

Antibiotic resistance pattern in urine cultures from community-dwelling women in southern Brazil – a cross-sectional study short communication

Padrão de resistência antimicrobiana em culturas ambulatoriais de urina em mulheres no sul do Brasil – estudo transversal comunicação breve


Patrón de resistencia a los antibióticos en cultivos de orina de mujeres que viven en la comunidad en el sur de Brasil – un estudio transversal comunicación corta


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ABSTRACT

Objective: The increase in antibiotic resistance (AR) is a global phenomenon with regional variation. This survey aims to describe the AR in urine cultures of women from the community in a southern Brazil city.

Methods: A retrospective cross-sectional single-center study in urine cultures of community dwelling individuals. The main outcome was the AR profile of bacterial isolates from women in outpatient care.

Results: From 4,011 urine cultures, 524 were positive (91% from women). The most frequently isolated bacteria were *Escherichia coli* (*E. coli*) (67.0%) and *Klebsiella spp.* (19.4%). *E. coli* presented low resistance to nitrofurantoin (3.7%), moderate to levofloxacin (15.6%), amoxicillin-clavulonate (16.4%) and ciprofloxacin (17.4%), and high to trimethoprim-sulfamethoxazole (26.9%).

Conclusions: Nitrofurantoin seems to be the best choice for the empirical treatment of low urinary tract infections in women, whereas sulfonamides are no longer an option, since *E. coli* resistance to this drug is above 20%.

Keywords: Urinary tract infections. Community-acquired infections. Drug resistance, microbial. Brazil. *Escherichia coli*.

RESUMO

Objetivo: O aumento da resistência aos antibióticos (AR) é um fenômeno global com variações regionais. Esta pesquisa tem como objetivo descrever a AR em culturas de urina de mulheres oriundas da comunidade em uma cidade sul-brasileira.

Métodos: Um estudo de centro único, transversal e retrospectivo em culturas de urina oriundas da comunidade. O principal desfecho foi o perfil de AR de bactérias isoladas de uroculturas ambulatoriais.

Resultados: De 4.011 culturas de urina, 524 foram positivas (91% de mulheres). As bactérias mais frequentemente isoladas em mulheres foram *Escherichia coli* (67,0%) e *Klebsiella spp.* (19,4%). *E. coli* apresentou baixa resistência à nitrofurantoína (3,7%), moderada a levofloxacina (15,6%), amoxicilina-clavulonato (16,4%) e ciprofloxacina (17,4%) e alta ao trimetoprim-sulfametoxazol (26,9%) entre mulheres.

Conclusões: A nitrofurantoína parece ser a melhor escolha para o tratamento empírico das infecções do trato urinário inferior em mulheres, enquanto as sulfonamidas não são mais uma opção, uma vez que a resistência de *E. coli* a essa droga é superior a 20%.

Palavras-chave: Infecções urinárias. Infecções comunitárias adquiridas. Resistência microbiana a medicamentos. Brasil. *Escherichia coli*.

RESUMEN

Objetivo: El aumento de la resistencia a los antibióticos (AR) es un fenómeno global con variaciones regionales. Esta investigación tiene como objetivo describir la AR en cultivos de orina de mujeres de la comunidad de una ciudad del sur de Brasil.

Métodos: Un estudio retrospectivo transversal, de un solo centro, de cultivos de orina de la comunidad. El resultado principal fue el perfil de AR de bacterias aisladas de estos cultivos de orina.

Resultados: De 4.011 cultivos de orina, 524 fueron positivos (91% de las mujeres). Las bacterias más frecuentemente aisladas en mujeres fueron *Escherichia coli* (67,0%) y *Klebsiella spp.* (19,4%). *E. coli* mostró baja resistencia a nitrofurantoína (3,7%), moderada a levofloxacina (15,6%), amoxicilina-clavulonato (16,4%) y ciprofloxacina (17,4%) y alta a trimetoprima-sulfametoxazol (26,9%) entre las mujeres.

Conclusiones: La nitrofurantoína parece ser la mejor opción para el tratamiento empírico de la infección de las vías urinarias inferiores en mujeres, mientras que las sulfonamidas ya no son una opción, ya que la resistencia de *E. coli* a este fármaco es superior al 20%.

Palabras clave: Infecciones urinarias. Infecciones comunitarias adquiridas. Farmacorresistencia microbiana. Brasil. *Escherichia coli*.

