The Increased Rate of Loss of Penicillinase Plasmids from
*Staphylococcus aureus* in the Presence of Rifampicin

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There have been a number of reports of the ability of various chemicals to increase the rate of loss of penicillinase and other plasmids from *Staphylococcus aureus*. Hashimoto, Kono & Mitsuhashi (1964) reported that 17 out of 18 staphylococcal strains grown overnight in the presence of 25 μg. acriflavine/ml. showed an increased number of penicillinase-less variants, and Harmon & Baldwin (1964) reported 6.2 % penicillin-sensitive cocci in a staphylococcal culture grown overnight in 10 μg. acridine orange/ml. However Novick (1963) and Richmond (1965) were unable to show any curing of staphylococcal strains using acridine dyes, and consequently the effect of these compounds must be regarded as rather variable from strain to strain. Recently Bouanchaud, Scavizzi & Chabbert (1969) have reported the elimination of penicillinase plasmids from certain staphylococcal strains with ethidium bromide, a drug known to intercalate between DNA base pairs, thus indirectly hindering the action of DNA and RNA polymerases (Waring, 1966).

The work presented here follows an observation that rifampicin (a rifamycin derivative—Maggi, Pasqualucci, Ballotta & Sensi, 1966) also has a curative effect on some staphylococcal extrachromosomal elements. This observation is of particular interest since the rifamycins bind directly to the RNA polymerase molecule itself—at least in *Escherichia coli* (Hartmann, Honikel, Knüsel & Staehelin, 1967; Wehrli, Nüesch, Knüsel & Staehelin, 1968; Wehrli, Knüsel, Schmid & Staehelin, 1968) and in *Staphylococcus aureus* (Wehrli, Knüsel & Staehelin, 1968)—rather than acting indirectly by intercalation.

In a preliminary experiment it was found that a culture of penicillin-resistant staphylococci grown at 35° in tryptone/soya broth containing 0.01 μg. rifampicin/ml. contained about 20 % of penicillinase-less variants after overnight growth while a culture incubated in the absence of the antibiotic contained 0.2 % at most. This concentration of rifampicin was about two-thirds of the growth-inhibitory concentration for the strain used here under these growth conditions.

To investigate this phenomenon further, inocula of various sizes of *Staphylococcus aureus* strain 8325 (α i−p* cad-r ero-r)—that is, a staphylococcal culture containing a Com I plasmid conferring resistance to penicillin, cadmium ions and to erythromycin (Richmond, 1969)—were made into batches of tryptone/soya broth each containing a different concentration of rifampicin and the cultures incubated at 35° overnight. A similar culture, inoculated with 10^6 organisms/ml. and incubated in the absence of rifampicin, acted as control. After overnight growth, samples from the cultures were plated onto nutrient agar to give single colonies, and after these colonies had grown
they were replica-plated on to further nutrient agar plates containing either $10^{-4}$ M-cadmium acetate or 10 μg. erythromycin/ml. to score for the presence of the plasmid cad-r and the ero-r markers respectively. After replication, the master plates were also stained to test for the presence of penicillinase—that is for the presence of the $i^{-}p^{+}$ gene group. The results of this experiment are shown in the Table. The incidence of penicillinase-less cocci was greatest with an inoculum of $10^{5}$ organisms/ml. and a concentration of 0.01 μg. rifampicin/ml. and reached a value 100 times higher than the level found in the control. All the penicillinase-less colonies that were detected had also lost the cad-r and ero-r markers, indicating that the whole penicillinase plasmid ($\alpha i^{-}p^{+} cad-r ero-r$), rather than the penicillinase genes alone, had been lost from the cocci.

Table 1. The incidence of plasmid-less cocci in a culture of S. aureus 8325 ($\alpha i^{-}p^{+} cad-r ero-r$) incubated in tryptone/soya broth containing various concentrations of rifampicin

<table>
<thead>
<tr>
<th>Inoculum size (cocci/ml.)</th>
<th>Rifampicin concn (μg./ml.)</th>
<th>Total no. of colonies tested</th>
<th>No. of plasmid-less colonies</th>
<th>No. of plasmid-less colonies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{4}$</td>
<td>0.0075</td>
<td>266</td>
<td>66</td>
<td>25</td>
</tr>
<tr>
<td>0.1</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^{5}$</td>
<td>0.0075</td>
<td>1011</td>
<td>163</td>
<td>16</td>
</tr>
<tr>
<td>0.01</td>
<td>498</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>861</td>
<td>19</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>1224</td>
<td>0</td>
<td>$&lt;0.1$</td>
<td></td>
</tr>
<tr>
<td>$10^{6}$</td>
<td>0.0075</td>
<td>863</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>962</td>
<td>662</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

One possible origin of the high proportion of plasmid-less variants in the treated culture was that rifampicin might select the plasmid-less variants known to occur spontaneously during the growth of this strain in liquid medium (Novick, 1963). To exclude this possibility, the rates of growth of the parent, a spontaneously occurring plasmid-less variant and a plasmid-less variant that had arisen previously in the presence of rifampicin were compared. In no case was there a detectable difference between the growth rates of the strains.

Similar experiments carried out with a Com II penicillinase plasmid, carried in this case in strain 147 (Richmond, 1969), have shown that a similar concentration of rifampicin to that used above produced 8.9 % of plasmid-less cocci after overnight growth against 0.11 % found in the control culture.

In view of the primary effect of rifampicin on RNA polymerase, the differential effect of rifampicin on bacterial growth and on plasmid survival suggests that there may be a specific RNA polymerase molecule or group of molecules involved in plasmid replication and plasmid distribution to daughter cells in Staphylococcus aureus.

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Short communications

REFERENCES


