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

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Assess the clinical features and epidemiology of toxic shock syndrome (TSS) in England, Wales, and Northern Ireland, based on a study using UK national surveillance data
- Identify the molecular epidemiology of TSS in England, Wales, and Northern Ireland
- Discuss superantigen production by dominant TSS strain types and antimicrobial sensitivity of isolates.

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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,

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most caused by burns and skin and soft tissue infections. Nonmenstrual TSS is now more common than menstrual TSS in the UK, although both types are strongly associated with the *tst*+ clonal complex (CC) 30 methicillin-sensitive *S. aureus* lineage, which accounted for 49.4% of all TSS and produced more TSST-1 and superantigen bioactivity than did *tst*+ CC30 methicillin-resistant *S. aureus* strains. Better understanding of this MSSA lineage and infections in children could focus interventions to prevent TSS in the future.

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 toxigenic 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, σ^B (*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* (SCC*mec*), 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 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 SCC*mec* 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 by 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 [^{3}H] 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.vicbioinformatics.com/software.prokka.shtml). We used Artemis (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 *ccpA* sequencing, we amplified and sequenced DNA using forward primer 1: 5′- CACAGTGTCGCGTGTTGTTA-3′ and reverse primer 1: 5′- TAAGCGCATCCCTACTGCAC-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 χ^2 test. We summarized nonparametric 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).

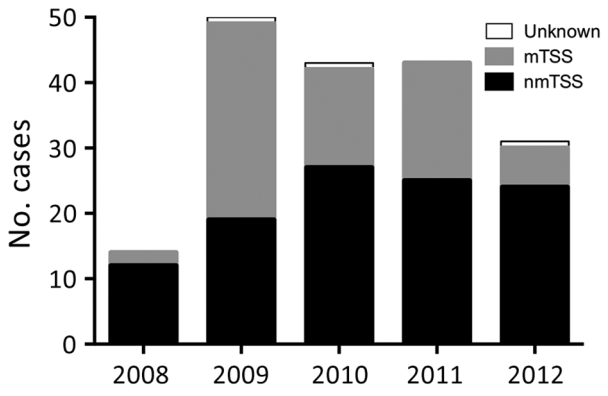

Figure 1. Staphylococcal TSS cases, England, Wales, and Northern Ireland, 2008–2012. The chart depicts the number of cases per year of total, menstrual, and nonmenstrual TSS cases reported to Public Health England. National guidance on toxin-producing *Staphylococcus aureus* disease affected reporting practice from November 2008. mTSS, menstrual TSS; nmTSS, nonmenstrual TSS; TSS, toxic shock syndrome.

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 2.3–64.3) years. Five isolates were identified as CC22-SCC*mec*IV, 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

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)	15.0 (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 patient isolates not assigned as menstrual or nonmenstrual due to lack of clinical data
‡Mann-Whitney U test comparing menstrual and nonmenstrual TSS cases.
§Fisher exact test comparing menstrual and nonmenstrual TSS cases.

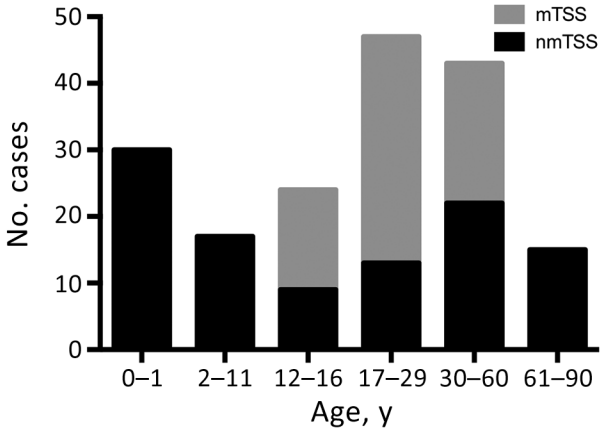

Figure 2. Age distribution of patients with staphylococcal TSS in England, Wales, and Northern Ireland, 2008–2012. mTSS, menstrual TSS; nmTSS, nonmenstrual TSS; TSS, toxic shock syndrome.

