ORIGINAL ARTICLE

Escherichia coli colonizing the neurogenic bladder are similar to widespread clones causing disease in patients with normal bladder function

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Study design: Clonal typing of neurogenic clones.

Objective: To determine whether neurogenic clones carried over weeks in the urine of asymptomatic children with neurogenic bladder were similar to known uropathogenic clones associated with disease.

Setting: Michigan State University; VA Medical Center, Minneapolis, MN, USA.

Methods: *Escherichia coli* isolates from the urine of 15 children previously followed were typed by multilocus sequence typing and compared to 2 human pyelonephritis genome strains, 29 pediatric or adult symptomatic urinary tract infection strains, 15 pediatric asymptomatic bacteriuria strains, 6 animal urinary tract infection strains and a neonatal meningitis-septicemia prototype K1 strain. Phylotypes and virulence genotypes were determined using PCR.

Results: Twenty-nine *E. coli* isolates were classified into 15 clones. Six of 15 clones were the same sequence type or a close relative to a clone that caused disease in a human or animal. These clones were considered uropathogens. The remaining nine clones were not closely related to a clone that caused disease and were considered commensal clones. Uropathogens were predominantly group B2, exhibited more virulence genes and were carried for more weeks in the neurogenic bladder compared to commensal clones. Nine of 15 children carried one or more uropathogenic clones; 4 children carried one or more commensal clones and 2 children carried both uropathogenic and commensal clones.

Conclusion: Children with neurogenic bladder most commonly carried commensal clones. Uropathogenic clones were associated with prolonged carriage, however, carriage was not associated with symptomatic disease or deterioration of the upper urinary tract.

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Introduction

The majority of children with neurogenic bladder have sensory deficits and loss of control over voiding. Clean in-and-out catheterization (CIC) of the bladder several times daily is currently the preferred method of bladder emptying in children with neurogenic bladder.¹ During CIC, periurethral bacteria are inoculated into the bladder urine and consequently, bacteriuria is common in this population.² In a previous longitudinal study, children with neurogenic bladder, a structurally normal upper urinary tract, receiving CIC were found to carry bacteria in their urine for weeks without symptoms of infection. The children remained clinically well and had no detectable deterioration of the upper urinary tract during 2 years.³ *Escherichia coli* was the most common organism causing

bacteriuria. Clonal typing of *E. coli* by multilocus enzyme electrophoresis revealed that children carried only one or two clones in their urinary tracts over months of surveillance. The same clone was often carried for 2–6 weeks.²

Recent data on long term outcomes in children with neurogenic bladder reveal that renal failure is a significant cause of morbidity during late adolescence.^{4,5} Whether chronic *E. coli* bacteriuria over decades contributes to progressive renal damage is unclear.^{4,5} Several studies have delineated characteristics of *E. coli* which cause extraintestinal disease.⁶ Clones belonging to phylogenetic group B2 and to a lesser extent group D express certain virulence genes (*pap*, *sfa/foc*, *hly* and *kps*) and are commonly isolated from patients with pyelonephritis, bacteremia and/or meningitis. In contrast, intestinal clones within phylogenetic groups A or B1 express few virulence factors and are commonly isolated from the fecal flora of healthy hosts.⁶

Multilocus sequence typing (MLST) has become the gold standard for investigating the genetic relationship between

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pathogenic strains of *E. coli* and reference strains.⁷ MLST uses nucleotide sequences of internal fragments of selected genes as the unit of comparison. As a result, MLST is highly reproducible and comparable between laboratories in different countries.⁸ There is no information on the sequence type (ST) or phylogenetic group of *E. coli* carried in the urine of patients with neurogenic bladder, nor how they are genetically related to other pathogens.⁹ Analysis of clones from the neurogenic bladder by MLST would allow comparison to known pathogenic strains of *E. coli* and would further our understanding of chronic bacteriuria in this population.

