# COMPARATIVE STABILITY OF PLASMID RESISTANCE TO ANTIBIOTICS *in vitro* AND *in vivo*

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The average percentage of spontaneous plasmid-negative variants in an initial resistant population of staphylococci developing *in vitro* was 0.9 ± 0.2 in broth and 1.2 ± 0.3 in exudate; developing *in vivo*, in a subcutaneous suppurative inflammatory focus it was 6.3 ± 2.0 and in the kidneys 9.5 ± 2.6. The average percentage of spontaneous plasmid-negative variants in purulent exudate *in vivo* was significantly higher than in purulent exudate *in vitro*. The frequency of appearance of these variants *in vivo* depends on the properties of the host.

KEY WORDS: plasmids; staphylococci; erythromycin.

Antibiotic-sensitive variants are often found in a population of staphylococci with plasmid drug resistance, kept on artificial nutrient media. The intensity of appearance of these variants depends on the keeping time [1, 2, 5, 11], the nature of the plasmid [10, 12], and the properties of the microbial cell [3, 7, 9].

The frequency of appearance of sensitive microbial cells in an initial resistant population of staphylococci *in vivo* has been the subject of a few investigations [6, 8]. Their results have shown that the intensity of the process *in vivo* is rather lower than *in vitro* [4].

This paper describes the results of a comparative study of the frequency of appearance of sensitive variants in an initial resistant population of straphylococci developing *in vitro* (broth, purulent exudate) and *in vivo* (subcutaneous suppurative-inflammatory focus, kidneys).

## EXPERIMENTAL METHOD

Strain *Staphylococcus aureus* 8325 Φ II de, containing the penicillinase plasmid recombined with temperate phage Φ II, was used. The gene controlling resistance to erythromycin is located on the plasmid. A culture of this staphylococcus is sensitive to phages Nos. 47, 53, 75, and 77. A suspension of an 18-h broth culture was added in the proportion of 1:10 to Hottinger's broth (pH 7.2; 128 mg % amino nitrogen). The resulting mixture was poured into tubes, 1 ml at a time, and incubated at 20-25°C.

Samples of 0.9 ml of exudate were taken from rats with aseptic suppurative-inflammatory foci by means of individual syringes and each sample was placed in an individual tube. To the extract was then added 0.1 ml of the 18-h broth culture and, after thorough mixing, the tubes were incubated at 20-25°C.

An 18-h broth culture, washed three times and concentrated by five times, was injected in a volume of 0.5 ml into aseptic suppurative-inflammatory foci created in rats [1]. Ten days later, exudate was obtained from each rat by means of an individual syringe and each sample of exudate was transferred to an individual tube and then seeded on agarized media.

Mice weighing 18-20 g were given an intravenous injection of 0.2 ml of the thrice washed and 5-times concentrated 18-h broth culture. The mice were decapitated after 10 days and the kidneys were removed, homogenized in physiological NaCl solution containing 10% horse serum. The resulting homogenate was seeded on agarized media.

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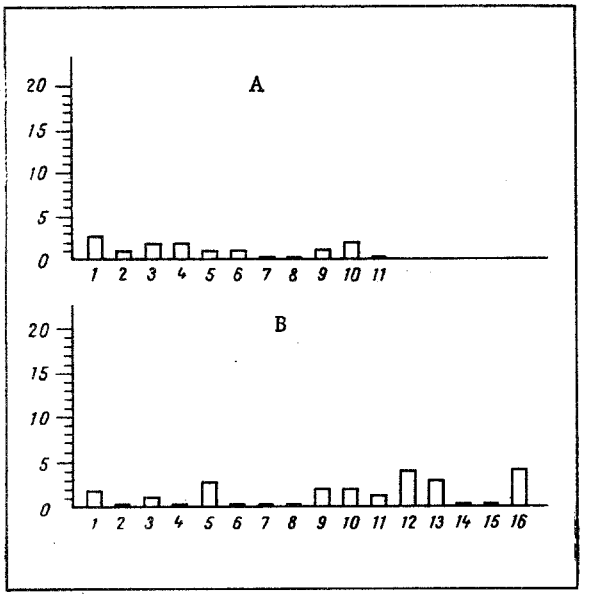


