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SUSCEPTIBILITY TO ANTIBIOTICS IN URINARY TRACT INFECTIONS IN A SECONDARY CARE SETTING FROM 2005-2006 AND 2010-2011, IN SÃO PAULO, BRAZIL: DATA FROM 11,943 URINE CULTURES

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SUMMARY

Introduction: Urinary tract infection (UTI) has a high incidence and recurrence, therefore, treatment is empirical in the majority of cases. **Objectives:** The aim of this study was to analyze the urine cultures performed at a secondary hospital, during two periods, 2005-2006 and 2010-2011, and to estimate the microbial resistance. **Patients and methods:** We analyzed 11,943 aerobic urine cultures according to basic demographic data and susceptibility to antibiotics in accordance with the Clinical and Laboratory Standards Institute (CLSI) for Vitek 1 and 2. **Results:** Most of our cohort consisted of young adult females that were seen at the Emergency Department. *E. coli* was the most frequent (70.2%) among the 75 species isolated. Resistance of all isolates was $\geq 20\%$ for trimethoprim/sulfamethoxazole (TMP/SMX), norfloxacin, nitrofurantoin, cefazolin and nalidixic acid. Although *E. coli* was more susceptible (resistance $\geq 20\%$ for TMP/SMX and nalidixic acid) among all of the isolates, when classified by the number and percentage of antibiotic resistance. Global resistance to fluoroquinolones was approximately 12%. Risk factors for *E. coli* were female gender and an age less than 65 years. Men and patients older than 65 years of age, presented more resistant isolates. Extended spectrum beta-lactamases (ESBL) were identified in 173 out of 5,722 Gram-negative isolates (3.0%) between 2010 and 2011. **Conclusion:** *E. coli* was the most frequent microbe isolated in the urine cultures analyzed in this study. There was a significant evolution of bacterial resistance between the two periods studied. In particular, the rise of bacterial resistance to fluoroquinolones was concerning.

KEYWORDS: Urinary tract infection; Bacteria; Susceptibility test; Urine culture; Brazil.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections diagnosed in patients, ranking first in the United States of America (USA). UTIs are characterized by high incidence, high recurrence and a mild severity of evolution in most cases. Because of the uncomfortable symptoms they cause, they often force physicians to introduce antibiotic treatment prior to obtaining the culture results^{14,15}. The resolution of between 25% and 42% of cystitis UTIs in the placebo arms of randomized controlled trials, with little incidence of evolution to complicated infections and a proportion of one case of pyelonephritis per every 28 cases of cystitis, confirms the mild character of UTIs¹⁵. Current guidelines establish that lower UTIs in young women should be treated empirically with short-term antibiotics. Therefore, it is important that the antimicrobial susceptibility data of major uropathogens are known.

In particular, given that it is an infection that is most often treated empirically, data on major uropathogens should be systematically and regularly analyzed. However, in Brazil, there is a paucity of such data.

Despite the guidelines, requests for urinalysis and microscopic examination of urine (pyuria, leukocyte esterase, nitrite, and the presence of bacteria) as well as requests for urine culture for diagnosis of uncomplicated UTIs, is common among physicians in Brazil^{14,23}. As a result, many cases that should be treated in a primary setting care are unnecessarily referred to the emergency department of secondary hospitals, resulting in increased health care costs. In clinical practice, physicians and their patients commonly insist that urine tests are necessary for the diagnosis of uncomplicated UTIs. The greatest proof of these assertions is evident from retrospective studies in several

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regions of Brazil, which were only possible because of this “breach of protocol”^{2,4,5,9,10,19,20,22,24,25,26}.

We hypothesized that, in recent years in our environment, the microbiological profile of isolated uropathogens has become increasingly worse. Recent literature¹⁵ postulates a fluoroquinolone sparing protocol; however, if more than 20% of uropathogens are resistant to a drug, it cannot be used empirically. Therefore, the aim of this study is to retrospectively analyze the bacterial susceptibility data of uropathogens isolated from patients at a university community hospital in the last decade and to establish a new treatment protocol in this healthcare setting.

MATERIALS AND METHODS

The University Hospital of São Paulo is a 246-bed secondary care setting, one of the teaching hospitals of the University of São Paulo School of Medicine, and serves a population of approximately 500,000 inhabitants in the western area of the city of São Paulo. The hospital Emergency Department serves approximately 800 patients a day.

This is a retrospective study, which included positive urine cultures from patients who were collected during the periods from 2005 to 2006 and from 2010 and 2011, at the University Hospital in the Emergency Department, Outpatient Clinics, and from external patients. The aim of the study was to compare resistance to antimicrobial agents during two time periods, regardless of whether more than one urine culture per patient was collected during those time periods. The samples were processed and analyzed at the Section of Microbiology of the Clinical Laboratory of the University Hospital.

Only aerobic bacterial infections were analyzed in this study. Anaerobic bacterial infection, fungal infection and negative cultures were excluded from this study. Positive urine cultures were obtained by consulting Apolo software (Oracle Corporation, Redwood City, California, USA), without consulting the medical records of the patients, except for information about patients with urine culture with count < 100,000 colony forming units (CFU) per milliliter of urine.

Urine collected by all methods during these time periods was included, although the standardized laboratory method is to collect the specimen urine midstream.

Urine cultures were taken quantitatively distributed on blood agar and MacConkey agar plates with a calibrated loop with a volume of 10 µL (references 35095 and 35092, respectively, bioMérieux, Rio de Janeiro, Brazil). CPS ID2 chromogenic agar (bioMérieux, Rio de Janeiro, Brazil) has been used in the laboratory since 2012. Urine cultures with a count ≥ 100,000 colony forming units (CFU) per milliliter of urine were considered positive after incubation in a bacteriologic incubator at 35 °C for at least 24 hours²⁷. This study also included as positive counts > 1,000 CFU and < 100,000 CFU of less than or equal to two infectious agents isolated (only pure or mixed culture) in the analysis because the patients presented symptoms of a UTI, which was documented in their chart¹⁶. Only one the most significant germs isolated between two agents from each agar plate was considered in the analysis.

Identification of bacterial species and an antibiogram was performed by Vitek 1 (from 2005 to 2006) and by Vitek 2 (from 2010 to 2011)

(bioMérieux, St. Louis, Missouri, USA). Card N104 was used in Vitek 2. Detection of extended spectrum beta-lactamases (ESBL) was performed on the samples from 2010 to 2011 by Vitek 2.

The standardization of the antibiogram followed the standards and recommendations of the Clinical and Laboratory Standards Institute (CLSI), documents M100-S15 (2005)⁵, M100-S16 (2006)⁶, M100-S20 (2010)⁷ and M100-S21 (2011)⁸. Antibiograms of nonfermenting Gram-negative agents were confirmed by the E-test® (AB Biodisk, Solna, Sweden) and/or Kirby-Bauer disk diffusion¹⁸ methods, according to the same standards. No value of the minimum inhibitory concentration was computed in the analysis due to the lack of reliability of the automated antibiogram, especially the values obtained by Vitek 1. ATCC (American Type Culture Collection) strains of *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* ESB (ATCC 700603), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853) and *Shigella sonnei* (ATCC 25931) were used for quality control of Vitek analysis. For analysis purposes, we considered the frequency of susceptibility rather than resistance, which is different from most studies in the literature because the semiquantitative antibiogram also measures intermediate susceptibility. For the purposes of the analysis, intermediate susceptibility was included as resistance, as occurs in clinical practice.

A review of the literature was performed in MEDLINE with the terms “outpatient UTI” and “resistance to antibiotics” and “Brazil”, as well as LILACS and SciELO (Scientific Electronic Library Online), which are Latin American scientific databases.

ETHICS

This study was approved by the Research Ethics Committee (Advisory Board) of the University Hospital of the University of São Paulo.

