

Escherichia coli Isolates Causing Asymptomatic Bacteriuria in Catheterized and Noncatheterized Individuals Possess Similar Virulence Properties[∇]

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Urinary tract infections (UTIs) are among the most common infectious diseases of humans, with *Escherichia coli* being responsible for >80% of all cases. Asymptomatic bacteriuria (ABU) occurs when bacteria colonize the urinary tract without causing clinical symptoms and can affect both catheterized patients (catheter-associated ABU [CA-ABU]) and noncatheterized patients. Here, we compared the virulence properties of a collection of ABU and CA-ABU nosocomial *E. coli* isolates in terms of antibiotic resistance, phylogenetic grouping, specific UTI-associated virulence genes, hemagglutination characteristics, and biofilm formation. CA-ABU isolates were similar to ABU isolates with regard to the majority of these characteristics; exceptions were that CA-ABU isolates had a higher prevalence of the polysaccharide capsule marker genes *kpsMT II* and *kpsMT K1*, while more ABU strains were capable of mannose-resistant hemagglutination. To examine biofilm growth in detail, we performed a global gene expression analysis with two CA-ABU strains that formed a strong biofilm and that possessed a limited adhesin repertoire. The gene expression profile of the CA-ABU strains during biofilm growth showed considerable overlap with that previously described for the prototype ABU *E. coli* strain, 83972. This is the first global gene expression analysis of *E. coli* CA-ABU strains. Overall, our data suggest that nosocomial ABU and CA-ABU *E. coli* isolates possess similar virulence profiles.

Urinary tract infections (UTIs) are among the most common infectious diseases acquired in nosocomial settings. UTIs may be symptomatic (e.g., cystitis and pyelonephritis) or asymptomatic. Asymptomatic bacteriuria (ABU) is an asymptomatic carrier state that resembles commensalism. ABU patients often carry >10⁵ CFU/ml of urine of a single bacterial strain without symptoms; in nosocomial settings, ABU frequently occurs in both catheterized and noncatheterized patients. In catheterized patients, the risk of bacteriuria is estimated to be 5 to 10% per day (46), and most patients with an indwelling urinary catheter for 30 days or longer develop bacteriuria (35). ABU in catheterized and noncatheterized patients may also lead to symptomatic UTIs, with patients experiencing fever, suprapubic tenderness, and, in some cases, bacteremia, acute pyelonephritis, and death (16, 45).

ABU is caused by a range of Gram-negative and Gram-positive organisms, including *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Staphylococcus epidermidis*, and *Enterococcus faecalis* (16, 46). In many cases, the bacteria that cause these infections are resistant to multiple antibiotics, and thus, they pose a serious threat to the safety and proper functioning of health care facilities. *E. coli* is one of

the most common causes of nosocomial UTIs (including ABU) (17, 36). Many ABU *E. coli* strains are phylogenetically related to virulent uropathogenic *E. coli* (UPEC) strains, with the differences in virulence properties being multifactorial and associated with various combinations of genotypic and phenotypic factors (26, 49). The prototype ABU *E. coli* strain, strain 83972, is a B2 clinical isolate capable of long-term bladder colonization, as shown in human inoculation studies (14, 38). Bladder infection with *E. coli* 83972 fails to induce a host inflammatory response, and this is associated with the attenuation of several virulence determinants, including type 1, P, and F1C fimbriae (24, 32, 47). The lack of fimbriae appears to be essential, as a P-fimbriated and adherent transformant of *E. coli* 83972 triggers inflammation in the human urinary tract (47).

The characteristics of *E. coli* that cause asymptomatic bacteriuria in catheterized patients (catheter-associated ABU [CA-ABU]) and their relationship to ABU *E. coli* in noncatheterized patients have not been investigated. The insertion and presence of an indwelling catheter predispose an individual to infection by causing damage to the uroepithelial mucosa and providing a surface for biofilm formation, which may thus affect the normal functioning and voiding of the bladder (7, 16). Therefore, *E. coli* strains causing asymptomatic bacteriuria in noncatheterized and catheterized patients may utilize different strategies to efficiently colonize the urinary tract. In the present study, we have compared the molecular characteristics, phenotypic properties and antimicrobial resistance profiles of

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176 consecutively isolated ABU *E. coli* strains (from noncatheterized patients) and CA-ABU *E. coli* strains from the same geographic area. Biofilm formation was identified as a common feature for both ABU and CA-ABU strains, and we explored this further by examining the transcriptome of two CA-ABU *E. coli* strains during biofilm growth and comparing this with the transcriptome for ABU *E. coli* 83972 grown under the same conditions.

MATERIALS AND METHODS

Patients and bacterial isolates. Eighty-eight *E. coli* ABU isolates and 88 *E. coli* CA-ABU isolates were obtained from urine samples of patients at the Princess Alexandra Hospital (Brisbane, Queensland, Australia) during the period from February to July 2006. ABU was defined as a clean-catch voided urine specimen with counts of $>10^5$ CFU/ml of a single organism. There were no ABU patients who were catheterized. CA-ABU was defined by the presence of an indwelling catheter and a urine specimen with counts of $>10^5$ CFU/ml. ABU and CA-ABU patients had no conditions indicative of symptomatic UTIs (i.e., fever, suprapubic tenderness, or costovertebral angle tenderness) in the 48-h period prior to or following urine collection. ABU patients (67% female) had a mean age of 66 years (range, 20 to 100 years); CA-ABU patients (46% female) had a mean age of 56 years (range, 17 to 94 years). The most common comorbid conditions were 23% endocrine, 19% cardiovascular dysfunction, 16% neurological, and 15% urological for patients with ABU and 51% neurological, 22% endocrine, 15% urological, 7% musculoskeletal, and 7% cardiovascular dysfunction for patients with CA-ABU. Urine cultures were performed as part of ward screening. All *E. coli* isolates were identified by typical morphology, lactose fermentation, and a positive spot indole test. Isolates that did not meet these criteria were identified in full by a Vitek 2-GN card (bioMérieux). Isolates were tested for resistance to common antimicrobial agents using the Vitek 2 system at the Princess Alexandra Hospital. *E. coli* 83972 is a prototype ABU clinical isolate originally isolated from a young Swedish girl who carried it for 3 years without adverse effects (1a, 25). All strains were routinely grown at 37°C on solid or in liquid Luria-Bertani (LB) medium.

PCR screening. The presence of the following virulence genes was assessed for each ABU and CA-ABU isolate: *fimH* (type 1 fimbrial adhesin), *papG I* to III (P fimbriae adhesins), *ftu* (antigen 43), *focG* (F1C fimbrial subunit protein), *sfaS* (S fimbrial adhesin), *afa/draBC* (Afa/Dr chaperone and anchoring proteins), *mrkB* (type 3 fimbrial chaperone protein), *hlyA* (hemolysin), *cnf1* (cytotoxic necrotizing factor 1), *fyuA* (yersiniabactin receptor), *iutA* (aerobactin receptor), *iroN* (salmonella receptor), *kpsMT II* (group II capsule), *kpsMT III* (group III capsule), *kpsMT K1* (K1 capsule), and *kpsMT K5* (K5 capsule). The methods for bacterial DNA preparation, primer sequences, and reaction conditions have been described previously (22, 23, 29, 42). The *E. coli* phylogenetic group of origin was determined using a three-locus PCR-based method (4). All strains were tested in duplicate with appropriate positive and negative controls.

