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Antimicrobial Resistance of *Escherichia coli* Strains Causing Community-Acquired Urinary Tract Infections Among Insured and Uninsured Populations in a Large Urban Center

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Summary

We assessed the susceptibility of *Escherichia coli* strains causing community-acquired urinary tract infection (UTI) in a large urban center in Brazil, comparing two different populations (patients with health insurance vs. uninsured). 581 non-duplicate strains of *E. coli* were isolated. The prevalence of antimicrobial resistance was greater than 20% for ampicillin (51%), trimethoprim-sulfamethoxazole (43%), tetracycline (41%) and chloramphenicol (22%). Overall, 12% of the *E. coli* isolates were resistant to ciprofloxacin. Resistance prevalences to most antimicrobials were similar in the two study populations. Our data provide much needed information on the prevalence of antimicrobial resistance among *E. coli* causing community-acquired UTI in Brazil. Antimicrobial resistance among strains of *E. coli* causing community-acquired UTIs was relatively high, particularly resistance to ciprofloxacin.

***Key words:* *Escherichia coli,* antimicrobial resistance, community-acquired, urinary tract infection, Brazil.**

INTRODUCTION

Uncomplicated community-acquired urinary tract infection (UTI) is one of the most common bacterial infections in outpatients, and one of the major reasons for antibiotic prescription [1]. UTIs result in approximately 8 million physician visits and more than 100,000 hospital admissions per year in the United States (US) [1]. Foxman *et al.* estimated that by age 24, one-third of American women will have had at least one physician-diagnosed UTI with prescription medication and that the annual cost of these UTIs is almost $1.6 billion, with a substantial burden upon society [2].

The majority of UTIs arise in female outpatients, many of whom are treated empirically if their symptoms suggest acute uncomplicated bacterial cystitis [1,3,4]. This management is based on the narrow and predictable spectrum of etiologic agents that cause UTIs and their susceptibility patterns, with *Escherichia coli* as the primary etiologic agent [4-6]. However, as with many community-acquired bacterial infections, antimicrobial resistance among the pathogens that cause community-acquired UTIs is increasing [3,7 8], highlighting the need for regular surveys of bacterial resistance in the community to ensure adequate empirical therapy for such infections.

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Such surveys conducted in different regions of the world, show for example, that the prevalence of *E. coli* resistant to sulfamethoxazole-trimethoprim (SXT) varies considerably, with estimates ranging from approximately 10 to 50% [4,5,9,10]. In addition, SXT resistance has been found to be associated with concurrent resistance to other antibiotics, resulting in multidrug-resistant uropathogens [11-13]. In Canada, Zhanel *et al*. have demonstrated a correlation between ampicillin and SXT resistance in *E. coli*, as well as ampicillin and SXT resistance for ciprofloxacin-resistant *E. coli* [11].

In Brazil, some studies have reported the antimicrobial resistance of bacteria isolated from UTI, however, in most of them, the bacterial isolates were recovered from hospitalized subjects [10,14]. The aim of this study was to assess the susceptibility pattern of *E. coli* isolated from outpatients in an urban center in northeastern Brazil, comparing the prevalence of drug-resistant UTI between two different populations—one comprised predominantly of patients who are uninsured, and another who have health insurance coverage.

PATIENTS AND METHODS

This study was performed in two clinical microbiology laboratories in Salvador, a city of 3 million people in northeastern Brazil. One laboratory is located at Hospital Santo Antônio (Lab A), a public non-government hospital, and the other at Hospital São Rafael (Lab B), a private, non-profit hospital. In addition to having two separate catchment geographic areas, these two study sites were chosen to compare the prevalence of resistant UTI among an uninsured population (Lab A), and among a population with health insurance and better access to health care (Lab B). These two hospitals were separated by about 20 kilometers in the city.

Demographic data

Demographic data and information on whether the UTI was a nosocomial or a community-acquired infection was obtained by in-person interview of the subjects (Lab A), or from the laboratory urine culture request form (Lab B, where only specimens with complete request forms are processed).

Collection and identification of E. coli strains

Consecutive strains of *E. coli* isolated from patients with UTIs from July/2001 to September/2002 in the two participating centers were collected for the study. UTI was defined as a urine sample with colony count of $\geq 10^5$ cfu/ml in the presence of $\geq 10^4$ leukocytes/ml. The samples were cultured on MacConkey agar and colonies that were positive for lactose and indole were presumptively identified as *E. coli*. Only strains isolated from outpatients were included in this study. We excluded patients who had undergone hospitalization or any urinary tract procedure within the previous 30 days of enrollment into this study (data obtained at the patient interview in Lab A, or from the lab request form and hospital database search in Lab B).

