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Ethidium Bromide Resistance, a New Marker on the Staphylococcal Penicillinase Plasmid

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A strain of *Staphylococcus aureus* resistant to ethidium bromide is described. The genetic determinants for the resistance are present on the same plasmid as the penicillinase genes.

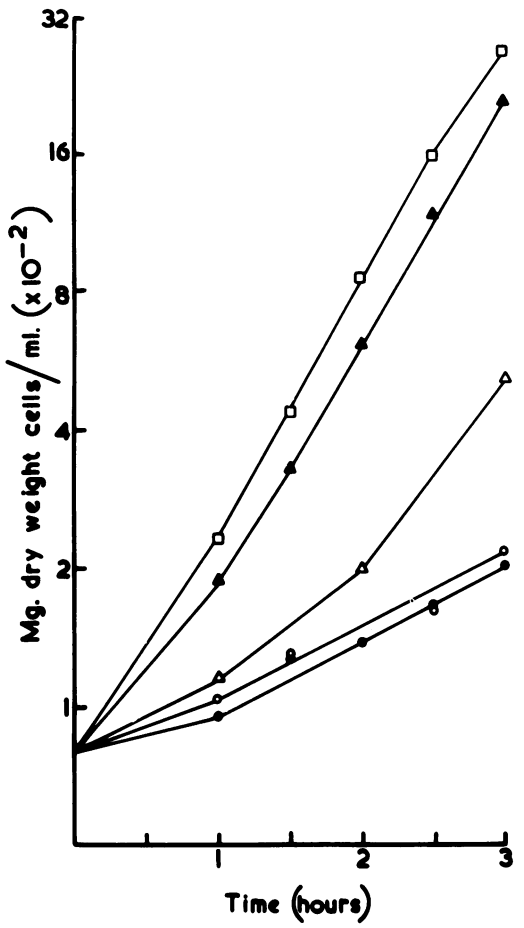

FIG. 1. *Effect of ethidium bromide on the growth of Staphylococcus aureus. Cultures grown in TSB at 37 C for 16 hr were diluted with fresh TSB. The diluted cultures were shaken at 37 C, and growth was followed by measurement of the extinction at 675 nm in a Unicam SP600 spectrophotometer. S. aureus 524 is a representative ethidium bromide-sensitive strain. Strains 1044, 1044N, and 524 in the absence of ethidium bromide (□). Strains 1044, (▲), 524 (●), and 1044N (○) in the presence of 8.0 μg of ethidium bromide/ml. Strain 1044 (△) in the presence of 80 μg of ethidium bromide/ml.*

Growth of certain strains of *Staphylococcus aureus* in the presence of ethidium bromide increases the rate of loss of their penicillinase plasmids (1). During the course of experiments designed to extend these observations, we found that one strain of *S. aureus*, 1044, could grow at a concentration of the drug (8 μg/ml) that was inhibitory to all other strains tested (Fig. 1). Even at an ethidium bromide concentration of 80 μg/ml, there was substantial growth of strain 1044.

Strain 1044 is resistant to penicillin, streptomycin, tetracycline, chloramphenicol, and oleandomycin; it produces C-type penicillinase (6) and is lysed only by phage 53 of the International Phage-Typing Set.

A penicillinase-negative variant of strain 1044 was isolated after growth in tryptone soy broth (TSB) containing 16 μg of acridine orange per ml (4). This penicillinase-negative variant (no. 1044N) was as sensitive to ethidium bromide as the other sensitive strains tested, in contrast to its parent strain 1044 (Fig. 1). Both 1044 and 1044N are sensitive to cadmium acetate, phenylmercuric nitrate, and sodium arsenate. Three additional independently isolated penicillinase-negative variants of 1044 were also found to have lost their resistance to ethidium bromide along with the ability to produce penicillinase. The derived penicillinase-negative variants were as resistant as the parent culture to streptomycin, tetracycline, chloramphenicol, and oleandomycin, and had not changed their phage-typing pattern. The minimum inhibitory concentration of ethidium bromide necessary to prevent growth for 24 hr was determined in TSB at 37 C with an inoculum of 4×10^6 cells/ml; strain 1044 grew

TABLE 1. *Uptake of ethidium bromide by strain 1044 and the derived penicillinase-negative variant 1044N*[a]

Time	Extinction at 480 nm of supernatant fluid after incubation with strain	
	1044	1044N
min		
0	1.20	1.20
30	1.20	0.78
60	1.20	0.70

[a] Bacteria [10 mg (dry wt)] were resuspended in 10 ml of a solution which contained 80 μg of ethidium bromide/ml in 0.1 M Na_2HPO_4—NaH_2PO_4 buffer (*p*H 7.0) and incubated at 37 C. Samples were taken at intervals and centrifuged at 2,000 × *g* for 15 min, and the extinction at 480 nm of the supernatant fluid was measured.

in 200 μg of ethidium bromide/ml but not in 240 μg/ml, whereas 1044N grew in 12 μg/ml but not in 14 μg/ml.

Phage 53, propagated in lytic cycle on 1044, was used to transduce ethidium bromide resistance back into 1044N (5). After 2 hr of growth at 37 C in casein hydrolysate medium, to allow phentotypic expression, transductants were selected on casein-hydrolysate agar containing 80 μg of ethidium bromide per ml, a concentration at which 1044 will grow but 1044N will not. All 52 ethidium bromide-resistant transductants tested had also acquired the ability to produce penicillinase as determined by a plate test (2).

In experiments in which uptake of ethidium bromide was measured, we found that strain 1044N was capable of removing the drug from the medium, but strain 1044 was not (Table 1). Thus, resistance to ethidium bromide may be due to this difference between the parent strain and the derived penicillinase-negative variant in the capacity to take up the drug.

Strain 1044 grows at a twofold higher concentration of proflavine in TSB than does 1044N. Ericson (3) has recently reported a strain of *S. aureus* resistant to a related acridine, acriflavine, in which the genetic determinants responsible for this resistance are carried on a penicillinase plasmid. We have not determined whether the gene determining the limited resistance to proflavine of strain 1044 is the same as that for ethidium bromide resistance, but the two resistances are present on the same penicillinase plasmid.

L. H. Johnston is a Rhodes Scholar and K. G. H. Dyke is a member of the Medical Research Council External Scientific Staff.

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