In this study, *E. coli* clones from the urine of children with neurogenic bladder were compared by MLST to banked clones associated with disease in human and animals without neurogenic bladder. In addition, the phylogenetic background and virulence characteristics of the neurogenic clones were assessed. We determined whether neurogenic clones carried over weeks in the urine and without symptoms of infection were similar to clones associated with disease.

Methods

Population and bacterial isolates

In a previously published study, 15 children with neurogenic bladder had weekly urine samples obtained by CIC for 6 months.^{2,3} At the University of Virginia Pediatric Microbiology Laboratory culture inoculation was performed less than 10 h after collection. Quantitative culture was done by inoculation of urine onto 5% sheep blood and MacConkey agar with a 0.001 ml calibrated loop. A positive urine culture (bacteriuria) was defined as $\geq 10^4$ CFU bacteria per milliliter of urine obtained by bladder catheterization. *E. coli* isolates were typed by multilocus enzyme electrophoresis. For this study 2–4 isolates per clone were retyped by MLST and compared to a set of well-characterized strains including two human pyelonephritis genome strains, 14 adult symptomatic urinary tract infection (UTI) strains, 15 pediatric symptomatic UTI strains, 15 asymptomatic bacteriuria strains from prepubertal girls, 6 animal UTI strains and a neonatal meningitis-septicemia prototype K1 strain. None of the isolates from patients with neurogenic bladder were associated with symptomatic UTI.

Multilocus sequence typing

Multilocus sequence typing uncovers allelic variation at seven conserved housekeeping genes by PCR amplification sequencing (3753 bp total).⁹ Two to four urine isolates per clone previously characterized by multilocus enzyme electrophoresis were re-classified into ST based on distinct combination of alleles at 7 MLST loci. Neighbor-joining trees based on p distance were constructed with the concatenated sequence data to assess phylogenetic relationships between strains.

Phylotypes and virulence genotypes

Major *E. coli* phylogenetic groups (A, B1, B2 or D) were determined by using a triplex PCR method.¹⁰ Virulence

genotypes for 42 uropathogen-associated markers and, for *pap* (P fimbriae)-positive isolates, 13 alleles of *papA* (Pfimbrial structural subunit) were determined by using established multiplex PCR assays.¹¹ Two representatives of each clone were tested in duplicate, using separately prepared bacterial lysates, together with appropriate positive and negative controls.

Bacterial carriage

Carriage was defined as the number of weeks during which a clone (ST) was isolated from the urine. If a clone was not detected in one weekly sample but was found in the sample before and the sample after the one showing negative results, the negative sample was disregarded in determination of length of carriage. For example, a clone isolated on weeks 1, 2, 3 and 5 of surveillance, but not on week 4, was said to be carried for 5 patient-weeks. If a sample was not obtained for 1 or 2 weeks but the clone was found in the sample before and the sample after the missing sample(s), the missing sample(s) was disregarded in determination of carriage. For example, if a clone was isolated on weeks, 1, 2, 3 and 6, but no sample was obtained on weeks 4 and 5, then the clone was considered to be carried for 6 weeks.

Results

Clonal analysis

Comparison of allelic variation among 7 conserved housekeeping genes revealed that 29 neurogenic *E. coli* isolates could be classified into 15 clonal types or STs (2–4 isolates/ST). Six clones (ST27, ST29, ST287, ST31, ST815 and ST223) of the total 15 neurogenic bladder clones were the same ST or a very close relative to a ST that caused disease including human UTI (Figure 1). These clones were considered uropathogens.

Sequence type 27 was the same ST as a human pyelonephritis genome strain CFT073, two human UTI strains and three symptomatic UTI strains from a healthy prepubertal girl.