Susceptibility testing

Lab A performed disk diffusion susceptibility testing as described by the National Committee for Clinical Laboratory Standards (NCCLS) [15] whereas Lab B conducted a proprietary susceptibility testing automated method produced by Vitek (bio Merieux, St. Louis, Mo.). Isolates of *E. coli* were tested against 16 antimicrobials (amikacin, ampicillin, cephalothin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, imipenem, nalidixic acid, nitrofurantoin, norfloxacin, tetracycline and SXT). Bacteria were classified as susceptible, intermediate or resistant in accordance with the criteria of the NCCLS (now CSLI) [15]. Multidrug resistance (MDR) was defined as resistance to three or more of the antimicrobials tested. For quality control purposes, the strain *E. coli* 25922 (from the American Type Culture Collection) was used as the reference strain. Intermediate susceptible strains were interpreted as being fully susceptible.

Statistical analysis

Completed questionnaires and laboratory reports were double entered into a computerized database to check for errors and internal consistency. The proportion of resistant strains was calculated by dividing the number of isolates that were resistant to each antimicrobial agent by the number of organisms that were tested against that antimicrobial agent. Statistical significance (two-tailed p-value <0.05) was assessed by χ^2 or Fisher's exact test for categorical variables.

RESULTS

A total of 581 non-duplicate, consecutive, strains of *E. coli* (Lab A, n=202; Lab B, n=379) were isolated from outpatients with UTIs in the study period. The sample was comprised of more women (81.8%) than men (17.7%). The age distribution of the patients ranged from 2 months to 95 years with a mean age of 43 years; no data were available on age for 37 patients. The age distribution by gender is summarized in *Table 1*. The majority of men were 60 years old or more (45.3%), while the most common age group among women was 20 to 39 years (31.3%). The age distribution was similar in the two study populations.

The prevalence of resistance for the *E. coli* isolates stratified by study site is provided in *Table 2*. The prevalence of antimicrobial resistance was

TABLE 1 - *Age distribution (%) of 544 study subjects by gender, Salvador, 2001-2002.*

Age (years)	Men (n=94)	Women (n=450)
≤6	7.5	10.3
7 to 19	5.4	8.3
20 to 39	18.3	31.3
40 to 59	23.6	25.7
≥60	45.3	24.5

Note: Age was unknown in 37 of 581 (6.4%) study participants.

greater than 20% for ampicillin (51%), SXT (43%), tetracycline (41%) and chloramphenicol (22%). Overall, 12% of the *E. coli* isolates were resistant to ciprofloxacin, ranging from 2% in children to 24% in men, with an intermediate value among women (11%). Resistance estimates did not vary significantly among men, women and children for ampicillin, SXT, tetracycline, chloramphenicol, gentamicin, amikacin, cefoxitin, ceftazidime, cefepime, imipenem and cephalothin, but resistance to norfloxacin (χ^2=9.93, p=0.007), ciprofloxacin (χ^2=17.7, p=0.0001), nitrofurantoin (χ^2=13.9, p=0.001) and nalidixic acid (χ^2=11.6, p=0.003) was significantly higher in men than in women or children. Resistance prevalences to some antimicrobials, including ciprofloxacin (11.2% in Lab A and 12.8% in Lab B) and norfloxacin (12.4% in both centers), were similar in the two study sites. However, among the male patients, resistance estimates to ampicillin, second/third/fourth generation cephalosporins, norfloxacin, ciprofloxacin, gentamicin, chloramphenicol, nalidixic acid, nitrofurantoin, and tetracycline were higher in those with health insurance coverage (Lab B) as compared to those uninsured (Lab A). Nevertheless, these differences were only significant for ampicillin (p<0.02) and tetracycline (p<0.004). In contrast, among female patients, resistance estimates to SXT and cephalothin were significantly higher in the public hospital (Lab A).

The number of agents to which isolates were resistant is shown in *Table 3*. Among the *E. coli* isolates tested, 32.5% were susceptible to all the agents studied, 13.6% were resistant to a single agent, and 17.9% were resistant to two antimicrobials (in both instances, predominantly ampicillin). MDR isolates accounted for 36.0% of the strains. The majority of the MDR strains were resistant to ampicillin (78 to 96%) or SXT (72 to 92%). Nearly one-third of the MDR isolates were resistant to ciprofloxacin (31%). The prevalence of MDR was comparable in the insured (34%) and uninsured (40%) study samples.