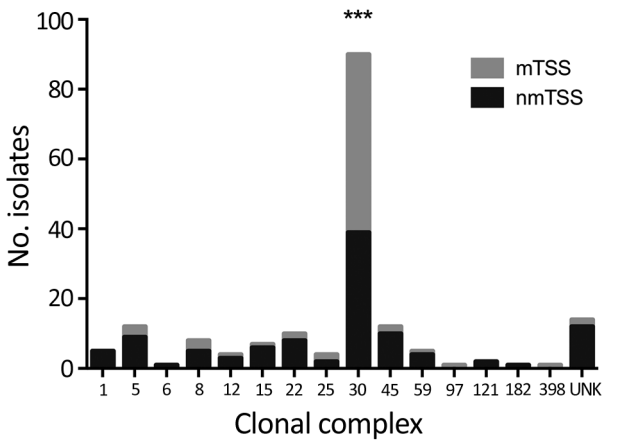

Figure 3. Number of isolates from each *Staphylococcus aureus* clonal complex causing staphylococcal toxic shock syndrome in England, Wales, and Northern Ireland, 2008–2012. ***p<0.0001 by Fisher exact test. mTSS, menstrual TSS; nmTSS, nonmenstrual TSS; TSS, toxic shock syndrome; UNK, unknown (isolates that failed to grow on subculture).

lineage (online Technical Appendix Table 4). The remaining CC22 isolate carried *tst* and belonged to a MRSA lineage frequently identified in the Middle East. Only 1 MRSA-TSS isolate was CC30-SCC*mec*II, corresponding to the UK HA-MRSA clade EMRSA-16. One isolate was CC6-SCC*mec*II 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 *tst.* We measured TSST-1 in broth-culture supernatants from *tst*-positive CC30 MSSA isolates from the TSS cohort and, for comparison, randomly selected clinical *tst*-positive MRSA isolates that belonged to the same lineage (CC30) (*20,29*) (online Technical Appendix Table 1).

Of note, 77/81 *tst*-positive CC30 MSSA isolates produced detectable TSST-1, compared with 9/39 *tst*-positive CC30 MRSA isolates. The *tst*-positive CC30 MSSA isolates produced more TSST-1 than did *tst*-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 *tst*-positive CC30 MSSA strains (164,893 ± 36,191 counts/min) was significantly greater than that of *tst*-positive CC30 MRSA strains (149,653 ± 30,412 counts/min; p = 0.02; Figure 4, panel B).

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

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

We detected mutations in TSST-1 regulator SaeRS in 2/4 *tst*-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.

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†	No. (%) cases			p value§
	Total, n = 180‡	Menstrual, n = 70	Nonmenstrual, n = 107	
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 *tst* 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.

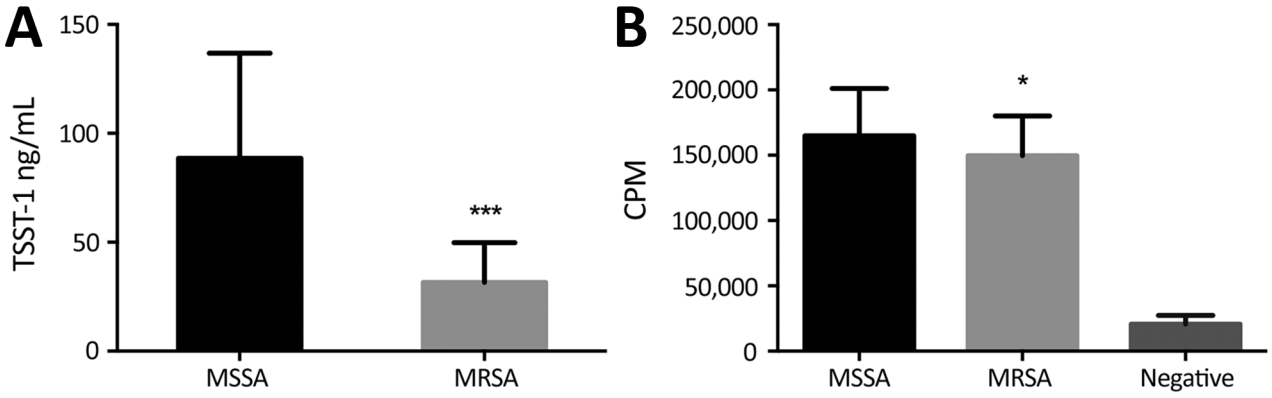

Figure 4. TSST-1 and total mitogen production in vitro by *tst*-positive clonal complex (CC) 30 MSSA and CC30 MRSA strains. A) Mean TSST-1 present in the culture supernatants of *tst*-positive CC30 MSSA (n = 81) and CC30 MRSA (n = 39) isolates measured by immunoblot after overnight culture in brain–heart infusion broth. B) Mean human PBMC proliferative response to culture supernatants of *tst*-positive CC30 MSSA (n = 77) and CC30 MRSA (n = 39) isolates. Negative indicates RPMI tissue culture medium (Invitrogen, Hemel Hempstead, UK) alone. Error bars indicate SDs. *p<0.05; ***p<0.0001 (both by 2-tailed t-test). cpm, counts per minute; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; PBMC, peripheral blood mononuclear cells; TSS, toxic shock syndrome; TSST-1, TSS toxin 1.

