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Decreased microbiota diversity associated with urinary tract infection in a trial of bacterial interference

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Abstract

Background—Patients with long-term indwelling catheters are at high risk of catheter-associated urinary tract infection (CAUTI). We hypothesized that colonizing the bladder with a benign *E. coli* strain (*E. coli* HU2117, a derivative of *E. coli* 83972) would prevent CAUTI in older, catheterized adults.

Materials and Methods—Adults with chronic, indwelling urinary catheters received study catheters that had been pre-coated with *E. coli* HU2117. We monitored the cultivatable organisms in the bladder for 28 days or until loss of *E. coli* HU2117. Urine from 4 subjects was collected longitudinally for 16S rRNA gene profiling.

Results—Eight of the ten subjects (average age 70.9 years) became colonized with *E. coli* HU2117, with a mean duration of 57.7 days (median: 28.5, range 0–266). All subjects also remained colonized by uropathogens. Five subjects suffered invasive UTI, 3 febrile UTI and 2 urosepsis/bacteremia, all associated with overgrowth of a urinary pathogen. Colonization with *E.*

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CONFLICT OF INTEREST STATEMENT

None of the authors have a conflict of interest with the information presented in this manuscript. The opinions expressed reflect those of the authors and not necessarily those of the Department of Veterans Affairs, the US government, or Baylor College of Medicine.

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Trial Registration: ClinicalTrials.gov, NCT00554996 <http://clinicaltrials.gov/ct2/show/NCT00554996>

coli HU2117 did not impact bacterial bladder diversity, but subjects who developed infections had less diverse bladder microbiota.

Conclusions—Colonization with *E. coli* HU2117 did not prevent bladder colonization or subsequent invasive disease by uropathogens. Microbial diversity may play a protective role against invasive infection of the catheterized bladder.

Keywords

Urinary tract infection; probiotics; microbiota; biodiversity; microbiome

INTRODUCTION

Catheter-associated urinary tract infection (CAUTI) is the most common infection in nursing homes and one of the top four healthcare-associated infections in acute care.^{1, 2} Patients with long-term indwelling catheters are chronically bacteriuric and are thus at high risk for CAUTI.³ No current approach is effective at preventing bladder colonization and subsequent CAUTI in persons who require long-term urinary catheters.⁴ Bacterial interference using potential bladder probiotic *Escherichia coli* 83972 has promise as a novel, antimicrobial sparing method of preventing CAUTI.⁵ In Sweden, this organism has been studied in several clinical trials.⁶⁻⁸ The version of *E. coli* 83972 used in clinical trials in the United States, HU2117, has a deletion in the P-fimbriae adhesion molecule (*papG*) as a safety measure.⁹ Trials of direct bladder inoculation with this organism in persons with neurogenic bladders suggested protection from symptomatic UTI, as did two studies of *E. coli* HU2117-coated catheters in persons with spinal cord injury (SCI).^{5, 10, 11} No prior trial was performed specifically in older residents of long-term care facilities, a population with high prevalence of long-term catheterization and symptomatic UTI.

CAUTI is often a polymicrobial infection, particularly in persons with indwelling catheters. This observation first arose through traditional microbiological methods and has been reinforced by more recent studies using 16S ribosomal RNA sequencing.¹²⁻¹⁵ However, the relationship between bladder bacterial diversity and predisposition to infection has not yet been explored. We hypothesized that such a relationship might exist, based on the established relationship between loss of gastrointestinal microbiota diversity and *Clostridium difficile* proliferation,¹⁶ and the fact that catheterized bladders normally have polymicrobial colonization.³

We performed a pilot clinical trial of urinary catheters coated with *E. coli* HU2117 in older adults. This trial allowed us to study clinical outcomes in parallel with both traditional microbiological culture and 16s rRNA sequencing of urine specimens. We hypothesized that urinary catheters coated with benign *E. coli* HU2117 would be a safe and effective means to colonize bladders of older patients requiring long-term indwelling urinary catheters and that rates of symptomatic UTI would decrease during bladder colonization with HU2117. We expected HU2117 to competitively exclude bladder uropathogens and induce favorable clinical outcomes.

