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Profile of the prevalence of enterococcal infections and bacterial resistance to antibiotics in public urinary infections

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Abstract

Urinary tract infections caused by antibiotic-resistant bacteria are the third most common type of infection in humans described worldwide. This is a time series study carried out from positive urine culture records, from January 1, 2011, to December 31, 2019, in the metropolitan region of the municipality of Goiania, Midwest Brazil, in people of all ages and sexes. The aim was to assess the prevalence of urinary tract infections, the pattern of resistance to antibiotics, and the trend of increasing resistance in *Enterococcus faecalis*. Descriptive analysis and Pearson's chi-square test were used to assess the growth in the prevalence of antibiotic resistance, and a correlation analysis by the *Poisson* method was used to assess the trend in the growth of bacterial resistance. Of 22,034 positive urine cultures, 646 (2.9%) were of *E. faecalis*. The findings showed that urinary tract infections are more prevalent in women aged over 19 years old. The prevalence of resistance was high for the fluoroquinolone drugs and a significant increase in resistance against Gentamicin (p=0.02%) and a decrease toward Ampicillin (p<0.001) and Tobramycin (p<0.001). The increasing trend was significant for Gentamicin and negative for Ampicillin and Tobramycin. The findings demonstrate that it is necessary to create surveillance programs that aim to monitor the growth of the resistance patterns in public UTIs, while considering the study site.

Keywords: *Enterococcus faecalis.* Antibiotics. Cystitis. Bacterial Pharmacoresistance.

INTRODUCTION

Enterococcal urinary tract infection occurs mainly in hospital environments but has also had an impact on public-acquired infections[1]. It is estimated that 250 million people are annually diagnosed with UTI, and when treated, the cost to the global economy is 6 billion dollars per year[2].

In Brazil, in 8% of clinical outpatient consultations, patients report signs and symptoms of UTI. Some risk factors for infection are associated with female vulnerability, inadequate hygiene, advanced age, and pregnancy. Adult women are 50 times more likely to acquire UTI than men and 30% of women have symptomatic UTI throughout their lives[3].

The causative agents of UTIs are diverse, both patients in hospital and public settings. UTIs can be caused by both Gram-positive and Gram-negative bacteria. Among Gram-positive bacteria, *E. faecalis* is the most prevalent species, accounting for 6-10% of all

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UTIs of public origin[4].

UTIs caused by *E. faecalis* have a low prevalence (2.9%) when compared to those caused by other enterobacteria, as demonstrated in a study carried out at the University of Szeged, Hungary[5]. However, when compared only with Gram-positive bacteria, *Enterococcus* spp. are one of the most prevalent[6].

The treatment of UTIs of public origin is often empirical, therefore, there is a risk of treatment failure and increased selective pressure. However, the excessive use of antibiotics in the treatment of infections caused by bacteria is associated with the selection of drug-resistant microorganisms[7]. It is known that bacteria of the genus *Enterococcus* are resistant to clindamycin, amoxicillin, and gentamicin, and to treat such infections the use of more potent antibiotics such as vancomycin and teicoplanin are necessary[8].

The growth of the antibiotic resistance pattern is considered a worldwide problem, as it favors the development of increasingly resistant microorganisms, threatening global health. According to the Pan American Health Organization, resistance to antibiotics affects about half a million people with bacterial infections annually, which requires efficient measures to combat this growth[9].

Studies show that the prevalence of UTIs has geographic variations and that the increase in infections by multidrug-resistant bacteria is a challenge for the treatment of these infections, requiring periodic analyses of the susceptibility profile of the microorganisms associated with these infections and even detecting the emergence of new UTI-causing pathogens[10,11].

Empirical therapy for UTIs of public origin is based on prior knowledge of the prevalence of certain organisms and their susceptibility to antibiotics.

The present study aims to evaluate the prevalence of UTIs, the pattern of resistance to antibiotics and the trend of increasing resistance of *E. faecalis* in public infections.

MATERIALS AND METHODS

This is a time series study conducted from January 2011 to December 2019, in the city of Goiania, located in the Midwest of Brazil, with a population of 1,302,000 inhabitants[12].

