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Enterotoxin production by strains of *Staphylococcus aureus* isolated from clinical and non-clinical specimens with special reference to enterotoxin F and toxic shock syndrome

By MARIANNE P. DE NOOIJ, WIJNANDA J. VAN LEEUWEN and SERVE NOTERMANS*

Laboratory for Zoonoses and Food Microbiology and Laboratory for Bacteriology, National Institute of Public Health, P.O. Box 1, Bilthoven, The Netherlands

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SUMMARY

Enterotoxin production by strains of *Staphylococcus aureus* isolated from clinical specimens of human and animal origin and from healthy human carriers was investigated. All nine patients admitted to hospital with symptoms of toxic shock syndrome (TSS) yielded enterotoxin-producing strains of *S. aureus*. Eight of these produced staphylococcal enterotoxin F (SEF). A significantly smaller proportion of strains (42% of 50 strains tested) isolated from other clinical specimens of hospitalized patients produced SEF. Production of SEF by strains isolated from clinical specimens of animal origin (48 strains) was not observed. Twenty-nine per cent of 24 *S. aureus* strains isolated from noses of hospital staff produced SEF. This result was not significantly different from that obtained from strains isolated from clinical specimens other than TSS. A similar percentage of strains isolated from healthy human carriers outside hospital produced SEF (25% of 24 strains tested).

The results indicated that enterotoxin production, especially that of SEF, is associated with *S. aureus* isolated from patients suspected of TSS. There was no indication of an association between *S. aureus* isolated from other staphylococcal infections and SEF production.

All strains were phage typed and 79% of the strains belonging to the international phage-group I produced SEF. All strains lysed by phage 187 were found to produce SEF.

INTRODUCTION

Todd *et al.* (1978) described a disease associated with *Staphylococcus aureus* called toxic shock syndrome (TSS). The symptoms observed included vomiting, diarrhoea, high fever, erythroderma, oedema, kidney-insufficiency and shock. Most of the patients described with this disease are women and most become ill during their menstrual period (Davis *et al.* 1980). Symptoms of TSS are also observed associated with other *S. aureus* infections such as abscesses and osteomyelitis (Bergdoll

* To whom off-print requests should be sent.

et al. (1981). These workers isolated strains producing an enterotoxin-like protein, which was tentatively named enterotoxin F (SEF), from 94% of patients. Their investigations demonstrated a clear association between TSS and SEF producing *S. aureus* strains. Schlievert *et al.* (1981) found the production of a toxic protein called exotoxin C in 100% of the *S. aureus* strains isolated from patients with TSS. Since both toxins have a similar molecular weight and an equal iso-electric point they may be identical (Schlievert *et al.* 1981; Bergdoll *et al.* 1981).

Bergdoll *et al.* (1981) examined 62 *S. aureus* strains from clinical sources other than TSS and from foods and animals. Only four of these strains produced SEF. Preliminary investigations in our laboratory indicated that SEF producing strains are frequently found in pus.

In this report we describe the enterotoxigenicity of *S. aureus* strains isolated from (i) patients suspected of TSS, (ii) other human clinical specimens from hospitalized patients, (iii) animal clinical specimens and (iv) healthy human carriers both in and outside hospital. All strains were also phage typed.

MATERIALS AND METHODS

Strains of S. aureus. Strains were isolated from patients with suspected TSS and from patients with wounds, furuncles, sepsis, mastitis and paronychia and were collected in 19 different hospitals in The Netherlands. Strains from animals were obtained from district animal health laboratories. These were isolated from chickens with sepsis (haemolytic strains were recognized as *S. aureus*), from cows with mastitis and from dogs with wounds. Strains were also obtained from the noses of hospital staff in 13 different hospitals and from the noses of people outside hospital. Eleven additional strains lysed by phage 187, and taken from the collection in our laboratory, were tested for enterotoxin production. These had been isolated from wounds and noses of hospital staff.

Enterotoxin production. Strains of *S. aureus* were tested for enterotoxin production using the sac-culture method of Donelly *et al.* (1967). After inoculation the sac-culture was incubated at 37 °C on a rotary shaker (120 r.p.m.) for 40h, and then centrifuged at 10000 *g* for 10 min. The supernatant was tested for the presence of enterotoxin using the optimal sensitive plate method (OSP-method) as described by Robbins, Gould & Bergdoll (1974). The OSP-method is a modified immuno-gel diffusion technique (Ouchterlony, 1958).

Reference toxins and antisera used in the OSP-method were kindly provided by Professor M. S. Bergdoll, Food Research Institute, Madison, Wisconsin, U.S.A. The tests for SEF production were also performed using SEF and anti-SEF prepared in our laboratory (Notermans and Dufrenne, in the press).

Phage-typing. All strains tested for enterotoxin production were phage typed using the international basic set of typing phages and phage 187 (Report Sub-committee Staphylococcal Phage-typing, 1975). If they were untypable with the international phages they were further tested with an additional set of phages (Van Leeuwen & Rost, 1976).

