Universidade de Aveiro Departamento de Biologia
2014

Tânia Alexandrina Ribeiro Costa

Frequency and antibiotic resistance of bacteria implicated in community urinary tract infections in North Aveiro (2011-2014)

Incidência e resistência a antibióticos em bactérias implicadas nas infeções urinárias no distrito de Aveiro Norte (2011-2014)

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de mestre em microbiologia, realizada sob a orientação científica da Professora Doutora Maria Adelaide de Pinho Almeida (CESAM) departamento de biologia da Universidade de Aveiro, e sob a coorientação do Dr. Ricardo Filipe Romão Ferreira, especialista no Centro Médico da Praça, Lda.

Dedico este trabalho à minha família pelo incansável apoio e a todos que acreditaram que era possível.

o júri

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agradecimentos

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palavras-chave Infeção do trato urinário, cistite, tratamento, resistência

resumo

A infeção do trato urinário é a segunda infeção mais comum na comunidade e a mais comum no contexto hospitalar a nível mundial. As crianças, grávidas, idosos, diabéticos, pacientes com deformidades urológicas, cateterizados e imunodeficientes são considerados população de risco e por isso são mais propensos a desenvolver infeção do trato urinário.

As amostras estudadas foram colhidas em regime de ambulatório no laboratório de análises clínicas, Centro Médico da Praça Lda, no município de São João da Madeira, distrito de Aveiro (Portugal), durante o período de estudo entre junho de 2011 a junho de 2014.

E. coli (64%) foi a bactéria patogénica mais frequente, seguida da *Klebsiella* spp (12%), de *Enterococcus* spp (7%) e *P. mirabilis* (6%).

Das 4270 urinas analisadas, 3561 (83%) foram colhidas em mulheres e apenas 709 (17%) em homens, num intervalo de idade entre 0 e os 104 anos. Desta amostra 1537 (37%) eram multirresistentes, entre elas 1099 foram colhidas de mulheres e 437 de homens. As bactérias patogénicas multirresistentes foram em média resistentes a 6 antibióticos e a 5 classes de antibióticos. Na generalidade os homens foram mais resistentes que as mulheres.

Os resultados do estudo mostraram que dos antibióticos de primeira linha de tratamento apenas a nitrofurantoína é apropriado no tratamento empírico para ambos os sexos. Dos antibióticos sugeridos pela EAU como segunda linha de tratamento, a Amoxicilina - ácido clavulânico e o sulfametoxazole - trimetoprim pode ser considerado apenas para tratar mulheres, por fim, a ampicilina não é adequada para aos pacientes deste estudo. Deste modo, é sugerido como alternativa os antibióticos imipenem e gentamicina para o tratamento empírico de ambos os sexos.

keywords Urinary tract infection, cystitis, antibiotic therapy, resistance.

abstract

The urinary tract infection is the second most common infection in community and the most common nosocomial infection worldwide. Specific subpopulations are more likely to have UTI, such as, infants, pregnant women, elderly, diabetics, patients with urologic abnormalities, patients with catheters and immunodeficients.

All the samples were collected at Centro Médico da Praça Lda on ambulatory system, located in São João da Madeira municipality, District of Aveiro north (Portugal) from June 2011 to June 2014.

From 4270 analysed urine samples, 3561 (83%) were collected from women and only 709 (17%) were collected from men, in a range age from 0 to 104 years old. *E. coli* was (64%) the most frequent uropathogen, followed by *Klebsiella* spp (12%), *Enterococcus* spp (7%) and *P. mirabilis* (6%).

From all samples, 1537 (37%) were multidrug resistant (MDR), 1099 were from women and 437 from men. The MDR uropathogens were resistant on average to a 6 antimicrobials and to a 5 antimicrobial classes of drugs. In general, men were more resistant to antimicrobials than women.

According the results of this study, among the first line drugs recommended by EUA for empirical treatment of UTI the antimicrobials only nitrofurantoin is suitable for both sexes and ciprofloxacin may be only considered to treat women. From EAU recommended second line therapy, ampicillin is not appropriated to empirical treatment for both sexes, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole should not be considered for men UTI empirical treatment, due the local high incidence of resistance. Thus, it is suggested imipenem and gentamicin as alternatives to treat both sexes.

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MAIN ACRONYMS

AES	Advanced Expert System
AMP	Ampicillin
AMX	Amoxicillin
AMX-CA	Amoxicillin-Clavulanic Acid
ASB	Asymptomatic Bacteriuria
AST	Antimicrobial Sensibility Test
CA-UTI	Catheter Associated Urinary Tract Infection
CFU	Colony-forming Unit
CIPRO	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
C-UTI	Complicated Urinary Tract Infection
DA-UTI	Diabetes-associated Urinary Tract Infection
DM	Diabetes mellitus
EAU	European Association of Urology
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESBL	Extended Spectrum β-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FOS	Fosfomycin
GEN	Gentamicin
GN	Gram-Negative
GP	Gram-Positive
ID	Identification

IDSA	Infection Disease Society of America
IMCI	Integrated Management of Childhood Illness
IMP	Imipenem
MIC	Minimal Inhibitory Concentration
MSU	Midstream Sample of Urine
NASA	National Aeronautics and Space Administration
NIT	Nitrofurantoin
QIRs	Quiescent Intracellular Reservoirs
R-UTI	Recurrent Urinary Tract Infection
SMX	Sulfamethoxazole
SWAB	*Stichting Werkgroep Antibioticabeleid*
TMP-SMX	Trimethoprim-Sulfamethoxazole
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary Tract Infection
U-UTI	Uncomplicated Urinary Tract Infection
WHO	World Health Organization

CHAPTER I – INTRODUCTION

CHAPTER I - INTRODUCTION

1. HISTORY

Urinary tract infection (UTI) is the second most common infection in community and the most common nosocomial infection worldwide (Girard, et al., 2002; Roriz-Filho, et al., 2010; Al-Badr, et al., 2013; Ott, et al., 2013; Sujatha, et al., 2014). Due to its prevalence, it has high impact on ambulatory healthcare costs, caused by many visits to a physician, diagnostic tests and prescriptions (Kapoor, et al., 2012; Al-Badr, et al., 2013).

TABLE 1 Classification of urinary tract infection episodes in six categories (Al-Badr, et al., 2013).

Classification	Description
Uncomplicated infection	The urinary tract is normal, both structurally and physiologically, and there is no associated disorder that impairs the host defence mechanisms
Complicated infection	When infection occurs within an abnormal urinary tract, such as when there is ureteric obstruction, renal calculi, or vesicoureteral reflux.
isolated infection	The first episode of UTI, or the episodes are 6 months apart. Isolated infections affect 25–40% of young females.
unresolved infection	When therapy fails because of bacterial resistance or due to infection by two different bacteria with equally limited susceptibilities.
reinfection	Occurs when there has been no growth after a treated infection, but then the same organism regrows two weeks after therapy, or when a different microorganism grows during any period of time.
relapse	When the same microorganism causes a UTI within two weeks of therapy; however, it is usually difficult to distinguish a reinfection from a relapse.

The episode of UTI is classified as lower and upper urinary tract infection according to where it occurs: urethritis in urethra, cystitis in bladder, bacteriuria in urine and pyelonephritis in kidney and ureters (Barber, et al., 2013). UTI may involve either lower and upper urinary tracts, or most often only the lower urinary tract (Rowe, et al., 2013). It is denominated as uncomplicated urinary tract infection (U-UTI) when it occurs in a young women non-pregnant with a normal genitourinary tract. Whereas the complicated urinary tract infection (C-UTI) occurs in a genitourinary tract with structural or functional abnormalities, including catheterized patients (Roriz-Filho, et al., 2010).

Typical UTI symptoms are pain, fever, urgency and frequency of micturition, dysuria (painful micturition), suprapubic cramping pain and sense of weight, turbid or cloudy urines, sometimes with an unpleasant smell, nocturia and haematuria. Usually the symptoms of lower urinary tract infection are slight, or absent in case of asymptomatic bacteriuria (ASB). In women, ASB is defined as the presence of two consecutive urine specimens positive for the same bacterial strain in quantities equal or superior to 10^5 CFU/ mL (Rowe, et al., 2013). In men, the microbiologic criteria for diagnosis of ASB is not as well rendered valid, some authors define as a single voided specimen with one bacterial isolate in quantities equal or superior to 10^4 CFU/mL (Grabe, et al., 2014).

Ascending infection causing pyelonephritis (upper urinary tract infection), causes back pain and costovertebral angle tenderness and can also be accompanied with symptoms of malaise, fever, nausea and vomiting in severe infections. If bacteria enters the blood stream it may lead to severe complications, including septicaemia, shock and, rarely, death (Kapoor, et al., 2012; Al-Badr, et al., 2013)

The UTI prevalence is significantly higher in women than men, likely as a result of anatomic differences, due the proximity of urethra to anus which provides the self-colonization by gastrointestinal pathogens (Al-Badr, et al., 2013). Also, the shorter

urethra in women can facilitate bacterial transit from the urethral opening to the bladder (Figure 1). Ureteral massage and trauma during sexual activity also enables the pathogens ascension to bladder (Roriz-Filho, et al., 2010; Kapoor, et al., 2012; Barber, et al., 2013). Urinary tract infections in men, elderly people, pregnant women, or patients who have an indwelling catheter or an anatomic or functional abnormality are considered complicated urinary tract infections (C-UTI) (Jancel, et al., 2002; Nicolle, et al., 2005).

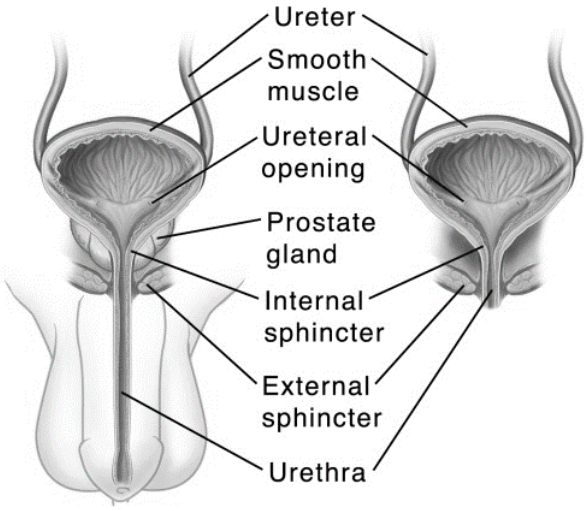

FIGURE 1 Urinary tract systems and their anatomic singular characteristics. Adapted from: http://healthcare.utah.edu/healthlibrary/related/doc, retrieved 9 24, 2014.

Recurrent urinary tract infection (R-UTI) is defined by three positive urine cultures during a 12-month period, or two infections during a 6 months period caused by the same pathogen (Graziottin, 2014). The incidence of R-UTI increases with age, sexual activity (post-coital cystitis) and medical illnesses complications such as diabetes mellitus (Al-Badr, et al., 2013).

Uncomplicated R-UTI associated with the uropathogenic *Escherichia coli* (UPEC) are common among healthy, sexual active and reproductive age women.

Uncomplicated R-UTI after intercourse is responsible for 60% of recurrent cystitis (Graziottin, 2014). Other factors contributing to R-UTI persistence are urinary tract obstruction, incomplete voiding, aberrant structural anatomy and accumulation of bacteria in unreachable locations for antibiotics, such as kidney stones (Al-Badr, et al., 2013; Barber, et al., 2013).

2. FACTORS OF SUSCEPTIBILITY

Specific subpopulations with increased risk of UTI include infants, elderly, pregnant women, patients with urologic abnormalities, patients with catheters, diabetes and other immunodeficiency diseases (Foxman, 2002).

2.1. INFANTS

UTI is a non-reported cause of childhood morbidity because it is not included in the current Integrated Management of Childhood Illness (IMCI), however it has been estimated that 1% of boys and 3% of girls are diagnosed with UTI (WHO, 2005). Contrary, in the first year of life UTI is more prevalent in boys with rates of 2.7% compared with 0.7% in girls, also uncircumcised boys have increased risk for developing UTI (Chang, et al., 2006; Edlin, et al., 2014).

Surveys have demonstrated that ASB in children of all ages, however it is accepted that ASB does not present a risk to a child. The detection of for ASB in child is not indicative of illness (WHO, 2005). Treatment of ASB can promote the growth of resistant organisms by selective pressure. Studies using components of human breast-milk have suggested that breastfeeding may provide some protection against UTI in childhood (Parida, et al., 2013; Edlin, et al., 2014).

2.2. ADULTS

The gender vulnerability is clear for UTI, women have 50 times more chances of acquiring UTI than men over all age groups (Graziottin, 2014). Most U-UTI occurs in women without any anatomic or functional abnormality and the seriousness varies from mild to severe. It is estimated that 50-60% of all women after puberty experience at least one UTI episode during their lifetime and in 8% of UTI episodes the pathogens may stay silent (Kapoor, et al., 2012; Al-Badr, et al., 2013; Barber, et al., 2013; Rowe, et al., 2013).

The UTI rates normally increase with the beginning of sexual activity in young patients, the post-coital symptoms in women last till 6 days on average (Graziottin, 2014). The major cause of U-UTI post-coital is due to the ascension of vaginal bacteria to the urinary tract (Rowe, et al., 2013). Also post-menopausal women have higher rates of UTI due to pelvic prolapse, lack of estrogen, loss of lactobacilli in the vaginal flora (Graziottin, 2014). Additional risk factors include comorbidity associations that increase UTI susceptibility, however the majority of UTI occurs in healthy women (Al-Badr, et al., 2013; Barber, Norton, et al., 2013).

The large gender difference in the UTI prevalence is caused by many factors including: the greater distance between urethra and anus the usual source of uropathogens; the drier environment surrounding the male urethra; the greater length of the male urethra; and the antibacterial activity of the prostatic fluid. The UTI in a healthy adult men between the ages of 15 and 50 years old is very uncommon, however, it is more common the UTI sepsis evolution in men than in women. The exact reasons for UTI infections in healthy men are not clear, however some sex-related behaviours, such as, unprotected intercourse with an infected partner, unprotected anal intercourse and even the lack of circumcision seem to be associated (Nicolle, et al., 2005; Roriz-Filho, et al., 2010; Grabe, 2008).

Usually, UTI in men are generally viewed as complicated because most of UTI episodes occur in new-born, infant or elderly and they are related to urological abnormalities, bladder outlet obstruction, instrumentation of genitourinary or several other comorbidities, such as, diabetes and HIV (Nicolle, et al., 2005; Roriz-Filho, et al., 2010; SIGN, 2012).

Among men the UTI incidence increases after the age of 50 and it is associated with the prostatic disease and urinary catheterization (Nicolle, et al., 2005; Roriz-Filho, et al., 2010). Conditions like prostatitis, chlamydial infection and epididymitis should be considered in the differential diagnosis of men with acute dysuria (SIGN, 2012).

In 90% of men the appearance of febrile UTI is associated to prostatitis, thus the urological evaluation should be carried out routinely in men whenever a complicating factor is suspected. Between 52% and 90% of men with a UTI have been reported to have prostatic involvement in the infection, which can result in prostatic abscesses or prostatitis (Grabe, et al., 2008).

2.3. PREGNANCY

Pregnant women in particular are vulnerable to UTI because pregnancy itself is an immunocompromised state (Kapoor, et al., 2012; Sujatha, et al., 2014). Pregnancy-UTI incidence elevates up to 20% and frequently causes of premature delivery. It also increases the risk between 25 to 40% chance of ascending to upper urinary tract infection, hypertensive disease, anaemia, postpartum complications and foetal mortality (Foxman, 2002; Kapoor, et al., 2012; Sujatha, et al., 2014)

Anatomical factors like the pressure of the enlarged uterus on the bladder, the physiological hormonal relaxant effect of progesterone on the smooth muscle of the urinary tract, the vesicoureteral reflux, may predispose to recurrent UTI (Figure 2) (Kapoor, et al., 2012).

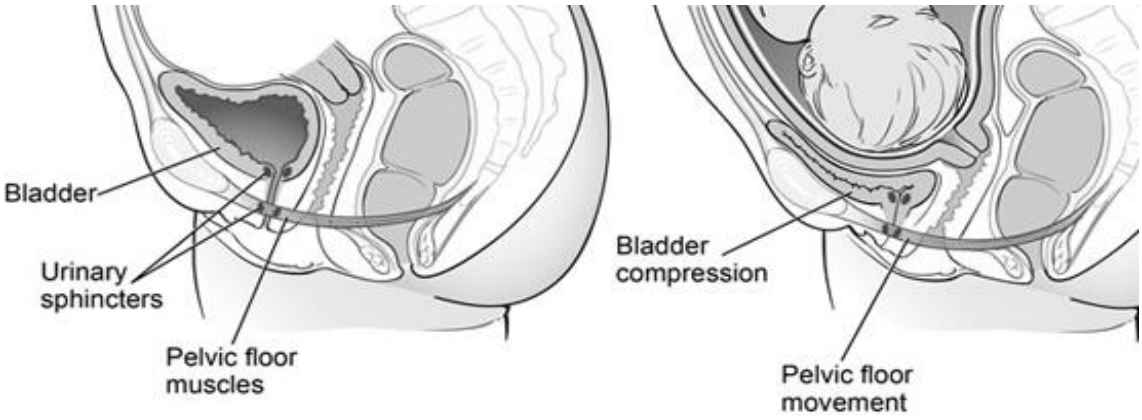

FIGURE 2 Bladder compression during pregnancy. Adapted from: http://my.clevelandclinic.org/health/diseases_conditions/hic_Am_I_Pregnant/hic_Coping_with_the_Physical_Changes_and_Discomforts_of_Pregnancy/hic-pregnancy-childbirth-bladder-control, retrieved 9 24, 2014.