STATISTICAL ANALYSIS

Demographic data (sex, age and setting of the healthcare entrance at the hospital) and periods were submitted to exploratory data analysis and the assumption of normality. Numerical data were presented as the medians and interquartile ranges (IQR: Q1 and Q3) because the data were non-normal. Categorical data (sex, setting and susceptibility to antibiotics) were presented as the number and percentage. The Mann-Whitney U test was used for comparison of numerical non-normal variables. The Pearson uncorrected chi-square (χ^2) or Fisher’s exact tests were used for comparison of susceptibility between the two periods (2005-2006 vs. 2010-2011). A crude (unadjusted or univariate) odds ratio, 95% confidence interval and *p*-value were used to assess risk factors for infection by the most frequently isolated bacteria. All tests were two-sided. Statistical significance was considered at *p* < 0.05. Statistical analysis was performed using the statistical software SPSS 10.0 for Windows (IBM, Chicago, IL, USA).

RESULTS

In both periods analyzed, 11,943 aerobic urine cultures were obtained, 5,755 (48.2%) from the period between 2005 and 2006 and 6,188 (51.8%) from the period between 2010 and 2011. This is an average of

7.88 positive urine cultures per day during the first period and 8.40 in the second. Only 849 (7.1%) cultures presented counts less than 100,000 UFC in the first period (2005-2006).

The median age of patients was 36 years old (IQR: 20-65 years), ranging from zero to 100 years of age. There was a statistically significant difference between the median age of men and women in the study: 57.5 years old (IQR: 14.25-76 years) vs. 32 years old (IQR: 21-59 years), respectively; $p < 0.0001$.

By age group, among patients 0 to 14 years old, the frequency of positive urine cultures was 2,007 (16.8%); 15 to 64 years, 6,834 (57.2%); and ≥ 65 years, 3,102 (26.0%). The female-to-male ratio per age strata was the following: 0.73 (206/283) for < 2 years old; 3.60 (267/74) for $\geq 2-5$ years; 5.20 (229/44) for $>5-14$ years; 6.25 (2,794/447) for $\geq 14-64$ years; and 1.64 (877/534) for > 65 years ($p < 0.0001$, Pearson chi-square).

The frequency of females was 77.6%, with females between the ages of 14 and 64 representing 23.4% of the total. Among the patients studied, the majority of samples were collected in the Emergency Department, 8,931 (74.8%). Other samples, 1,944 (16.3%), were collected from the outpatient Clinic, and others, 1,059 (8.9%), were considered "external" (patients from primary care units in the western region of São Paulo).

It was not possible to calculate the positivity rate of the urine cultures. Of all of the isolates, 10,891 (91%) were Gram-negative, of which, 10,704 were Enterobacteriaceae (98.3%) and 187 (1.7%) were nonfermenting Gram-negative bacteria (mainly *Pseudomonas aeruginosa*, 132; *Acinetobacter baumannii*, 36; and *Stenotrophomonas maltophilia*, 7). Seventy-five different bacterial species were isolated from the urine cultures (Table 1), but the most frequently observed species were *Escherichia coli* (8,376, 70.2%). The order of frequency of bacteria was statistically significant and varied between men and women ($p < 0.0001$) (Table 2).

The main risk factor for *E. coli* and *Staphylococcus saprophyticus* infection was female sex (Table 2) [crude odds ratio (OR) = 3.60, 95% CI (confidence interval): 3.30 to 3.90; $p < 0.0001$ and OR = 10.37, 95% CI: 3.84-28.0; $p < 0.0001$, respectively]. Both bacteria were also more common in younger patients. *E. coli* was more common in patients less than 65 years old [(OR) = 1.98 (95% CI: 1.81 to 2.16); $p = 0.033$], and *S. saprophyticus* was more prevalent in patients aged 14 to 65 years [OR = 8.62 (95% CI: 4.77-15.58); $p < 0.0001$]. For *Proteus mirabilis*, the main risk factor was male sex, even though an age of less than 65 years (OR = 1.20, 95% CI: 1.01 to 1.44; $p = 0.033$) was also a risk factor. In the stratum of ages 0-14, we observed the largest male-to-female ratio (1.7), whereas in the 15-64 year stratum it was 0.15, and in the older age stratum (≥ 65 years) it was 0.61.

Male sex was also a risk factor for the following less frequent bacteria: *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *P. aeruginosa*, *Enterobacter cloacae*, *Citrobacter freundii* and *Morganella morganii* (Table 2). The crosstabulation between stratum of age and gender resulted in a male-to-female rate in the age range of 65 years or greater divided per bacteria: 0.32 (*E. coli*), 1.023 (*E. faecalis*), 1.65 (*E. cloacae*), 1.625 (*C. freundii*), 3.25 (*Staphylococcus epidermidis*), 1.81 (*Klebsiella oxytoca*), 2.22 (*E. aerogenes*), 2.26 (*S. aureus*), and 1.875 (*Citrobacter koseri*).

Table 1

Frequency of species among 11,943 bacteria isolated from urine cultures collected between 2005-2006, and 2010- 2011 at the University Hospital of the University of São Paulo (HU-USP)

Order	Bacteria isolated in urine culture	Frequency (n)	Percentage (%)
1°	<i>Escherichia coli</i>	8,376	70.2%
2°	<i>Proteus mirabilis</i>	795	6.8%
3°	<i>Klebsiella pneumoniae</i>	692	5.9%
4°	<i>Enterococcus faecalis</i>	322	2.8%
5°	<i>Enterobacter aerogenes</i>	187	1.7%
6°	<i>Staphylococcus saprophyticus</i>	148	1.2%
7°	<i>Pseudomonas aeruginosa</i>	132	1.1%
8°	<i>Enterobacter cloacae</i>	126	1.1%
9°	<i>Citrobacter freundii</i>	122	1.0%
10°	<i>Morganella morganii</i>	104	0.9%
11°	<i>Staphylococcus aureus</i>	98	0.8%
12°	<i>Staphylococcus epidermidis</i>	85	0.7%
13°	<i>Citrobacter koseri</i>	82	0.7%
14°	<i>Staphylococcus</i> sp. DNase negative	65	0.5%
15°	<i>Staphylococcus warneri</i>	62	0.5%
16°	<i>Serratia marcescens</i>	62	0.5%
17°	<i>Klebsiella oxytoca</i>	58	0.5%
18°	<i>Streptococcus agalactiae</i>	43	0.4%
19°	<i>Staphylococcus hominis</i>	38	0.3%
20°	<i>Acinetobacter baumannii</i>	36	0.3%
21°	Others	310	2.1%
Total		11,943	100.0%

Notes: 1: neg.: negative, or *Staphylococcus* spp., neither classified as *S. aureus* (DNase positive) nor had a specie discrimination, due to lack of discriminatory power by automated method (Vitek 1 or 2).

Appendix: Another isolates, in order of frequency, from the twenty-first: *Staphylococcus haemolyticus*, *Enterococcus faecium*, *Streptococcus viridans*, *Streptococcus mitis*, *Proteus vulgaris*, *Staphylococcus simulans*, *Providencia stuartii*, *Providencia rettgeri*, *Staphylococcus sciuri*, *Salmonella* spp., *Stenotrophomonas maltophilia*, *Staphylococcus auricularis*, *Staphylococcus capitis*, *Citrobacter amalonaticus*, *Proteus penneri*, *Raoultella ornithinolytica*, *Raoultella planticola*, *Staphylococcus xylosus*, *Enterobacter asburiae*, *Enterococcus* spp., *Serratia fonticola*, *Serratia liquefaciens*, *Streptococcus anginosus*, *Streptococcus sanguinis*, *Aeromonas* spp., Gram-negative (not discriminated), *Burkholderia cepacia*, *Citrobacter braakii*, *Citrobacter* spp., *Enterobacter gergoviae*, *Enterobacter* spp., *Pseudomonas* spp., coagulase-negative *Staphylococcus* spp., *Staphylococcus intermedius*, *Staphylococcus lentus*, *Staphylococcus lugdunensis*, *Streptococcus bovis*, *Streptococcus* spp. galloyticus var. galloyticus, *Acinetobacter haemolyticus*, *Acinetobacter lwoffii*, *Acinetobacter* spp., nonfermenting Gram-negative bacteria, *Enterobacter sakazaki*, *Enterococcus hirae*, *Klebsiella ornithinolytica*, *Pantoea* spp., *Proteus* spp., *Providencia* spp., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Staphylococcus cohnii* spp. *cohnii*, *Streptococcus gordonii*, *Streptococcus parasanguinis*, *Streptococcus pyogenes*, beta-hemolytic group G *Streptococcus* spp.

