Universidade de Aveiro
2020

ANDREIA CRISTINA MIGUEL DA SILVA

REVISITING THE FREQUENCY AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA IMPLICATED IN COMMUNITY URINARY TRACT INFECTIONS

REVISITANDO A FREQUÊNCIA E PADRÕES DE RESISTÊNCIA ANTIMICROBIANA DE BACTÉRIAS ASSOCIADAS A INFEÇÕES URINÁRIAS NA COMUNIDADE

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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 Doutora Adelaide Almeida, Professora Catedrática do Departamento de Biologia de Universidade de Aveiro.

Dedico este trabalho aos meus pais, sem eles nada disto seria possível.

o júri

presidente

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palavras-chave

Infeções do trato urinário, infeções adquiridas na comunidade, uropatogénicos, antimicrobianos, resistência antimicrobiana.

resumo

A infeção do trato urinário (ITU) é uma das doenças infeciosas mais comuns tanto a nível comunitário como a nível hospitalar. O contínuo mau uso de antimicrobianos tem como consequência um aumento na resistência bacteriana, sendo este um problema a nível mundial. O objetivo deste trabalho foi estudar a incidência e o padrão de resistência antimicrobiana das principais bactérias responsáveis por infeções do trato urinário na comunidade do centro e norte de Portugal, e estabelecer um tratamento empírico apropriado. As amostras de urina estudadas foram colhidas no Avelab – Laboratório Médico de Análises Clínicas, em regime ambulatório, durante um período de 5 anos (2015-2019). Das 106019 amostras analisadas de pacientes ambulatórios, 15439 tinham infeção urinária. As infeções urinárias foram mais frequentes em mulheres (79,6%) do que em homens (20,4%), e os pacientes mais afetados foram os idosos (56,9%), sendo responsáveis por mais de metade das amostras com infeção. *Escherichia coli* (70,1%) foi o uropatógeno mais frequente, seguido de *Klebsiella pneumoniae* (8,9%), *Proteus mirabilis* (5,5%) e *Enterococcus faecalis* (3,2%). As bactérias responsáveis por UTI variaram com o sexo do paciente, sendo que foram observadas as maiores diferenças para *E. Faecalis* e *P. aeruginosa*, estando estas mais incidentes em homens. De um modo geral, observou-se um aumento da resistência bacteriana à medida que a idade dos pacientes aumentava. Na generalidade, as bactérias de Gram-negativo mostraram ser mais resistentes que as bactérias de Gram-positivo. Apesar de *E. coli* ser o uropatógeno mais responsável por ITU, este encontrou-se entre as bactérias mais suscetíveis. Comparando os nossos resultados com resultados de há 10 anos atrás, registou-se de um modo geral um aumento na resistência para alguns antimicrobianos e bactérias. Baseando-nos nos antibióticos recomendados para tratamento de ITUs não complicadas pela European Association of Urology, os antibióticos de primeira linha (nitrofurantoína e fosfomicina), e os antibióticos alternativos cefalosporinas, nomeadamente a cefotaxima e cefuroxima, podem ser considerados apropriados para o tratamento empírico de infeções urinárias adquiridas na comunidade. O trimetoprim-sulfametoxazol, a amoxicilina/ácido clavulânico e a ciprofloxacina não devem ser prescritos empiricamente para a região estudada.

keywords

Urinary tract infection, community adquired infections, uropathogens, antimicrobials, antimicrobial resistance.

abstract

Urinary tract infections (UTI) are one of the most common infectious diseases at both community and hospital levels. The continue misuse of antimicrobials is leading to an increase in bacterial resistance, which is a worldwide problem. The objective of this work was to study the incidence and pattern of antimicrobial resistance of the main bacteria responsible for urinary tract infections in the community of central and northern Portugal and establish an appropriate empirical treatment. The studied urine samples were collected in Avelab – Laboratório Médico de Análises Clínicas, in outpatients, over a period of 5 years (2015-2019). Of the 106019 samples analysed, 15439 had urinary infection. Urinary infections were more frequent in females (79.6%) than in males (20.4%), and the most affected patients were the elderly (56.9%), being responsible for more than half of the samples with infection. *Escherichia coli* (70,1%) was the most frequent uropathogen, followed by *Klebsiella pneumoniae* (8,9%), *Proteus mirabilis* (5,5%), and *Enterococcus faecalis* (3,2%). The bacteria responsible for UTI varied according to the patient's gender, with the greatest differences being observed for *E. faecalis* and *P. aeruginosa*, these being more prevalent in men. In general, there was an increase in bacterial resistance as the age of patients increased. Generally, Gram-negative bacteria proved to be more resistant than Gram-positive bacteria. Although *E coli* was the most responsible uropathogen for UTI, it was among the most susceptible. Comparing our results with results from 10 years ago, there was generally an increase in resistance for some antimicrobials and bacteria. Based on the antibiotics recommended for the treatment of uncomplicated UTIs by the European Association of Urology, first-line antibiotics (nitrofurantoin and fosfomycin) and alternative cephalosporin antibiotics, namely cefotaxime and cefuroxime, can be considered appropriate for the empirical treatment of community-acquired urinary infections in the area studied. Trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, and ciprofloxacin should not be prescribed empirically for the region studied.

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Abbreviations

AMX-CLA - Amoxicillin-clavulanic acid

AST - Antimicrobial susceptibility test

CEP - Cephalosporins

CFU - Colony forming unit

DHFA - Dihydrofolic acid

EAU - European Association of Urology

EUCAST - European Committee on Antimicrobial Susceptibility Testing

ESBLs - Extended-spectrum β-lactamases

FOM - Fosfomycin

MDR - Multidrug resistance

NIT - Nitrofurantoin

PABA - Para-aminobenzoic acid

PBPs - Penicillin binding proteins

PIP-TAZ - Piperacillin-tazobactam

THFA - Tetrahydrofolic acid

QUI - Quinolones

SPSS - Statistical Package for the Social Sciences

SXT - Trimethoprim-sulfamethoxazole

UTI - Urinary tract infection

WHO - World Health Organization

Chapter 1

1. Introduction

1.1 Urinary tract infections

Urinary tract infections (UTIs) are among the most frequent bacterial diseases, affecting 150 million people worldwide every year (McLellan & Hunstad, 2016). A urinary tract infection is an infection of the urethra, bladder or kidney. Bacteria can get into the urethra and travel to the bladder and kidneys, causing an infection (Gupta et al., 2017). These infections can develop disease in a variety of forms, including chronic, recurrent infection and acute. The main symptoms are recurrent and urgent urination, pain or burning when urinating, hematuria, pain in the lower back or pelvic area, fever, nausea, nocturia and malaise (Gupta et al., 2017; McLellan & Hunstad, 2016). The occurrence of three or more UTIs per year, as well as 2 or more UTIs in less than 6 months is considered as the recurrent UTI, which is the main challenge in treatment of UTI patients (Gupta et al., 2017; Karam et al., 2019). With the large number of existing urinary infections, a high economic impact of its diagnosis and treatment is to be expected, resulting in great cost for health care annually (Flores-Mireles et al., 2015).

1.1.1 Risk factors

UTI is considerably more common in women than in men, due to anatomic and physiological motives. However, in subjects aged 65 or older, both genders have a similar incidence. The vaginal cavity and rectal opening (where potential uropathogens live) are closer to the urethral opening in females, plus, women have extra moister periurethral areas where bacteria multiply (Foxman, 2014). On entering the urethra, the bacteria are more likely to rise to the female bladder than the male bladder, due to the smaller urethral length. Those who are incapable of urinate frequently and empty their bladder fully have higher possibilities of immune-response engagement (Foxman, 2014).