It has been estimated that between 2% to 10% of women in developed countries will experience ASB or UTI in pregnancy. Unlike infants, the detection and treatment of ASB in pregnancy in several developed countries has resulted in birth outcomes improvement. Therefore, the ASB detection was recommended by the World Health Organization (WHO) antenatal care package to reduce prematurity, low birth weight, the neonatal morbidity and mortality (Gilbert, et al., 2013).

2.4. ELDERLY

ASB and UTI are the second most common infections in people over the age of 65 years. In elderly group, particularly those living in long-term care facilities, are less likely to present genitourinary symptoms. In both men and women, the incidence of UTI increases substantially in elderly (Rowe, et al., 2013). Between 25% to 50% of elderly women and 15% to 40% of elderly men in long-term care facilities usually develop ASB and the majority of these elderly people have chronic neurologic illnesses (Nicolle, et al., 2005). In younger women, the estimated prevalence of ASB is 1–5%, increasing to an estimated 6–16% in women over 65 years of age.

The treatment for ASB is only recommended in elderly prior to transurethral resection of the prostate or any urologic procedures for which mucosal bleeding is anticipated. The differentiation of UTI from ASB must be looked at very carefully, because misclassification may occurs (Rowe, et al., 2013).

Age-associated changes in immune function, exposure to nosocomial pathogens in case of institutionalized patients and the increasing number of comorbidities put the elderly at an increased risk for developing infection. Medical comorbidities, such as stroke and dementia, may predispose individuals to bowel and bladder incontinence, which have been associated with symptomatic UTI and persistent ASB (Rowe, et al., 2013).

In older women, some studies suggest an association with menopause, due to the loss of estrogens and the worsening of constipation with age (Graziottin, 2014). In older men, prostatic hypertrophy causing obstruction to the normal flow of urine leads to high post void residual and it has been postulated to be a risk factor for UTI in old-aged (Rowe, et al., 2013).

2.5. CATHETER-ASSOCIATED UTI

The catheter-associated UTI (CA-UTI) is a very common nosocomial infection and indwelling urethral catheter is a procedure exceedingly used in health care facilities, with 17.5% of patients in 66 European hospitals having a catheter (Nicolle, 2014). Indwelling urethral catheter is generally considered to be short term if it is *in situ* for less than 30 days and chronic or long term when it is *in situ* for 30 days or more, in case of institutionalized adults (Nicolle, 2014).

Catheterization is also a major cause of hospital-acquired UTI, which may be associated with enhanced nosocomial mortality rates (Parida, et al., 2013). The risk increases between 3% to 10% per day of catheterization, and at the 30th day of catheterization the infection rates are about 100%, which is an additional cost per

admission (Meddings, et al., 2014) (Parida, et al., 2013; Nicolle, 2014). For indwelling urethral catheter patients, ASB is defined as a positive urinary culture for one bacterial isolate in quantities $\geq 10^2$ CFU/mL, in the absence of symptoms (Rowe, et al., 2013).

Prevention of CA-UTI has recently become an important goal of health-care infection prevention programs. These programs criteria include aseptic insertion of urinary catheters, minimizing the use and duration of catheters, which has led to a decrease in the incidence of CA-UTI. In adults who require catheterization, the use of antimicrobial-coated catheters may delay bacterial colonization and thus decreases the incidence of CA-UTI (Rowe, et al., 2013; Nicolle, 2014)

Potential risk factors resulting from prolonged catheterization and catheter insertion outside a protected environment, also put the patient at an increased risk (Parida, et al., 2013). A recent study about cleaning the urinary catheter prior to the catheterization showed significant evidences that the use of water or saline solution reduces rates of UTI and suggests the realization and implementation of a new guideline to prevent CA-UTI. The aseptic insertion and maintenance of urinary catheter and system and its correct removal are relevant from the point of view of prevention and control of UTI (Figure 3). Also guidelines and previous studies indicate that urinary meatus should be cleaned with water or saline solution (Cunha, et al., 2013). Alternate voiding management strategies such as intermittent catheterization or/and, external condom catheters for men, should be used when possible (Nicolle, 2014).

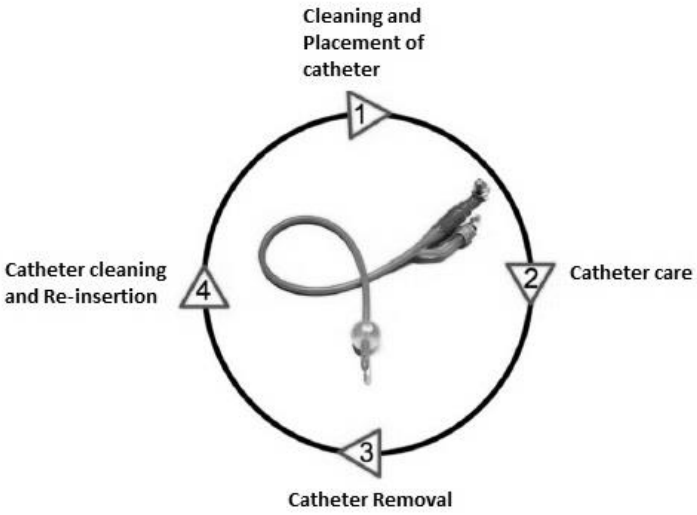

FIGURE 3 The four stages of the urinary catheter lifecycle to decrease catheter use and CA-UTI. Adapted from (Meddings, et al., 2014).

2.6. DIABETES-ASSOCIATED UTI

Patients with *Diabetes mellitus* (DM) have a higher risk of ASB, R-UTI and pyelonephritis (Al-Badr, et al., 2013). DM is a progressive disease that is characterized by a state of chronic hyperglycaemia. Findings suggest that UTI are more commonly experienced by those with DM compared to those without DM. The exact mechanism is unknown, but several possibilities have been proposed to explain the association between these two diseases. The presence of higher glucose concentrations in the urine might promote UTI development by amplifying bacterial reproduction and creating a favourable environment for infections (Fu, et al., 2014). Women are more prone to ASB than men with diabetes, but in both sexes the progression to clinical pyelonephritis is more probable than in normal individuals. Women with type 1 diabetes are particularly at risk if they have had diabetes for a long time or have developed complications, particularly peripheral neuropathy and proteinuria. The risk factors for developing ASB differ between type 1 and type 2 diabetes, usually the

patients with type 2 diabetes were old age, proteinuria, a low body mass index and a past history of R-UTI (Grabe, et al., 2014).

Diabetes-associated UTI (DA-UTI) increases the risk of acute pyelonephritis by Enterobacteriaceae that usually infects the lower tract, also *Klebsiella* infection is particularly common (25% compared with 12% in non-diabetics). Other patient-related factors such as age, metabolic control and duration of diabetes have also been suggested as increasing the risk of infection among those with diabetes (Fu, et al., 2014).

3. DIAGNOSIS

Urine contains an enormous amount of information, despite being a waste product. Urine bladder culture and microscopic urine sediment analysis have been the gold standards to urinalysis. Well-standardized procedures are the basis of an effective diagnostic strategy for urinalysis (Delanghe, et al., 2014; Sujatha, et al., 2014).

The laboratory is responsible for giving the correct information to the patient about the best sampling procedures, including biological collection, conservation, storage and specimen transport to the laboratory. Since the patients themselves often collect the urine specimen, a flawless pre-analytical urinalysis depends on well-standardized procedures. Therefore, for being a very susceptible procedure, the requirements of urinalysis have gained importance and have become stricter (Delanghe, et al., 2014).

3.1. SPECIMEN COLLECTION

To avoid contamination with urogenital flora the collection of urine specimen should be achieved by cleaning the hands and washing the glans penis of men or the introitus of women (Demilie, et al., 2014). Due to the influence on the viability of

bacteria, the use of soap or antiseptics is not recommended. Clean-catch urine or midstream portions of first morning urine samples (not less than 4 hours storage in the bladder) should be collected to a sterile urine container in order to avoid contaminations. The volume should be between 15 ml and 20 ml in a sterile urine container provided by the laboratory or purchased in a pharmacy. Samples are transported in the primary containers and stored at 4°C until be processed, the lack of temperature control can lower the quality of urinary test results (European Confederation of Laboratory Medicine, 2000).

Catheter-urine specimens for culture should be collected directly from the catheter or tubing, to maintain a closed drainage system. These may be collected either through the catheter collection port or through puncture of the tubing with a needle (Nicolle, 2014).

In case of an incorrect specimen collection, the urine collection must be repeated. The conservation procedure is also a limiting factor for the over-all diagnostic accuracy, urine samples must be refrigerated so that a precise urinalysis can be processed within 24 hours (Delanghe, et al., 2014). A proper pre-analytical procedure is crucial to urinalysis.

3.2. URINALISYS

The experimental and analytical procedures follow the norms from the Clinical and Laboratory Standards Institute (CLSI, 2010).

On arrival at the laboratory the samples are triaged and 10-15 mL from the original sample are transferred into one examination tube to the dipstick analysis in order to detect nitrite (indicative of microbial activity) and leucocyte esterase (indicative of pyuria).

Microscopic analysis of the urine sediment is performed after centrifugation at 1500 rpm for 5 minutes, haematuria (erythrocytes) and pyuria (neutrophils) are searched, if superior to 10 cells per high power field may be diagnosed with bacteriuria. This optional analysis contributes to the diagnosis of UTI and it may suggest abnormalities and vulnerabilities that could lead to a complicated UTI (Barber, et al., 2013).

Then, gram stain is used to differentiate bacterial species into gram-positive and gram-negative. Dipstick test, that detects nitrite and leucocyte esterase, is also used to diagnose UTI (Gieteling, et al., 2014).

The urine culture procedure is performed by vertical immersion with a disposable plastic loop of 1 µl into the original sterile container and spread horizontally on the surface of the chromID™ CPS® plates (bioMérieux SA, 2013). Then, plates are incubated at 37 °C in aerobic atmosphere for 18 hours. After incubation, all the negative cultures, if growth is inferior to 10^3 CFU/mL, are excluded.

ChromID™ CPS® is an isolation and identification medium that is used for urinary samples and enables the microbial count of the sample through the standardized seeding method. The colonies of *E.coli* are pink and red; *Enterococcus* are turquoise colonies; *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter* are brown-greenish colonies; *Proteus*, *Providencia* and *Morganella* are dark brown colonies expressing desaminase.

The microbial criteria in urine culture is defined as a positive result to UTI, If growth is equal or superior to 10^5 CFU/mL in mid-stream sample of urine (MSU), with a maximum of 2 isolated microbial species. It is considered contamination if it has been observed 3 or more specimens in the culture, or if growth is equal or inferior to 10^3 CFU/mL (Girard, et al., 2002; Roriz-Filho, et al., 2010). ASB is defined by the presence of bacteria in urine culture, if growth is inferior to 10^5 CFU/mL in MSU, without clinical

symptoms of infection and should not be treated in young non-pregnant women (Roriz-Filho, et al., 2010). To the subpopulations with increased risk of UTI and symptomatic should be considered positive culture, if growth is equal or superior to 10^4 UFC/mL, such as, catheter samples, pregnant women, children and elderly. For suprapubic aspirated urine should be considered positive culture, if growth is equal or superior to 10^2 UFC/mL (Kapoor, et al., 2012).

3.3. VITEK 2 DIAGNOSIS TOOL

VITEK 2 system (bioMérieux) is an accurate and reproducible diagnostic tool that allows the determination of the exact etiology of patient's infectious. With great automation, safety and minimal manual operations provides microbial identification *in vitro* on short time compared with conventional methods (Khardori, 2014)

Nowadays Vitek 2 system is directed to clinical laboratories professionals, but it was originated with the National Aeronautics and Space Administration (NASA) space program to identify infections in astronauts (bioMérieux SA, 2009). The VITEK 2 system cards are the size and shape of a playing card and contain 64 microwells (Figure 4). Each well contains identification substrates or known antimicrobials in different concentrations in association with mass spectrometry and bioinformatics make the identification possible (bioMérieux SA, 2014 b; Khardori, 2014).

FIGURE 4 VITEK® 2 identification and antimicrobial susceptibility test cards. Adapted from: http://www.biomerieux-diagnostics.com/vitek-2-identification-cards, retrieved 10 17, 2014.

3.3.1. VITEK 2 IDENTIFICATION

For bacteria identification, the isolate is collected from a pure culture and inoculated in a saline suspension, with a density between 0.5-0.65 in McFarland scale. According to the colonies characteristics (colour, form, size and smell), it is chosen the VITEK® 2 GN ID Card to identify lactose fermenting and lactose non-fermenting gram-negative bacilli, or VITEK® 2 GP ID Card to Identify gram-positive bacteria (Funke, et al., 2004; bioMérieux SA, 2014 b).

3.3.2. VITEK 2 ANTIMICROBIAL SUSCEPTIBILITY TEST (AST)

In addition to VITEK® 2 ID Cards, it is performed the VITEK® 2 AST Card 3 mL saline suspension, which is prepared from the VITEK® 2 ID Card suspension based on established concentrations. From gram-negative it should be taken 145 µl of the VITEK® 2 ID Card suspension, and from gram-positive it should be taken 280 µl of the VITEK® 2 ID Card suspension. After the preparation of both suspensions, VITEK® 2 ID Card and VITEK® 2 AST Card are introduced into the respective saline suspensions and

incubated at 36°C in the VITEK® 2 incubator, the results are available within 10 hours (bioMérieux SA, 2014 a).

The antimicrobial susceptibility testing (AST) results are validated by the Advanced Expert System (AES) program, and follow the European Committee on Antimicrobial Susceptibility Testing (EUCAST) program. The determination of the phenotypic AST depends on minimal inhibitory concentration (MIC) breakpoints. The breakpoints allow the bacteria grouping into the categories: susceptible, intermediate, and resistance. Meanwhile it is also performed the ESBL-confirmation tests (bioMérieux SA, 2014 a; Stokkou, et al., 2014).

The VITEK® 2 AST Cards most used in Portugal urinalysis are: VITEK® 2 AST-GN26 (Portuguese card of urines), VITEK® 2 AST-GN86 VITEK® 2 AST-N113 for gram negative bacteria with resistance to majority of the antibiotics from the VITEK® 2 AST-GN26 (Table 2); VITEK® 2 AST-P586 for gram positive E*nterococcus* and *Streptococcus* and VITEK® 2 AST P619 for Gram positive *Staphylococcus* (Table 3) (bioMérieux Portugal, 2014).

TABLE 2 Content of VITEK® 2 AST gram-negative most used cards for UTI diagnosis (bioMérieux Portugal, 2014)

AST-GN26	AST-GN86	AST-N113
Ampicillin	AMX-CA	Ampicillin
AMX-CA	Ampicillin	AMX-CA
Pip/tazo	Cefazolin	Cefuroxime
Cefalotin	Cefepime	Cefotaxime
Cefuroxime	Ceftazidime	Ceftazidime
Cefpodoxime	Ceftriaxone	Cefepime
Ceftazidime	Cefuroxime	Cefoxitin
Cefotaxime	Ciprofloxacin	Imipenem
Cefepime	Ertapenem	Ertapenem
Cefoxitin	ESBL Confirmation Test	Amikacin
Meropenem	Gentamicin	Gentamicin
Amikacin	Imipenem (new formula)	Tobramycin
Gentamicin	Nitrofurantoin	Ciprofloxacin
Norfloxacin	Tetracycline	Nitrofurantoin
Ciprofloxacin	Tobramycin	TMP-SMX
Nitrofurantoin	TMP-SMX	Fosfomycin
TMP-SMX	----------	ESBL Confirmation Test

TABLE 3 Content of VITEK® 2 AST Gram Positive (GP) most used cards for UTI diagnosis (bioMérieux Portugal, 2014)

AST-P586 (*Streptococcus* and *Enterococcus*)	**AST P619 (*Staphylococcus*)**
Benzilpenicillin	Benzilpenicillin
Ampicillin	Oxacillin
Cefuroxime	Vancomycin
Imipenem	Teicoplanin
Vancomycin	Daptomycin
Teicoplanin	Gentamicin
Streptomycin High Conc.	Levofloxacin
Gentamicin High Conc.	Moxifloxacin
Moxifloxacin	Tetracycline
Levofloxacin	Tigecycline
Tetracycline	Eritromicin
Tigecycline	Clindamycin
Clindamycin	Mupirocin
Quinopristina/Dalfopristina	Linezolid
Linezolid	Rifampicin
Nitrofurantoin	Fusidic acid
TMP-SMX	Fosfomycin
----------	NIT
----------	TMPSMX

4. TREATMENT OF UTI

The aim of antimicrobial UTI treatment is the eradication of the current microbial infection by applying effective antimicrobial therapy. To relief from recurrence and minimize collateral damages, it is strongly advised to whenever possible avoid the empirical treatment and perform the antimicrobial susceptibility testing.

4.1. EMPIRICAL TREATMENT

The early treatment of UTI is crucial to reduce the rate of morbidity, empirical antimicrobial therapy guided by local resistance rates should be the primary influence on clinician's choices (Rodrigues, et al., 2011; Barber, Norton, et al., 2013; Linhares, et al., 2013).