MATERIALS AND METHODS

Participants

Participants were adults over age 50 residing in a long-term care facility, with a requirement for urinary catheter use, at least one prior symptomatic UTI, and pre-existing bladder colonization. Participants with bladder anatomic abnormalities were excluded, as well as people with active malignancies, immunocompromised states such as AIDS/HIV, or uncontrolled diabetes.

Study Design

Subjects received 7-10 days of antibiotics targeted to Gram-negative urinary pathogens and enterococci present in their urine. The existing urinary catheter was replaced with a standard urinary catheter during the course of antibiotics. Antibiotic therapy was followed by a 2-4 day washout period prior to placement of a study catheter that had been pre-coated with *E. coli* HU2117 (see catheter preparation below). Subjects had UTI symptoms assessment and serial urine cultures in a clinical microbiology laboratory on study days 0, 1, 3, 7, 14, 21, and 28. On study day 0, the day of catheter placement, urine was collected for culture pre and post catheter insertion if possible. The study catheter was removed and processed (see below) on day 28. Monthly urine samples were collected after study catheter removal until HU2117 did not grow from 2 consecutive cultures. Symptomatic CAUTI was defined by the Infectious Diseases Society of America criteria,¹⁷ using our diagnostic algorithm.¹⁸ This protocol was approved by the FDA (IND 14007), the Baylor College of Medicine IRB, and the Houston VA Research & Development committee (NCT00554996).

Preparation of the study catheter

Following a standardized protocol,^{10, 11} a Foley catheter (Bardex; Lubricath) was placed into a sterile plastic bottle containing Luria Bertani (LB) broth (Lennox, Fisher Scientific). The broth was inoculated with one colony of *E. coli* HU2117 growing on LB agar and incubated for 48 hours at 37°C. At 24 and 48 hours, a sample of the broth was streaked on various selective media. Bottles that contained contaminants were discarded. Two study catheters were prepared in parallel for each patient: one was inserted on study day 0 and the other was processed for quality control.

Processing of study catheter

The balloon tip was cut off and discarded. The proximal 7-10 cm of the catheter was further processed. This catheter section was washed three times for 30 seconds in 45 mL 1X phosphate buffered saline (PBS), then flushed with 30 mL 1x PBS using a needle and syringe. Three 1 cm pieces were cut from that section, placed into 1.5 mL tubes with 1 mL PBS + 0.1% SDS, and sonicated for 10 minutes. The sonicated sample was serially diluted and plated onto selective agar. Bacterial colonies were quantified after overnight incubation at 37°C.

Bacterial Culture, Enumeration, and Identification

Bacteria were cultured aerobically on various agar media, including those selective for Gram-negative, Gram-positive, and fungal organisms. Two representative colonies with morphology consistent with *E. coli* HU2117 were picked and inoculated in LB with 10% glycerol and frozen at -80°C for subsequent molecular analysis. DNA was extracted using UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's instructions. The identity of the HU2117-consistent colonies was verified by PCR with three HU2117-identifying primer sets for regions unique to *E. coli* HU2117/83972 (*fim*, *papG*, and O-antigen) (Invitrogen).⁹ PCR was performed with Sapphire Amp Fast PCR Master Mix (Takara Bio, Otsu, Japan) using the manufacturer's recommended reaction mixture and a GeneAmp System 9700 thermocycler (Applied Biosystems, Foster City, CA).

16S rRNA gene sequencing and profiling

Sequential urine samples collected from the final four subjects in the trial before closure were frozen at -80°C . Bacterial DNA was extracted from thawed urine following methods adapted from the Human Microbiome Project.¹⁹ Briefly, the V4 region of the 16S rRNA gene was amplified through PCR and sequenced on the MiSeq platform (Illumina) using the 2×250bp protocol.²⁰ Sequencing read pairs were demultiplexed based on unique molecular barcodes, filtered for PhiX using Bowtie2 v2.2.1, and merged with SeqPrep. Sequences were demultiplexed using QIIME v1.8.0¹⁹ and clustered using the UCLUST pipeline.²⁰ Operational taxonomic unit (OTU) classification was achieved by mapping reads to the SILVA database,²¹ and abundances were recovered by mapping the demultiplexed reads to the UCLUST OTUs. The resulting OTU table was used in QIIME to calculate alpha-diversity, beta-diversity, and taxonomic summaries.^{22, 23}