Phenotypic assays. The capacity of bacteria to express type 1 fimbriae was assayed by determining their ability to agglutinate yeast cells on glass slides (34). Those strains that reacted negative in this assay were retested after three successive rounds of 48 h of static growth in LB medium. Mannose-resistant hemagglutination (MRHA) was assessed as described previously (9). Hemolysin production was detected on sheep blood agar plates as described previously (21). Strains that produced a clear zone of lysis after incubation for 24 h at 37°C were considered positive. Motility was assayed by inoculating 0.25% LB agar plates with 2 μ l of overnight liquid cultures and measuring the diameter of bacterial growth 16 h after inoculation.

Biofilm assays. For screening of the isolate collection, biofilm formation on polyvinyl chloride (PVC) surfaces was monitored using 96-well microtiter plates (Falcon), following growth in synthetic urine, as described previously (26). Isolates were considered positive for biofilm formation if the stained biofilm had an absorbance at 595 nm equal to or greater than 0.5. The results represent the averages of three independent experiments, each with four replicates per strain. *E. coli* M366 and MS2034 and their respective *ybtS* deletion mutants were assayed for biofilm formation in the same manner. Biofilm growth on Bard Biocath Foley latex catheters was assayed for 10 ABU and 10 CA-ABU strains selected as representatives for the different levels of biofilm growth observed on the PVC microtiter plates. The strains were grown statically for 24 h at 37°C in 3 ml of pooled human urine in the presence of a catheter section (1 cm long, vertically dissected). The urine was then removed and replaced with fresh pooled human urine from the same original source, and the incubation was continued as

described above for a further 24 h. Following this incubation, the catheter sections were removed, washed twice with phosphate-buffered saline (PBS) and transferred to 1 ml sterile PBS, and the biofilm was disrupted by vigorous vortexing using a TissueLyser (Qiagen). The bacteria were enumerated using quantitative colony counts on agar to determine the amount of biofilm growth. All experiments were repeated at least twice.

Microarray experiments. DNA microarrays were performed for CA-ABU *E. coli* strains M366 and MS2034, as described previously (11). Briefly, M366 and MS2034 were grown to mid-exponential phase in morpholinepropanesulfonic acid (MOPS) minimal medium (supplemented with 0.2% glucose) or pooled human urine or as a biofilm in urine in 94-mm petri dishes (Greiner Bio-One). Each combination of strain and growth condition was assayed in triplicate. Samples for RNA isolation were extracted and mixed directly with 2 volumes of RNeasy Protect bacterial reagent (Qiagen AG). Total RNA was isolated, and on-column DNase digestion was performed using an RNeasy minikit and an RNase-free DNase set (Qiagen AG). Purified RNA (10 μ g per sample) was converted to cDNA, and microarray analysis was performed according to the guidelines of the *GeneChip Expression Analysis Technical Manual 701023*, revision 4 (Affymetrix Inc., Santa Clara, CA) (1). Labeled cDNA was hybridized to *GeneChip E. coli* Genome 2.0 arrays (Affymetrix). Overall, nine samples were hybridized to nine chips for each strain, with triplicates for the three growth conditions being used. Hybridization, washing, and staining were performed as described in the *GeneChip Expression Analysis Technical Manual 701023* (1), with the microarrays being scanned using a GeneChip Scanner 3000 scanner. The 18 arrays for strains M366 and MS2034 were normalized with the *E. coli* 83972 arrays previously analyzed under the same growth conditions (11, 33). Data analysis was performed as described previously (11). For each strain, the fold changes were calculated for biofilm growth compared to either planktonic growth in MOPS or planktonic growth in urine. Randomly permuting the samples 200 times gave false discovery rates of 0.0 to 0.7% (1 to 22 false-positive genes) for the different comparisons.

Construction of *ybtS* deletion mutants. Deletion mutants of the yersiniabactin synthesis gene *ybtS* in strains M366 and MS2034 were constructed using the bacteriophage λ red recombinase gene inactivation method, as described previously (5). Plasmid pKD4 was used as the template with the deletion primers previously employed by Henderson et al. (12). The PCR product was transformed into M366(pKD46) and MS2034(pKD46), respectively, and mutants were selected following growth on LB agar containing kanamycin (50 μ g/ml). Deletion mutants were verified by PCR with the *ybtS* flanking primers 5'-GCA CCGCCTTATACAATTGG and 5'-GTATTCGGTGGCGTTGTTG.

Statistical analysis. Comparisons of prevalence data were tested using the χ^2 test and Fisher's exact test with MiniTab (version 15) statistical software. The median virulence scores were compared using the Mann-Whitney U test. Differences in catheter biofilm growth were tested using a Student *t* test. The level of statistical significance was set at $P < 0.05$.

Microarray data accession number. The microarray data have been deposited in Array Express (<http://www.ebi.ac.uk/arrayexpress>) under accession number E-MEXP-2298.

RESULTS

Phylogenetic grouping of ABU and CA-ABU strains. Our collection comprised 88 ABU and 88 CA-ABU *E. coli* strains isolated from the same geographic location. The majority of the strains belonged to phylogenetic group B2 (68% versus 8% for group A, 5% for group B1, and 19% for group D) (Table 1). The distribution of phylogenetic groups did not differ significantly between the ABU and CA-ABU strains (for ABU strains, 8% A, 6% B1, 68% B2, and 18% D; for CA-ABU strains, 9% A, 3% B1, 68% B2, and 19% D).

Resistance to antibacterial agents. We compared the antibiogram of each ABU and CA-ABU *E. coli* strain by examining for resistance to ampicillin, ceftazidime, cephalothin, cefazolin, ciprofloxacin, ceftriaxone, cefepime, ceftoxitin, gentamicin, cephalixin, meropenem, nitrofurantoin, norfloxacin, trimethoprim-sulfamethoxazole, trimethoprim, tobramycin, and piperacillin-tazobactam. There was no significant difference in the number of ABU and CA-ABU strains resistant to each antibiotic (Fig.

