

## Correlation of Periurethral Bacterial Flora with Bacteriuria and Urinary Tract Infection in Children with Neurogenic Bladder Receiving Intermittent Catheterization

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Periurethral bacteria are inoculated daily into the urine of children with neurogenic bladder during clean intermittent catheterization (CIC). We examined how frequently periurethral bacterial species produced bacteriuria in children followed longitudinally. When *Escherichia coli* was detected on the periurethra, bacteriuria was also present 93% of the time. When *Klebsiella*, *Pseudomonas*, or *Enterococcus* species or nonpathogens were detected on the periurethra, bacteriuria was present 80%, 40%, 40%, and 25% of the time, respectively. Clonal typing of multiple colonies of *E. coli* from each periurethral and urine culture revealed that children carried only one or two *E. coli* clones in their urinary tracts over months of surveillance. When *E. coli* was detected in the urine, the identical clone was on the periurethra. *E. coli* persisted for weeks in the urine without causing symptoms. Occasionally the same *E. coli* clone carried for weeks caused a urinary tract infection. Bacteriuria frequently occurs after inoculation of periurethral *E. coli* into the urine during CIC.

The majority of children with neurogenic bladder have sensory deficits and loss of control over voiding. Clean in-and-out or intermittent catheterization (CIC) of the bladder several times daily is currently the preferred method of bladder emptying in patients with neurogenic bladder [1]. Bacteriuria is common in patients receiving CIC without urinary symptoms [2]. In a longitudinal study, children with neurogenic bladder receiving CIC were found to carry bacteria in their urine for weeks without symptoms of infection [3]. The children remained clinically well and had no detectable deterioration of their upper urinary tract.

During CIC, periurethral bacteria are inoculated into the bladder urine each day. In this longitudinal study, we examined how frequently individual bacterial species on the periurethra produced bladder bacteriuria in children receiving CIC and how often a species carried in bladder urine caused a symptomatic urinary tract infection (UTI). *Escherichia coli* is the most common cause of bacteriuria and UTI in this population [3, 4]. Clonal typing of *E. coli* was done by use of multilocus enzyme electrophoresis on strains isolated from the periurethra and urine to determine the clonal diversity of *E. coli* carried over time by each individual and to investigate whether specific clones caused UTI.

### Methods

*Population and surveillance cultures.* Twenty-five children (13 girls, 12 boys; 1–18 years of age) with neurogenic bladder receiving CIC four to six times a day for bladder emptying were visited weekly in their homes during 6 months of surveillance as described [3]. Twenty-one children had neurogenic bladder due to myelomeningocele (10 lumbar, 6 sacral, and 5 thoracic); the four children with neurogenic bladder caused by a traumatic spinal cord injury were studied at least 6 months after the injury. Fourteen patients were not receiving antimicrobial prophylaxis during the study period and 11 were (seven, nitrofurantoin; three, trimethoprim-sulfamethoxazole once daily; one, bladder wash with silver nitrate twice daily). The use of antimicrobial prophylaxis was not randomized but was based on physician preference. In this study, we describe the bacterial species on the periurethra and how frequently these species produced bacteriuria and UTI.

Each week a periurethral swab sample was collected. Periurethral specimens were obtained by separating the vaginal labia or retracting the foreskin and circumferentially swabbing the periurethral mucosa one time with a single sterile, dry, cotton-tipped swab. The swab was placed in 2 mL of sterile saline, mixed, rolled against the side of the glass vial to remove liquid, and discarded. For isolation of bacteria from the periurethral specimens, 10  $\mu$ L of eluate was spread onto 5% sheep blood agar plates and MacConkey agar plates, which were incubated at 37°C and examined after 24 and 48 hours for organisms. We estimated that the minimum density of bacteria detected by our sampling method of the periurethral region was 10<sup>4</sup> organisms per swab (assuming a dilution of 0.02 mL of liquid per swab into 2 mL of saline solution and an inoculum of 0.01 mL/plate). After the periurethral sample was obtained, a urine sample was collected by catheterization. Specimens were immediately refrigerated, transported on ice, and plated

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Informed consent was obtained from the patients or their parents or guardians, and guidelines for human experimentation of the U.S. Department of Health and Human Services and the University of Virginia Human Investigation Committee were followed in the conduct of the clinical research.

