 23–64.3) years. Five isolates were identified as CC22-SC-seg-tst (Table 2) and 4 carried sec, corresponding to the healthcare-associated MRSA clade dominant in the UK, EMRSA-15; MRSA-TSS cases showed a clear association with this clade. The mecA-positive isolates had only 2 superantigen genes (seg-tst) whereas one isolate was mecA-negative and carried only tst. Resistance to clindamycin was 29.4% (4/13) and to erythromycin 46.2% (6/13) for mecA-positive isolates and 0% (0/5) for mecA-negative isolates. Four isolates were mecA-negative and resistant to clindamycin (30.8%); two were mecA-negative and resistant to erythromycin (15.4%). The resistance rate to ciprofloxacin was 76.9% (10/13) for mecA-positive isolates and 0% (0/5) for mecA-negative isolates. As mecA-bearing isolates were also mecA-negative, we included mecA-negative isolates in the analysis.

Table 1. Clinical characteristics of staphylococcal toxic shock syndrome cases, United Kingdom, 2008–2012

| Characteristics       | All patients, n = 180† | Menstrual, n = 70 | Nonmenstrual, n = 107 | p value |
|-----------------------|------------------------|-------------------|-----------------------|---------|
| Median age, y (IQR)   | 19.0 (9.0–38.3)        | 21.5 (17–35.3)    | 15.9 (1–43.5)         | 0.01‡   |
| Sex, no. (%)          |                         |                   |                       |         |
| F                     | 128 (71.1)             | 70 (100)          | 55 (51.4)             | 0.0001§ |
| M                     | 51 (28.3)              | 0                 | 51 (47.7)             |         |
| Unknown               | 0                      | 0                 | 1 (0.9)               |         |
| Deaths, no. (%)       | 9 (5.0)                | 4 (5.7)           | 5 (4.7)               | 0.74§   |

†Boldface indicates a statistically significant result, p<0.05. IQR, interquartile range.
‡3 Mann-Whitney U test comparing menstrual and nonmenstrual TSS cases.
§Fisher exact test comparing menstrual and nonmenstrual TSS cases.
lineage (online Technical Appendix Table 4). The remaining CC22 isolate carried ts and belonged to a MRSA lineage frequently identified in the Middle East. Only 1 MRSA-TSS isolate was CC30-SCCmecII, corresponding to the UK HA-MRSA clade EMRSA-16. One isolate was CC6-SCCmecII and lacked all superantigen genes tested.

**TSST-1 Production by CC30 S. aureus**

The strong association of CC30 with TSS was unsurprising because of the presence of ts. We measured TSST-1 in broth-culture supernatants from ts-positive CC30 MSSA isolates from the TSS cohort and, for comparison, randomly selected clinical ts-positive MRSA isolates that belonged to the same lineage (CC30) (20,29) (online Technical Appendix Table 1).

Of note, 77/81 ts-positive CC30 MSSA isolates produced detectable TSST-1, compared with 9/39 ts-positive CC30 MRSA isolates. The ts-positive CC30 MSSA isolates produced more TSST-1 than did ts-positive CC30 MRSA isolates, albeit with marked variability (88.5 ± 48.3 vs. 31.4 ± 18.1 ng/mL; p<0.0001; Figure 4, panel A). Furthermore, the superantigenic activity of isolates, measured by T-cell proliferation in response to broth-culture supernatants, of ts-positive CC30 MSSA strains (164,893 ± 36,191 counts/min) was significantly greater than that of ts-positive CC30 MRSA strains (149,653 ± 30,412 counts/min; p = 0.02; Figure 4, panel B).

**tst-positive CC30 MRSA and Mutation in ts Regulator, CcpA**

To ascertain the basis for the observed variability in TSST-1 production among CC30 S. aureus, we subjected 4 ts-positive CC30 MSSA isolates from the TSS cohort and 5 ts-positive CC30 MRSA clinical isolates to whole-genome sequencing. The ts gene, promoter, and regulator sequences, including SarA, SrrAB, agr, and σB, were identical among the 9 sequenced strains and reference isolates (MN8/MRSA252).

We detected mutations in TSST-1 regulator SaeRS in 2/4 ts-positive CC30 MSSA isolates; a synonymous SNP C481T in SaeR in 1 strain and a nonsynonymous SNP in SaeS in another resulted in a change from asparagine to serine at aa residue 218. Because these strains produced abundant TSST-1 (online Technical Appendix Table 1), we did not study these mutations further.

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**Table 2. Frequency of major superantigen genes among Staphylococcus aureus isolates associated with menstrual and nonmenstrual toxic shock syndrome, United Kingdom, 2008–2012**

| Superantigen gene† | Total, n = 180‡ | Menstrual, n = 70 | Nonmenstrual, n = 107 | p value§ |
|--------------------|----------------|------------------|-----------------------|---------|
| *sea and *tst combined* | 54 (30.0) | 27 (38.6) | 25 (23.4) | 0.04 |
| *tst* alone | 37 (20.5) | 23 (32.9) | 13 (12.1) | 0.001 |
| *sea* alone | 12 (6.7) | 4 (5.7) | 8 (7.5) | 0.77 |
| *seb* alone | 11 (6.1) | 3 (4.3) | 8 (7.5) | 0.53 |
| *sec* alone | 14 (7.8) | 1 (1.4) | 13 (12.1) | 0.01 |
| *sed* alone | 4 (2.2) | 0 | 4 (3.7) | 0.15 |

*Boldface indicates a statistically significant result.
†Does not include 48 TSS isolates that did not have *sea*, *seb*, *sec*, *sed*, or *ts* in isolation.
‡Three additional TSS isolates could not be classified as menstrual or nonmenstrual due to lack of clinical data.
§By Fisher exact test comparing the percentage carriage of a given superantigen gene among menstrual and nonmenstrual isolates.
We detected a nonsynonymous SNP in the sequence of regulator ccpA in all 5 tst-positive CC30 MRSA isolates but not in any tst-positive CC30 MSSA isolate. This difference translated into a change from threonine (A\(_{\text{TSS-1}}\)) to isoleucine (A\(_{\text{TSS-2}}\)) at aa residue 87/329 (online Technical Appendix Figure 3).

To determine the prevalence of the ccpA (T87I) variant in CC30, we sequenced ccpA in an additional 34 tst-positive CC30 MRSA and 19 tst-positive CC30 MSSA isolates (online Technical Appendix Table 1). Including genome-sequenced isolates, 33/39 tst-positive CC30 MRSA isolates had ccpA (T87I), compared with 0/23 tst-positive CC30 MSSA isolates, confirming an association of ccpA (T87I) with CC30 MRSA strains. Furthermore, ccpA (T87I) was strongly negatively associated with production of TSST-1 in tst-positive CC30 S. aureus: 26/33 ccpA (T87I) isolates did not produce TSST-1, compared with only 1/23 wild-type ccpA isolates (p<0.0001 by Fisher exact test).