The WHO Global Strategy recommends that the choice of empirical treatment should be guided by local or national resistance surveillance data, in the absence of community regional level surveillance study. Also the local surveillance data should be used to guide the drugs management, educate prescribers and guide infection control policies. But unfortunately, there are few publications about the main uropathogens in the community-acquired UTI and their antimicrobial resistance profile, when compared with UTI acquired at hospital level (WHO, 2001; Girard, et al., 2002; WHO, 2014)

The Infectious Diseases Society of America (IDSA) Practice Guidelines in collaboration with European Society for Microbiology and Infectious Diseases (ESCMID) and European Association of Urology (EAU) recommends as first-line therapy of U-UTI, fosfomycin due to minimal resistance and propensity for collateral damage (Gupta, et al., 2011). Ciprofloxacin is suggested as an effective treatment, but among

men the treatment should be extended to 10-14 days (Roriz-Filho, et al., 2010). Nitrofurantoin is also an appropriate choice for therapy due to minimal resistance and secondary effects (Gupta, et al., 2011).

The second-line therapy recommended is fluoroquinolones, which contain 6-fluoro substituents when compared to quinolones and it is highly efficacious in 3-day regimens, but have a propensity for collateral damages and resistance, so they should only be used if sensitivity testing is performed (Rowe, et al., 2013). The β-lactam agents are appropriate choices for therapy when other recommended agents cannot be used, because of their inferior efficacy and more adverse effects. Trimethoprim-Sulfamethoxazole (TMP-SMX) in spite of being widely diffused in the international guidelines should be based on AST and only prescribed if local resistance rates of uropathogens do not exceed 20%, due to its high resistance rates in *E. coli* bacterium (Linhares, et al., 2013). According to the 1999 guidelines, amoxicillin or ampicillin should not be used for empirical treatment given the relatively poor efficacy and the very high prevalence of resistance to these agents worldwide (Gupta, et al., 2011)

In placebo-controlled studies of U-UTI spontaneous clinical cure rate was from 25% to 42%, it was significantly higher compare to symptomatic and bacteriological cure rates, however the patients treated with antibiotic therapy had a better prevention of reinfection (Wagenlehner, et al., 2011).

4.2. EXCEPTIONAL TREATMENT

4.2.1. CHILDHOOD RECOMMENDED TREATMENT

Children with U-UTI are likely to respond to amoxicillin, sulphonamides, TMP-SMX or cephalosporins, due to their good concentration in the lower urinary tract (WHO, 2005).

4.2.2. PREGNANCY RECOMMENDED TREATMENT

In pregnant women, treatment of UTI deserves special attention because of the perinatal risks involved. During pregnancy treatment is mandatory and the AST should guide the treatment rather than empirical treatment (Calderón-Jaimes, et al., 2013; Kapoor, et al., 2012).

The first-line antibiotics include all β-lactam agents, nitrofurantoin. Second-line antibiotics include fosfomycin, trimethoprim (TMP) and the fluoroquinolones, and should never be considered during pregnancy antibiotics such as sulphonamides, chloramphenicol and tetracycline (Roriz-Filho, et al., 2010; Kapoor, et al., 2012).

In the first trimester of pregnancy, TMP and fluoroquinolones cannot be considered because they affect the normal growth and formation of the foetus. Nitrofurantoin is restricted during the last few weeks due to the risk of haemolytic anaemia of foetus or neonate (Kapoor, et al., 2012).

4.2.3. DIABETES RECOMMENDED TREATMENT

For DA-UTI patients are recommended two weeks (7-14 days) of oral antimicrobial therapy and hyperglycaemic control (Mnif, et al., 2014). Fosfomycin, TMP-SMX and nitrofurantoin are safe and effective antimicrobial methods to cure and prevent UTI in patients with DM, and fosfomycin is associated with rarely recurrence of UTI (Ruxer, et al., 2007).

Antibiotic treatment of ASB significantly increases the risk of adverse events without significant clinical benefit, and also increases resistance (SIGN, 2012). However ASB is common in women with DM, and if left untreated, it may lead to renal

functional impairment, so the clinicians must measure the cost-benefit according to each patient criteria (Grabe, et al., 2014).

4.2.4. ELDERLY RECOMMENDED TREATMENT

TMP-SMX should have the preference for treatment of UTI in old-aged (Rowe, et al., 2013). Among elderly males it is recommend the prostate examination and antimicrobial sensibility test (AST) (Roriz-Filho, et al., 2010).

Nitrofurantoin has low resistance rates in *E. coli*, although other *Enterobacteriaceae* species are more common in elderly and may have intrinsic resistance to nitrofurantoin. In addition, this antimicrobial is contra-indicated in patients with chronic kidney disease (Rowe, et al., 2013).

5. ETIOLOGY

The literature is unanimous, *E. coli* is the most common UTI bacterium. This bacterium is responsible for 75% to 95% of community-acquired infections and for 50%-60% of the hospital-acquired UTI (Roriz-Filho, et al., 2011; Gilbert, et al., 2013; Landry, et al., 2014; Nicolle, 2014). The uropathogenic *Escherichia coli* (UPEC) is known to invade urothelium cells and form quiescent intracellular reservoirs (QIRs). It is thought that QIRs may provide a source for bacterial persistence and R-UTI (Hickling, et al., 2013)

Other significant common bacteria that can cause UTI are *Staphylococcus saprophyticus, Staphylococcus epidermidis, also* other *Enterobacteriaceae*, such as *Proteus mirabilis*, *Klebsiella* and *Providentia* species. Gram-positive organisms, like methicillin-resistant *Staphylococcus aureus* and *Enterococcus,* are less common overall, but are seen with increasing frequency in healthcare settings and in adults with chronic

indwelling (Roriz-Filho, et al., 2010; Al-Badr, et al., 2013; Gilbert, et al., 2013; Rowe, et al., 2013).

For susceptible subpopulations, such as DM patients, the incidence of *Klebsiella spp* and S*treptococcus sp* infections are more common. However *Pseudomonas sp* infections are more common in chronically-catheterised patients (Al-Badr, et al., 2013). In the C-UTI patients and R-UTI are very frequent the appearance of multidrug resistant *E. coli* with extended spectrum β-lactamases (ESBL), which difficult the treatment (Roriz-Filho, et al., 2010). For men, coagulase-negative staphylococci are also common, in addition to gram-negative bacilli and *Enterococcus sp* and *Proteus mirabilis* (Nicolle, et al., 2005).

6. UROPATHOGEN RESISTANCE TO ANTIMICROBIALS

The emergence of antimicrobial resistance is a growing problem in medicine worldwide (WHO, 2001). The consumption and misuse of antimicrobials are directly related to the increased of bacteria resistance, infected patients morbidity and mortality, and consequently to the rising of health-care costs (Edlin, et al., 2014; Landry, et al., 2014).

The nosocomial ASB constitutes a major pool of antibiotic-resistant strains of pathogens (Parida, et al., 2013). Multi-resistant gram-negative bacteria accounted for a higher number of nosocomial infections than resistant gram-positive bacteria (Ott, et al., 2013).

In community, in spite of being well known that excessive use of antimicrobials compromise its efficacy and lead to its resistance, the continuous antibiotic prophylaxis therapy is usually used as an effective measure to prevent UTI (Kapoor, et al., 2012).

E. coli resistance rates have been reported to ampicillin (39-45%), TMP-SMX (14-31%), nitrofurantoin (1.8-16%) and fluoroquinolones (0.7-10%) (WHO, 2005). According to WHO in the Global Report on Surveillance, the *E.coli* is resistant to the 3rd generation of cephalosporins and fluoroquinolones and also multidrug resistant *Klebsiella* spp (Girard, et al., 2002; WHO, 2014). The ciprofloxacin is in continuous decrease for urine isolates from outpatients (from 90% to 88%) and inpatients (from 85% to 82%) (Landry, et al., 2014). Among C-UTI patients and R-UTI it is very common the appearance of *E. coli* with extended spectrum β-lactamases (ESBL), which implies a difficult treatment with broad-spectrum antibiotic (Roriz-Filho, et al., 2010). Even in childhood antimicrobial resistance is alarming. Edling and his colleagues reported in a study about antimicrobial resistance in paediatric urology that in 2009 TMP-SMX resistance rates for *E. coli* pediatric UTI increased in both sexes, boys (from 23% up to 31%) and girls (from 20% up to 23%) (Edlin, et al., 2014).

TMP-SMX is widely diffused in international guides, however, they should only be prescribed after antimicrobial sensibility tests and not as an empirical treatment, due to its high rates of resistance in *E. coli* isolates (Roriz-Filho, et al., 2010). Fluoroquinolones have played an important role in the treatment of infectious disease, with their wide spectrum of activity, convenient dosing, and good patient tolerability (Landry, et al., 2014).

6.1. CLASSES AND ANTIMICROBIAL AGENTS

Antibiotics were discovered in the middle of the 19th century and soon after the discovery of penicillin a number of treatment failures with some bacteria, such as staphylococci, which were no longer sensitive to penicillin (Byarugaba, et al., 2009).

Over the years, the continued use of various antimicrobial agents lead to the development of bacteria resistance mechanisms (Giedraitienė, et al., 2011). The multidrug resistance emergence contributes to a global economic and health-care

crisis, listed by the World Health Organization as one of the top 3 threats to global public health (Brooks, et al., 2014). And the main problem is the increasing resistance of antibiotic resistance while the number of new antibiotics is decreasing (Figure 5).

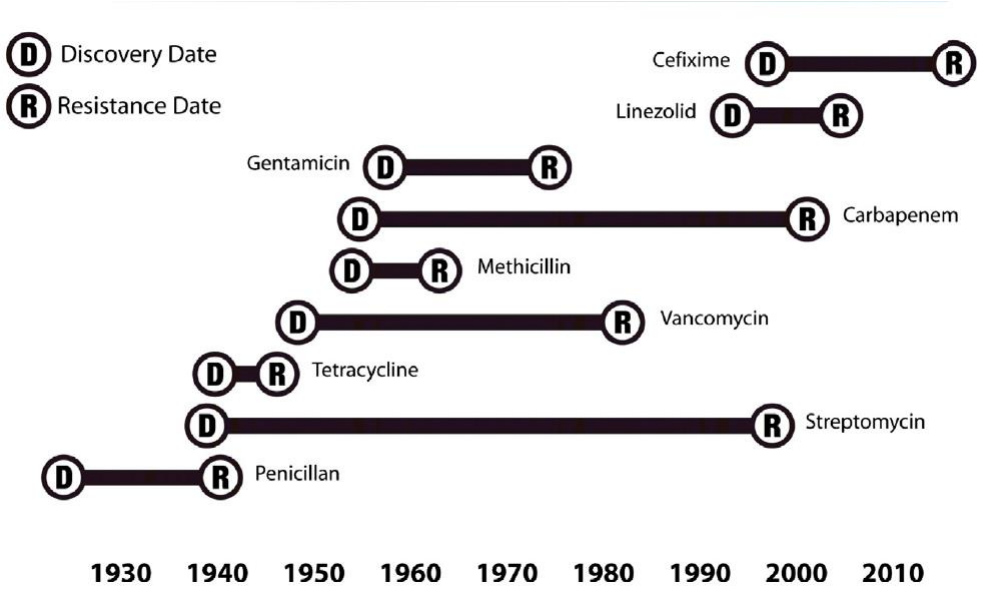

FIGURE 5 Antibiotic resistance is increasing while the number of antibiotics is decreasing. Adapted from Brooks (2014).

The antimicrobial agents can be classified based on the cellular components or system they affect, such as: interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, and inhibition of a metabolic pathway (Tenover, 2006). In addition if they induce cell death (bactericidal drugs) or only inhibit cell growth (bacteriostatic drugs). According to the spectrum of activity can be also classified as: broad spectrum, when effective a variety of gram negative or gram positive bacteria; narrow spectrum, when effective only against gram negative or gram positive (Kohanski, et al., 2010) (Table 4).

TABLE 4 Antimicrobials classification and targets (bioMérieux, Inc, 2008; Range, et al., 2007).

Class		Substance	Target
β-lactam Ring	Penicillins	• Ampicillin • Amoxicillin	These semi-synthetic antibiotics are bactericidal. Responsible for the inhibition of peptidoglycan synthesis from the cell wall and the autolytic activation, which will determine the lyses and posteriori death.
	1st Generation Cephalosporins	• Cefalotin	Good Gram-positive activity and relatively modest Gram-negative activity. Their target is the cell wall lysis.
	2nd Generation Cephalosporins	• Cefaclor • Cefuroxime • Cefoxitin	Better Gram-negative coverage, however less Staphylococcal activity. Their target is the cell wall lysis.
	3rd Generation Cephalosporins	• Cefotaxime • Cefixime • Ceftazidime	Bactericidal action after the linkage with the cytoplasmic membrane. Wide spectrum of action, good activity against Gram-negative and less active against Gram-positive.
	Carbapenems	• Imipenem	Synthesis inhibition of the cell wall. A broad spectrum of action. Wide diffusion in the body, especially in the cerebrospinal fluid.
		• Clavulanic Acid	β-lactamase[1] enzymatic inhibitor, have weak or poor antibacterial activity alone. Should be used in association with β-lactams.
Quinolones	• Ciprofloxacin • Levofloxacin		Interfere with the bacterial DNA synthesis, blocking the twisting of the strands of DNA to form double-stranded, inhibitor of the DNA-girase. Bactericidal action.
Aminoglycosides	• Gentamicin • Tobramycin • Amikacin		Inhibitors of protein synthesis, their penetration depends on the active transportation of oxygen, thus have a slow rate against anaerobic microbial. Therapeutic drug monitoring is mandatory to control side effects. Hospital use only, bactericidal action.
Macrolides	• Eritromicin		Inhibitor of the protein synthesis by binding to the 50S subunit of bacterial ribosomes.
Tetracyclines	• Tetracycline		Inhibitor of protein synthesis in both Gram-positive and Gram-negative bacteria. It has a bacteriostatic action.
Glycopeptides	• Vancomycin		Bactericidal for Gram-positive only. Their complex chemical structure also Inhibit the cell wall synthesis, but at a different site than the β-lactam agents. Good diffusion in all tissues and high toxic effects: ears and kidneys.
Lincosamides	• Clindamycin		Inhibits the synthesis of proteins, similar to chloramphenicol and macrolides.
Fosfomycins			Inhibition of the cell wall synthesis at a stage earlier than the penicillins or cephalosporins.

6.2. MECHANISMS OF RESISTANCE

In order to survive, the bacteria develop mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. Antimicrobial resistance is a natural biological phenomenon and is often a consequence of microbial adaptation to antimicrobials exposure (Byarugaba, et al., 2009).

Different antimicrobials have different spectrum of action against the microbes. Some are specific for gram positive while other are specific for gram negative, no antimicrobial is effective to all microbes (Vishwas, et al., 2012) (Table 5).

TABLE 5 Resistance mechanisms found in common bacteria pathogens (Poole, 2007; Vishwas, et al., 2012; Mandell, et al., 2004)

Bacteria	**Method of resistance**	**Antimicrobials**
Escherichia coli	Active efflux system	Tetracyclines
Klebsiella pneumoniae	Enzymatic inhibition Alteration of ribosomal target sites	B- lactams Aminoglycosides
Pseudomonas aeruginosa	Enzymatic inhibition Active efflux system	B- lactams Aminoglycosides Quinolones Tetracyclines Trimethoprim
Enterococci	Alteration of target enzymes Altered ribosomal target site mutations Enzymatic inhibition Alteration of cell-wall precursor targets Alteration of ribosomal target sites	Ampicillin Aminoglycosides Vancomycin Linezolid
Streptococcus pneumonia	Alteration of target enzymes Alteration of ribosomal target sites Protection of ribosomal target sites	B- lactams Macrolides Lincosamides Tetracyclines Trimethoprim Sulfamides
Staphylococcus aureus	Enzymatic inhibition Alteration of target enzyme Alteration of cell-wall precursor targets	Penicillin MRSA GISA GRSA

Bacteria may be naturally resistant (vertical evolution) by the lack of transport system for an antibiotic, the lack of the target of the antibiotic molecule, or the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic (gram negative).

Or may develop resistance to antibiotics by mutating existing genes (vertical gene transfer). When exposed to unfavourable environmental conditions the bacteria enter into a "hypermutable state" model, which the rate of mutations increase from 10 to 50 up to 10 000 times during a prolonged nonlethal selection of microorganisms. This adaptive process is the only and main source of the antibiotic-resistant mutants to originate under normal conditions and they are called "adaptive mutations" (Giedraitienė, et al., 2011; Vishwas, et al., 2012; Brooks, et al., 2014).

The acquiring resistance (horizontal gene transfer) beyond spontaneous mutation requires either the modification of existing genetic material or the acquisition of new genetic material from another source, strain or specie. The horizontal gene transfer occurs by many different mechanisms, mobile genetic elements including: phages, plasmids and transposons (Giedraitienė, et al., 2011; Vishwas, et al., 2012).

There are at least three possible mechanisms: transduction, (via bacteriophages and integrons) occurs when DNA are transferred between two closely related bacteria; transformation (via plasmids and conjugative transposons) is a process where parts of DNA are taken up by the bacteria normally present in the external environment due to the death and lysis of another bacterium; conjugation (via incorporation of chromosomal DNA, plasmids into a chromosome) occurs when there is direct cell-cell contact between two bacteria closely related and transfer small pieces of DNA (Giedraitienė, et al., 2011; Vishwas, et al., 2012).

The transfer of resistance genes is more effective than chromosomal mutation. Most plasmids are double-stranded circular DNA whose size may vary from 2–3 kb, which encode up to 10% of the host cell chromosome and confer resistance to main classes of antimicrobial agents (cephalosporins, fluoroquinolones, and aminoglycosides) (Giedraitienė, et al., 2011).

