SHORT ARTICLES

PREVALENCE OF EPIDERMOLYTIC TOXIN IN CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS*

JOYCE DE AZAVEDO AND J. P. ARBUTHNOTT

Department of Microbiology, Moyne Institute, Trinity College, Dublin 2

SUMMARY. One hundred and sixteen strains of *Staphylococcus aureus* isolated from exfoliative skin lesions were screened for their ability to produce different serotypes of epidermolytic toxin (ET). Toxin production was assessed by immunodiffusion, analytical isoelectric focussing and examination for the Nikolsky sign in mice. Of 84 strains of phage group II, 72 (85.7%) were toxinogenic as were 10 of 32 (31.3%) non-group-II strains. The ability to produce ET serotypes A and B was not confined to a particular phage group.

INTRODUCTION

Two antigenically distinct serotypes of staphylococcal epidermolytic toxin (exfoliative toxin or ET), referred to here as ETA and ETB, have been reported (Kondo, Sakurai and Sarai, 1974; Arbuthnott and Billcliffe, 1976; Wiley and Rogolsky, 1977). These toxins are well characterised (Kondon, Sakurai and Sarai, 1976; Johnson *et al.*, 1979; Bailey, de Azavedo and Arbuthnott, 1980) and comparisons of amino-acid content indicate that serotypes purified in different laboratories are identical (Bailey *et al.*, 1980).

Production of toxin was initially thought to be confined to *Staphylococcus aureus* of phage group II (Melish and Glasgow, 1970) and therefore, in some surveys, strains belonging to this group alone were screened for toxin production (Miller and Kapral, 1972; Galinski, 1976). However, non-group-II strains have been isolated from patients with staphylococcal scalded-skin syndrome (SSSS) (Faden *et al.*, 1974; Rasmussen, 1975) and it is now known that strains from other phage groups are capable of ET synthesis (Kapral, 1974; Kondo *et al.* 1974; Arbuthnott and Billcliffe, 1976). In the only survey of the incidence of toxin serotypes so far carried out, Kondo *et al.* (1975) reported that only strains of phage group II produced ETA alone whereas production of ETB alone was a feature of non-group-II strains. However, in a study of assay methods for the detection of ET, Arbuthnott and Billcliffe (1976) noted that ETA was produced by a few non-group-II strains.

This study was made to investigate the prevalence of the two known serotypes of ET in strains isolated from patients with SSSS and other superficial staphylococcal skin lesions in Britain and Ireland.

MATERIALS AND METHODS

Bacterial strains. One hundred and sixteen isolates of *Staphylococcus aureus* from patients with superficial skin lesions were supplied by hospitals in Britain and Ireland and by the Central Public Health Laboratory, Colindale. Of these strains, 33 (28.4%) were from patients with SSSS, 38 (32.8%) from patients with impetigo and 45 (38.8%) from patients with other superficial skin conditions. The strains were lyophilised on receipt and stored until required.

Preparation of culture concentrates. Batches of 16 lyophilised strains were reconstituted in

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1-ml amounts of Bernheimer's yeast-diffusate medium (Bernheimer and Schwartz, 1963) and plated on 1% (w/v) agar (Difco) in Bernheimer's medium; three of the 16 strains were controls comprising one ETA producer, one ETB producer and one mixed producer. Several colonies from each plate were inoculated into 75-ml volumes of Bernheimer's medium in 250-ml flanged flasks. Cultures were incubated at 37°C for 40 h with constant shaking at 150 oscillations/min, and 25-ml samples were taken from them at 20 h and 40 h. ETA production reached a peak at 20 h in control cultures whereas ETB production was maximal at 40 h. ET was precipitated from supernates by dialysis against 90%-saturated ammonium sulphate at 4°C. Precipitates were redissolved in 0.5 ml of 0.07M phosphate-buffered saline at pH 7.2; concentrates were dialysed against this buffer and stored at -20°C until required for analysis.

Screening tests for epidermolytic toxin production. Concentrates were tested for the presence of the two serotypes of ET by Ouchterlony double-diffusion tests against specific antiserum, thin-layer-gel analytical isoelectric focussing and by neonatal-mouse assay. These tests have been described in detail in a previous paper (Arbuthnott and Billcliffe, 1976).

