

## Studies on staphylococci from toxic shock syndrome in France, 1981–1983

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### SUMMARY

Staphylococci from 22 cases of toxic shock syndrome with onsets between 1981 and March 1983 have been studied. Another four cases were detected by abstract surveillance. Three of these patients died. The case histories show that the syndrome occurs in women during menstruation as well as in males and in children, and is associated with *Staphylococcus aureus* infections.

The production of enterotoxins (A, B, C) and toxic shock toxin by *S. aureus* isolates from toxic shock syndrome was investigated. Twenty-two of the 23 isolates were found to be toxigenic: 7 produced enterotoxin A, 8 produced enterotoxin B, 3 produced enterotoxin C and 13 produced toxic shock toxin. The latter was found with enterotoxin A in five cases, and with enterotoxins A and B in only one case.

Sixty-three percent of 46 *S. aureus* strains isolated from the vagina of patients with diseases other than toxic shock syndrome produced toxin; eight of these strains produced toxic shock toxin.

### INTRODUCTION

Toxic shock syndrome (TSS) was first described by Todd *et al.* in 1978 as an illness associated with *Staphylococcus aureus* infections. The syndrome is clinically characterized by sudden onset of high fever, hypotension, vomiting, diarrhoea, rash and subsequent desquamation of the skin of the palms and soles (Todd *et al.* 1978; Tofte & Williams 1981; Davis *et al.* 1982; Shands *et al.* 1982; Reingold *et al.* 1982). The importance of this illness was recognized in the United States during the summer of 1980, when it was described as a potentially fatal illness affecting healthy young women who used tampons during menstruation (Shands *et al.* 1980; Davis *et al.* 1980). Yet this syndrome may also be associated with other staphylococcal infections in men, women and children (Reingold *et al.* 1982; Bartlett *et al.* 1982).

Two *S. aureus* toxins, enterotoxin F (Bergdoll *et al.* 1981), recently named toxic shock toxin (TST) (Reiser *et al.* 1983), and pyrogenic exotoxin C (Schlievert *et al.* 1981) have been isolated from TSS patients' strains, both are thought to have a role in the aetiology of the sickness. Since these two toxins have almost similar characteristics they are considered to be the same toxin (Bonventre *et al.* 1983; Reiser *et al.* 1983).

In contrast to the great numbers of TSS reported from the USA (Centers for Disease Control, 1983), there have been few case reports from France (Abadie *et al.* 1981; Rapin, Denfert & Cabane, 1981; Granthil, Dumont & Brun 1982; Letellier *et al.* 1982; Rouffineau *et al.* 1982; Floret *et al.* 1983). Staphylococci isolated from TSS patients were received in the Centre National de Référence des Staphylocoques for toxin detection. Phage typing and serotyping of these isolates have also been investigated. An attempt was made to detect toxigenicity in vaginal isolates of *S. aureus* from patients with histories other than TSS.

#### MATERIALS AND METHODS

*Strains.* Twenty-three *S. aureus* strains isolated from 22 TSS cases, identified according to the criteria of Reingold *et al.* (1982), were investigated. Clinical and epidemiological information on these patients was obtained by correspondence with the physicians concerned.

In addition 46 *S. aureus* vagina isolates from patients with infections other than TSS were also detected.

*Toxin production.* The cellophane agar overlay method (Hallander, 1965) modified by us (Melconian, Brun & Fleurette, 1983) was used.

*Toxin detection.* The microslide immunodiffusion method was employed (Melconian, Flandrois & Fleurette, 1983). Reference toxins (A, B, C enterotoxins and TST) and antitoxin sera were kindly donated by M. S. Bergdoll (Food Research Institute, Wisconsin, Madison).

*Phage typing.* Isolates of *S. aureus* were tested with the international basic set of typing phages (De Saxe & Rosendal, 1982) provided by Dr. J. Fouace of the Institut Pasteur, Paris. Cultures were typed at both routine test dilution (RTD) and 100 RTD.

*Serotyping.* This was performed according to the Oeding, Haukenes, Grün system modified by Fleurette & Modjadedy (1976).