Extended spectrum beta-lactamases (ESBL) were identified in 173 of the 5,722 Gram-negative isolates (3.0%) that were collected between 2010 and 2011 and analyzed by the Vitek 2. Of these, 108 were

Table 2
Frequency by gender of species among 11,943 bacteria isolated from urine cultures collected between 2005-2006, and 2010- 2011 at the University Hospital of the University of São Paulo (HU-USP)

Order	Male, n = 2,673	Female, n = 9,270	Odds ratio*	CI 95%	p
1°	<i>Escherichia coli</i> , 1,271 (47.5%)	<i>Escherichia coli</i> , 7,103 (76.6%)	3.60 (F)	3.30-3.90	<0.0001
2°	<i>Proteus mirabilis</i> , 302 (11.3%)	<i>Proteus mirabilis</i> , 493 (5.31%)	2.27 (M)	1.94-2.64	<0.0001
3°	<i>Klebsiella pneumoniae</i> , 215 (8.04%)	<i>Klebsiella pneumoniae</i> , 477 (5.1%)	1.61 (M)	1.36-1.91	<0.0001
4°	<i>Enterococcus faecalis</i> , 151 (5.65%)	<i>Enterococcus faecalis</i> , 171 (1.8%)	3.19 (M)	2.53-4.01	<0.0001
5°	<i>Morganella morganni</i> , 77 (2.88%)	<i>Staphylococcus saprophyticus</i> , 144 (1.5%)	-	-	-
6°	<i>Pseudomonas aeruginosa</i> , 76 (2.84%)	<i>Enterobacter aerogenes</i> , 131 (1.4%)	-	-	-
7°	<i>Enterobacter cloacae</i> , 59 (2.20%)	<i>Enterobacter cloacae</i> , 67 (0.72%)	3.09 (M)	2.17-4.41	<0.0001
8°	<i>Citrobacter freundii</i> , 56 (2.10%)	<i>Citrobacter freundii</i> , 66 (0.71%)	2.98 (M)	2.08-4.27	<0.0001
9°	<i>Enterobacter aerogenes</i> , 56 (2.10%)	<i>Staphylococcus warneri</i> , 59 (0.63%)	-	-	-
10°	<i>Staphylococcus aureus</i> , 52 (1.94%)	<i>Pseudomonas aeruginosa</i> , 56 (0.60%)	-	-	-

Notes: *(F): female, reference is male; (M): male, reference is female (reference is the counterpart of the gender indicated in parentheses). *Morganella morganni*: OR = 10.15 (95% CI: 6.53-15.76), $p < 0.0001$ (male); *Pseudomonas aeruginosa*: OR = 4.81 (95% CI: 3.40-6.82), $p < 0.0001$ (male); *Staphylococcus saprophyticus*: OR = 10.37 (95% CI: 3.84-28.0), $p < 0.0001$ (female); *Enterobacter aerogenes*: OR = 1.48 (95% CI: 1.08-2.02), $p = 0.012$ (male).

Escherichia coli (1.3% of total per specie), 59 were *K. pneumoniae* (8.5%), four were *K. oxytoca* (6.9%), and two were *P. mirabilis* (0.25%). Of these, 4.6% (n = 8) of patients were 0-14 years old; 38.7% (n = 67) were 15-64 years; 56.7% (n = 98) were ≥ 65 years old; 60.1% (n = 104) were female; and 74% (n = 128) were from the Emergency Department. Of the patients ≥ 65 years old, 55.1% were female and 44.9% were male.

Although it was neither carried out using the Hodge test nor another method for detecting carbapenemases, including KPC (*K. pneumoniae* carbapenemase), the susceptibility to ertapenem was high among isolates of Enterobacteriaceae (*E. coli*, 100%; *Proteus mirabilis*, 99.5%; *K. pneumoniae*, 98.5%; and *E. aerogenes*, 100%) during the period between 2010 and 2011. Global analysis of the isolates demonstrated a susceptibility of 99.8% to ertapenem (2010-2011). Susceptibility to vancomycin among isolates of *E. faecalis* was 98.6% and 97.3% ($p = 0.699$), in the 2005-2006 and 2010-2011 periods, respectively.

SUSCEPTIBILITY

In an attempt to select an appropriate antibiotic for empirical treatment, we performed a comprehensive analysis of the antibiograms of 11,943 isolates from urine cultures from the two periods studied, despite differences in interpretation of susceptibility, particularly among Gram-positive and Gram-negative bacteria. Global susceptibility was 83.4% (67,864 out of 81,406), in 2005-2006 vs. 83.5% (72,442 out of 86,770) in 2010-2011, $p = 0.499$. Only clindamycin, ceftazidime, erythromycin, gentamicin (high level resistance, tested for *Enterococcus* spp. infection), linezolid, penicillin G (parenteral), tetracycline and vancomycin had no statistically significant differences in antimicrobial susceptibility in both periods. All of these antimicrobial agents are used for covering Gram-positive bacterial infections. All of the following antimicrobial agents were highly statistically significant different in the two periods ($p < 0.0001$): nalidixic acid (88.7 vs. 78.1), amikacin (98.4 vs. 99.5), amoxicillin/clavulanate (98.4 vs. 99.5), ampicillin/sulbactam (21.9 vs. 43.6), cephalothin (77.9 vs. 64.7), cefepime (98.0 vs. 95.4), ceftriaxone (98.2 vs. 95.9), ciprofloxacin (90.1 vs. 83.4), gentamicin (94.4 vs. 93.3), imipenem (99.8 vs. 93.1), meropenem (89.0 vs. 99.8),

nitrofurantoin (86.4 vs. 77.4), norfloxacin (91.3 vs. 75.6) and piperacillin/tazobactam (72.9 vs. 94.4). The difference was minor in the following cases: ampicillin (45.1 vs. 44.0, $p = 0.037$), aztreonam 57 (48.3 vs. 61.5, $p = 0.048$), cefuroxime axetil (92.1 vs. 86.9, $p = 0.004$), oxacillin (22.4 vs. 35.6, $p = 0.001$), rifampin (96.4 vs. 97.7, $p = 0.002$) and trimethoprim/sulfamethoxazole (TMP/SMX) (68.0 vs. 65.9, $p = 0.035$). Global resistance to fluoroquinolones (levofloxacin, norfloxacin and ciprofloxacin) was 12%.

Among *E. coli*, the most commonly isolated agent, only TMP/SMX and nalidixic acid showed a resistance rate of $\geq 20\%$, in the period from 2010 to 2011. The global susceptibility was 88.3% (50,142/56,764) vs. 86.1% (55,436/64,400); $p < 0.0001$. Among *P. mirabilis*, except for colistin and nitrofurantoin, which were antibiotics against which this species has intrinsic resistance (0% susceptibility in this study) and TMP/SMX (susceptibility of 77.8% and 74.5% in the first and second periods, respectively), all other antibiotics had $< 20\%$ resistance in both periods. *K. pneumoniae* had a global susceptibility of 80.8% (3,242/4,013) vs. 76.8% (4,719/6,150); $p < 0.0001$. Various antibiotics showed a resistance rate higher than 20%, but norfloxacin was a good option in both periods that were studied (89.7% vs. 82.6%). Among *E. faecalis*, the global susceptibility was 77.2% (880/1,141) vs. 82.2% (1,328/1,616); $p = 0.001$, which indicated an improvement in susceptibility during the two periods.

The most resistant species was *E. aerogenes*, with a global susceptibility of 70.9% (878/1,237) vs. 68.9% (1,012/1,468); $p = 0.674$. In contrast to the aforementioned bacteria, *S. saprophyticus* presented a favorable profile with an improvement in global susceptibility during the two periods, 61.1% (1,249/2,043) vs. 88.2% (15/17); $p = 0.042$. The reason that the number of samples that were obtained during the second period was small is because the laboratory is no longer disclosing the antibiogram for *S. saprophyticus*, as recommended by the CLSI. Among *P. aeruginosa*, the global susceptibility was 48.3% (532/1,102) in 2005-2006 vs. 85.6% (640/748) in 2010-2011; $p < 0.0001$.