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 (ACA) to isoleucine (ATA) 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 SCC*mec* 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 SCC*mec*II; 7/11 SCC*mec*II isolates had *ccpA* (T87I), compared with 0/4 SCC*mec*IV isolates (p = 0.03 by χ^2 test). This finding highlights the possibility that reduced TSST-1 production might be attributable to either SCC*mec*II 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 association with TSS. We linked *tst* to mTSS and CC30. Several groups have demonstrated similar associations of staphylococcal superantigen 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 carriage, infection type, and disease presentations have not been made. A recent study of atopic dermatitis that examined the relationship of ethnicity and staphylococcal virulence 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 characteristics. The ethnicity of the patients with TSS referred to PHE in this study was not recorded, and such bacterial genetic associations with disease could not be made but may merit consideration in future studies.

Among MSSA isolates, resistance rates to key antimicrobial drugs were similar to reported UK MSSA bacteremia isolates (*38*). Notably, teicoplanin resistance was detected, 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-synthesis 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 recognized 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 national 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 superantigenic 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 SCC*mec*II (EMRSA-16; ST36-SCC*mec*II) that may reduce cytolytic toxin production and, in association with *fudoh* gene carriage by this element, reduce hemolytic activity and virulence (*43,44*). Our findings suggest an association between SCC*mec*II 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 Technical Appendix Figure 3) that could influence *tst* promoter binding and affect TSST-1 secretion. Such SNPs in virulence regulators may have had a role in shaping the healthcare-associated phenotype of EMRSA-16 (*20*). New tools that allow manipulation of previously nontransformable lineages such as CC30 will facilitate investigating such genetic mechanisms in *S. aureus* (*45*).

Our study shows that the ability to produce TSST-1 varies widely within the *tst*-positive CC30 lineage and impaired expression is associated with the presence of SCC*mec*II and *ccpA* (T87I), underlining the potential for genomic 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 transmission, 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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About the Author

Dr. Sharma is a physician in infectious diseases, microbiology, and virology and a clinical research fellow at Imperial College London. Her primary research interests relate to the pathogenesis of staphylococcal disease.

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

Technical Appendix

Methods

Bacterial Culture

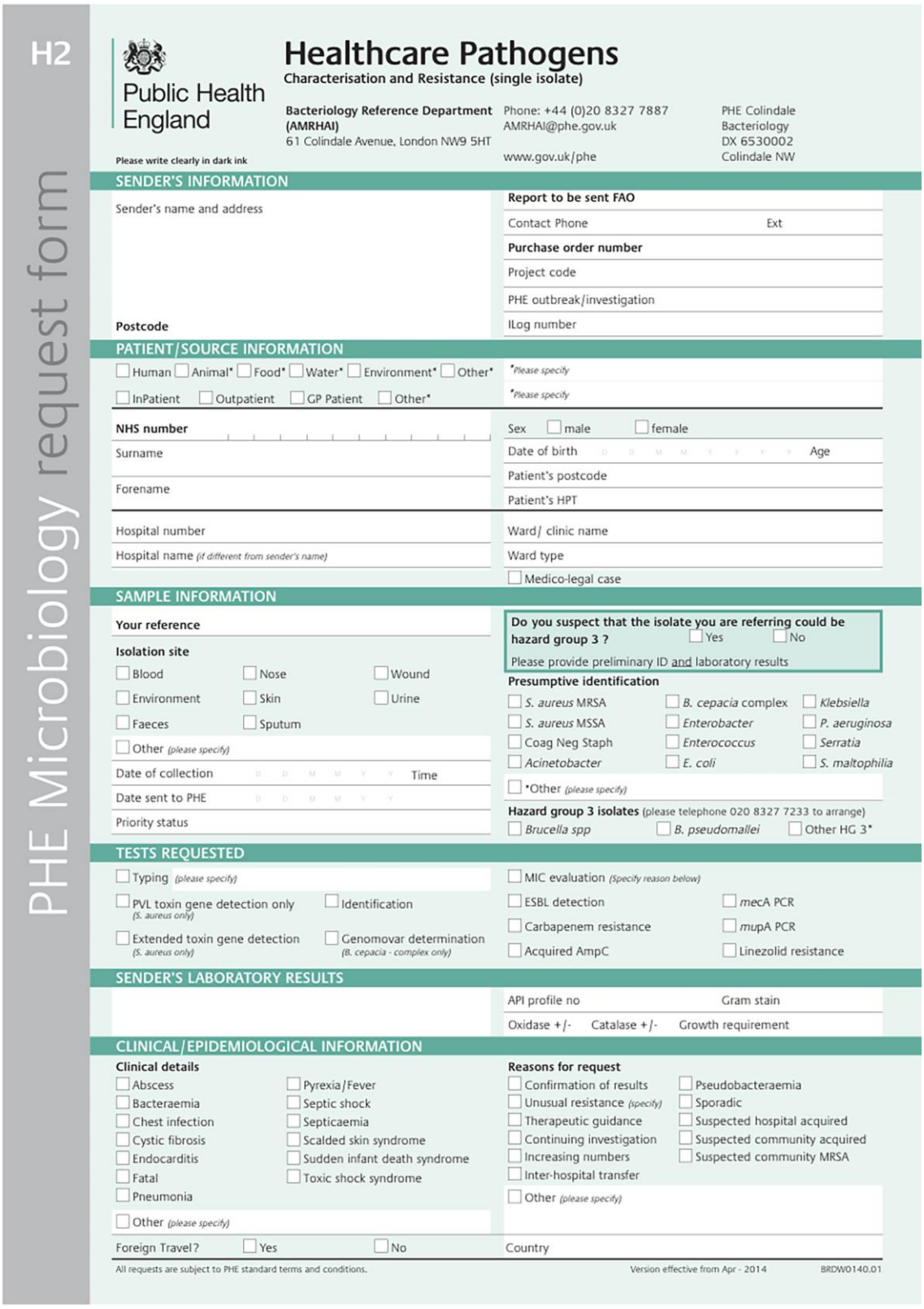
We cultured bacterial isolates in 5 mL of brain heart infusion (BHI) for Western blotting or Roswell Park Memorial Institute (RPMI) media supplemented with 10% fetal calf serum (FCS) for proliferation assays, at 37°C with agitation. We removed bacterial cells from supernatants by centrifugation and 0.2µM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