Sequence type 29 was the same ST isolated as a neonatal meningitis/septicemia prototype strain K1, three strains causing human UTI and a strain causing an animal UTI. ST31 was the same ST as a human pyelonephritis genome strain J96, a human UTI strain and was closely related to two strains from a healthy prepubertal girl with asymptomatic bacteriuria. ST287 was closely related to ST31. ST815 was the same ST as two strains isolated from a prepubertal female with asymptomatic bacteriuria and was closely related to a known strain causing human UTI. ST223 was the same ST isolated from three animal UTIs and one pediatric UTI. The remaining 9 clones (ST 319, ST359, ST288, ST365, ST827, ST367, ST369, ST386 and ST85) were not closely related to a strain that caused disease and were considered commensal strains (Figure 1).

Phylogenetic groups and virulence genes

All major phylogenetic groups of *E. coli* were represented among neurogenic bladder clones (Table 1).⁶ Group B2

Figure 1 Dendrogram of 106 strains and sequence types (STs) based on multilocus sequence typing. Strains from patients with neurogenic bladder in which the clone are associated with human pyelonephritis strains are shaded.

accounted for six clones, group B1 for seven clones and groups D and A for one clone each. The six clones (ST 223,815,27,29,31,287) considered uropathogens (labeled UTI-associated in Table 1) were predominately Group B2. Four clones (ST27, ST29, ST31 and ST287) belonged to group B2 and two (ST223 and ST815) belonged to group B1. In contrast, only two of nine clones not related to UTI belonged to group B2, whereas five belonged to group B1 and one each belonged to group D and A.

Of the 42 virulence genes tested, 32 were detected among the 15 neurogenic bladder clones ranging from *sfa/foc*, *sfaS*, *cnf1* and *pic* being detected in one clone each to *fimH* being detected in all clones. Clones related to UTI or neonatal bacteremia/meningitis exhibited more virulence genes (median, 12) than did commensal clones (median, 7). Virulence genes were also more prevalent among clones belonging to groups B2 or D (median 11.5) compared to clones belonging to groups B1 or A (median 2).

Carriage of clones

The clonal (ST) type by surveillance week for each of the 15 children in the longitudinal study is displayed in Figure 2.

Once a clone was detected in the urine, the same clone was often carried for 2 weeks or longer. Nine of the 15 children carried one or more uropathogenic clones; 4 children carried one or more commensal clones and 2 children carried both uropathogenic and commensal clones. Overall, carriage of a uropathogenic clone was more common (median 4 weeks) than carriage of a commensal clone (median 1 week). For example, ST29, a clone associated with infant urosepsis and/or meningitis¹² was the most common clone isolated in children with neurogenic bladder. Child no. 2 carried ST29 during the first 10 weeks of study then again during weeks 22–24 weeks (Figure 2). Child no. 3 carried ST29 during weeks 22–24. Child no. 4 carried ST29 over 24 weeks of study. Child no. 8 carried ST29 during weeks 1, 2 and 6–9 weeks of study. Finally, child no. 11 carried ST29 during 15 and 16 weeks of study.

Discussion

In our study, children with neurogenic bladder commonly carried clones known to cause disease in the urinary tract, blood and/or meninges of hosts with normal bladder

Table 1 Virulence genes in 15 *Escherichia coli* clones that colonized the urinary tract of children with neurogenic bladder