Transposons can be integrated into plasmids or the host's chromosome, encompass small elements called insertion sequence elements and transposing bacteriophages. They have terminal repeat sequences that play a role in recombination and recognize a protein (for example, transposase or recombinase) that is necessary to insert or remove a transposon from specific genome regions. Transposons are transferred by conjugation, transformation, or transduction and spread quicker than genes in chromosomes. Conjugative transposons have characteristic features of plasmids and can help to transfer endogenic plasmids from one microorganism to another (Giedraitienė, et al., 2011).

When resistance determinants are on plasmids, they will spread quickly within the genus or even unrelated bacterial genera. When resistance is associated with genes on chromosomes, resistant microorganisms will spread more slowly (Giedraitienė, et al., 2011). Also, the presence of low levels of the antibiotic in the environment promotes gene transfer.

The combined effects of fast growth rates to large densities of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution of bacteria (Vishwas, et al., 2007).

6.3. ANTIBIOTIC STEWARDSHIP

Bacterial resistance is closely associated with the use of antimicrobial agents in clinical practice. Prolonged therapy with antibiotics may lead to the development of

resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt gradually and develop resistance to antibiotics (Giedraitienė, et al., 2011). Also, the use in nonhuman niches is another important reason for the spread of resistant bacteria, such for growth promotion, feed efficiency, and routine disease prevention purposes in animal agriculture it is a factor of increasing resistance (IDSA, 2011; Giedraitienė, et al., 2011). For example, *Salmonella* and *Campylobacter* acquire resistance to antibiotics and have transferred antibiotic resistance to natural human flora. The *E. coli* resistance to ciprofloxacin is associated with the use of fluoroquinolones in aviculture (Giedraitienė, et al., 2011).

Some conditions that may promote the acquiring of antimicrobial resistance that should be avoided, such as:

- Exposure to non-lethal levels of antimicrobials;
- Exposure to bacteria with acquired resistance genes;
- Over- the-counter purchases without medical supervision;
- Antimicrobials prescriptions for non-bacterial infections;
- Antimicrobials self-medication without medical supervision;
- Growth promotion, feed efficiency, and routine disease prevention in animal agriculture without veterinarian prescription;

Considering the rising of multidrug resistant bacteria, preventative measures are required (Byarugaba, et al., 2009). To minimize the medication errors in clinical practice were suggested the 10 tips for safe prescribing (National Prescribing Centre, 2011):

1. Keep yourself up-to-date in your knowledge of therapeutics, especially for the conditions you commonly see;

2. Before prescribing, make sure you have all the information you need about the patient, including co-morbidities and allergies;

3. Before prescribing, make sure you have all the information you need about the drug(s) you are considering to prescribe, including side effects and interactions;

4. Sometimes the risks of prescribing outweigh the benefits and before doing so, think: 'Do I need to prescribe this drug at all?;

5. Check computerised alerts in case you have missed an important interaction or drug allergy;

6. Always carefully check prescriptions for errors before signing them;

7. Involve patients in prescribing decisions and give them the information they need in order to take the medicine as prescribed, to recognise important side-effects and to know when to return for monitoring;

8. Have systems in place for ensuring that patients receive essential laboratory test monitoring for the drugs they are taking, and that they are reviewed at appropriate intervals;

9. Make sure that high levels of safety are built into your repeat prescribing system;

10. Make sure you have safe and effective ways of communicating medicines information between primary and secondary care, and act on medication changes suggested by secondary care clinicians;

7. PREVENTION

7.1. PREVENTIVE ANTIMICROBIAL MEASURES

Appropriate prescribing is essential to improve patient outcomes and to help prevent the emergence of resistant organisms. Risk versus benefits should be weighed carefully before using antibiotics. Poor empiric prescribing practices, lack of urine testing, and nonselective use of prophylaxis exacerbate this problem. It is also strongly recommended some practice patterns, such as: urine antimicrobial susceptibility testing in order to only treat when indicated; high selective application of antibiotic prophylaxis; local surveillance programs with inpatient *versus* outpatient data should be implemented (Edlin, et al., 2014).

The adoption of conservative measures by subpopulations at increased risk is very important to counter the prevalence of community-acquired UTI. Some hygiene modifications, such as: limiting of spermicide use; urinating and washing the genitals pre- and post-coital; wiping from front to back after toilet and every 3-4 hours; shower instead of immersion bath and avoid the perfumed soaps; acquisition of cotton underwear instead nylon. These preventive behavioural actions can avoid many episodes of R-UTI in the long-term (Kapoor, et al., 2012).

7.2. PREVENTIVE NON-ANTIMICROBIAL FORMULATIONS

Prevention strategies for R-UTI in subpopulations with increased risk have been studied using non-antimicrobial therapies in place of antibiotic prophylaxis, such as:

estrogen replacement therapy, lactobacilli formulation and cranberry formulations (SIGN, 2012; Hickling, et al., 2013).

Estrogen is thought to play an important role in maintaining a low vaginal pH in pre-menopausal women. The low estrogen in postmenopausal women promotes changes in the vaginal flora and the predominant flora in younger women often disappear. This leads to an increase in vaginal pH and promotes colonization of uropathogens (Rowe, et al., 2013). The application of intravaginal estrogen, as topical vaginal estrogen cream, proved to be effective and safe (Hickling, et al., 2013).

Oral lactobacilli formulation has been tested as a prevention strategy in postmenopausal women with recurrent UTI. The hypothesis was that oral lactobacilli may repopulate the vagina with premenopausal vaginal flora, thereby preventing UTI (Rowe, et al., 2013).

The intake of Cranberry products is a non-antimicrobial approach, which has been used for prevention and treatment of UTI. Cranberry proanthocyanidin is the active ingredient in cranberries that inhibits adherence of P-fimbriated *E.coli* to the bladder epithelium. In contrast, cranberry formulations interfere with certain medications and should be taken after medical advice only (SIGN, 2012; Hickling, et al., 2013; Jepson, et al., 2013; Rowe, et al., 2013).

Other formulations, such as ascorbic acid (vitamin C) intake to acidify urine and make it hostile for bacterial growth, *in vitro* data suggest a bacteriostatic effect (Hickling, et al., 2013). Another example is methenamine salts intake, these salts are hydrolysed in the urine to form ammonia and formaldehyde. Formaldehyde is widely bacteriostatic and does not allow the development of bacterial resistance. In a study, they have compared continuous methenamine hippurate and continuous antimicrobial prophylaxis in healthy adult women and found no significant difference between prophylactic trimethoprim and methenamine hippurate (Hickling, et al., 2013).

Fluid intake, especially water, helps dilute urine and promotes frequently voiding, which allows bacteria to be flushed from your urinary tract before an infection can begin (Beetz, 2003). Well as the limitation of the intake of substances that irritate the bladder epithelium, like caffeine and chocolates may help to prevent UTI (Kapoor, et al., 2012).

8. RESISTANCE AND FUTURE PERSPECTIVES

The antimicrobial resistance is a global problem that calls for a global response, and the implementation of a global strategy and preventive guidelines to slow down the antimicrobial resistance is crucial to its success (WHO, 2014).

The local surveillance implementation is very important to promote effective empirical treatments. However, the main clinical diagnostic dilemma persists, the distinguishing of symptomatic UTI from ASB and which patients to treat with antibiotics (Barber, et al., 2013; Hickling, et al., 2013; Rowe, et al., 2013).

Future studies to evaluate non-antimicrobial formulations in order to stop the increasing resistance rates remain important to prevention of UTI (Barber, et al., 2013; Hickling, et al., 2013; Rowe, et al., 2013). Like the implementation of CA-UTI prevention guidelines, which should be included into an effective program. Including indications of catheter selection, catheter insertion/maintenance and dates of insertion and removal should be established. And alternative strategies, such as substitution of indwelling urethral catheters for external condom catheters in men, and the staff education in health care facilities are essential to minimize the risk of CA-UTI (Nicolle, 2014).

The application of preventive practices and renewed treatment approaches can reduce significantly the prevalence of UTI, even as the resistance rates worldwide in the future.

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CHAPTER II - FREQUENCY AND ANTIBIOTIC RESISTANCE OF BACTERIA IMPLICATED IN COMMUNITY URINARY TRACT INFECTIONS IN NORTH AVEIRO BETWEEN 2011 AND 2014

FREQUENCY AND ANTIBIOTIC RESISTANCE OF BACTERIA IMPLICATED IN COMMUNITY URINARY TRACT INFECTIONS IN NORTH AVEIRO BETWEEN 2011 AND 2014.

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1. ABSTRACT

Urinary tract infection (UTI) is the second most common infection in community and the most common nosocomial infection worldwide. The main goal of this study is to evaluate the predominance of uropathogens responsible for urinary tract infection and determine their resistance patterns, in order to assess if the recommended empirical treatment is appropriated for this population. Samples were collected at Centro Médico da Praça Lda on ambulatory system, in São João da Madeira municipality (Aveiro, Portugal) from June 2011 to June 2014. From 4270 analysed urine samples, 3561 (83%) were from women and only 709 (17%) were from men, from 0 to 104 years of age. *E. coli* was the most frequent uropathogen, followed by *Klebsiella* spp and *Enterococcus* spp and *P. mirabilis*. From all data 37% were MDR isolates and from these 30.9% were from women and 61.6% were from men. In general men were more resistant to antimicrobials than women. From first line drugs recommended by EAU to empirical treatment of uncomplicated UTI only nitrofurantoin is appropriated for both sexes and ciprofloxacin may be only considered to treat women. In the second line antibiotics, ampicillin is not an appropriate drug to empirical treatment of uncomplicated UTI, however the antimicrobials AMX-CA and TMP-SMX should not be used to empirical treatment of men, due the local high

incidence of resistance, over 20% for men. Thus, it is suggested the use of imipenem and gentamicin as an alternative to treat both sexes.

2. INTRODUCTION

Urinary tract infection (UTI) is the second most common infection in community and the most common nosocomial infection worldwide (Al-Badr, et al., 2013; Ott, et al., 2013; Sujatha, et al., 2014). The episode of UTI is classified as lower (bladder, urethra and prostate) or upper (Kidneys) urinary tract infection considering the affected organs. Uncomplicated urinary tract infection (U-UTI) occurs in non-pregnant young women with a normal genitourinary tract. Complicated urinary tract infection (C-UTI) was diagnosed in genitourinary tract with structural, functional abnormalities or developed in healthcare settings (including catheterized patients) (Roriz-Filho, et al., 2010). Recurrent urinary tract infection (R-UTI) results from three positive urine cultures during a 12-month period, or two infections during the previous 6 months and it is mainly caused by reinfection of the same pathogen (Graziottin, 2014). Symptoms are usually slight in U-UTI or absent in case of asymptomatic bacteriuria (ASB). The diagnosis of infection is defined as the presence of two consecutive positive urine specimens with the same bacterial strain in quantities equal or superior to 10^5 CFU mL^{-1} (Rowe, et al., 2013).

Community-acquired urinary tract infections are mainly uncomplicated, colonizing preferably the bladder causing cystitis (Stamm, 2006; Wiles, Kulesus, et al., 2008). However, some bacteria may ascend through the ureters to the kidneys and cause more severe infections such as pyelonephritis (Stamm, 2006; Wiles, et al., 2008). Moreover, specific subpopulations are more likely to have UTI, such as, patients with catheters, urologic abnormalities, diabetics, immunodeficiency, infants, pregnant women and elderly, to these patients UTI can be more severe (Foxman, 2002).

Undesirably, there is little knowledge about the main uropathogens and their antimicrobial resistance pattern implicated in community-acquired UTI compared with UTI acquired at hospital level. The importance of this information is the periodic monitoring of the changes over the years, in order to decrease the number of therapeutic failures (Linhares, et al., 2013).

The few studies carried out in the community have shown that uropathogens, such as, *Escherichia coli* (46.4 - 74.2%), *Klebsiella* spp (6.0 - 13.45%), *Proteus* spp (4.7 - 11.9%) and *Enterococcus* spp (5.3 - 9.54%) are the most prevalent in UTI (Neto, et al., 2003; Akram, et al., 2007; Correia, et al., 2007; Francesco, et al., 2007; Laupland, et al., 2007; Pires, et al., 2007; Mendo, et al., 2008; Costa, et al., 2009; Rahman, et al., 2009; Martins, et al., 2010; Nimri, et al., 2010). *E. coli* has been indicated as the most frequent uropathogen involved in the community-acquired UTI (Francesco, et al., 2007; Laupland, et al., 2007; Mendo, et al., 2008; Costa, et al., 2009) probably because it belongs to the normal flora of the human intestine and therefore easily colonize the urinary tract.

According to the European Association of Urology (EAU) the empirical first line treatment recommended to acute U-UTI is ciprofloxacin, fosfomycin and nitrofurantoin, followed by ampicillin, amoxicillin-clavulanic acid (AMX-CA) and trimethoprim-sulfamethoxazole (TMP-SMX) as second line treatment. The European Society for Microbiology and Infectious Diseases (ESCMID) recommends as first line therapy for acute U-UTI, fosfomycin due to minimal resistance and collateral damage (Gupta, et al., 2011). Ciprofloxacin is a suggested as an effective 3-day regiment, while men treatment should be extended to 10-14 days (Roriz-Filho, et al., 2010). Nitrofurantoin is also an appropriate choice for therapy due to minimal resistance and collateral damages (Gupta, et al., 2011).

The second line therapy recommended the class of β-lactam agents when first line recommended agents cannot be used, due to their inferior efficacy and more

adverse effects. TMP-SMX in spite of being widely diffused in the international guidelines must be based on AST and it should only be prescribed if local resistance rates of uropathogens do not exceed 20% (Grabe, et al., 2014). According to the 1999 Uncomplicated Urinary Tract Infection Guidelines by the IDSA, AMX or AMP should not be used for empirical treatment given the relatively poor efficacy and the very high prevalence of resistance to these agents worldwide (Gupta, et al., 2011).

The high rates of resistance among pathogenic bacteria suggest the failure of the past preventive guidelines to slow down the antimicrobial resistance (WHO, 2001; Francesco, et al., 2007; Koningstein, et al., 2013; Linhares, et al., 2013, WHO, 2014). The empirical treatment, used in an early disease phase to decrease the rate of morbidity, should be guided by national resistance surveillance data, in the absence of surveillance community studies at regional level (WHO, 2001; Linhares, et al., 2013; WHO, 2014). The surveillance should be continuous, the bacterial resistance patterns change over the time. In order to administer an appropriate empirical therapy it is crucial to know not only the main bacteria usually involved in the UTI but also their respective antibiotic resistance patterns. This surveillance procedure controls the increase of antimicrobial resistance and the spread of resistant bacterial strains that represent a public health problem worldwide.

The goal of this study is to evaluate the predominance of uropathogens responsible for urinary tract infection and their resistance patterns, in North Aveiro (Portugal) between 2011 and 2014 in order to assess if the empirical recommended treatment of UTI is appropriate for this population.

3. METHODS

3.1. DATA SAMPLES

All the samples were collected in the ambulatory system from June 2011 to June 2014 at Centro Médico da Praça Lda, located in São João da Madeira municipality, district of Aveiro in the north-western region of Portugal. Positive UTI samples at the reception were registered with code and associated to the patient's process number. To build the database was registered the gender, age, bacterial strain and the corresponding antimicrobial susceptibility testing (AST) from the VITEK© 2 (bioMérieux SA) report. This study was approved by the clinical analysis laboratory Centro Médico da Praça Lda.

3.2. SAMPLING

First morning urine samples (not less than 4 hours storage in bladder) were collected by the patients after the daily hygiene at home. The first and final urine was rejected, only the midstream portion was collected to a sterile urine container to avoid contaminations. Samples were transported in their primary containers and stored at 4°C until they can be processed (European Confederation of Laboratory Medicine, 2000).

3.3. URINALYSIS

In the laboratory, samples were triaged and 10-15 mL from the sterile containers were transferred into examination tubes to the dipstick analysis, to nitrite and leucocyte esterase detection. Then the examination tubes were centrifuged at 1500 rpm for 5 minutes to the microscopic analysis.

One microliter of urine sample was inoculated with a disposable plastic loop on CLED media or chromID™ CPS® plates (bioMérieux) and incubated at 37 °C in aerobic atmosphere for 18 hours. These culture media allow direct presumptive identification

of *E. coli*, *Proteus* spp and *Enterococcus* spp having in consideration the sizes, colours and shapes of the colony-forming units (CFU) (bioMérieux SA, 2013). After incubation, all the negatives cultures, bacteria growth inferior to 10^3 CFU/mL, were excluded. Urine culture was defined as positive if bacterial growth was equal or superior to 10^5 CFU/mL, with a maximum of 2 isolated species. Samples were considered contaminated if 3 or more different colonies grown in the culture medium, these samples were classified as "polymorph flora" and a second sample was requested from the patient. Bacteriuria was defined when bacterial growth was inferior to 10^5 CFU/mL and, in these cases, it was only preformed the identification (ID) and antimicrobial sensibility test (AST) for pregnant women or children.

3.4. IDENTIFICATION OF BACTERIAL ISOLATES

Identification was performed by the VITEK© 2 ID card, after a gram stain to differentiate bacterial species into gram-positive or gram-negative. The isolates were inoculated in a saline suspension, with a density between 0.5-0.65 in McFarland scale. According to the colonies characteristics, it was chosen the VITEK® 2 GN ID Card to identify lactose fermenting and non-fermenting gram-negative bacilli, or VITEK® 2 GP ID Card to identify gram-positive bacteria (bioMérieux SA, 2014 b). The VITEK® 2 ID cards were introduced into the saline suspension and incubated at 36 °C in the VITEK® incubator, and the results were available within 10 hours (Funke, et al., 2004; bioMérieux SA, 2014 b).