RESULTS

Clinical outcomes: Bladder Colonization and UTI

Ten subjects were enrolled (8 male, 2 female, Table 1). All subjects, except subject 9 who had $<10,000$ CFU/mL in the baseline urine culture, received antibiotics before the insertion of the study catheter. We planned to enroll 20 subjects but ended the trial early after consultation with our safety monitoring board. Average age of subjects was 70.9 (range 57-88). Eight of the ten subjects (80%) became colonized with *E. coli* HU2117 for a mean duration of 57.7 days (median: 28.5, range 0-266). All subjects remained co-colonized with other microorganisms throughout the study (Table 2). All the subjects had microorganisms present in the urine immediately before study catheter insertion despite the use of targeted antibiotics and catheter change during treatment (Table 2). In all but 1 of the subjects, at least one of the organisms present at enrollment appeared in a subsequent urine culture, and in subject 9, the original organism (*Acinetobacter*) was subsequently identified by 16S rRNA sequencing, although not detected by standard microbiology culturing.

Five subjects suffered invasive disease from the co-colonizing bacteria (3 febrile UTI and 2 urosepsis/bacteremia, Table 1). All five cases were associated with the overgrowth of another urinary pathogen. In the three cases of febrile UTI, HU2117 was only detected in the urine of one of these subjects, who developed UTI after his catheter became obstructed,

likely related to crystal formation from the *Proteus* in his urine. HU2117 had not been found in previous urine specimens from this subject since insertion day, but the agar plate in the microbiology lab had a single colony that appeared to be HU2117 and was verified as such by PCR. In the other 2 cases of febrile UTI, HU2117 was replaced by a pathogenic *E. coli*, as demonstrated by morphology, susceptibility, and PCR. In one case of urosepsis, HU2117 was detected on the removed study catheter but was not present in the subject's urine. Another pathogenic *E. coli* was detected in that subject's urine and blood. The second case of urosepsis was caused by *Pseudomonas*, and no HU2117 was detected during infection.

In the year before enrollment, our 10 subjects had a rate of 7 UTIs per 1000 patient-days. During bladder colonization with HU2117, the rate was 8.5 UTIs, and in the year after our study, the rate was 6 UTIs per 1000 patient-days. Rates of UTI did not differ before, during, and after bladder colonization in all 10 subjects ($p=0.5$).

PCR analysis of 102 bacterial isolates confirmed that each colony identified as HU2117 by visual inspection was genetically HU2117 (94 colonies), and colonies that were identified as *E. coli* other than HU2117 by visual inspection were indeed not HU2117 by PCR (8 colonies).

Impact of *E. coli* HU2117 on bladder microbiota—Each of the four subjects whose urine was sent for microbiome analysis had a different clinical outcome in terms of bladder colonization with HU2117 and onset of symptomatic UTI: not colonized/not infected, colonized/not infected, not colonized/infected, and colonized/infected (Table 3). We analyzed 26 urine samples from the four patients, generating a minimum of 14,750 sequences per sample. Communities were dominated by Proteobacteria, which was present in 100% of the samples. Firmicutes, Bacteroidetes, and Actinobacteria were also identified.

Bacterial diversity indices revealed a significant difference in bacterial richness of the urine microbiota between patients who developed a symptomatic infection during the trial and patients who did not get an infection (Figure 1). Overall, infection was associated with significantly lower diversity by observed OTUs and by the Shannon Diversity Index (Figure 1A and 1C). Bacterial diversity was not significantly affected by bladder colonization with HU2117 (Figure 1B and 1D). We repeated the diversity analyses after excluding the urine samples that had been collected on the day of and after antibiotics were given for UTI; no differences were observed. Beta diversity analysis using UniFrac based principle coordinate analysis (PCoA) revealed clustering of samples from patients who developed an infection during the study (Figure S1), indicating a higher degree of phylogenetic similarity between those microbial communities. Supporting previous findings, there was no apparent clustering in patients who were colonized with HU2117 and patients who were not.