TABLE 1. Distribution of phylogenetic groups and virulence factors among ABU and CA-ABU *E. coli* isolates

Phylogenetic group, virulence gene, or phenotype	All strains				Ampicillin resistance				Cephalothin resistance						
	No. (%) of strains		<i>P</i> ^b	No. (%) of ABU strains		<i>P</i> for ABU strains	No. (%) of CA-ABU strains		<i>P</i> for CA-ABU strains	No. (%) of ABU strains		<i>P</i> for ABU strains	No. (%) of CA-ABU strains		<i>P</i> for CA-ABU strains
	ABU (<i>n</i> = 88)	CA-ABU (<i>n</i> = 88)		Susceptible (<i>n</i> = 45)	Resistant (<i>n</i> = 43)		Susceptible (<i>n</i> = 44)	Resistant (<i>n</i> = 44)		Susceptible (<i>n</i> = 40)	Resistant (<i>n</i> = 48)		Susceptible (<i>n</i> = 36)	Resistant (<i>n</i> = 52)	
Phylogenetic group															
A	7 (8)	8 (9)	NS	3 (7)	4 (9)	NS	3 (7)	5 (11)	NS	1 (3)	6 (13)	NS	4 (11)	4 (8)	NS
B1	5 (6)	3 (3)	NS	3 (7)	2 (5)	NS	3 (7)	0 (0)	NS	2 (5)	3 (6)	NS	2 (6)	1 (2)	NS
B2	60 (68)	60 (68)	NS	33 (73)	27 (63)	NS	28 (64)	32 (73)	NS	35 (88)	25 (52)	<0.001	22 (61)	38 (73)	NS
D	16 (18)	17 (19)	NS	6 (13)	10 (23)	NS	10 (23)	7 (16)	NS	2 (5)	14 (29)	0.005	8 (22)	9 (17)	NS
Virulence gene															
<i>fimH</i>	86 (98)	86 (98)	NS	45 (100)	41 (95)	NS	43 (98)	43 (98)	NS	40 (100)	46 (96)	NS	35 (97)	51 (98)	NS
<i>papG</i> I	3 (3)	0 (0)	NS	1 (2)	2 (5)	NS	0 (0)	0 (0)	NS	0 (0)	3 (6)	NS	0 (0)	0 (0)	NS
<i>papG</i> II	16 (18)	26 (30)	NS	8 (18)	8 (19)	NS	6 (14)	20 (45)	0.001	8 (20)	8 (17)	NS	7 (19)	19 (37)	NS
<i>papG</i> III	21 (24)	17 (19)	NS	13 (29)	8 (19)	NS	8 (18)	9 (20)	NS	15 (38)	6 (13)	0.006	9 (25)	8 (15)	NS
<i>papG</i> (all alleles)	37 (42)	38 (43)	NS	21 (47)	16 (37)	NS	14 (32)	24 (55)	0.031	22 (55)	15 (31)	0.025	15 (42)	23 (44)	NS
<i>fliC</i>	51 (58)	55 (63)	NS	25 (56)	26 (60)	NS	21 (48)	34 (77)	0.004	27 (68)	24 (50)	NS	23 (64)	32 (62)	NS
<i>focG</i>	14 (16)	20 (23)	NS	9 (20)	5 (12)	NS	9 (20)	11 (25)	NS	11 (28)	3 (6)	0.009	11 (31)	9 (17)	NS
<i>stxS</i>	8 (9)	7 (8)	NS	4 (9)	4 (9)	NS	5 (11)	2 (5)	NS	6 (15)	2 (4)	NS	2 (6)	5 (10)	NS
<i>afaldatABC</i>	7 (8)	7 (8)	NS	6 (13)	1 (2)	NS	2 (5)	5 (11)	NS	4 (10)	3 (6)	NS	4 (11)	3 (6)	NS
<i>mnbB</i>	1 (1)	3 (3)	NS	0 (0)	1 (2)	NS	2 (5)	1 (2)	NS	1 (3)	0 (0)	NS	2 (6)	1 (2)	NS
<i>hlyA</i>	17 (19)	24 (27)	NS	12 (27)	5 (12)	NS	12 (27)	12 (27)	NS	13 (33)	4 (8)	0.006	11 (31)	13 (25)	NS
<i>cbfI</i>	24 (27)	23 (26)	NS	12 (27)	12 (28)	NS	11 (25)	12 (27)	NS	15 (38)	9 (19)	0.049	10 (28)	13 (25)	NS
<i>fyuA</i>	69 (78)	73 (83)	NS	34 (76)	35 (81)	NS	34 (77)	39 (89)	NS	31 (78)	38 (79)	NS	26 (72)	47 (90)	0.026
<i>iutA</i>	31 (35)	43 (49)	NS	10 (22)	21 (49)	0.009	13 (30)	30 (68)	<0.001	15 (38)	16 (33)	NS	14 (39)	29 (56)	NS
<i>trnV</i>	44 (50)	37 (42)	NS	27 (60)	17 (40)	NS	17 (39)	20 (45)	NS	30 (75)	14 (29)	<0.001	16 (44)	21 (40)	NS
<i>kpsMT</i> II	45 (51)	63 (72)	0.005	18 (40)	27 (63)	0.033	29 (66)	34 (77)	NS	18 (45)	27 (56)	NS	20 (56)	43 (83)	0.006
<i>kpsMT</i> III	1 (1)	4 (5)	NS	0 (0)	1 (2)	NS	2 (5)	2 (5)	NS	0 (0)	1 (2)	NS	2 (6)	2 (4)	NS
<i>kpsMT</i> K1	11 (13)	28 (32)	0.002	7 (16)	4 (9)	NS	16 (36)	12 (27)	NS	5 (13)	6 (13)	NS	9 (25)	19 (37)	NS
<i>kpsMT</i> K5	24 (27)	34 (39)	NS	9 (20)	15 (35)	NS	12 (27)	22 (50)	0.029	10 (25)	14 (29)	NS	11 (31)	23 (44)	NS
Virulence gene score, median	5	6	NS	5	5	NS	4	6	0.017	7	4	0.004	6	6	NS
Phenotype															
Yeast agglut ^a	82 (93)	81 (92)	NS	43 (96)	39 (91)	NS	41 (93)	40 (91)	NS	37 (93)	45 (94)	NS	33 (92)	48 (92)	NS
MRHA	30 (34)	13 (15)	0.003	16 (36)	14 (33)	NS	7 (16)	6 (14)	NS	18 (45)	12 (25)	0.049	9 (25)	4 (8)	0.033
Hemolysis	18 (20)	16 (18)	NS	13 (29)	5 (12)	0.045	8 (18)	8 (18)	NS	14 (35)	4 (8)	0.003	8 (22)	8 (15)	NS
Mortality	60 (68)	63 (72)	NS	30 (67)	30 (70)	NS	34 (77)	29 (66)	NS	25 (63)	35 (73)	NS	24 (67)	39 (75)	NS
Biofilm	52 (59)	42 (48)	NS	26 (58)	26 (60)	NS	27 (61)	15 (34)	0.010	24 (60)	28 (58)	NS	20 (56)	22 (42)	NS

^a agglut., agglutination.
^b Only significant *P* values (*P* < 0.05) have been shown; nonsignificant values are labeled NS.