Specific subpopulations at bigger risk of UTI include infants, pregnant women and the elderly, as well as those with spinal cord injuries, indwelling catheters, diabetes,

multiple sclerosis, immunodeficiency, underlying urologic abnormalities and people who are sexually active or use certain types of birth control such as a spermicide (Gupta et al., 2017; Martínez et al., 2007).

1.1.2 Uncomplicated UTI

UTIs can be clinically classified as complicated and uncomplicated (Karam et al., 2019; Zacchè & Giarenis, 2016). Uncomplicated UTI's are usually found in patients with a healthy urinary tract system, which is frequently seen in community-acquired infections (Karam et al., 2019). These infections can be distinguished into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Flores-Mireles et al., 2015). The clinical situation of the patient will be more serious as the higher up will be the microorganism invasion of the urinary tract (Bjerklund Johansen et al., 2014).

Uncomplicated UTIs begin when pathogens that live in the gut contaminate the periurethral region and can colonize the urethra (Figure 1). After that, they migrate to the bladder, resulting in colonization and invasion of the superficial umbrella cells. Later, host inflammatory responses begin to clear extracellular bacteria, however, some bacteria evade the immune system, either over host cell invasion or through morphological changes, that results in resistance to the immune responses, making these bacteria undergo in multiplication and biofilm creation. These uropathogens are able to produce toxins and proteases that induce host cell damage, releasing essential nutrients that promote bacterial survival and can help their ascension to the kidneys (Flores-Mireles et al., 2015).

1.1.3 Complicated UTI

Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence (e.g. underlying diabetes or immunosuppression) and are attributed to patients with weakened or obstructed urinary tract system, or patients that use medical devices such as catheters, which make their treatment sometimes more difficult for physicians (Karam et al., 2019).

The initial steps described for uncomplicated infections are the same used by pathogenic bacteria responsible for complicated UTIs (Figure 1). Although, for the infection to appear, the bladder must be compromised. The most frequent reason of a compromised bladder is catheterization. Due to the strong immune response stimulated by catheterization, fibrinogen accumulates on the catheter, providing an ideal environment for the attachment of uropathogens that express fibrinogen-binding proteins (Flores-Mireles et al., 2015).

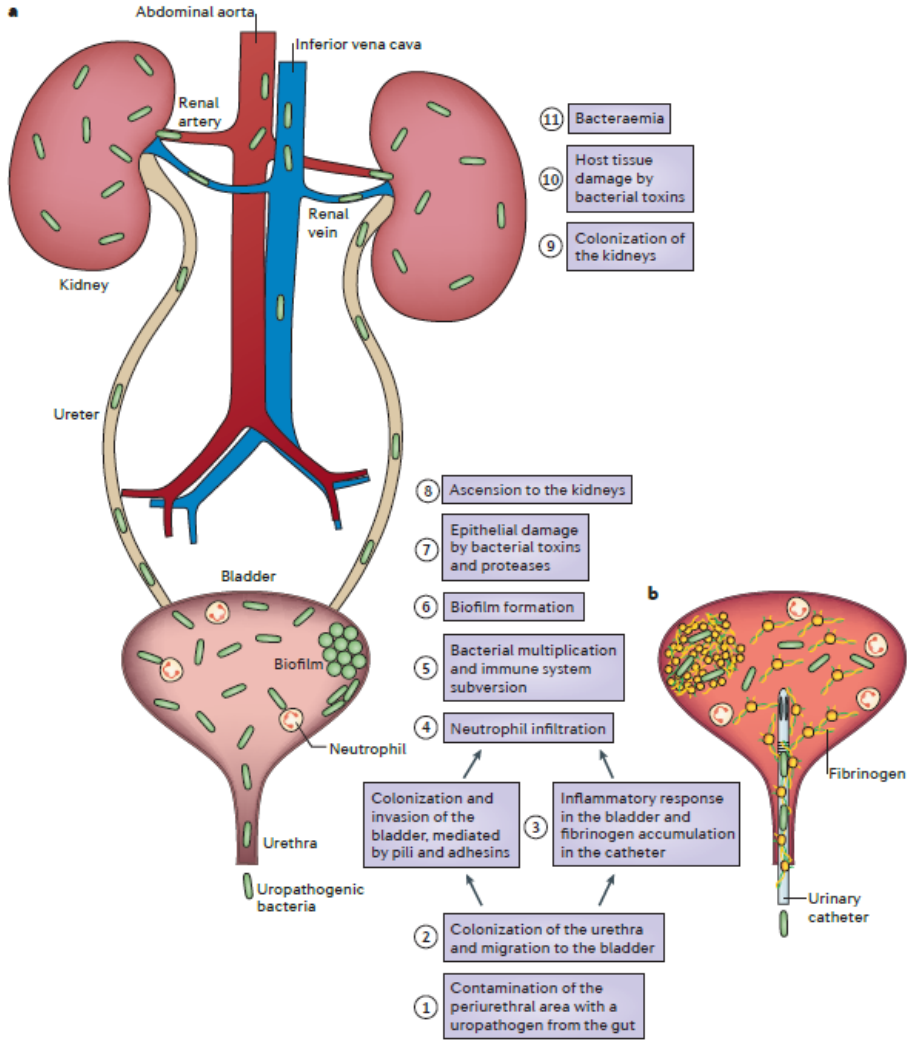

Figure 1 Pathogenesis in urinary infections. a) uncomplicated urinary tract infections; b) complicated urinary tract infections (Flores-Mireles et al., 2015).

1.1.4 Commensal flora in the urinary tract

Commensal species within the urinary tract and urogenital tract microbiomes act as a protection against colonization by uropathogens (Neugent et al., 2020), therefore, the presence of bacteria in the lower urinary tract should not be taken as proof of infection.

The most common bacteria found is *Lactobacillus crispatus* (Mueller et al., 2017; Neugent et al., 2020). This bacterium has been associated with the lack of UTI symptoms and it was reported that intravaginal administration of the probiotic strain of *L. crispatus* reduces episodes of recurrent UTI (Mueller et al., 2017). These findings suggest that *L. crispatus* may be advantageous to preserving the health of the bladder.

1.1.5 Frequent microorganisms responsible for UTIs

The bacteria responsible for UTIs can be either Gram-negative or Gram-positive. The most common cause of UTI is the Gram-negative *Escherichia coli*, causing approximately 70-80% community-acquired infections (Foxman, 2014; Ny et al., 2019), which are transmitted by the fecal-oral route and direct contact person to person. The *E. coli* strains causative of UTI are different, changing in the presence of known uropathogenic factors and represent various genetic lineages (Foxman, 2014).

Besides *E. coli*, the most frequent bacteria found in UTIs are *Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus saprophyticus* and *Staphylococcus aureus* (Flores-Mireles et al., 2015; Sorlozano et al., 2014; Zacchè & Giarenis, 2016).

Males with recurrent UTI or with urinary catheter are more likely to have a UTI caused by a non-*E coli* uropathogen (Amna et al., 2013).

1.1.6 Urinary tract infection diagnostic

When patients show typical symptoms of UTI and an infection is suspected, an urinalysis and urine culture is typically performed. Dipsticks are often used, since they are inexpensive, convenient and useful (Leung et al., 2019). The leukocyte esterase dipstick test gives information about the presence of pyuria by histochemical methods, that detect this enzyme in neutrophils. Esterase gives positive results even if the leucocytes are lysed, however, a positive result does not always mean positive UTI. This test can also be falsely negative if leucocytes are present in low concentration (Leung et al., 2019). The dipstick also gives the information about the presence of blood and nitrite, which can be an indicative of the existence of bacteria (Bonkat et al., 2018). Microscopy should also be performed for the detection of bacteriuria and pyuria. Bacteria may be not so visible due to the presence of significant number of urothelial cells, vaginal cells, red blood cells and crystals in the urine (Leung et al., 2019). The presence of numerous vaginal cells often corresponds to a bad sample due to poor sample collection.