We conducted SCCmec typing on a subset of tst-positive CC30 MRSA strains (n = 15; online Technical Appendix Table 1). Results demonstrated an association of ccpA (T87I) with SCCmecII; 7/11 SCCmecII isolates had ccpA (T87I), compared with 0/4 SCCmecIV isolates (p = 0.03 by \(\chi^2\) test). This finding highlights the possibility that reduced TSST-1 production might be attributable to either SCCmecII or ccpA (T87I).

**Discussion**

We provide a substantive national clinical and microbiological overview of staphylococcal TSS cases in the United Kingdom. TSS incidence was 0.07/100,000 population, nmTSS cases now outnumber mTSS cases, and nmTSS affects younger persons. The tst-positive CC30 S. aureus lineage was linked strongly with TSS and almost all mTSS cases. CC30 MSSA is a prevalent lineage in the United Kingdom (16), so ongoing surveillance and clinical vigilance for TSS are important.

Our findings may underestimate TSS incidence because notification of TSS is voluntary in the United Kingdom and we included only microbiologically confirmed cases. These factors increase diagnostic confidence, but TSS is a syndromic condition not requiring bacteriological confirmation. Overall TSS incidence was low but similar to rates in the United States (2); improvements in care may account for low overall incidence of TSS, because patients may not fulfill all of the criteria required by the case definition of TSS. The overall TSS incidence in children contrasts with findings from a British Pediatric Surveillance Unit study in which a higher incidence of combined streptococcal and staphylococcal TSS cases was reported (30).

The number of cases of mTSS fell from 2009 to 2012, such that nmTSS cases are now more common than mTSS cases, mirroring US trends (31). Patients in our study were younger than in US cohorts (31,32), and nmTSS patients were younger than those with mTSS. Most nmTSS cases occurred in children, with burns and SSTIs as the cause in 51.8% (29/56) of these cases. An association between nmTSS and increased mortality rate has been reported, although a high incidence of bacteremia may have affected the findings of that study (33). It is possible that we did not ascertain all cases of TSS, although we found no difference in reported deaths between mTSS and nmTSS cases or associations with age; the overall death rate was 5%.

The association of TSS, and particularly mTSS, with a single lineage corresponding to CC30 S. aureus has been described in diverse geographic localities (14,15). The tst-positive CC30 MSSA clone has recently been named epidemic MSSA-ST30 because it is responsible for a substantial
amount of *S. aureus* disease and is a precursor to the HA-
MRSA clone, EMRSA-16, which has been responsible for
major national UK MRSA outbreaks (29).

The *tst* gene was the predominant superantigen gene
among TSS isolates, excluding *seg* and *sei*, which were
also previously implicated in TSS (34). The superantigens
*seg* and *sei* are carried on the *egc*, which is widespread in
*S. aureus* (5), and are unlikely to have any specific associa-
tion with TSS. We linked *tst* to mTSS and CC30. Several
groups have demonstrated similar associations of staphy-
lococcal superantigens genes with specific lineages (35,36),
due to clonal associations, superantigen arrangements, and
transmission via mobile genetic elements, although other
firm associations linking lineage, superantigen gene car-
riage, infection type, and disease presentations have not
been made. A recent study of atopic dermatitis that exam-
ined the relationship of ethnicity and staphylococcal viru-
ulence factors found a lack of *tst*-positive *S. aureus* atopic
dermatitis in African American persons that was consistent
with an absence of *tst*-positive *S. aureus* mTSS among
this group, suggesting differences in disease presentation
among disparate ethnic groups (37) based on host charac-
teristics. The ethnicity of the patients with TSS referred to
PHE in this study was not recorded, and such bacterial ge-
netic associations with disease could not be made but may
merit consideration in future studies.

Among MSSA isolates, resistance rates to key antimicro-
bial drugs were similar to reported UK MSSA bacteremia
isolates (38). Notably, teicoplanin resistance was detec-
ted, although rarely. This finding circumvents any need
to change current recommendations for antimicrobial drugs
for TSS that include a bactericidal cell wall inhibitor (e.g.,
β-lactamase–resistant antistaphylococcal) and protein-syn-
thesis inhibitor (e.g., clindamycin) along with intravenous
immunoglobulin for severe cases unresponsive to first-line
therapy and source control (39). No vaccines are available
to prevent TSS, although a recombinant TSST-1 variant
vaccine has shown promise in a recent human clinical trial
and was found to be safe and immunogenic (40).

The MRSA-TSS rate in this study was lower than rates
in the United States (32), perhaps reflecting the low UK
community-associated MRSA prevalence (41). All MRSA
cases were nonmenstrual and mostly associated with recog-
nized healthcare-associated MRSA clones, although we did
not record the mode of acquisition. Only 1 CC30 MRSA
(EMRSA-16) isolate caused TSS, even though CC30 is the
main TSS-associated lineage; this finding mirrors the na-
tional decline in UK EMRSA-16 over time (42).

Isolates of *tst*-positive CC30 MSSA were more likely
to produce TSST-1 in vitro and secreted almost 3 times
more TSST-1 than did *tst*-positive CC30 MRSA isolates,
which translated into a functional difference in superanti-
genic activity. We do not know whether such a difference
would extend to the in vivo setting. Our study of TSST-1
production was limited by availability of clinical *tst*+ CC30
strains; clinical TSS CC30 MSSA strains were therefore
compared with clinical non-TSS CC30 MRSA strains and
not to clinical TSS MRSA strains. Thus, more MSSA than
MRSA strains were from the genital tract or from burns,
potentially confounding phenotypic differences observed.
Defining the precise comparator group for TSS CC30
MSSA isolates is challenging because of lack of TSS
CC30 MRSA isolates and suitable non-TSS strains referred
to PHE.

Bacterial acquisition of antimicrobial drug resistance
elements can be associated with a fitness cost. In the United
Kingdom, most CC30 HA-MRSA strains carry SCCmecII
(EMRSA-16; ST36-SCCmecII) that may reduce cytolytic
toxin production and, in association with *fudoh* gene car-
riage by this element, reduce hemolytic activity and viru-
ulence (43,44). Our findings suggest an association between
SCCmecII and reduced TSST-1 production that might be
linked to a SNP in a regulatory gene, *ccpA*. The resulting
mutation in CcpA occurs adjacent to a co-repressor binding
site in the transcriptional regulation region (online Techni-
cal Appendix Figure 3) that could influence *tst* promoter
binding and affect TSST-1 secretion. Such SNPs in viru-
ulence regulators may have had a role in shaping the health-
care-associated phenotype of EMRSA-16 (20). New tools
that allow manipulation of previously nontransformable
lineages such as CC30 will facilitate investigating such ge-
netic mechanisms in *S. aureus* (45).

Our study shows that the ability to produce TSST-1
varies widely within the *tst*-positive CC30 lineage and im-
paired expression is associated with the presence of SC-
CmecII and *ccpA* (T87I), underlining the potential for ge-
nomic approaches to contribute to greater understanding
of patterns of clinical disease. Given the prevalence of *tst*-
positive CC30 MSSA causing TSS and its role as a dominant
UK lineage of *S. aureus*, active surveillance of this lineage
is required. Clarification of the particular modes of trans-
mission, acquisition, and pathogenesis of this lineage may
identify susceptible persons, such as younger persons with
burns and SSTIs, who might benefit from interventions
such as vaccination with recombinant TSST-1 or *S. aureus*
screening and decolonization in the future to prevent the
occurrence of this life-threatening syndrome.