*Resistance to antibiotics and heavy metals.* The TSS strains were screened by disk diffusion on Müller Hinton agar (bio-Mérieux) at 35°C (30°C for methicillin) for susceptibility to penicillin G (6 µg), oxacillin (1 µg), cefamandole (30 µg), cephalothin (30 µg), kanamycin (30 µg), gentamicin (10 µg), sisomicin (10 µg), dibekacin (10 µg), tobramycin (10 µg), amikacin (30 µg), netilmicin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), minocycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), pristinamycin (15 µg), rifampicin (30 µg); cotrimoxazole (1,25/23,75 µg), vancomycin (30 µg), fosfomycine (50 µg). *S. aureus* strain CNCM 7625 (ATCC 25923) was used as a drug-sensitive control.

The disk diffusion method of EL Solh *et al.* (1984) was used to test for resistance to cadmium (cadmium acetate, 53 µg/disk), arsenate (sodium arsenate, 624 µg/disk) and mercury (mercuric nitrate, 112 µg/disk) ions. Throughout the test two strains of *S. aureus* were used as sensitive (NCTC 8325) and resistant (NCTC 9789) controls.

## RESULTS

During the period from January 1981 to March 1983 strains of *S. aureus* isolated from identified cases of toxic shock syndrome sent by different hospitals had reached our laboratory with a request for toxin detection. Details of some cases were published after our confirmation; others were published without confirmation by us. Details of all these cases are given in Table 1.

Table 1. Details of 26 toxic shock syndrome cases in France

Case no.	Date of onset	Age (years) and sex	Site of isolation	Reference
Not checked in our laboratory				
1	20 March 1976	36 F	Wound	Rapin <i>et al.</i> 1981
2	5 June 1978	20 M	Wound	Rapin <i>et al.</i> 1981
3	8 Jan. 1981	17 F	Vagina and pharynx	Rapin <i>et al.</i> 1981
4	NK	20 M	Pus (humerus)	Letellier <i>et al.</i> 1982
Checked in our laboratory				
5 (died)	23 Jan. 1981	17 M	Sputum	Granthil <i>et al.</i> 1982
6 (died)*	17 May 1981	53 M	Blood	†
7	8 June 1981	70 M	Skin	Abadie <i>et al.</i> 1981
8	6 Nov. 1981	38 F	Vagina	Rouffineau <i>et al.</i> 1982
9	25 Dec. 1981	16 M	Wound	†
10 (died)	Dec. 1981	22 M	Blood	†
11	Jan. 1982	NK M	Wound (knee)	†
12	Feb. 1982	18 F	Vagina	†
13	March 1982	5 M	Blood	Floret <i>et al.</i> 1983
14	March 1982	1·7 F	Wound	Floret <i>et al.</i> 1983
15	May 1982	16 M	Stool and urine	†
16	14 June 1982	8 F	Wound (scalp)	†
17	26 Nov. 1982	24 F	Pus	†
18	18 Dec. 1982	21 M	Pus (knee)	†
19	Dec. 1982	28 F	Vagina and blood	†
20	Jan. 1983	31 F	Vagina, nose and trachea	†
21	21 Feb. 1983	20 F	Vagina and urine	†
22	March 1983	67 F	Skin graft (burn)	†
23	March 1983	18 F	Uterus (postpartum)	†
24	March 1983	NK F	Skin abscess	†
25	March 1983	NK F	Skin abscess	†
26	March 1983	35 M	Pus (femur)	†

F, Female; M, male; NK, not known.

\* Absence of one of the major criteria.

† Unpublished cases.

Fourteen of the 26 reported cases were females of varying ages; there were two children, one of 19 months and the other of 8 years. A five-year-old male child was also reported (case 13, Table 1). Patients 5, 6 and 10 died (fatality ratio 11·6%), whereas all the others recovered.

Twenty-one of the 22 cases checked in our laboratory produced one or more toxins: 7 (32%) produced enterotoxin A, 8 (36%) produced enterotoxin B, 3 (14%) produced enterotoxin C and 13 (59%) produced TSS. This last toxin was found in association with enterotoxin A in five cases and with the enterotoxins A and B in only one case.

