Methicillin-induced lysis of some methicillin-resistant strains of *Staphylococcus aureus*

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The effects of inhibitory and sub-inhibitory doses of methicillin on five strains of *Staphylococcus aureus* have been studied at different temperatures. Cells grown overnight at 37° were treated with methicillin at 25, 30 or 37°, and cells grown overnight at 25 or 30° were treated at 37°. The strains of *S. aureus* consisted of two methicillin resistant (MR) β-lactamase producers, 9254 + and 7270 +; a MR non β-lactamase producer, 7270–; and the penicillin-sensitive, methicillin-sensitive Oxford strain, N.C.T.C. 6571. Inhibitory and sub-inhibitory doses of methicillin used were calculated as fractions (8/5, 3/5 and 1/5) of the minimum inhibitory concentration (MIC) of each strain at 37°. Methicillin was added to cultures at zero time, or to shaken logarithmic phase cultures.

The initial inoculum in each experiment was standardized to give $\sim 5 \times 10^8$ viable cells ml^{-1}. Growth and lysis were followed spectrophotometrically, and viable counts were made by the pour-plate technique. From the results obtained, the temperature of treatment with methicillin, rather than the pretreatment temperature at which the cells were grown, was the important factor. Annear (1968) has previously shown that the resistance of MR strains to methicillin increases with a lowering of the incubation temperature. Inhibitory doses of methicillin caused some lysis of shaken MR cultures, followed by regrowth; preliminary findings have indicated that a higher MIC value of methicillin is obtained against shaken than against non-shaken cultures. With the shaken MR cultures in the present experiments there was an increase in viable numbers after the addition of methicillin, followed by a decrease in viability; with the Oxford strain, in contrast, an initial rapid decrease in viability with concurrent lysis occurred following treatment with methicillin.

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REFERENCE

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Inactivation of β-lactam antibiotics by *Pseudomonas aeruginosa*

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Eight strains of *Pseudomonas aeruginosa* have been examined for their ability to destroy ampicillin, carbenicillin, cephaloridine and cephacetrile. Antibiotics were added to suspensions of whole cells, broken cells and to cell filtrates from overnight cultures grown in a synthetic medium; drug concentrations used were approximately half the minimum inhibitory concentration (MIC) for each particular strain. Cephaloridine and cephacetrile were assayed spectrophotometrically at 255 nm and 263 nm, respectively (Russell & Furr, 1973) whilst the penicillins were heated in an acid buffer containing a trace of copper salt and assayed at 320 nm (Smith, de Grey & Patel, 1967).

Antibiotic concentrations did not decrease in any of the cell-free filtrates, thus ruling out the presence of a constitutive extracellular β-lactamase. Four strains (NCTC 8203 and 6750 and the two highly carbenicillin-sensitive strains NCTC 10701 and 10490), whether as whole or disrupted cell preparations, caused no antibiotic destruction, despite the fact that cells of these strains are highly resistant to ampicillin, cephaloridine and cephacetrile (Russell & Mills, 1974). Thus, no constitutive β-lactamase is present, and resistance in these strains appears to be due to the inability of the drugs to enter the cells.

Two R-factor bearing strains, 1822 R^+ and 3425 R^+, and two substrains (designated 1822 R^- and 3425 R^-) which have been derived from these strains and which have either lost the R-factor or the ability to transfer it, were able, when used as whole or disrupted cell preparations, to reduce markedly the antibiotic concentrations. These results might suggest that the so-called R-strains still possessed the R-factor but were unable to transfer it to our