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References
1. Osterholm MT, Forfang JC. Toxic-shock syndrome in Minnesota: results of an active-passive surveillance system. J Infect Dis. 1982;145:458–64. http://dx.doi.org/10.1093/infdis/145.4.458
2. Adams DA, Thomas KR, Jajosy RA, Foster L, Sharp P, Onweh DH, et al.; Nationally Notifiable Infectious Conditions Group. Summary of notifiable infectious diseases and conditions—United States, 2014. MMWR Morb Mortal Wkly Rep. 2016;63:1–152. http://dx.doi.org/10.15585/mmwr.mm6354a1
3. Fraser JD, Profi T. The bacterial superantigen and superantigen-like proteins. Immunol Rev. 2008;225:226–43. http://dx.doi.org/10.1111/j.1600-065X.2008.00681.x
4. Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol. 1990;17:251–72. http://dx.doi.org/10.3109/10408419009105728
5. Grumann D, Nübel U, Bröker BM. Staphylococcus aureus toxins—their functions and genetics. Infect Genet Evol. 2014;21:583–92. http://dx.doi.org/10.1016/j.meegid.2013.03.013
6. Whiting JL, Rosten PM, Chow AW. Determination by western blot (immunoblot) of serovерosions to toxic shock syndrome (TSS) toxin 1 and enterotoxin A, B, or C during infection with TSS- and non–TSS-associated Staphylococcus aureus. Infect Immun. 1989;57:231–4.
7. Novick RP. Mobile genetic elements and bacterial toxinoses: the chromosomal islands of Gram-positive bacteria. Nat Rev Microbiol. 2007;51:3374–7. http://dx.doi.org/10.1128/AAC.00275-07
8. Novick RP, Christie GE, Penadés JR. The phage-related chromosomal islands of Gram-positive bacteria. Nat Rev Microbiol. 2010;8:541–51. http://dx.doi.org/10.1038/nrmicro2393
9. Li Z, Stevens DL, Hamilton SM, Parimon T, Ma Y, Kearns AM, et al. Fatal S. aureus hemorrhagic pneumonia: genetic analysis of a unique clinical isolate producing both PVL and TSST-1. PLoS One. 2011;6:e27246. http://dx.doi.org/10.1371/journal.pone.0027246
10. Pragman AA, Schlievert PM. Virulence regulation in Staphylococcus aureus: the need for in vivo analysis of virulence factor regulation. FEMS Immunol Med Microbiol. 2004;42:147–54. http://dx.doi.org/10.1016/j.femsimm.2004.05.005
11. Seidl K, Bischoff M, Berger-Bächi B. CcpA mediates the catabolite repression of tuf in Staphylococcus aureus. Infect Immun. 2008;76:5093–9. http://dx.doi.org/10.1128/IAI.00724-08
12. Andrey DO, Jousselin A, Villanueva M, Renzoni A, Monod A, Barras C, et al. Impact of the regulators sigB, rot, sarA and sarS on the toxic shock tst promoter and TSST-1 expression in Staphylococcus aureus. PLoS One. 2015;10:e0135579. http://dx.doi.org/10.1371/journal.pone.0135579
13. Baroja ML, Herfst CA, Kasper KJ, Xu SX, Gillett DA, Li J, et al. The SaeRS two-component system is a direct and dominant transcriptional activator of toxic shock syndrome toxin 1 in Staphylococcus aureus. J Bacteriol. 2016;198:2732–42. http://dx.doi.org/10.1128/JB.00425-16
14. Musser JM, Schlievert PM, Chow AW, Ewan P, Kreiswirth BN, Rosdahl VT, et al. A single clone of Staphylococcus aureus causes the majority of cases of toxic shock syndrome. Proc Natl Acad Sci U S A. 1990;87:225–9. http://dx.doi.org/10.1073/pnas.87.1.225
15. Fitzgerald JR, Sturdevant DE, Mackie SM, Gill SR, Musser JM. Evolutionary genomics of Staphylococcus aureus: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc Natl Acad Sci U S A. 2001;98:8821–6.
16. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, et al. How clonal is Staphylococcus aureus? J Bacteriol. 2003;185:3307–16. http://dx.doi.org/10.1128/JB.185.11.3307-3316.2003
17. Hiramatsu K, Ito T, Tsukaboriishi S, Sasaki T, Takeuchi F, Morimoto Y, et al. Genomic basis for methicillin resistance in Staphylococcus aureus. Infect Chemother. 2013;45:117–36. http://dx.doi.org/10.3947/ic.2013.45.2.117
18. Wu Z, Li F, Liu D, Xue H, Zhao X. Novel type XII staphylococcal cassette chromosome mec harboring a new cassette chromosome recombinase, CerC2. Antimicrob Agents Chemother. 2015;59:7597–601. http://dx.doi.org/10.1128/AAC.01692-15
19. Subedi A, Ubeda C, Adhikari RP, Penadés JR, Novick RP. Sequence analysis reveals genetic exchanges and inaspecific spread of SaPI2, a pathogenicity island involved in menstrual toxic shock. Microbiology. 2007;153:3325–45. http://dx.doi.org/10.1099/mic.0.2007/006932-0
20. McAdam PR, Templeton KE, Edwards GF, Holden MT, Feil EJ, Aanesen DM, et al. Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant Staphylococcus aureus. Proc Natl Acad Sci U S A. 2012;109:9107–12. http://dx.doi.org/10.1073/pnas.120869109
21. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol. 2004;42:792–9. http://dx.doi.org/10.1128/JCM.42.2.792-799.2004
22. Milheiro C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in Staphylococcus aureus. Antimicrob Agents Chemother. 2007;51:1374–7. http://dx.doi.org/10.1128/AAC.00725-07
23. Boakes E, Kearns AM, Ganner M, Perry C, Warner M, Hill RL, et al. Molecular diversity within clonal complex 22 methicillin-resistant Staphylococcus aureus encoding Panton-Valentine leukocidin in England and Wales. Clin Microbiol Infect. 2011;17:140–5. http://dx.doi.org/10.1111/j.1469-0691.2010.01399.x
24. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(Suppl 1):5–16. http://dx.doi.org/10.1093/jac/48.suppl_1.5
25. Unnikrishnan M, Altmann DM, Profi T, Wahid F, Cohen J, Fraser JD, et al. The bacterial superantigen streptococcal mitogenic exotoxin Z is the major immunogenic agent of Streptococcus pyogenes. J Immunol. 2002;169:2561–9. http://dx.doi.org/10.4049/jimmunol.169.5.2561
26. Pospiech B, Neumann B. A versatile quick-prep of genomic DNA from Gram-positive bacteria. Trends Genet. 1995;11:217–8. http://dx.doi.org/10.1016/S0168-9525(00)98052-6
27. Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, et al. Complete genomes of two clinical Staphylococcus aureus strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci U S A. 2004;101:9786–91. http://dx.doi.org/10.1073/pnas.0402521101
28. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9. http://dx.doi.org/10.1093/bioinformatics/btp352
29. Aanensen DM, Fell EJ, Holden MT, Dordel J, Yeats CA, Fedosejev A, et al.; European SRL Working Group. Whole-genome sequencing for routine pathogen surveillance in public health: a population snapshot of invasive Staphylococcus aureus in Europe. MBio. 2016;7:e00444-16. http://dx.doi.org/10.1128/mBio.00444-16
30. Adalat S, Dawson T, Hackett SJ, Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Holtfreter S, Grumann D, Schmudde M, Nguyen HT, Eichler P, Jarraud S, Cozon G, Vandenesch F, Bes M, Etienne J, Lina G. Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. J Clin Microbiol. 1999;37:2446–9.
31. Hajjeh RA, Reingold A, Weil A, Shutt K, Schuchat A. In association with the British Pediatric Surveillance Unit. Toxic shock syndrome surveillance in UK children. Arch Dis Child. 2014;99:1078–82. http://dx.doi.org/10.1136/archdischild-2013-304741
32. DeVries AS, Lesher L, Schlievert PM, Rogers T, Villaume LG. One in five mortality in menstrual cases in a balanced French series versus no mortality in non-menstrual toxic shock syndrome causing bacteraemias in the UK between 2001 and 2007. J Antimicrob Chemother. 2010;65:446–8. http://dx.doi.org/10.1093/jac/dkp448
33. Aanensen DM, Feil EJ, Holden MT, Dordel J, Yeats CA. In association with the European SRL Working Group. Whole-genome sequencing for routine pathogen surveillance in public health: a population snapshot of invasive Staphylococcus aureus in Europe. MBio. 2016;7:e00444-16. http://dx.doi.org/10.1128/mBio.00444-16
34. DeVries AS, Lesher L, Schlievert PM, Rogers T, Villaume LG, Danila R, et al. Staphylococcal toxic shock syndrome 2000–2006: epidemiology, clinical features, and molecular characteristics. PLoS One. 2011;6:e22997. http://dx.doi.org/10.1371/journal.pone.0022997
35. Descloux E, Perpoint T, Ferry T, Lina G, Bes M, Vandenesch F, et al. Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome. J Clin Microbiol. 1999;37:2446–9.
36. Holtfreter S, Grumann D, Schmudde M, Nguyen HT, Eichler P, Jarraud S, Cozon G, Vandenesch F, Bes M, Etienne J, Lina G. Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. J Clin Microbiol. 1999;37:2446–9.
37. Merriman JA, Mueller EA, Cahill MP, Beck LA, Paller AS, Hanifin JM, et al. Temporal and racial differences associated with atopic dermatitis Staphylococcus aureus and encoded virulence factors. mSphere. 2016;1:e00295-16.
38. Public Health England. Voluntary reporting of Staphylococcus aureus bacteraemia in England, Wales, and Northern Ireland, 2013 [cited 2015 Mar 6]. http://www.gov.uk/government/uploads system/uploads/attachment_data_file/346324/Voluntary_reporting_S._aureus_bacteraemia_England_Wales_Northern_Ireland_2013.pdf
39. American Academy of Pediatrics. Staphylococcal infections. In: Kimberlin DW, Brady MT, Jackson MA, Long SS. Red book: 2015 report of the Committee on Infectious Diseases. Elk Grove Village (IL): American Academy of Pediatrics, 2015. p. 715–32.
40. Schwameis M, Roppensber B, Firbas C, Gruen G, Model N, Stich N, et al. Safety, tolerability, and immunogenicity of a recombinant toxic shock syndrome toxin (rTSST)-1 variant vaccine: a randomised, double-blind, adjuvant-controlled, dose escalation first-in-man trial. Lancet Infect Dis. 2016;16:1036–44. http://dx.doi.org/10.1016/S1473-3099(16)30115-3
41. Ellington MJ, Hope R, Livermore DM, Kearns AM, Henderson K, Cookson BD, et al. Decline of EMRSA-16 amongst methicillin-resistant Staphylococcus aureus causing bacteraemias in the UK between 2001 and 2007. J Antimicrob Chemother. 2010;65:446–8. http://dx.doi.org/10.1093/jac/dkp448
42. Rudkin JK, Edwards AM, Bowden MG, Brown EL, Pozzi C, Waters EM, et al. Methicillin resistance reduces the virulence of healthcare-associated methicillin-resistant Staphylococcus aureus by interfering with the agr quorum sensing system. J Infect Dis. 2012;205:798–806. http://dx.doi.org/10.1093/jinf/dis685
43. Elston JW, Barlow GD. Community-associated MRSA in the United Kingdom. J Infect. 2009;59:149–55. http://dx.doi.org/10.1016/j.jinf.2009.07.001
44. Kaito C, Omae Y, Matsumoto Y, Nagata M, Yamaguchi H, Aoto T, et al. A novel gene, fudoh, in the SCCmec region suppresses the colony spreading ability and virulence of Staphylococcus aureus. PLoS One. 2008;3:e3921. http://dx.doi.org/10.1371 journal. pone.0003921
45. McKee MR, Shah IM, Xu M, Tan MW, Foster TJ. Transforming the untransformable: application of direct transformation to manipulate genetically Staphylococcus aureus and Staphylococcus epidermidis. MBio. 2012;3:e00277-11. http://dx.doi.org/10.1128/mBio.00277-11