H2

PHE Microbiology request form

Public Health England

Healthcare Pathogens

Characterisation and Resistance (single isolate)

Bacteriology Reference Department (AMRHAI)
61 Colindale Avenue, London NW9 5HT

Phone: +44 (0)20 8327 7887
AMRHAI@phe.gov.uk
www.gov.uk/phe

PHE Colindale
Bacteriology
DX 6530002
Colindale NW

Please write clearly in dark ink

SENDER'S INFORMATION

Sender's name and address

Postcode

Report to be sent FAO

Contact Phone Ext

Purchase order number

Project code

PHE outbreak/investigation

ILog number

PATIENT/SOURCE INFORMATION

☐ Human ☐ Animal* ☐ Food* ☐ Water* ☐ Environment* ☐ Other* *Please specify

☐ InPatient ☐ Outpatient ☐ GP Patient ☐ Other* *Please specify

NHS number

Surname

Forename

Sex ☐ male ☐ female

Date of birth D D M M Y Y Y Y Age

Patient's postcode

Patient's HPT

Hospital number

Hospital name *(if different from sender's name)*

Ward/ clinic name

Ward type

☐ Medico-legal case

SAMPLE INFORMATION

Your reference

Isolation site

☐ Blood ☐ Nose ☐ Wound
☐ Environment ☐ Skin ☐ Urine
☐ Faeces ☐ Sputum
☐ Other *(please specify)*

Date of collection D D M M Y Y Time

Date sent to PHE D D M M Y Y

Priority status

Do you suspect that the isolate you are referring could be hazard group 3 ? ☐ Yes ☐ No

Please provide preliminary ID and laboratory results

Presumptive identification

☐ *S. aureus* MRSA ☐ *B. cepacia* complex ☐ *Klebsiella*
☐ *S. aureus* MSSA ☐ *Enterobacter* ☐ *P. aeruginosa*
☐ Coag Neg Staph ☐ *Enterococcus* ☐ *Serratia*
☐ *Acinetobacter* ☐ *E. coli* ☐ *S. maltophilia*
☐ *Other *(please specify)*

Hazard group 3 isolates (please telephone 020 8327 7233 to arrange)

☐ *Brucella spp* ☐ *B. pseudomallei* ☐ Other HG 3*

TESTS REQUESTED

☐ Typing *(please specify)*

☐ PVL toxin gene detection only *(S. aureus only)* ☐ Identification

☐ Extended toxin gene detection *(S. aureus only)* ☐ Genomovar determination *(B. cepacia - complex only)*

☐ MIC evaluation *(Specify reason below)*

☐ ESBL detection ☐ *mecA* 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

Clinical details

☐ Abscess ☐ Pyrexia/Fever
☐ Bacteraemia ☐ Septic shock
☐ Chest infection ☐ Septicaemia
☐ Cystic fibrosis ☐ Scalded skin syndrome
☐ Endocarditis ☐ Sudden infant death syndrome
☐ Fatal ☐ Toxic shock syndrome
☐ Pneumonia

☐ Other *(please specify)*

Foreign Travel? ☐ Yes ☐ No

Reasons for request

☐ Confirmation of results ☐ Pseudobacteraemia
☐ Unusual resistance *(specify)* ☐ Sporadic
☐ Therapeutic guidance ☐ Suspected hospital acquired
☐ Continuing investigation ☐ Suspected community acquired
☐ Increasing numbers ☐ Suspected community MRSA
☐ Inter-hospital transfer

☐ Other *(please specify)*

Country

All requests are subject to PHE standard terms and conditions.