Table 1. *Enterotoxin production by strains of* S. aureus *isolated from patients with suspected TSS*

Patient*	Site of isolation	Phage-group (phage pattern)	Production of enterotoxin
1	Throat	I(52)	F
2	Vagina	I(29/52/81)	F
3	Vagina	I(29/80)	F
4	Cervix, ureter, anus, urine	non-typable	F
5	Vagina	I(29)	AF
6	Vagina, cervix	I(29/52/79)	AF
7	Nose, pustule, cerebro spinal fluid	I(29/52/79/81)III(54/42E)	F
	Blood	II(3A/55/71)	—
		XIII(94/96/14/15)	—
8	Throat	I(81)	F
		I(29/52/80/81)	F
		I(29/81)/III(6/47/53/54/75/83A/85)	A
9	Nose, throat	I(81)	ABD
	Wound	II(36C'/55)	—

* Patient 1–7: menstruating women; patient 8: girl (11 years old); patient 9: girl (7 years old).

RESULTS

Enterotoxin-producing strains of *S. aureus* were isolated from all of the patients with suspected TSS (Table 1). Strains from eight cases produced SEF alone or together with SEA. No attempts were made to isolate *S. aureus* from the vagina of patient 1, though a strain of *S. aureus* isolated from the throat was available. From patient 4 strains of *S. aureus* were also isolated from the ureter, anus, and urine and they were indistinguishable from the strain isolated from the cervix. Strains with an identical phage pattern were obtained from the vagina of patient 6 and from her cervix, and from menstrual discharge. Similar strains of *S. aureus* were isolated from the nose, from a pustule and from cerebro-spinal fluid of patient 7. These strains had the same phage type and produced SEF. Two different strains were isolated from her blood, one belonged to phage group II and the other to phage group XIII, and they did not produce SEF.

Patient 8 (a non menstruating girl of 11 years) yielded *S. aureus* from the throat only. Three different strains were obtained; two of these produced SEF, one was lysed by phage 81 (group I) and the other by phages 29, 52, 80, 81 (group I). The third strain produced SEA and was lysed by phages 29, 81, 6, 47, 53, 54, 75, 83A, 85 (group I/III).

Strains of *S. aureus* sensitive to phage 81 were isolated from the nose and throat of patient 9 and they produced SEA, SEB and SED. A strain of *S. aureus* isolated from a knee wound belonged to phage group II and did not produce enterotoxin.

Enterotoxin production by strains of *S. aureus* isolated from human clinical specimens not associated with TSS is given in Table 2. Forty-two per cent of the strains isolated from infections other than TSS produced SEF, which is significantly lower ($P = 0{\cdot}05$) than the frequency of SEF production by strains isolated from patients with suspected TSS. Twenty-nine per cent of strains isolated from noses of hospital staff produced SEF (Table 3, upper panel). Of strains isolated from nasal

Table 2. *Enterotoxin production by strains of* S. aureus *isolated from clinical specimens*

Source of specimen	No. of strains tested	No. of strains producing enterotoxins A–F	Enterotoxin produced					
			A	B	C	D	E	F
Wounds	29	18	2	4	2	—	—	12
Furuncles	6	5	2	1	—	1	—	2
Paronychia	8	8	3	1	1	1	—	5
Mastitis	2	2	—	—	—	—	—	2
Tracheitis	1	1	—	—	—	1	—	—
Septicaemia	2	1	—	1	—	—	—	—
Cerebro-spinal fluid	2	1	—	1	—	—	—	—
TSS	9	9	3	1	—	1	—	8

carriers outside hospital 25% produced SEF, which is not significantly lower than SEF production by strains isolated from carriers in hospital and from human clinical specimens. Strains of *S. aureus* isolated from clinical specimens of animals did not show SEF production (Table 3, lower panel).

Production of enterotoxins other than SEF is also shown in the tables. Some strains produced a combination of two or more enterotoxins. Of note was the combination of SEF production with SEA and SEC. Of the 55 strains of *S aureus* which produced SEF, 31% produced SEF only, 14% SEF and SEA, 5% SEF and SEC and 5% SEF, SEA and SEC.

Phage-typing results showed that these animal strains were different from those isolated from human beings (Table 4). There was no great difference between the incidence of different phage groups among strains isolated from human clinical specimens, from carriers inside hospital and from carriers outside hospital.

Enterotoxin production by strains isolated from human beings (patients and carriers in and outside hospital) and the phage groups to which these strains belonged are presented in Table 5 (together with enterotoxin production by the additional strains lysed by phage 187). Seventy-nine per cent of strains which belonged to phage group I produced SEF. All strains that were sensitive to phage 187 produced SEF, usually in combination with SEA and SEC. No clear relationship between SEF production and susceptibility to any particular phage in phage group I was found.

