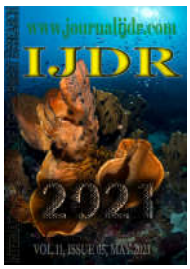
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 11, Issue, 05, pp. 47029-47033, May, 2021
https://doi.org/10.37118/ijdr.21754.05.2021



RESEARCH ARTICLE OPEN ACCESS

ANTIMICROBIAL SUSCEPTIBILITY PROFILE FOR ESCHERICHIA COLI IN URINE CULTURE OF PATIENTS FROM A HOME HEALTH PROGRAM

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ARTICLE INFO

Article History:
Received 20th February, 2021
Received in revised form
14th March, 2021
Accepted 18th April, 2021
Published online 22th May, 2021

Key Words:
Urinary Tract Infection; Urine; Microbiology; Antibiotic Resistance; Home Health Care.

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ABSTRACT

Abstract: There is a high frequency ofE.coli genitourinary infections in patients of home care programs. The susceptibility profile of infectious agents in such patients needs to be studied toestablish effective preventive measures and treatment. **Objective:** We aimed to evaluate the profile of antimicrobial susceptibility of the E. coli isolated from patients assisted by a Home Care Program of health. **Methods:** Home visits were conducted and 112 urine samples were collected. Bacteria were isolated and identified through specific tests for each bacterial class. Susceptibility tests were performed for various classes of antibiotics. **Results:** Of the 112 cultures tested, 61 (54.5%, CI = 45.28 to 63.72) exhibited growth of an infectious agent, 46 from males (75.4%, CI = 67.42 to 83.38) and 15 from females (24.6%, CI = 16.62 to 32.58). Of the 61 cultures, 17 (27.87%) showed the presence of E. coli. Antibiogram analysis revealed that of the 17 samples tested 14 showed bacterial multiresistance. **Conclusion:** This was the first report that linkedbacterial multiresistance of urine cultures from patients to the home health program. Further studies are needed to define the genotypic associations and the resistance mechanisms of these agents.

Citation: Camila Lima Guimarães, Carolina Braga Borges, Maria Clara Ribeiro Figueiredo, Matheus Medeiros Aguiar, Elisa Lima Vieira, Melissa Carvalho Martins de Abreu1, Marcelo Costa Araújo, Carlo José Freire Oliveira, Wellington Francisco Rodrigues and Camila Botelho Miguel, 2021. "Antimicrobial susceptibility profile for Escherichia coli in urine culture of patients from a home health program", *International Journal of Development Research*, 11, (05), 47029-47033.

INTRODUÇÃO

The changing demographic profile in Brazil has led to adjustments in the health care delivery model. This included the emergence of the Home Care program, which enabled dehospitalization, decreased the use of hospital beds, decreased costs, and resulted in centralized care for individuals(Rajao, F.L& Martins M., 2020). The Home Care service started in the 1960s and was regulated by Decree No. 825/2016. It is a modality of health care integrated into the Health Care Network that aims at prevention, treatment, rehabilitation, palliation, and health promotion, guaranteeing effective clinical care in the patient's home, therebylowering the need for hospital admissions (De Souzaet al., 2019). To meet the demand of the population, some regulations were implemented and/or reformulated. On November 8, 2011, the federal government implemented the Best at Home Program, to meet different levels of health care (Ministério da Saúde do Brasil, 2013). This program is aimed at individuals who have difficulty moving from their homes to a healthcare facility, or those who require multiprofessional home care.

The Best at Home program is supported by Ordinance No. 963, 2013, which standardizes the actions and strategies related to Home Care (Oliveira Neto A.V, & Dias, M.B., 2014). The Best at Home Program also allows the individual to recover in a known environment, leading to the decreased use of hospital beds and consequently, reducing hospitalization expenses. In 2011, this reduction was approximately 4.7%, however it increased to 5.8% and 10.2% in 2012 and 2013, respectively(Nishimura F, 2019). The individuals served by the program are often bedridden, and the vast majority has a basic pathology, which has left them disabled, fragile, and prone to acquiring infections (Petrovici, C.Get al., 2011). Urinary tract infections (UTIs) and their associated complications are a major cause of mortality and morbidity worldwide. *Escherichia coli* is responsible for approximately 80% of UTIs in outpatients and it is usually treated empirically. UTIs are increasingly associated with microorganisms that are resistant to the treatments of choice. This is mainly identified in hospitalized patients, both by the individual's immune response capacity and by antimicrobial susceptibility profiling. Individuals with reduced mobility have a higher frequency