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within 10 hours of collection. Organisms were identified by use of standard methods [5].

**Definitions.** A positive urine culture (bacteriuria) was defined as  $\geq 10^4$  cfu of bacteria per milliliter of urine obtained by bladder catheterization [6]. Symptomatic UTI was diagnosed in accordance with guidelines developed at the Round Table Discussion of Symptoms of UTI in Neurogenic Bladder at the October 1990 meeting of the American Academy for Cerebral Palsy and Developmental Medicine [3]. The definition of a UTI using these guidelines included symptoms or signs of infection (fever, abdominal pain, change in continence pattern, or change in color or odor of urine) associated with a positive urine culture. Pathogens were defined as the Enterobacteriaceae, *Enterococcus* species, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and group B streptococci. Nonpathogens included coagulase-negative staphylococci,  $\alpha$ -hemolytic or nonhemolytic streptococci, and *Corynebacterium* species.

**E. coli isolates.** Isolates of *E. coli* detected on the original plate from urine or periurethral swab were saved. Up to five single colonies from the original blood agar plate were picked when there were sufficient numbers of colonies. When there were colonies with different morphologies, five colonies of each morphological type were picked. Each colony picked was called an isolate. A total of 719 isolates (362 periurethral, 357 urine) were stored in 50% glycerol in trypticase soy broth at  $-70^\circ\text{C}$ . For study, stored organisms were inoculated onto nutrient agar and incubated overnight at  $37^\circ\text{C}$ .

**Enzyme polymorphisms.** The degree of genetic relatedness among isolates was assessed by enzyme polymorphism determined by multilocus enzyme electrophoresis as described [7]. Electromorphs (mobility variants) of each enzyme were equated with alleles at the corresponding chromosomal genetic locus. Distinctive combinations of alleles, marking strains with distinct multilocus genotypes, were designated as an electrophoretic type (ET) or clonal type. Isolates of the same clonal type were considered to be of the same naturally occurring clone or cell line.

## Results

Four hundred twenty-one periurethral samples and 427 urine samples were collected from the 25 children during the 24 weeks of surveillance of each child. Of the periurethral samples, 56% were positive for a pathogen (range,  $10^2$ – $10^5$  cfu of bacteria/mL of diluent; median,  $10^3$  cfu of bacteria/mL), and 61% of urine samples were positive for a pathogen. *E. coli* was the most common pathogen, followed by *Klebsiella*, *Pseudomonas*, and *Enterococcus* species. Coagulase-negative staphylococci and *Corynebacterium* species were the most common nonpathogens detected. *E. coli* was detected in 101 episodes of bacteriuria and caused 12 symptomatic infections. *Klebsiella* species were detected in 37 episodes of bacteriuria and caused two symptomatic infections. Twenty-five episodes of bacteriuria and one UTI each were due to *Pseudomonas* and *Entero-*

*coccus* species. Nonpathogens were detected in 57 episodes of bacteriuria but caused no infections.

It is of interest that antimicrobial prophylaxis was not associated with a lower rate of bacteriuria or symptomatic UTI. From the 14 patients not receiving prophylaxis, 48% of 225 periurethral samples and 62% of 244 urine samples were positive for a pathogen. From the 11 patients receiving prophylaxis, 66% of 196 periurethral samples (129 of 196 vs. 108 of 225 [48%];  $P = .0002$ ) and 59% of 183 urine samples (108 of 183 vs. 151 of 244 [62%];  $P = .6$ ) were positive for a pathogen. Six symptomatic UTIs were diagnosed during the 336 patient-weeks of surveillance in the 14 patients not receiving prophylaxis (0.9 infections/patient-year), compared with 10 symptomatic infections during 264 patient-weeks of surveillance in the 11 patients receiving prophylaxis (1.9 infections/patient-year). Discussion of the effect of prophylaxis in this study is not warranted because patients were not randomized and were receiving different forms of prophylaxis.

**Concordance of bacterial species on periurethra and in urine.** Since periurethral bacteria are inoculated into the bladder during CIC, organisms in bladder urine might be expected to reflect bacteria on the periurethra. We examined the concordance or likelihood that a bacterial species on the periurethra would also be in the urine obtained on the same day (table 1). The concordance of species in the periurethral and urine sample pairs was calculated by determining whether a species detected on the periurethra was also detected in the urine. If more than one species was on the periurethra, concordance was calculated for each.

