Antibiotic Resistance Patterns of Clinical Isolates of _Serratia marcescens_

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Antimicrobial susceptibility patterns of 102 clinical isolates of _Serratia marcescens_ from three medical centers were studied by using disk sensitivity and agar dilution methods. The least resistance was demonstrated against gentamicin, nalidixic acid, chloramphenicol, and sulfisoxazole, all of which inhibited more than 80% of the strains. Cephalothin was completely ineffective, and more than 90% of strains were resistant to ampicillin and tetracycline. As demonstrated by the agar dilution method, the minimal inhibitory concentration of nalidixic acid, gentamicin, tobramycin, and chloramphenicol for most strains fell within therapeutically attainable concentrations. The prevalence of resistance to ampicillin, cephalothin, and tetracycline was nearly the same at all three medical centers, whereas there appeared to be patterns characteristic for each center with regard to the other drugs used. Eleven of the isolates produced pigment and exhibited patterns similar but not identical to those of the nonpigmented strains, all 11 being resistant to between three and six drugs. Half of the strains were resistant to five or more antibiotics, indicating that some _Serratia_ exhibit resistance to an unusually broad range of therapeutic agents.

The problems associated with hospital infections caused by _Serratia marcescens_ have become increasingly evident (3, 5, 15). The spectrum of infections includes meningitis, pulmonary infections, septicemia, endocarditis, and a variety of localized infections. The ability of this opportunistic pathogen to acquire resistance to a broad range of antibiotics has made effective therapy more difficult. Several recent investigations have dealt with the problem of antibiotic resistance in _Serratia_ (3, 4, 6, 10–13). The overall findings of these studies indicate that the polymyxins and cephalosporins are the least effective agents against _Serratia_ in vitro, and although gentamicin, kanamycin, chloramphenicol, and nalidixic acid are the most effective, resistance to kanamycin and chloramphenicol, as well as the β-lactam antibiotics, has increased during the last decade. _Serratia_ strains isolated from different hospitals often appear to have characteristic resistance patterns (13). Other studies have provided conflicting results concerning resistance patterns of pigmented versus nonpigmented strains (2, 11).

It is evident that patterns of drug resistance in _Serratia_ may vary over even short periods of time and may be characteristic for different hospitals. For this reason and because of the rapid development of new antibiotics, periodic evaluation of antibiotic resistance in _Serratia_ is needed. This study was undertaken to obtain up-to-date information regarding the resistance of _S. marcescens_ to antimicrobial agents currently available.

MATERIALS AND METHODS

A total of 102 strains of _S. marcescens_ isolated between June and December 1973 was examined. Sixty-two strains were isolated at the Diagnostic Bacteriology Laboratory of the Medical University of South Carolina, 22 were provided by J. Shulman of Emory University, and 18 were made available by D. Gröschel of the M. D. Anderson Hospital and Tumor Institute in Houston, Tex. Each was referred to the South Carolina State Board of Health for species confirmation or subjected to biochemical tests including triple sugar-iron, arabinose, indole, motility, and deoxyribonuclease. Sources of isolation included urine, blood, throat, sputum, stool, exudate, and wounds. Strains were inoculated onto Trypticase soy agar slants and incubated overnight to detect pigment production and then stored at 4 °C until needed.

Disk sensitivity testing was performed by the single-disk technique described by Bauer et al. (1) on Mueller-Hinton agar with the following antibiotics: streptomycin (10 μg), sulfisoxazole (0.25 mg), tetracycline (30 μg), cephalothin (30 μg), kanamycin (30 μg), ampicillin (10 μg), nalidixic acid (30 μg), gentamicin (10 μg), carbenicillin (50 μg), colistin (10 μg), tobramycin (10 μg), and chloramphenicol (30 μg). The minimum inhibitory concentration (MIC) was deter-
mained by the agar dilution method (7) on Mueller-Hinton agar, using the Steers inocula-replicating apparatus (12) with an inoculum of approximately 5 \times 10^4 bacteria. The MIC was defined as the lowest concentration of drug preventing visible growth after 18 h of incubation at 37 C.

RESULTS

Table 1 gives the results of disk sensitivity testing for the 102 Serratia strains isolated. All strains tested were resistant to cephalothin. Ninety-three percent of the strains were resistant to ampicillin and tetracycline. The most effective drugs were nalidixic acid (8% of strains resistant), gentamicin (14% resistant), sulfisoxazole (15% resistant), and chloramphenicol (16% resistant). Next in order of increasing resistance were kanamycin (22% resistant), tobramycin (26% resistant), streptomycin (34% resistant), carbenicillin (39% resistant), and colistin (57% resistant). Multiple resistance was common, as demonstrated by the observation that 56 of the 102 isolates were resistant to at least 5 of the drugs tested, 14 were resistant to at least 8, and 5 were resistant to all 12. Resistance varied among the three hospitals (Table 1), with strains from M. D. Anderson Hospital and Tumor Institute being more frequently resistant to every drug tested except colistin and cephalothin. The strains from Emory University were more resistant to colistin than the strains from either of the other two hospitals, and the Emory strains were more resistant to kanamycin, carbenicillin, sulfisoxazole, gentamicin, and streptomycin than were the strains from the Medical University of South Carolina.