The gold standard for the diagnosis of UTI is quantitative urine culture. According to Bonkat et al. (2018), a colony count $\geq 10^3$ cfu/mL of uropathogens confirms microbiologically the diagnosis. But since this approach is not always economically reasonable nor practical in daily routine because of the delay of the results, the empirical treatment is most of the times applied, which can lead to the wrong antibiotic prescription and consecutively to the spreading of bacterial resistances.

1.2 Treatment

The basic therapy of UTI is centred on the use of antibiotics. Sadly, the widespread and misuse of these antibiotics resulted in the increasing rate of resistance to them in the society (Cortes-Penfield et al., 2017). Antibiotics should be properly selected by considering characteristics such as the antibiotic susceptibility pattern of the infectious bacteria, infection type (community-acquired or hospital-acquired), and some patients conditions including age, gender, previous antibiotic consumption, history of previous UTIs and location of UTI (Karam et al., 2019), however, this does not happen most of the

times, as most antibiotics are prescribed empirically, with the intention of starting the therapy as fast as possible.

The prescription of the right antibiotic is essential since if not correctly selected, antibiotic may not only not destroy the reservoirs of bacteria, but it can also act as a shelter for the survival of the bacteria in the bladder cells (Karam et al., 2019).

1.2.1 Antibiotics recommend for UTIs

UTIs result in significant economic and public health problems and substantially affect the life quality of afflicted individuals. Currently, the antibiotics for uncomplicated cystitis suggested by the European Association of Urology (EAU) are the fosfomycin, nitrofurantoin and pivmecillinam as drugs of first choice when available (Bonkat et al., 2018). Alternative antimicrobials include trimethoprim-sulfamethoxazole (SXT) and cephalosporins if local resistance is lower than 20% (Bonkat et al., 2018). It is also used quinolones as an alternative antimicrobial in some countries, but it is not so recommended due to the adverse effects such as being sometimes especially toxic (Bonkat et al., 2018; Cortes-Penfield et al., 2017; Karam et al., 2019). Aminopenicillins are still used for alternative treatment, but they are no longer the most suitable for empirical therapy since there is a worldwide high *E. coli* resistance, although they can still be used in selected cases. Aminopenicillins in combination with a beta-lactamase inhibitor, such as amoxicillin plus clavulanic acid are not so effective as short-term therapy but can still be used in select cases (Bonkat et al., 2018).

For pyelonephritis, the most used antimicrobial agents for empirical oral treatment are the quinolones and cephalosporins since they can reach adequate renal tissue levels. However, oral cephalosporins achieve significantly lower concentrations than intravenous cephalosporines. Local resistance to quinolones and cephalosporins should be less than 10% to treat pyelonephritis empirically. First line antimicrobials for uncomplicated cystitis should be avoided in these cases, since they cannot achieve enough renal tissues levels (Bonkat et al., 2018). Some others appropriate choices are trimethoprim and an oral beta-lactam, if previously known to be susceptible, if not, the

administration should be intravenous. It can also be used aminoglycosides such as amikacin and gentamicin for alternative treatment (Bonkat et al., 2018).

In complicated UTIs, for patients requiring hospitalisation is suggested the use initially of an intravenous antimicrobial, such as amoxicillin plus an aminoglycoside or second/third generation cephalosporins also with a combination of an aminoglycoside. It can also be used quinolones, but only if the local resistance percentages are less than 10%, if the treatment is given orally, if patients do not require hospitalization, and if the patient hasn't used quinolones in the last six months (Bonkat et al., 2018).

When in all situations there is a case indicating the presence of a multi-drug resistance organism it should be considered the use of carbapenems. The choice between these agents should be based on local resistance patterns and based on drug susceptibility results (Gupta & Bhadelia, 2014).

1.2.2 Mechanisms of Antibiotics

Antibiotics do not use all the same mechanisms to fight bacteria. Knowledge of the mechanisms of action of each antimicrobial against bacteria is crucial to understand how bacteria develop resistance to these therapeutic drugs as antibiotic resistance increasingly limits the success of antibiotic treatments.

1.2.2.1 Cell wall synthesis inhibitors

Gram-negative bacteria tend to be more resistant to antimicrobial agents than Gram-positive bacteria, because of the presence of the extra protection offered by the outer membrane. Additionally to this outer membrane, both Gram-positive and Gram-negative bacteria have a cell wall made of peptidoglycan, which consists of long sugar polymers (Epand et al., 2016). The peptidoglycan goes through cross-linking of the glycan strands, and the peptide chains expand from the sugars in the polymers and form cross links, one peptide to another (Kapoor et al., 2017). The D-alanyl-alanine portion of

peptide chain is cross linked by glycine residues, in the presence of penicillin binding proteins (PBPs) (Kapoor et al., 2017).

A large portion of antimicrobials used in the treatment of UTIs are β-lactam antibiotics, which act by inhibiting the cell wall synthesis (Epand et al., 2016). β-lactam agents target the PBPs, mimicking the D-alanyl-alanine portion of peptide chain. The β-lactam ring interacts with PBPs, making them not available for the synthesis of the of new peptidoglycan (Kapoor et al., 2017).

Another type of this antibiotics is fosfomycin. This antibiotic acts as bactericidal, presents very low toxicity and inhibits the cell wall enzyme MurA (UDP-N-acetylglucosamine-enolpyruvyltransferase), which is responsible for catalysing the first committed step in peptidoglycan synthesis (Silver, 2017).

1.2.2.2 DNA replication inhibitors

Quinolones, another class of antibiotics, prevents DNA replication and transcription by inhibiting the enzyme DNA gyrase, which nicks the double stranded DNA, introduces negative supercoils and then reseals the nicked ends (Kapoor et al., 2017). They also inhibit topoisomerase IV, the main target of third generation quinolones in Gram positive bacteria, which nicks and separates daughter DNA strand after DNA replication (Kapoor et al., 2017).

1.2.2.3 Folic acid metabolism inhibitors

Antimetabolite antibiotics, composed of sulphonamides/trimethoprim, prevent bacterial growth due to a lack of folate cofactors, which are essential for the synthesis of nucleic acids and proteins (Sousa, 2006). Each of these drugs inhibits distinct steps in folic acid metabolism. Trimethoprim binds and inhibits the enzyme dihydrofolate reductase, blocking the conversion of dihydrofolic acid (DHFA) to its functional form. This reduces the quantity of folate, an essential cofactor in the biosynthesis of nucleic acids, resulting in the interference either nucleic acid and protein production (Kapoor et al., 2017).

Sulfonamides inhibit the enzyme dihydropteroate synthetase, responsible for incorporation of para-aminobenzoic acid (PABA) into dihydrofolic acid , the immediate precursor of folic acid (Kapoor et al., 2017).

1.2.2.4 Inhibitors of protein biosynthesis

Another mechanism of antibiotics is inhibiting the protein synthesis. These types of antibiotics interfere with the processes at the 30S subunit or 50S subunit of the 70S bacterial ribosome (Kapoor et al., 2017). Examples of these antibiotics include aminoglycosides such as gentamicin, amikacin and tobramycin.

Aminoglycosides inhibit protein synthesis by binding, with great affinity, to the A-site on the 16S ribosomal RNA of the 30S ribosome (Krause et al., 2016). As a result of this interaction, the antibiotic encourages improper translation by provoking codon misreading on delivery of the aminoacyl transfer RNA. This results in error in the protein synthesis, allowing for incorrect amino acids to assemble into a polypeptide that is subsequently released to cause damage to the cell membrane (Krause et al., 2016).

