Clonal Composition of *Escherichia coli* Causing Community-Acquired Urinary Tract Infections in the State of Rio de Janeiro, Brazil

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Recent studies from North America and Europe have demonstrated community-wide clonal spread of uropathogenic *Escherichia coli* (UPEC). To investigate if a similar pattern of spread occurs in Brazil, we characterized UPEC from women with community-acquired urinary tract infection (UTI) in Rio de Janeiro. *E. coli* isolates from women with UTI in one public outpatient clinic were evaluated for antibiotic susceptibility, *E. coli* phylogenetic grouping, enterobacterial repetitive intergenic consensus (ERIC) 2 PCR and pulsed-field gel electrophoresis fingerprinting, and multilocus sequence typing. From March 2005 to November 2006, 344 patients were studied. Of these, 186 (54%) had confirmed UTI, 118 (63.4%) of which were caused by *E. coli*. More than 50% of these isolates were resistant to ampicillin and trimethoprim/sulfamethoxazole. Of these, 96 (81%) belonged to 19 ERIC2 clonal groups. The largest group included 15 isolates, all belonging to multilocus sequence typing group ST69 and phylogenetic group D; they had pulsed-field gel electrophoresis patterns sharing at least 89% similarity compared with the CgA reference strain ATCC BAA-457. CgA strains have been found to be widespread in the United States in the early 2000s. Clonal group *E. coli* strains accounted for a large proportion (52%) of all UTIs and 82% of the trimethoprim/sulfamethoxazole-resistant *E. coli* UTIs. Thus, as in North America and Europe, UPECs that cause UTI in Rio de Janeiro also show clonal distribution, and a substantial proportion of drug-resistant UTI is caused by a small set of genetically related *E. coli* strains.

Introduction

URINARY TRACT INFECTIONS (UTI) are among the most common bacterial infections worldwide. One-third of all women will have at least one UTI episode by the age of 24 years, and about 50% will have at least one episode of UTI in their lifetime.^{8,14,27} At the community level, UTI typically occurs as an endemic infection, but outbreaks caused by *Escherichia coli*, the most common etiologic agent, have been documented.²⁵ During the late 1980s, *E. coli* O15:K52:H1 caused a community-wide outbreak of UTI in South London,²⁵ United Kingdom, and Barcelona, Spain.²⁶

In 2001 Manges and colleagues described an *E. coli* clonal group, designated CgA, based on a characteristic enterobacterial repetitive intergenic consensus (ERIC)-PCR electrophoresis banding pattern in women with acute uncomplicated UTI at three different university communities in the United

States.¹⁸ CgA isolates belong to ST69 by multilocus sequence typing (MLST),³¹ ECOR phylogenetic group D,¹² have identical *fimH* single-nucleotide polymorphism,³² and share closely related pulsed-field gel electrophoresis (PFGE) patterns.¹⁸ CgA strains caused nearly 50% of the trimethoprim/sulfamethoxazole (SXT)-resistant *E. coli* UTI in one university community.¹⁸

CgA has been subsequently recovered from women with cystitis, pyelonephritis, and bloodstream infections from several regions of the United States and Canada.^{12,17,18,20,24} In 2005 Johnson and colleagues reported finding CgA among *E. coli* isolates from other parts of the world, including 3 from a collection of 60 *E. coli* isolates from urine samples of unknown origin obtained in Curitiba, Brazil.¹³ The observation of widespread community distributions of phylogenetically related strains of uropathogenic *E. coli* (UPEC) suggests that transmission could have occurred by person-to-person in the

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community or hospital spread, or from a common source in the community such as food and water. Whether this observation is limited to North America and Europe is not known.