Eleven of the 102 isolates produced pigment and were resistant to cephalothin, ampicillin, and tetracycline. All were susceptible to chloramphenicol, kanamycin, nalidixic acid, sulfisoxazole, and gentamicin, as were the majority of nonpigmented strains. However, eight of the pigmented isolates were resistant to streptomycin, eight were resistant to colistin, and five were resistant to tobramycin, indicating that a higher percentage of pigmented strains were resistant to these drugs than were the nonpigmented strains.

Figure 1 shows the cumulative percentage of strains inhibited in vitro by 11 drugs tested at various concentrations in agar. The figures in parentheses represent approximate serum concentrations achieved with recommended dosages except for nalidixic acid, for which approximate achievable urinary tract concentration is listed (8, 9). Tobramycin, gentamicin, and chloramphenicol inhibited greater than 90% of the strains by concentrations equal to or less than obtainable serum levels, and nalidixic acid inhibited at least 90% of the strains at a concentration achievable in the urine. Cephalothin and colistin failed to inhibit any of the isolates at the achievable serum concentrations. Levels of resistance of the 11 pigmented strains did not appear to differ significantly from those of the nonpigmented strains except for streptomycin, which was found to have MICs of between 20 and 40 \(\mu\)g/mL. Sixty-five percent of the nonpigmented Serratia had MICs of streptomycin that were between 5 and 20 \(\mu\)g/mL. The MICs for ampicillin, tetracycline, and cephalothin (antibiotics to which the highest percentages of strains were resistant) were higher for those strains that were resistant to more than five drugs, indicating that multiresistant

<table>
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<tr>
<th>Antibiotic</th>
<th>MUSC* (62 strains)</th>
<th>Emory (22 strains)</th>
<th>M. D. Anderson (18 strains)</th>
<th>Total (102 strains)</th>
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</table>

*Medical University of South Carolina.
strains are often more resistant to these three drugs than are strains resistant to fewer than five antimicrobial agents.

DISCUSSION

Our results indicate that recently isolated strains of S. marcescens are susceptible to gentamicin, chloramphenicol, sulfisoxazole, and nalidixic acid at therapeutically achievable concentrations. These findings correspond to previous reports concerning the efficacy of these antibiotics (4, 6, 10, 12). Ampicillin, cephalothin, tetracycline, and colistin are ineffective in vitro and do not appear to be useful in the treatment of Serratia infections. The susceptibility of these strains to kanamycin, streptomycin, tobramycin, and carbenicillin was highly variable.

Characteristic resistance patterns were found for each of the three hospitals with regard to every antibiotic tested except ampicillin, tetracycline, and cephalothin. The strains from the M. D. Anderson Hospital and Tumor Institute were more frequently resistant than the strains from either of the other two hospitals to every drug tested except colistin. The high degree of overall resistance demonstrated by these strains may be related to selective pressure by long-term antibiotic therapy in cancer patients. The Serratia that were isolated at the Medical University of South Carolina were more susceptible than those from Emory to every drug tested except cephalothin, tobramycin, and chloramphenicol. The observation that at least 90% of the 102 strains were resistant to ampicillin, tetracycline, and cephalothin may indicate innate resistance to these drugs in this species.

A greater percentage of pigmented than nonpigmented strains were resistant to tobramycin, streptomycin, and colistin. Pigmented strains had higher MICs of streptomycin (20 to 40 \( \mu g/\text{ml} \) versus 5 to 20 \( \mu g/\text{ml} \) for most of the nonpigmented strains). Previous reports have provided conflicting results regarding antimicrobial susceptibility of nonpigmented and pigmented Serratia on the basis of differences in cell wall lipid (2, 11). However, this premise has been recently challenged by Winsell and Neu (16), who found no appreciable differences in lipid content among resistant nonpigmented, susceptible nonpigmented, and susceptible pigmented groups of S. marcescens.

Medeiros and O'Brien (10) demonstrated transferable resistance due to R factors in 21 of 22 strains of Serratia resistant to five or more antibiotics. They concluded that R factors contribute additional degrees of resistance to tetracycline and ampicillin in strains already moderately resistant to these drugs. We have also found R factors among our multiple-resistant strains of S. marcescens, and are currently investigating further the properties and significance of these plasmids in this species.
ACKNOWLEDGMENTS

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LITERATURE CITED