1.2.2.5 Nitrofurantoin

Nitrofurantoin has a bacteriostatic and bactericide mechanism of action, and affects several bacterial enzymatic systems, which will affect metabolisms, DNA and RNA synthesis. Due to its multiple activity, there is lower chances of acquiring resistance to this drug (Sousa, 2006). This antibiotic is converted by bacterial nitroreductases to highly reactive electrophilic intermediates, which inhibit the citric acid cycle as well as synthesis of DNA, RNA and protein (Roemhild et al., 2020). The particularity of nitrofurantoin is that it is only used to treat urinary infections.

1.2.3 Resistance and mechanisms of resistance

With the increasing use of antibiotics nowadays, the resistance to these antimicrobials is increasingly worrying. Antimicrobial resistance is a serious health

problem that is growing and has already been described by the World Health Organization (WHO) as one of the biggest health problems facing our generation (WHO, 2019). This resistance is mainly due to the misuse and overuse of antibiotics. Antibiotics have been so overprescribed that resistant bacteria have made the treatment of UTI's a complicated task. The prevalence of resistance in community populations has increased and must be considered, even in outpatients (Gupta et al., 2017; Phamnguyen et al., 2019).

Antibiotic resistance can be obtained through different mechanisms, being those mechanisms possibly changes in regulatory locus on the chromosome of the bacteria, or acquisition of resistance genes via mobile genetic elements such as plasmids, gene cassettes in the integrons and transposons (Karam et al., 2019).

1.2.3.1 Modifications of the antibiotic molecule

Producing enzymes that inactivate the drug is one of the most successful bacterial strategies to achieve resistance. This is most of times achieved by adding specific chemicals to the antibiotic molecule or that destroy the compound itself, making the antibiotic incapable to interact with its target (Munita & Arias, 2016).

The creation of enzymes capable of introducing chemical changes to the antimicrobial molecule is a mechanism antibiotic resistance in both Gram-negative and Gram-positive bacteria. Most of the antimicrobials affected by these enzymatic modifications exert their mechanism of action by inhibiting protein synthesis at the ribosome level, like aminoglycosides (Krause et al., 2016). The central mechanism of β-lactam resistance relies on the destruction of antibiotic molecules by the action of β-lactamases (Munita & Arias, 2016).

1.2.3.2 Decreased antibiotic penetration and efflux

As many antibiotics have intracellular bacterial targets, many microorganisms develop ways to decrease their permeability to the compound. This is accomplished by

decreasing the uptake of the antimicrobial molecule, and is particularly important in Gram-negative bacteria, by limiting the influx of substances from the external medium (Munita & Arias, 2016).

The production of complex bacterial systems able to expel a toxic compound out of the cell, called efflux plums, can also result in antimicrobial resistance. Many classes of efflux pumps have been characterized in both Gram-negative and Gram-positive bacteria (Munita & Arias, 2016). These systems can be substrate specific or with board substrate specific, found in multidrug resistance (MDR) bacteria. This machinery affects a wide range of antimicrobial classes including protein synthesis inhibitors, β-lactams, fluoroquinolones and carbapenems (Munita & Arias, 2016).

1.2.3.3 Changes in target sites

Interfering with target site of antibiotics is a mechanism of resistance. To accomplish this, bacteria created different tactics including protecting the target and modifications of the target site, resulting in decreased affinity to the antimicrobial molecule. Inducing modifications of the target is a very common mechanism of resistance in pathogens, and its target alterations may consist of point mutations in the genes encoding the target site, enzymic alterations of the binding site, and replacement or bypass of the original target (Munita & Arias, 2016).

1.2.4 Multidrug resistance in uropathogenic pathogenic bacteria

Handling of patients with infections in the ambulatory setting is growing particularly challenging because of the increase in resistance and lack of oral treatment options (Gupta & Bhadelia, 2014). Multidrug resistant uropathogenic organisms are becoming a growing public health threat, as Enterobacteriaceae family members gradually acquire extended-spectrum β-lactamases (ESBLs) (Flores-Mireles et al., 2015). As with other antimicrobial classes, the extensive use of β-lactams has led to the

emergence and dissemination of resistance. β-lactamases work by parting the amide link of the β-lactam ring, thus inactivating β-lactam antimicrobials (Gupta & Bhadelia, 2014).

ESBLs had their starting point in *K. pneumoniae* and *E. coli*, however, they are now prevalent throughout the Enterobacteriaceae family. ESBLs are plasmid-encoded or chromosomally encoded β-lactamases. To make things worse, ESBLs are encoded on plasmids that typically carry other resistance genes against quinolones, sulfonamides, and aminoglycosides, making the bacteria that gain these plasmids multidrug resistant. *E. coli* ESBLs producers are now present in patients with no evident health care exposure or danger factors which is of great concern (Gupta & Bhadelia, 2014).

1.3 Regional importance

Even though antibiotic resistances of uropathogens have increased over the past years all over the world, resistance patterns are variable, depending on patient population and geographic region (Foxman, 2014). One of the factors to be considered when antibiotic therapy is started empirically is the regional prevalence of antimicrobial resistance among common pathogenic bacteria. The knowledge of the susceptibility pattern of the most common microorganisms to antimicrobial agents helps in the correct selection of an appropriate treatment for outpatients with UTIs (Mamani et al., 2015).

To this day, there are still only a few publications about the most common bacteria implicated in community acquired UTI and their antimicrobial resistance pattern, when compared to UTI acquired at hospital level. Therefore, monitoring this information periodically is very important because it reflects the changes over the years and it helps to decrease the number of failures during therapy (Kranz et al., 2017; Linhares et al., 2013).

If we base ourselves on the susceptibility patterns of each region when choosing the antibiotic to prescribe, it is possible to help prevent the development and spread of new multidrug resistance. Therefore, it is important to treat patients with narrow spectrum antibiotics that show good susceptibility to the uropathogens population in order to decrease the number of therapeutic failures (Ny et al., 2019).

Chapter 2

2. Objective

Nowadays, antibiotics are prescribed in an empirical way, without initially carrying out laboratory analyses to obtain precise information regarding the microorganism responsible for the infection in order to start treatment as soon as possible. Time required to obtain results regarding patient's illness are too wide, which makes doctors prescribe the antibiotics before the results are known. Because of this, sometimes the antimicrobial prescribed is not the best option.

Urinary tract infection is one of the most common infections in the community, and for that reason, it is essential to have a prior knowledge of the regional susceptibility pattern of microorganisms to antimicrobials, in order to choose the best antimicrobial to prescribe empirically.

The aim of this study was:

1. Evaluate, within a five-year period, the prevalence and the antimicrobial resistance pattern of the main bacteria accountable for urinary tract infection in the central and northern community of Portugal.
2. Establish an appropriate empirical therapy. For that, it was used data and samples from Avelab Laboratório Médico de Análises Clínicas.

Chapter 3

3. Materials and Methods

3.1 Characterization of Avelab Laboratório Médico de Análises Clínicas

Avelab was founded in 1956 and has more than 60 posts of collection, distributed by various counties in central region and north region, namely Aveiro, Viseu, Porto, Coimbra, Vila Real, Bragança and Guarda. The headquarter laboratory is located in Forca, Aveiro, where it was built in 1996.

In Avelab, numerous analysis are carried out on various biological products, such as blood, urine, faeces, expectoration, vaginal exudates, sperm, among others.

3.2 Samples

All urine samples from patients in ambulatory regime of the north and central region were analysed at Avelab Laboratório Médico de Análises Clínicas (Aveiro, Portugal), during the period 2015-2019. The samples were collected from patients presenting clinical symptoms of UTI, pregnant women and urinary tract infection post-treatment patients.

The following data were registered for each patient, sex, age, urine culture results, identification of the bacterial strain responsible for UTI and the corresponding antimicrobial susceptibility test (AST) results.