Public-sourced positive urine culture records that resulted in the isolation and identification of *E. faecalis* were analyzed in people of all ages. These records were issued by four laboratories that serve patients in the private, public, and health insurance systems, which have clinical sample collection centers, with wide territorial coverage in the city of Goiania, Goias, Brazil and in the cities of the metropolitan region.

Only the results of the first record of each patient were considered to avoid duplication of results unless there was an episode of reinfection three months after the first one. Results that, regardless of the reason, were not completed and the patient did not return to provide a new sample were excluded.

The cultures, performed by the laboratories participating in the study, were obtained from the mid-stream of urine, which were processed and incubated in Agar CLED, at a temperature of 35°C, for 18 hours. A urine culture was considered positive when it showed a bacterial count greater than 100,000 colony forming units per milliliter of urine (CFU/mL). The identification of the isolate and the antibiotic susceptibility test were performed using the VITEK2 auto-

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mated system (BioMérieux).

The following antibiotics were evaluated: Vancomycin, Gentamicin, Amikacin, Tobramycin, Ciprofloxacin, Norfloxacin, Nalidixic Acid, Trimethoprim/Sulfamethoxazole, Amoxicillin/Clavulanate, Ampicillin, Tetracycline and Nitrofurantoin. The interpretation of the results followed the recommendations of the Clinical and Laboratory Standards Institute, in their respective version up to December 30, 2017[13]. For the purposes of this study, susceptibility was classified as sensitive or resistant. The intermediate resistance, in this study was considered as resistant.

Demographic data and the susceptibility profile were stored in a Microsoft Excel database and analyzed using SPSS, version 20.0 and Stata, version 15.0.

The prevalence of resistance in E. faecalis was determined using absolute and relative frequencies and presented using multiple contingency tables. In order to verify if there was a significant increase in any of the evaluated years, the chi-square test was applied. The association of the prevalence of resistance in *E. faecalis* toward each antibiotic with gender and age range was performed using the Pearson/Post hoc chi-square test. To assess whether there was a linear relationship in the resistance growth trend for each antibiotic, a correlation analysis was performed using the Poisson method. The statistical package used was IBM SPSS Statistics for Windows and the significance level was set at $p<0.05$.

The study was carried out in accordance with the principles expressed in the Declaration of Helsinki and approved by the Human Ethics Committees, opinion nº 348.549.

RESULTS

A total of 22,034 positive urine culture reports were identified. However, only 646 (2.9%) were infections caused by *E. faecalis*, and the remaining 21,388 (97.1%) by other pathogens.

The study showed that the difference between the genders of service users in the historical series was not significant; however, the prevalence of infections in the period under study was higher among women ($p <0.001$) (Table 1).

Higher, significant prevalence of bacterial resistance occurred in females aged over 19 years old and in males when age was greater than or equal to 60 years old (Table 2).

The study demonstrated that the difference in the antibiotic resistance pattern between the two sexes was significant only for Gentamicin (Table 3).

The data demonstrated that the prevalence of resistance was high toward the fluoroquinolone drugs and resistance increased significantly toward Gentamicin ($p=0.02\%$) and decreased against Ampicillin ($p<0.001$) and Tobramycin ($p<0.001$) (Table 4).

The prevalence of resistance in *E. faecalis* was high toward Ciprofloxacin and Norfloxacin with a significant difference between age groups (Table 5).

The resistance trend was negative and significant toward Ampicillin and Tobramycin. On the other hand, the increasing trend was significant against Gentamicin (Table 6).

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Table 1 - Public-borne urinary infections caused by *E. faecalis* distributed by year and sex in the metropolitan region of Goiania, Brazil, 2011 to 2019.