3.5. ANTIMICROBIAL SUSCEPTIBILITY TEST (AST)

The AST was performed with VITEK® 2 AST Card. The VITEK® 2 AST-GN26 (Portuguese card for urine samples) and VITEK® 2 AST-N113 to gram negative bacteria with resistance to the majority of the antibiotics from the VITEK® 2 AST-GN26; VITEK® 2 AST-P586 to gram positive E*nterococcus* spp and *Streptococcus* spp and VITEK® 2 AST P619 to Gram positive *Staphylococcus* spp (bioMérieux Portugal, 2014) were used.

Three millilitres of a commercial saline (0.45%) liquid medium, recommend for procedures that require the use of a diluent with a VITEK® system, were prepared for each respective ID card suspension, based on the established concentrations (Thermo Fisher Scientific Inc., 2008; Thermo Fisher Scientific Inc., 2009). From gram-negative (GN) suspensions 147 µl of the VITEK® ID suspension were taken and from gram-positive (GP) 270 µl of the VITEK® ID suspension were used. Both suspensions, ID cards and AST cards were introduced into respective saline suspensions and incubated at 36 °C in the VITEK® incubator for 10 hours (Funke, et al., 2004; bioMérieux SA, 2014 a).

The AST results were validated by advanced expert system (AES) program, which follows the European committee on antimicrobial susceptibility testing (EUCAST). The phenotypic antimicrobial susceptibility testing determination depends on the minimal inhibitory concentration (MIC) breakpoints. Based on breakpoints, bacteria were grouped into three categories: susceptible, intermediate and resistance. Meanwhile, the ESBL-confirmation test was also performed (bioMérieux SA, 2014 a; Stokkou, et al., 2014)

3.6. STATISTICAL ANALYSIS

The data base was done using the Microsoft Excel program and treated using the Statistical Package for the Social Sciences (SPSS) 16.0 for Windows. The normality, homogeneity and independence of the variants were checked before analysis. Since the data failed the normality assumptions, non-parametric methods were used to detect significant differences. Mann–Whitney U test was used to evaluate differences between genders. The Kruskal-Wallis test was used to evaluate the age and study period influence in the UTI incidence. The Chi-square test was used to evaluate the incidence of isolates into the age groups. The significant level established was $p>0.05$. The main bacteria responsible for UTI, such as, *E.coli*, *Enterococcus* spp*, Klebsiella* spp, *P. mirabilis*, *P. aeruginosa*, *Staphylococcus* spp and *S. agalactiae* were identified as responsible for at least 1% of the study infections (representing 93.5% of the total

urinary tract infections). The remaining bacteria were grouped and classified as "other bacteria" (representing 6.5% of the total infections) and were included the uropathogens: *Acinetobacter baumannii, Burkholderia cepacia, Citrobacter* spp*, Enterobacter* spp*, Hafnia alvei, Morganella* spp*, Proteus* spp*, Providencia* spp*, Pseudomonas* spp*, Raoultella* spp*, Salmonella enteretidis, Serratia* spp*, Shigella* spp*, Sphingomonas paucimobilis, Yersinia enterocolitica.* The resistant uropathogens to three or more antimicrobial classes were considered multi-drug resistant (MDR).

4. RESULTS

4.1. CHARACTERIZATION OF THE UTI SAMPLES

From 4270 analysed urine samples, 3561 (83%) were from women and only 709 (17%) were from men. The age range varied from 0 to 104 with an average of 61 years of age. The average age for women was 59 years of age and for men 68 years of age. The incidence of UTI increased with the age (Chi-square, $p<0.05$), being the elderly (≥ 65) age group the most affected with 2219 (52%) urine samples (78,1% corresponding to women and 21,9% corresponding to men), followed by adults (35-64) with 1381 (32.3%) samples (87% corresponding to women and 13% corresponding to men), young adults with 516 (12.1%) samples (97,9% corresponding to women and 2,1% corresponding to men), adolescents (13-18) with 53 (1.2%) samples (94,3% corresponding to women and 5,7% corresponding to men), and the last age group, children (0-12) with 101 (2.4%) samples (70,3% corresponding to women and 29,7% corresponding to men) (Figure 1a). However, between children and the adolescent groups a 1% decrease in women was observed, the decreasing tendency was also observed between children and the young adults groups (2% to 0%, respectively) in men (Figure 1a).

In general, women was the most affected in all age groups and the incidence of UTI increased with the patient's age (Figure 1a). The largest gender differences among age groups occurred in elderly, adults and young adults (differences about 30%, 24% and 12%, respectively) (Figure 1b).

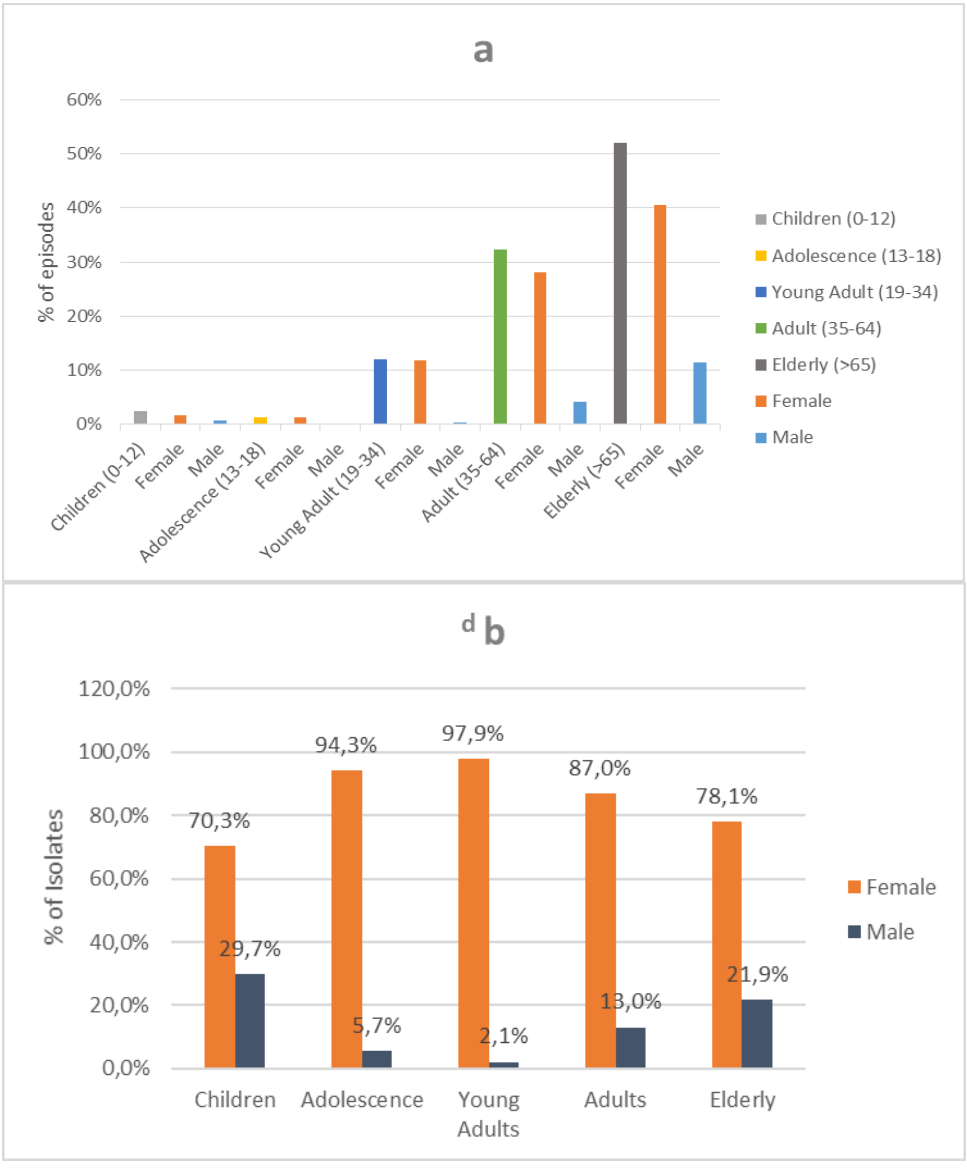

FIGURE 1 a - The incidence of UTI by age groups considering the genders; d Significant statistically differences between age groups distribution (Chi-square, $p < 0.05$); Percentage in relation to all UTI episodes (N=4270). **b** – ^{d} Significant statistically

4.2. BACTERIAL IMPLIED IN UTI

The predominant uropathogens were *E. coli* (63.8%), *Klebsiella* spp (11.8%), *Enterococcus* sp (6.8%), *P. mirabilis* (5.6%), *Staphylococcus* spp (2.4%), *P. aeruginosa* (1.9%) and *S. agalactiae* (1.2%). The isolates under 1% of UTI classified as "other bacteria" correspond to a 6.5% of the samples (Table 1).

TABLE 1 Frequency of isolates responsible for at least 1%; N – frequency of isolates; Total % - percentage of isolates in relation to N; Female (%) - percentage of isolates in relation to n women; Male (%) - percentage of isolates in relation to n men.

Order	Bacteria	Total (%)		Female (%)	Male (%)
		N = 4270		n = 3561	n = 709
1º	*Escherichia coli*	2723	63.8	67.80	43.40
2º	*Klebsiella* spp	502	11.8	11.30	14.00
3º	*Enterococcus* spp	290	6.8	6.20	9.60
4º	*Proteus mirabilis*	240	5.6	4.60	10.70
5º	*Staphylococcus* spp	103	2.4	2.20	3.40
6º	*Pseudomonas aeruginosa*	83	1.9	1.30	5.10
7º	*Streptococcus agalactiae*	50	1.2	1.30	0.70
…	Other Bacteria	279	6.5	5.20	13.10

The main bacteria implicated in women UTI was statistically different from the main bacteria implicated in men UTI (Mann-Whitney U test, $p< 0.05$). *E. coli* was the bacterium most implicated in both genders UTI, however its incidence was higher in women, 67.8%, than in men, 43.4% (Table 2). To the remaining bacteria in women, the second most implicated bacterium was *Klebsiella* spp (11.3%) followed by *Enterococcus* sp (6.2%), *P. mirabilis* (4.6%), *Staphylococcus* spp (2.2%), *P. aeruginosa* (1.3%) and *S. agalactiae* (1.3%), other bacteria represented 5.2% of the isolates. For men, the second uropathogen most frequent was *Klebsiella* spp (14%) followed by *P. mirabili*s (10.7%), *Enterococcus* sp (9.6%), *P. aeruginosa* (5.1%), *Staphylococcus* spp (3.4%) and *S. agalactiae* (1.3%), other bacteria represented 13.1% of the isolates (Table 2).

The analysis of aetiology has shown statistical significant differences between age groups (Kruskal-Wallis test, $p<0.05$). An increasing incidence of *Klebsiella* spp over the age groups was reported. The incidence of *P. mirabilis* was substantially higher in children compared to the remaining age groups. *Staphylococcus* spp and *Enterococcus* spp had higher incidence in adolescence and young adults compared to the remaining age groups. For young adults and adults, *E.coli* was responsible for more than half of the UTI infections (Figure 2).

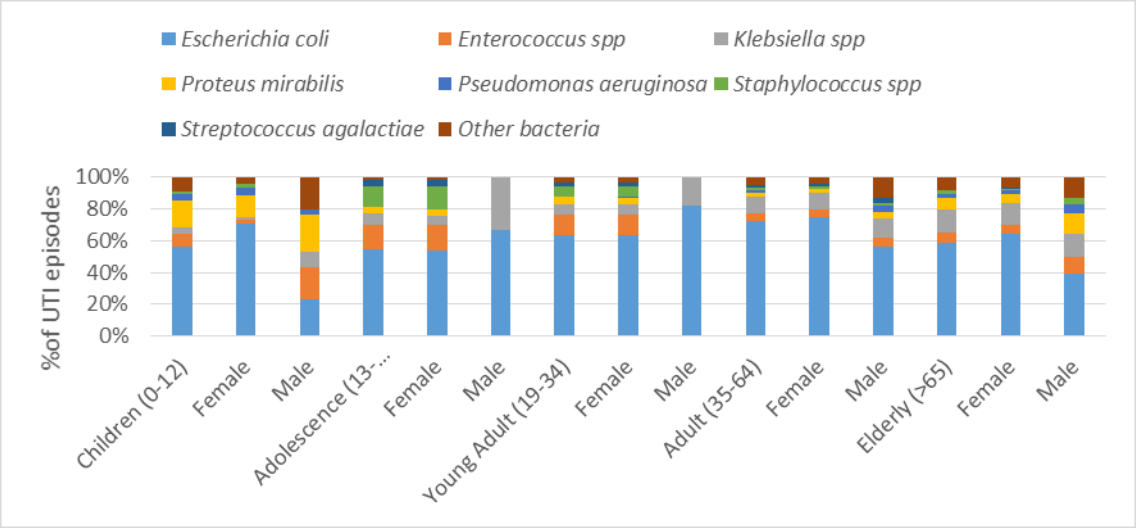

FIGURE 2 Study of aetiology of UTI isolates by age groups considering the genders; d significant differences between age groups (Kruskal-Wallis test, $p<0.05$); d Significant statistically differences between sexes (Mann-Whitney U test, $p< 0.05$).

Significant differences in the UTI main bacteria were observed throughout the study period (Kruskal-Wallis test, $p< 0.05$). During the study period the incidence of *E. coli* has varied slightly between 60.8%-66.0%, it was always responsible for more than half of the UTI episodes (Figure 3a). The incidence of *Klebsiella* spp has shown a 3% decrease over the study period (variation 14.8%-10.7%). However, *Enterococcus* spp has registered a 5.3% increase during the same period (variation 4.4%-9.7%). The incidence of *P. aeruginosa* (variation 1.7%-2.2%), *Staphylococcus* spp (variation 2.1%-2.6%), *S. agalactiae* (variation 0%-2.2%) and *P. mirabilis* (variation 4.9%-6.6%) were

irregular but almost constant over the study period (Figure 3b). Significant gender differences in the main bacteria responsible for UTI were observed during the study period into the years 2011 and 2013 (Mann–Whitney U test, $p < 0.05$) (Figure 4).

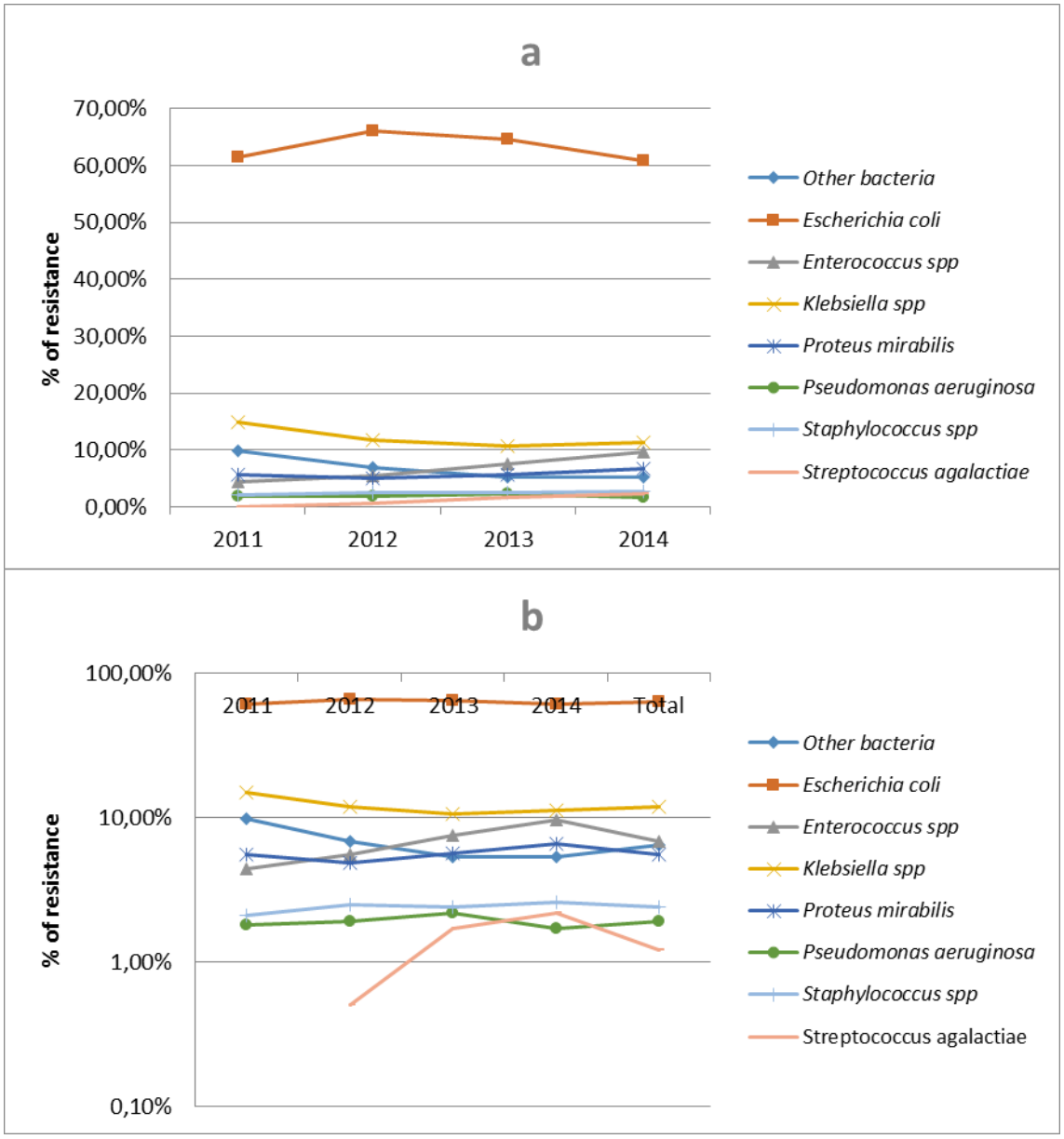

FIGURE 3 Etiology over the study period; a Percentage scale; b Logarithmic scale; d significant statistical differences between the main bacteria throughout the study period (Kruskal-Wallis test, $p < 0.05$).