3.2.1 Sampling

Applying the Avelab protocol, early urine was collected by midstream clean-catch technique after patient daily hygiene. The initial portion of the micturition was discarded and the middle jact was collected right into the sterile recipient. A collection bag surrounding the enthral area was used for collecting the urine for children under two years old. The bag was under control every fifteen minutes and after micturition, the bag

was removed, sealed and stored at 4 °C until processing. The samples were analysed within one hour after collection, when not possible the samples were stored at 4 °C until processing.

3.2.2 Microscopic examination

Firstly, the samples were homogenized and transferred to a conical tube of 10 mL. Secondly, the urine was centrifuged at 2500 rpm for five minutes and the supernatant was decanted. Lastly the pellet was homogenized and put in slides that were directly exanimated, where it was searched the presence of bacteria, leucocytes, erythrocytes, cells, and crystals. Slides were also stained by the Gram technique to differentiate from Gram-positive and Gram-negative.

3.2.3 Urine culture

Different culture media was used and inoculated. A calibrated loop of 1 µL was dipped in vertical position in the urine and the loop was used to inoculate the mediums using the streak plate method. The Levine medium (Biokar Diagnostics, BK056HA) was used for the detection of aerobic Gram-negative bacilli. For Gram positive cocci, the urine samples were spread in Mannitol Salt Agar (Biokar Diagnostics, BK030HA) for the detection of *Staphylococcus*, in Blood Agar for the detection of *Streptococcus* and in bile Esculin Agar (BD BBL, 212205) for the detection of *E. faecalis.*

The plates were incubated during 24 h at 37 °C. The plates of Blood Agar were incubated in 5-10% CO_2 atmosphere, while the others were incubated at O_2 atmosphere. After incubation, the samples were classified as positive, negative, and contaminated. Contaminated classification was determined when polymorphic bacterial growth (growth of three or more bacterial species) was observed. When growth was lower than 10^3 CFU/mL the urine cultures were categorized as negative. The urine cultures were classified as positive when bacterial growth was equal or higher than 10^3 CFU/mL, and only for these cases the antimicrobial susceptibility test was done.

3.2.4 Identification of bacterial isolates

For the identification of the bacterial isolates further biochemical tests were done when the urine culture was positive. These identifications were performed based on the morphology of the isolated bacteria, biochemical profile, and on the results of the microscopic examination of the Gram stained smear. The following media, the Kigler (BD BBL, 211317), Tryptone (BD BBL, 264410), Simmons Citrate (BD BBL, 211620) and Urea (Oxoid, CM00539), were used to differentiate Enterobacteriaceae. The catalase test was used to distinguish *Staphylococcus* from *E. faecalis* and *Streptococcus*. The coagulase test (Biomérieux, Slidex Staph plus, 73115) was used to identify *S. aureus*. *S. saprophyticus* was identified using the novobiocin susceptibility test (BD BBL Sensi-Disc, 231314). The identification of *Streptococcus agalactiea* was done with the chromogenic medium granada (Biomérieux). To identify Pseudomonaceae, the oxidase test (BD BBL, 231746) was used. *P. aeruginosa* was also identified by production of diffusible pigments on Mueller-Hinton Agar (Biokar Diagnostics, BK048HA) and for a grape-like odour released.

Reference strains *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *S. aureus* ATCC 29123, *P. aeruginosa* ATCC 29123, *S. agalactiae* ATCC 13813, *E. faecalis* ATCC 29212, *S. saprophyticus* ATCC 43867, *P. mirabilis* ATCC 35659 and *Proteus vulgaris* ATCC 6380 were used as positive control.

3.2.5 Antimicrobial susceptibility

The modified Kirby-Bauer disk diffusion method was used for the AST. A bacterial suspension was prepared for in physiological saline solution, with a turbidity of 0.5 on McFarland scale, by using 1-2 colonies from pure cultures. For spreading the suspension on Mueller-Hinton Agar a swab was used. Antimicrobial-impregnated disks (BD BBL, Sensi-Disc) were placed onto the cultures Muellller-Hinton surface using an automated disk dispenser.

For *E. coli*, the antibiotics amoxicillin, amoxicillin-clavulanic acid (AMX-CLA), cefazolin, cefuroxime, cefotaxime, nitrofurantoin, fosfomycin, ciprofloxacin, trimethoprim-

sulfamethoxazole and amikacin were tested (Table 1). For the others Enterobacteriaceae the antibiotics tested were the same as *E. coli* except for fosfomycin. When *E. coli* and *K. pneumoniae* were resistant to five or more antibiotics, it was also tested gentamicin, tobramycin, imipenem, ceftazidime, cefepime, aztreonam and piperacillin-tazobactam (PIP-TAZ) (Table 1). *P. aeruginosa* was tested for amikacin, gentamicin, tobramycin, ceftazidime, cefepime, aztreonam, piperacillin-tazobactam and ciprofloxacin (Table 1).

Table 1 Antibiotics tested for each uropathogen.

Uropathogen	Antibiotics
E. coli	Amoxicillin, AMX-CLA, Cefazolin, Cefuroxime, Nitrofurantoin, Fosfomycin, Ciprofloxacin, SXT, Amikacin, Gentamicin*, Tobramycin*, Imipenem*, Ceftazidime*, Cefepime*, Aztreonam* and PIP-TAZ*
K. pneumoniae	Amoxicillin, AMX-CLA, Cefazolin, Cefuroxime, Nitrofurantoin, Ciprofloxacin, SXT, Amikacin, Gentamicin*, Tobramycin*, Imipenem*, Ceftazidime*, Cefepime*, Aztreonam* and PIP-TAZ*
P. mirabilis	Amoxicillin, AMX-CLA, Cefazolin, Cefuroxime, Nitrofurantoin, Ciprofloxacin, SXT and Amikacin
P. vulgaris	Amoxicillin, AMX-CLA, Cefazolin, Cefuroxime, Nitrofurantoin, Ciprofloxacin, SXT and Amikacin
Enterobacter	Amoxicillin, AMX-CLA, Cefazolin, Cefuroxime, Nitrofurantoin, Ciprofloxacin, SXT and Amikacin
K. oxytoca	Amoxicillin, AMX-CLA, Cefazolin, Cefuroxime, Nitrofurantoin, Ciprofloxacin, SXT and Amikacin
P. aeruginosa	Amikacin, Gentamicin, Tobramycin, Ceftazidime, Cefepime, Aztreonam, Piperacillin-tazobactam and Ciprofloxacin
E. faecalis	Amoxicillin, Ampicillin, Nitrofurantoin, Fosfomycin, Ciprofloxacin and Levofloxacin
S. agalactiae	Cefotaxime, Nitrofurantoin, Ampicillin, Amoxicillin, Ciprofloxacin, Levofloxacin, Ofloxacin and SXT
S. aureus	Nitrofurantoin, AMX-CLA, Ciprofloxacin, SXT and Gentamicin
S. saprophyticus	Nitrofurantoin, AMX-CLA, Amoxicillin, Ciprofloxacin and SXT

*Antibiotics only tested when bacteria were resistant to 5 or more antibiotics previously.

For *E. faecalis,* amoxicillin, ampicillin, nitrofurantoin, fosfomycin, ciprofloxacin and levofloxacin were used. *S. agalactiae* had the following antibiotics tested, cefotaxime, nitrofurantoin, ampicillin, amoxicillin, ciprofloxacin, levofloxacin, ofloxacin and SXT (Table 1). For *Staphylococcus* nitrofurantoin, amoxicillin-clavulanic acid, ciprofloxacin, and SXT were tested when in *S. aureus* was also tested gentamicin and in *S. saprophyticus* was

also tested amoxicillin (Table 1). The concentration of each disk containing the antibiotic tested is presented at table 2.