Table 2. Properties of *Staphylococcus aureus* strains isolated from 22 toxic shock syndrome patients

Case no.	Antigens detected	Phage typing pattern	Toxins detected*	Resistance to antibiotics and heavy metals†
5	h <sub>2</sub>	Group II 3A/3C/55/71	B	P, OX
6	m/263 <sub>1</sub> /263 <sub>2</sub> /1	Group I:29	TST	Cd
7	h <sub>2</sub> /k <sub>1</sub> k <sub>2</sub> /m/263 <sub>2</sub>	Group I:(79)	A, TST	Cd
8	a <sub>3</sub> /k <sub>1</sub> k <sub>2</sub> /m/263 <sub>1</sub> /263 <sub>2</sub>	Mixed:(29)/81	A, TST	Cd
9	c <sub>1</sub> /o	Mixed:(84)/94/96	B	Cd
10	c1/o	Mixed:(47)/(29)/(42E)/(75)/(81)/(95)/(84)/53/96	TST	—
11	c1/o	Group III:(53)	B	Cd
12	a <sub>3</sub> /h <sub>2</sub> /k <sub>1</sub> k <sub>2</sub> /m/263 <sub>1</sub>	Non-typable	TST	P, E, TE, TM, K
13	a <sub>3</sub> /k <sub>1</sub> k <sub>2</sub> /m/263 <sub>1</sub> /263 <sub>2</sub>	Group I:52	TST	Cd
14	m/263 <sub>1</sub> /263 <sub>2</sub>	Mixed:52/81	TST	Cd
15	h <sub>2</sub> /m	Group I:79	TST	Cd
16	a <sub>4</sub> /a <sub>3</sub> /b <sub>1</sub> /1	Group III:53/83A	A, C	Cd
17	b <sub>1</sub> /c <sub>1</sub> /o	Complex:94/96	B	Cd
18	c <sub>1</sub> /o	Complex:94/96	B, C	Cd
18	18	Group I:(79)	C	TE
19	h2/1	Group II:3C/71	B	P, OX
20	a <sub>3</sub> /h <sub>2</sub> /m/1	Group I:29/52/80	A, TST	Cd
21	a <sub>3</sub> /m/263 <sub>2</sub>	Group I:29/52	A, TST	Cd
22	c <sub>1</sub> /h <sub>1</sub> /o/18	Group III:(83A)	TST	P, K, AN, OX, E, TE
23	a <sub>3</sub> /h <sub>2</sub> /k <sub>1</sub> k <sub>2</sub> /m/1	Group I:(52)	A, TST	Cd
24	(a <sub>4</sub> )/(b <sub>1</sub> )/c <sub>1</sub> /o/18	Group III:(47)/53/75/83A/85	B	Cd
25	a <sub>3</sub> /k <sub>1</sub> k <sub>2</sub> /m	Non-typable	A, B, TST	Cd
26	b <sub>1</sub> /1/o/p	Non-typable	None	—

\* A, B, C enterotoxins and toxic shock toxin (TST).

† Antibiotics tested: penicillin (P), oxacillin (OX), cefamandole, cephalothin, kanamycin (K), gentamicin (GM), sisomicin (SIS) dibekacin (DBK), tobramycin (TM), amikacin (AN), netilmicin, chloramphenicol, tetracycline (TE), minocycline erythromycin (E), clindamycin, pristinamycin, rifampicin, cotrimoxazole, vancomycin, fosfomycin. Heavy metals tested: mercury (mercuric nitrate), cadmium (cadmium acetate) Cd, and arsenate (sodium arsenate) As.

— Susceptible to all antibiotics or heavy metals tested.

Production of TST was correlated with susceptibility to group I-phages and production of the antigens  $a_5$ ,  $h_2$ ,  $k_1k_2$ ,  $m$ ,  $263_1$ ,  $263_2$ , whilst enterotoxin B appeared in strains lysed by phages of the complex 94/96 and with the antigens  $C_{1/0}$  (Table 2).