The global frequency of intermediate susceptibility was the following: 1.7% (2005-2006) and 2.7% (2010-2011) (global); 1.6%

Table 3 Susceptibility of species among 11,943 bacteria isolated from urine cultures collected between 2005-2006, and 2010-2011 at the University Hospital of the University of São Paulo (HU-USP)

Antibiotic	<i>Escherichia coli</i>			<i>Proteus mirabilis</i>			<i>Klebsiella pneumoniae</i>			<i>Enterococcus faecalis</i>			<i>Enterobacter aerogenes</i>			<i>S. saprophyticus</i>			<i>Pseudomonas aeruginosa</i>		
	2005-2006	2010-2011	P	2005-2006	2010-2011	P	2005-2006	2010-2011	P	2005-2006	2010-2011	P	2005-2006	2010-2011	P	2005-2006	2010-2011	P	2005-2006	2010-2011	P
Nalidixic acid	90.1%	78.1%	<0.0001	91.6%	85.9%	0.011	87.1%	74.9%	<0.0001	86.2%	90%	0.422	86.2%	90%	0.422	86.2%	90%	0.422	42.9%		
Amikacin	99.8%	99.8%	0.783	98.8%	99.7%	0.316	96.7%	99.7%	0.006	95.3%	96.9%	0.932	95.3%	96.9%	0.932	95.3%	96.9%	0.932	78.6%	95.5%	0.004
Amoxicillin/clavulanate	83.5%	86.8%	<0.0001	91.9%	78.6%	<0.0001	90.4%	84.9%	0.034	5.7%	1%	0.155	5.7%	1%	0.155	5.7%	1%	0.155	28.6%		
Ampicillin							100%	90.2%	0.004												
Cefazolin	95.2%	91.9%	0.040	95.6%	76.2%	0.023	95.3%	79.2%	0.054	17.4%	0%	0.888	17.4%	0%	0.888	17.4%	0%	0.888			
Cefepime	99.6%	97.2%	<0.0001	98.2%	95.4%	0.035	94.8%	84.2%	<0.0001	95.3%	97.9%	0.643	95.3%	97.9%	0.643	95.3%	97.9%	0.643	80.3%	92.4%	0.076
Ceftazidime				100%	95.4%	1.000	50%	85.5%	0.073	93%	100%	1.000	93%	100%	1.000	93%	100%	1.000	75.8%	92.4%	0.017
Ceftriaxone	99.6%	97.8%	<0.0001	98.5%	81%	<0.0001	95.9%	91.7%	0.328												
Cefuroxime axetil	96.8%	92.3%	<0.0001	96.9%	81%	0.003	88.6%	87.5%	1.000												
Ciprofloxacin	92.9%	84.9%	<0.0001	95.1%	87.9%	<0.0001	89.9%	78.1%	<0.0001	73.9%	74.8%	0.859	92%	92%	0.991	100%	100%	1.000	68.2%	89.4%	0.003
Clindamycin										99.3%											
Colistin	100%			0%			66.7%														
Erythromycin										79.5%											
Ertapenem	100%			99.5%			98.5%			100%											
Streptomycin							68.8%														
Streptomycin (high level resistance)							78.8%														
Gentamicin	96.6%	94.3%	<0.0001	95.8%	91.5%	0.013	94.9%	90.1%	0.021	100%	97%	1.000	96.6%	97%	1.000	96.6%	97%	1.000	61.5%	89.4%	<0.0001
Gentamicin (high level resistance)							78.8%			79.8%	0.206										
Imipenem	100%	100%	1.000	100%	100%	1.000	100%	100%	1.000	100%			100%						89.4%	91.2%	0.762
Levofloxacin	100%						100%														
Linezolid	100%			97.5%	98.9%	1.000	97.5%	98.9%	1.000										85%	92.4%	
Meropenem	100%	100%	1.000	100%	100%	1.000	100%	100%	1.000	100%			100%								
Minozocline																					
Nitrofurantoin	98.4%	92.3%	<0.0001	1%	0%	0.147	63%	32.7%	<0.0001	100%	47.1%	5%	<0.0001	99.3%	28.6%	47.1%	5%	<0.0001	28.6%		
Norfloxacin	92.7%	85.7%	<0.0001	94.8%	100%	0.695	89.7%	82.6%	0.479	92%	100%	1.000	92%	100%	1.000	92%	100%	1.000	67.9%	86.7%	0.037
Oxacillin																					
Penicillin G										89.8%	85.9%	0.294									
Piperacillin/tazobactam	100%	96.9%	1.000	100%	96.2%	1.000	16.7%	88.7%	<0.0001	87.5%			87.5%						89.4%	94%	0.588
Rifampin							26.8%	18.9%	0.326	99.3%	100%	1.000	99.3%	100%	1.000	99.3%	100%	1.000			
Tetracycline										81.5%			81.5%								
Trimethoprim/sulfamethoxazole	65%	62.9%	0.023	77.8%	74.5%	0.268	82.7%	77.9%	<0.0001	92%	94%	0.583	92%	94%	0.583	92%	94%	0.583	3.1%		
Vancomycin							98.6%	97.3%	0.699	100%	100%	1.000	100%	100%	1.000	100%	100%	1.000			

Note: * $p < 0.05$ compared between gender within each stratum of age indicated (Pearson uncorrected chi-square test or Fisher's exact test when values ≤ 5).

and 2.7% (*E. coli*); 0.8% and 2.5% (*Proteus mirabilis*); 3.0% and 3.5% (*K. pneumoniae*); 0.5% and 2.0% (*E. faecalis*); 3.6% and 3.7% (*E. aerogenes*); 0.5% and 0% (*S. saprophyticus*); and 4.6% and 5.3% (*P. aeruginosa*).

The comparison among the age strata, 0-14, 15-64 and ≥ 65 years old, versus gender, among the 45 antibiotics that were tested (Table 4), showed statistically significant differences for 25 of the antibiotics between males and females, with greater rates of resistance in men and in patients older than 65 years. The antibiotics demonstrating these differences were the following: nalidixic acid, amikacin, amoxicillin/clavulanate, ampicillin, ampicillin/sulbactam, aztreonam, cephalothin, cefazolin, cefepime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime axetil, ciprofloxacin, carbenicillin, ertapenem, streptomycin (high level resistance), gentamicin, imipenem, meropenem, nitrofurantoin, piperacillin/tazobactam, ticarcillin, TMP/SMX and vancomycin.

DISCUSSION

This is the third largest study of susceptibility to antibiotics in urinary tract infections in Brazil (Table 5). All studies of this type in Brazil, except for one¹⁰, are retrospective and exclude many demographic variables, except gender and age, as well as clinical, radiological or laboratory variables, which does not allow multivariate analysis, and thus, greatly reduces the impact of these publications^{1,2,4,9,19,20,25,26}.

Initially, this study had the goal of guiding the implementation of a protocol for the treatment of UTIs; however, the lack of clinical data (mainly regarding the uncomplicated infections) and the large number of isolates, preclude such an achievement.

Considering that \$5.30 US dollars was paid for each urine culture and antibiogram according to the table of Brazilian Unified Health System, the "estimated cost" of this study was \$63,297.90²⁷. Taking into account that in the same period approximately 60,000 urine cultures were requested, of which, only 20% were positive, the total cost to the health system was about \$183,000.00 just in the University Hospital of the University of São Paulo, which is one of 6,753 hospitals in Brazil, of which only 30% are public. This justifies conducting a prospective study to address the clinical characteristics of patients in Brazil.

The biggest (multicenter) impact study involving Brazilian cases of UTI isolates was the SENTRY¹³, which was published a decade ago and involved four centers in the country but only included inpatients. The methodology included the use of the gold standard procedure of microdilution for the preparation of the antibiograms and involved 1,961 urine isolates. *E. coli* was the most frequent pathogen that was isolated followed by *Klebsiella* spp., *P. aeruginosa*, and *P. mirabilis*. At that time, the amount of detected resistance was $> 45\%$ to TMP/SMX and a resistance to fluoroquinolones of 17.5-18.9% was detected. Furthermore, the susceptibility to broad-spectrum antibiotics (91.0-100.0%) and nitrofurantoin (87%) remained high, including piperacillin/tazobactam, aztreonam, extended-spectrum cephalosporins (third and fourth generations), carbapenems and amikacin. At that time, the biggest problems in terms of resistance was carbapenem-resistant *P. aeruginosa*, ciprofloxacin-resistant *E. coli* and ESBL-producing *K. pneumoniae*. The Enterobacteriaceae producing carbapenemases were not even mentioned in that study¹³.

