Resistance Plasmid Transfer by *Serratia marcescens* in Urine

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Resistance plasmids were transferred in urine from a multi-drug-resistant *Serratia marcescens* to *Escherichia coli*. Transfer of resistance to kanamycin, tetracycline, chloramphenicol, streptomycin, ampicillin, and carbenicillin occurred readily after 4 h of incubation at room temperature (25°C). The urinary catheter collection bag is postulated as a potential site for extraintestinal resistance plasmid transfer in the *Enterobacteriaceae*, especially for pathogens such as *Serratia*, which do not frequently colonize the intestinal tract.

Serratia marcescens is predominately a nosocomial pathogen and frequently has a high level of antimicrobial resistance (2). *Serratia* often derives resistance to antimicrobial agents from resistance (R) plasmids (5). An unusually resistant strain was the cause of a large outbreak of nosocomial infections in Nashville, Tenn., and most of the resistance of that strain was plasmid mediated (6). Although it could have been a naturally occurring strain that was subsequently introduced into the hospital, another explanation is that a partially resistant *Serratia* strain acquired one or more R-plasmids in the hospital, which then mediated the majority of the strain's drug resistance.

The postulated site for R-plasmid transfer between most gram-negative bacilli is the gastrointestinal tract. For *S. marcescens* this is less likely, since intestinal colonization with this organism is rarely demonstrated (4). In the Nashville investigation, we did not detect gastrointestinal colonization with *Serratia* in any instance despite the use of selective media (6).

The lack of intestinal colonization suggested the possibility that extraintestinal R-plasmid transfer might have been involved in contributing to the resistance of this strain. Numerous extraintestinal sites for transfer were possible, but because of the marked predilection of this strain for the urinary tract, we investigated the possibility that urine of catheterized patients might be a site for R-plasmid transfer among gram-negative bacteria.

MATERIALS AND METHODS

All *Serratia* isolates from patients with urinary tract infections in the Nashville outbreak were serotype O1:H7 and phage type 186 (6). The antibiotic resistance patterns of all isolates were determined by a standardized disk technique (1). Levels of resistance to antimicrobial agents were determined by

a standard twofold dilution method using Mueller-Hinton broth (BBL).

R-plasmid transfer was determined by a modification of the method of Schroeder et al. (7), using ATCC 26772 *Escherichia coli* K-12, F⁻ (Lac⁺ Nal) as the recipient strain. Donor strains were *S. marcescens* N35 and N61, which were multiply resistant but susceptible to nalidixic acid. A control strain, *S. marcescens* N10, which was multiply resistant including nalidixic acid, was plated simultaneously on all plates to insure that each plate would support growth of transconjugants.

A single colony each of R-donor *Serratia* and recipient *E. coli* were inoculated into 5 ml of sterile urine obtained from catheterized patients and incubated at room temperature (25°C) overnight. Mating cultures were prepared by adding 0.5 ml of the donor and 0.5 ml of the recipient overnight urine growth to 4 ml of sterile urine. After mixing, the cultures were incubated at room temperature. At 0, 4, 8, 12, and 24 h of incubation, 0.1 ml of the mixture and appropriate controls was spread on MacConkey agar containing nalidixic acid (25 µg/ml) and either tetracycline (8 µg/ml), streptomycin (25 µg/ml), gentamicin (15 µg/ml), or ampicillin (10 µg/ml), which was used for counterselection of transconjugants. After overnight incubation at 37°C, potential transconjugant colonies that grew on the selective plates were picked for biochemical identification and antimicrobial susceptibility testing.

RESULTS

R-plasmid transfer in urine was readily demonstrated between *S. marcescens* and *E. coli*. Transfer frequency was 2.6×10^{-6} per donor cell at 4 h of incubation and 1.6×10^{-5} per donor cell at 8 h. The pattern of resistance transferred was similar regardless of selecting drug (Table 1). Resistance to gentamicin, tobramycin, and colistin was not transferred to any of the transconjugants. A consistent observation in all experiments was a reduction in zone size around the 30-µg cephalothin disk from 22 to 24 mm in

TABLE 1. Minimum inhibitory concentration of antibiotics for recipient, donor, and transconjugants mated in urine

Antibiotic	Recipient, <i>E. coli</i> K-12 26772	R-donor		Transconjugant	
		<i>S. marcescens</i> N-35	<i>S. marcescens</i> N-61	26772/N-35	26772/N-61
Nalidixic acid	500.0	3.9	1.9	500.0	500.0
Kanamycin	3.9	500.0	250.0	500.0	500.0
Tetracycline	0.4	62.5	15.6	62.5	62.5
Gentamicin	0.4	250.0	1.9	3.9	0.9
Chloramphenicol	3.9	125.0	62.5	500.0	250.0
Streptomycin	3.9	250.0	250.0	250.0	125.0
Tobramycin	0.4	15.6	7.8	3.9	3.9
Ampicillin	3.9	>1,000.0	>1,000.0	>1,000.0	>1,000.0
Carbenicillin	3.9	>1,000.0	>1,000.0	>1,000.0	>1,000.0
Cephalothin	15.6	>1,000.0	>1,000.0	62.5	125.0

the recipient *E. coli* to 14 to 16 mm in the transconjugant. The donor *Serratia* gave no zone around cephalothin disks. The minimum inhibitory concentration of cephalothin for *E. coli* K-12 was 15.6 $\mu\text{g/ml}$. For the transconjugants, minimum inhibitory concentrations were 62.5 and 125 $\mu\text{g/ml}$, whereas that for the R-donor *Serratia* was >1,000 $\mu\text{g/ml}$.

DISCUSSION

The problem of drug-resistant nosocomial infections, especially those caused by gram-negative rods, is assuming increased importance. The contribution of plasmid-mediated resistance to the overall spectrum of drug resistance in these pathogens is well known. Extraintestinal R-plasmid transfer might account for a portion of this resistance.

Smith has shown that R-plasmid transfer in the intestinal tract can occur, but that unusual circumstances are necessary (8). Urine, on the other hand, is a good growth medium, and bag urine in catheterized patients is free of even the local antibacterial properties of the urinary bladder. Catheterized patients are frequently acutely ill and frequently receive antimicrobial therapy. This therapy would select for those strains that acquire resistance. In addition, bag urine is emptied at infrequent intervals, usually once at the end of an 8-h shift or "whenever full." We have shown R-plasmid transfer occurring within 4 h at room temperature. Multiple organisms are frequently cultured from urine of catheterized patients. This mixed-culture setting would provide an opportunity within the hospital for exchange of genetic information. Also, urine collection bags are usually not changed and complete emptying is difficult, so residual urine could provide an inoculum that would multiply rapidly to concentrations optimal for R-plasmid transfer.

R-plasmid transfer in urine among *Enterobacteriaceae* can occur. How frequently this oc-

curs in the actual hospital setting requires further study. Like Gardner and Smith (3), we feel that, once established, multi-drug-resistant strains persist because of cross-infection, colonization, and selection by drug therapy and other factors. However, the control measures directed at preventing cross-infection may have an additional benefit. Proper insertion of catheters and care directed at minimizing the introduction of bacteria into the urinary drainage system may have as an added benefit the prevention of the opportunity for a drug-resistant strain to transfer its R-plasmids to other bacteria that might be present simultaneously in the urine.

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