NOTES

Antibiograms and Lipid Contents of Pigmented and Nonpigmented Strains of Serratia marcescens

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The anti-gram of pigmented 08 and nonpigmented Bizio strains of Serratia marcescens was determined. Their lipid content was also analyzed.

Nonpigmented strains of Serratia marcescens have been isolated with increasing frequency from clinical specimens (4, 5, 10, 12). Edwards and Ewing (7) estimated that only approximately one-fourth of S. marcescens isolates recovered were pigmented, and Wilkowski et al. (13) reported the incidence to be as low as 6%. Several plausible explanations exist for the recent reports of greater frequency in the recovery of nonpigmented variants. Technological improvements in identification of these organisms in the laboratory certainly may be a contributing factor (10). However, consideration must also be given to the possibility that nonpigmented varieties may indeed be more resistant, for various reasons, than pigmented ones (8). Inasmuch as certain penicillin-resistant gram-negative bacteria contained no penicillinase, it has been suggested that resistance was probably due to inability of the antibiotic to penetrate to the site of action in the bacterial cell. Recent studies have strengthened this hypothesis and indicated that increased synthesis of lipid in the cell envelope may be a factor in the resistance of certain gram-positive and gram-negative bacteria to antibacterial substances (2, 6, 11). It was the purpose of this preliminary study to determine the antibiograms of representative pigmented (08) and nonpigmented (Bizio) strains of S. marcescens and correlate any differences in susceptibility patterns with the lipid content of the two strains.

The cultures of the pigmented strain (08) and nonpigmented strain (Bizio) were originally received as a subculture from A. Nowotny, Temple University, Philadelphia, Pa. These two strains were selected for study because of the significant difference in the composition of the lipopolysaccharide components in their outer membranes (1).

A serial twofold broth dilution method was employed to determine minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) in tryptic soy broth (9). Six-hour cultures were diluted 1:1,000 before addition of 0.05 ml (10⁸ cells) to the twofold antibiotic dilution. The drug concentrations tested ranged from 1,000 to 1.95 µg/ml except when MICs were >1,000 µg/ml, when the range of 10,000 to 19.5 µg/ml was included. Cells for lipid extractions were grown in an inorganic medium and harvested at late log phase (1). Total extractable lipids were obtained by extraction from dried cells with chloroform-methanol (2:1, vol/vol), and the phospholipids were separated by preparative thin-layer chromatography with the solvent system petroleum ether-ether-acetic acid (95:5:0.1, vol/vol). Phosphorus analysis was used to estimate the content of the phospholipids (3).

The results of tests for in vitro susceptibility by broth dilution of pigmented 08 and nonpigmented Bizio to 14 drugs are summarized in Table 1. Ampicillin and carbenicillin were found to be active against the nonpigmented Bizio strain (MIC, 3.9 and 1.95 µg/ml) and pigmented 08 strain, although the MICs for 08 were at least four times greater. Both 08 and Bizio strains were highly resistant to cephalo-
thin and cephalaridine. A ninefold greater MIC, however, was exhibited by 08 for cephalaridine compared with nonpigmented Bizio. Both organisms demonstrated an MIC of 10,000 μg/ml for cephalothin. The aminoglycosides (streptomycin, kanamycin, and gentamicin) were found to be almost equally effective against strains 08 and Bizio. An eightfold greater activity, however, was demonstrated by kanamycin against strain Bizio (MIC, 1.95 μg/ml) when compared with strain 08 (MIC, 15.6 μg/ml). Chloramphenicol and erythromycin had no activity against either organism, whereas both were significantly susceptible to the inhibitory action of tetracycline. High concentrations of two urinary antiseptics, naladixic acid and nitrofurantoin, can be achieved; therefore, the MICs obtained for these drugs would indicate effectiveness against strains 08 and Bizio.

Studies with the polypeptide antibiotics polymyxin B and colistin revealed no activity against pigmented 08. Colistin was equally ineffective against Bizio (MIC, 10,000 μg/ml), whereas polymyxin B demonstrated significantly greater activity (MIC, 7.8 μg/ml) against this nonpigmented strain. Cross-resistance to the polymyxins, although usually inferred, was not demonstrated. It was noteworthy that the nonpigmented strain with three times as much total extractable lipid and phospholipid (Table 2) as the pigmented strain was extremely susceptible to the membrane-active antibiotic polymyxin B. Further, it would appear that increased lipid in nonpigmented Bizio did not confer a concomitant permeability barrier to the cell wall-inhibiting antibiotics. The pigmented strain was more resistant in specific instances when compared with the nonpigmented strain, which is in marked contrast to what has been implied in the literature (4). The preliminary findings of this study indicated that specific differences in antibiotic resistance existed between a pigmented and nonpigmented strain of Serratia; however, these differences could not be attributed to quantitative variations in total extractable lipid and phospholipid content. Further studies are warranted to ascertain the consistency of these antibiotics in additional clinical isolates of both pigmented and nonpigmented strains of S. marcescens. The influence of the pigment (prodigiosin and/or its derivatives) on susceptibility patterns remains to be demonstrated. Nonpig-
mented variants from pigmented 08 strain have been isolated, and work is in progress to establish their antibiograms and lipid content.

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LITERATURE CITED