of UTIs and users of devices for urinary incontinence and/or catheters have a higher recurrence of infection and complications (Jones, L.Fet al., 2019, Ahmed, S.S. et al., 2019). The use of diapers in adult patients is indicated for cases of incontinence or severe mobilization restriction; however, when used improperly, it can lead to compromised skin integrity, as well as an increased risk of infection. UTIs were identified in 30.2% of the samples analyzed from patients in diapers for more than three days (Silva, T.Cet al., 2015). Evidence also shows that UTIs related to the use of bladder catheters were present in 80% of cases. These were found to be directly related to the time of catheterization, with the chance of developing a UTI increasing by 5% for each day of use. It is estimated that 4% of individuals using a bladder catheter will progress to secondary sepsis within 28 days, which has an associated mortality rate of up to 30% (Mota, E.C. & Oliveira, A.C. et al., 2019). The intrinsic defense mechanism against UTI development, such as urination, is nullified using the bladder catheter, thus predisposing the patient to the risk of infections. In a study where individuals using a bladder catheter were evaluated, no changes in urine were identified within 84 h after catheterization (Souza Neto, J.Let al., 2008). According to epidemiological data, approximately 35–45% of UTIs are acquired from hospital infections, and of these, 80% are related to catheter use (Cyrino, A.C.T. & Stuchi, R.A.Get al., 2016).

The treatment of UTIs typically requires the use of antibiotics; however, studies in recent years have shownanincreasing rate of resistance to antibiotics (Makanjuola, O.B. et al., 2018). This makes treatment increasingly difficult, exacerbated by the indiscriminate use of antibiotics, a high number of bacterial mutations, and the high cost of prescribed medications (Rodrigues, W.F et al., 2016). UTIs are predominantly caused by gram-negative aerobic bacteria from the intestinal flora; the most common being*Escherichia coli*(*E. coli)*. Some strains of *E. coli* are resistant to various drugs and antibiotics. This happens through the production of extended spectrum beta-lactamase, which hydrolyzes the beta-lactam ring of penicillin, cephalosporins, and other antibiotics. *E. coli* also has the ability to form micro-colonies (biofilms) in the bladder mucosa that can induce resistance to the host immune response and treatment. Thus, there are several mechanisms that lead to therapeutic failure (Rodrigues, W.F et al., 2016). In this study we aimed to assess the antibiotic resistance profiles of *E. coli*in urinary samples to assess the health conditions of individuals participating in a home care program in the city of Mineiros, Brazil.

MATERIALS AND METHODS

Ethical aspects of research: Data and biological material (urine) were obtained and evaluated from participants who had signed consent forms. This study followed the ethical principles contained in the Declaration of Helsinki, as well as Resolution 466/12 of the National Health Council. All research procedures were assessed and approved by the Research Ethics Committee of Federal University of Goiás, under protocol number 3,739,612.

Study design: This was a cross-sectional observational analytical study. The study population resided in the Mineiros municipality in the central-west region of Brazil, in the State of Goiás. The municipality has approximately 68,154 inhabitants and is recognized for its outpatient and hospital care facilities. The individuals selected for this study are part of a home health care program that is offered by the federal government. Second jet urine samples were collected and evaluated for the presence of *E coli*. The GPower 3.1 program calculated the sample size needed to ensure greater sensitivity to the experiment. In addition, patients were allocated to the experimental group by randomizing the homes visited during the study period.

Inclusion and exclusion criteria: Samples were collected from individuals aged 18 years or older. Samples with a negative urine culture (<100,000 CFU / mL) or positive for bacteria other than *E. coli*, were excluded. To minimize confounding factors, patients who were recently discharged from hospitals were not included. In total, 112 urine samples were collected and analyzed between January 2018 and March 2020. Analysis of samples took place in the microbiology laboratory of the Clinical Pathology Service at the Federal University of Triângulo Mineiro (UFTM). Sample inclusion and exclusion criteria were adopted according to the flowchart in (Figure 1).

