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Some Properties of the Beta-Lactamase Genes in *Staphylococcus epidermidis*

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Eighty-three per cent of 200 different freshly isolated cultures of *Staphylococcus epidermidis* produced beta lactamase. Growth in the presence of acridine orange or ethidium bromide or growth at 44 C resulted in a high frequency of loss of the beta lactamase genes in some strains of *S. epidermidis*. The relationship between beta-lactamase production and resistance to mercuric, cadmium and arsenate ions differed from that observed in *Staphylococcus aureus*. It is postulated that the genes for beta lactamase in certain strains of *S. epidermidis* are on a plasmid.

The genes controlling formation of the penicillinase, a β-lactamase, produced by *Staphylococcus aureus* occur in most strains on a plasmid (3, 7). Also on this plasmid are found the genes determining resistance to the heavy metals Hg, Cd, Zn, and As (8), and in a few strains, to erythromycin (6). Although several investigators have reported β-lactamase production by *Staphylococcus epidermidis* (4, 11) it is not known whether the genes controlling its production in this organism are extrachromosomal or chromosomal. Richmond (10) reported that the β-lactamase from one strain of *S. epidermidis* tested was immunologically similar to both enzyme type A and C of *S. aureus*. This paper describes some preliminary studies on the nature of the β-lactamase genes in *S. epidermidis* in light of current understanding of the β-lactamase of *S. aureus*.

MATERIALS AND METHODS

Isolation of cultures. Nasal swabs obtained from 200 young adults were streaked onto Phenol Red Mannitol Agar (Difco) containing 7.5% NaCl (PRSM) and PRSM containing 0.1 unit per ml benzyl penicillin (Eli Lilly Co., Indianapolis, Ind.). After incubation for 48 hr, the plates were scored for colonies that produced β-lactamase by a modification of the starch-iodine technique of Perret (9). The PRSM agar was layered with 4 ml of a solution containing 1% agar-agar, 1% soluble starch and 10 mg per ml benzyl penicillin. The plates were incubated at ambient temperature for 10 min and then flooded with a solution of iodine (2.0 g of I, 4.0 g of KI, 1000 ml of distilled water). Colonies producing moderate amounts of β-lactamase were white and surrounded by zones of clearing against an intense blue background. With strains producing small amounts of β-lactamase the colony was initially blue but gradually changed to white. Growth of representative colonies was inoculated into Brain Heart Infusion broth (BHI; Difco). The BHI cultures after incubation at 37 C for 24 hr were used to test for coagulase production and to prepare stock cultures. Classification of an organism as *S. epidermidis* was based on the source, morphology, the nature of the growth on a medium containing 7.5% NaCl, and the failure to produce coagulase.

Curing of the plasmid state. Small inocula (10^5 cells) of penicillinase-positive strains were incubated for 24 hr in Trypticase Soy Broth (TSB) containing either 12.5 μg of acridine orange per ml or 2.5 to 5.0 μg of ethidium bromide (3,8-diamino-5 ethyl-6-phenylphenanthridinium) per ml at 37 C (2) or for 5 hr at 44 C (4). Mutants that lost the β-lactamase genes were detected by streaking appropriate dilutions of the TSB cultures onto a medium (YTS) consisting of 0.3% yeast extract (Difco), 3% TSB, and 1.5% agar-agar (Difco). After incubation for 24 hr, the colonies were sufficiently large to be tested by the starch-iodine technique. Parent strains and mutants that had lost the capacity to produce β-lactamase were tested for resistance to Hg, Cd, and As on YTS agar containing 0.1 mM $HgCl_2$, 0.1 mM $CdCl_2$ or 0.02 M Na_2HAsO_4.

RESULTS AND DISCUSSION

Table 1 shows the frequency of isolation of *S. epidermidis* from nasal cultures and the percentage of these strains producing a β-lactamase. No attempts were made to classify the strains of *S. epidermidis* into the subgroups described by Baird-Parker (1). Ninety-three per cent of the nasal cultures yielded *S. epidermidis*. All of the cultures negative for *S. epidermidis* yielded such a heavy growth of *S. aureus* on the PRSM plates that colonies of *S. epidermidis*, if present, would have been masked. Thirty-six per cent of the cultures were positive for *S. aureus*. Eighty-three per cent of the cultures of *S. epidermidis* produced β-lactamase while only 40% of the cultures of

TABLE 1. *Incidence of penicillinase-positive staphylococci in nasal cultures of young adults*

No. cultured	200
Percentage of nasal cultures positive for	
S. aureus	36
S. epidermidis	93
Percentage of staphylococci producing penicillinase	
S. aureus	40
S. epidermidis	83