The data found in this study are similar to those found in previous literature, with some minor peculiarities. The proportion of females (77.6% vs. 69%-88.8% in studies), the female-to-male ratio by age group, the age group most affected (15-64 years: 57.2%) and the first isolated agent (*E. coli*, 70.2%) and frequency is very similar to that found in many published studies^{2,4,9,10,15,19,20,25,26}. The percentage of *E. coli* that is isolated from UTIs, according to international literature, is 75% to 95%¹⁵. In Brazil, the frequency varied from 48.2%⁹ to 71.6%¹⁹ in São Paulo. The proportion of Gram-negative isolates varied from 84.4% to 87.3%^{4,19,24}.

The second most frequent agent presented a variation in different studies in Brazil. Except in studies by COSTA *et al.*⁹ and KOCH *et al.*²⁰, in which *Proteus mirabilis* was the second most frequently isolated uropathogen, the second most commonly isolated pathogen was *Enterococcus* spp.²⁵, *K. oxytoca* and *S. agalactiae*²², *Enterobacter* spp.², and *K. pneumoniae*^{4,11,19,22}.

In the one Brazilian study to date¹¹ about ESBL-producing Enterobacteriaceae in community-acquired UTIs, which evaluated 78,964 urine cultures in Juiz de Fora, State of Minas Gerais, Southeastern of Brazil, from 2001 to 2009, detected a frequency of 0.66% (n = 512) over nine years in the majority of patients older than 65 years (female, 52.6% and male, 48.1%), which is similarly to the results of this study. The percentage reported in the literature varied from 0.2% to 3.5%¹¹, which is close to the value of 3.0% among Gram-negative tested between 2010 and 2011, in this study. Although the most frequently isolated bacterium that produces ESBL is *Klebsiella* spp. (24 to 60%), DIAS *et al.* also detected *E. coli* (72.4%), followed by *Klebsiella* spp. (19.8%), which is similar to this study in which *E. coli* (62.4%), *Klebsiella* spp. (36.4%) and *Proteus* spp. (1.2%) were isolated. This study did not perform a confirmatory test for ESBL, which can result in false positives¹².

Global resistance was $\geq 20\%$ for TMP/SMX, tetracycline, norfloxacin, nitrofurantoin, erythromycin, cefazolin and nalidixic acid, especially leveraged by other bacteria than *E. coli*. Global resistance was $\geq 20\%$ for TMP/SMX, tetracycline, norfloxacin, nitrofurantoin, erythromycin, cefazolin and nalidixic acid in the isolated bacteria other than *E. coli*. There was significant difference between virtually all antibiotics in the two periods studied, which was commonly highly significant ($p < 0.0001$); however, no difference was observed between the antibiotics used for coverage of Gram-positive bacteria, except for rifampin and oxacillin. The fact that there was no statistically significant difference between the two periods in the overall susceptibility to key antibiotics by Gram-positive bacteria reveals the minor importance of these bacteria in the pathogenesis of UTIs in the study population, due to the reduced presence of selective pressure, which occurs with antibiotics that are used to treat Gram-negative bacteria.

The study by KOCH *et al.*²⁰ assessed the temporal trends of antimicrobial resistance, from 2000 to 2004 of gentamicin ($p = 0.98$), cephalothin ($p = 0.29$), cefoxitin ($p = 0.16$), TMP/SMX ($p = 0.40$), nalidixic acid ($p = 0.02$), ceftriaxone ($p = 0.12$) and ciprofloxacin ($p = 0.07$). Therefore, unlike this study, only nalidixic acid had statistical significance and ciprofloxacin had a statistical trend, which demonstrates the selective pressure that was exerted by quinolones in Rio Grande do Sul, southern Brazil. The same study also showed that older patients showed increased resistance to nalidixic acid, ciprofloxacin and

Table 4

Susceptibility to antibiotics of all 11,943 bacteria isolated from urine cultures, between 2005 and 2006, and 2010 and 2011 at the University Hospital of the University of São Paulo (HU-USP), stratified by gender and stratum of age

Antibiotic, susceptible, n(%)	Male gender			Female gender		
	0-14 years	15-64 years	≥65 years	0-14 years	15-64 years	≥65 years
Nalidixic acid	603 (97.3)*	532 (74.2)*	506 (59.3)*	1.198 (94.1)*	4.794 (88.1)*	1.220 (69.8)*
Amikacin	528 (100)	635 (96.1)*	794 (95.0)*	1.107 (100)	4.740 (99.8)*	1.569 (99.2)*
Amoxicillin/clavulanate	454 (73.2)*	490 (68.3)*	530 (62.1)*	1.050 (82.5)*	4.637 (85.2)*	1.395 (79.8)*
Ampicillin	253 (38.8)	304 (37.7)*	318 (31.3)*	539 (41.2)	2.802 (48.6)*	880 (46.5)*
Ampicillin/sulbactam	3 (16.7)	17 (29.8)*	40 (44.9)*	2 (11.1)	39 (17.1)*	14 (23.3)*
Aztreonam	9 (100)	25 (49.0)	31 (41.9)*	18 (85.7)	11 (45.8)	27 (64.3)*
Cephalothin	457 (73.2)*	448 (60.3)*	443 (49.4)*	964 (75.4)*	4.154 (76.2)*	1.185 (66.9)*
Cefazolin	87 (77.0)*	102 (68.5)*	106 (70.7)*	178 (87.7)*	741 (73.0)*	217 (83.8)*
Cefepime	527 (99.8)	604 (91.4)*	725 (86.5)*	1.095 (98.9)*	4.692 (98.7)*	1.499 (94.8)*
Cefotaxime	230 (95.0)	296 (81.8)*	374 (73.3)*	549 (95.8)	2.717 (97.2)*	854 (88.5)*
Cefoxitin	0	4 (14.8)*	3 (6.8)	5 (45.5)	17 (65.4)*	4 (16.0)
Ceftazidime	254 (98.8)	349 (87.5)*	444 (81.0)*	609 (97.9)	2.902 (97.7)*	1.383 (87.7)*
Ceftriaxone	383 (98.7)*	374 (91.9)*	382 (91.0)*	705 (100)*	2.677 (99.5)*	807 (97.6)*
Cefuroxime axetil	272 (93.2)*	243 (77.9)*	227 (70.7)*	528 (97.8)*	1.954 (96.1)*	557 (88.8)*
Ciprofloxacin	657 (98.8)	641 (76.2)*	692 (63.0)*	1.308 (98.2)	5.519 (92.5)*	1.446 (74.7)*
Clindamycin	12 (100)	21 (63.6)*	41 (75.9)	14 (100)	263 (98.9)*	25 (58.1)
Chloramphenicol	-	-	1 (100)	-	2 (100)	-
Carbenicillin	32 (37.6)	52 (61.9)	32 (45.7)*	59 (39.6)	306 (55.8)	97 (60.2)*
Colistin	-	3 (75.0)	2 (100)	1 (100)	4 (80.0)	4 (100)
Erythromycin	9 (75.0)	16 (48.5)*	31 (57.4)	13 (86.7)	216 (81.2)*	23 (53.5)
Ertapenem	231 (100)	318 (99.4)	446 (98.9)*	563 (100)	2778 (99.9)	933 (99.9)*
Streptomycin	7 (58.3)	10 (62.5)	38 (73.1)	5 (55.6)	14 (63.6)	30 (62.5)
Streptomycin (high level resistance)	10 (83.3)	15 (57.7)	39 (76.5)*	8 (100)	35 (72.9)	18 (34.0)*
Gentamicin	623 (97.0)	702 (86.8)*	855 (84.5)*	1.271 (96.6)	5.617 (96.4)*	1.680 (91.0)*
Gentamicin (high level resistance)	21 (87.5)	27 (64.3)*	80 (77.7)	17 (100)	64 (91.4)*	70 (70.0)
Imipenem	296 (100)	338 (98.5)*	383 (98.0)*	545 (100)	1.975 (100)*	646 (99.5)*
Levofloxacin	-	-	-	-	7 (100)	3 (100)
Linezolid	24 (100)	55 (100)	117 (99.2)	22 (100)	205 (98.6)	96 (99.0)
Meropenem	241 (100)	362 (98.4)*	509 (97.7)*	582 (100)	2.800 (100)*	968 (99.7)*
Minocycline	50 (58.8)*	58 (69.0)	47 (67.1)	115 (77.2)*	393 (71.7)	115 (71.4)
Nitrofurantoin	389 (60.2)*	584 (74.8)*	660 (67.7)*	1.119 (86.2)*	5.010 (86.8)*	1.515 (81.7)*
Norfloxacin	400 (98.0)	363 (80.6)*	338 (65.6)*	733 (98.4)	2.664 (94.7)*	712 (79.3)*
Oxacillin	5 (29.4)	16 (39.0)*	43 (51.8)	7 (31.8)	67 (18.6)*	21 (37.5)
Penicillin G	23 (54.8)	38 (44.2)*	89 (47.1)	19 (49.6)	112 (23.9)*	95 (57.6)
Piperacillin/tazobactam	131 (97.8)	184 (84.4)*	258 (81.9)*	306 (96.5)	1.326 (96.4)*	503 (93.8)*
Polymyxin B	2 (100)	15 (100)	23 (100)	13 (100)	4 (100)	11 (100)
Quinupristin/dalfopristin	3 (50.0)	11 (68.8)	17 (56.7)	1 (33.3)	28 (93.3)	10 (45.5)
Rifampin	17 (100)	35 (85.4)*	78 (94.0)	22 (100)	361 (99.7)*	51 (89.5)
Teicoplanin	16 (100)	27 (100)	59 (89.4)	17 (100)	135 (99.3)	61 (98.4)
Tetracycline	20 (47.6)*	50 (54.9)*	81 (54.4)	45 (71.4)*	350 (66.5)*	78 (48.4)
Ticarcillin	73 (81.1)*	86 (78.9)*	82 (73.2)*	141 (91.0)*	520 (93.4)*	162 (89.5)*
Tigecycline	-	3 (75.0)	2 (100)	1 (100)	4 (80.0)	3 (100)
Tobramycin	85 (100)	83 (98.8)	71 (98.6)	147 (98.7)	547 (99.5)	159 (98.8)
Trimethoprim/sulfamethoxazole	415 (65.5)	485 (63.0)*	564 (59.2)*	807 (62.4)	4.007 (70.6)*	1.176 (65.1)*
Vancomycin	43 (100)	93 (98.9)	190 (94.1)*	43 (100)	496 (100)	170 (98.8)*