In *Table 4*, we listed the most common MDR phenotypes that were identified. Concurrent resistance to SXT, ampicillin, and tetracycline accounted

TABLE 2 - *Prevalence (%) of antimicrobial resistance in 544[a] community-acquired Escherichia coli urinary tract isolates in two populations by health insurance status, Salvador, Brazil, 2001-2002.*

Antimicrobials	Uninsured population (n=194)			Insured population (n=350)			Total (n=544)		
	Men (n=28)	Women (n=149)	Children[b] (n=17)	Men (n=58)	Women (n=255)	Children[b] (n=37)	Men (n=86)	Women (n=404)	Children[b] (n=54)
Amikacin	11.5	3.5	5.9	3.4	1.2	2.7	6.0	2.0	3.7
Ampicillin	28.6	55.0	58.8	55.2	49.0	62.2	46.5	51.2	61.1
Cefepime	0.0	1.3	0.0	1.8	1.2	0.0	1.2	1.3	0.0
Cefoxitin	3.6	2.7	5.9	5.3	1.6	2.8	4.7	2.0	3.8
Ceftazidime	0.0	0.7	0.0	1.8	0.8	0.0	1.2	0.8	0.0
Ceftriaxone/Cefotaxime	0.0	1.3	0.0	3.4	0.0	0.0	0.0	0.9	0.0
Cephalothin	14.8	19.3	25.0	13.8	6.5	19.4	14.1	11.2	21.2
Chloramphenicol	7.4	26.2	23.5	24.5	21.0	14.3	18.4	23.1	17.3
Ciprofloxacin	19.2	11.0	5.9	26.8	11.4	0.0	24.4	11.3	1.9
Gentamicin	0.0	0.7	0.0	7.0	2.3	0.0	4.7	1.7	0.0
Imipenem	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Nalidixic acid	17.9	12.4	5.9	31.3	15.2	5.7	26.3	14.0	5.8
Nitrofurantoin	3.7	0.7	0.0	14.9	2.9	6.1	10.8	2.0	4.1
Norfloxacin	14.3	13.1	5.9	25.0	12.1	0.0	21.1	12.5	1.9
Sulfamethoxazole-trimethoprim	39.3	50.0	29.4	39.7	39.2	56.8	39.5	43.2	48.1
Tetracycline	15.4	44.6	26.7	49.0	40.4	37.1	37.3	42.1	34.0

[a] Out of the 581 isolates, data on patient's age was available for 544. [b] Subjects age 6 years old or less.

TABLE 3 - *Resistance to antimicrobials among 581 Escherichia coli urinary tract isolates, Salvador, Brazil, 2001-2002.*

N. of agents to which isolates were resistant	Total % of isolates (n.)	% Isolates (n.) resistant to:				
		Ampicillin	Sulfamethoxazole-trimethoprim	Cephalothin	Ciprofloxacin	Nitrofurantoin
0	33 (189)					
1	14 (79)	43 (34)	15 (12)	3 (2)	1 (1)	3 (2)
2	18 (104)	78 (81)	63 (66)	10 (10)	3 (3)	0
3	15 (84)	86 (72)	76 (64)	20 (17)	10 (8)	0
4	10 (58)	86 (50)	84 (49)	35 (20)	16 (9)	3 (2)
5	3 (18)	83 (15)	72 (13)	44 (8)	56 (10)	17 (3)
6	4 (23)	78 (18)	78 (18)	17 (4)	57 (13)	4 (1)
≥7	4 (26)	96 (25)	92 (24)	31 (8)	92 (24)	31 (8)

Note: 36.0% of isolates were resistant to three or more antimicrobials and defined as multidrug resistant.