Fig. 1

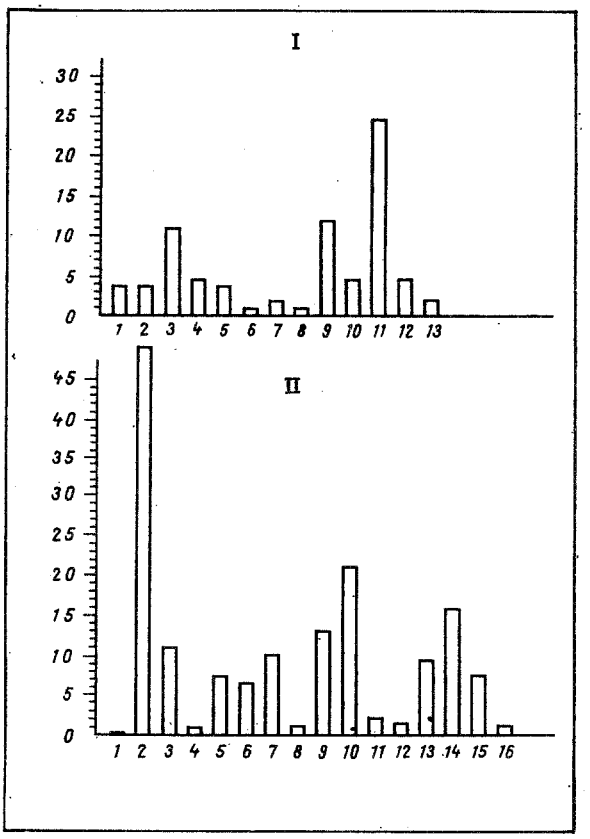


Fig. 2

Fig. 1. Frequency of appearance of erythromycin-sensitive variants in population of *S. aureus* 8325 Φ II de, developing *in vitro*. Abscissa, No. of sample; ordinate, frequency of appearance (in %). Columns denote number of erythromycin-sensitive cells in each sample. A) Broth, B) exudate.

Fig. 2. Frequency of appearance of erythromycin-sensitive variants in population of *S. aureus* 8325 ΦII de developing *in vivo*. I) Focus, II) kidney. Remainder of legend as in Fig. 1.

Erythromycin-sensitive and erythromycin-resistant variants were discovered in populations of staphylococci which had developed for 10 days under different conditions both *in vitro* (broth or aseptic purulent exudate) and *in vivo* (subcutaneous suppurative-inflammatory focus or in the kidney).

Sensitive variants were detected by the replica method. For this purpose each sample, in an appropriate dilution, was seeded on agarized medium without the antibiotic. After incubation of the dishes for 24 h at 37°C the colonies were replicated on medium containing 100 μg/ml erythromycin and on medium without the antibiotic. The plates were incubated for 24 h at 37°C, after which the percentage of sensitive cells in the population was calculated by the usual method. The identity of the sensitive and resistant variants was verified by phage typing.

## EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the frequency of appearance of spontaneous sensitive variants in broth was very low, not more than 1.8%. In 3 of the 10 tubes no erythromycin-sensitive variants were found.

In exudate *in vitro* erythromycin-sensitive cells appeared rather more frequently: the maximal number of these cells in some samples was 3.6%. However, the results given in Table 1 indicate that differences between the mean number of sensitive variants in these two media were not statistically significant. It will also be clear from Table 1 that in purulent exudate *in vivo* spontaneous erythromycin-sensitive variants appeared more frequently ($P < 0.05$) than in purulent exudate *in vitro*. This was shown by significant differences between the

TABLE 1. Number of Spontaneous Sensitive Variants in Initial Resistant Population of Staphylococci Developing *in vitro* and *in vivo* (M ± m)

| Experimental conditions | Medium in which staphylococci developed | Number of samples studied | Mean number of sensitive variants |
|---|---|---|---|
| in vitro | Broth | 10 | 0,9±0,2 |
| | Exudate | 17 | 1,2±0,3 |
| in vivo | Focus | 13 | 6,3±2,0 |
| | Kidneys | 19 | 9,5±2,6 |

Number of tubes (*in vitro*) and number of animals (*in vivo*) shown in table.

mean values of this index *in vitro* and *in vivo*. The mean number of erythromycin-sensitive variants found in kidney homogenate (9.5 ± 2.6) was a little higher than the mean number of these variants discovered in the subcutaneous focus (6.3 ± 2.0). However, the differences were not statistically significant.

The results are thus evidence that spontaneous sensitive microbial cells appear significantly more frequently in an original resistant population of staphylococci developing *in vivo* than in the same population but developing *in vitro*.

Attention is also drawn to what, in the writers' opinion, is a very important fact, namely the considerable fluctuation in the number of sensitive cells in individual animals (Fig. 2). For instance, the percentage of sensitive cells in the purulent exudate *in vivo* varied in individual animals from 0 to 25.0, and in the kidneys from 0 to 49.0. Meanwhile fluctuations of this index *in vitro* were 0-1.8% in broth and 0-3.6% in exudate. In the writers' view these observations may be evidence that the stability of plasmid drug resistance *in vivo* is largely dependent on the properties of the host.

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