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Clinical and Molecular Epidemiology of Staphylococcal Toxic Shock Syndrome in the United Kingdom

Technical Appendix

Methods

Bacterial Culture

We cultured bacterial isolates in 5 mL of brain heart infusion (BHI) for Western blotting or Roswell Park Memorial Institute (RPMI) media supplemented with 10% fetal calf serum (FCS) for proliferation assays, at 37°C with agitation. We removed bacterial cells from supernatants by centrifugation and 0.2µM filtration.

Analysis of TSST-1 by Western Blot

tst+ CC30 methicillin-sensitive S. aureus (MSSA) TSS-isolates (n = 81) and randomly selected tst+ CC30 methicillin-resistant S. aureus (MRSA) isolates (n = 39) (Technical Appendix Table 1) were cultured to stationary phase in BHI and supernatants prepared as above and then concentrated x10 using a 10 kDa spin column (Amicon, Merck Millipore, Nottingham, UK). Standard concentrations of purified TSST-1 (Toxin Technology, Sarasota, FL, USA) and bacterial supernatants were diluted 2:1 with NuPAGE LDS sample buffer (4x) (Life Technologies, Hemel Hempstead, UK) and 100 mM dithiothreitol then heated to 70°C for 10 minutes. A 15 µL sample was loaded onto 10% NuPAGE novex bis-tris gels. After electrophoresis, we transferred proteins to a PVDF membrane (Amersham Hybond-LFP, GE Healthcare Life Sciences, Amersham, UK) then blocked with 5% milk (Sigma, Dorset, UK) with 0.05% Tween-20 (Sigma, Dorset, UK). We incubated the samples overnight at 4°C with rabbit anti-TSST-1 polyclonal primary antibody (Abcam, Cambridge, UK) diluted 1:10,000; washed the blots and incubated them with anti-rabbit-HRP conjugated secondary antibody (Life Technologies) diluted 1:50,000; then developed them using Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare Life Sciences, Amersham, UK). We determined TSST-1...
concentration in supernatants by comparing them with a TSST-1 standard curve by densitometry (LabWorks, UVP, Upland, CA, USA).
Referral Form