In most communities, first-line empiric antimicrobial regimens have included SXT or fluoroquinolones.^{7,35} However, decreasing antibiotic susceptibility has become a worldwide concern.¹⁸ In Brazil data on antimicrobial susceptibility of UPEC compiled from clinical centers in 2003 showed resistance prevalence as high as 49.6% (ampicillin), 42.4% (SXT), and 10.1% (ciprofloxacin).¹ We hypothesized that in Brazil, the rapid rise in drug-resistant UTI incidence may be accounted for by the spread of a limited number of drug-resistant clonal UPEC strains. We conducted a study to examine the resistance prevalence and clonal composition of UPEC that cause community-acquired UTI in one outpatient clinic in Rio de Janeiro, Brazil.

Materials and Methods

Study design

A prospective cross-sectional study was conducted among women seeking care in one public walk-in clinic in Rio de Janeiro. The study included sampling from March to November 2005 and from March to November 2006.

Attending physicians were asked to refer to our study team members on site all patients older than 14 years with suspected and symptomatic UTI, Monday to Thursday from 7 AM to 5 PM. Written informed consent was obtained from each patient enrolled into the study. Subjects were asked about age, gender, UTI symptoms, UTI history, and medical history. Urine samples were collected from every subject for microbiologic and strain typing analyses. UTI was defined as complicated or uncomplicated, as described below. Study protocols were approved by the Committee for Protection of Human Subjects at the University of California at Berkeley and by the study site institutions' ethics committees.

Definitions

UTI was defined as the presence of typical symptoms of UTI and growth of one or two uropathogens in urine cultures with $>10^3$ colony-forming units (CFU)/ml.¹¹ Patients reporting a functional or structural urinary abnormality, a urinary stent or indwelling catheter, immunosuppression, diabetes, and pregnancy or any healthcare assistance were classified as having a complicated UTI. Patients reporting none of these symptoms were classified as having uncomplicated UTI.^{11,23,29}

Microbiological procedures

Urine specimen collection and bacterial identification. Immediately after collection, midstream urine samples were inoculated onto cystine–lactose–electrolyte-deficient agar medium and incubated at $36 \pm 1^\circ\text{C}$ for 24–48 h. Final culture results were recorded as positive (growth of one or two uropathogens), contaminated (growth of more than two bacterial species), or negative (no growth). Biochemical testing was used to identify the most common bacterial species known to cause UTI.^{16,22,36} Isolates were stored in suspensions containing skimmed milk (10%) and glycerol (10%) at -20°C for further testing.

Antimicrobial susceptibility testing. Susceptibility was determined by the disk diffusion test⁵; isolates exhibiting intermediate susceptibility were interpreted as resistant. Minimal inhibitory concentrations of SXT were determined by Etest (AB BIODISK, Solna, Sweden). Isolates were tested for extended-spectrum β -lactamase production with disks containing the combination of clavulanic acid and a third-generation cephalosporin.⁶ Multidrug resistance was defined as resistance to ≥ 3 of the nine groups of antimicrobial agents tested (aminoglycoside, carbapenem, cephem, folate pathway inhibitor, fosfomycin, β -lactam/ β -lactamase inhibitor combination, penicillin, nitrofurantoin, and quinolone).

Strain typing. *E. coli* isolates were typed by ERIC2-PCR and PFGE fingerprinting, phylogenetic grouping, and MLST. ERIC2-PCR was performed as previously described⁶; isolates were considered to belong to a single clonal group if they had an indistinguishable PCR banding pattern on visual inspection. PFGE of the *Xba*I-digested DNA was carried out as previously described,² and results were interpreted by visual inspection³³ and computer-assisted analysis with GelCompar II, version 4.01 (Applied Maths, Courtrai, Belgium), by the Dice index and the unweighted pair group method with arithmetic averages. *E. coli* main reference collection (ECOR) phylogenetic groups A, B1, B2, or D were determined by a previously reported triplex PCR-based assay.^{4,9} MLST was performed by a standard protocol (<http://mlst.ucc.ie/>). The CgA reference strain ATCC BAA-457 was included in all typing experiments for comparison.