The Shannon diversity index was determined for the urine specimens from each subject. Samples from the 2 subjects who did not develop a symptomatic infection had a mean Shannon-diversity score of 2.256, while samples from the other 2 patients who developed an infection had a mean score of 0.6207. These differences in diversity are apparent in Figure 2. Interestingly, both infected subjects had the same identified genera: *Acinetobacter*, *Escherichia-Shigella*, *Pseudomonas*, and *Staphylococcus*.

Using standard microbiological techniques we identified 28 species across 17 samples. 100% of organisms identified by microbiological methods were also detected by sequencing, but the 28 microbiology laboratory identifications represented only a fraction of the genera identified by sequencing. The organisms that were identified by both methods included *E. coli*, *Staphylococcus*, *Enterococcus*, *Proteus*, *Pseudomonas*, *Alcaligenes*, *Corynebacterium*, and *Ochrobactrum*. Organisms frequently missed by standard microbiology included *Actinobaculum*, *Aerococcus*, *Campylobacter*, *Lactobacillus*, *Prevotella*, *Porphyromonas*; the latter two are obligate anaerobes. In many cases the bacteria missed by standard cultures had higher relative abundance in the genetic analysis than those identified by culture methods. For example, in subject 10 on day 51 (Table 3), *Campylobacter* and *Actinobaculum* had 40% and 32% relative abundance by 16s identification, respectively, in comparison to the organisms identified by microbiology cultures: *Proteus* (11%), *Staphylococcus* (0.5%), and *Enterococcus* (7%).

DISCUSSION

In this trial of bladder inoculation with *E. coli* HU2117, the colonization rate in the study population (80%) was similar to the colonization rate in a previous trial of *E. coli*-coated catheters in SCI subjects (83%).¹¹ However, bacterial interference was not an effective means for preventing bladder colonization by pathogenic bacteria or for preventing CAUTI. Our sequencing analysis offers some insight on this negative result. Bladder flora diversity was associated with protection from symptomatic infection, and colonization with HU2117 did not increase the diversity of the bladder microbiota, nor did colonization decrease pathogen presence in the bladder.

The rate of symptomatic UTI in our patients, 0.85 per 100 patient days, was comparable but potentially higher than what we observed in prior studies in the SCI population (0.77 and 0.15 per 100 patient-days),^{10, 11} and 5 of our 10 subjects (50%) developed symptomatic UTI. The high incidence of UTI was probably related to the advanced age and co-morbidities of our study population, but it is difficult to draw conclusions without a control group. In all 5 episodes of symptomatic UTI another pathogen was implicated; the two cases of bacteremia were definitively caused by an organism other than *E. coli* HU2117, as demonstrated by speciation and sensitivities. In contrast, in a recent Swedish study of bacterial interference by *E. coli* 83972 in non-catheterized subjects with recurrent UTIs, 4 subjects developed 5 uncomplicated symptomatic UTIs caused by *E. coli* 83972.⁶ This number was still significantly fewer than the rate of UTIs in the Swedish control group, supporting the study's conclusion that *E. coli* 83972 colonization did provide a protective effect in non-catheterized patients. Analysis of the *E. coli* 83972 isolates recovered during these symptomatic UTIs revealed that the organisms had not acquired established genetic virulence factors during colonization, although each isolate exhibited a unique gene expression profile. Our *E. coli* HU2117 differs from *E. coli* 83972 only in the deletion of the *papG* cluster, but since *papG* is not functional in wild-type *E. coli* 83972, this modification alone cannot account for the lack of symptomatic UTI with *E. coli* HU2117 in our studies. Patient factors may have also accounted for the different outcomes of our study compared to the Swedish study, as the older subjects in our study are probably less likely to express bladder symptoms.