Correlation was observed between the production of the TST and resistance to cadmium and arsenate (Table 2): 12 of 13 TSS-TST producer strains were resistant to cadmium and 6 were also resistant to arsenate. However, two strains-resistant to cadmium and arsenate did not produce TST (produce enterotoxin B) and one strain sensitive to these two metals produced TST. None of the TSS strains was resistant to mercury.

Twenty of the 23 TSS strains were resistant to penicillin. All except one TST producer strains were resistant to this antibiotic.

Twenty-nine (63%) of the 46 vaginal isolates from non-TSS patients were toxigenic: 16 strains produced enterotoxin A, 4 produced enterotoxin B, 8 produced enterotoxin C, and 8 strains (17%) produced TST. Enterotoxin A production was associated with enterotoxin C in four strains and with TST in three strains. Similar associations were observed between toxin production, phage groups and agglutinogens of these vaginal strains as with the TSS toxigenic isolates.

#### DISCUSSION

Compared with the large numbers reported from the United States (2204 cases, Centers for Disease Control, 1983) TSS is less frequently observed in France (26 cases) or in other countries such as the United Kingdom (De Saxe *et al.* 1982) and Canada (Clayton, Peacocke & Ewan, 1982). This difference is probably due to the absence of publicity, inefficient clinical surveillance, fewer tampon users or to the different tampon brands available locally.

Although this syndrome usually manifests itself during menstruation (Davies *et al.* 1980; Shands *et al.* 1980), in our study it appeared in men and children, and in women whether menstruating or not. This divergence has already been reported (Bartlett *et al.* 1982; Reingold *et al.* 1982). *S. aureus* was isolated from all our TSS patients and the toxigenicity of the 22 case isolates was tested in our laboratory. All but one of these strains produced one or more toxins (A, B, C enterotoxin or TST) TST being the most common (59%). Bergdoll *et al.* (1981) reported the association of enterotoxin F (now TST) and TSS isolates (94%) and Bonventre *et al.* (1983) found almost the same relationship (92%) when testing 136 isolates of *S. aureus* from TSS patients. Some authors (e.g. Bergdoll *et al.* 1981) observed that some strains implicated in TSS produced enterotoxins A, B or C but not TST. In our study, six of the 23 TSS strains produced enterotoxin B alone, one produced B and C, one produced A and C and one produced enterotoxin C alone. The implications of production of these enterotoxins by the TSS strains without the production of TST are not yet clear.

One of our TSS strains was a non-toxin producer; this could be explained by an isolation error, production of a toxin other than the ones tested or of a toxin not yet identified.

Serotyping and phage typing of TSS strains showed an association between the TST and the  $a_5$ ,  $k_1k_2$ ,  $m$ ,  $263_1$ ,  $263_2$  antigens with lysis by the phages of group I.

Melconian, Brun & Fleurette in 1983 found the same relation when testing *S. aureus* strains isolated from various clinical sources of non-TSS patients. Altmeier *et al.* (1982) have earlier observed certain correlation between enterotoxin F (TST) and lysis by phages of group I.

All but two TSS-TST producer strains were resistant to cadmium and arsenate and all these were resistant to penicillin except one. De Saxe *et al.* (1982) reported similar findings: all their TSS-enterotoxin F producer strains were resistant to penicillin, cadmium and arsenate.

The case fatality ratio in our study (11.6%) is high compared with some series (5.6 and 6%) (Centers for Disease Control 1982, Paris *et al.* 1982) but similar to others (13%) (Tofte & Williams, 1981).

Vaginal isolates of non-TSS patients were also toxigenic; of these, 17% produced TST. Linnemann *et al.* (1982) recovered *S. aureus* from vaginal cultures in 31 (5%) of the 600 women examined; seven (1%) were enterotoxin F (TST) producers. In the studies of Bonventre *et al.* (1983), two of 48 strains (4%) were positive for the same toxin. The importance of the toxigenic strains in the vaginas of the patients with histories other than TSS is not known, but obviously the implications should be considered.