Data analysis

Data were stored and analyzed with Epi Info version 3.3.2 (CDC, Atlanta, GA). Differences in categorical variables were analyzed by the chi-square test or Fisher's exact test. Distribution of patient's age was analyzed as a numerical variable by analysis of variance and Mann–Whitney *U* tests. A two-tailed *p*-value of ≤ 0.05 was defined as statistically significant.

Results

From March 2005 to November 2006, 344 of the 345 identified female outpatients agreed to enroll into the study (one patient declined to participate). A symptomatic UTI was confirmed in 186 (54.1%) of the 344 subjects. *E. coli* comprised 118 (63.4%) of the positive urine samples: 81 (68.6%) were uncomplicated UTI and 37 (31.4%) were complicated UTI (15 patients were exposed to healthcare assistance, 7 were pregnant, 6 had diabetes, 5 had urinary calculi, 2 had cancer, 1 had immunosuppressive disease, and 1 had urinary calculi and immunosuppressive disease). Patients with uncomplicated UTI were younger (median age 31 years old) than those with complicated UTI (median age 38 years old) ($p = 0.062$).

Antimicrobial susceptibility

High resistance prevalence was observed for ampicillin (77%) and SXT (52%) among *E. coli* isolates from patients with uncomplicated UTI, as for patients with complicated UTI (60% and 38%, respectively) (Table 1). All *E. coli* isolates resistant to SXT by disk diffusion had SXT minimal inhibitory concentrations $\geq 32 \mu\text{g/ml}$. A total of 28 *E. coli* isolates

TABLE 1. PREVALENCE OF RESISTANCE TO ANTIMICROBIAL AGENTS IN UROPATHOGENIC *ESCHERICHIA COLI*

Antimicrobial agent	Number (%) of resistant isolates ^a		
	Total (n = 118)	Uncomplicated UTI (n = 81)	Complicated UTI (n = 37)
Ampicillin	84 (71)	62 (77)	22 (60)
SXT	56 (48)	42 (52)	14 (38)
SXT and ampicillin ^b	51 (43)	38 (47)	13 (35)
Amoxicillin/ clavulanic acid	22 (19)	16 (20)	6 (16)
Nalidixic acid	12 (10)	5 (6)	7 (19)
Cefoxitin	9 (8)	4 (5)	5 (14)
Fluoroquinolones ^c	7 (6)	4 (6)	3 (8)
Cefuroxime	5 (4)	3 (4)	2 (5)
Fosfomycin	4 (3)	1 (1)	3 (8)
Nitrofurantoin	4 (3)	1 (1)	3 (8)
Amikacin	3 (3)	3 (4)	0
Gentamicin	3 (3)	2 (3)	1 (3)
Cefotaxime	2 (2)	1 (1)	1 (3)

^aIsolates exhibiting intermediate susceptibility were interpreted as resistant.

^bCoresistance to SXT and ampicillin.

^cFluoroquinolones: ciprofloxacin, levofloxacin, or norfloxacin.

All *E. coli* isolates were susceptible to ceftazidime and meropenem. *p* values are all >0.05.

UTI, urinary tract infection; SXT, trimethoprim/sulfamethoxazole.

were multidrug resistance: 18 (22.2%) of the 81 causing uncomplicated UTI and 10 (27.0%) of the 37 causing complicated UTI ($p=0.737$). Extended-spectrum β -lactamase production was not observed in any isolate.

Strain typing

ERIC typing revealed 19 distinct clonal groups among the 118 *E. coli* isolates. Each clonal group included 2–15 isolates. These groups accounted for 96 (81.4%) of the 118 isolates: 69% from uncomplicated UTI and 31% from complicated UTI (Table 2). The two largest groups had 15 (12.7%) and 14 (11.9%) isolates each (CgA and RCg1, respectively). All isolates that had the CgA by ERIC2-PCR pattern were typed by PFGE. The clonal group designated as CgA had an ERIC2-PCR band pattern indistinguishable (100% similarity) and PFGE patterns sharing at least 89% similarity compared with the CgA reference strain ATCC BAA-457 (11 isolates had ≤ 6 PFGE band differences). Comparisons within CgA isolates from Rio de Janeiro revealed five PFGE groups (total of 13 isolates) sharing >96% similarity. Among these groups, four were composed of two to four isolates with up to four different bands.