Version effective from Apr - 2014 BRDW0140.01

Technical Appendix Table 1. *Staphylococcus aureus* strains that caused toxic shock syndrome in the United Kingdom, 2008–2012*

Strain	Site of infection†	*mecA*	SCC*mec*	MLST-CC	*ccpA* (T87I)	Superantigen genes‡	TSST-1 ng/mL§
MSSA							
¶/#HSS354	mTSS	–	–	30	–	*seg, seh, sei, tst*	49.0
¶/#HSS355	mTSS	–	–	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, tst*	57.5
#HSS394	Abscess	–	–	30	ND	*sea, seg, sei, tst, pvl*	87.0
#HSS395	mTSS	–	–	30	–	*sea, seg, sei, tst*	124.8
#HSS397	Burn	–	–	30	–	*sea, seg, sei, tst*	73.3
#HSS398	Burn	–	–	30	ND	*seg, sei, tst*	38.8
#HSS405	Abscess	–	–	30	ND	*sea, seg, sei, tst*	57.1
#HSS409	mTSS	–	–	30	ND	*seg, sei, tst*	32.8
#HSS412	mTSS	–	–	30	–	*seg, sei, tst*	46.3
#HSS413	mTSS	–	–	30	ND	*seg, sei, tst*	40.1
#HSS414	mTSS	–	–	30	ND	*seg, seh, sei, tst*	42.3
#HSS416	mTSS	–	–	30	ND	*sea, seg, seh, sei, tst*	106.7
#HSS417	Skin	–	–	30	–	*seg, sei, tst*	25.8
#HSS419	mTSS	–	–	30	ND	*seg, sei, tst*	<25.0
#HSS422	mTSS	–	–	30	ND	*seg, sei, tst*	83.0
#HSS423	URT	–	–	30	–	*sea, seg, seh, sei, tst*	126.9
#HSS425	mTSS	–	–	30	ND	*sec, tst*	196.4
#HSS426	mTSS	–	–	30	ND	*sea, seg, sei, tst*	182.9
#HSS427	Skin	–	–	30	ND	*seg, sei, tst*	96.9
#HSS428	mTSS	–	–	30	ND	*sea, sec, seg, sei, tst*	57.0
#HSS429	mTSS	–	–	30	–	*seg, seh, sei, tst*	48.3
#HSS430	mTSS	–	–	30	ND	*seg, seh, sei, tst*	<25.0
#HSS431	mTSS	–	–	30	ND	*sea, seg, sei, tst*	55.2
#HSS432	mTSS	–	–	30	ND	*seg, seh, sei, tst*	120.0
#HSS434	Skin	–	–	30	ND	*seg, seh, sei, tst*	<25.0
#HSS435	mTSS	–	–	30	ND	*sea, seg, sei, tst*	54.9
#HSS436	mTSS	–	–	30	ND	*sea, seg, sei, tst*	125.2
#HSS437	Bacteremia	–	–	30	ND	*sea, seg, sei, tst*	161.4
#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, sei, tst*	108.2
#HSS445	UK	–	–	30	ND	*seg, seh, sei, tst*	105.7
#HSS446	LRT	–	–	30	ND	*sea, seg, sei, tst*	54.6
#HSS449	mTSS	–	–	30	–	*sea, seg, sei, tst*	134.3
#HSS451	mTSS	–	–	30	ND	*seg, sei, tst*	57.4
#HSS454	mTSS	–	–	30	ND	*sea, seg, sei, tst*	49.1
#HSS456	mTSS	–	–	30	ND	*seg, sei, tst*	51.9
#HSS457	mTSS	–	–	30	ND	*seg, sei, tst*	78.0
#HSS459	Skin	–	–	30	ND	*sea, seg, sei, tst*	54.2
#HSS463	mTSS	–	–	30	–	*seg, seh, sei, tst*	57.9
#HSS468	UK	–	–	30	ND	*sec, seg, sei, tst, pvl*	26.8
#HSS469	Bacteremia	–	–	30	–	*sea, seg, sei, tst*	133.6
#HSS470	mTSS	–	–	30	ND	*sea, seg, sei, tst*	96.0
#HSS473	Skin	–	–	30	–	*seg, seh, sei, tst*	92.4
#HSS474	mTSS	–	–	30	–	*sea, seg, sei, tst*	<25.0
#HSS475	mTSS	–	–	30	ND	*sec, seg, sei, tst*	94.4
#HSS476	mTSS	–	–	30	ND	*sec, seg, sei, tst*	108.3
#HSS478	mTSS	–	–	30	ND	*seg, sei, tst*	66.3
#HSS479	Skin	–	–	30	ND	*seg, sei, tst*	204.6
#HSS481	Skin	–	–	30	ND	*sea, seg, sei, tst*	40.0
#HSS485	mTSS	–	–	30	ND	*sea, seg, sei, tst*	68.7
#HSS490	UK	–	–	30	ND	*sea, seg, sei, tst*	174.8
#HSS492	Burn	–	–	30	ND	*seg, sei, tst*	82.9
#HSS493	Eye	–	–	30	ND	*sea, seg, sei, tst*	65.8
#HSS494	Bacteremia	–	–	30	ND	*sea, seg, sei, tst*	116.9
#HSS496	mTSS	–	–	30	ND	*sea, seg, sei, tst*	44.7
#HSS497	Bacteremia	–	–	30	ND	*seg, seh, sei, tst*	90.8
#HSS499	Abscess	–	–	30	ND	*sea, seg, sei, tst*	133.4
#HSS501	Skin	–	–	30	–	*seg, seh, sei, tst*	122.8
#HSS502	mTSS	–	–	30	ND	*seg, sei, tst*	98.0
#HSS503	Skin	–	–	30	ND	*sea, seg, sei, tst*	111.6
#HSS504	mTSS	–	–	30	ND	*sea, seg, sei, tst*	60.0
#HSS512	mTSS	–	–	30	–	*sea, seg, seh, sei, tst*	37.6
#HSS513	Burn	–	–	30	–	*sea, seg, sei, tst*	66.9