TABLE 2. *Loss of β-lactamase production after growth of S. epidermidis in the presence of ethidium bromide*

Culture	Percentage of colonies negative for β-lactamase	
	Control	Ethidium bromide
P-1	<0.05	7.4
P-12	<0.1	<0.1
P-14	<0.1	<0.1
P-23	<0.2	0.6
P-40	<0.1	2.5
P-45	<0.1	5.1

S. aureus produced the enzyme. The frequency of β-lactamase-positive cultures was higher than frequencies reported by other investigators (3, 10). This we feel was due to the use of the starch penicillin overlay technique. On many PRSM plates, only 1 to 10 β-lactamase-positive colonies were detected among several hundred β-lactamase-negative colonies. Furthermore, on PRSM agar containing 0.1 unit of penicillin per ml, many strains that produced small detectable amounts of β-lactamase failed to grow. Rountree and Kjellander, Klein, and Finland (4, 11) isolated strains of *S. epidermidis* that produced such small amounts of β-lactamase they were considered sensitive to penicillin. The starch penicillin overlay technique should be extremely useful in clinical laboratories since the resistance to penicillin of colonies on primary isolation on many different types of media can be determined in 15 min without further subculturing.

Growth in the presence of ethidium bromide (Table 2) or acridine orange resulted in a high frequency of loss of the β-lactamase genes by certain strains. With four strains, the percentage of β-lactamase-negative colonies detected after exposure to ethidium bromide varied from 0.6 to 7.4%. The loss of capacity to produce β-lactamase in these four strains was irreversible. In two other strains tested, ethidium bromide caused no loss of β-lactamase production. When P-1 and P-40 were grown in the presence of acridine orange, 1.4% of the cells of P-40 and <0.02% of the cells of P-1 lost the penicillinase gene. This was interesting since both lost the β-lactamase genes at a high frequency (P-40, 5.1%; P-1, 7.5%) when exposed to ethidium bromide. In inducing a loss of penicillin resistance in *S. epidermidis* by acridine orange or ethidium bromide, irregular results were obtained with a given strain. It was important that the cells be taken from a tube showing partial inhibition of growth; usually 5.0 to 15 μg of acridine orange or 2 to 5 μg of ethidium bromide per ml of TSB were best. S. Rubin (*personal communication*) also found that cultures growing well in the presence of ethidium bromide failed to yield a high frequency of cured cells.

In 7 of 17 cultures, the percentage of cells losing the β-lactamase genes after growth at 44 C was 0.1 or greater (Table 3). The frequency of sensitive cells in cultures of P-1 was 0.1%, whereas the frequency for P-40 was 1.0%. The high frequency of loss of the β-lactamase gene by certain strains following growth in the presence of acridine orange or ethidium bromide or at 44 C indicated that the β-lactamase genes in these strains of *S. epidermidis* were carried on a plasmid as in most strains of *S. aureus*. The genes controlling resistance to Hg, Cd, and As are also carried on this plasmid in *S. aureus* (7). However, in *S. epidermidis*, resistance to these heavy metals was not always associated with β-lactamase produc-

TABLE 3. *Loss of β-lactamase production after growth of S. epidermidis at 44 C*

No. of cultures	Percentage of colonies negative for β-lactamase
2	1.0–1.6
5	0.1–0.9
10	<0.1

TABLE 4. *Resistance of cultures of S. epidermidis to heavy metals*

No. of cultures	Pattern of resistance[a]		
	Hg	Cd	As
18	R	R	R
11	S	S	R
10	R	S	R
2	R	R	S
2	S	R	S
1	S	R	R
1	S	S	S

[a] Symbols: R, resistant; S, sensitive.

tion. After curing of penicillin resistance by growth in acridine orange, or ethidium bromide, or growth at 44 C, the patterns of sensitivity to Hg, Cd, and As were the same as in the β-lactamase-producing parents shown in Table 4. Although 18 of the parent cultures were resistant to all 3 of the heavy metal ions tested, the majority were sensitive to 1 or more. β-Lactamase-negative strains of *S. epidermidis* isolated from nature showed similar variations in the patterns of sensitivity to Hg, Cd, and As. These findings indicate conclusively that the genes controlling resistance to these heavy metals are not on the β-lactamase plasmid.

It seems possible to us that the high frequency of penicillinase-positive strains of *S. epidermidis* might be involved in the emergence of penicillinase-producing strains of *S. aureus* in nature by in vivo transduction of this plasmid. We have isolated from *S. epidermidis* several phages active for *S. aureus* and will report the results of this type of experiment at a later date.

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