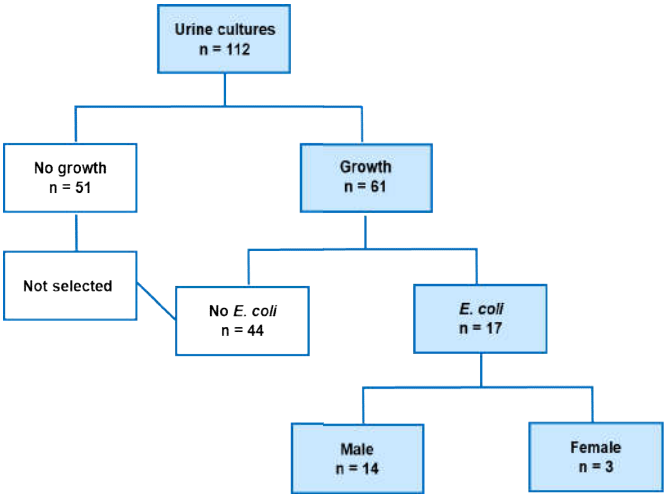

Figure 1. Inclusion and exclusion criteria used in this study. A total of 17 urine cultures testing positive for E. coli were ultimately selected for further analysis in this study

Bacterial identification and antibiotic susceptibility test: Bacterial identification and antibiotic susceptibility tests were performed according to Rodrigues et al. (2016). The antibiotics tested included amikacin, amoxicillin + clavulanic acid, ampicillin, cefaclor, cefalexin, cefazolin, cefotaxime, cefoxitin, ceftriaxone, ciprofloxacin, fosfomycin, nitrofurantoin, norfloxacin, and sulfa-trimethoprim.

Statistical analysis: Statistical analysis was performed using Prism program of "GraphPad" (http://www.graphpad.com). In all variables, normal distribution (Shapiro-Wilk test) and homogeneous variance (Bartlett's test) were tested. When the distribution was considered normal and variance was homogeneous, unpaired *t* tests were used. Results are expressed as mean ± SEM. The observed differences were considered significant when $p<0.05$ (Arango, H.G., 2001). The other comparisons (sensitive and resistant antibiotics, as well as the number of antibiograms performed with a resistance profile) are described in absolute values and percentages.

RESULTS

A total of 112 urinary cultures with antibiograms of patients treated by the home care program from January 2018 to March 2020, were evaluated. Of these, 77 (69.2%) were obtained from male subjects and 35 from female subjects (30.8%). Upon analysis, 51 of the 112 cultures exhibited no bacterial growth, 31 (60.8%) of which were from males and 20 (39.2%) from females. In contrast, 61 of 112 cultures showed growth ofsome infectious agent in the urine, with 46 (75.4%) from males and 15 (24.6%) from females. Of these 61 cultures, 17 tested positive for *E. coli*, including 14 (82.4%) from males and 3 (17.6%) from females (Figure 1).

Whileassessing the age of individuals who participated in the home care program and those who tested positive for bacteria (n = 61), it was observed that females who presented with UTI were on average significantly older than males (81.36 vs. 63.31 years) ($p = 0.0091$; Figure 2). From the results of positive urine cultures, the participants positive for the *E. coli* bacterium were stratified; most positive results were found among males (82.35%; 17.65% for females) (Figure 3). After evaluating the urine cultures for the presence of *E. coli*, the sensitivity and resistance profiles of 14 antibiotics were analyzed. *E.coli*isolated from the 17 cultures showed high resistance to the antibiotics that were tested and are usually used in the empirical treatment for*E.coli* infections (Table 1).

