Antibiogram and Lipid Analysis of a Pigmented Strain of
Serratia marcescens and Its Nonpigmented Variants

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Antibiograms and lipid analyses of Serratia marcescens pigmented strain 08 and its nonpigmented variants are compared. The overall lack of significant differences between pigmented and nonpigmented strains suggests that the role of pigment formation may not be related to antibiotic susceptibility.

In recent years, Serratia marcescens has been increasingly incriminated in serious infections in man (5, 7, 13). Most strains responsible for the nosocomial diseases are predominantly nonpigmented (5, 12, 17, 18) and are also resistant to multiple antibiotics (6, 12). The incidence of pigmented isolates was reported to be as low as 6% (20). This unusually low incidence of pigmented strains in clinical isolates suggests that they are probably more susceptible to antibiotics (5, 22), and the pigment, prodigiosin, and its metabolites may play some possible role in this increased susceptibility. Other differences, such as lipid content or fatty acid composition or both, have also been associated with an increased antibiotic resistance (2, 8, 15, 16). However, the divergence of results reported does not permit any generalization to be drawn with regard to the role of lipid (4, 22). A more recent publication claims that, even though pigmented strains of S. marcescens are more susceptible to antibiotics, neither lipid content of the cell wall nor pigment production has any relation to the antibiotic susceptibility patterns of the strains studied (22). Most studies, attempting to correlate lipid content and pigment production, were carried out using hospital isolates in which the susceptible and resistant strains were not closely related. We have, therefore, compared the antibiograms and lipid analyses of a pigmented strain of S. marcescens and its nonpigmented variants, grown under identical conditions. The study of a pigmented and an unrelated nonpigmented strain was reported previously (15).

The nonpigmented variants were isolated from tryptic soy agar plates streaked with the parent pigmented strain S. marcescens 08. Biochemical differentiation studies were carried out for identification purposes. Cells for lipid extraction were grown on an enriched medium (1, 19), harvested at late log phase, and lyophilized. Total extractable lipids were obtained by extraction from dried cells with chloroform:methanol (2:1, vol/vol) by the method of Huston and Albro (10) and washed by the method of Folch et al. (9). Phospholipids were separated from total extractable lipids by preparative thin-layer chromatography with the solvent system hexane:diethyl ether:acetic acid (90:10:0.1, vol/vol). Phosphorus analysis (3) was used to estimate the content of phospholipid. Fatty acid composition was determined by gas chromatography of the methyl esters (14).

A serial twofold broth dilution method was used to determine minimal inhibitory concentration (MIC) in tryptic soy broth (11, 15). Six-hour cultures were diluted 1:1,000 before addition of 0.05 ml (10^4 cells) to the twofold

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Pigmented 08</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>15.6</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>15.6</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>3.9</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>15.6</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>3.9</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
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</tr>
<tr>
<td>Nalidixic acid</td>
<td>7.8</td>
</tr>
<tr>
<td>Colistin</td>
<td>10,000.0</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>1,000.0</td>
</tr>
<tr>
<td>Cephalothin</td>
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</tr>
<tr>
<td>Cephaloridine</td>
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<tr>
<td>Chloramphenicol</td>
<td>250.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>125.0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7.8</td>
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</tbody>
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Table 1. MIC of Serratia marcescens pigmented strain 08 and its two nonpigmented variants.

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antibiotic broth dilution. The drug concentrations tested ranged from 1.95 to 1,000 μg/ml except when MIC’s were greater than 1,000 μg/ml when the range of 19.5 to 10,000 μg/ml was included.

By using standard bacteriologic procedures, the nonpigmented variants were shown to give the same biochemical characteristics as their parent pigmented strain 08. Results of the MIC’s are given in Table 1. All strains were equally susceptible to semisynthetic penicillins, aminoglycosides, and the urinary antiseptics. The polymyxins, cephalosporin, chloramphenicol, erythromycin, had no activity against any of the three strains. The MIC’s of kanamycin and cephaloridine, however, were greater for parent strain 08 when compared with the nonpigmented variants.

The growth patterns, total extractable lipids, phospholipid contents, and the fatty acid compositions of the nonpigmented variants closely resembled those of the parent pigmented strain (Table 2). The proportion of the individual phospholipids was unaltered in the variants, and fatty acid analyses of the total extractable lipids failed to detect any significant differences between the pigmented and nonpigmented cells. Our lipid analytical results are consistent with those reported by Kates et al. (12) who suggested that cells of S. marcescens, whether pigmented or nonpigmented, depending on their physiological age, contained similar amounts of total extractable lipids as well as phospholipids. Although the proportions of monoenoic and cyclopropane acids were reported to vary greatly with age of the cultures (12), in this study we analyzed the monoenoic acids only, since all the strains were grown under identical conditions.

In this study we have attempted to correlate (i) pigment formation with antibiotic susceptibility, and (ii) pigment formation with lipid content of S. marcescens. However, our results of this and other studies (15) do not seem to strongly support the implication that pigmented cells in general are more susceptible to antibiotics (5, 22). The study of Winshell and Neu (22) seemed to indicate that clinical isolates of pigmented strains of different bacteriocin types were generally more susceptible to diverse antibiotics than were nonpigmented strains. Normally in S. marcescens a nonpigmented organism is not necessarily more resistant (15). When a pigmented organism is resistant to multiple antibiotics, such as strain 08, the loss of the ability for pigment formation in its variants (isolates M-1 and M-2) does not concomitantly induce any change in the level of antibiotic susceptibility. The overall lack of significant differences between the strains in both lipid content and composition as well as antibiograms leads us to believe that, at least in the strains examined, the role of pigment formation (or the lack of) may not be related to antibiotic susceptibility. The function of the pigment, prodigiosin, and its metabolites, has been speculated upon as a secondary metabolite in S. marcescens (21), but their actual physiological and biochemical roles in the cell envelope of the organism remain unclear.

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LITERATURE CITED

3. Bartlett, G. R. 1958. Phosphorus assay in column chro-