Year	Sex N (%)		Total N (%)	p (p<0.05)
	Female N (%)	Male N (%)		
2011	14 (3.0)	4 (2.3)	18 (2.8)	
2012	22 (4.7)	16 (9.2)	38 (5.9)	
2013	16 (3.4)	11 (6.3)	27 (4.2)	
2014	52 (11.0)	18 (10.3)	70 (10.8)	0.05
2015	89 (18.9)	20 (11.5)	109 (16.9)	
2016	90 (19.1)	24 (13.8)	114 (17.6)	
2017	171 (36.2)	74 (42.5)	245 (37.9)	
2018	11 (2.3)	3 (1.7)	14 (2.2)	
2019	7 (1.5)	4 (2.3)	11 (1.7)	
Total	472 (73.1)	174 (26.9)	646 (100.0)	<0.001

Source: Data obtained from laboratories participating in the study.

Table 2 - Positive urine cultures for *E. faecalis* distributed by age and sex, in the metropolitan region of Goiania, Brazil, from 2011 to 2019.

	Sex n (%)		Total n (%) n = 646	p (p<0.05)
	Female	Male		
Age Group				
≤ 18	23 (4.9)	14 (8.0)	37 (5.7)	0.09
19 to 59	302 (64.0)	56 (32.2)	358 (55.4)	<0.001
≥ 60	147 (31.1)	104 (59.8)	251 (38.9)	<0.001

Source: Data obtained from laboratories participating in the study.

Table 3 - Resistance of *E. faecalis* to antibiotics distributed by sex, in the metropolitan region of Goiania, Brazil, from 2011 to 2019.

Antibiotic	Sex		p (p<0.05)
	Female N (%)	Male N (%)	
Nalidixic Acid	0 (0.0)	1 (50.0)	0.38
Amikacin	0 (0.0)	1 (25.0)	0.15
Amoxicillin Clavulanate	1 (20.0)	0 (0.0)	0.49
Ampicillin	22 (4.8)	15 (8.8)	0.06
Ciprofloxacin	96 (21.0)	47 (27.6)	0.07
Gentamicin	4 (9.1)	4 (40.0)	0.01
Nitrofurantoin	19 (26.0)	8 (36.4)	0.34
Norfloxacin	66 (32.5)	36 (39.6)	0.24
Tetracycline	5 (2.5)	0 (0.0)	0.12
Tobramycin	55 (18.6)	19 (17.8)	0.83
Trimethoprim Sulfamethoxazole	0 (0.0)	0 (0.0)	na*
Vancomycin	0 (0.0)	1 (20.0)	0.06

Source: Data obtained from laboratories participating in the study.

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Table 4 - Prevalence and growth of resistance in *E. faecalis* to different antibiotics isolated from urinary tract infections of public origin in the metropolitan region of Goiania, Brazil, 2011 to 2019.

Antibiotics	2011 N (%)	2012 N (%)	2013 N (%)	2014 N (%)	2015 N (%)	2016 N (%)	2017 N (%)	2018 N (%)	2019 N (%)	2011 to 2015 (A)	2016 to 2019 (B) N (%)	Total N (%)	P (p<0.05) (A vs B)
Nalidixic Acid	1 (33.3)	-	-	-	-	-	-	-	-	1 (33.3)	-	1 (33.3)	na*
Amikacin	1 (20.0)	-	-	-	-	-	-	-	-	1 (10.0)	-	1 (8.3)	0.64
Amoxicillin/ Clavulanate	-	1 (100.0)	-	-	-	-	-	-	-	1 (20.0)	-	1 (14.3)	0.49
Ampicillin	2 (12.5)	6 (20.0)	5 (19.2)	6 (8.6)	9 (8.3)	6 (5.4)	3 (1.2)	-	-	28 (11.2)	9 (2.4)	37 (5.9)	**<0.001**
Ciprofloxacin	3 (21.4)	9 (25.0)	5 (18.5)	15 (21.4)	18 (16.5)	45 (39.8)	43 (17.8)	3 (21.4)	2 (50.0)	50 (19.5)	93 (25.0)	143 (22.8)	0.10
Gentamicin	1 (20.0)	-	-	-	2 (6.5)	1 (33.3)	1 (14.3)	1 (100.0)	2 (40.0)	3 (7.9)	5 (31.3)	8 (14.8)	**0.02**
Nitrofurantoin	1 (33.3)	1 (100.0)	-	-	4 (12.9)	11 (39.3)	10 (43.5)	-	-	6 (17.1)	21 (35.0)	27 (28.4)	0.06
Norfloxacin	3 (33.3)	14 (40.0)	7 (30.4)	25 (39.1)	18 (26.5)	19 (38.0)	11 (33.3)	5 (45.5)	-	67 (33.7)	35 (36.8)	102 (34.7)	0.59
Tetracycline	-	-	-	-	2 (6.1)	3 (4.5)	-	-	-	2 (1.2)	3 (2.5)	5 (1.7)	0.41
Tobramycin	5 (45.5)	12 (57.1)	2 (16.7)	14 (32.6)	22 (40.0)	19 (33.3)	-	-	-	55 (38.7)	19 (7.3)	74 (18.4)	**<0.001**
Trimethoprim/ Sulfamethoxazole	-	-	-	-	-	-	-	-	-	-	-	-	na*
Vancomycin	-	-	-	1 (25.0)	-	-	-	-	-	1 (6.3)	-	1 (4.5)	0.53