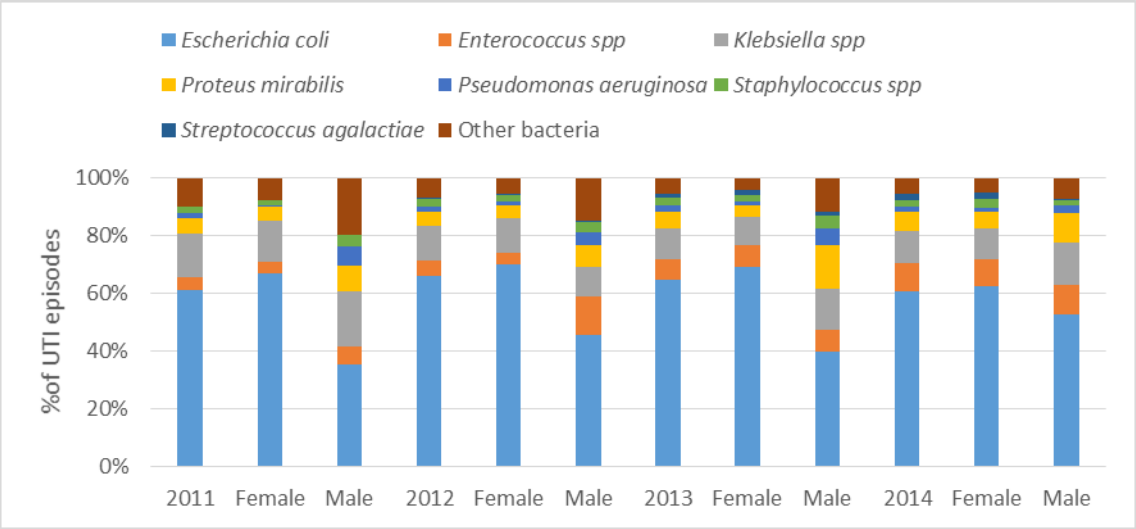

FIGURE 4 Etiology over the study period considering the genders; d etiology significantly statistically differences between genders into the years 2011 and 2013 (Mann-Whitney U test, $p< 0.05$).

4.3. ANTIMICROBIAL RESISTANCE PATTERN OF THE MAIN BACTERIA IMPLIED IN UTI

Gram-negative bacteria, on average, presented high resistance to penicillins, 1st generation cephalosporins, 2nd generation cephalosporins, fosfomycin, nitrofurantoin and TMP-SMX (Table 2). *E. coli* has shown high resistance to ampicillin (47.9%) and TMP-SMX (25.1%) for both genders. *Klebsiella* spp presented high resistance to ampicillin (98.6%), 1st (22.9-23.1%) and 2nd generation (25.3%) cephalosporins, ciprofloxacin (25.3%), fosfomycin (51.5%) and nitrofurantoin (28.9%). *P. mirabilis* presented high rates of resistance to nitrofurantoin (97.4%), TMP-SMX (39.3%), ampicillin (48.1%), fosfomycin (34.9%), ciprofloxacin (30.4%). *P. aeruginosa* presented high rates of resistance to TMP-SMX (95.8%), ampicillin (95.6%), AMX-CA (93.3%), fosfomycin (81.0%), nitrofurantoin (95.7%), cefalotin (93.3%), cefixime (91.3%), cefotaxime (88.9%) (Table 2).

Gram-positive bacteria, on average, presented high resistance to cephalosporins 2nd generation, carbapenems, ciprofloxacin, eritromicin, tetracycline and clindamycin. *Enterococcus sp* presented the highest rates of resistance to TMP-

SMX (94.8%), cefaclor (94.3%), cefuroxime (94.4%), clindamycin (92.7%) and tetracycline (80.9%). *Staphylococcus* spp showed the highest resistance to cefotaxime (100%), cefuroxime (85.7%), imipenem (83.9%), cefaclor (82.5%) and fosfomycin (80.0%). *S. agalactiae* showed high resistance to tetracycline (87.8%), ciprofloxacin (100%), clindamycin (51.1%) and eritromicin (46%) (Table 2).

The other bacteria group, on average, showed the highest resistances to tetracycline (100%), vancomycin (100%), linezolid (100%), ampicillins (87.5%), cefalotin (72.7%) and AMX-CA (60.7%) (Table 2).

The average resistance presented by bacteria implicated in UTI was statistically different (Mann-Whitney U test, $p< 0.05$) between genders. *E. coli*, *Klebsiella* spp, *P. mirabilis* and *Staphylococcus* spp, were the bacteria responsible for the major difference between genders. No differences in the resistance patterns between women and men were observed for *S. agalactiae* and the other bacteria group (Table 2).

Considering all the uropathogens isolated, on average, they were resistant to 3 antimicrobials and to 2 antimicrobial of different classes of drug. Considering the isolates from both genders: the isolates from women were resistant on average to 2 antimicrobials and to 2 antimicrobial classes of drugs; the isolates from men were resistant, on average, to 5 antimicrobials and to 4 antimicrobial classes of drug.

The bacterial resistance changed significantly over the study period (Kruskal-Wallis test, $p < 0.05$) (Figure 5). The bacterial resistance to penicillins, 2nd and 3rd generation cephalosporins, lincosamides, fosfomycins, carbapenems and macrolides decreased. However, for *S. agalactiae* was observed an increase in resistance to the antibiotics tetracyclines, glycopeptides, lincosamides and macrolides over the study period. The pattern of resistance of *P. aeruginosa* was different from that of the other gram-negative (*E.coli*, *P. mirabilis*, *Klebsiella spp*) from 2011-2013 for 1st to 3rd

generation cephalosporins, fosfomycins and sulfamides. The resistance of *E coli* was constant throughout the study period to each antimicrobials tested (Figure 5).

The pattern of bacterial resistance to antibiotics was significantly different among age groups (Kruskal-Wallis test, $p<0.05$). The pattern of resistance of *P. aeruginosa* was different from that of the other gram-negative (*E. coli*, *P. mirabilis*, *Klebsiella* spp), namely in the group of young adults, for penicillins, 1st to 3rd generation cephalosporins, fosfomycins, carbapenems, quinolones, nitrofurans and sulfamides (Figure 6).

Since 63.8% of the infections were caused by *E. coli* and 36.2% by the remaining bacteria, resistance were achieved through the weighted average. The weighted average was calculated through arithmetic average, but having in to account the incidence of each bacterium. The values of antimicrobial resistance of each bacterium were multiplied by its incidence.

Considering the first line treatment recommended by EAU, the weighted average for ciprofloxacin was > 20%, exceeding the recommended value. However, considering only the women, the resistance was < 20%. For fosfomycin the resistance was > 20% for isolates of both genders. Contrarily, the resistance to nitrofurantoin was < 20% for isolates of both genders.

Considering the second line treatment recommended by EAU, the weighted average resistance to ampicillin for both sexes was > 20%, higher than the value recommended value by EAU. The weighted average resistance to TMP-SMX and AMX-CA was > 20% considering all the isolates but it was < 20% when only the women were considered. The resistance to imipenem and gentamicin was < 20% for both genders, below the recommended value of the EAU (Table 3).

TABLE 2 Arithmetic averaged of antimicrobial resistance in the main bacteria for women and men; TMP-SMX, trimethoprim-sulfamethoxazole; AMX-CA, amoxicillin-clavulanic acid; n total number of resistant bacteria; F – Female; M- Male; ● Higher than the recommendable value (>20%).

			E. coli (%)			*Klebsiella spp* (%)			P. mirabilis (%)			*P. aeruginosa* (%)		
Class	Antimicrobial	N = 4270	n = 2723	F = 2415	M =308	n = 502	F = 403	M = 99	n = 240	F = 164	M = 76	n = 83	F = 47	M = 36
Penicillins	Ampicillin	3028	47,9%	45,8%	66,5%	98,6%	98,3%	100,0%	48,1%	46,8%	51,0%	95,6%	95,5%	95,7%
	AMX-CA	3778	11,4%	10,3%	19,8%	18,1%	13,3%	37,4%	17,3%	14,9%	22,4%	93,3%	95,5%	91,3%
Cephalosporins 1st G	Cefalotin	3757	16,9%	15,3%	29,7%	26,5%	20,0%	52,5%	23,9%	19,5%	19,5%	93,3%	95,5%	91,3%
Cephalosporins 2nd G	Cefaclor	4080	10,9%	9,3%	23,8%	22,9%	16,7%	47,5%	19,6%	16,9%	25,3%	95,6%	95,5%	95,7%
	Cefuroxime	4098	10,9%	9,3%	23,8%	23,1%	17,0%	47,5%	21,3%	19,0%	26,3%	95,7%	95,7%	95,7%
	Cefoxitin	3766	4,7%	3,9%	11,7%	11,3%	8,6%	22,2%	10,7%	9,4%	13,3%	93,3%	95,5%	91,3%
Cephalosporins 3rd G	Cefotaxime	3766	4,0%	3,4%	8,9%	12,1%	9,6%	22,2%	5,5%	4,3%	8,0%	88,9%	95,5%	82,6%
	Cefixime	3776	4,3%	3,7%	8,5%	11,8%	9,0%	23,2%	6,8%	5,6%	9,2%	91,3%	95,7%	87,0%
	Ceftazidime	2288	2,8%	2,2%	7,5%	4,6%	2,8%	13,0%	7,1%	4,6%	12,8%	19,4%	12,1%	27,6%
Carbapenems	Ertapenem	3728	,5%	,3%	2,0%	2,0%	2,0%	2,0%	3,4%	3,1%	4,0%	60,0%	75,0%	42,9%
	Imipenem	2476	,6%	,4%	2,3%	,3%	,4%		40,9%	39,1%	45,0%	2,0%		4,2%
Quinolones	Ciprofloxacin	3827	18,7%	16,0%	39,6%	25,3%	19,7%	48,5%	30,4%	24,1%	44,0%	29,6%	26,1%	34,3%
	Levofloxacin	435												
Aminoglycosides	Gentamicin	3823	7,0%	6,6%	9,7%	15,2%	13,3%	23,2%	14,8%	14,4%	15,8%	8,5%	10,9%	5,6%
	Tobramycin	2271	7,1%	7,0%	8,1%	15,2%	14,1%	20,8%	12,6%	12,6%	12,5%	5,6%	7,4%	3,7%
	Amikacin	93				4,3%		10,0%				12,0%	12,5%	11,1%
Macrolides	Eritromicin	1433	,9%	,5%	3,9%	3,0%	2,9%	3,3%	4,2%	4,2%	4,2%			
Tetracyclines	Tetracycline	432												
Glycopeptides	Vancomycin	422												
Lincosamides	Clindamycin	350												
Fosfomycins	Fosfomycin	3797	4,7%	3,7%	12,4%	51,5%	52,3%	48,5%	34,9%	30,9%	43,4%	81,0%	87,9%	73,3%
Nitrofurans	Nitrofurantoin	4202	5,0%	4,1%	12,7%	28,9%	28,1%	32,3%	97,9%	97,5%	98,7%	95,7%	95,8%	95,7%
Sulfamides	TMP-SMX	4249	25,1%	,1%	36,4%	24,0%	18,4%	46,5%	39,3%	38,7%	40,8%	95,8%	97,4%	93,9%
Oxazolidinones	Linezolid	422												

			Enterococcus sp (%)			*Staphylococcus spp* (%)			*S. agalactiae* (%)			Other bacteria (%)		
Class	Antimicrobial	N = 4270	n = 290	F =222	M = 68	n = 103	F = 79	M = 24	n =50	F = 45	M = 5	n = 279	F = 186	M = 93
Penicillins	Ampicillin	3028	38,6%	35,2%	48,1%	28,6%	33,3%	20,0%	0,0%			87,8%	87,9%	87,5%
	AMX-CA	3778	33,3%		100,0%	0,0%			0,0%			60,7%	61,5%	59,1%
Cephalosporins 1st G	Cefalotin	3757	66,7%	50,0%	100,0%	0,0%			0,0%			72,7%	74,6%	69,0%
Cephalosporins 2nd G	Cefaclor	4080	94,3%	93,7%	96,2%	82,5%	81,4%	85,7%	6,5%	7,4%		49,2%	44,7%	58,6%
	Cefuroxime	4098	94,4%	93,7%	96,4%	85,7%	83,9%	90,9%	6,3%	7,1%		48,3%	44,1%	56,8%
	Cefoxitin	3766	33,3%		100,0%	0,0%			0,0%			41,7%	43,5%	37,9%
Cephalosporins 3rd G	Cefotaxime	3766	33,3%		100,0%	100,0%	100,0%		0,0%			9,0%	7,2%	12,6%
	Cefixime	3776	50,0%	50,0%	50,0%	60,0%	75,0%		0,0%			8,7%	7,3%	11,5%
	Ceftazidime	2288										12,3%	9,2%	18,6%
Carbapenems	Ertapenem	3728	25,0%		100,0%	100,0%	100,0%		0,0%			5,0%	2,9%	9,3%
	Imipenem	2476	34,8%	33,7%	37,5%	83,9%	85,4%	80,0%	0,0%			2,9%	,9%	7,0%
Quinolones	Ciprofloxacin	3827	25,0%	33,3%		0,0%			100,0%		100,0%	18,1%	14,1%	26,4%
	Levofloxacin	435	18,0%	16,1%	23,9%	19,8%	10,4%	50,0%	2,1%	2,3%				
Aminoglycosides	Gentamicin	3823	16,7%	20,0%	0,0%							17,6%	16,6%	19,8%
	Tobramycin	2271										15,3%	15,3%	15,5%
	Amikacin	93										6,3%		11,1%
Macrolides	Eritromicin	1433	54,1%	52,3%	59,7%	53,1%	51,4%	58,3%	46,0%	44,4%	60,0%	7,7%		25,0%
Tetracyclines	Tetracycline	432	80,9%	78,7%	88,1%	38,1%	32,9%	54,2%	87,8%	88,9%	75,0%	100,0%		100,0%
Glycopeptides	Vancomycin	422	25,7%	24,3%	30,3%	20,8%	16,7%	33,3%	9,1%	10,0%		100,0%		100,0%
Lincosamides	Clindamycin	350	92,7%	92,1%	94,3%	57,0%	55,1%	62,5%	51,1%	46,5%	100,0%			
Fosfomycins	Fosfomycin	3797	100,0%	100,0%	100,0%	80,0%	75,0%	100,0%				48,9%	45,6%	55,7%
Nitrofurans	Nitrofurantoin	4202	8,7%	8,2%	10,3%	15,4%	10,1%	31,8%	4,2%	4,7%		46,7%	38,5%	63,6%
Sulfamides	TMP-SMX	4249	94,8%	95,5%	92,6%	3,9%	2,5%	8,3%	4,0%	2,2%	20,0%	21,7%	18,4%	28,3%
Oxazolidinones	Linezolid	422	18,8%	17,2%	23,9%	7,7%	7,1%	9,5%	8,5%	9,3%		100,0%		100,0%

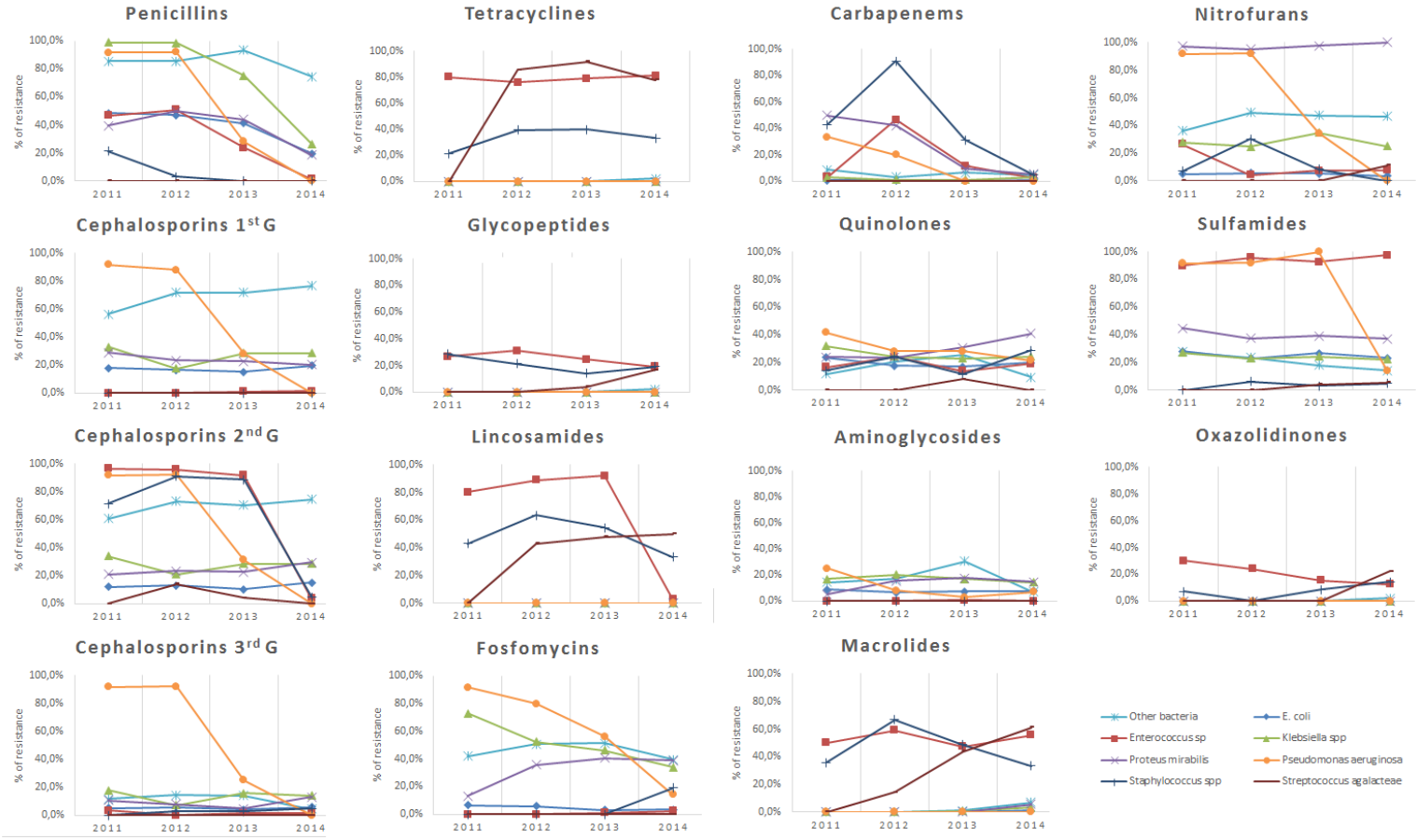

FIGURE 5 Variation of antimicrobial resistance pattern of bacteria during the study period (2011-2014).

