Specific Distribution of R Factors in Serratia marcescens Strains Isolated from Hospital Infections

S. SCHAEFLER, J. WINTER, A. CATELLI, J. GREENE, AND B. TOHARSKI

Department of Health, Bureau of Laboratories and Public Health Research
Institute of the City of New York, Inc., New York, New York 10016

Received for publication 30 April 1971

Serratia marcescens strains from three hospitals in the city of New York were tested for antibiotic susceptibility patterns and the presence of transmissible antibiotic resistance factors. There appears to be a pattern characteristic for each hospital with regard to the sensitivity to nalidixic acid, tetracycline, chloramphenicol, and sulfonamides, whereas the resistance to ampicillin, cephalothin, and streptomycin is similar in the strains isolated from all three hospitals. In one hospital, a single type of R factor was found which transfers resistance to streptomycin, kanamycin, ampicillin, and sulfonamides, whereas strains isolated from a second hospital transfer only ampicillin resistance. No R factors could be detected in multiply resistant Serratia strains isolated in a third hospital. The presence of a single type of R factor probably reflects the relative ecological isolation of S. marcescens and could be useful for epidemiological studies of hospital infections with Serratia.

Certain characteristics of nonpigmented Serratia marcescens make this organism particularly suited to the epidemiological study of resistance transfer (R) factors. Infections with Serratia are almost entirely limited to the hospital environment. These infections are primarily found in the urinary tract, although respiratory infections, wound infections, and cases of septicemia have been reported. Furthermore, Serratia strains are rarely isolated from the stool, suggesting a relative ecological isolation and limited contact with the Enterobacteriaceae of the intestinal flora. One might therefore expect that the distribution of R factors in Serratia would be subject to a smaller number of determining factors than in Escherichia coli, Klebsiella, or Salmonella.

A study of R factors detected in strains isolated in Peter Bent Brigham Hospital in Boston indicated the transfer of a variety of resistance determinants from Serratia to E. coli. In several strains tested in this respect, the R factors were unstable in both the original Serratia strains and the E. coli strain to which they were transferred. To our knowledge, no systematic data are available on Serratia strains isolated in other hospitals. The present study was made with Serratia strains isolated in three hospitals in the city of New York. It appears that both the antibiotic susceptibility patterns and the resistance determinants transmitted by R factors are specific for each hospital.

MATERIALS AND METHODS

Bacterial strains. One hundred and thirty-eight S. marcescens strains isolated in three hospitals in the city of New York were studied. The origin of the strains is indicated in Table 1. In addition, 16 other strains were investigated, originating from four different hospitals in the city of New York. When several isolates from the same patient were received, only a single strain was used. No differences in antibiotic susceptibility were found among successive isolates from the same patient.

Testing of antibiotic susceptibility. Antibiotic susceptibility was tested by the base and seed layer method which is routinely used in our laboratory. The susceptibility to tetracycline, streptomycin, chloramphenicol, kanamycin, ampicillin, gentamycin, colistin, and nalidixic acid was tested with standard low- and high-potency discs (BBL). The susceptibility to cephalothin and gentamycin was tested only with high-potency discs. The discs were applied to a seed layer of 5 ml of beef heart infusion agar containing approximately 5 × 10^6 bacteria per ml, overlaid on a base layer of 10 ml of the same medium in petri dishes (100 by 15 mm). Mueller-Hinton agar was used for testing of the susceptibility to sulfonamides. Bacteria were considered resistant when no visible zone could be detected with low-potency discs, and

1 Mount Sinai School of Medicine, City University of New York, and Beth Israel Medical Center, New York, N.Y. 10029.
2 Bellevue Hospital, New York, N.Y. 10016.
the clear zone with high-potency discs was less than 10 mm for tetracycline, chloramphenicol, kanamycin, gentamicin, nalidixic acid, and ampicillin and less than 8 mm for gentamycin, streptomycin, and colistin. A study made with various Enterobacteriaceae, including Serratia (Schaefer and Catelli, unpublished data), indicated that the results obtained with the base and seed layer method are consistent with these obtained with the Kirby-Bauer method (2). Minimal inhibitory concentrations (MIC) for tetracycline, streptomycin, cephalothin, ampicillin, and kanamycin were determined by the plate dilution method by using the replicating apparatus of Steers et al. (13). In addition, MIC values were determined for nalidixic acid, colistin, sulfadiazine, and chloramphenicol with all strains isolated in Beth Israel Hospital and selected strains from the other hospitals.

