Lack of Correlation between Methicillin Resistance and Susceptibility to Lysostaphin in *Staphylococcus aureus*

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INTRODUCTION

Naturally occurring methicillin-resistant staphylococci consist of populations of bacteria with varying levels of resistance to the drug (for example, see Dyke, 1969). Sabbath, Leaf, Gerstein & Finland (1970) and Sabath & Wallace (1971) have shown that organisms uniformly resistant to methicillin, selected from such heterogeneous populations with cloxacillin, are also relatively more resistant to lysis by lysostaphin than cultures of predominantly methicillin-sensitive organisms. Although decreased susceptibility to lysis in these strains is probably the result of membrane changes (Sabath & Wallace, 1971), the relationship of such alterations to the mechanism of methicillin resistance is unclear. Since the strains were probably non-isogenic (i.e. differed in properties other than variation in methicillin resistance) it is possible that the observed resistance to lysostaphin was unrelated to methicillin resistance. However, to establish whether or not a correlation does exist, we have compared the rates of lysis by lysostaphin of pairs of strains differing only in the possession of the gene(s) for methicillin resistance. Acquisition of methicillin resistance did not affect susceptibility to lysis by lysostaphin.

METHODS

Methicillin resistance was transduced from strain DU4916 (Dornbusch, Hallander & Löfquist, 1969) to two recipients: strain 11164 *met-s*, a methicillin-sensitive derivative of strain 1164 (Lacey, 1972), and strain 649MR, a strain that harbours six plasmids (Lacey & Chopra, 1974). A transducing lysate was prepared by propagation of typing phage 88 in three successive cycles, followed by filtration three times through a Millipore filter of 0.45 µm pore size. Transduction was by the method of Lacey (1972) with a phage-to-cell ratio of 0.1. Transductants were selected on nutrient agar containing 25 µg methicillin/ml after incubation at 30 °C for 72 h. They resembled the parent strains in phenotypic properties, including phage typing pattern, except in the acquisition of heterogeneous resistance to methicillin. Thus, strains 11164 *met-s* and 11164 *met-r*, and 649MR and 649MR *met-r*, were considered isogenic pairs except for the variation in methicillin resistance.

Estimation of the susceptibility of strains to lysostaphin was performed as follows. Mid-logarithmic phase cultures (at cell densities equivalent to 0.33 mg dry wt cells/ml) grown either at 30 or 37 °C in nutrient broth, were harvested by centrifugation (15,000 g, 20 min, 4 °C) and washed twice in cold tris-saline buffer (50 mM-tris, 100 mM-NaCl, pH 7.5). Cultures were resuspended in the above buffer to give an extinction at 675 nm of 0.40, and equilibrated to either 30 or 37 °C before the addition of lysostaphin (1 µg/ml final). Incubation was continued at the respective temperature with gentle swirling, and samples were removed at intervals for estimation of extinction at 675 nm. From these data, a series of curves was constructed from which the rate of 50 % lysis could be computed.

Table 1. *The rate of lysis of methicillin-sensitive and resistant Staphylococci by lysostaphin*

Strain	Period (min) required for 50 % reduction in E_{675} *		Minimal inhibitory concentration† (μ g methicillin/ml)
	Growth and lysis at (°C)		
	30	37	
649MR <i>met-r</i>	15.2	6.4	> 1000
649MR	16.4	8.1	2
11164 <i>met-r</i>	22.0	17.2	1000
11164 <i>met-s</i>	21.0	14.8	2

* Values quoted are the means of at least two independent experiments.

† To methicillin at 30 °C; performed by the method of Lacey (1972).

RESULTS AND DISCUSSION

Methicillin resistance was only exhibited by about one cell in 10^5 when cultures were grown in nutrient broth at 37 °C. Thus, even if methicillin resistance was correlated to the rate of lysis by lysostaphin, the rates of lysis of methicillin-resistant or sensitive strains grown at 37 °C in nutrient broth should be similar. Indeed, the rate of lysis of methicillin-resistant strains grown and lysed at 37 °C was very similar to that of their methicillin-sensitive counterparts (Table 1). It is notable that the 11164 strains lysed at markedly lower rates than the 649 strains.

However, the rate of lysis of methicillin-resistant strains grown and lysed at 30 °C (at this temperature every cell shows resistance to methicillin) was virtually identical to that of their methicillin-sensitive counterparts (Table 1). As at 37 °C, the 11164 strains were lysed at lower rates than the 649 strains. It seems, therefore, that methicillin resistance is unrelated to resistance to lysis by lysostaphin.

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