TABLE 4 - *Antimicrobial resistance phenotypes of multidrug resistant (MDR) Escherichia coli urinary tract isolates (n=209), Salvador, Brazil, 2001-2002.*

N. of agents to which isolates were resistant	Most common resistant pattern combinations	N. of isolates	% within group	% MDR isolates	% Total isolates*
3 (n=84)	SXT + AMP + TET	35	42	16.7	6.0
	SXT + AMP + CHL	10	12	4.7	1.7
4 (n=58)	SXT + AMP + TET + CHL	27	47	12.9	4.6
	SXT + AMP + TET + CFL	14	24	6.6	2.4
5 (n=18)	SXT + AMP + TET + CHL + CFL	04	22	1.9	0.6
	AMP + TET + CFL + CIP + NOR + NAL	02	11	0.9	0.3
6 (n=23)	SXT + TET + CHL + CIP + NOR + NAL	03	13	1.4	0.5
	SXT + AMP + TET + CHL + NOR + NAL	03	13	1.4	0.5
≥7 (n=26)	SXT + AMP + TET + CHL + CIP + NOR + NAL	14	54	6.6	2.4
	SXT + AMP + TET + CHL + CIP + NOR + NAL + NIT	04	15	1.9	0.6

*Total number of isolates=581.

SXT= Sulfamethoxazole-trimethoprim; AMP=Ampicillin; TET=Tetracycline; CHL=Chloramphenicol; CFL=Cephalothin; CIP=Ciprofloxacin; NOR=Norfloxacin; NAL=Nalidixic acid; NIT=Nitrofurantoin.

for 48.3% of the MDR isolates. Interestingly, among the *E. coli* resistant to three, four, and seven or more agents, 42%, 47% and 54%, respectively, had the same antimicrobial resistance phenotype. Of ciprofloxacin-resistant (n=68), norfloxacin-resistant (n=64) and nitrofurantoin-resistant (n=16) isolates, 94.1%, 98.4% and 87.5% were MDR, respectively. In contrast, for ampicillin-resistant (n=295) and SXT-resistant (n=246) isolates, 61% and 68.3% were MDR, respectively. The frequency of MDR phenotypes did not vary significantly between the two study sites.

DISCUSSION

The purpose of the present study was to describe the susceptibility pattern of *E. coli* isolated from two groups of outpatient populations (insured and uninsured) in a large urban center in Brazil, focusing on the prevalence of MDR isolates and on quinolone resistance. As the majority of therapy for UTIs is empiric and the urinary tract pathogens have shown increasing antimicrobial resistance in Brazil in the past years [10,14], continuous surveillance of antimicrobial susceptibility patterns would be beneficial to the management of community-acquired UTIs.

The overall prevalence of resistance to ampicillin (51%), SXT (43%) and ciprofloxacin (12%) found in our study was substantially higher than those reported by other studies from Canada (SXT 19% and ciprofloxacin 1%)[11], United States (SXT 16-18% and ciprofloxacin 0%) [3,6] and northern Europe (SXT 13% and norfloxacin 0%) [16-18]. However, they were similar to those reported from Spain (SXT 32% and

ciprofloxacin 13%) [18,19] and lower than those from Bangladesh (SXT 60% and ciprofloxacin 18%) [20]. The prevalence of ciprofloxacin-resistant isolates was higher among males than females. This may reflect the greater risk of males to present more often with complicated UTI, which may be associated with increased antimicrobial use and selection of resistant pathogens. It may also be that females and children have had less exposure to quinolones, as usually these compounds are not recommended in such populations (especially children). The current data also demonstrate that a ciprofloxacin-resistant phenotype without MDR is uncommon, as described previously by US investigators [12]. The level of quinolone resistance in our study reinforces the caution given concerning the use of quinolones in uncomplicated UTIs and the need for further risk factor investigation [18,21,22].

Our data provide much needed information on the prevalence of antimicrobial resistance among *E. coli* causing community-acquired UTI in Salvador, Brazil. In particular, we demonstrate distinct differences in the pattern of resistance between populations with or without health insurance. The prevalence of resistance to ciprofloxacin or norfloxacin was similar in the two populations overall. However, among men, resistance estimates to ampicillin and tetracycline were significantly higher in the insured population, whereas among women resistance estimates to SXT and cephalothin were significantly higher in the uninsured population. These differences in resistance prevalence may be important, because the availability of health insurance could influence the choice or availability of antimicrobial drugs to these populations, which in turn, could affect the pattern of drug susceptibility of *E. coli* isolates from these two populations. However, these differences could also reflect differences in the antimicrobial drug prescribing patterns of the physicians in the two hospitals that are separated geographically. Lastly, these differences might also be the result of chance alone. In any case, the knowledge of these differences in resistance prevalence is important for the empirical decision to initiate UTI treatment for patients who attend these two hospitals. Further studies are needed to investigate these specific questions.

The continued evolution of antimicrobial resistance and the current prevalence of MDR among community-acquired *E. coli* isolates are worrisome and mandate both further surveillance and new approaches to slow the emergence or resistance.

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