The AST plates were incubated at 37 °C for 18-24 h. Following incubation, the diameter of the zones of inhibition were measured determining the antimicrobials efficacy (European Committee on Antimicrobial Susceptibility Testing, 2014; European Committee on Antimicrobial Susceptibility Testing, 2019). According to the inhibition zone measure the bacterial strains were classified as susceptible (S), intermediate (I) or resistance (R) (EUCAST, 2014; EUCAST, 2019).

Table 2 Concentration of antibiotic tested.

Antibiotic	Disk content (µg)
Ampicillin	10
Amoxicillin	20
Amoxicillin-clavulanic acid	20-10
Piperacillin-tazobactam	30-6
Cefazolin	10
Cefepime	30
Cefotaxime	5
Ceftazidime	10
Cefuroxime	30
Imipenem	10
Aztreonam	30
Ciprofloxacin	5
Levofloxacin	5
Ofloxacin	5
Amikacin	30
Gentamicin	10
Tobramycin	10
Fosfomycin	200
Nitrofurantoin	100
Trimethoprim-sulfamethoxazole	1.25-23.75

3.2.6 Statistical analysis

For the statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 26.0 for Windows was used. To make easier the treatment of the data, the main bacteria responsible for UTI were selected, namely *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E.*

faecalis, *P. aeruginosa*, *S. aureus*, *S. agalactiae*, *Enterobacter spp*, *P. vulgaris, S. saprophyticus* and *Klebsiella oxytoca*. These selected bacteria represented 97.4% of all positive urines while the non-selected represented 2.6%. The Chi-squared test and the Binomial test were used. The significant level established was 0.05.

Uropathogens resistant to three or more antimicrobial classes were considered multidrug resistance (Magiorakos et al., 2012).

Chapter 4

4. Results

From the 5-year period study, 106019 samples of ambulatory patients were analysed, and 15439 had urinary tract infection. The annual average of urine analyses was 21201.8, with a bigger number of analyses being done in 2015 (24543), and a smaller number of analyses in 2017 (19201) (Table 3).

Table 3 Annual number of bacteriological tests on urine done during the study period.

	Nº of analysis	**%**
2015	24543	23.1
2016	22120	20.9
2017	19201	18.1
2018	20156	19.0
2019	19999	18.9
Total	106019	100.0
Average and standard deviation	21203.8±2153.5	-

4.1 Characterization of patients with bacterial UTI

The female patients with UTI represented 79.0% in total while males represented 21.0%. The age of the patients ranged from 1 to 106 years old, with a mean of 64.0 years. For female UTI patients, the average age was 62.3 years, while for male patients was 70.6 years (Table 4). From the 15025 positive bacteriological tests, 11959 (79.6%) were from female patients and 3066 (20.4%) were from male patients (Table 5).

The group most affected by UTI were the elderly with a frequency of 56.9% (42.1% for females and 14.8% for males). The group less affected by the UTI were the adolescents, showing the lowest frequency, 1.0% (0.94% corresponding to females and 0.06% to male patients) (Table 5). Children were responsible for 1.9% of the infections, the young adults for 8.5% and the adults for 31.6% (Table 5). A higher prevalence of

female infections was detected for all the group ages, however, the males showed a higher prevalence in the elderly when compared to other age groups (Table 5).

Table 4 Age of patients with urinary infection.

Age of patients with UTI		
Number of samples		15439
Minimum		1
Maximum		106
Average		64.0±21.4
Female	Average	62.3±22.0
Male	Average	70.6±17.3

4.2 Bacteria implied in UTI

The eleven bacteria more implicated in the UTI, during the 5 years period, correspond annually to more than 95% of the bacterial isolates. The main bacteria achieved the higher percentage in the year of 2016, representing 98.1% of the total and in 2015 reached the lowest percentage, being 96.6% of the isolates.

The more predominant agents were *E. coli* (70.1%), *K. pneumoniae* (8.9%), *P. mirabilis* (5.5%), *E. faecalis* (3.2%), *P. aeruginosa* (2.8%), *S. aureus* (2.5%), *S. agalactiea* (1.1%), *Enterobacter spp* (0.9%), *P. vulgaris* (0.8%), *S. saprophyticus* (0.8%) and *K. oxytoca* (0.8%) (Table 5).

The incidence of the main bacteria responsible for UTI varied significantly (Chi-Square test, $p < 0.05$) along the study period. Overall, the incidence of *K. pneumonia*, *K. oxytoca* and *S. saprophyticus* increased and the incidence of *S. agalactiea, P. aeruginosa* decreased (Figure 2). *E. coli* was always the pathogen most implicated in the UTI, followed by *K. pneumoniae* and *P. mirabilis.* The presence of the same bacteria between male and

Table 5 Incidence of the main bacteria implicated in urinary tract infection during the study period.

	Children			*Adolescents*			*Young adults*			*Adults*			*Elderly*			*Isolates in the 5 years (%)[a] (N= 15439)*	*Female (%)[a] (N= 11959)*	*Male (%)[a] (N= 3066)*	*MDR (%)*
	0-12 years			13-18 years			19-34 years			35-64 years			> 65 years						
Bacteria	Total[a] (N= 282)	F[b] (n= 232)	M[b] (n=50)	Total[a] (N= 157)	F[b] (n= 147)	M[b] (n=10)	Total[a] (N= 1277)	F[b] (n= 1206)	M[b] (n=71)	Total[a] (N= 4753)	F[b] (n= 4044)	M[b] (n= 709)	Total[a] (N= 8556)	F[b] (n= 6330)	M[b] (n= 2226)				
E. coli	74.8	67.4[c]	7.4	63.1	59.3[c]	3.8	75.3	71.1[c]	4.2	77.1[d]	67.2[c]	9.9	68.7	55.2[c]	13.5	70.1	76.2[c]	55.6	23.3
Klebsiella pneumoniae	1.8	1.1	0.7	6.4	5.8[c]	0.6	4.5	4.1[c]	0.4	6.2	4.9[c]	1.3	11.7[d]	8.3[c]	3.4	8.9	8.4	11.7[c]	40.4
P. mirabilis	16.3[d]	8.9	7.4	10.2	9.6[c]	0.6	5.3	5.2[c]	0.1	5.2	4.4[c]	0.8	5.5	3.0[c]	2.5	5.5	5.4	6.7[c]	10.0
P. vulgaris	1.1	0.4	0.7	0.6	0.6	0.0	0.1	0.1	0.0	0.4	0.2	0.2	1.2[d]	0.7	0.5	0.8	0.6	1.9	19.4
Enterobacter spp	0.0	0.0	0.0	1.3[d]	1.3	0.0	0.4	0.4	0.0	0.8	0.6[c]	0.2	1.2	0.6	0.6	0.9	0.8	1.8[c]	29.9
Klebsiella oxytoca	0.4	0.0	0.4	0.0	0.0	0.0	0.3	0.2	0.1	0.5	0.4[c]	0.1	1.1[d]	0.7[c]	0.4	0.8	0.7	1.3[c]	24.2
P. aeruginosa	2.1	1.8	0.4	0.0	0.0	0.0	0.2	0.1	0.1	1.3	0.3[c]	1.3	4.2[d]	1.9	2.3	2.8	1.6	8.1[c]	34.7
E. faecalis	1.1	0.7	0.4	0.6	0.6	0.0	2.5	2.1[c]	0.4	2.1	1.2	0.9	4.1[d]	1.5[c]	2.6	3.2	1.8	8.8[c]	18.3
S. aureus	1.8	1.8	0.0	14.0[d]	12.7[c]	1.3	7.0	6.8[c]	0.2	3.5	3.2[c]	0.3	1.2	0.4[c]	0.8	2.5	2.5	2.8[c]	13.6
S. saprophyticus	0.7	0.7	0.0	3.2[d]	3.2	0.0	3.1	3.0[c]	0.1	1.3	1.3[c]	0.0	0.1	0.1	0.0	0.8	1.0[c]	0.2	5.8
S. agalactiae	0.0	0.0	0.0	0.6	0.6	0.0	1.3	1.2[c]	0.1	1.6[d]	1.4[c]	0.2	1.0	0.7[c]	0.3	1.1	1.2[c]	1.1	6.8
Total of UTI (%)	1.9	1.6	0.3	1.0	0.9	0.1	8.5	8.0	0.5	31.6	26.9	4.7	56.9	42.1	14.8		79.6	20.4	

The incidence of UTI by sex, age group and multidrug resistance percentage.