Healthcare Pathogens
Characterisation and Resistance (single isolate)

Bacteriology Reference Department
(AVRHBC)
61 Colindale Avenue, London NW9 5HR

Phone: +44 (0)20 8327 7887
PHE Cilindale
AMR@phee.gov.uk
Bacteriology
tox 65300002
www.gov.uk/phe
Colindale NW

SENSE’S INFORMATION
Sender’s name and address

Report to be sent FAO
Contact Phone
Ext.
Purchase order number
Project code
PHE outbreak/investigation
Log number

POSTAL/ SOURCE INFORMATION

*Please specify
*Please specify

In Patient
Out Patient
GP Patient
Other

NHS number
Sex
male
female
Surname

NHS number
Surname
Forename

Hospital number
Ward/clinic name
Hospital name (different from sender’s name)
Ward type
Medical-legal case

SALVAGE INFORMATION
Your reference
Do you suspect that the isolate you are referring could be hazard group 3
Yes
No

Isolation site
Blood
Nose
Wound
Environment
Skin
Meal
Other (please specify)

Date of collection

Date sent to PHE

Priority status

TESTS REQUESTED

Presumptive identification
S. aureus MRSA
B. cepacia complex
Klebsiella
S. aureus MSSA
Enterobacter
P. aeruginosa
Coag Neg Staph
Enterevoccus
S. maltophilia
A. Acinetobacter
E. coli
S. maltophilia

MIC evaluation (specify reason below)
ESBL detection
VRE A PCR
Carbapenem resistance
MupA PCR
Acquired AmpC
Linezolid resistance

SENDER’S LABORATORY RESULTS
API profile no
Gram stain
Oxidase +:
Catalase +:
Growth requirement

CLINICAL/Epidemiological information

Pick one

Clinical details
Bacteremia
Pyrexia/Fever
Septic shock
Septicemia
Sudden infant death syndrome
Toxic shock syndrome
Pulmonary

Reasons for request
Confirmation of results
Unusual resistance (please specify)
Sporadic
Therapeutic guidance
Continuing investigation
Increasing numbers
Inter-hospital transfer
Other (please specify)

Foreign Travel
Yes
No
Country

All requests are subject to PHE standard terms and conditions.
Version effective from Apr - 2014
BRIND140201
**Technical Appendix Table 1. Staphylococcus aureus strains that caused toxic shock syndrome in the United Kingdom, 2008–2012**