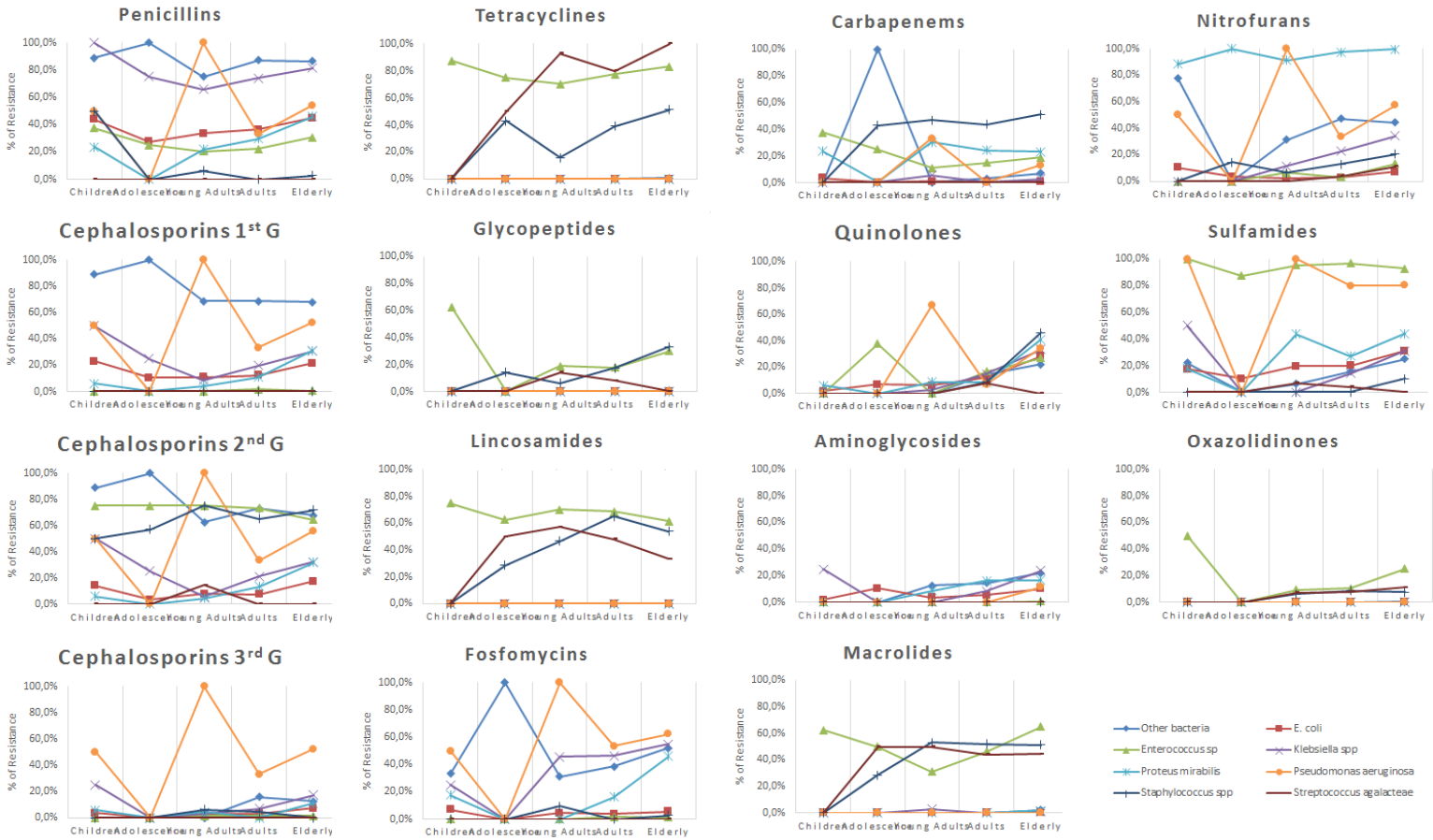

FIGURE 6 Variation of antimicrobial resistance pattern of bacteria over the age groups (children - adolescence - young adults - adults - elderly).

TABLE 3 Weighted average of bacterial resistance considering the weight of the total incidence of each uropathogens for women and men ; - Not considered, natural resistance; T- Total average resistance; F – Female's average resistance; M- Male's average resistance; CIPRO – ciprofloxacin; FOS - fosfomycin; NITRO - Nitrofurantoin; AMP – ampicillin ; TMP-SMX – trimethoprim-sulfamethoxazole; AMX-CA – amoxicillin-clavulanic acid; FOS – fosfomycin; IMP - imipenem; GEN – gentamicin;● Higher than the recommendable value (>20%).

		CIPRO			FOS			NITRO			AMP			TMP-SMX			AMX-CA			IMP			GEN		
		T	F	M	T	F	M	T	F	M	T	F	M	T	F	M	T	F	M	T	F	M	T	F	M
E. coli	64	19	16	40	5	4	12	5	4	13	48	46	67	25	0	36	11	10	20	1	0	2	7	7	10
Klebsiella spp	12%	25	20	49	52	52	52	29	28	32	99	98	100	24	18	47	18	13	37	0	0	0	15	13	23
P. mirabilis	6%	30	24	44	35	31	43	-	-	-	48	47	51	39	15	22	17	15	22	41	39	45	15	14	16
P. aeruginosa	2%	30	26	34	81	88	73	-	-	-	96	96	96	96	96	91	93	45	58	2	0	4	9	11	6
Enterococcus sp	7%	25	33	0	100	100	100	9	9	8	39	35	48	95	96	93	33	13	37	35	34	34	17	20	0
Staphylococcus spp	1%	0	0	0	80	75	100	15	10	32	29	33	20	4	3	8	0	0	0	84	85	80	0	0	0
S. agalactiae	0%	100	0	100	0	100	100	4	5	0	0	0	0	4	2	20	0	0	0	0	0	0	0	0	0
Other bacteria	7%	18	14	26	49	46	56	47	39	64	88	88	88	22	18	28	61	62	59	3	1	7	18	17	20
Weighted Average		20,6	18	36,7	24,1	23,1	30,3	10,7	9,4	17,4	56,7	55,1	69,8	31,5	12,9	40,9	18,8	15,1	25,2	9,1	6	8	9,8	9,5	11,5

4.4. MULTIDRUG RESISTANT BACTERIA IMPLIED IN UTI

Multidrug resistance to three or more antimicrobial classes was considered and defined as MDR. The total percentage of MDR isolates were 1536 (36%), including 1099 (30.9%) women and 437 (61.6%) men. The percentage of MDR isolates varied from 22.4% to 85.2% in all MDR bacteria (Figure 7). The most frequent MDR isolates were *Enterococcus* spp (85.2%), *Staphylococcus* spp (62.1%), *P. mirabilis* (58.3%) and *P. aeruginosa* (57.8%). The less frequent MDR isolates were *E. coli* (22.4%), Klebsiella spp (39.8%) and *S. agalactiae* (44.0%). For the other bacteria group73.1% of isolates were MDR (Figure 7).

The percentage of MDR isolates implicated in UTI were statistically different between women and men, considering: *E. coli*, *Klebsiella spp*, *P. mirabilis* and *Staphylococcus spp* (Mann-Whitney U test, $p< 0.05$). The MDR isolates frequency in women was: *E. coli* (19.7%), *Klebsiella sp* (32.5%), *P. mirabilis* (52.4%) and *Staphylococcus spp* (55.7%). The MDR isolates frequency in men was: *E. coli* (43.8%), *Klebsiella sp* (69.7%), *P. mirabilis* (77.1%) and *Staphylococcus spp* (83.3%)(Figure 7).

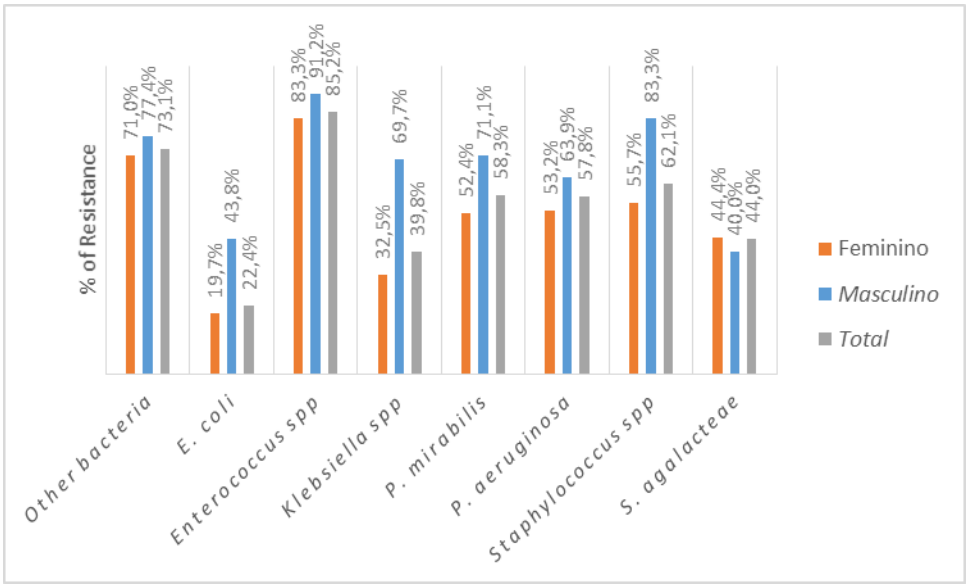

FIGURE 7 Variation of MDR isolates by age groups; d statistical significant differences between genders (Mann–Whitney U test, p <0.05);

The MDR isolates were resistant, on average, to 6 antimicrobials and to 5 antimicrobial classes of antibiotics. There were also statistical significant differences (Mann–Whitney U test, ρ <0.05) between genders. For women, the MDR uropathogens were resistant on average to 6 antimicrobials and to 5 antimicrobial classes of antibiotics. For men, the MDR uropathogens were resistant on average to 7 antimicrobials and to 5 antimicrobial classes of antibiotics.

The prevalence of MDR isolates varied significantly among the age groups (Kruskal-Wallis test, p < 0.05). The highest percentages of resistance were observed in the elderly (45.2%) and children (31.7%) (Figure 8). The lowest percentages were observed in adults (24.9%) and young adults (27.1%) (Figure 8). Significant gender differences were also observed in children, adults and elderly (Mann–Whitney U test, p <0.05) (Figure 8).

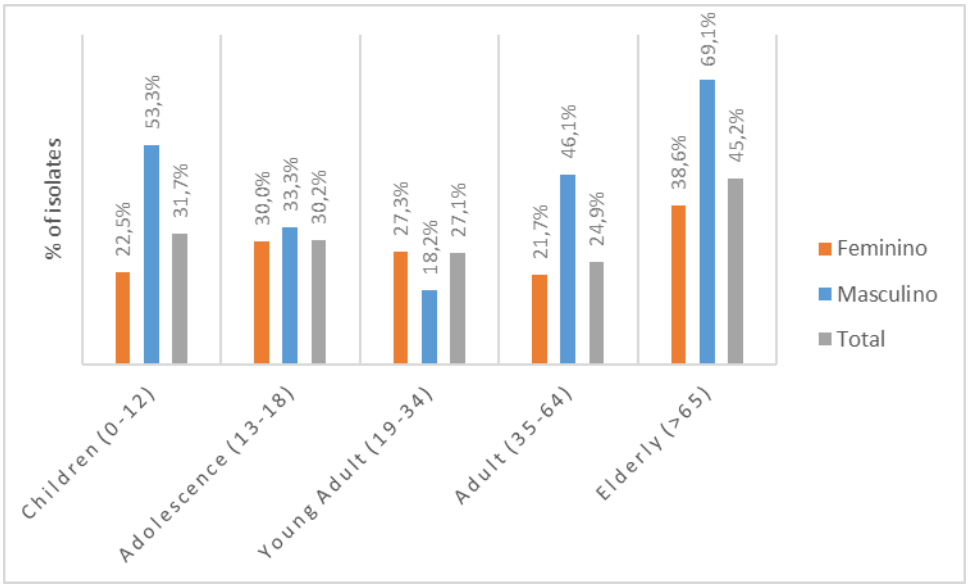

FIGURE 8 Variation of MDR isolates by age groups; d statistical significant differences between genders (Mann–Whitney U test, p <0.05); d statistical significant differences over the age groups (Kruskal-Wallis test, p < 0.05)

The percentage of MDR isolates presented a significant decrease throughout the study period (10.4%) (Kruskal-Wallis test, p < 0.05). The highest percentage was observed in 2011 (40.8%) and the lowest percentage was observed in 2014 (30.4%) (Figure 9). During the study period there were also significant gender differences from 2011 to 2014 (Mann–Whitney U test, ρ <0.05) (Figure 9).

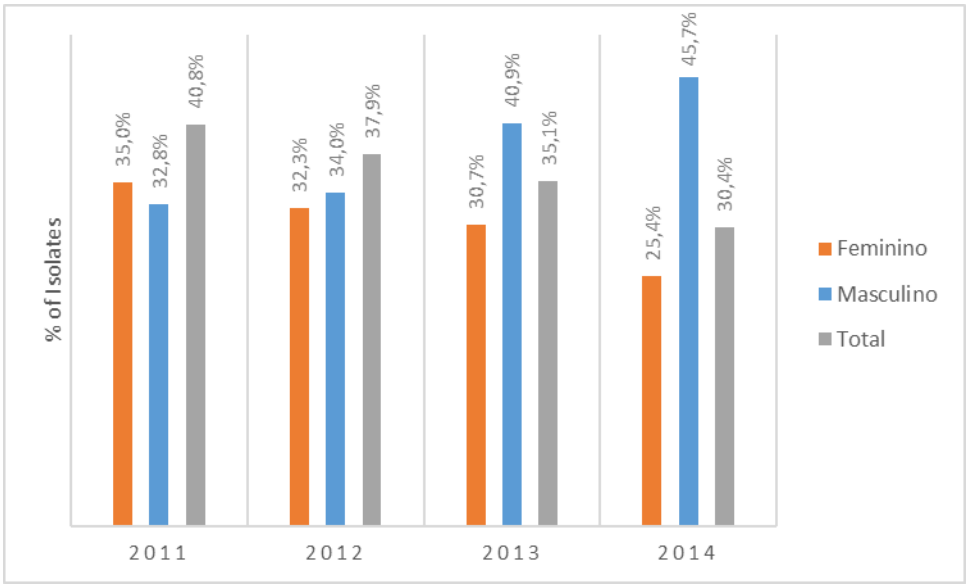

FIGURE 9 Variation of MDR isolates over the study period; d statistical significant differences between genders (Mann–Whitney U test, $p < 0.05$); d statistical significant differences throughout the study period (Kruskal-Wallis test, $p < 0.05$).

The highest rates of MDR were observed to ampicillins (83.4%), cefalotin (65.5%), tetracycline (77.9%), clindamycin (92.4%) and TMP-SMX (61.4%) for both genders. There were statistical significant differences on MDR isolates between genders, with exception of amikacin, tetracycline and linezolid (Figure 10).