Reproducibility of the results. Twenty-five strains chosen at random were tested a second time for enterotoxin production. In this experiment the whole procedure (including growth in sac-cultures) was repeated. Twenty-three strains gave the same results as in the first experiment. Once SEC production was not observed and once, instead of SEF, production of SEC was observed. Anti-SEF serum produced in our laboratory gave the same results as anti-SEF serum obtained from Professor M. S. Bergdoll, when used to detect the production of SEF by 20 strains.

Table 3. *Enterotoxin production by strains of* S. aureus *isolated from human clinical infections, excluding TSS, from carriers in and outside hospital and from animals*

Strains isolated from	No. of strains tested	No. of strains producing enterotoxins A–F	Enterotoxin produced					
			A	B	C	D	E	F
Clinical infections	50	36	7	8	3	3	—	21
Carriers in hospital	24	15	7	2	2	1	—	7
Carriers outside hospital	24	16	3	2	3	4	—	6
Cows (mastitis)	24	1	—	—	1	—	—	—
Chickens (sepsis)	14	2	1	—	—	1	—	—
Dogs (wounds)	10	4	—	—	4	—	—	—

Table 4. *Phage-groups of strains of* S. aureus *isolated from carriers, patients and animals*

Phage-group*	No. of carriers in hospital	No. of carriers outside hospital	No. of patients	No. of animals
I	7	7	23	—
II	1	4	8	—
III	1	2	3	10
XI	4	5	3	—
XIII	3	2	7	—
XVI	3	—	4	—
I/III	2	2	5	12
mixed†	2	—	1	9
non-typable	1	2	6	17

* Phage groups XI, XIII and XVI are described by Van Leeuwen & Rost (1976).
† Other phage groups.

Table 5. *Enterotoxin production by strains of* S. aureus *isolated from human beings in relation to phage-groups*

Phage-group*	No. of strains tested	No. of strains producing enterotoxin A–F	Enterotoxin produced					
			A	B	C	D	E	F
I	37	35	10	2	2	2	—	29
II	13	3	1	2	—	—	—	—
III	6	5	2	1	1	2	—	—
187	13	13	9	—	7	—	—	13
XI	12	7	2	—	6	1	—	1
XIII	12	7	—	6	—	—	—	1
XVI	7	7	—	—	—	1	—	6
I/III	9	7	2	1	1	2	—	2
Mixed†	3	0	—	—	—	—	—	—
Non-typable	9	6	1	1	—	1	—	3

* Phage groups XI, XIII and XVI are described by Van Leeuwen & Rost (1976).
† Other phage groups.

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DISCUSSION

Enterotoxin-producing strains of *S. aureus* were isolated from all nine patients with suspected TSS and strains from eight of the patients produced enterotoxin F (SEF). These findings are in agreement with the results of Bergdoll *et al.* (1981) indicating that enterotoxins, particularly SEF, may be a cause of the signs and symptoms of TSS. In constrast with the findings of Bergdoll *et al.* (1981) we found that a high percentage of strains isolated from clinical specimens not associated with TSS also produced SEF (42% of the strains tested). However, the results may suggest that SEF production was not associated with *S. aureus* isolated from patients with symptoms other than those of TSS: (i) an equal percentage of strains isolated from clinical infections and from human carriers in and outside hospital produced SEF and (ii) SEF production was not observed among strains isolated from animal clinical specimens. Production of enterotoxin, particularly that of SEF, can be regarded as an important virulence factor of *S. aureus*. However, the question arises as to whether strains which are able to produce SEF in broth also produce the toxin under other conditions. It is known that enterotoxin production of *S. aureus* under anaerobic conditions is much reduced (Baird-Parker, 1971). Under strict anaerobic conditions we found that SEF producing *S. aureus* strains failed to produce this toxin *in vitro* (results not presented). As SEF-producing strains are commonly found in human carriers both outside and in hospital more investigations are needed regarding SEF production *in vivo* Also, other factors which may limit the occurrence of TSS should be studied, for instance, the antibody production against SEF.

The production of enterotoxins A to E by strains isolated from human infections corresponded with the results of Wieneke (1974) and Berman, Gilpil & Knight (1981). The enterotoxin production by strains isolated from animals agreed with the results of Casman *et al.* (1967), Brückler *et al.* (1981) and Shiozawa, Kato & Shimiza (1980). With regard to the phage-typing patterns of SEF-producing strains a strong association was found with lysis by phage 187 and by phages of group I. Strains sensitive to phage 187 are rarely isolated; about 1% of strains from clinical specimens and human carriers in the Netherlands are lysed by this phage (unpublished results). About 30% of human strains belong to phage group I. All strains lysed by phage 187 and nearly 80% of strains belonging to phage group I produced SEF. The observation that SEF is produced as a single toxin or only in combination with SEA or SEC may also be important with regard to the genetic mechanism of SEF production by *S. aureus*.

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