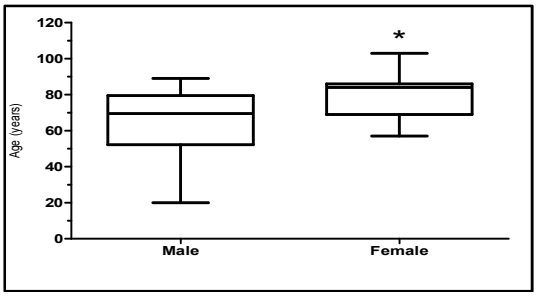

Figure 2. Age of participants in the home care program who tested positive for any bacteria, stratified by sex (males, n = 46; females, n = 15). Mann Whitney test, p = 0.0091

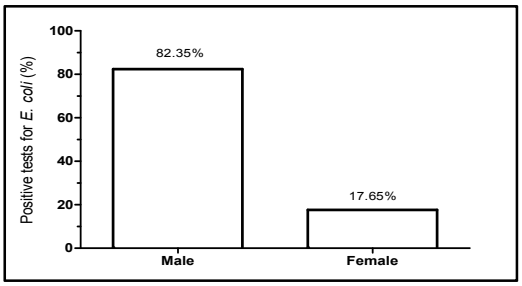

Figure 3. Percentage of positive tests for E. coli in individuals treated by a home care program

Table 1. Profile of antibiotic sensitivity and resistance tested against 17 urine cultures that tested positive for *E. coli*.

Antibiotics	Sensitive		Resistant	
	Absolute	%	Absolute	%
Amikacin	17	100.0	0	0.00
Amoxicillin + Clavulanic Acid	6	35.3	11	64.7
Ampicillin	0	0.0	17	100.00
Cefaclor	11	64.7	6	35.3
Cefalexin	11	64.7	6	35.3
Cefazolin	11	64.7	6	35.3
Cefotaxime	6	35.3	11	64.7
Cefoxitin	17	100.0	0	0.0
Ceftriaxone	10	58.8	7	41.2
Ciprofloxacin	3	17.6	14	82.4
Fosfomycin	17	100.0	0	0.0
Nitrofurantoin	17	100.0	0	0.0
Norfloxacin	3	17.6	14	82.4
Sulfa-trimethoprim	8	47.1	9	52.9

This shows thatsuch UTIs are difficult to treat, asthe potential for selecting antibiotics is reduced. Analysis of the profile quantifying antibiotic resistance testing for positive *E. coli* cultures did not show a significant difference compared to the sensitivity profile (p = 0.0545) (Figure 4).

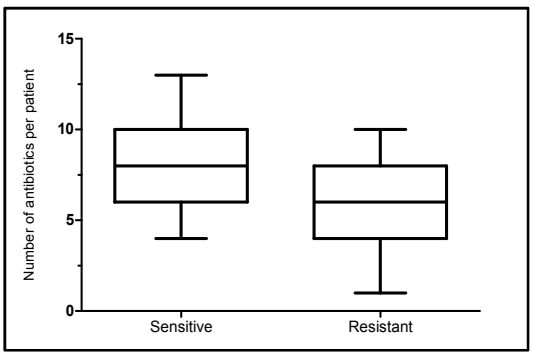

Figure 4. Sensitivity and resistance profile of the antibiotics tested for positive E. coli urine cultures. Mann Whitney test, p = 0.0545

The resistance profile revealed that 5 samples were resistant to 6 antibiotics; 3 were resistant to 8 antibiotics, 2 were resistant to 9 antibiotics, and 1 sample was resistant to 10 antibiotics (Table 2). This shows that there is a high number of patients with multidrug resistance to the possible treatments of UTI caused by *E. coli*.

Table 2. Resistant samples

N° of antibiotics tested	N° of mono or multidrug tests
1 drug	2
2 drugs	0
3 drugs	2
4 drugs	0
5 drugs	2
6 drugs	5
7 drugs	0
8 drugs	3
9 drugs	2
10 drugs	1
11 drugs	0
12 drugs	0
13 drugs	0
14 drugs	0