Note: * $p < 0.05$ compared between gender within each stratum of age indicated (Pearson uncorrected chi-square test or Fisher's exact test when values ≤ 5).

Table 5
Brazilian retrospective surveillance studies of community-acquired urinary tract infections

	Rocha JL <i>et al.</i> ²⁵	Costa LC <i>et al.</i> ⁹	Bravo A <i>et al.</i> ⁴	Koch CR <i>et al.</i> ²⁰	Kiffer CR <i>et al.</i> ¹⁹	Santo E <i>et al.</i> ²⁶	Dias RC <i>et al.</i> ¹⁰	Bail L <i>et al.</i> ²	Dias VC <i>et al.</i> ¹¹	Andrade SS <i>et al.</i> ¹	
Positive urine cultures (n)	9,798	566	1,252	957	37,261	78	186: 118 <i>E. coli</i>	106	78,964	611	
Setting of study	Curitiba, Southern of Brazil	Campina Grande, Northeastern of Brazil	Presidente Prudente, Southeastern of Brazil	Porto Alegre, Southern of Brazil	São Paulo, Southeastern of Brazil	Ribeirão Preto, Southeastern of Brazil	Rio de Janeiro, Southeastern of Brazil	Ponta Grossa, Southern of Brazil	Juiz de Fora, Southeastern of Brazil	Brasília, Florianópolis, Porto Alegre, São Paulo, Brazil	
Period of study	May to Dec 2009	Jan 2006 to Jun 2008	Jan 2006 to Dec 2007	Jan 2000 to Dec 2004	Jan 2000 to Dec 2003	Jul 2000 and Jul 2003	Mar 2005 to Nov 2006	Jan to Dec 2001	2001 to 2009	Jan to Dec 2013	
Year of publication	2012	2010	2009	2008	2007	2007	2009	2006	2012	2006	
Antibiogram method	Vitek 2	disk diffusion	disk diffusion	disk diffusion	disk diffusion, Vitek 1	disk diffusion	disk diffusion and E-test	disk diffusion	disk diffusion	microdilution	
Standardization	CLSI	CLSI	CLSI	CLSI	CLSI	CLSI	CLSI	CLSI	CLSI	CLSI	
Females (%)	88%	85.2%	69.01%	81.3%	88.8%	not available	100%	70%	69.8%	75.6%	
Most frequent age strata	21-40 years (36.9%)	> 60 years (36.9%)	20-49 years (52.95%)	not available	13-60 years (60.0%)	not available	not available	not available	not available	not available	
Gram-negatives (%)	not available	not available	84.42%	not available	87.3%	100%	100%	92.5%	100%	not available	
<i>E. coli</i> (%)	66.1%	48.2%	65.97%	66.2%	71.6%	100%	100%	58.6%	72.4%	66%	
Other frequent agents (%)	<i>Enterococcus spp</i> (8.1), <i>Klebsiella spp.</i> (5.9), <i>S. saprophyticus</i> (3.9), <i>Proteus mirabilis</i> (3.9), <i>Enterobacter spp.</i> (2.7), <i>Pseudomonas aeruginosa</i> (1.2)	<i>P. mirabilis</i> (11), <i>K. pneumoniae</i> (9.9), <i>S. saprophyticus</i> (5.5), <i>Enterobacter sp.</i> (5.3), <i>S. epidermidis</i> (5.1), <i>S. aureus</i> (3.5), <i>K. oxytoca</i> (3.5), <i>Paeruginosa</i> (2.5)	<i>Klebsiella pneumoniae</i> (6.07), <i>Proteus mirabilis</i> (5.51), <i>Enterobacter sp</i> (2.88), <i>Pantoea agglomerans</i> (1.92), <i>Citrobacter sp</i> (1.36)	<i>Proteus mirabilis</i> (8.4), <i>Klebsiella sp</i> (5.6)	<i>K. pneumoniae</i> (6.4), <i>P. mirabilis</i> (6.1), <i>E. faecalis</i> (4.8), <i>P. aeruginosa</i> (1.8), <i>S. saprophyticus</i> (1.6), <i>E. aerogenes</i> (1.6), <i>E. cloacae</i> (1.1)	No	No	<i>Enterobacter sp.</i> (11.8), <i>Proteus sp.</i> (4.4), <i>Serratia sp</i> (2.9), <i>Klebsiella sp.</i> (2.9)	<i>Enterobacter sp.</i> (11.8), <i>Proteus mirabilis</i> (2.5%), <i>Proteus mirabilis</i> (1.9%), <i>Klebsiella oxytoca</i> (1.3%), <i>Klebsiella ozaenae</i> (1.1%).	<i>Klebsiella pneumoniae</i> (17.4%), <i>Morganella morganii</i> (2.5%), <i>Proteus mirabilis</i> (1.9%), <i>Klebsiella oxytoca</i> (1.3%), <i>Klebsiella ozaenae</i> (1.1%).	<i>Klebsiella spp.</i> (7%), <i>P. mirabilis</i> (6.4%), <i>Enterococcus spp.</i> (5.6%), <i>P. aeruginosa</i> (4.6%).
Clinical variables present	no	no	no	no	no	no	yes	no	no	no	
<i>E. coli</i> susceptibility	Ampicillin (55.9), nitrofurantoin (96.0), TMP-SMX (65.8), fluoroquinolones ¹ (82.2), nitrofurantoin extended-spectrum cephalosporins ² (96.8), Gentamicin (93.1).	Amoxicillin/clavulanate (94), ampicillin (48), cephalotin (59), cefuroxime (86), ceftriaxone (90), ceftazidime (97), cefepime (97), ciprofloxacin (86), gentamicin (96), nitrofurantoin (96), TMP/SMX (62)	Ciprofloxacin (78.8), norfloxacin (77), nitrofurantoin (93), TMP/SMX (54.3)	Gentamicin (88), cephalotin (55), nitrofurantoin (86), ceftriaxone (98), ciprofloxacin (87), TMP/SMX (54)	Ampicillin (57), cephalotin (86), ceftriaxone (99), ciprofloxacin (88), gentamicin (97), nitrofurantoin (97), norfloxacin (88), TMP/SMX (66), tetracycline (69)	Ampicillin (63), cephalotin (92), cefuroxime (99), ceftriaxone (99), gentamicin (100), nitrofurantoin (97), norfloxacin (88), TMP/SMX (71), tetracycline (69)	Ampicillin (29), TMP/SMX (52), amoxicilline/flucloxacillin (81), vancomycin (94), roquinolones ³ (94), cefuroxime (96), fosfomicin (97), nitrofurantoin (97), gentamicin (88), cefotaxime (98)	Ampicillin (49), cephalotin (74), TMP/SMX (56), nitrofurantoin (95), norfloxacin (88), gentamicin (88)	2009: Amikacin (94), ciprofloxacin (24), gentamicin (69), nitrofurantoin (95), TMP/SMX (24), imipenem (100)	Ampicillin (50), amoxicillin/ clavulanate (89), cefuroxime (99), ciprofloxacin (90), levofloxacin (91), nitrofurantoin (98), TMP/SMX (58)	
<i>Proteus sp.</i> susceptibility	-	Ciprofloxacin (91.2), norfloxacin (91.2), nitrofurantoin (58.1), TMP/SMX (58.1)	Gentamicin (91), cephalotin (44), nitrofurantoin (40), ceftriaxone (91), cefepime (95), ciprofloxacin (83), gentamicin (93), nitrofurantoin (83), TMP/SMX (82)	Gentamicin (91), cephalotin (44), ceftriaxone (91), ciprofloxacin (91), TMP/SMX (47), tetracycline (0)	Ampicillin (81), cephalotin (97), ceftriaxone (99), ciprofloxacin (96), gentamicin (98), nitrofurantoin (0), norfloxacin (96), TMP/SMX (78), tetracycline (0)	2009: Amikacin (100), ciprofloxacin (100), gentamicin (0), nitrofurantoin (0), TMP/SMX (50), imipenem (100)	-	-	-	-	