N: total number of bacteria for each age group; n: total number of bacteria for each sex; [a] Percentage determined in relation to N; [b] Percentage determined in relation to n; [c] Statistically significant differences of frequency between sex; [d] Statistically significant differences between age groups; M – male; F – female.

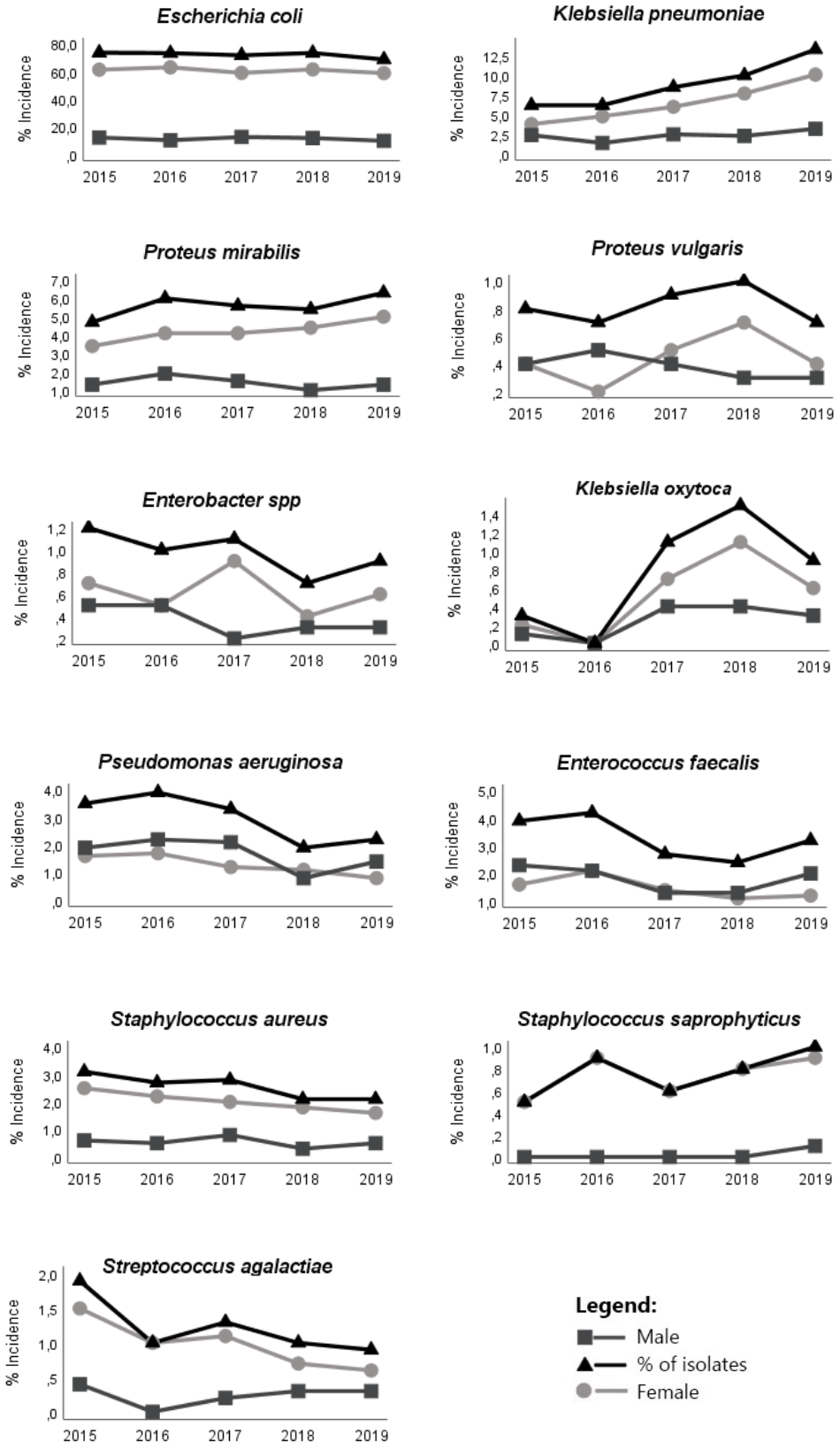

Figure 2 Incidence of the main bacteria implicated in UTI by sex during the study period.

female was evident, but the relative proportions were different (Binomial test $p < 0.05$). *E. coli* was the most present bacteria in both male and female, but females had an average of 76.2% and males an average of 55.6% (Table 5). The average of *E. faecalis* between female and male was distinct, being *E. faecalis* the third responsible (8.8%) of UTI in males and only fifth (1.8%) in females (Table 5). The same happened with *P. aeruginosa,* as a difference was observed, being in fourth cause for males (8.1%) and six for females (1.6%) (Table 5).

The incidence of bacteria in the different age groups increased significantly with the patient age (Chi-Square test, $p < 0.05$). Significant differences (Binomial test $p < 0.05$) were also observed when samples from females and males were analysed separately, showing that the difference also increased with the age (Table 5).

4.3 Antimicrobial resistance pattern of the main bacteria implicated in UTI

With the exception of *E. coli*, the Gram-negative bacteria exhibited higher resistance to penicilins, quinolones, SXT, cephalosporins of 1st and 2nd generation, and nitrofurantoin when compared with the other tested antimicrobials (Table 6). With the exception for *P. aeruginosa*, which showed a resistance of 17.3% for amikacin, the studied bacteria showed the smallest resistance to amikacin and fosfomycin, having a resistance rate lower than 4.5% (Table 6). The isolates of *K. pneumoniae, P. vulgaris* and *Enterobacter* showed high resistance to the several tested antimicrobials (some due to natural resistances) and the isolates of *E. coli* the less resistance to the studied antimicrobials (Table 6). In general, Gram-negative bacteria exhibited higher resistance than Gram-positive bacteria.

The bacterial isolates (*E. coli* and *K. pneumoniae*) that were tested with extra antimicrobials when they presented resistance to at least 5 previous antimicrobials, showed high resistance to these drugs. The imipenem was an exception, as only 7% of these isolates presented resistance against this drug (Table 6).

Table 6 Average antimicrobial resistance of the main Gram-negative uropathogens for female and male patients.