Source: Data obtained from laboratories participating in the study.

Table 5 - Distribution of antibiotic resistance in *E. faecalis* by age group, in the metropolitan region of Goiania, Brazil, from 2011 to 2019.

Antibiotic	Age Group			P (p<0.05)
	≤ 18 N (%)	19 to 59 N (%)	≥ 60 N (%)	
Nalidixic Acid	0 (0.0)	0 (0.0)	1 (100.0)	0.08
Amikacin	0 (0.0)	0 (0.0)	1 (20.0)	0.21
Amoxicillin Clavulanate	1 (33.3)	0 (0.0)	0 (0.0)	0.45
Ampicillin	4 (11.4)	20 (5.7)	13 (5.3)	0.34
Ciprofloxacin	9 (25.7)	66 (19.0)	68 (27.8)‡	0.03
Gentamicin	0 (0.0)	4 (13.8)	4 (19.0)	0.60
Nitrofurantoin	1 (20.0)	16 (28.1)	10 (30.3)	0.88
Norfloxacin	8 (42.1)	43 (28.1)	51 (41.8)‡	0.04
Tetracycline	0 (0.0)	3 (2.2)	2 (1.6)	0.74
Tobramycin	3 (15.0)	36 (15.9)	35 (22.4)	0.26
Trimethoprim Sulfamethoxazole	0 (0.0)	0 (0.0)	0 (0.0)	na*
Vancomycin	0 (0.0)	1 (9.1)	0 (0.0)	0.59

Source: Data obtained from laboratories participating in the study.

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Table 6 - Trend in antibiotic resistance growth in the metropolitan region of Goiania, Brazil, from 2011 to 2019.

Antibiotic	Resistance prevalence / year	
	r	p*
Nalidixic Acid	-0.54	0.12
amikacin	-0.55	0.11
Amoxicillin Clavulanate	-0.41	0.27
Ampicillin	-0.94	<0.001
Ciprofloxacin	0.18	0.63
gentamicin	0.68	0.04
Nitrofurantoin	-0.31	0.41
Norfloxacin	-0.15	0.69
Tetracycline	0.09	0.81
Tobramycin	-0.81	0.008
Trimethoprim Sulfamethoxazole	0.01	0.98
Vancomycin	-0.13	0.73

Source: Data obtained from laboratories participating in the study.

DISCUSSION

In this study (Table 1) and in others, higher rates of UTIs in women were also identified and they are related to specific risk factors, such as anatomical conditions (short urethra) or circumstances related to age, pregnancy, recent sexual intercourse, use of diaphragm or spermicides in young and adult women or induced by hormones or anatomical changes due to menopause in older women[14-16].

In the female population (Table 2), higher rates of infection occurred in the age group between 19 and 59 years old. On the other hand, in the male population (Table 2), the highest rates were found in users aged at least 60 years old, as demonstrated in another study[17]. This age group has characteristics that favor the development of UTIs. Among young women and men, the use of contraceptives, spermicides, high sexual activity, variety of partners, and other forms of sexual activity, such as anal intercourse, increase the risk of urinary tract infections in both sexes[18,19]. Among women, the postmenopausal period, genitourinary and immunological disorders may be related to a higher prevalence of UTIs[20].