Table 5
Brazilian retrospective surveillance studies of community-acquired urinary tract infections (cont.)

	Rocha JL <i>et al.</i> ²⁵	Costa LC <i>et al.</i> ⁹	Braios A <i>et al.</i> ⁴	Koch CR <i>et al.</i> ²⁰	Kiffer CR <i>et al.</i> ¹⁹	Santo E <i>et al.</i> ²⁶	Dias RC <i>et al.</i> ¹⁰	Bail L <i>et al.</i> ²	Dias VC <i>et al.</i> ¹¹	Andrade SS <i>et al.</i> ¹
<i>Klebsiella</i> sp. susceptibility	-	Ciprofloxacin (82.1), norfloxacin (78.6), nitrofurantoin (76.8), TMP/SMX (71.4)	Amoxicillin/clavulanate (90), ampicillin (42), cephalotin (80), cefuroxime (84), ceftriaxone (88), ceftazidime (94), cefepime (97), ciprofloxacin (94), gentamicin (94), nitrofurantoin (12), TMP/SMX (96)	Gentamicin (85), cephalotin (43), nitrofurantoin (42), ceftriaxone (94), ciprofloxacin (79), TMP/SMX (53)	Ampicillin (0), cephalotin (92), ceftriaxone (98), ciprofloxacin (94), gentamicin (97), nitrofurantoin (79), norfloxacin (91), TMP/SMX (82), tetracycline (80)	-	-	-	2009: Amikacin (94), ciprofloxacin (13), gentamicin (63), nitrofurantoin (44), TMP/SMX (24), imipenem (100)	-
<i>E. faecalis</i> susceptibility	-	-	-	-	Ampicillin (99), ciprofloxacin (84), nitrofurantoin (99), tetracycline (40)	-	-	Nitrofurantoin (100), norfloxacin (100), gentamicin (100)	-	-
<i>E. aerogenes</i> susceptibility	-	Ciprofloxacin (85.7), norfloxacin (81), nitrofurantoin (66.7), TMP/SMX (47.6)	Amoxicillin/clavulanate (94), cephalotin (86), ciprofloxacin (94), clindamycin (91), oxacillin (91), ciprofloxacin (94), gentamicin (97), nitrofurantoin (97), norfloxacin (97), TMP/SMX (94), vancomycin (100)	-	Ampicillin (0), cephalotin (0), ceftriaxone (98), ciprofloxacin (95), gentamicin (98), nitrofurantoin (79), norfloxacin (95), TMP/SMX (92), tetracycline (93)	-	-	-	2009: Amikacin (76), ciprofloxacin (50), gentamicin (75), nitrofurantoin (0), TMP/SMX (50), imipenem (100)	-
<i>S. saprophyticus</i> susceptibility	-	Ciprofloxacin (73.3), norfloxacin (72.4), nitrofurantoin (86.7), TMP/SMX (39.3)	-	-	Ciprofloxacin (99), nitrofurantoin (99), tetracycline (84)	-	-	-	-	-
<i>P. aeruginosa</i> susceptibility	-	-	-	-	Ceftriaxone (11), ciprofloxacin (37), gentamicin (52), norfloxacin (38)	-	-	Norfloxacin (33), gentamicin (33)	-	-
Temporal trend of resistance	-	-	-	Ciprofloxacin ($p=0.07$), nalidixic acid ($P=0.02$)	Ciprofloxacin, norfloxacin, tetracycline, nalidixic acid (all: $p<0.0001$)	-	-	-	-	-

Notes: 1: fluoroquinolones are ciprofloxacin and levofloxacin; 2: extended-spectrum cephalosporins includes ceftriaxone and ceftaxime; 3: includes norfloxacin, ciprofloxacin and levofloxacin.

nitrofurantoin, and in men, for many of the antibiotics that were tested, with the exception of TMP/SMX²⁰. KIFFER *et al.*¹⁹ demonstrated a temporal trend of a decreased susceptibility to quinolones from 2000 to 2003 including ciprofloxacin, norfloxacin, tetracycline, and nalidixic acid ($p < 0.0001$). An earlier study, that was conducted two decades ago in Salvador, Northeastern Brazil, showed a reported resistance to norfloxacin that reached 9.1% and to ciprofloxacin of 16.5%²¹.

The study by DIAS *et al.*¹¹ evaluated the temporal trend of resistance among ESBL-producing bacteria and showed a peak of occurrence in 2005 (2000-2009) for all antibiotics close to 80% (amikacin, ciprofloxacin, gentamicin, nitrofurantoin, TMP/SMX and imipenem) and a second peak in 2008 (ciprofloxacin and TMP/SMX were close to 80% and nitrofurantoin was close to 40%).

The susceptibility of *E. coli* in 10 Brazilian studies of community-acquired UTIs showed that only TMP/SMX, cephalothin and norfloxacin presented < 80% susceptibility^{1,2,4,9,10,11,19,20,25,26}. In addition to nitrofurantoin, to which *P. mirabilis* presents intrinsic resistance, TMP/SMX and cephalothin were not good options for treatment of UTIs. According to other studies in Brazil, *K. pneumoniae* retains relative susceptibility to fluoroquinolones, as shown in this study, and only TMP/SMX and nitrofurantoin were not good options for treatment of UTIs. The profile found in the literature for *E. aerogenes* is similar to that found in our study, although the literature data indicate a decreased susceptibility to TMP/SMX^{9,11,19}.

Except for lower levels of susceptibility to tetracycline and ciprofloxacin, the data regarding *E. faecalis* from this study resembles that found in the study by KIFFER *et al.*¹⁹. Data regarding *S. saprophyticus* demonstrated distinct profiles of susceptibility, and are similar to the data found in this study. Finally, there is a notable discrepancy between the data regarding the susceptibility of *P. aeruginosa* in this study and that of KIFFER *et al.*¹⁹, the latter being characterized by bacteria with more resistance.