Strain	Site of infection†	*mecA*	SCC*mec*	MLST-CC	*ccpA* (T87I)	Superantigen genes‡	TSST-1 ng/mL§
#HSS514	mTSS	–	–	30	ND	*sea, seg, seh, sei, tst*	74.9
#HSS517	mTSS	–	–	30	ND	*sea, seg, sei, tst*	30.4
#HSS518	mTSS	–	–	30	ND	*sea, seg, sei, tst*	70.2
#HSS520	mTSS	–	–	30	ND	*sea, seg, sei, tst*	79.8
#HSS521	mTSS	–	–	30	–	*sea, seg, sei, tst*	61.8
#HSS522	Skin	–	–	30	ND	*sea, seg, sei, tst*	75.8
#HSS525	Skin	–	–	30	ND	*sea, seg, sei, tst*	168.4
#HSS527	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							
¶HSS360	Urine	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS361	Bone and joint	+	ND	30	+	*sea, seg, sei, tst*	<25.0
¶/#HSS362	Abscess	+	II	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	+	II	30	+	*seg, sei, tst*	<25.0
HSS537	Skin	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS538	Skin	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS539	Sputum	+	ND	30	+	*seg, sei, tst*	<25.0
HSS540	Bacteremia	+	ND	30	+	*sea, seg, tst*	<25.0
HSS542	Nose	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS543	Nose	+	II	30	+	*sea, seg, sei, tst*	94.5
HSS544	Bacteremia	+	II	30	+	*sea, seg, sei, tst*	63.1
HSS545	UK	+	ND	30	+	*seg, sei, tst*	<25.0
HSS546	Nose	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS548	Bacteremia	+	II	30	–	*sea, seg, sei, tst*	<25.0
HSS549	Sputum	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS550	Skin	+	IV	30	–	*seg, sei, tst*	76.3
HSS551	Skin	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS552	Nose	+	IV	30	–	*sea, seg, sei, tst*	97.5
HSS553	Eye	+	ND	30	+	*sea, seg, sei, tst*	25.8
HSS555	Skin	+	II	30	+	*sea, seg, sei, tst*	31.2
HSS556	Abscess	+	ND	30	+	*seg, sei, tst*	<25.0
HSS558	Throat	+	ND	30	+	*sea, seg, tst*	<25.0
HSS559	Skin	+	II	30	+	*sea, seg, sei, tst*	38.5
HSS560	UK	+	II	30	–	*sea, seg, sei, tst*	<25.0
HSS561	Bacteremia	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS562	Throat	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS563	Nose	+	II	30	+	*sea, seg, tst*	27.0
HSS564	Throat	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS565	Skin	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS566	Sputum	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS570	Nose	+	II	30	+	*sea, seg, sei, tst*	<25.0
HSS571	Bacteremia	+	II	30	+	*sea, seg, sei, tst*	26.7
HSS573	Nose	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS574	Nose	+	ND	30	+	*sea, seg, sei, tst*	<25.0
HSS575	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.