To investigate if CgA occurred in hospitals before the current study, we examined a collection of *E. coli* hospital isolates from a previous survey of β -lactamase-mediated resistance at a large urban university hospital⁶ after the results of this study were obtained. We found three CgA strains (7%) among 42 hospital-acquired *E. coli* isolates (obtained from UTI and central-vascular catheter-associated infections) in 2001. These three isolates were identical to each other by ERIC2-PCR and PFGE banding patterns and were 100% similar in ERIC2-PCR banding pattern and 89% similar

in the PFGE pattern to that of the ATCC BAA-457 strain. They also belonged to *E. coli* phylogenetic group D and ST69.

In the present study, women with UTI caused by CgA in the community were significantly younger (median age 25 years, range 15–45 years) compared to those with UTI caused by other isolates (median age 34 years, range 16–86 years, $p=0.022$). Only 1 woman with CgA and 28 women with non-CgA strains were >44 years of age. Among clonal groups with six or more isolates, CgA and RCg3 were each significantly ($p \leq 0.043$) more likely to be resistant to SXT and ampicillin than other groups; 93% of the CgA strains were resistant to SXT compared with 41% for all the other isolates ($p=0.0004$). On the other hand, only 14% of the RCg1 strains were resistant to SXT, compared with all the other clonal groups combined (52%, $p=0.001$).

Use of any antibiotic in the 6 months preceding the UTI was reported by 61 (52%) of the 118 women who had *E. coli* UTI, compared with 124 (55%) of the 226 women without UTI who were recruited into the study ($p=0.898$). The prevalence of resistance to ampicillin, cefoxitin, cefuroxime, fluoroquinolones, gentamicin, nalidixic acid, nitrofurantoin, and SXT was higher in patients who reported previous use of antibiotics ($\geq 58\%$) compared with those who denied previous use ($\leq 41\%$). This difference was not statistically significant for SXT-resistant *E. coli* ($p=0.639$), even after excluding CgA ($p=0.917$), but was significant for *E. coli* resistant to fluoroquinolones ($p=0.016$) (data not shown). For SXT, our study should have had enough power to detect a significant association: 30 of the 56 patients with SXT-resistant UTI, versus 31 of the 62 patients with a SXT-susceptible UTI used this antimicrobial agent. Eighty-one (69%) patients reported a previous history of UTI; 23 (28%) had UTI in the previous year. Resistance prevalences of isolates obtained from women with first UTI episode were not significantly different from those of women with recurrent UTI.

The occurrence of temporal clustering of patients within an ERIC2 clonal group (defined as two or more patients infected with the same clonal group visiting the walk-in clinic on the same day) showed that there were three such instances involving clonal groups CgA, RCg1, and RCg3. These clusters belonged to patients living at least 6 km apart.

Phylogenetic analysis

Most of the 118 *E. coli* isolates belonged to ECOR group B2 (51, 43.2%) and D (43, 36.4%) (Table 2). All phylogenetic group identifications were internally consistent within each ERIC-PCR clonal group. As expected, all 15 CgA isolates belonged to group D.¹² Indeed, isolates belonging to clonal groups were significantly associated with phylogenetic group D (41.7%) or A (17.7%) compared with nonclonal isolates (13.6%; $p=0.027$ and 0%; $p=0.039$, respectively). However, the association of clonal isolates with phylogenetic group D was entirely due to CgA. On the other hand, non-clonal isolates belonged significantly more often to group B1 (31.8%), compared with clonal isolates (0%; $p < 0.001$).