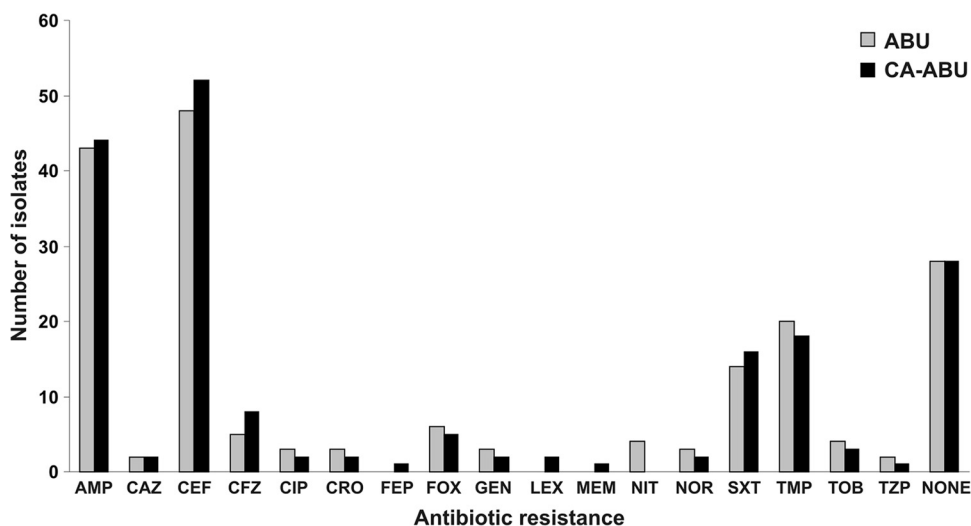


FIG. 1. Numbers of ABU and CA-ABU *E. coli* isolates resistant to each antibiotic included in the antibiotic screening. The “none” category indicates isolates that were susceptible to all antibiotics included in this study. There were no statistically significant differences between the numbers of resistant isolates in the ABU and CA-ABU groups for any antibiotic. AMP, ampicillin; CAZ, ceftazidime; CEF, cephalothin; CFZ, ceftazolin; CIP, ciprofloxacin; CRO, ceftriaxone; FEP, cefepime; FOX, ceftoxitin; GEN, gentamicin; LEX, cephalixin; MEM, meropenem; NIT, nitrofurantoin; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; TMP, trimethoprim; TOB, tobramycin; and TZP, piperacillin-tazobactam.

1). The antibiotics to which there was most commonly resistance were cephalothin (57% of all strains) and ampicillin (49% of all strains); 32% of the strains were susceptible to all of the antibiotics tested.

Distribution of virulence genes. The prevalence of 18 defined virulence marker genes in the 176 ABU and CA-ABU *E. coli* strains was assessed by PCR (Table 1). The median numbers of virulence genes for the ABU and CA-ABU strains were similar: five and six, respectively. The *fimH* gene was the most common virulence gene and was present in 98% of both ABU and CA-ABU strains. The *papG* gene was detected in 42% of ABU strains and 43% of CA-ABU strains. Genes for F1C fimbriae, S fimbriae, and the Afa/Dr family of adhesins were present in 8 to 23% of strains, with similar numbers observed for ABU and CA-ABU strains. The gene encoding the auto-transporter adhesin Ag43 (*flu*) was present in 60% of all strains, and the distributions were similar among the ABU and CA-ABU groups. Genes encoding the UPEC toxins hemolysin (*hlyA*) and cytotoxic necrotizing factor 1 (*cnf1*) were also found in similar numbers in ABU strains (19% *hlyA* positive, 27% *cnf1* positive) and CA-ABU strains (27% *hlyA* positive, 26% *cnf1* positive). Several siderophore receptor-encoding genes were examined; the *fyuA* gene (encoding the yersiniabactin receptor) was the most prevalent (78% of ABU strains, 83% of CA-ABU strains), while the *iutA* (encoding the aerobactin receptor) and *iroN* (encoding the salmochelin receptor) genes were present in 35 to 50% of ABU and CA-ABU strains (the differences were not significant). The only statistically significant differences in prevalence were observed for the *kpsMT* II gene, specific for group II capsule biosynthesis, and the *kpsMT* K1 gene, which were more frequently associated with CA-ABU isolates than with ABU isolates ($P = 0.005$ and $P = 0.002$, respectively). Overall, the virulence factor loads of isolates from both the ABU and CA-ABU groups were similar. The median numbers of virulence factors for each phylogenetic

group were as follows: for ABU strains, three virulence factors for group A, two for B1, seven for B2, and four for D; for CA-ABU strains, two virulence factors for group A, one for B1, seven for B2, and six for D.

Distribution of virulence genes among ampicillin- and cephalothin-resistant strains. Ampicillin and cephalothin were the antibiotics to which the *E. coli* strains examined in this study were most commonly resistant. There was a significant difference in the median number of virulence factors between ampicillin-resistant and ampicillin-susceptible CA-ABU strains (six versus four; $P = 0.017$). Ampicillin-resistant CA-ABU *E. coli* had a significantly higher prevalence of the *papG*, *flu*, *iutA*, and *kpsMT* K5 virulence genes (Table 1). There was no difference in the median number of virulence factors between ampicillin-resistant and ampicillin-susceptible ABU strains. The comparison between cephalothin-resistant ABU and CA-ABU strains revealed the opposite distribution: the median numbers of virulence factors for cephalothin-resistant and cephalothin-susceptible ABU strains were 4 and 7, respectively ($P = 0.004$), while there was no difference between cephalothin-resistant and cephalothin-susceptible CA-ABU strains. Genes found more commonly in cephalothin-susceptible strains than in cephalothin-resistant strains included *papG*, *focG*, *hlyA*, *cnf1*, and *iroN*.

Phenotypic characterization of ABU and CA-ABU strains. Many UTI *E. coli* strains carry virulence genes but fail to express the associated phenotype (26). Phenotypic assays were therefore performed to assess the expression of type 1 fimbriae, P fimbriae, and hemolysin (Table 1). The majority of *fimH*-positive ABU and CA-ABU strains agglutinated yeast cells (95% and 94%, respectively), demonstrating functional type 1 fimbrial expression. MRHA can be promoted by several different fimbrial types, including P, S, G, and M fimbriae, as well as the Afa/Dr family of adhesins (19). A higher percentage of ABU strains than CA-ABU strains showed an MRHA phenotype (34% and 15%, respectively; $P = 0.003$). More strains

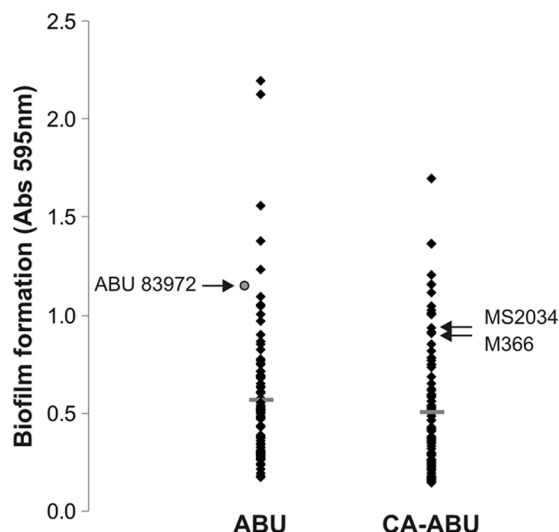


FIG. 2. Biofilm formation of ABU and CA-ABU *E. coli* isolates in synthetic urine. Strains were grown in PVC microtiter plates, washed, and stained with 0.1% crystal violet. The absorbance at 595 nm correlates to the amount of biofilm formed. The results are the averages of three independent experiments, each with four replicates. Horizontal bars indicate the mean for each infection category. The CA-ABU *E. coli* isolates selected for transcriptome analysis (M366 and MS2034) and the relative position of ABU *E. coli* strain 83972 are indicated.