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#### REFERENCES

- ABADIE, J. C., GOUET, D., MARECHAUD, R., BECQ-GIRAUDON, B. & SUDRE, Y. (1981). Syndrome de choc toxique staphylococcique à porte d'entrée extragynécologique. *La Nouvelle Presse Médicale* **10**, 3793.
- ALTEMEIER, W. A., LEWIS, S. A., SCHLIEVERT, P. M., BERGDOLL, M. S., BJORNSON, H. S., STANECK, J. L. & CRASS, B. A. (1982). *Staphylococcus aureus* associated with toxic shock syndrome. *Annals of Internal Medicine* **96**, 978-982.
- BARTLETT, P., REINGOLD, A. L., GRAHAM, D. R., DAN, B. B., SELINGER, D. S., TANK, G. W. & WICHTERMAN, K. A. (1982). Toxic shock syndrome associated with surgical wound infections. *Journal of American Medical Association* **247**, 1448-1450.
- BERGDOLL, M. S., CRASS, B., REISER, R. F., ROBBINS, R. & DAVIS J. P. (1981). A new staphylococcal enterotoxin, enterotoxin F, associated with toxic shock syndrome *Staphylococcus aureus* isolates. *Lancet* **i**, 1017-1021.
- BONVENTRE, P. F., WECKBACH, L., STANECK, J., SCHLIEVERT, P. M. & THOMPSON, M. (1983). Production of staphylococcal enterotoxin F and pyrogenic exotoxin C by *Staphylococcus aureus* isolates from toxic shock syndrome associated sources. *Infection and Immunity* **40**, 1023-1029.
- Centers for Disease Control (1983). Update: toxic-shock syndrome - United States. *Morbidity and Mortality Weekly Report* **32**, 398-400.
- CLAYTON, A. J., PEACOCKE, J. E. & EWAN, P. E. (1982). Toxic shock syndrome in Canada. *Canadian Medical Association Journal* **126** 776-779.
- DAVIS, J. P., CHESNEY, P. J., WAND, P. J. & LA VENTURE, M. and THE INVESTIGATION AND LABORATORY TEAM (1980). Toxic-shock syndrome. Epidemiologic features, recurrence, risk factors, and prevention *New England Journal of Medicine* **303**, 1429-1435.
- DAVIS, J. P., OSTERHOLM, M. T., HELMS, C. M., VERGERONT, J. M., WINTERMEYER, L. A., FORFANG, J. C., JUDY, L. A., RONDEAU, J., SCHELL, W. L. and the Investigation Team (1982). Tri-State toxic-shock syndrome study. II. Clinical and laboratory findings. *Journal of Infectious Diseases* **145**, 441-448.
- DE SAXE, M. J. & ROSENDAL, K. (1982). International committee on systematic bacteriology.