Serological typing. The O and H serotypes of representative R+ and R− strains from each hospital was determined at the Center for Disease Control, through the courtesy of W. Ewing and G. J. Herman.

Media. LB broth [20 g of tryptone (Difco), 10 g of yeast extract (Difco), 5 g of NaCl, and 1,000 ml of water; made to pH 7.0 with NaOH] and LB-2 1/2% agar were used in all experiments. Strains were maintained by periodic transfers on LB agar slants.

Mating experiments. The multiple antibiotic-resistant Serratia strains described in this study were used as donors, whereas an F− E. coli K-12 strain served as the acceptor. The latter is sensitive to all of the antibiotics tested in this study but resistant to 150 μg of sodium azide per ml, a concentration inhibitory for the tested Serratia. In addition, the E. coli strain is constitutive for phospho-β-glucosidase and β-glucoside permease (11), which are absent in the Serratia strains. Media containing sodium azide and the antibiotics to which the E. coli acceptor strain is sensitive will eliminate the parental E. coli and Serratia strains, allowing only the growth of E. coli cells which received resistance determinants from Serratia. Logarithmic-phase cultures of the donor and acceptor strain were mixed in a ratio of 1 donor to 10 acceptor cells. An equal volume of LB broth was added. After overnight incubation at 37 C, 0.05-ml amounts of 1:10 and 1:25 dilutions of the mixture were plated on LB agar containing 150 μg of sodium azide per ml and one of the following antibiotics: tetracycline, 20 μg/ml; chloramphenicol, 20 μg/ml; ampicillin, 50 and 100 μg/ml; cephalothin, 30 μg/ml; streptomycin, 20 and 100 μg/ml; and kanamycin, 25 μg/ml. The plates were incubated for 48 hr, and recombinants were tested for their E. coli characteristics by applying a drop of p-nitrophenyl-β-glucoside (2 × 10−2 M in water) and by slide agglutination with E. coli K-12 antisera. Development of an intensive yellow color within 3 to 5 min after the addition of p-nitrophenyl-β-glucoside indicates constitutive phospho-β-glucosidase activity. Recombinant colonies were re-isolated and tested for antibiotic susceptibility.

Elimination of extrachromosomal resistance. Attempts to eliminate extrachromosomal resistance were made by (i) growth in LB medium for 48 hr at 45 C; (ii) growth in LB medium (pH 8.0) at 37 C in the presence of 50 μg of acriflavine per ml; (iii) growth in the presence of 100 and 200 μg of ethidium bromide per ml; (iv) growth in the presence of 10′, (w/v) dodecyl sulfate. After streaking on LB agar, the elimination of antibiotic resistance was tested by replica plating from master plates without antibiotics onto plates with 50 μg of kanamycin per ml, 100 μg of streptomycin per ml, and 50 μg of ampicillin per ml.

RESULTS

The resistance spectrum and the distribution of transmissible resistance among the analyzed strains are indicated in Fig. 1. The data shown in Fig. 1 indicate that all strains exhibit multiple antibiotic resistance with characteristic differences in the resistance to tetracycline, chloramphenicol, sulphonamides, and nalidixic acid between the strains isolated in each of the three hospitals.

Strains isolated in Beth Israel Hospital. All 63 strains tested were resistant to tetracycline (MIC > 500 μg/ml), chloramphenicol (50 μg/ml), ampicillin (2,000 μg/ml), and cephalothin (2,000 μg/ml). The majority were resistant to streptomycin (1,000 μg/ml), nalidixic acid (100 μg/ml), colistin (50 μg/ml), and sulfonamides (1,000 μg/ml). Of the 63 tested strains, 18 were resistant to kanamycin (500 μg/ml). All strains tested were sensitive to gentamycin.