We suspect that the advanced age and high co-morbidity of our study population accounts for the high rate of invasive UTI, but we also believe that the pre-insertion antibiotics may have played a role in this outcome. An increased frequency of UTI in young women treated with antibiotics for asymptomatic bacteriuria has been reported previously.²⁴ Furthermore, our results confirm the futility of treating asymptomatic bacteriuria in catheterized patients.²⁵ In all subjects, at least one of the organisms cultured in the enrollment urine sample reappeared. These resurgent organisms may be coming from bladder intracellular biofilms, small renal stones, or chronic pyelonephritis related to indwelling catheter use.²⁶⁻²⁹ Pre-treatment antibiotics may have caused harm by decreasing the diversity of bladder microbiota.

Our comparison of the species identified by standard microbiology and by 16S rRNA gene profiling demonstrates that culturing finds the organisms typically expected to be in the bladder and those that grow well under standard microbiology conditions (*E. coli*, *Enterococcus*). As molecular identification methods and genetic sequencing become more common, we may need to reassess what constitutes typical urinary flora, particularly in a catheterized bladder. Species identified by sequencing but not by culturing often had a higher genetic representation in our sample than those found by both approaches. However, in our two subjects who had both 16s rRNA analysis and symptomatic UTI, the causative organism was identified by both culturing and sequencing. A recent comparison of 16s sequencing to urine culture in patients with UTI also found that the dominant uropathogen identified by culture was the most abundant organism identified by sequencing.³⁰ Thus, the clinical relevance of the additional species picked up by 16s rRNA analysis is not clear.

CONCLUSIONS

Our trial was a small study without a control group, so we cannot determine whether the high incidence of UTI observed was typical for our older study population. Unnecessary use of antimicrobials to treat asymptomatic bacteriuria was not only ineffective at preventing UTI but may have caused harm by shifting the urinary microbiome away from a commensal state of diversity. The bladder microbiome diversity index may eventually become a useful biomarker to distinguish asymptomatic bladder colonization from impending symptomatic UTI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

We performed a clinical trial of bacterial interference as a means to prevent urinary tract infection in older, catheterized adults with chronic bladder colonization.

Direct bladder inoculation with *E. coli* HU2117 (derived from 83972) did not prevent subsequent symptomatic infection of the urinary tract by uropathogens.

Microbial diversity, as measured by 16S rRNA profiling, revealed that subjects who developed symptomatic infections had less diverse bladder microbiota.

Standard microbiological culture techniques identified only a fraction of the organisms present by sequencing.

Figure 1.

Subjects who developed infections had lower microbial diversity. A. Observed OTUs (alpha diversity) in urine samples from subjects that developed an infection compared to samples from subjects who did not develop an infection. B. Observed OTUs in urine samples from subjects colonized and not colonized by *E. coli* HU2117. C. Shannon Diversity Index of urine samples from subjects that developed infection compared to subjects who did not develop an infection. D. Shannon Diversity Index of samples from subjects colonized and not colonized by *E. coli* HU2117. P values were obtained using the Mann Whitney test.

Figure 2.

Relative abundance of identified genera. A. Identified genera in urine samples from Subject 3, colonized but not infected. B. Identified genera in samples from Subject 9, both colonized and infected. C. Identified genera in samples from Subject 10, neither colonized nor infected. D. Identified genera in samples from Subject 11, not colonized but became infected. Other refers to grouped organisms with species abundance of < 0.5%. Pre and post refer to collection relative to insertion of a new catheter (study or standard) on that day.

Table 1

Clinical characteristics and clinical outcomes of subjects

Subject Number	Age	Gender	Reason for catheter dependence	# Days colonized with HU2117	Clinical outcome	Was <i>E. coli</i> HU2117 present at time of UTI?
1	80	M	Bed-bound Healing sacral wound	114	Colonization	
2	88	M	Bed-bound Healing sacral wound	58	Colonization Catheter blockage day 6	
3	60	F	Hemiparesis	300	Colonization	
4	58	M	Urinary retention	14	Colonization	
5	64	M	Healing sacral wound	43	Colonization Pseudomonas urosepsis and death day 53	No
6	66	M	Bed-bound Incontinence	11	Colonization Catheter blockage and febrile Proteus UTI day 11	Yes
7	87	M	Obstruction secondary to prostatic enlargement	14	Colonization E. coli urosepsis day 22	Yes (on catheter)
9	70	M	Neurogenic bladder	57	Colonization Febrile E. coli UTI day 76	No
10	67	M	Urinary retention	0	No colonization	
11	74	F	Areflexic bladder	0	No colonization Febrile E. coli UTI day 2	No