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INTRODUCTION

According to the Global Burden of Disease study, there were an estimated 274 million new cases of UTIs globally in 2017, across all ages and both sexes⁽¹⁾. The estimated lifetime prevalence of UTI in women is above 50,000 cases per 100,000 and around 10,000 cases per 100,000 in men⁽²⁾. Urinary tract infection (UTI) severity ranges from cystitis, impacting quality of life and leading to lost working days, to pyelonephritis, with risk of sepsis and even death⁽³⁾.

Lower urinary tract infection in healthy and non-pregnant women is considered uncomplicated and may allow a clinical diagnosis. All other conditions classify as complicated UTI, requiring urine culture⁽³⁾. However, the initial treatment is frequently empirical, given the length of time needed to isolate the agent and to test the antibiotic resistance (AR)⁽³⁾. The empirical treatment should be based on the up-dated local AR pattern, due to its great variability according to geographic regions and periods of time⁽⁴⁾. There are a series of determinants for local AR, such as health policies regulating local antibiotic practices, use of antibiotics in husbandry, and control over counterfeit medicines^(5,6). Host factors may also determine the susceptibility to UTI and the species of microorganism invading the urinary tract.

Therefore, a rational and cost-effective choice of the antibiotic to be used in empirical therapy of UTI depends on the knowledge of local AR pattern. Periodic reports on local AR have not been provided by the Brazilian public health system, which would be the most suitable data to base this clinical decision. The relative scarcity of scientific publications on updated AR of uropathogens in southern Brazil does not contribute to filling this gap⁽⁷⁾. Brazil is a large country, with continental dimension and large socioeconomic heterogeneity, precluding that the AR described for one region could be extrapolated to all the country.

The continuous increase in AR has been described around the world, causing that prescribing on base of outdated data led to higher risk of therapeutic failure, with consequent social and economic costs⁽⁸⁾. As Brazilian nurses integrated in health care teams are allowed to prescribe drugs available in public health programs and recommended by the health institution guidelines⁽⁹⁾, this issue concern nurses as well as clinicians. The aim of the current report is to provide local AR patterns of uropathogens isolated from urine cultures of community women in a city from southern Brazil.

METHODS

Study Design: This is a cross-sectional, observational and retrospective study, done through the revision of the results of urine cultures from the Microbiology Laboratory of a University Hospital in Southern Brazil. The study design and report were performed according to *Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)* recommendations. The study protocol was evaluated and approved by the University Ethics Committee under number 3.233.494.

Setting: The results of all urine cultures collected from ambulatory patients between January 1 and December 31, 2018, in the Microbiology Laboratory of the Catholic University of Pelotas Hospital, were collected from the laboratory's electronic database. The laboratory serves hospitalized and ambulatory patients, in addition to the local emergency unit. The exams are funded by the Brazilian public health system, private health insurance or patient out-of-pocket payment. In the studied city, another sixteen laboratories perform urine cultures.

Participants: Data on sex, age, bacteria strain and antibiotic resistance pattern from urine cultures of individuals from the community were collected. The urine cultures were eligible if there is bacterial growth above 10⁽⁵⁾ colony forming units per milliliter (CFU/mL), with antibiotic susceptibility test, collected from community-dwelling individuals during the year of 2018. Urine cultures with polymicrobial or fungus growth, or collected from hospitalized or institutionalized, were excluded.

Outcomes: The main outcome was the antibiotic resistance profile of bacterium strains isolated in urine cultures. Secondary outcomes include AR patterns difference according to age.

Measurements: The laboratory studied use bacterium identification and susceptibility tests protocol according the PNCQ – National Program of Quality Control, from the Brazilian Health Regulatory Agency, following the Resolution RDC n° 153. The process began with the sewing of urine in Petry plaque with agar-blood, McConkey and agar-chocolate. Next, if there is bacteria growth after 24 hours of incubation, proceed to automated bacteria identification and susceptibility tests, using the system Phoenix 100 BD. This system identifies the germ through the detection of products of microbial metabolism and tests the susceptibility screening the bacterial growth in presence of graded concentration of antibiotics in panel holes, which are continuously incubated

and read. The automated system provides fast results on growth and AR of most aerobic and facultative anaerobic, Gram- positive and Gram-negative bacteria that infect humans. The system provides the AR results as resistant, intermediary and sensible according to minimum inhibitory concentration (MIC).

The results of the urine cultures and demographic data were collected through a structured questionnaire elaborated for the study. As the collection was based in secondary data archived in the digital database of the laboratory, clinical and socioeconomic data, not originally recorded, could not be recollected.

Statistical Analysis: Descriptive statistics on isolated bacteria strains and their AR patterns presented mean and standard deviation, median and interquartile range, or absolute number and percentual. The difference in positive urine cultures, patterns of isolated bacteria and AR according to age was analyzed by chi-square, t-student, or Mann-Whitney test. Intermediary susceptibility was included as resistant in the analysis. The statistical analysis package STATA 15.2 was used for statistical analysis.