DISCUSSION

This study assessed the presence of UTIs caused by *E. coli* in individuals participating in a home care program in the municipality of Mineiros. A total of 112 urine cultures were examined, with bacterial growth detected in 61 samples, and of these, 17 were found to be positive for *E. coli*. Our results are similar to those of other studies, where *E. coli* was isolated (Rodrigues, W.F. et al., 2016). This high incidence of UTIs can be explained by the colonization of pathogenic bacteria that rise from the urethra, through the bladder and kidneys, causing cystitis and pyelonephritis, and proteins found on the bacterial surface that guarantee the colonization of uroepithelial cells (Dale, A.P. & Woodford, N et al., 2015 –Shigemura, K et al., 2020). The individuals analyzed in this study who were participants ofa home care program, were bedridden and the vast majority used diapers and/or a device for incontinence and external urinary drainage. The use of these devices raises the risk of developing a UTI (Shigemura, K et al., 2020). The age of the individuals participating in this study showed a prevalence of elderly people, with an average age of 81.36 years for women and 63.31 years for men. In addition to the presence of basic diseases in these individuals, several other factors could have impacted their well-being. These include the fact that many are bedridden, the devices for urinary and fecal containment generated a hot and humid microenvironment, their immune response may be deficient, many have abnormalities of the urinary tract, urinary incontinence is common, and anatomical changes in the prostate in men and hormonal changes in women are all observed (Folliero, V. et al., 2020). When evaluating the groups with respect togender, in theindividuals that tested positive for *E. coli*, there was a higher incidence ofmen (75.41%) than women (24.59%). The male gender has a greater tendency for anatomical occlusion of the bladder neck, causing a greater chance of increased bladder pressure (Marchisio, M. et al., 2015). Another important factor is the anatomy of the longer urethra and the presence of the prostate, which also contributes to the accumulation of urine residues and urethral injury, which can lead to UTIs(Shigemura, K. et al., 2020). It is also worth noting that in the present study, a larger number of samples were evaluated from males (n = 14) than from females (n = 3). The presence of *E. coli* reveals an important and concerning reduction in sensitivity to antibiotics, especially with the most commonly usedones. This resistance can occur due to several factors, the main one being gene mutations and transfers due to the indiscriminate use of antibiotics (Rodrigues, W.F. et al., 2016). High rates of antibiotic resistance were noted for ampicillin (100%), ciprofloxacin and norfloxacin (both 82.4%), amoxicillin + clavulanic acid (64.3%), cefotaxime (62.5%), and sulfa-trimethoprim (52.9%).
With the indiscriminate use of antibiotics, there is an increase in resistance being observed more frequently in men than in women. Our findings are in line with other studies wherein the resistance rate in outpatients gradually increased over the years, from 16% in 2010 to 36% in 2014. An increase in resistance to fluoroquinolones in nursing homes has also been reported(Marchisio, M, et al., 2015,Reis,

A.C.C et al., 2016,Taniece, R.E et al., 2020). When assessing the number of different resistant antibiotics for each sample tested, we found that 13 of 17 samples were resistant to a minimum of 5 of 14 antibiotics, and one sample was resistant to 10 antibiotics. Such results are in line with the findings of other studies in which the number of resistant antibiotics was high (Kengne, M. et al., 2017, Shigemura, K. et al., 2018, Aguilar-Santelises M. et al., 2020). Thus, antibiotics widely used for empirical treatment of UTIs are already exhibiting a high degree of resistance, which corroborates with other literature reports (Marchisio, M.et al., 2015, Reis, A.C.C. et al., 2016, Aguilar-Santelises, M. et al., 2020–De Santana, T.C.F.S et al., 2012). Despite these observations and trends, a comparison of the quantification of antibiotic sensitivity and resistance profiles in this study revealed no significant difference. Thus, despite the high antibiotic resistance rate measured in this study, it is still possible to observe the positive effect of therapy efficient medications. On the other hand, further studies are needed to define the genotypic associations and the resistance mechanisms of these agents present in the community. The present study showed a high resistance rate of *E. coli* to the main antibiotics used in the treatment of UTIs. In addition, elderly, bedridden males using diapers or catheters were especially susceptible to UTIs and consequently, to multi-resistant bacteria. Therefore, it is extremely important to control the use of antibiotics, since indiscriminate use is directly related to increased resistance.

Acknowledgments

This work was supported by the Centro Universitario de Mineiros (UNIFIMES) and Universidade Federal do Triangulo Mineiro (UFTM).

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