A preliminary conclusion of the risk factors could indicate a predominance of *E. coli* in young women and a predominance of other more resistant isolates, particularly of *Proteus* spp. in men, which, according to literature, are most affected by urinary infections when they develop benign prostatic hyperplasia (BPH), even though the odds ratio is statistically significant for patients younger than 65^{14,15}. Data from a higher incidence of resistance in elderly men are corroborated by three other Brazilian studies^{19,20,25}. ROCHA *et al.*²⁵ also demonstrate a statistically significant difference of the frequency of bacteria between men and women. They found a predominance of *Enterococcus* spp., *Enterobacter* spp. and *Pseudomonas* spp., but not *Proteus* spp. in men and a predominance of *S. saprophyticus* and *E. coli* in women. The same study also demonstrated a highly statistically significant susceptibility to antibiotics by *E. coli* and others, which corroborates the data from this study.

The favorable resistance profile of *E. coli* results in an expanded list of oral therapeutic options for cystitis treatment in young women, including pregnant women, such as fluoroquinolones for not pregnant patients, and cephalexin, cefuroxime axetil and amoxicillin/clavulanate for pregnant patients. However, a policy of sparing fluoroquinolones in such cases is urgently needed. Although it was previously a good option

for young women, nitrofurantoin had low levels of susceptibility by the bacteria that were frequently isolated, such as *Proteus* spp. (intrinsically resistant), *Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas* spp., as well as *M. morgani* and *S. marcescens* (both intrinsically resistant).

Therefore, the biggest problem with making recommendations for empiric antibiotics according to this casuist is men and the elderly. *P. mirabilis*, a urease-producing bacteria that is capable of causing struvite urolithiasis and was the second most frequent isolate, presents intrinsic resistance to several antibiotics, such as cefazolin, tigecycline, polymyxins and nitrofurantoin. Therefore, any empirical recommendation for men and elderly people, cannot dispense with fluoroquinolones or a reassessment based on early urine cultures.

For complicated infections and pyelonephritis, fluoroquinolones, which have renal penetration, such as ciprofloxacin, moxifloxacin and levofloxacin (although the latter two are reserved for respiratory infections because of their expanded spectrum for Gram-positive bacteria and atypical bacteria) are also good choices, although third and fourth generation cephalosporins, as well as aminoglycosides, are a second option and are preferred in cases of severe sepsis and septic shock. Amoxicillin/clavulanate is a good oral treatment option, especially after a loading dose of extended-spectrum cephalosporins or aminoglycosides.

Fosfomycin, although not evaluated by the antibiograms in this study, had a good susceptibility profile in a small Brazilian study (97.0% of susceptibility, n = 118)¹⁰ and is an option for multidrug-resistant infections, especially for ESBL- and carbapenemase-producing bacteria.

An elegant study from the viewpoint of mathematical modeling in the city of São Paulo, Brazil, by KIFFER *et al.*¹⁸ in 2002, demonstrated the correlation between the probability of resistance of strains of *E. coli* in urine and the "density the ciprofloxacin usage for the previous three months" (defined as D_DDDA), $p = 0.023$. Additionally, ciprofloxacin D_DDDA was found to be related to clusters¹⁸: the western region of São Paulo was deemed a hot spot for the use of ciprofloxacin (average probability of 0.8808). This study is an elegant demonstration of how the indiscriminate use of ciprofloxacin against uropathogens may result in the loss of a medication to treat a broad spectrum of uropathogens. Furthermore, taking into account that approximately 30% to 45% of ciprofloxacin ingested is excreted in a non-metabolized form in the urine, widespread use can have environmental consequences due to presence of the antibiotic in the sewage and exposure of Enterobacteriaceae to the antibiotic in that environment. This finding only reinforces the urgent need, based on studies, for an efficient policy of reducing the use of fluoroquinolones and broader-spectrum antibiotics.

A recent study in the United States¹⁷ showed a higher risk of levofloxacin-resistant *E. coli* related to previous use of this antibiotic (adjusted OR = 7.6, 9% CI: 2.1-27.5, $p = 0.002$) and previous hospitalization (OR = 2.0, 95% CI: 1.0-3.9, $p = 0.04$) as well as a statistically significant risk to resistance to other antibiotics in isolates resistant to this fluoroquinolone. Another interesting finding was increased rates of resistance to TMP/SMX despite a 50% decrease in the selective pressure, which is most likely due to multidrug resistance plasmids or multidrug efflux pumps.

This study has several limitations, even though it has the merit of

being one of the few and largest of its type in Brazil: there is one small study of the antimicrobial susceptibility profile according to clinical presentation. In our analysis, we do not separate nosocomial infections from those acquired in the community. The samples were collected not only from midstream but also by other techniques, such as indwelling catheter, which can increase the chance of isolating hospital germs. Despite this, our resistance rates are similar to studies that included only community isolates. Our analysis was based on retrospective data and focused on microbiological aspects. Reproducibility problems can arise due to differences in interpretation of the minimum inhibitory concentration (MIC) between CLSI and EUCAST (European Committee on Antimicrobial Susceptibility Testing) documents.

Studies assessing other demographic, clinical, radiological and laboratory risk factors (including multivariate analysis to control for confounding factors) for infections by germs other than *E. coli*, such as *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp., are urgently needed, to make treatment recommendations that can save broad-spectrum drugs, such as fluoroquinolones, and third and fourth generations of cephalosporins, carbapenems, fosfomicin and aminoglycosides.

CONCLUSIONS

The most favorable scenario is the treatment of *E. coli*. TMP/SMX and nalidixic acid were only two whose resistance exceeds the threshold of 20%, which contraindicates their use according to the guidelines, in our study. The rise of resistance to fluoroquinolones is concerning and averaged 12%. Furthermore, the 20% threshold resistance was exceeded by norfloxacin in the overall analysis, mainly at the expense of bacteria other than *E. coli*. In this study, a high frequency of community-acquired ESBL bacteria was found, which is greater than that found in a study in Brazil, but within the range described in the literature. Most isolates were observed in the elderly. As in other studies, the main problem of resistance was noted in men and in the elderly, which limits most conservative treatment recommendations that save broad-spectrum antibiotics such as fluoroquinolones, aminoglycosides and third and fourth generations cephalosporins. Well-controlled prospective studies that evaluate the risk factors for UTIs by bacteria other than *E. coli* and resistance in Brazil are greatly needed.

RESUMO

Suscetibilidade a antibióticos em infecções de trato urinário em um hospital secundário, 2005-2006 e 2010-2011, em São Paulo, Brasil: dados de 11.943 uroculturas

Introdução: A infecção do trato urinário (ITU) tem alta incidência e recorrência, e o tratamento é empírico na maioria dos casos. **Objetivos:** O objetivo deste estudo foi analisar as culturas de urina realizadas em um hospital secundário, durante dois períodos: 2005-2006 e 2010-2011, para estimar a resistência microbiana. **Pacientes e métodos:** Foram analisadas 11.943 culturas aeróbicas de urina de acordo com um conjunto de dados demográficos básicos e susceptibilidade aos antibióticos, obedecendo às normas do *Clinical and Laboratory Standards Institute* (CLSI) para Vitek 1 e 2. **Resultados:** A maioria dos participantes era adulta e jovem atendida no Serviço de Emergência. *E. coli* foi a mais freqüente (70,2%) entre as 75 espécies isoladas. Resistência de todos os isolados foi $\geq 20\%$ para

sulfametoxazol/trimetoprim (SMX/TMP), norfloxacin, nitrofurantoína, cefazolina e ácido nalidíxico, apesar de *E. coli* ter sido mais suscetível (resistência $\geq 20\%$ apenas para SMX/TMP e ácido nalidíxico) entre todos os isolados, levando em conta a porcentagem de resistência e o número de antibióticos testados. Resistência às fluoroquinolonas foi de 12%. Fatores de risco para *E. coli*: sexo feminino e idade < 65 anos. Homens e pacientes com mais de 65 anos apresentaram isolados mais resistentes. Beta-lactamases de espectro estendido (ESBL) foram identificadas em 173 de 5.722 isolados Gram-negativos (3,0%), 2010-2011. **Conclusões:** *E. coli* foi o isolado mais sensível a antibióticos. Houve uma evolução significativa da resistência antimicrobiana entre os dois períodos. Foi preocupante o aumento da resistência às fluoroquinolonas.

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CONFLICTS OF INTEREST (DISCLOSURES)

No potential conflict of interest are declared for this paper.

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