■ RESULTS

A total of 4,011 urine cultures of community-dwelling individuals were performed during the year of 2018 in the studied microbiology laboratory, from which 524 (13.1%)

were eligible for this survey. There were 3,374 (84.1%) exclusion due to no bacteria growth, 46 (1.2%) with polymicrobial growth, 9 (0.2%) without antibiotic susceptibility tests, 10 (0.2%) due to insufficient urine to culture, and 47 (1.2%) could not be used due to technical problems in the electronic database. From the 524 cultures included, 478 were from women, which presented a median of age of 51 (IQR 31 – 64) years, with most positive cultures from women between 18 and 59 years old, comparing with younger and older age groups ($p < 0.001$).

The bacteria most frequent was *Escherichia coli*, that was isolated from urine cultures of 67.0% of female individuals, including 1.3% of *E. coli* producing extended spectrum beta lactamase (ESBL). Other bacteria frequently isolated were *Klebsiella* spp 19.4%, *Enterobacter* spp 4.2%, *Staphylococcus* spp 4.6%, *Streptococcus* spp 4.3%, *Proteus mirabilis* 2.9%, *Enterococcus* spp 3.4%, *Citrobacter* 2.7%, *Acinetobacter baumannii* 1.3% e *Pseudomonas aeruginosa* 0.4%.

Regarding AR, *E. coli* presented low resistance to nitrofurantoin (3.7%), moderate to levofloxacin (15.6%), amoxicillin-clavulonate (16.4%) and ciprofloxacin (17.4%), and high resistance to trimethoprim-sulfamethoxazole (26.9%). *E. coli* isolated was highly resistant to ampicillin (48.1%) (Table 1).

Enterobacter spp. presented high AR to most antibiotics tested, with the lower resistance to levofloxacin (5.7%), ceftriaxone (16.7%) and ciprofloxacin (10.0%). The AR of *Enterobacter* spp to amikacin (5.5%) was lower. (Table 1)

Table 1 – Antibiotic resistance according to uropathogens

Pathogens	Antibiotic Resistance (%)						Ami	Cef
	Amp	Ax/Cl	Cip	Lev	Nit	S/T		
<i>E. coli</i>	48.1	16.4	17.4	15.6	3.7	26.9	0.3	3.4
<i>Enterococcus</i> spp	7,7	0	27.3	0	20	25	NT	33.3
<i>Proteus mirabilis</i>	42.9	28.6	7.1	0	100	14.3	0	20.0
<i>Klebsiella</i> spp	100	28.6	22.2	22.5	39.5	9.1	2.3	26.7
<i>Enterobacter</i>	89.5	100	10.1	5.7	42.1	15.8	5.5	16.7
<i>Staphylococcus</i> spp	94.1	NT	0	0	13.6	36.4	NT	NT
<i>Citrobacter</i> spp	100	61.5	15.4	23.1	36.4	30.8	0	0
<i>Streptococcus</i> spp	28.6	NT	33.3	18.2	36.4	64.3	NT	33.3

Source: Research data.

Amp: ampicillin; Ax/Cl: amoxicillin clavulanate; Cip: ciprofloxacin; Lev: levofloxacin; Nit: nitrofurantoin; S/T: sulfametoxazole thrimetropin; Ami: amikacin; Cef: ceftriaxone; NT: not tested

Klebsiella spp. also presented high rates of AR, being the lower to trimethoprim-sulfamethoxazole (9.1%), ciprofloxacin (22,2%) and levofloxacin (22.5%) in urine from female individuals. The unique antibiotic to which *Klebsiella spp* presented low resistance was amikacin (2,3%) (Table 1).

■ DISCUSSION

Escherichia coli was the most prevalent uropathogen isolated in this sample of urine cultures from community-dwelling women in southern Brazil, presenting low resistance to nitrofurantoin, intermediate resistance to quinolones and amoxicillin-clavulonate and high resistance to sulfonamide.

The high resistance of *E. coli* to sulfonamide is occurring worldwide⁽⁴⁾. The continuous use of sulfonamide for almost a century in humans and animals, associated with the easy transference of mobile resistance genes between *E. coli* isolates, could easily explain this resistance pattern. Nitrofurantoin is also an old drug, in clinical use since the 1950s. However, its multifactorial mechanism of action imposes difficulties in the acquisition of resistance⁽¹⁰⁾. In addition, the drug only reaches therapeutic concentration in the urinary tract, restricting its widespread clinical use.