In women, UTIs are more recurrent than in men; however, the prevalence of resistant bacteria is higher in men (Table 03)[21]. Among men, UTIs are more complicated and are often associated with urinary tract abnormalities, such as prostate enlargement or urethral stricture, requiring a longer period of antibiotic use, which may contribute to the development of bacterial resistance to antibiotics[22,23]. For the empirical therapy of UTIs of public origin, it is relevant to consider the local patterns of susceptibility, which may vary with the microorganism and the patient's characteristics.

Fluoroquinolones are antibiotics with good activity against Gram-positive bacteria; however, it is observed that their re-

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sistance rate is growing, due to over-prescription, mainly in the population up to 18 years old or at least 60 years old (Table 04 and 05)[24]. This class of antibiotics is widely prescribed for the treatment of UTIs, both in nosocomial and public infections; however, our results showed that resistance rates are higher than the 20% limit recommended for its use in the empirical treatment of UTIs of public origin[25]. This indicates that Norfloxacin and Ciprofloxacin are not an available option for the empirical treatment of UTIs, as therapeutic failure may occur, and their use should be guided by an antibiogram.

Enterococci are intrinsically resistant to a number of antibiotics and can acquire resistance genes from other pathogens resulting in a high level of resistance to Gentamicin (Table 06)[26]. The high level of resistance to Gentamicin by this pathogen is worrying, as in severe infections the combined use of Gentamicin with another antibiotic that acts upon the cell wall, such as Penicillin, Ampicillin or Vancomycin, will be necessary[27]. In this study, the low rate of resistance to Gentamicin was similar to that described in a study carried out in Freiburg, Germany[28]. However, a high level of resistance to Gentamicin in *Enterococci* isolated from UTIs of public origin has already been described in India[29]. These results show that permanent monitoring of resistance among *Enterococci* involved in UTIs of public origin is necessary.

Nitrofurantoin, one of the oldest oral antibiotics with a low rate of resistance in most parts of the world, was also identified in this study (Table 04). This drug is equally active *in vitro* in multidrug resistant (MDR) *Enterococci* as well as in non-MRD *Enterococci*[30,31]. In addition, Nitrofurantoin has fewer side effects and low selective pressure due to the broad mechanism of action that may explain the absence of acquired bacterial resistance to this drug. The guidelines of the American Society of Infectious Diseases recommend Nitrofurantoin as one of the compounds for the empirical treatment of uncomplicated cystitis[32]. Therefore, this drug can be considered as an excellent choice of first-line, economical, and effective oral therapy in patients with UTIs of public origin. Therefore, one of the ways to combat the development of increasingly resistant microorganisms is based on the development of studies that determine the sensitivity of each microorganism to antibiotics, taking into account the location and period of the study[33].

The creation of a bacterial resistance monitoring system is of interest to public health, since it is possible to detect the emergence of pathogens resistant to antibiotics that are used in the treatment of UTIs. These measures have a social and economic impact, as they reduce costs as they reduce the use of more expensive and more toxic drugs, as well as hospitalization, morbidity, and mortality.

The main strength of this study is that it is based on a large database provided by large clinical and reference laboratories with wide territorial coverage. However, the study has some limitations in relation to the lack of some relevant data, such as clinical history, lack of information on previous infections, recent use of antibiotics, associated comorbidities, and recent hospitalization. Another limiting factor was that some of the positive samples were not tested for susceptibility to all antibiotics available for the treatment of UTIs.

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CONCLUSION

From the study, it can be concluded that the prevalence of resistance among UTIs by *E. faecalis* is low for most of the antibiotics tested; however, there was a significant increase in resistance against Ampicillin and Tobramycin, and a significant decrease for Gentamicin. The negative growth trend was significant for Ampicillin and Tobramycin, and negative for Gentamicin. They are more prevalent in women aged over 19 years old.

Locally, this study could be useful in defining public policy for the empirical use of antibiotics in the treatment of public borne UTIs and in evaluating their antibiotic formulary guidelines and raising awareness. Annual reports supported by data from this survey will help prevent emerging strains from spreading within the public.

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