contained the *papG* allele than expressed the associated MRHA phenotype: 65% ABU and 29% CA-ABU *papG*-positive strains expressed MRHA ($P = 0.002$). Hemolysin assays were also performed to determine if strains possessing *hlyA*

were hemolytic. Three *hlyA*-positive strains were nonhemolytic, while four strains that screened negative for *hlyA* were hemolytic. There was no significant difference in the number of hemolytic ABU strains (20%) and CA-ABU strains (18%).

Biofilm formation. The ability of each ABU and CA-ABU *E. coli* isolate to form a biofilm was assessed using the microtiter plate biofilm assay. The CA-ABU strains displayed no significant difference from the ABU strains in the biofilm formation phenotype; 48% of CA-ABU and 59% of ABU strains formed a biofilm (Table 1). The mean biofilm formation values were similar for the ABU and CA-ABU groups (0.58 versus 0.54; $P = 0.391$) (Fig. 2). To examine how these results compared with biofilm formation on a catheter surface, we selected 10 ABU and 10 CA-ABU strains that encompassed the range of biofilm formation observed on PVC microtiter plates and examined their biofilm formation phenotype using a catheter biofilm assay. Under these experimental conditions, all of the ABU strains formed a biofilm equivalent to that produced by the prototype ABU *E. coli* strain, 83972 (Fig. 3). In contrast, four of the CA-ABU strains showed significantly higher levels of biofilm growth on the catheter surface than *E. coli* 83972 ($P < 0.05$) (Fig. 3).

Global gene expression of CA-ABU *E. coli* strains during biofilm formation. To examine the similarities between the ABU and CA-ABU strains further, two CA-ABU strains (M366 and MS2034) with a limited repertoire of known adhesins and biofilm growth similar to that of prototype ABU *E. coli* strain 83972 were selected for further analysis (Table 2; Fig. 2). We examined the global gene expression profile of *E. coli* M366 and MS2034 during biofilm growth in pooled human urine as well as during planktonic growth in pooled human

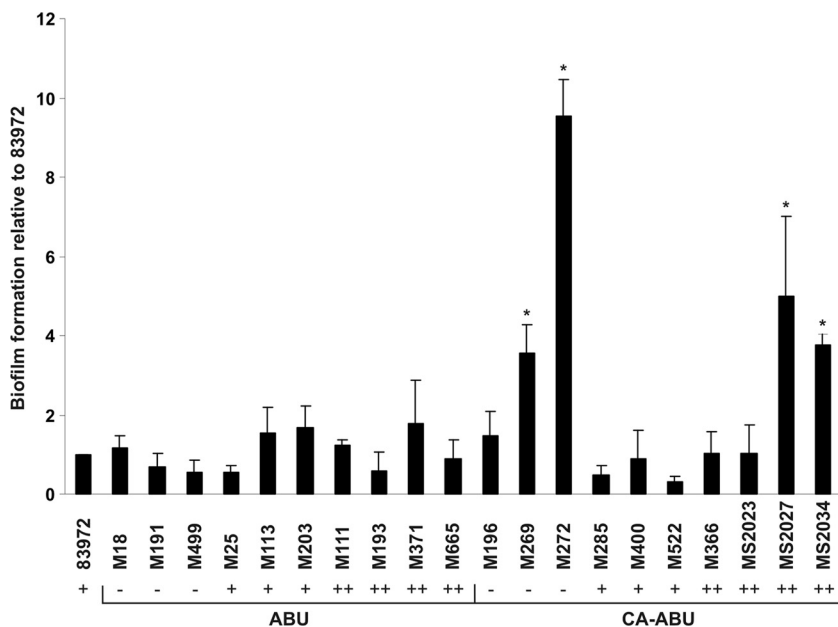


FIG. 3. Biofilm formation of selected ABU and CA-ABU *E. coli* isolates on Foley latex catheters. Catheter sections were incubated with isolates in human urine, and biofilm formation was assessed via colony counting. Biofilm formation as the number of CFU per 1-cm catheter section was standardized to that of ABU *E. coli* strain 83972. The results are the averages of at least duplicates, with error bars indicating standard deviations. The four CA-ABU isolates which had significantly higher levels of biofilm growth than ABU *E. coli* 83972 are marked with asterisks ($P < 0.05$). The corresponding amount of biofilm growth for each strain on PVC microtiter plates is also indicated and expressed as either low (-), medium (+), or high (++) .

TABLE 2. Characteristics of CA-ABU *E. coli* isolates selected for transcriptome analysis compared with those of ABU *E. coli* strain 83972^a

Characteristic	M366	MS2034	83972
Adhesins			
<i>fimH</i>	+	+	NF
<i>papG</i> I	-	-	-
<i>papG</i> II	-	-	-
<i>papG</i> III	-	-	NF
<i>flu</i>	-	-	+
<i>focG</i>	-	-	NF
<i>sfaS</i>	-	-	-
<i>afa/draBC</i>	-	-	-
<i>mrkB</i>	-	-	-
Toxins			
<i>hlyA</i>	-	+	NF
<i>cnfI</i>	-	+	+
Iron uptake systems			
<i>fyuA</i>	+	+	+
<i>iutA</i>	-	-	+
<i>iroN</i>	-	+	+
Capsular types			
<i>kpsMT</i> II	+	+	+
<i>kpsMT</i> III	-	-	-
<i>kpsMT</i> K1	+	+	-
<i>kpsMT</i> K5	-	-	+
Agglutination			
Yeast	+	+	-
MRHA	-	-	-
Hemolysis	-	-	-
Biofilm formation on PVC	+	+	+
Motility	+	+	-
Antibiotic resistance	CEF	AMP/CEF	-

^a All three strains are phylogenetic group B2. Abbreviations: NF, nonfunctional; CEF, cephalothin; AMP, ampicillin.

urine and in MOPS minimal medium. We then compared these data with those previously obtained for *E. coli* 83972 grown under the same conditions (11, 33).