Strains giving negative results in the in-vitro tests were injected into neonatal mice at an inoculum of 5×10^8 organisms and the mice were examined for wrinkling of the skin when lightly stroked (a positive Nikolsky sign) at 4, 8 and 24 h.

Preparation of antisera. Highly purified preparations of ETA and ETB (Bailey *et al.*, 1980) were used to prepare specific antisera in rabbits. A strain of phage group III (type 54/85) described previously (Arbuthnott and Billcliffe, 1976) was selected for ETA production and purification; this strain gave consistently high yields of this toxin serotype alone. ETB was purified from cultures of a strain of phage group II (type 71) which produced large amounts of this toxin. Antisera raised from these preparations were specific for each serotype and did not react with the heterologous ET type.

RESULTS

Of the 116 strains tested, concentrates from 68 (58.6%) gave positive results in all three tests and 82 (70.7%) strains were positive in one or more tests (table I). One concentrate, from a strain of phage group II gave a positive Nikolsky sign in mice but was negative in other tests. Immunodiffusion was a good indicator of toxin production; 81 (98.8%) of 82 mouse-positive concentrates were positive by this test. Analytical isoelectric focussing was relatively insensitive, detecting only 68 (82.9%) of mouse-positive strains. Of the 116 strains, 34 were non-toxinogenic in in-vitro tests and also when injected *in vivo* into neonatal mice.

Phage typing showed that of the 116 strains tested, 84 were of group II, four of group I, nine of group III, 17 of group I/III and two were not typable.

Of the 84 strains of phage group II, 72 (85.7%) produced ET whereas only 10 (31.3%) of the 32 non-group-II strains produced the toxin as indicated by mouse assay. The prevalence of the two serotypes of ET produced by phage-group-II and non-group-II strains as determined by double-diffusion tests, is shown in table II. Of the 71 group-II toxin-producing strains that were positive by serological testing, 31 (43.7%) produced ETA, 12 (16.9%) produced ETB and 28 (39.4%) produced ETA and ETB. The distribution of serotypes among 10 non-group-II toxin producers was: 6 (60%) ETA, 1 (10%) ETB and 3 (30%) ETA and ETB.

TABLE I

Presence of epidermolytic toxin in culture concentrates from 116 strains of Staphylococcus aureus

Detection system	Number (%) of strains giving positive results
Mouse assay	82 (70.7)
Immunodiffusion	81 (69.8)
Analytical isoelectric focussing	68 (58.6)
All three tests	68 (58.6)

TABLE II

Prevalence of the two serotypes of epidermolytic toxin (ET) produced by phage group II and non-group-II strains of Staphylococcus aureus

Toxin serotype	Number (%) of	
	phage-group-II strains	Non-phage-group-II strains
ETA	31 (36.9)	6 (18.8)
ETB	12 (14.3)	1 (3.1)
ETA + B	28 (33.3)	3 (9.4)
ET not detected	13 (15.5)	22 (68.8)
Total	84	32

ETA = epidermolytic toxin type A; ETB = epidermolytic toxin type B.

DISCUSSION

Our results show that ET production is not confined to strains of phage group II, confirming the findings of Kondo *et al.* (1974) and Kapral (1974). The prevalence of toxin production was found to be much higher among strains of phage group II (85.7%) than among non-group-II strains (31.3%). However, a large proportion of the latter were isolated from superficial skin infections other than impetigo or SSSS. Kondo *et al.* (1975) reported ET production by 95.8% and 89.5% of group-II and non-group-II strains respectively but almost all these strains were isolated from SSSS patients. Our results also indicate that production of either ETA or ETB is not confined to particular phage groups.

The high prevalence of non-toxinogenic strains in our survey could be due to the use of strains isolated from a wider variety of skin infections. Of the 34 strains not producing toxin, as determined by mouse assay, four were isolated from patients with SSSS, 11 from patients with impetigo and 19 from patients with other superficial infections. Possibly ET production in these strains was weak and rapidly lost in subculture or, in the case of isolates from patients with conditions other than SSSS and impetigo, skin lesions may not have been caused by ET.

Only one concentrate that was negative in the in-vitro tests gave a positive Nikolsky sign when injected into neonatal mice. As a screening technique, therefore, immunodiffusion seems to be very sensitive, and has an advantage over the mouse assay of allowing identification of serotypes. However, it must be borne in mind that only existing serotypes for which antisera are available can be identified by this method and it is thus imperative that negative strains be screened *in vivo*.

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