The most recent emergence of resistance to quinolones, however, is alarming. Quinolones have represented a therapeutic reserve for pyelonephritis⁽³⁾. Most other drugs do not acquire adequate levels in the kidney or are only available for parenteral use, precluding out-of-hospital treatment. In this sample from southern Brazil, 17.4% and 15.6% of the community-acquired *E. coli* strains were resistant to ciprofloxacin and levofloxacin, respectively. The rate of community-acquired *E. coli* resistance to ciprofloxacin described worldwide ranges from 11% in Switzerland⁽¹¹⁾ to 53% in India⁽¹²⁾ and 55% in Mexico⁽¹³⁾. The use of over-the-counter or non-prescription antibiotics, a practice more frequent in developing world, proved to be an important predictor for higher resistance⁽¹⁴⁾. The ARESC study⁽⁷⁾ enrolled seventy centers in nine European countries and in Brazil between 2003 and 2006, including healthy adult women with symptoms of uncomplicated lower UTI. They found that *E. coli* isolated in Brazil presented 10,8% resistance rate to ciprofloxacin. Kiffer et al⁽¹⁵⁾. evaluated *E. coli* resistance to ciprofloxacin in Brazil in 2007 and found a resistance rate of 12%. Although these studies have included different regions of Brazil, a continental dimensions country, with great socioeconomic and cultural differences, the resistance rate found in the present sample is only 5% higher than that found 10 years ago, a change lower than that found in other developing countries. Since 2010, the Brazilian Health Regulatory Agency has banned the sale of

non-prescription antibiotics, which could have contributed to minimize the increase in *E. coli* resistance to quinolones.

Another important step has been planned by the Brazilian Health Regulatory Agency: providing traceability for medicines marketed in Brazil. The lack of quality control of antibiotics also contributes to the increasing in AR, due to exposure to subtherapeutic levels. A recent report by the World Health Organization (WHO)⁽¹⁶⁾ describes that about 10% of medicines marketed in low-to-middle income countries fulfilled the criteria for substandard/spurious/false-labelled /falsified/ counterfeit medical products. However, the controversy remains that patent protection and economic interests are the main driver for the seizure of drugs considered counterfeit. A recent investigation⁽¹⁷⁾ found that antimalarials and antibiotics were the medicines most commonly sold in substandard or falsified conditions in low-income African and Asian countries. In 2008, 500,000 units of medicines suspected of falsification were seized in Brazil. This number staggered to 18.000.000 units in 2010. Among the drugs seized for falsification between 1998-2015 in Brazil, 8% were antibiotics⁽¹⁸⁾.

The extensive antimicrobial use in livestock has also been associated with antimicrobial resistance globally. A recent publication in Science journal⁽¹⁹⁾ has drawn the global map of AR in husbandry, describing hotspots of resistance exactly in India and Mexico, the same countries with the highest rates of resistance to ciprofloxacin among human community-acquired *E. coli*^(12,13). The same global map found hotspots in the southeast region of Brazil, but Rio Grande do Sul presented low AR in livestock. However, the exact amount of transmission of AR between human and livestock pathogens is still a matter of debate.

The lack of access to relevant clinical information, such as symptoms, previous exposure to antibiotics and associated comorbidities, or previous hospitalizations is a weakness of this report, which was based on secondary data from a microbiology laboratory. The urine cultures were performed for clinical indication. Since uncomplicated UTI in women should be treated empirically, this sample may predominantly include cases of complicated UTI, which can overestimate resistance rates. Finally, the absence of resistance test for fosfomicin, a drug that has also been described⁽¹⁰⁾ as highly effective against *E. coli* and other uropathogens, is another flaw.

■ CONCLUSIONS

This report helps to fill a gap in antibiotic resistance surveys in southern Brazil, data on which health care teams (including nurses) and policy makers should base their

decisions. According to the present findings, nitrofurantoin is the best choice for the empirical treatment of lower urinary tract infections in women. Sulfonamides are no longer an option, since *E. coli* resistance to this drug is above 20%, the recommended limit to allow use in empirical treatment. Quinolones should be preserved from the escalating resistance of *E. coli*, restricting its use, in women, to complicated or upper urinary tract infections.

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■ **Authorship contribution:**

Claudia Rejane Mews Peter, Josiane Cristine dos Passos Krause Braga, Lourdes Helena de Araújo Rodrigues and Mauricio Parcio Arrieira collected the data and contributed to data analysis, writing and revision of the paper.

Maristela Böhlke conceived, designed the methodology, supervised the project, performed the analysis and revised the paper.

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