| Strain | Site of infection† | mecA | SCCmec | MLST-CC | ccpA (T87I) | Superantigen genes‡ | TSST-1 ng/mL§ |
|--------|-------------------|------|--------|---------|-------------|---------------------|--------------|
| MSSA   |                   |      |        |         |             |                     |              |
| HSS354 |                   |     -|        | 30      |             | seg, seh, sei, tst  | 49.0         |
| HSS355 |                   |     -|        | 30      |             | sec, tst           | 39.2         |
| HSS356 | mTSS              |     -|        | 30      |             | sea, seg, sei, tst | 112.5        |
| HSS357 | mTSS              |     -|        | 30      |             | seg, sei, tst      | 187.3        |
| HSS358 | mTSS              |     -|        | 30      |             | seg, sei, tst      | 154.5        |
| HSS359 | Burn              |     -|        | 30      |             | seg, sei           | 67.6         |
| HSS394 | Abscess           |     -|        | 30      | ND          | sea, seg, sei, tst | 87.0         |
| HSS395 | mTSS              |     -|        | 30      |             | sea, seg, sei      | 124.8        |
| HSS397 | Burn              |     -|        | 30      |             | seg, sei, tst      | 73.3         |
| HSS398 | Burn              |     -|        | 30      | ND          | seg, sei           | 38.8         |
| HSS405 | Abscess           |     -|        | 30      | ND          | sea, seg, sei, tst | 57.1         |
| HSS409 | mTSS              |     -|        | 30      | ND          | seg, sei           | 32.8         |
| HSS412 | mTSS              |     -|        | 30      |             | seg, sei           | 46.3         |
| HSS413 | mTSS              |     -|        | 30      | ND          | seg, sei           | 40.1         |
| HSS414 | mTSS              |     -|        | 30      | ND          | sec, seh, tst      | 42.3         |
| HSS416 | mTSS              |     -|        | 30      | ND          | sea, seg, seh, tst | 106.7        |
| HSS417 | Skin              |     -|        | 30      |             | seg, sei           | 25.8         |
| HSS419 | mTSS              |     -|        | 30      | ND          | seg, sei           | <25.0        |
| HSS422 | mTSS              |     -|        | 30      | ND          | seg, sei           | 83.0         |
| HSS423 | URT               |     -|        | 30      | ND          | sea, seg, seh, tst | 126.9        |
| HSS425 | mTSS              |     -|        | 30      | ND          | sea, seg, sei      | 186.4        |
| HSS426 | mTSS              |     -|        | 30      | ND          | sea, seg, sei, tst | 139.9        |
| HSS427 | Skin              |     -|        | 30      | ND          | seg, sei           | 96.9         |
| HSS428 | mTSS              |     -|        | 30      | ND          | sea, seg, seg, sei | 57.0         |
| HSS429 | mTSS              |     -|        | 30      | ND          | seg, seh, tst      | 48.3         |
| HSS430 | mTSS              |     -|        | 30      | ND          | seg, seh, tst      | <25.0        |
| HSS431 | mTSS              |     -|        | 30      | ND          | sea, seg, sei      | 55.2         |
| HSS432 | mTSS              |     -|        | 30      | ND          | seg, seh, tst      | 120.0        |
| HSS434 | Skin              |     -|        | 30      | ND          | seg, seh, tst      | <25.0        |
| HSS435 | mTSS              |     -|        | 30      | ND          | sea, seg, sei      | 54.9         |
| HSS436 | mTSS              |     -|        | 30      | ND          | sea, seg, sei, tst | 125.2        |
| HSS437 | Bacteremia        |     -|        | 30      | ND          | sea, seg, sei, tst | 137.4        |
| HSS438 | mTSS              |     -|        | 30      | ND          | sea, seg, seh, sei, tst | 173.8 |
| HSS439 | mTSS              |     -|        | 30      | ND          | sea, seg, sei, tst | 186.3        |
| HSS440 | Burn              |     -|        | 30      | ND          | sea, seg, sei, tst | 129.9        |
| HSS441 | mTSS              |     -|        | 30      | ND          | sea, seg, sei, tst | 61.8         |
| HSS443 | mTSS              |     -|        | 30      | ND          | seg, seh, tst      | 108.2        |
| HSS445 | UK                |     -|        | 30      | ND          | seg, seh, tst      | 105.7        |
| HSS446 | LRT               |     -|        | 30      | ND          | sea, seg, sei      | 54.6         |
| HSS449 | mTSS              |     -|        | 30      | ND          | sea, seg, sei, tst | 134.3        |
| HSS451 | mTSS              |     -|        | 30      | ND          | seg, sei           | 57.4         |
| HSS454 | mTSS              |     -|        | 30      | ND          | seg, sei           | 49.1         |
| HSS456 | mTSS              |     -|        | 30      | ND          | seg, sei           | 51.9         |
| HSS457 | mTSS              |     -|        | 30      | ND          | seg, sei           | 78.0         |
| HSS459 | Skin              |     -|        | 30      | ND          | sea, seg, sei      | 54.2         |
| HSS463 | mTSS              |     -|        | 30      | ND          | seg, seh           | 57.9         |
| HSS468 | UK                |     -|        | 30      | ND          | sec, seh, tst, pvβ | 26.8         |
| HSS469 | Bacteremia        |     -|        | 30      | ND          | sea, seg, sei, tst | 133.6        |
| HSS470 | mTSS              |     -|        | 30      | ND          | sea, seg, sei, tst | 96.0         |
| HSS473 | Skin              |     -|        | 30      | ND          | seg, seh           | 92.4         |
| HSS474 | mTSS              |     -|        | 30      | ND          | sea, seg, tst      | <25.0        |
| HSS475 | mTSS              |     -|        | 30      | ND          | sec, seg, sei      | 94.4         |
| HSS476 | mTSS              |     -|        | 30      | ND          | sec, seg, sei      | 108.3        |
| HSS478 | mTSS              |     -|        | 30      | ND          | seg, sei           | 66.3         |
| HSS479 | Skin              |     -|        | 30      | ND          | seg, sei           | 204.6        |
| HSS481 | Skin              |     -|        | 30      | ND          | sea, seg, sei      | 40.0         |
| HSS485 | mTSS              |     -|        | 30      | ND          | sea, seg, sei      | 68.7         |
| HSS490 | UK                |     -|        | 30      | ND          | sea, seg, sei      | 174.9        |
| HSS492 | Burn              |     -|        | 30      | ND          | seg, sei           | 82.9         |
| HSS493 | Eye               |     -|        | 30      | ND          | sea, seg, sei      | 65.8         |
| HSS494 | Bacteremia        |     -|        | 30      | ND          | sea, seg, sei, tst | 116.9        |
| HSS496 | mTSS              |     -|        | 30      | ND          | sea, seg, sei      | 44.7         |
| HSS497 | Bacteremia        |     -|        | 30      | ND          | seg, seh, tst      | 90.8         |
| HSS499 | Abscess           |     -|        | 30      | ND          | sea, seg, sei, tst | 114.4        |
| HSS501 | Skin              |     -|        | 30      |             | seg, seh, tst      | 122.8        |
| HSS502 | mTSS              |     -|        | 30      | ND          | seg, sei           | 98.0         |
| HSS503 | Skin              |     -|        | 30      | ND          | sea, seg, sei      | 111.6        |
| HSS504 | mTSS              |     -|        | 30      | ND          | sea, seg, sei      | 60.0         |
| HSS512 | mTSS              |     -|        | 30      | ND          | sea, seg, seh, sei, tst | 57.6 |
| HSS513 | Burn              |     -|        | 30      |             | sea, seg, sei      | 66.9         |
| Strain | Site of infection† | mecA | SCC/mec | MLST-CC | ccppA (T87I) | Superantigen gene‡ | TSST-1 ng/ml§ |
|--------|-------------------|------|---------|---------|-------------|------------------|-------------|
| HSS514 | mTSS              | –    | –       | 30      | ND          | sea, seg, sei, tst | 74.9        |
| HSS517 | mTSS              | –    | –       | 30      | ND          | sea, seg, sei, tst | 70.2        |
| HSS518 | mTSS              | –    | –       | 30      | ND          | sea, seg, sei, tst | 79.8        |
| HSS520 | mTSS              | –    | –       | 30      | ND          | sea, seg, sei, tst | 61.8        |
| HSS521 | mTSS              | –    | –       | 30      | –           | sea, seg, sei, tst | 75.8        |
| HSS522 | Skin              | –    | –       | 30      | ND          | sea, seg, sei, tst | 168.4       |
| HSS525 | Skin              | –    | –       | 30      | ND          | sea, seg, sei, tst | 134.9       |
| HSS530 | Abscess           | –    | –       | 30      | ND          | seg, sei, tst     | 45.1        |
| HSS533 | UK                | –    | –       | 30      | –           | sea, seg, sei, tst | 114.6       |
| HSS535 | mTSS              | –    | –       | 30      | –           | sec, tst          | 191.6       |

MRSA

| Strain | Site of infection† | mecA | SCC/mec | MLST-CC | ccppA (T87I) | Superantigen gene‡ | TSST-1 ng/ml§ |
|--------|-------------------|------|---------|---------|-------------|------------------|-------------|
| HSS360 | Bone and joint    | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS361 | Bone and joint    | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS362 | Bacteremia        | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS363 | Bacteremia        | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS364 | Bacteremia        | +    | ND      | 30      | +           | seg, sei, tst     | <25.0       |
| HSS377 | UK                | +    | IV      | 30      | –           | seg, sei, tst     | <25.0       |
| HSS378 | UK                | +    | IV      | 30      | –           | seg, sei, tst     | <25.0       |
| HSS379 | UK                | +    | IV      | 30      | –           | seg, sei, tst     | <25.0       |
| HSS380 | Skin              | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS381 | Sputum            | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS382 | Nose              | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS383 | Nose              | +    | II      | 30      | +           | sea, seg, sei, tst | 94.5       |
| HSS384 | Bacteremia        | +    | II      | 30      | +           | sea, seg, sei, tst | 63.1       |
| HSS385 | UK                | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS386 | Nose              | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS387 | Bacteremia        | +    | II      | 30      | –           | sea, seg, sei, tst | <25.0       |
| HSS388 | Sputum            | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS389 | Skin              | +    | IV      | 30      | –           | seg, sei, tst     | 96.3        |
| HSS390 | Skin              | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS391 | Nose              | +    | IV      | 30      | –           | sea, seg, sei, tst | 97.5        |
| HSS392 | Eye               | +    | ND      | 30      | +           | sea, seg, sei, tst | 25.8        |
| HSS393 | Eye               | +    | ND      | 30      | +           | sea, seg, sei, tst | 31.2        |
| HSS394 | Skin              | +    | II      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS395 | Abscess           | +    | ND      | 30      | +           | seg, sei, tst     | <25.0       |
| HSS396 | Throat            | +    | ND      | 30      | +           | sea, seg, tst     | <25.0       |
| HSS397 | Skin              | +    | ND      | 30      | +           | sea, seg, tst     | 38.5        |
| HSS398 | UK                | +    | II      | 30      | –           | sea, seg, tst     | <25.0       |
| HSS399 | Bacteremia        | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS400 | Throat            | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS401 | Nose              | +    | II      | 30      | +           | sea, seg, sei, tst | 27.0        |
| HSS402 | Throat            | +    | ND      | 30      | +           | sea, seg, tst     | <25.0       |
| HSS403 | Skin              | +    | ND      | 30      | +           | sea, seg, tst     | <25.0       |
| HSS404 | Sputum            | +    | ND      | 30      | +           | sea, seg, tst     | <25.0       |
| HSS405 | Nose              | +    | II      | 30      | +           | sea, seg, tst     | <25.0       |
| HSS406 | Nose              | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |
| HSS407 | Nose              | +    | ND      | 30      | +           | sea, seg, sei, tst | <25.0       |