Concordance for *E. coli* and *Klebsiella* species in patients not receiving prophylaxis was 80%–90%. Concordance for *Pseudomonas* and *Enterococcus* species was 40%. When a nonpathogen was detected on the periurethra, it was not commonly detected in the urine on the same day. Antimicrobial prophylaxis did not significantly alter the concordance of species on the periurethra and in the urine except for that of

**Table 1.** Concordance of species in periurethral and urine sample pairs in children with neurogenic bladder receiving clean intermittent catheterization.

Species	Concordance	
	No prophylaxis (n = 14)	Antibiotic prophylaxis (n = 11)
<i>Escherichia coli</i>	42/45 (93)	28/57 (49)
<i>Klebsiella</i> species	18/23 (78)	10/13 (77)
<i>Pseudomonas</i> species	2/5 (40)	6/7 (86)
<i>Enterococcus</i> species	7/17 (40)	15/25 (60)
Coagulase-negative staphylococci	28/89 (31)	17/72 (24)
<i>Corynebacterium</i> species	8/67 (12)	4/45 (9)
Alpha- or nonhemolytic streptococci	10/39 (26)	17/45 (38)

NOTE. Data are number of urine samples in which the species was present/number of sample pairs in which species was present on periurethra (%).

*E. coli*. Prophylaxis did not appear to affect periurethral colonization with *E. coli* but did inhibit the growth of *E. coli* in urine [8, 9]. In 42 (93%) of 45 episodes during which *E. coli* was detected on the periurethra, it was also found in urine. For patients receiving prophylaxis, in 28 (49%) of 57 episodes during which *E. coli* was detected on the periurethra, it was also found in urine ( $P = .0001$ ).

**Clonal typing and carriage of *E. coli*.** *E. coli* was detected at least once in 18 of the 25 children studied and caused 12 of the 16 symptomatic UTIs. Comparison of the allele profiles across 20 enzyme loci revealed that 719 periurethral and urine isolates of *E. coli* could be classified into 37 clonal types or ETs (10–141 isolates/ET). ETs differed on average at 47% of the enzyme loci.

In general, a small number of *E. coli* clones colonized the urinary tract of each patient with neurogenic bladder (table 2). The median number of ETs per patient was 2; the range was 1 ET (patients 3, 8–10, 13, 14, 18) to 10 ETs (patient 16). When one or more clonal types were isolated from the periurethra, the same clonal type was isolated from the urine on the same day 95% of the time in the eight patients not receiving prophylaxis (figure 1A). The high concordance of clonal type carriage between periurethral and urine sample pairs dropped in 5 of the 11 patients receiving antimicrobial prophylaxis (figure 1B, patients 1, 2, 10, 15, 17). This reduction in concordance was due to reduced detection of *E. coli* in urine. Prophylaxis appeared to have no effect on periurethral organisms.

**Table 2.** *Escherichia coli* detected in cultures and number of clones characterized from 18 patients with neurogenic bladder.

Prophylaxis, patient no.	Periurethral cultures positive	Urine cultures positive	Isolates saved	ETs
No Prophylaxis				
4	8	8	58	2
5	9	16	99	3
6	1	1	10	2
8	15	17	141	1
9	8	13	16	1
14	1	2	15	1
16	0	5	11	10
18	6	9	11	1
Prophylaxis				
1	9	3	44	3
2	2	1	12	3
21	6	5	16	1
7	3	2	17	2
10	1	2	12	1
11	8	8	62	3
12	10	10	91	5
13	1	1	10	1
15	15	0	61	3
17	3	5	33	2

NOTE. Clonal type = electrophoretic type (ET); the same clonal type may be detected in more than one patient.