By using an F− derivative of E. coli K-12 as acceptor, 18 strains transferred resistance to kanamycin, streptomycin, ampicillin, and sulphonamides. Since all R+ strains transfer the same group of resistance determinants, this seems to indicate the presence of a single type of R factor (Amp Kan Str Su) in the R+ strains isolated in Beth Israel Hospital. The level of resistance transferred is 500 to 1,000 μg/ml for kanamycin and streptomycin, >2,000 μg/ml for ampicillin, and 500 to 1,000 μg/ml for sulfonamides. No transfer of resistance to tetracycline and chloramphenicol could be detected, although all tested Serratia strains were resistant to these antibiotics. By comparing the antibiotic susceptibility of the R+ and R− strains, the only significant difference consisted in their susceptibility to kanamycin, only the R+ strains being resistant to kanamycin (Fig. 1). No detectable differences between R−
and R⁻ strains were found in their susceptibility to streptomycin, ampicillin, and sulfonamides.

Attempts to eliminate kanamycin resistance from six R⁺ Serratia strains by the procedures outlined above were unsuccessful. The Amp Kan Str Su R factor could also not be eliminated after its transfer into E. coli. In this respect, the R factor found in the analyzed Serratia strains is quite different from that found in strains described by Medeiros and O'Brien (9).

By analyzing the distribution of the Serratia strains and the relationship between their serotype and the presence of the R factor, it was found that the R⁺ strains were isolated mainly in one of the pavilions of the hospital and that the same R factor can be found in strains belonging to two different serotypes. The distribution of the R⁺ and R⁻ Serratia strains was 16 R⁺ and 4 R⁻ strains in one pavilion of the hospital and 2 R⁺ and 44 R⁻ strains in the other. The antigenic structure of six R⁺ strains isolated in the pavilion with predominantly R⁺ strains was four strains 03:nonmotile and two strains 02:nonmotile. No detectable differences in the resistance pattern or the level of resistance transferred to E. coli could be found with Serratia strains of the two antigenic types.

Strains isolated in Bellevue Hospital. The 34 strains isolated in Bellevue Hospital were resistant to ampicillin, cephlothin, and streptomycin, and most of these strains were resistant to kanamycin and colistin. The level of resistance is similar to that found for the Serratia strains isolated in Beth Israel Hospital. With the exception of one strain, the strains isolated in Bellevue Hospital were sensitive to tetracycline, and most of the strains were also sensitive to chloramphenicol, sulfonamides, and nalidixic acid (Fig. 1).

An R⁺ factor transferring resistance to only ampicillin was found in 27 of the 34 strains analyzed. The R⁺ strains were of the serotypes 014:H 12 and 014:h undetermined, and no detectable difference was found in the ampicillin resistance of the R⁺ and R⁻ strains.

Attempts to eliminate ampicillin resistance were successful after its transfer into E. coli but not in the original Serratia strains. In E. coli, the Amp R factor is lost spontaneously at a rate of 0.03%. The rate of elimination of ampicillin resistance is increased to 3.9% by acriflavine, 5.8% by ethidium bromide, 7.3% by dodecyl sulfate, and 4.1% by growth at 44 C. The lack of elimination of ampicillin resistance in the original Serratia strains is possibly due to the presence of a second β-lactamase which determines non-transmissible resistance to cephlothin but which also hydrolyzes ampicillin.

R factors transferring resistance to ampicillin alone are less frequent in Enterobacteriaceae than R factors which transfer multiple resistance (1, 10); therefore, the presence of the Amp R factor in Enterobacteriaceae isolated together with the Serratia strain could be indicative of a genetic exchange in vivo. A preliminary study was made with 11 E. coli, 2 Klebsiella, and 2 Enterobacter strains isolated from urine samples from which Amp R⁺ Serratia strains were isolated. Five strains possessed three types of R factors transferring resistance to ampicillin, streptomycin, and...
tetracycline and tetracycline, streptomycin, and kanamycin. No R factor transferring only ampicillin resistance was found in these strains.

**Strains isolated from Veterans Administration Hospital.** With the exception of one strain, all 41 *Serratia* strains analyzed were resistant to streptomycin, ampicillin, and cephalothin and 38 strains were resistant to colistin. Approximately 60% of the strains were resistant to chloramphenicol, tetracycline, and kanamycin. Most of the strains were sensitive to nalidixic acid and sulfonamides (Fig. 1). There appears to be a linkage in the resistance to chloramphenicol, tetracycline, and kanamycin, 19 out of 26 strains resistant to chloramphenicol being resistant to the other two antibiotics.