†MLST-CC, multilocus sequence type-clonal complex (inferred from spa typing data); MRSA, methicillin-resistant S. aureus; MSSA, methicillin-sensitive S. aureus; mTSS, menstrual toxic shock syndrome; ND, not done; SCC/mec, staphylococcal cassette chromosome mec element; UK, unknown. Boldface indicates MSSA or MRSA isolates.
‡Toxin gene profile was determined by multiplex PCR that detected sea, seg, sej, tst and pvl.
§TSST-1 production measured by immunoblot, limit of detection 25ng/mL.
¶Strains that were subject to whole genome sequencing; data deposited in the GenBank short read archive (accession no. SRP023205).
#CC30 strains tested for antimicrobial susceptibility.
Technical Appendix Table 2. Clinical characteristics of fatal cases of toxic shock syndrome in England, Wales, and Northern Ireland, 2008–2012*

| Characteristics | Fatal cases n = 9 | Nonfatal cases n = 171 | p-value |
|-----------------|------------------|------------------------|---------|
| Median age, y (IQR) | 36 (6–51) | 19 (9–38) | 0.39† |
| Sex, no. (%) | | | |
| Female | 7 (77.8) | 121 (70.8) | 1.00‡ |
| Male | 2 (22.2) | 49 (28.6) | 1.00‡ |
| Unknown | 0 | 1 (0.6) | |

Type of TSS, no. (%)§

| Menstrual | 4 (44.4) | 102 (56.7) | 0.74‡ |
| Nonmenstrual | 5 (55.6) | 66 (36.7) | |

1*IQR, interquartile range; TSS, toxic shock syndrome.
†Mann-Whitney U test comparing fatal and nonfatal cases.
§Fisher exact test comparing fatal and nonfatal cases.

Technical Appendix Table 3. The clonal complexes and associated spa-types of isolates causing menstrual and nonmenstrual toxic shock syndrome in England, Wales, and Northern Ireland, 2008–2012*

| MLST-CC | spa types | no. (%) | spa types | no. (%) | p-value† |
|---------|-----------|---------|-----------|---------|---------|
| Unknown | NA | 2 (2.9) | NA | 12 (11.2) | |
| 1 | NA | 0 | t127, t1292 | 5 (4.7) | |
| 5 | t002, t6614 | 3 (4.3) | t002, t045, t548, t688, t7348 | 9 (8.4) | 0.37 |
| 6 | NA | 0 | t304 | 1 (0.9) | |
| 8 | t197, t1188, t12650 | 3 (4.3) | t008, t1104, t1273 | 5 (4.7) | |
| 12 | t160 | 1 (1.4) | t156, t160 | 3 (2.8) | |
| 15 | t084 | 1 (1.4) | t084, t085, t1091, t1774 | 6 (5.6) | |
| 22 | t223 | 2 (2.9) | t005, t020, t022, st032, t1223, t1379, t1966 | 8 (7.5) | 0.32 |
| 25 | t078 | 2 (2.9) | t167, t1937 | 2 (1.9) | |
| 30 | t012, t018, t021, t089, t1136, t1166, t1338, t1400, t1582, t1622, t1705, t1704, t2018, t2387, t2868, t3072, t3368, t3867, t4242, t4359, t112601, t112649 | 51 (72.9) | t012, t018, t019, t021, t1122, t1166, t1275, t1388, t1411, t1298, t1675, t168, t3233, t3800, t4077, t5753, t6364, t6424, t11323 | 39 (36.4) | <0.0001 |
| 45 | t026, t130 | 2 (2.9) | t015, t065, t1230, t1382, t1465, t1583, t2642, t12887 | 10 (9.3) | 0.12 |
| 59 | t7467 | 1 (1.4) | t216, t1437, t1747 | 4 (3.7) | |
| 97 | t359 | 1 (1.4) | NA | 0 | |
| 121 | NA | 0 | t171, t1314 | 2 (1.9) | |
| 182 | NA | 0 | t264 | 1 (0.9) | |
| 398 | t651 | 1 (1.4) | NA | 0 | |

*MLST-CC, Multilocus sequence type-clonal complex (inferred from spa typing data); NA, not applicable. Boldface indicates a statistically significant result. 3 case isolates not assigned as mTSS or nmtSS due to lack of clinical data.
†Fisher exact test comparing MLST-CC between menstrual and nonmenstrual toxic shock syndrome case-patients in MLST-CC groups with ≥10 isolates.

Technical Appendix Table 4. The contribution of methicillin-resistant S. aureus isolates to toxic shock syndrome cases, 2008–2012∗

| Attribute | MRSA | MSSA | p-value |
|-----------|------|------|---------|
| Clinical characteristics, no. (%) | | | |
| Menstrual | 0 (0) | 70 (40.5) | |
| Nonmenstrual | 7 (100) | 100 (57.8) | 0.04† |
| Median age, y (IQR) | 34 (2.3–64.3) | 19 (10–38.5) | 0.39‡ |
| Sex, no. (%)§ | | | |
| Female | 2 (28.6) | 126 (72.8) | |
| Male | 5 (71.4) | 46 (26.5) | 0.02† |
| Molecular characteristics, no. (%) | | | |
| MLST-CC/SCC/mec | | | |
| 6/II | 1 (14.3) | 16 (8.7) | 1.00 |
| 22/IV | 5 (71.4) | 5 (2.9) | <0.0001† |
| 30/II | 1 (14.3) | 91 (52.6) | 0.06† |
| Superantigens, no. (%) | | | |
| sea and tst | 1 (14.3) | 51 (29.5) | 0.68† |
| sec | 4 (57.1) | 10 (5.8) | 0.0007† |
| tst | 2 (28.6) | 101 (59.4) | 0.14† |