†MSSA isolates were all from TSS cases. Site of infection specified only for nonmenstrual TSS isolates.

‡Toxin gene profile was determined by multiplex PCR that detected *sea-see, 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. SRP082305).

#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)	
Nonmenstrual	5 (55.6)	66 (36.7)	0.74‡

*IQR, interquartile range; TSS, toxic shock syndrome.
†Mann-Whitney U test comparing fatal and nonfatal cases.
‡Fisher exact test comparing fatal and nonfatal cases.
§3 case isolates not assigned as mTSS or nmTSS due to lack of clinical data.

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*

	Menstrual (n = 70)		Nonmenstrual (n = 107)		
MLST-CC	***spa* types**	**no. (%)**	***spa* types**	**no. (%)**	p-value†
Unknown	NA	2 (2.9)	NA	12 (11.2)	
1	NA	0	t127, t922	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, t104, t723	5 (4.7)	
12	t160	1 (1.4)	t156, t160	3 (2.8)	
15	t084	1 (1.4)	t084, t085, t091, t774	6 (5.6)	
22	t223	2 (2.9)	t005, t020, t022, st032, t223, t379, t9606	8 (7.5)	0.32
25	t078	2 (2.9)	t167, t937	2 (1.9)	
30	t012, t018, t019, t021, t089, t136, t166, t338, t399, t440, t582, t862, t870, t942, t2018, t2387, t2868, t3072, t3368, t3687, t4242, t6359, t12601, t12649	51 (72.9)	t012, t018, t019, t021, t122, t166, t275, t338, t414, t1298, t1675, t2895, t3233, t3800, t4077, t5753, t6364, t6424, t11323	39 (36.4)	**<0.0001**
45	t026, t230	2 (2.9)	t015, t065, t230, t383, t465, t583, t2642, t2887	10 (9.3)	0.12
59	t7467	1 (1.4)	t216, t437, t471	4 (3.7)	
97	t359	1 (1.4)	NA	0	
121	NA	0	t171, t314	2 (1.9)	
182	NA	0	t364	1 (0.9)	
398	t571	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 n = 7	MSSA n = 173	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 (26.6)	**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 (58.4)	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*

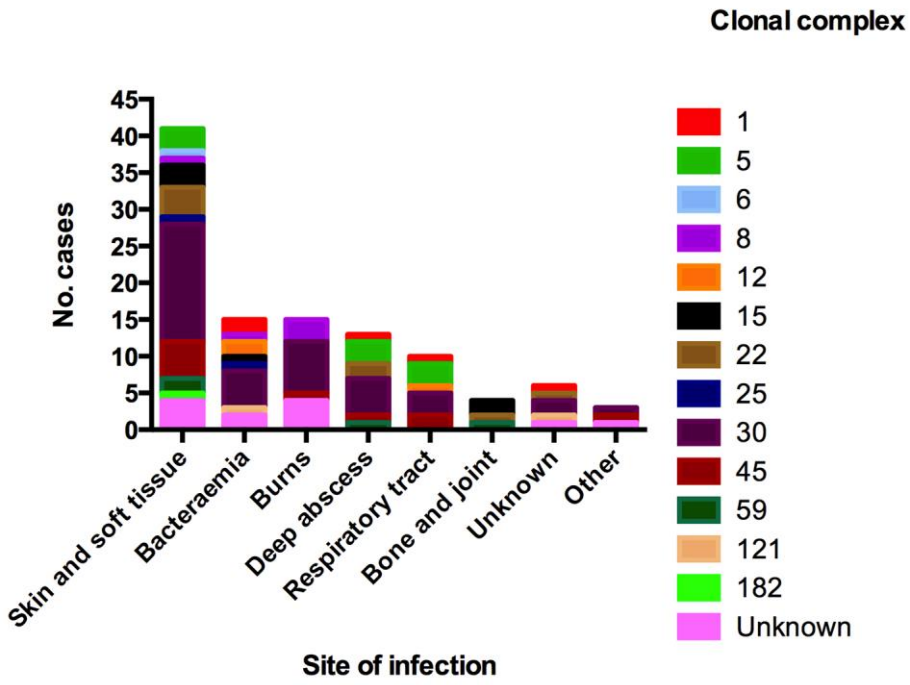
MLST-CC	No. (%) isolates n = 180	Mean no. superantigen genes/CC	Superantigen, no. (%) positive isolates			
			sea	*seb*	*sec*	*sed*
1	5 (2.8)	2.6	4 (80.0)	1 (20.0)	0	0
5	12 (6.7)	3.1	0	0	2 (16.7)	4 (33.3)
6	1 (0.6)	0.0	0	0	0	0
8	8 (4.4)	1.4	4 (50.0)	1 (12.5)	0	1 (12.5)
12	4 (2.2)	1.8	1 (25.0)	2 (50.0)	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 (1.1)	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	2 (1.1)	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<0.0001 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< 0.001 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.