Isolates in each of the 19 ERIC2-PCR clonal groups fell into 19 distinct sequence types (STs) by MLST (Table 2). All STs were internally consistent within the ERIC2-PCR clonal group. As expected, all but one *E. coli* CgA strains belonged to ST69³¹; one isolate (HMMC366) had a polymorphism in the *icd* gene and was classified as ST828, an ST69 single-locus

TABLE 2. CHARACTERISTICS OF ERIC-2-PCR *ESCHERICHIA COLI* CLONAL GROUPS AMONG 118 *ESCHERICHIA COLI* ISOLATES

Clonal group ^a	Number (%)	ECOR (%)	ST	Number (%) of isolates resistant to the antimicrobial(s) indicated							
				AMP	AMC	SXT	FQ	NAL	NIT	MDR	Pan-S
CgA ^b	15 (12.7)	D	69 ^c	14 (93)	3 (20)	14 (93)	0	1 (7)	0	5 (33)	0
RCg1 ^b	14 (11.9)	B2	73	9 (64)	5 (36)	2 (14)	0	0	0	1 (7)	4 (29)
RCg2 ^b	11 (9.3)	A	10	8 (73)	2 (18)	4 (36)	0	0	0	3 (27)	3 (9)
RCg3	11 (9.3)	D	62	10 (91)	2 (18)	6 (55)	0	1 (9)	0	4 (36)	1 (9)
RCg4 ^b	6 (5.1)	B2	127	0	0	0	0	0	0	0	6 (100)
RCg5	5 (4.2)	D	59	1 (20)	0	0	0	0	0	0	4 (80)
RCg6	5 (4.2)	D	38	5 (100)	0	4 (80)	0	3 (60)	1 (20)	5 (100)	0
RCg7 ^b	4 (3.4)	B2	144	4 (100)	0	4 (100)	0	0	0	1 (25)	0
RCg8	4 (3.4)	A	93	4 (100)	0	4 (100)	0	0	0	0	0
RCg9	3 (2.5)	B2	550	2 (67)	2 (67)	1 (33)	0	0	0	1 (33)	0
RCg10 ^b	2 (1.7)	D	405	1	0	1	0	0	0	0	0
RCg11	2 (1.7)	B2	14	2	0	1	0	0	0	0	0
RCg12	2 (1.7)	B2	95	1	0	1	0	0	0	0	0
RCg13	2 (1.7)	B2	131	2	2	1	0	0	0	1	0
RCg14 ^b	2 (1.7)	D	354	2	2	2	2	2	2	2	0
RCg15 ^b	2 (1.7)	B2	421	1	0	0	0	0	0	0	1
RCg16	2 (1.7)	A	697	2	0	0	0	0	0	0	0
RCg17	2 (1.7)	B2	706	2	0	1	0	0	0	1	0
RCg18 ^b	2 (1.7)	B2	827	0	0	0	0	0	0	0	2
Nonclonal isolates	22 (18.6)	D (13.6) B2 (54.5) A (0) B1 (31.8)	ND	14 (64)	4 (18)	10 (46)	5 (23)	5 (23)	1 (5)	4 (18)	4 (18)

^aAll isolates were typed by ERIC2-PCR.

^bClonal groups with one to three isolates were from patients exposed to any healthcare assistance.

^cOne isolate showed identical sequences in all housekeeping genes but one (*icd*) that differed by one nucleotide, resulting in a new ST (828), single-locus variant of ST69.

ECOR, *E. coli* reference collection phylogenetic grouping (A, B1, B2, or D); ST, sequence type; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; FQ, fluoroquinolones (ciprofloxacin, levofloxacin, or norfloxacin); NAL, nalidixic acid; NIT, nitrofurantoin; MDR, multidrug resistant (resistant to three or more of the nine groups of antimicrobial agents tested); Pan-S, isolate susceptible to all 16 antimicrobial agents tested; isolates exhibiting intermediate susceptibility were interpreted as resistant; ND, not determined.

variant. Four new STs (ST697, ST706, ST827, and ST828) were detected among seven isolates, including each of two STs with a new allele (*mdh101* and *icd154*). These data were deposited into the *E. coli* MLST website database (<http://mlst.ucc.ie/>).