Transcriptomic analysis of M366 and MS2036 revealed that there was considerable overlap with *E. coli* 83972 in the genes up- or downregulated during biofilm growth versus planktonic growth in urine and MOPS minimal medium (Fig. 4). Comparing biofilm and planktonic growth in urine, 69% and 57% of the upregulated genes in M366 were also upregulated in MS2034 and 83972, respectively; under the same conditions, 77% and 57% of the downregulated M366 genes were shared with MS2034 and 83972, respectively. Overall, the three strains shared 443 genes significantly upregulated and 498 genes downregulated during biofilm growth in urine compared to the level of gene expression during planktonic growth in urine (data not shown). The three strains also shared 420 genes significantly upregulated and 709 genes downregulated during biofilm growth in urine compared to the level of gene expression during planktonic growth in MOPS (data not shown). The majority of the top 50 upregulated genes of *E. coli* M366 during biofilm growth compared to the level of gene expression during planktonic growth were also upregulated in strains MS2034 and 83972. These genes included those encoding cold

and heat shock proteins, transcriptional regulators, enzymes, putative transport proteins, and iron uptake proteins.

We also compared the gene expression profiles of M366, MS2034, and 83972 according to functional group classifications (Table 3). The two CA-ABU strains had similar numbers of genes up- or downregulated as ABU strain 83972 for most functional groups. The most upregulated functional groups were transcription, intracellular trafficking and secretion, and signal transduction mechanisms. In contrast, the most downregulated functional groups were translation; amino acid transport and metabolism; and secondary metabolite biosynthesis, transport, and catabolism.

Upregulation of genes involved in iron uptake. We specifically examined the transcription of genes involved in iron uptake by *E. coli* M366 and MS2034 during biofilm growth in urine and compared this to transcription during planktonic growth in MOPS minimal medium (Fig. 5). We observed a consistent pattern of increased gene expression, with the most strongly expressed genes being associated with the enterobactin, SitABCD, and yersiniabactin pathways (for example, 12/19 yersiniabactin genes and *sitA* were in the top 50 upregulated genes of M366 during biofilm formation compared to the gene expression during planktonic growth in MOPS). We next compared these data with the transcription profile for *E. coli* 83972 grown under the same conditions. Unlike *E. coli* 83972, *E. coli* M366 and MS2034 did not express the genes encoding aerobactin synthesis, which is consistent with our PCR typing of these strains as *iutA* negative. *E. coli* M366 was also *iroN* negative, and we did not detect transcription of the *iroBCDEN* genes. However, a major difference between the M366 and MS2034 CA-ABU strains and ABU *E. coli* strain 83972 was the level of expression of the yersiniabactin genes. Most of the

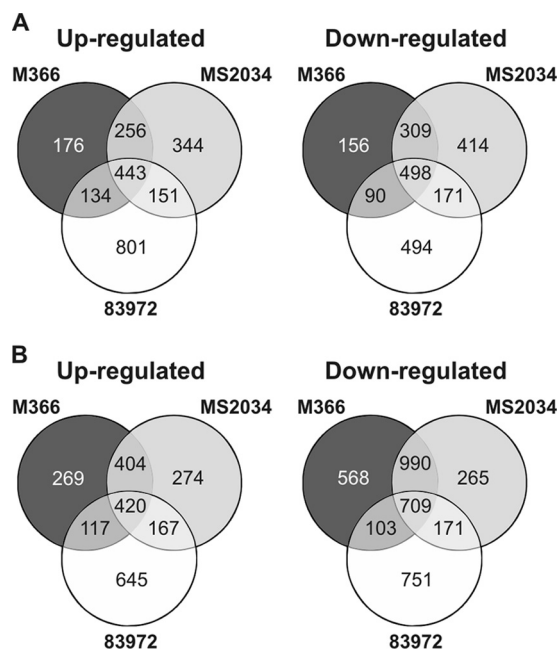


FIG. 4. Numbers of significantly up- and downregulated genes of *E. coli* strains 83972, M366, and MS2034 during biofilm growth compared to the level of gene expression during planktonic growth in urine (A) and MOPS minimal medium (B).

TABLE 3. Numbers of significantly up- and downregulated *E. coli* MG1655 genes in each functional group for the *E. coli* strains during biofilm growth in urine compared to the level of expression during planktonic growth in urine

Functional group	No. (%) of genes ^a						Total
	Upregulated genes in biofilm			Downregulated genes in biofilm			
	M366	MS2034	83972	M366	MS2034	83972	
Amino acid transport and metabolism	28 (9)	39 (13)	36 (12)	93 (31)	102 (34)	85 (29)	296
Carbohydrate transport and metabolism	39 (13)	40 (13)	37 (12)	81 (27)	94 (32)	90 (30)	298
Cell cycle control, mitosis, and meiosis	6 (19)	9 (29)	7 (23)	8 (26)	9 (29)	6 (19)	31
Cell motility	4 (4)	7 (8)	14 (15)	4 (4)	4 (4)	3 (3)	91
Cell wall/membrane biogenesis	25 (13)	28 (14)	28 (14)	45 (23)	54 (27)	37 (19)	200
Coenzyme transport and metabolism	22 (20)	24 (22)	23 (21)	16 (15)	24 (22)	16 (15)	108
Defense mechanisms	2 (6)	3 (9)	9 (26)	6 (17)	9 (26)	1 (3)	35
Energy production and conversion	25 (10)	21 (9)	32 (13)	71 (30)	84 (35)	74 (31)	240
Function unknown	45 (18)	52 (21)	65 (26)	50 (20)	61 (25)	43 (17)	247
General function prediction only	48 (18)	45 (17)	68 (25)	48 (18)	61 (23)	42 (16)	269
Inorganic ion transport and metabolism	24 (15)	32 (20)	40 (25)	22 (14)	34 (21)	18 (11)	160
Intracellular trafficking and secretion	9 (25)	13 (36)	8 (22)	5 (14)	4 (11)	7 (19)	36
Lipid transport and metabolism	12 (17)	12 (17)	11 (15)	12 (17)	18 (25)	17 (24)	71
Nucleotide transport and metabolism	19 (24)	19 (24)	14 (18)	10 (13)	20 (26)	13 (17)	78
Posttranslational modification, protein turnover, and chaperones	19 (16)	25 (21)	31 (26)	22 (18)	31 (26)	19 (16)	119
Replication, recombination, and repair	26 (16)	30 (18)	33 (20)	20 (12)	23 (14)	18 (11)	165
Secondary metabolites biosynthesis, transport, and catabolism	4 (7)	5 (9)	5 (9)	16 (30)	20 (37)	16 (30)	54
Signal transduction mechanisms	19 (16)	19 (16)	37 (32)	19 (16)	28 (24)	11 (9)	116
Transcription	65 (28)	73 (31)	74 (31)	24 (10)	35 (15)	26 (11)	235
Translation	40 (26)	48 (31)	26 (17)	20 (13)	22 (14)	55 (35)	156
Not in COGs ^b	175 (16)	190 (18)	217 (20)	100 (9)	164 (15)	126 (12)	1,065
MG1655 (total of above genes)	656 (16)	734 (18)	815 (20)	692 (17)	901 (22)	723 (18)	4,070

^a Values in boldface indicate the functional groups with the highest percentage of differentially regulated genes for each *E. coli* strain.