Table 2

Microbiologic outcomes using standard culture methods

Subject	Organisms at Enrollment	Antibiotics Pre-Inoculation	Organisms at Inoculation	Organisms During Colonization with <i>E. coli</i> HU2117	Organisms After Loss of <i>E. coli</i> HU2117
1	Proteus Enterococcus Pseudomonas Mixed Gram positive	Cefepime	<i>E. coli</i> other ^a Enterococcus MRSA <i>E. coli</i> (HU2117)	Proteus <i>E. coli</i> other Enterococcus Providencia Mixed Gram positive HU2117	Serratia Providencia <i>S. epidermidis</i>
2	Proteus	Amoxicillin	Proteus Mixed Gram positive Group B Strep <i>E. coli</i> (HU2117)	Proteus <i>E. coli</i> other, MRSA Mixed Gram positive Group B Strep <i>E. coli</i> (HU2117)	Proteus Alcaligenes Morganella Mixed Gram positive
3	<i>E. coli</i> other	Sulfa methoxazole- Trimethoprim	Enterococcus <i>E. coli</i> (HU2117)	Enterococcus Mixed Gram positive Group B Strep Aerococcus <i>E. coli</i> (HU2117)	<i>E. coli</i> other Mixed Gram positive
4	<i>E. coli</i> other Enterococcus Streptococcus, alpha hemolytic	Nitrofurantoin	<i>E. coli</i> other <i>E. coli</i> (HU2117)	<i>E. coli</i> other Enterococcus <i>E. coli</i> (HU2117)	<i>E. coli</i> other Enterococcus Mixed Gram positive
5	<i>E. coli</i> other Pseudomonas Klebsiella	Ertapenem	<i>E. coli</i> other Pseudomonas <i>E. coli</i> (HU2117)	Pseudomonas Enterococcus Mixed Gram positive Klebsiella <i>E. coli</i> (HU2117)	Pseudomonas Klebsiella Providencia
6	Proteus Mixed Gram positive Providencia sp.	Sulfa methoxazole- Trimethoprim	Proteus Mixed Gram positive <i>E. coli</i> (HU2117)	Proteus <i>E. coli</i> (HU2117)	Proteus Alcaligenes
7	Proteus	Ertapenem	MRSA <i>E. coli</i> (HU2117)	HU2117 Pseudomonas <i>E. coli</i> other Mixed Gram positive	Pseudomonas <i>E. coli</i> other <i>S. epidermidis</i> Proteus ^b
9	Rhizobacterium Acinetobacter sp.	None (species <10,000)	<i>E. coli</i> (HU2117)	Pseudomonas <i>E. coli</i> (HU2117)	<i>E. coli</i> other
10	Enterococcus Pseudomonas Mixed Gram positive <i>S. aureus</i> Enterobacter	Ciprofloxacin	Alcaligenes <i>S. epidermidis</i> Corynebacterium sp. <i>E. coli</i> (HU2117)		Enterococcus Ochrobactrum Bordetella Mixed gram positive Proteus
11	<i>E. coli</i> other Enterococcus	Amoxicillin	<i>E. coli</i> other		<i>E. coli</i> other

^a *E. coli* other denotes a strain of *E. coli* other than HU2117, as demonstrated by PCR.

^b This organism appeared after additional antibiotics had been given.

Species names have been listed whenever available based on identification techniques.

Table 3

Clinical, microbiological and sequencing outcomes of four subjects.