- Subcommittee on phage-typing of staphylococci. Minutes of the meeting 2 Sept. 1978. *International Journal of Systematic Bacteriology* **32**, 253-254.
- DE SAXE, M. J., WIENEKE, A., AZAVEDO, J. & ARBUTHNOTT, J. P. (1982). Toxic shock syndrome in Britain. *British Medical Journal* **284**, 1641-1642.
- EL SOLH, N., BISMUTH, R., ALLIGNET, J. & FOUACE, J. M. (1984). Résistance à la pristinamycine (ou virginiamycine) des souches de *Staphylococcus aureus*. *Pathologie Biologie* **32**, 362-368.
- FLEURETTE, J. & MODJADEDY, A. (1976). Attempts to combine and simplify two methods for serotyping of *Staphylococcus aureus*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektion Krankheiten und Hygiene I. Abteilung. Supplement* **5**, 71-80.
- FLORET, D., WRIGHT, C., FEBVEY, L., REVERDY, E., BRUN, Y. & MONNET, P. (1983). Le syndrome de choc toxique staphylococcique. A propos de deux observations chez l'enfant. *Lyon Médical* **250**, 35-40.
- GRANTHIL, C., DUMONT, J. C. & BRUN, Y. (1982). Syndrome de choc toxique à staphylocoque doré group II, producteur d'entérotoxine B. *La Nouvelle Presse Médicale* **11**, 54.
- HALLANDER, H. O. (1965). Production of large quantities of enterotoxin B and other staphylococcal toxins on solid media. *Acta Pathologica et Microbiologica Scandinavica* **63**, 299-305.
- LETELLIER, N., COULOMB, F., LEBEC, C., BRENET, J. M., LABABIDI, A., THERY, P. & TINEL, G. (1982). Choc toxique staphylococcique. *La Nouvelle Presse Médicale* **11**, 1080.
- LINNEMANN, C. C., STANECK, J. L., HORNSTEIN, S., BARDEN, T. P., RAUH, J. L., BONVENTRE, P. F., BUNCHER, C. R. & BEITING, A. (1982). The epidemiology of genital colonization with *Staphylococcus aureus*. *Annals of Internal Medicine* **96**, 940-944.
- MELCONIAN, A. K., BRUN, Y. & FLEURETTE, J. (1983). Enterotoxin production, phage typing and serotyping of *Staphylococcus aureus* strains isolated from clinical materials and food. *Journal of Hygiene* **91**, 235-242.
- MELCONIAN, A. K., FLANDROIS, J. P. & FLEURETTE, J. (1983). Modified method for production and purification of *Staphylococcus aureus* enterotoxin B. *Applied and Environmental Microbiology* **45**, 1140-1143.
- PARIS, A. L., HERWALDT, L. A., BLUM, D., SCHMID, G. P., SHANDS, K. N. & BROOME, C. V. (1982). Pathologic findings in twelve fatal cases of toxic shock syndrome. *Annals of Internal Medicine* **96**, 852-857.
- RAPIN, M., DENFERT, J. & CABANE, J. (1981). Le syndrome de choc toxique staphylococcique. *La Nouvelle Presse Médicale* **10**, 2167-2170.
- REINGOLD, A. L., HARGRETT, N. T., DAN, B. B., SHANDS, K. N., STRICKLAND, B. Y. & BROOME, C. V. (1982). Nonmenstrual toxic shock syndrome. *Annals of Internal Medicine* **96**, 871-874.
- REINGOLD, A. L., HARGRETT, N. T., SHANDS, K. N., DAN, B. B., SCHMID, G. P., STRICKLAND, B. Y. & BROOME, C. V. (1982). Toxic shock syndrome surveillance in the United States, 1980, to 1981. *Annals of Internal Medicine* **96**, 875-880.
- REISER, R. F., ROBBINS, R. N., KHOE, G. P. & BERGDOLL, M. S. (1983). Purification and some physicochemical properties of toxic-shock toxin. *Biochemistry* **22**, 3907-3912.
- ROUFFINEAU, J., DUCROZ, B., BAUFLE, J. L., PONCIN, E., LHUISSIER, D. AND BECQ-GIRAUDON, B. (1982). Oedème pulmonaire lésionnel au cours d'un syndrome de choc toxique staphylococcique. *La Nouvelle Presse Médicale* **11**, 676-677.
- SCHLIEVERT, P. M., SHANDS, K. N., DAN, B. B., SCHMID, G. P. & NISHIMURA, R. D. (1981). Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic shock syndrome. *Journal of Infectious Diseases* **143**, 509-516.
- SHANDS, K. N., SCHLECH III, W. F., HARGRETT, N. T., DAN, B. B., SCHMID, G. P., BENNETT, J. V. & THE INVESTIGATION TEAM (1982). Toxic shock syndrome: case-control studies at the Centers for Disease Control. *Annals of Internal Medicine* **96**, 895-898.
- SHANDS, K. N., SCHMID, G. P., DAN, B. B., BLUM, D., GUIDOTTI, R. J., HARGRETT, N. T., ANDERSON, R. L., HILL, D. L., BROOME, C. V., BAND, J. D. & FRASER, D. W. (1980). Toxic-shock syndrome in menstruating women. Association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. *New England Journal of Medicine* **303**, 1427-1442.
- TODD, J., FISHAUT, M., KAPRAL, F. & WELCH, T. (1978). Toxic-shock syndrome associated with phage group I staphylococci. *Lancet* **25**, 1116-1118.
- TOFTE R. W. & WILLIAMS, D. N. (1981). Toxic shock syndrome. Evidence of a broad clinical spectrum. *Journal of the American Medical Association* **246**, 2163-2167.