^b COGs, clusters of orthologous groups.

genes involved in the yersiniabactin biosynthetic pathway, as well as the *fyuA* receptor-encoding gene, were significantly upregulated in *E. coli* M366 and MS2034 compared to the level of expression in *E. coli* 83972 (Fig. 5). To examine if the biosynthesis of yersiniabactin affected biofilm formation, we deleted the *ybtS* gene from *E. coli* M366 and MS2034. There was no difference in biofilm growth between wild-type and *ybtS* mutant strains, suggesting that while the level of transcription of the yersiniabactin biosynthesis genes was increased, this was not an absolute requirement for biofilm growth by *E. coli* M366 and MS2034 under the experimental conditions employed.

DISCUSSION

In the present study, we compared *E. coli* strains that cause ABU and CA-ABU for differences in phylogenetic group, antibiotic resistance, virulence factors, and phenotypic properties (including biofilm formation). We then selected two CA-ABU strains and examined their global gene expression profile during biofilm growth in urine and compared this to the profile for the prototype ABU *E. coli* strain 83972.

Our analysis revealed that the majority of the ABU and CA-ABU strains belonged to phylogenetic groups B2 and D. These phylogenetic groups are most commonly associated with extraintestinal pathogenic *E. coli* (2, 23, 30, 39). In support of this typing, we also observed a widespread occurrence of virulence factors for ABU and CA-ABU strains. Of the 18 UTI-associated virulence genes examined, there was a similar prevalence rate for all genes except the group II capsule-specific *kpsMT* II gene and the K1 capsule marker gene *kpsMT* K1,

which were more prevalent within CA-ABU strains. This difference may reflect polysaccharide production during catheter colonization and biofilm growth by CA-ABU strains. Although there was no increased expression of capsule genes during biofilm growth *in vitro* for CA-ABU isolates M366 and MS2034, capsule production may be increased during host infection.

An interesting finding in our study was the observation that ABU and CA-ABU strains displayed very similar profiles with respect to antibiotic resistance. Ampicillin, cephalothin, trimethoprim, and trimethoprim-sulfamethoxazole were the antibiotics to which resistance was most commonly detected. This is consistent with the use of these antibiotics to treat nosocomial and community-acquired UTIs. The low levels of resistance to gentamicin and quinolone antibiotics reflect their restricted use in Australia. Previous studies have epidemiologically linked antimicrobial resistance in extraintestinal pathogenic *E. coli* strains with reduced inferred virulence, often due to a decreased prevalence of phylogenetic group B2 within antimicrobial-resistant strains (20, 37, 44). In our study, cephalothin-resistant ABU strains had a lower median number of virulence factors and a decreased prevalence of phylogenetic group B2 compared to the cephalothin-susceptible ABU strains. However, analysis of group B2 ABU strains independently revealed that the difference in phylogenetic grouping between resistant and susceptible strains did not account for the difference in inferred virulence (five and seven median virulence factors for group B2 cephalothin-resistant and -susceptible strains, respectively; $P = 0.016$). Interestingly, resistance to ampicillin among the CA-ABU strains followed a reverse trend with respect to the me-

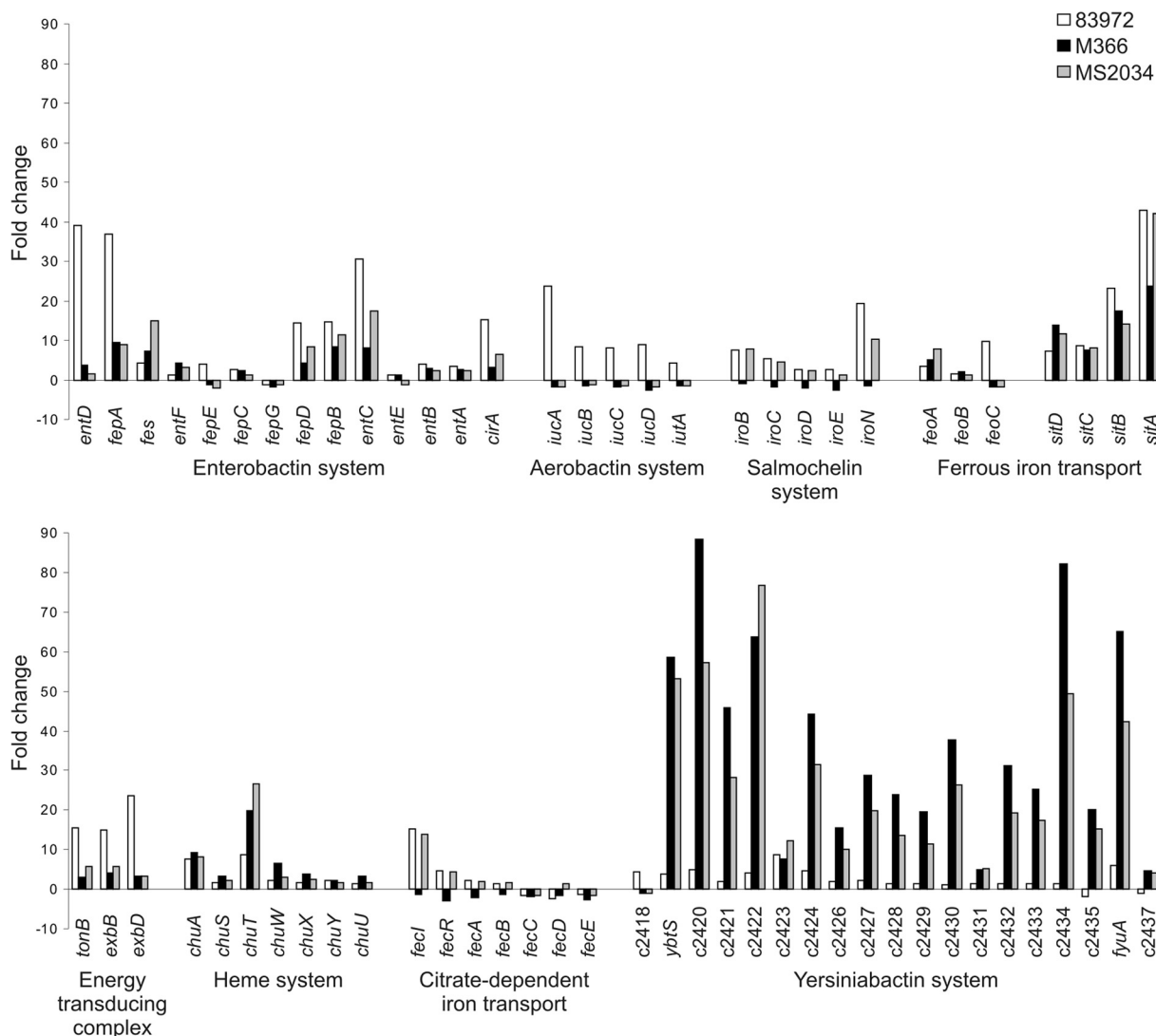


FIG. 5. Fold changes in the levels of expression of iron acquisition genes of *E. coli* strains 83972, M366, and MS2034 during biofilm growth in urine compared to the levels during planktonic growth in MOPS minimal medium.

dian number of virulence factors (four versus six for susceptible and resistant strains, respectively). Overall, the relationship between antimicrobial resistance and virulence in our isolate collection varied not only with the antimicrobial agent but also with the strain clinical origin.