ST	Phylo. group	UTI- assoc.	Virulence genes ^{a,b}																				Miscellaneous											
			Adhesins										Toxins					Siderophores				Capsule												
			pap				sfa/foc	sfaS	hra	iha	fimH	hlyD	hlyF	cnf1	sat	astA	vat	iroN	fyuA	ireA	iutA	kpsM II	K1	K2	cvaC	usp	traT	ibeA	pic	ompT	iss	H7	malX	
			A allele	C	EF	G allele																												
223	B1	+	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—		
815	B1	+	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—		
27	B2	+	—	—	—	—	—	—	—	+	+	—	—	—	+	—	+	—	+	—	+	+	+	—	+	+	—	+	—	+	—	—	+	
29A ^c	B2	+	11	+	+	II	—	—	—	—	+	—	+	—	—	+	+	+	+	+	+	+	—	+	+	+	—	—	+	+	+	+	+	
29B ^c	B2	+	11	+	+	II	—	—	—	—	+	—	—	—	—	+	—	+	+	—	+	+	—	—	+	+	—	—	+	—	—	+	+	
31	B2	+	16	+	—	—	+	+	—	—	+	—	—	—	+	+	+	—	—	+	—	—	—	—	+	+	—	—	+	—	—	+		
287	B2	+	48	+	+	III	—	—	+	—	+	—	—	+	—	+	—	—	—	+	—	—	—	—	+	+	—	—	+	—	—	+		
85	A	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+	+	—	+	—	—	+	—	—	—	—	—	—		
365	B1	—	—	—	—	—	—	—	—	+	—	+	—	—	+	—	+	—	—	+	—	—	—	+	—	+	—	—	+	+	—	—		
367	B1	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—		
827	B1	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	
369	B1	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
386	B1	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
319	B2	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	+	+	—	—	+	+	—	—	+	—	+	—	+	—	+	—	+	
359	B2	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	+	+	—	—	+	+	—	—	+	—	+	—	+	—	+	—	+	
288	D	—	7-2	+	+	II	—	—	+	+	+	+	—	—	—	+	—	—	+	—	+	—	+	—	—	+	—	—	—	—	—	—	+	

Abbreviations: Phylo. group, phylogenetic group; ST, sequence type; UTI-assoc., urinary tract infection-associated.

^a+, present; —, absent; *pap*, P fimbriae operon; A allele, *papA* (P fimbriae structural subunit) variants; *papC*, P fimbriae assembly; *papEF*, P fimbriae minor subunits; G allele, *papG* (P adhesin molecule) variants II and III; *sfa/foc*, S and F1C fimbriae; *sfaS*, S fimbriae; *hra*, heat-resistant agglutinin; *iha*, adhesin-siderophore; *fimH*, type 1 fimbriae; *hlyD*, alpha hemolysin; *hlyF*, hemolysin F; *cnf1*, cytotoxic necrotizing factor 1; *sat*, secreted autotransporter toxin; *astA*, enteroaggregative *E. coli* stable toxin; *vat*, vacuolating autotransporter (serine protease); *iroN*, siderophore receptor; *iutA*, aerobactin receptor; *kpsM* II, group 2 capsules; K1 and K2, group 2 capsule variants; *cvaC*, microcin V; *usp*, uropathogenic-specific protein; *traT*, serum resistance-associated; *ibeA*, invasion of brain endothelium A; *pic*, protein involved in intestinal colonization (serine protease); *ompT*, outer membrane protease T; *iss*, increased serum survival; H7, flagellar (*fliC*) variant; *malX*, pathogenicity island marker.

^bVirulence genes sought but not detected: *afa/draBC* and *afaE8*, Dr-binding adhesins; *focG*, F1C fimbriae; *bmaE*, M fimbriae; *gafD*, G fimbriae; F17 and *clpG*, other non-P adhesins; *cdtB*, cytolethal distending toxin; *kpsM* III, group 3 capsules; and *rbc*, O4 lipopolysaccharide.

^cClones 29A and 29B, which occurred in different subjects, exhibited the same ST (ST29), but differed reproducibly according to virulence gene content (*hlyF*, *iroN*, *iutA*, *cvaC* and *iss*) and multi-locus enzyme electrophoresis profile (data not shown).