*MLST-CC, Multilocus sequence type-clonal complex (inferred from spa typing data); MRSA, Methicillin-resistant S. aureus; MSSA, Methicillin sensitive S. aureus; SCC/mec, Staphylococcal Cassette Chromosome mec element. Boldface indicates a statistically significant result. 3 case isolates not assigned as mTSS or nmtSS due to lack of clinical data.
†Fisher exact test.
‡Mann-Whitney U test.
§Sex of 1 nmTSS case-patient is unknown.
### Technical Appendix Table 5. Superantigen gene frequency of sea-sed in each S. aureus clonal complex causing toxic shock syndrome, 2008–2012*

| MLST-CC | No. (%) isolates | Mean no. superantigen genes/CC | Superantigen, no. (%) positive isolates |
|---------|------------------|---------------------------------|-----------------------------------------|
|         | n = 180          |                                 | sea | seb | sec | sed |
| 1       | 5 (2.8)          | 2.6                             | 4 (80.0) | 1 (20.0) | 0 | 0 |
| 5       | 12 (6.7)         | 3.1                             | 0 | 0 | 2 (16.7) | 4 (33.3) |
| 6       | 1 (0.6)          | 0.0                             | 0 | 0 | 0 | 0 |
| 8       | 8 (4.4)          | 1.4                             | 4 (50.0) | 1 (12.5) | 0 | 1 (12.5) |
| 12      | 4 (2.2)          | 1.8                             | 1 (25.0) | 2 (50.0) | 1 (25.0) | 0 |
| 15      | 7 (3.9)          | 0.0                             | 0 | 0 | 0 | 0 |
| 22      | 10 (5.6)         | 2.9                             | 0 | 0 | 4 (40.0) | 0 |
| 25      | 4 (2.2)          | 2.0                             | 0 | 2 (50.0) | 0 | 0 |
| 30      | 92 (51.1)        | 3.7                             | **48 (52.2)**† | 1 (11.1) | 8 (8.7) | 0 |
| 45      | 12 (6.7)         | 2.8                             | 0 | 1 (8.3) | **7 (58.3)**‡ | 1 (8.3) |
| 59      | 5 (2.8)          | 1.2                             | 1 (20.0) | 2 (40.0) | 0 | 0 |
| 97      | 1 (0.6)          | 0.0                             | 0 | 0 | 0 | 0 |
| 121     | 4 (2.1)          | 2.0                             | 0 | 0 | 0 | 0 |
| 182     | 1 (0.6)          | 3.0                             | 0 | 0 | 0 | 0 |
| 398     | 1 (0.6)          | 0.0                             | 0 | 0 | 0 | 0 |
| Other   | 15 (8.3)         | 2.7                             | 6 (40.0) | 3 (20.0) | 1 (6.6) | 4 (26.7) |

*MLST-CC, Multilocus sequence type-clonal complex (inferred from spa typing data). The superantigen gene see was not detected in any isolate. Boldface indicates a statistically significant result. Percentages may not total 100 due to rounding.

†p<0.001 by Fisher exact test comparing the percentage carriage of a given superantigen gene by an individual CC to percentage carriage of the same superantigen gene by all other CCs combined.

‡p<0.005 by Fisher exact test comparing the percentage carriage of a given superantigen gene by an individual CC to percentage carriage of the same superantigen gene by all other CCs combined.

### Technical Appendix Table 6. Superantigen gene distribution of seg-tst in each S. aureus clonal complex causing toxic shock syndrome, 2008–2012*

| MLST-CC | No. (%) isolates | seg | seh | sei | sej | tst |
|---------|------------------|-----|-----|-----|-----|-----|
|         | n = 180          |     |     |     |     |     |
| 1       | 5 (2.8)          | 1 (20.0) | 5 (100.0) | 1 (20.0) | 0 | 1 (20.0) |
| 5       | 12 (6.7)         | 12 (100.0) | 0 | 12 (100.0) | 4 (33.3) | 3 (25.0) |
| 6       | 1 (0.6)          | 0 | 0 | 0 | 0 | 0 |
| 8       | 8 (4.4)          | 0 | 0 | 3 (37.5) | 2 (25.0) |     |
| 12      | 4 (2.2)          | 1 (25.0) | 0 | 1 (25.0) | 0 | 1 (25.0) |
| 15      | 7 (3.9)          | 0 | 0 | 0 | 0 | 0 |
| 22      | 10 (5.6)         | 10 (100.0) | 0 | 10 (100.0) | 0 | 5 (50.0) |
| 25      | 4 (2.2)          | 3 (75.0) | 0 | 3 (75.0) | 0 | 0 |
| 30      | 92 (51.1)        | **87 (94.6)**† | 18 (19.6)**‡ | **87 (94.6)**† | 0 | **89 (96.7)**† |
| 45      | 12 (6.7)         | 11 (91.7) | 0 | 12 (100.0) | 1 (8.3) | 1 (8.3) |
| 59      | 5 (2.8)          | 1 (20.0) | 0 | 1 (20.0) | 0 | 1 (20.0) |
| 97      | 1 (0.6)          | 0 | 0 | 0 | 0 | 0 |
| 121     | 2 (1.1)          | 2 (100.0) | 0 | 2 (100.0) | 0 | 0 |
| 182     | 1 (0.6)          | 1 (100.0) | 1 (100.0) | 1 (100.0) | 0 | 0 |
| 398     | 1 (0.6)          | 0 | 0 | 0 | 0 | 0 |
| Other   | 15 (8.3)         | 9 (60.0) | 1 (6.6) | 9 (60.0) | 4 (26.7) | 3 (20.0) |

*MLST-CC, Multilocus sequence type-clonal complex (inferred from spa typing data). The superantigen gene see was not detected in any isolate. Boldface indicates a statistically significant result. Percentages may not total 100 due to rounding.

†p<0.0001 by Fisher exact test comparing the percentage carriage of a given superantigen gene by an individual CC to percentage carriage of the same superantigen gene by all other CCs combined.

‡p< 0.05 by Fisher exact test comparing the percentage carriage of a given superantigen gene by an individual CC to percentage carriage of the same superantigen gene by all other CCs combined.
Technical Appendix Figure 1. Foci of infection and clonal complexes of S. aureus isolates causing nonmenstrual toxic shock syndrome in England, Wales, and Northern Ireland, 2008–2012. The figure shows numbers of isolates from each clonal complex causing 107 nonmenstrual TSS cases by focus of infection. “Other” includes one of each of eye, gastrointestinal, and genitourinary tracts. “Clonal complex: Unknown” refers to isolates that failed to grow on sub-culture.

Technical Appendix Figure 2. Superantigen gene frequency and clonal complexes of S. aureus isolates causing toxic shock syndrome in England, Wales and Northern Ireland, 2008–2012. The figure shows number of isolates from each clonal complex carrying each superantigen gene in A) menstrual and B) nonmenstrual TSS cases; see was not detected in any isolate. mTSS, menstrual toxic shock syndrome; nmTSS, nonmenstrual toxic shock syndrome “Clonal complex: Unknown” refers to isolates that failed to grow on subculture.
Technical Appendix Figure 3. The amino acid sequence of CcpA in CC30 MSSA and CC30 MRSA. Region A represents the helix-turn-helix DNA binding domain of the LacI family of transcriptional regulators to which CcpA belongs. Region B represents the ligand binding domain, which is the major transcriptional regulator. The solid vertical bars represent potentially important residues. Effector binding site 1 at position 84 is one of 8 residues where the key co-repressor phosphoprotein (HPr) binds to CcpA; adjacent to this at position 87 is the amino acid change from Threonine in CC30 MSSA to Isoleucine in CC30 MRSA (T87I). This has been expanded to illustrate the change in the nucleotide sequence from C in CC30 MSSA to T in CC30 MRSA at base pair 257 from the transcriptional start site.