Since UTI *E. coli* strains may possess virulence genes but fail to express the associated phenotype, several virulence-related phenotypes were examined. ABU and CA-ABU *E. coli* strains differed only with regard to MRHA, possibly due to the decreased number of *papG*-positive CA-ABU strains capable of MRHA under the conditions used for testing. While P fimbriae are not associated with persistence in healthy or catheterized urinary tracts, they have been shown to enhance the establishment of bacteriuria by ABU strain 83972 (1a, 28, 48). Type 1 fimbriae, as determined by yeast agglutination, were expressed by the majority of the ABU and CA-ABU *E. coli* strains. This fimbrial type, unlike P fimbriae, is associated with catheter colonization and persistence in the catheterized urinary tract

(28, 41). Furthermore, the introduction of a plasmid containing type 1 fimbrial genes increased catheter adherence of ABU strain 83972 up to 100-fold and improved its ability to prevent colonization by pathogenic *E. coli* (41).

CA-ABU results from the growth of bacterial biofilms on the inner surface of the urinary catheter. Although the molecular mechanisms of biofilm formation by *E. coli* in the catheterized urinary tract are not fully understood, surface adhesins such as type 1 fimbriae, type 3 fimbriae, and curli have been shown to contribute to biofilm growth (28, 29, 41, 43). In contrast, ABU *E. coli* strains are often associated with poor epithelial cell adhesion properties, and thus, the role of biofilm growth in colonization by these strains is less clear. Despite this, we found that the ABU and CA-ABU *E. coli* strains examined in this study possessed similar biofilm formation properties, as assessed by the PVC microtiter plate assay. Furthermore, we did not observe any significant difference in the adhesin gene repertoires of the ABU and CA-ABU strains. It

is possible that some ABU strains form biofilms within the bladder without invoking host responses through direct attachment to mucosal cell receptors or that biofilm growth in the urinary tract leads to reduced virulence gene expression and thus a decreased interaction with the host. Indeed, we previously found several ABU strains able to adhere to bladder epithelial cells without provoking a cytokine response (26).

To examine biofilm growth in more detail, we also tested a representative selection of ABU and CA-ABU strains for their ability to form a biofilm on a catheter surface in pooled human urine. All of the ABU strains formed a biofilm similar to that formed by *E. coli* 83972 in this assay. Among the CA-ABU strains examined, four strains displayed a significant increase in the level of biofilm formation relative to that of *E. coli* 83972. We have previously reported that *E. coli* strains which cause symptomatic and asymptomatic infections display different biofilm formation phenotypes in human urine on polystyrene microtiter plates and catheter surfaces (6). The results presented here suggest that while there is some degree of congruence between these two assays, some differences are also apparent at the strain level.

We further examined biofilm formation for selected CA-ABU strains using global gene expression analysis with comparison to the gene expression of prototype ABU *E. coli* strain 83972. The two CA-ABU strains (M366 and MS2034) selected were from the same phylogenetic group as ABU 83972 and also had a limited adhesin repertoire. There was a significant overlap in the genes up- or downregulated during biofilm growth compared to the gene expression during planktonic growth for the three *E. coli* strains, with 420 to 709 genes being shared between the three strains, depending on the comparison.

We focused on the transcription of genes involved in iron uptake, since free iron is strictly limited in the urinary tract. Efficient iron uptake has been suggested to be a mechanism by which ABU strains grow in urine and are maintained in the bladder (31, 33). One widespread method for iron acquisition is the production of siderophores, low-molecular-mass iron-chelating compounds, and their associated membrane receptors (27). We examined the transcription of genes involved in iron uptake by CA-ABU *E. coli* M366 and MS2034 with comparison to the transcription by ABU strain 83972. The majority of these genes were upregulated during biofilm growth compared to their level of expression during planktonic growth in MOPS minimal medium for all three *E. coli* strains. Most notably, the genes involved in the synthesis and transport of the siderophore yersiniabactin (including the receptor-encoding *fyuA* gene) were upregulated up to 88-fold in strains M366 and MS2034, with smaller changes (up to 9-fold) occurring in strain 83972. Increased expression of the yersiniabactin pathway genes has previously been shown for ABU *E. coli* 83972 and VR50 during biofilm formation (10, 11) and for *E. coli* 83972 during colonization of the human urinary tract (31). Additionally, the yersiniabactin receptor *FyuA* is required for efficient biofilm formation by strains 83972 and VR50 in urine (10). However, when the yersiniabactin synthesis gene, *ybtS*, was deleted in strains M366 and MS2034, no change in biofilm growth was observed. Although the siderophore yersiniabactin appears to be important for biofilm formation by ABU strain 83972, this was not seen in the CA-ABU strains examined here. Of note also is the fact that *E. coli* 83972 is unable to

express type 1, P, and F1C fimbriae (24, 32). In contrast, 93% and 34% of the ABU isolates in the present study expressed type 1 and MRHA fimbriae, respectively. This suggests that *E. coli* 83972 may use a different than that utilized strategy to colonize the urinary tract by other ABU strains.

Overall, the *E. coli* ABU and CA-ABU isolates in our collection had similar virulence profiles. Previous studies have compared the virulence properties of *E. coli* ABU isolates in noncatheterized patients to those of fecal isolates and isolates from patients with other clinical syndromes, including cystitis and pyelonephritis. Urinary isolates, including ABU *E. coli*, typically have higher inferred virulence than fecal isolates from healthy individuals (3, 18). Pyelonephritis isolates display the most consistent differences in virulence properties from those of ABU isolates, specifically, a higher prevalence of P-fimbrial genes and an increased ability to cause MRHA (18, 26). There is limited research on the virulence properties of *E. coli* isolates colonizing the catheterized urinary tract, despite the high numbers of catheterized patients and the high risk of developing bacteriuria once one is catheterized. The catheter literature has also been complicated by the lack of differentiation between bacterial isolates causing CA-ABU and catheter-associated symptomatic urinary tract infection (CAUTI), with guidelines recently being published to rectify this (13, 40). Comparative studies have been carried out mainly with patients with bladder dysfunction and intermittent catheterization where there were limited or no differences between the *E. coli* strains causing asymptomatic and symptomatic infections (8, 15). Since the insertion and presence of an indwelling catheter substantially change the urinary tract environment, we compared the virulence properties of *E. coli* isolates causing asymptomatic bacteriuria in catheterized and noncatheterized patients. An indwelling catheter predisposes an individual to infection by introducing bacteria into the bladder, causing damage to the uroepithelial mucosa, and providing a surface for biofilm formation and can also affect normal functioning and voiding of the bladder (7, 16). Despite this, we found little difference in the virulence properties between the *E. coli* ABU and CA-ABU isolates.

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