Subject	Colonized with HU2117	Invasive UTI	Microbiological Outcomes				
			Day	Colonization	UTI	Micro Lab Identified	16S rRNA gene profiling Identified (sequence abundance)
3	Yes (266 days)	No	202	Yes	No	<i>E. coli</i> (HU2117) Gram positives	Escherichia-Shigella (92%) Prevotella (5%) Dialister (.8%) Lactobacillus (.5%)
			300	No	No	<i>E. coli</i> (other) Gram positives (x3 species) <i>S. aureus</i> (1 colony)	Prevotella (34%) Actinobaculum_schaalii (25%) Finegoldia (8%) Dialister (6%) Lactobacillus (5%) Escherichia-Shigella (4%) Varibaculum_cambriense (4%) Fastidiosipila (3%) Aerococcus (2%) Campylobacter (2%) Porphyromonas (1%) Peptoniphilus (1%) Anaerococcus (.8%) Jonquetella (.6%) Staphylococcus (.5%)
			335	No	No	<i>E. coli</i> Gram positives	Escherichia-Shigella (96%) Prevotella (1%) Lactobacillus (.8%)
9	Yes (57 days)	Yes (day 76)	0	Yes	No	<i>E. coli</i> (HU2117)	Escherichia-Shigella (99%) Enterobacter (.5%)
			7	Yes	No	<i>E. coli</i> (HU2117) Pseudomonas	Escherichia-Shigella (98%) Acinetobacter (1%) Pseudomonas (.5%)
			28 post ^a	Yes	No	<i>E. coli</i> (HU2117)	Escherichia-Shigella (99%)
			57	Yes	No	<i>E. coli</i> (HU2117)	Escherichia-Shigella (99%)
			76	No	Yes	<i>E. coli</i> (other)	Escherichia-Shigella (99%)
			104	No	No	Staphylococcus ^b	Staphylococcus (96%) Escherichia-Shigella (2%)
10	No	No	0	Yes	No	<i>E. coli</i> (HU2117) Alcaligenes fecalis Corynebacterium <i>S. epidermidis</i>	Escherichia-Shigella (60%) Staphylococcus (20%) Actinobaculum_schaalii (12%) Bifidobacterium_breve (3%) Alcaligenes (1%) Corynebacterium_striatum (1%)
			8	No	No	Enterococcus (2 types) Ochrobactrum Anthropi Bordetella bronchiseptica	Actinobaculum_schaalii (36%) Alcaligenes (22%) Ochrobactrum (17%) Enterococcus (9%) Bifidobacterium_breve (7%) Corynebacterium_striatum (5%) Brevundimonas (.9%) Vagococcus (.8%) Actinobaculum_suis (.5%)

Subject	Colonized with HU2117	Invasive UTI	Microbiological Outcomes				
			Day	Colonization	UTI	Micro Lab Identified	16S rRNA gene profiling Identified (sequence abundance)
11	No	Yes (day 1)	21 pre ^c	No	No	Enterococcus Alcaligenes <i>fecalis</i> Gram positives	Bosea (.5%) Actinobaculum_schaalii (47%) Enterococcus (31%) Bifidobacterium_breve (14%) Alcaligenes (4%) Lactobacillales (1%) Corynebacterium_striatum (.8%) Bacillus (.6%)
			51	No	No	Proteus vulgaris S. aureus Enterococcus	Campylobacter (40%) Actinobaculum_schaalii (34%) Proteus (11%) Enterococcus (7%) Bifidobacterium_breve (5%) Morganella (1%) Staphylococcus (.5%)
			0 post ^d	No	No	E. coli (other)	Escherichia-Shigella (99%)
			1	No	Yes	E. coli (other)	Escherichia-Shigella (99%)
			28	No	No	S. epidermidis (post ^a)	Staphylococcus (99%) (pre ^c)
11	No	Yes (day 1)	58	No	No	P. aeruginosa Gram positives	Acinetobacter (91%) Pseudomonas (8%) Staphylococcus (.6%)

^aPost removal of study catheter.

^bIdentified by visual morphology, not confirmed by micro lab.

^cPre removal of study catheter.

^dPost insertion of study catheter.

Species names have been listed whenever available based on identification techniques.