No transferrable antibiotic resistance could be detected with the strains isolated from the Veterans Administration Hospital. Attempts to mobilize nontransmissible resistance determinants by using the R-100 RTF strain lacking resistance determinants (5) and an *E. coli* F\(^+\) strain have been unsuccessful thus far.

Among *Serratia* strains isolated from other hospitals in the city of New York, six strains originating from three hospitals possessed R factors with the following resistance determinants: tetracycline, kanamycin, chloramphenicol, and streptomycin; tetracycline, ampicillin, and streptomycin; and only ampicillin. In one hospital, strains carrying two different R factors were isolated.

**DISCUSSION**

The investigation of the antibiotic susceptibility and of the presence of transferrable R factors in *Serratia* strains isolated in three hospitals in the city of New York indicated a characteristic antibiotic susceptibility pattern of strains isolated in each of the three hospitals and the presence of a single type of R factor in strains isolated from two hospitals: with Amp Kan Str Su determinants for strains isolated in Beth Israel Hospital and the Amp determinant for strains isolated in Bellevue Hospital. Since penicillin and its derivatives can be hydrolyzed by several β-lactamases (12), the classification of the Amp R factor as a single type of R factor is only tentative. A manifest contribution of the R factors to the antibiotic resistance of the R\(^+\) strains was found only for the kanamycin resistance of strains possessing the Amp Kan Str Su R factor. No differences in the resistance to ampicillin, streptomycin, or sulfonamides were found between R\(^+\) and R\(^-\) strains isolated in Beth Israel Hospital or for ampicillin resistance between strains isolated in Bellevue Hospital.

By comparing the antibiotic susceptibility of the strains isolated in the three hospitals, it appears that the *Serratia* strains isolated in all three hospitals are resistant to streptomycin, ampicillin, and cephalothin. There are, however, marked differences in their susceptibility to chloramphenicol, tetracycline, sulfonamides, and nalidixic acid (Fig. 1). Most of the strains isolated in Beth Israel Hospital were resistant to chloramphenicol, tetracycline, sulfonamides, and nalidixic acid, whereas most of the strains isolated in Bellevue Hospital were sensitive to these agents. Among the strains isolated in the Veterans Administration Hospital, only 5% were resistant to nalidixic acid and 15% were resistant to sulfonamides. The resistance to chloramphenicol and tetracycline varied with the strain; in most instances, strains resistant to chloramphenicol were also resistant to tetracycline. The reasons for these differences are difficult to assess and can apparently not be directly related to the use of these antibiotics in these hospitals.

Among the resistance determinants reported to be transferrable by R factors (1, 10) are those for tetracycline, chloramphenicol, kanamycin, streptomycin, ampicillin, and sulfonamides. No transfer of tetracycline or chloramphenicol resistance was observed with any of the resistant *Serratia* strains isolated in this study. The strains isolated in the Veterans Administration Hospital contain nontransferrable determinants for the above antibiotics and also ampicillin. Attempts to mobilize potential nontransferrable determinants by the addition of F\(^+\) and RTF cells to the mating mixture have been unsuccessful thus far. This could be due either to the chromosomal nature of the resistance determinants or to the inability of the transfer factor used to recombine with the extrachromosomal resistance determinants.

Compared to *E. coli* or *Klebsiella* (1, 10), the investigated *Serratia* strains are characterized by the presence of a rather limited number of transferrable R factors. The comparatively small number of different transferrable R factors observed in *Serratia* could be partly the result of a relative ecological isolation of *Serratia* from the intestinal flora and their prevalence in hospital infections. The presence of a limited number of R factors, especially when combined with serological typing, makes a useful object for the study of the ecology of R factors. The prevalence of R\(^+\) strains in only one of the two main pavilions in Beth Israel Hospital could be explained either by the spread of bacteria carrying the R factor among the patients of this pavilion or by the spread of R factors among strains already present in this pavilion. The serological data obtained indicate that the same type of R factor can be
R FACTORS IN S. MARCESCENS STRAINS

present in strains belonging to two different serotypes. This seems to indicate the spread of R factors among strains already present in these hospitals.

LITERATURE CITED