Discussion

In the present study, we observed that the resistance prevalence in uncomplicated UTI caused by *E. coli* in a community in Rio de Janeiro is among the highest described in the literature: > 51% for ampicillin and SXT, and 20% for amoxicillin/clavulanic acid. Our data show that these antimicrobial agents are no longer appropriate as empirical regimens. On the other hand, *E. coli* resistance prevalence for gentamicin, fosfomycin, nitrofurantoin, quinolones, and second- and third-generation cephalosporins were all ≤ 6%. Studies in 16 European countries, Canada, United States, Japan, and Russia between 1999 and 2006 have found these resistance rates to be substantially lower: 26–37% for ampicillin, 11–21% for SXT, and 3–16% for amoxicillin/clavulanic acid.^{10,15,21,30}

A distinct feature of the present study design was that we examined all urine samples from consecutively recruited women with suspected symptomatic UTI. Thus, the study avoided the usual sampling bias that arises in studies where

samples are collected only from women with complicated or UTI that did not respond to empirical treatment. No significant associations between resistant UTI and previous use of antimicrobial agents were detected for several drugs. On the other hand, the significant association between antimicrobial use and fluoroquinolone-resistant UTI indicates that this particular type of resistance may be emerging and being selected by the use of this drug in the human population.

The surprising finding in this study was the identification of clonally related UPEC strains called CgA among young women with UTI in Rio de Janeiro. Strains belonging to this group were first described in the United States in the early 2000s, from cases of cystitis, pyelonephritis, and hospitalized blood stream infections.^{12,17,18,20,24} They have also been detected in sewage effluents in the United States,³ as well as from patients with UTI in other countries.¹³ In the United States, a suggestion was made that CgA strains may have been spread by contaminated food products.¹⁹

CgA is defined as a clonal group because isolates show a high degree of genotypic similarity. The most likely explanation for this identity is a common ancestral origin within a reasonable, arbitrary, time span. However, the PFGE results lead to interesting speculations. Recently, a panel of experts proposed that isolates differing by up to four PFGE bands should be assigned to the same type.³⁴ By this criterion, four PFGE types were observed within the CgA isolates in the

community. CgA isolates may have been introduced several times in the community or a single ancestral introduced in a more distant timeframe led to the emergence of different PFGE types by the accumulation of mutations. However, at this time, we have no evidence of the mode of CgA introduction and spread in the community in Rio de Janeiro. An additional finding of the present study was the hospital spread of CgA: three hospital-acquired CgA isolates were clustered in time and had indistinguishable PFGE patterns. In this case, a common source or cross-transmission of CgA may have occurred among patients in the hospital. Community-acquired CgA and possibly other UPEC clonal groups may pose another source of resistant infections in hospitals.

In the present study, 81% of the 118 *E. coli* UTI during an 18-month period were caused by isolates included in 19 ERIC2 clonal groups; only 4 of these clonal groups accounted for 43% of the infections. In a university community at Berkeley, up to 57% of the 584 UTI during a 14-month period were caused by 35 *E. coli* ERIC2 clonal groups,²⁸ whereas in a university community in Montreal, only 14% of the 256 belonged to an ERIC clonal group during a 12-month study period.²⁰ Manges and colleagues pointed out that there is a correlation between an increased prevalence of clonal group UTI and the total number of UPEC isolates sampled.²⁰ Here even in a smaller number of UTI cases we studied, the prevalence of clonal groups was higher than that in Montreal or Berkeley. Drug resistance prevalence was also substantially higher in our samples than those in Montreal or Berkeley. Our observation suggests that the circulating clonal composition of UPEC can greatly affect drug resistance prevalence, and that the prevalence of clonal groups correlates with drug resistance prevalence. Thus, as in North America and Europe, community-acquired UTI, especially drug-resistant UTI, can occur as outbreaks in Brazil.

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Disclosure Statement

No competing financial interests exist.

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