Distribution of STs by Week of Study

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
2	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
5	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
6	369	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386	386
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
13	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287	287
14	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319	319
15	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288

Figure 2 Isolation of *Escherichia coli* by clonal (ST) type from urine cultures collected over 24 weeks in 15 children (no.) with neurogenic bladder. 0 = culture showed <10⁴ CFU bacteria per milliliter urine, blank = culture not done. ST represents ≥10⁴ cfu *E. coli* per milliliter urine. ST in bold represents a clone associated with disease in patients with normal bladder function.

function. Although group B2, virulence genes and prolonged carriage were more common among uropathogens compared to commensals, these factors were not associated with symptomatic UTI. By itself, the presence of a uropathogen was not associated with disease in children with neurogenic bladder. Another population where uropathogenic clones were not associated with disease were prepubertal girls with asymptomatic bacteriuria. Five clones (ST825, ST824, ST826, ST815 and ST814) from prepubertal girls with asymptomatic bacteriuria were related by MLST to prepubertal girls with symptomatic UTI or adults with pyelonephritis. These clones were able to infect the bladder of prepubertal girls with asymptomatic bacteriuria but at the time of study were not associated with symptoms/signs of UTI.¹³

Bladder-emptying is a life-long problem in children with neurogenic bladder.¹⁴ Although CIC is performed several times a day to empty the bladder, residual urine remains after catheterization. In addition, periurethral bacteria are inoculated into the bladder urine each time CIC is performed. In the previous study, we found that when *E. coli* was detected in the urine of children with neurogenic bladder on CIC, the identical clone was present on the periurethra.² These findings suggested that the origin of *E. coli* isolated from the urine of children receiving CIC is the periurethra.

Once periurethral bacteria are inoculated by catheterization into the bladder urine, carriage may be enhanced by bacterial factors as phylogenetic group or virulence genes. Alternatively, commensal strains may be able to persist in the neurogenic bladder due to the presence of residual urine and compromised bladder function. Wullt *et al.*¹⁵ inoculated, *E. coli* strain 83972 isolated from a patient with asymptomatic bacteriuria, into the bladder of 16 adults with recurrent UTI. *E. coli* 83972 possesses several virulence genes but does not express any.¹⁶ Long-term colonization was established in 6 of 12 patients with neurogenic bladder and residual urine. In contrast, long-term bacteriuria was not established in the four patients with normal bladder function. The investigators concluded that urodynamic defects permit a strain not associated with symptomatic infection to establish in the urinary tract.

Further work with this strain was conducted in adults with spinal cord injury. In a prospective pilot trial, 27 adults with spinal cord injury were randomized to receive bladder

instillation of *E. coli* 83972 or sterile saline.¹⁷ Thirteen of 21 patients in the experimental group became colonized with *E. coli* 83972 for 1 month or more. Urine cultures obtained during period of colonization revealed high titers of *E. coli* 83972 alone or in combination with other organisms. Six of 13 patients colonized developed a symptomatic UTI during the 1 year follow-up compared to 13 of 14 non-colonized patients. The investigators hypothesized that *E. coli* 83972 colonization exerted a protective effect against infection by inhibiting growth of other pathogens or by blocking binding sites on the urothelium.

E. coli colonization of the neurogenic bladder is to be expected due to the fact that *E. coli* are inoculated daily during CIC and remain in residual urine following bladder catheterization. The persistence of a 1–2 *E. coli* clones in the neurogenic bladder may be due to *E. coli*'s growth rate in the urine over other species¹⁸ and/or its ability to evade the host's innate immune response.¹⁹ In addition, phylogenetic group and/or virulence genes may promote selection and persistence of specific clones.

As Ronald suggests,²⁰ bladder instillation with a 'safe' strain of *E. coli* may be the most effective strategy to date, in preventing disease in a bladder that is always colonized with bacteria. Antimicrobial therapies aimed at eradicating bladder bacteriuria have only served to increase the frequency of bacterial resistance to standard antibiotics.

In our study, uropathogenic and commensal clones were able to successfully colonize the neurogenic bladder. Carriage of a uropathogen was not associated with symptomatic disease or deterioration of the upper urinary tract over 2 years of observation. Bacterial and host factors which promote colonization and prevent disease of the urinary tract in patients with neurogenic bladder remains to be elucidated.

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