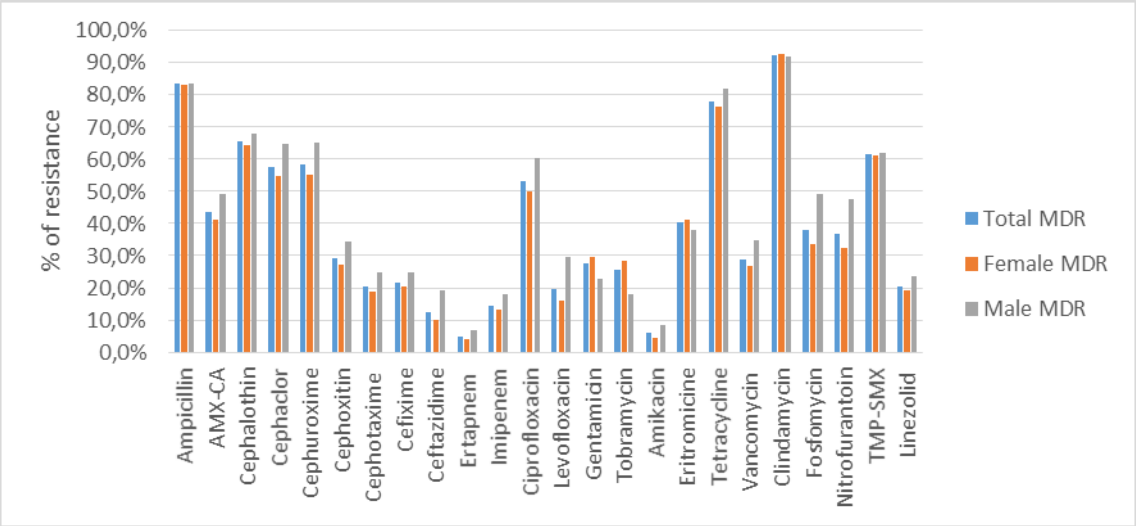

FIGURE 10 Distribution of MDR isolates resistance to the antimicrobials tested - total number of bacteria multidrug resistant; Total MDR, N=1536; Female MDR, n=1099; Male MDR, n=437; d statistical significant different between genders (Mann–Whitney U test, $p<0.05$).

5. DISCUSSION

As observed in other studies, *E. coli* was the most common uropathogen implicated in the UTI covering the population of Aveiro North. *E. coli* bacterium was responsible for more than an half of the UTI (64%). Moreover, *Klebsiella spp, P. mirabilis* and *Enterococcus spp* were also frequently involved in UTI (6 – 12% respectively), similar to the observed in other studies performed at community level worldwide (Francesco, et al., 2007; Mendo, et al., 2008; Costa, et al., 2009; Narciso, et al., 2012; Chatterjee, et al., 2014; Miranda, et al., 2014; Moayednia, et al., 2014). The high frequency of these bacteria can be an indicative of self-colonisation by gastrointestinal pathogens, since these bacteria are very common in gastrointestinal flora and are reported as the main responsible for R-UTI (Al-Badr, et al., 2013). *Enterobacteriaceae* have several factors responsible for their attachment to

urothelium, which transform them into the main bacteria responsible for UTI (Dash, et al., 2013). Being the three most reported UTI main bacteria (81.2%) in the community studies worldwide, would be significant for the empiric treatment success an action spectrum for them (Costa, et al., 2009).

As stated before UTI is more frequent in women than in men (67.8% and 43.4%, respectively), and the highest difference between women and men patients was observed for the *P. mirabilis* (incidence 10.7%, 2.3 times higher in men) and *P. aeruginosa* (incidence 5.1%, 3.9 times higher in men). *E. coli* bacterium was the most predominant in both genders, however its incidence was significantly higher in women (67.8%) than in men (43.4%) (Mann-Whitney U test, $p<0.05$), which was also observed in other similar studies (Linhares, et al., 2013; Chatterjee, et al., 2014). According to the literature, *P. aeruginosa* is more frequent in men due to characteristics inherent to the patient, including sex, age, previous use of antimicrobials, previous interventions in the urinary tract and patients with neurogenic bladder (Grabe, et al., 2008; Koeijers, et al., 2010; Rodrigues, et al., 2011; Linhares, et al., 2013).

The higher incidence of *P. mirabilis* in men than in women was consistent with published literature (Orrett, et al., 2001). *P. mirabilis* due to biofilms production capacity is a serious medical problem in catheter-associated UTI, which is consistent with its higher rates in elderly (Czerwonka, et al., 2014). As reported by Nicolle (2014). The elderly age group, especially in men (incidence in men 16% and women 3%), were the most frequent catheter-associated UTI patients (Nicolle, 2014). Moreover, *Pseudomonas* and *Proteus* spp have been found with high frequency in complicated UTI, which may justify their high incidence in men, as well as their higher rates in children and elderly age groups (Grabe, et al., 2008).

The average age of men with UTI was significantly higher than women (averaged age 68 and 59 years, respectively), which can also explain the aetiological differences among genders (Mann–Whitney U test, ρ-value <0.05). The higher

incidence of UTI in older men can be due to benign prostate hyperplasia (BPH) and neurogenic bladder more common at this age (Koeijers, et al., 2010; Dash, et al., 2013). Moreover, the higher incidence of UTI caused by *P. aeruginosa* bacterium is frequently associated with the hospital environment, in men seems to be related to the increased hospitalizations due to their higher age (Koeijers, et al., 2010; Nicolle, 2014). Despite hospital-acquired urinary infection caused by *P. aeruginosa* in community is still uncommon (1.9% in this study). However, it should not be ignored, since other community-acquired UTI studies have reported its increasing incidence (Koeijers, et al., 2010; Rodrigues, et al., 2011; Linhares, et al., 2013).

The distribution into age groups was not random (Chi-square test, $p<0.05$), the incidence of UTI increased with age in both genders. Being the elderly the most affected (52%), followed by adults (32%), young adults (12%), children (2%) and adolescents (1%). In the literature, it is well documented that the incidence of community-acquired UTI increases with age, affecting both genders in the elderly population (Cove-Smith, et al., 2007; Linhares, et al., 2013; Chatterjee, et al., 2014). The increase in young adult and adult women is well established with the sexual activity, the use of contraceptives (spermicides, diaphragm and oral contraceptives), the use of antimicrobials and pregnancy (Narciso, et al., 2012). In adult and elder women, the increase of UTI has been explained by menopause. After menopause the estrogen levels and *Lactobacilli* number drop and makes post-menopausal women susceptible to UTI (Al-Badr, et al., 2013). Also to elder women the frequent use of antimicrobials, the increase of surgical urogenital interventions, urinary incontinence, post void residual (PVR), more frequent hospitalization and other pathologies associations explain the (Molander, et al., 2000; Rowe, et al., 2013; Rowe, et al., 2014). In adult and elder men, the increase of urinary infections has been mainly related to benign prostate enlargement causing voiding obstruction which leads to PVR, the use of antimicrobials, more frequent hospitalization, urogenital interventions, neurogenic

bladder and other pathologies (Narciso, et al., 2012; Dash, et al., 2013; Rowe, et al., 2014).

According to the literature (Weir, et al., 2000; Nwokocha, et al., 2014), there is an increase of urinary tract infections from children to adolescence, related to the onset of sexual activity, which was not observed in this study. The number of urine samples from adolescents was much lower compared to those performed by children, which may explain the higher frequency of urinary infections in this age group compared to the adolescent group. Despite the low incidence, it was observed the highest prevalence of gram positive infection in this age group. According to Blumer (2009), older children and adolescents also share many features commonly seen in adult patients with infections at the same sites, however gram-positive organisms an opportunistic agent are the most common cause of bacterial infections in infants, children and adolescents, taking advantage of their vulnerability (Blumer, 2009). Following the Blumer (2009) study, the most responsible gram negative uropathogen in cystitis is the *Staphylococci* coagulase-negative, which may justify the increase of *Staphylococcus* spp in the adolescent group in this study.

The incidence of *E. coli*, responsible for more than an half of the UTI isolates, was constant during the study period (variation 60.8-66.0%) but *Klebsiella* spp has shown a 3% decrease and, contrarily, *Enterococcus* spp registered a 5.3% increase. Francesco et al (2007) also registered a slight increase for *Enterococcus* (0.7%) in his 4 years study (2002-2005) among the community outpatients. In the study of Kuzdan (2014) was also observed a decrease and the increase, respectively, for the species *Klebsiella pneumonia* and *Enterococcus faecium* (Kuzdan, et al., 2014). For *E. coli* and the remaining bacteria were almost constant throughout the study period.

The more predominant uropathogen, *E. coli,* presented the lowest antimicrobial average resistance comparing with the remaining bacteria implicated in UTI. The resistance to the first line treatment recommended by EAU for uncomplicated

UTI was tested for *E. coli* and revealed low resistance to the three antimicrobials recommended (5.0% nitrofurantoin, 18.7% ciprofloxacin and 4.7% fosfomycin). To the second line treatment recommended by EUA, *E. coli* isolates exceeding the recommendable value (<20%) for two of the second line treatment, ampicillin and TMP-SMX (31.5% and 25.1% respectively), contrary to AMX-CA (11.4%). *E. coli* high resistance to ampicillin and moderate resistance to AMX-CA and SMX have been also reported in other studies (Francesco, Ravizzola, Peroni, Negrini, & Manca, 2007).

Having into account the high incidence of *E. coli* (63.8%) and the drug resistance pattern of the most implicated bacteria in UTI, it was calculated the weighted average of resistance. Among the first line choice, that cause little collateral damage, only the nitrofurantoin is suitable to be used in the empiric treatment of UTI in the community (weighted average resistance of 10.7%, 20.6% and 24.1%, respectively, for nitrofurantoin, ciprofloxacin and fosfomycin). The weighted average of nitrofurantoin resistance was not determined for *P. mirabilis* and *P. aeruginosa* because they are naturally resistant to this antimicrobial. Considering the second line therapy recommended by EAU, the ciprofloxacin and TMP-SMX are not appropriate to treat UTI in the community but the antimicrobial AMX-CA is appropriated to be used in UTI empiric treatment (weighted average resistance of 56.7%, 31.5% and 18.8%, respectively for ampicillin, TMP-SMX and AMX-CA).

The antimicrobial resistance also suggest that the choice of empirical antimicrobial therapy should have into account the sex of the patient. The uropathogens isolated from men presented, on average, resistance to a higher number of antibiotics (on average 5) compared to those isolated from women (on average 4). This difference was clearly evident in the average resistance of *E. coli* and *Klebsiella* spp, which presented significant differences for at least 12 of the 18 antimicrobials tested (corresponding to 66.7% and 72.2% of the antibiotics, respectively). For the remaining bacteria the difference between women and men was also evident, namely

for the antibiotics ciprofloxacin, AMX-CA and TMP-SMX. Consequently, the antimicrobials AMX-CA and TMP-SMX should be suitable to treat women (resistance <20% for the bacteria more implicated in UTI), but should not be used to treat men (resistance >20% for all of the bacteria more implicated in UTI, except *E. coli*). Two recent studies also found differences in antimicrobial resistance among genders. In a recent Portuguese study the fluoroquinolones were found unsuitable to treat men, and also in an Indian study *E.coli* isolates showed resistance to the majority of antibiotics in men (Linhares, et al., 2013; Chatterjee, et al., 2014).

The resistance of the main bacteria among age groups has presented significant differences to the antimicrobial classes tested on young adults and elderly (Kruskal-Wallis test, $p<0.05$). For these two age groups, significant statistical differences were observed in all antimicrobials classes. As it was observed before in other studies, the resistance tendency significantly increases with age (Cove-Smith, et al., 2007; Linhares, et al., 2013). The higher rates of resistance in elder patients can be due to the more frequent hospitalization of this age group, as a result of the increase of average life expectancy, weak immune system and recurring infections. It is recognized that hospitalizations increase the transmission of bacterial strains between hospital and community (Ott, et al., 2013).

In general, the resistance by the main bacteria implicated in UTI to penicillins, 2nd and 3rd generation cephalosporins, lincosamides, fosfomycins, carbapenems and macrolides decreased during the study period. However, for glycopeptides, quinolones, aminoglycosides, nitrofurans, sulfamides and oxazolidinones the resistance was almost constant throughout the study period to the main uropathogens. Nevertheless, *S. agalactiae* presented a resistance increase over the study period to the tetracyclines, glycopeptides, lincosamides and macrolides. *P. aeruginosa* presented a different pattern of resistance relatively to the antimicrobials 1st to 3rd generation cephalosporins and sulfamides, comparing to the other gram-

negative (*E .coli*, *P. mirabilis*, *Klebsiella spp*). *E coli* resistance was constant throughout the study period to all antimicrobials tested. According to Linhares et al (2013), the pattern of resistance to the main bacteria also showed significant changes during a ten years period, but *E. coli* did not show significant changes in the resistance to the tested antimicrobials. Similarly, *P. aeruginosa* had also a different behaviour compared to the gram negative bacteria (*E.coli*, *P. mirabilis*, *P. vulgaris*, *Klebsiella* spp, *Enterobacter* spp and *Providencia* spp) throughout the study period, specially observed in the classes of cephalosporins and quinolones (Linhares et al., 2013).

The resistance to first and second line therapy it is probably the reflection of their misuse and/or overuse (Bartoloni, et al., 2004). Although most organisms remain susceptible to nitrofurantoin, Pallett (2010) defends that this agent is licensed for lower UTI only and it has shown that resistance may develop during treatment (Pallett, et al., 2010). The oral options available for the treatment of UTI with simultaneous resistance to trimethoprim and quinolones are small. As a suitable alternative to first and second line recommended therapy, this study recommends imipenem and gentamicin to empiric treatment, due to the low bacterial resistance to these drugs in both genders, and also considering the low percentage of bacterial resistance to both antimicrobials in the MDR isolates. However, gentamicin is contraindicated in patients with significant renal impairment, which is more common in the elderly. It is also required the regular monitoring of pre-dose serum concentrations to assess further dosing (Pallett, et al., 2010). Imipenem can be used in empiric treatment of complicated UTI in view its broad spectrum of action and wide diffusion in the body (intravenous) (Pallett, et al., 2010). These drugs involve the inconvenience of parenteral administration and it can be more expensive than those indicated for UTI.

The multidrug resistant isolates were observed among the most prevalent bacteria involved in the community-acquired UTI, being a 36% of these bacteria resistant to three or more antimicrobials of distinct classes. However, the most

implicated bacteria in UTI, *E. coli*, responsible for more than half of the UTI of the studied patients, presented the lowest MDR bacterial percentage (22.4%). The second bacteria more implicated in the community-acquired UTI, *Klebsiella* spp, presented also a low percentage of MDR isolates (39.8%). Considering the main bacteria more implicated in UTI, the percentage of MDR bacteria in this study was higher than that found in the Antimicrobial Resistance Epidemiological Survey on Cystitis (ARESC) study, which 10.3% of bacteria isolated at community level in nine European countries and in Brazil were MDR (Heijer, et al., 2010). However, it was very similar to that obtained in a similar study performed at the community level in which 36.7% of isolates responsible for UTI were MDR (Heijer, et al., 2010; Sheerin, 2011). Keeping in view the more implicated bacterium in UTI, *E. coli*, higher percentages of MDR isolates have been detected in other studies (35.9-72.7%) done at the community level (Sulikowska, et al., 2001; Narciso, et al., 2011; Sheerin, 2011). In this study, the most predominant gram negative bacteria and *S. agalactiae* presented lower percentages of MDR isolates (between 22.4% and 44%). The highest percentages were observed among the other gram positive bacteria (Enterococcus spp, *P. aeruginosa* and *Staphylococcus* spp) and the other bacteria group (between 62.1% and 85.2%).

The MDR bacteria isolated from men were, on average, resistant to a higher number of antimicrobial classes than those isolated from women and the higher percentage of MDR was also observed in men (61.6%). Most of the UTI in men are complicated (associated with structural or functional abnormality in urinary tract), requiring prolonged antimicrobial therapy and antibiotics able to reach high therapeutic concentrations in the prostatic tissues (Hooton, 2000; Kuroda, et al., 2005; Magiorakos, et al., 2011). For these reasons, men are more likely to develop resistance to other antibiotics, usually used with success in the treatment of UTI in women (Sheerin, 2011). Corroborating this hypothesis, Baral and colleagues showed that the percentage of MDR was higher when bacteria were isolated from men (58.7%) (Baral, et al., 2012).

Nearly 45.2% of the bacteria isolated from elderly patients were MDR. This may be explained by the increase of number and duration of hospital admissions with the increasing age of the patient. During hospitalization some invasive procedures as catheterization and other interventions may be associated with the transmission of resistant hospital bacteria to the elderly patients. The immune system fragility of the elderly patients may also explain the increase of urinary infection incidence and also of UTI caused by MDR bacteria (Kisich, et al., 2008). In this study, patients with community and hospital-acquired UTI were included which can validate the MDR high percentages in isolates from men.

The incidence of MDR bacteria decreased about 10.4% throughout the study period which may be related with the decrease of the antimicrobial resistance of some MDR bacteria during the study period, such as, *Enterococcus* spp, *Staphylococcus* spp and other bacteria group that showed high averaged percentage of MDR, 85.2% and 62.1%, respectively.

6. CONCLUSIONS

The most prevalent bacteria responsible for UTI were gram negative *Enterobacteriaceae* (*E.coli*, *Klebsiella* spp and *P. mirabilis*) and *Enterococcus* spp. Being *E.coli*, *Klebsiella* spp and *P. mirabilis* the three most common bacteria responsible for UTI in the community studies worldwide, would be significant for the empiric treatment success an action spectrum for them.

The results of this study emphasized the relevance to consider sex and age as differentiator factors in the choice of UTI empirical treatment, due to differences in aetiology and in antimicrobial resistance. The episodes of UTI are undoubtedly higher in women and in the elderly group, increasing the differences in the incidence between women and men with age. In general, men were more resistant to

antimicrobials (on average 5) and to antimicrobial classes resistance (on average 4) than women (2 and 2, respectively).

The results obtained in this study suggest that from first line treatment recommended by the EAU to empirical treatment of uncomplicated UTI only nitrofurantoin is appropriated for both sexes and ciprofloxacin may be only considered to treat women. In the second line antibiotic, ampicillin is not an appropriate drug to empirical treatment of uncomplicated UTI, though the antimicrobials AMX-CA and TMP-SMX should not be used to empirical treatment of men, due to the local high incidence of resistance (> 20%). As alternative to first and second line recommended treatment, imipenem and gentamicin should be used due to their low weighted average percentage of resistance.

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