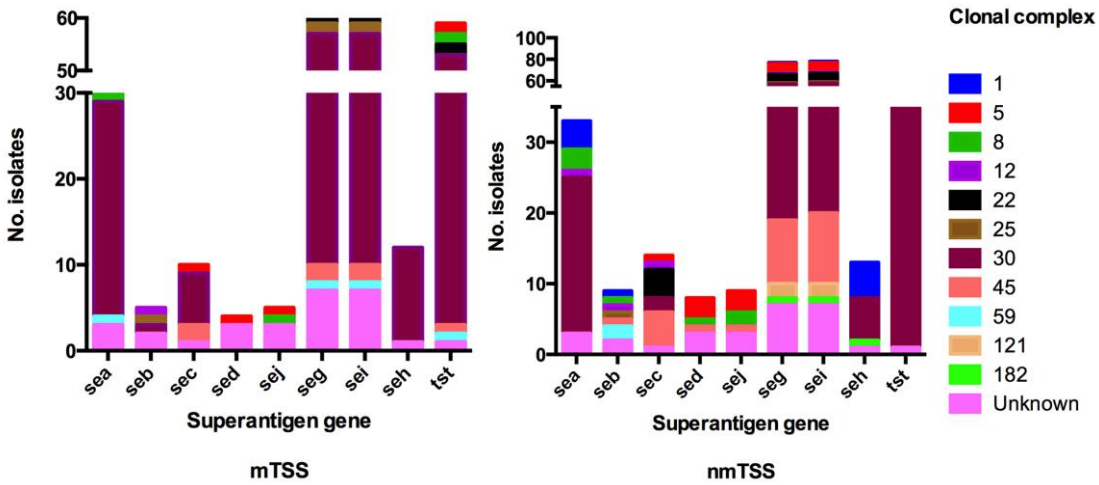
Technical Appendix Table 6. Superantigen gene distribution of *seg-tst* in each *S. aureus* clonal complex causing toxic shock syndrome, 2008–2012*

MLST-CC	No. (%) isolates n = 180	Superantigen, no. (%) positive isolates				
		seg	*seh*	*sei*	*sej*	*tst*
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	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)

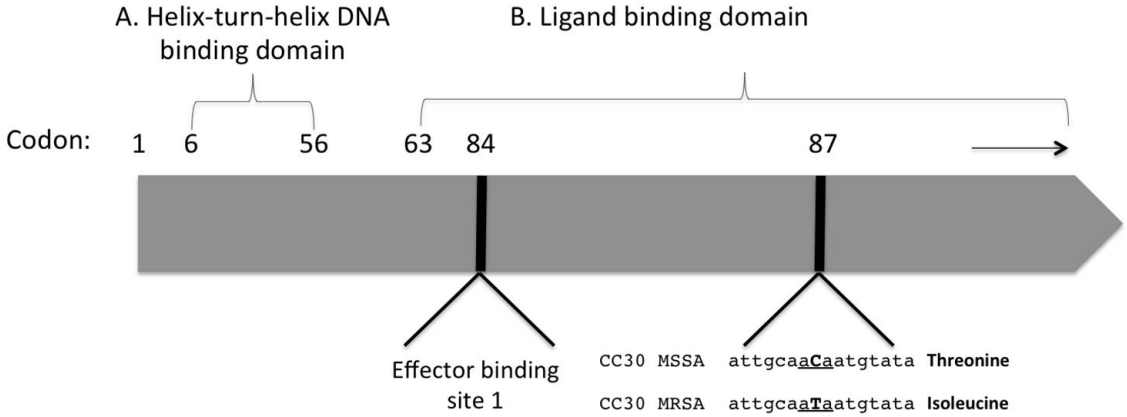
*MLST-CC, Multilocus sequence type-clonal complex (inferred from *spa* typing data). The superantigen gene *see* was not detected in any isolate. Boldface indicates a statistically significant result.
†p < 0.0001 by Fisher exact test comparing the percentage carriage of a given superantigen gene by an 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.