Carriage of a clone for several weeks was common (figure 1). For example, ET 9 was recovered over 12 weeks from patient 5 (figure 1A), ET 23 was recovered over 20 weeks from patient 15 (figure 1B), and patient 8 carried ET 2 exclusively over 24 weeks of surveillance (figure 1A). Five ETs (2, 4, 9, 10, 13) were found in samples from more than one patient (figure 1). Three of these patients (patients 5, 7, 8) lived together in a group-home setting, which may have resulted in sharing of clonal types. However, the other six patients who had one of the shared clones had no known contact with the three in the group home or with each other. Seven patients (patients 7, 9–12, 17, 18) each had one or two symptomatic UTIs during surveillance (figure 1). Seven of eight clones of *E. coli* associated with symptomatic infection (ET 2, 10, 13, 18, 35–37) were also detected on the periurethra or in urine at another time without accompanying symptomatic UTI. For example, ET 2 was carried for 24 weeks by patient 8 without an associated symptomatic illness (figure 1A) but was associated with symptomatic UTI at the single time it was detected in patient 7 (figure 1B). ET 10 was detected in patients 5 and 7 (figure 1), but only patient 7 experienced a symptomatic infection with it. ET 13 was detected in three patients (patients 9–11) (figure 1B); all three experienced symptomatic illness due to ET 13. The single clone (ET 20) that was detected only during a symptomatic infection was found in patient 12 (figure 1B).

## Discussion

Our study demonstrated frequent carriage of bacterial pathogens in high titers on the periurethral mucosa of children with neurogenic bladder. Since bacteria on the periurethra are inoculated daily into the bladder urine during CIC, the bacterial species in the urine would be expected to reflect bacteria on the periurethra. In this study, concordance of species in periurethral and urine sample pairs was high for *E. coli* and *Klebsiella* species, but concordance was only 40% or less when *Pseudomonas* or *Enterococcus* species or nonpathogens were detected on the periurethra. This discrepancy in concordance of the different species may be due to the survival capability of the species in urine. Several investigators [10–13] have shown that urine is a good culture medium for bacteria. When Stamey and Mihara [11] incubated different species in urine for 24 hours, *E. coli* grew 3 logs ( $10^2$  cfu/mL to  $10^5$  cfu/mL), and *Enterococcus* species and coagulase-negative staphylococci grew 2 logs ( $10^2$  cfu/mL to  $10^4$  cfu/mL). Titers of streptococci and *Corynebacterium* species declined. When voiding was added to the experimental model, survival in urine for pathogens and coagulase-negative staphylococci changed. Voiding was simulated in an in vitro model [13]. Bacteria grown overnight in urine were diluted with urine to  $10^4$  cfu/mL (time 0). At 3 and 6 hours, bacteria were transferred to fresh urine so as to reduce the bacterial titer by 2 logs, as would occur during voiding. In this voiding model, coagulase-negative staphylococci declined in titer, whereas *E. coli* and

Clonal type of <i>Escherichia coli</i>		Week of study																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<b>A</b>																									
Pt. 4																									
Urine	4	4	4	0	0	0	0	8	8	8	0	0													
Peri	0	4	4	0	0	0	0	8	8	8	8	8													
Pt. 5																									
Urine	9	9	9	9	9	9	9	9	10,11	10,11	0	9	9												
Peri	0	9	9	9	9	9	9	9	0	0	0	9	9												
Pt. 6																									
Urine	0	0	0	0	0	0	0	0	0	0	0	9													
Peri	0	0	0	0	0	0	0	0	0	0	0	9,12	0												
Pt. 8																									
Urine	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Peri	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
Pt. 9																									
Urine	13	13	13	13	13	0	0	13*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	0	13	13	13	13	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pt. 14																									
Urine	22	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	22	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pt. 16																									
Urine	0	0	0	0	0	0	0	25-29	0	0	30-33	0	0												
Peri	0	0	0	0	0	0	0	0	0	0	33,34	0	0												
Pt. 18																									
Urine	0	0	37*	0	37	37	37	37	37	37	37	37	37												
Peri	0	0	37	0	37	37	37	37	37	37	37	37	37												
<b>B</b>																									
Pt. 1																									
Urine	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	1,2	2	0	2	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pt. 2																									
Urine	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0	0	0	0	0	0	0	
Peri	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4,5,6	0	0	0	0	0	0	0	0	
Pt. 3																									
Urine	0	0	0	0	0	7	7	7	7	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pt. 7																									
Urine	0	0	0	0	10*	0	0	0	0	2*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	0	0	0	0	10	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pt. 10																									
Urine	0	0	0	0	0	0	0	0	13*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	0	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	
Pt. 11																									
Urine	14,15	0	0	0	13*	13	13	13	0	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	
Peri	13	0	0	0	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	
Pt. 12																									
Urine	0	0	16	16	16,17	18	18	19	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	
Peri	0	0	16	16	16	18	18	19	18	18	19	18	18	18	18	18	18	18	18	18	18	18	18	18	
Pt. 13																									
Urine	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	0	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pt. 15																									
Urine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	23	23	23,24	23,24	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	25	
Pt. 17																									
Urine	0	0	0	0	35	35*	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Peri	36	0	0	0	35	35	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

**Figure 1.** Isolation of *Escherichia coli* by clonal type from periurethral (peri) and urine cultures over 24 weeks. *A*: Eight patients not receiving prophylaxis; *B*: 10 patients receiving prophylaxis. 0 = culture obtained, *E. coli* not detected; \* = symptomatic urinary tract infection diagnosed, based on  $\geq 10^4$  cfu of *E. coli*/mL of urine, plus symptoms of infection. Boxes indicate patient receiving antibiotic treatment for urinary tract or respiratory infection.

*Klebsiella* species increased in titer by 1 log and *Enterococcus* species increased in titer by 0.4 log [13]. Thus, the dominance of *E. coli* and *Klebsiella* species in bacteriuria in patients with neurogenic bladder may be due to the ability of these species to increase in titer despite voiding.

Bacteriuria caused by species that do not survive voiding may be due to the presence of residual urine in the neurogenic bladder after CIC. In work by Stamey and Mihara [11], *Enterococcus* species and nonpathogens incubated in stationary urine without voiding over a period of 8 hours increased in titer.

Complete emptying of the bladder does not occur in most patients with neurogenic bladder, even with CIC [2]. Residual urine after catheterization may allow the bacterial titers of *Pseudomonas* and *Enterococcus* species and coagulase-negative staphylococci to increase to a titer that is detectable on culture.

Although a variety of species caused bacteriuria in our patients with neurogenic bladder, 75% of symptomatic infections were due to *E. coli*. On average, one symptomatic infection occurred for every 8 weeks in which *E. coli* was carried in the urine. In contrast, an infection due to *Klebsiella* species occurred for every 19 weeks of carriage, and an infection due to *Pseudomonas* or *Enterococcus* species occurred once in every 25 weeks. UTIs were not caused by nonpathogens despite 50 weeks of carriage in bladder urine. In summary, multiple species are inoculated into the urine of patients with neurogenic bladder during CIC. Several of these species cause bacteriuria lasting for weeks, but infection is largely due to *E. coli*.

*E. coli* is the most common cause of UTI in children with neurogenic bladder receiving CIC, just as it is in patients with normal urinary tracts [3, 4]. The mechanism by which *E. coli* causes symptomatic infection in the neurogenic bladder, however, is unclear. Specific bacterial virulence factors are believed to be a prerequisite for UTI in the normal host [14]. In contrast, virulence factors may not be required for *E. coli* infection in the compromised patient [14–18]. For example, Domingue et al. [15] reported that 100% of infecting *E. coli* in nonobstructive pyelonephritis were P-fimbriated, whereas in the presence of obstructive uropathic conditions, a significant number of infecting *E. coli* strains lacked P adhesin. Elsewhere we will examine the prevalence of virulence factors among *E. coli* carried in or infecting the urinary tracts of children with neurogenic bladder.

Although clonal typing was done on multiple colonies for each periurethral and urine culture, we found that children with neurogenic bladder typically carried one or two *E. coli* clones in their urinary tract over months of surveillance. When *E. coli* was detected in the urine, the identical clone was present on the periurethra. These findings suggest that the origin of *E. coli* isolated from the urine of children with neurogenic bladder receiving CIC is the periurethra. Although typing was not done on other bacterial species, it seems reasonable to conclude that bacteriuria due to *Klebsiella* or *Pseudomonas* species also results from inoculation of the same species from the periurethra.

*E. coli* persisted for weeks, both on the periurethra and in the urine, without causing symptomatic infection. Occasionally the same *E. coli* clone carried for weeks in a patient without symptoms caused a UTI. Host factors may be as important as or more important than putative virulence factors of *E. coli* in

the pathogenesis of symptomatic UTI in patients with neurogenic bladder.

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