Antimicrobial group		Antimicrobials	*E. coli*				*K. pneumoniae*				*P. mirabilis*				*P. vulgaris*				*Enterobacter spp*				*K. oxytoca*				*P. aeruginosa*			
			N	%	F	M	N	%	F	M	N	%	F	M	N	%	F	M	N	%	F	M	N	%	F	M	N	%	F	M
Aminoglycosides		Amikacin	7246	1.6	1.5*	2.4	952	3.9	3.3	5.2	573	0.7	0.6	0.0	100	2.0	0.0	3.3	139	4.3	2.3	7.5	104	1.9	1.4	3.1	424	17.3	13.6	19.9
		Gentamicin	326	50.6	46.2*	60.2	356	59.0	59.0	58.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	425	31.1	27.9	33.3
		Tobramycin	299	63.5	60.0*	72.6	354	63.0	61.4	65.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	416	21.2	15.5*	25.2
β-lactam	Carbapenems	Imipenem	311	7.1	7.9	5.2	354	6.8	7.9	5.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	430	15.3	17.4*	26.0
	Cephalosporins 1st G	Cefazolin	9474	14.9	13.1*	24.7	1105	39.3	31.8*	58.6	737	15.2	14.0	18.8	111	100.0	100.0	100.0	125	100.0	100.0	100.0	102	32.4	28.2	41.9	-	-	-	-
	Cephalosporins 2nd G	Cefuroxime	10814	10.0	8.5*	17.9	1368	33.4	26.7*	52.2	848	4.0	3.3	6.3	124	100.0	100.0	100.0	144	77.8	75.6	81.5	120	16.7	15.0	20.0	-	-	-	-
	Cephalosporins 3rd G	Cefotaxime	10796	7.2	6.1*	13.2	1360	28.8	22.8*	45.7	844	1.5	1.6	1.5	123	4.9	4.5	5.6	142	21.1	13.5*	34.0	120	12.5	10.0	17.5	-	-	-	-
		Ceftazidime	338	68.3	64.1*	77.6	359	83.4	82.6	85.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	431	26.9	24.3	28.9
	Cephalosporins 4th G	Cefepime	329	63.5	59.0*	73.5	360	71.4	71.0	71.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	431	20.6	19.5	21.5
	Monobactams	Aztreonam	257	68.5	64.4*	77.5	311	85.2	83.0	87.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	428	16.4	17.5	15.5
	Penicilins	Amoxicillin	10816	46.3	44.1*	57.7	1366	100.0	100.0	100.0	847	36.6	34.3*	43.7	124	100.0	100.0	100.0	144	100.0	100.0	100.0	120	100.0	100.0	100.0	-	-	-	-
		AMX-CLA	10807	20.3	18.8*	28.5	1365	69.4	65.7*	79.7	846	9.9	7.8*	16.6	124	58.9	56.1	62.1	144	100.0	100.0	100.0	120	55.0	51.3	62.5	-	-	-	-
		PIP-TAZ	334	37.1	35.9	39.8	356	46.6	46.5	46.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	432	20.1	17.3	22.3
Quinolones		Ciprofloxacin	10489	20.5	18.0*	33.7	1351	34.6	27.8*	53.7	788	21.6	18.6*	31.0	120	42.5	42.2	42.9	142	21.1	11.4*	37.0	119	20.2	16.3	28.2	430	44.0	39.9	47.0
Miscellaneous agents		Nitrofurantoin	10804	7.0	6.4*	10.2	1273	81.7	79.8*	86.8	843	100.0	100.0	100.0	124	100.0	100.0	100.0	138	73.9	72.1	76.9	113	77.9	73.7	86.5	-	-	-	-
		Fosfomycin	9829	1.4	1.4	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		SXT	10811	24.8	23.3*	33.1	1365	36.5	30.4*	53.3	846	28.8	28.2	30.7	124	41.9	47.0	36.2	144	22.2	15.6*	33.3	120	22.5	18.8	30.0	-	-	-	-

N: total number of bacteria tested against each antimicrobial; - Antimicrobial not tested; M – male; F – female; * Statistically significant differences (p-value < 0.05) of antimicrobial resistance between female and male patients.

Most Gram-positive bacteria did not show high resistance. However, some bacteria presented higher resistance to SXT, quinolones, and amoxicillin (Table 7). *E. faecalis* showed high resistance to ciprofloxacin and levofloxacin, showing a resistance of 46.4% and 38.3%, respectively (Table 7). *S. agalactiea* presented low resistance to cefotaxime, less than 3%, and also low resistance to nitrofurantoin and fosfomycin, that resistance being smaller than 6%. However, a high resistance for SXT was found (Table 7).

Table 7 Average antimicrobial resistance of the main Gram-positive uropathogens for female and male patients.

Antimicrobial group		Antimicrobials	*E. faecalis*				*S. aureus*				*S. saprophyticus*				*S. agalactiae*			
			N	%	F	M	N	%	F	M	N	%	F	M	N	%	F	M
Aminoglycosides		Gentamicin	-	-	-	-	270	4.1	2.0*	10.4	-	-	-	-	-	-	-	-
β-lactam	Cephalosporins 3rd G	Cefotaxime	-	-	-	-	-	-	-	-	-	-	-	-	176	2.8	2.8	2.9
	Penicilins	Ampicillin	465	7.5	8.1	7.1	-	-	-	-	-	-	-	-	175	1.1	0.7	3.0
		Amoxicillin	436	8.3	8.2	8.3	-	-	-	-	103	48.5	47.4	66.7	166	2.4	2.3	3.0
		AMX-CLA	-	-	-	-	379	11.6	6.4*	29.8	120	6.7	6.1	16.7	-	-	-	-
Quinolones		Ciprofloxacin	485	46.4	43.1	49.1	363	15.7	10.3*	33.7	114	5.3	4.7	14.3	116	8.6	8.5	9.1
		Levofloxacin	472	38.3	29.0*	45.8	-	-	-	-	-	-	-	-	173	7.5	7.1	9.4
		Ofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	144	8.3	8.3	8.7
Miscellaneous agents		Nitrofurantoin	470	3.2	3.3	3.1	374	5.9	2.7*	17.7	120	4.2	3.5	14.3	146	0.7	0.9	0.0
		Fosfomycin	450	5.3	5.0	5.6	-	-	-	-	-	-	-	-	-	-	-	-
		SXT	-	-	-	-	383	14.6	9.7*	31.8	120	10.8	10.5	16.7	134	39.6	38.0	46.2

N: total number of bacteria tested against each antimicrobial; - Antimicrobial not tested; M – male; F – female; * Statistically significant differences (p-value < 0.05) of antimicrobial resistance between female and male patients.

During the study period, the bacterial resistance changed significantly (Chi-squared test, $p < 0.05$). *E. coli* showed a slight increase of resistance to amikacin and cephalosporins from first generation throughout the years (Figure 3). *Enterobacter* showed a resistance increase to cephalosporins from 3rd generation, more precisely to cefotaxime (Figure 3). *P. aeruginosa* showed a slight resistance increase to aztreonam. In general, the resistance registered to ciprofloxacin was constant during the study period,

only the *K. oxytoca* isolates showed a slight decrease. *P. mirabilis* and *P. vulgaris* showed a decrease in resistance to SXT (Figure 3).

Gram-positive bacteria *S. aureus* showed an increase of resistance to the aminoglycoside during the studied years (Figure 4). *E. faecalis* and *S. aureus* also showed a slight increase in the resistance to nitrofurantoin. *S. saprophyticus* presented a decrease in the resistance to this antimicrobial (Figure 4). *E. faecalis* and *S. saprophyticus* showed an increase to penicilins. *S. agalactiea* presented the biggest increase in resistance to SXT, and *S. saprophyticus* also showed a resistance increase to this antibiotic (Figure 4).

The resistance of bacteria implicated in UTI for male patients, was, in most cases, statistically different (Chi-Square test, $p < 0.05$) from that observed for bacteria isolated from female patients. In general, resistance was higher in male patients (Tables 6 and 7). The bacterial strains isolated from female patients were, on average, resistant to approximately 2 antimicrobials while the male bacterial strains were, on average, resistant to 3 antimicrobials (Table 8).

Table 8 Average number of antimicrobials, to which the main uropathogens were resistant, according to the patient's sex.

Sex	N	Average	Standard deviation
Female	11959	1.67	2.04
Male	3066	2.66	2.54
Total	15025	1.87	2.19

For *E. coli* and *K. pneumoniae*, in aminoglycosides, cephalosporins 3rd generation, and penicilins, only amikacin, cefotaxime, amoxicillin and AMX-CLA were taken into account.

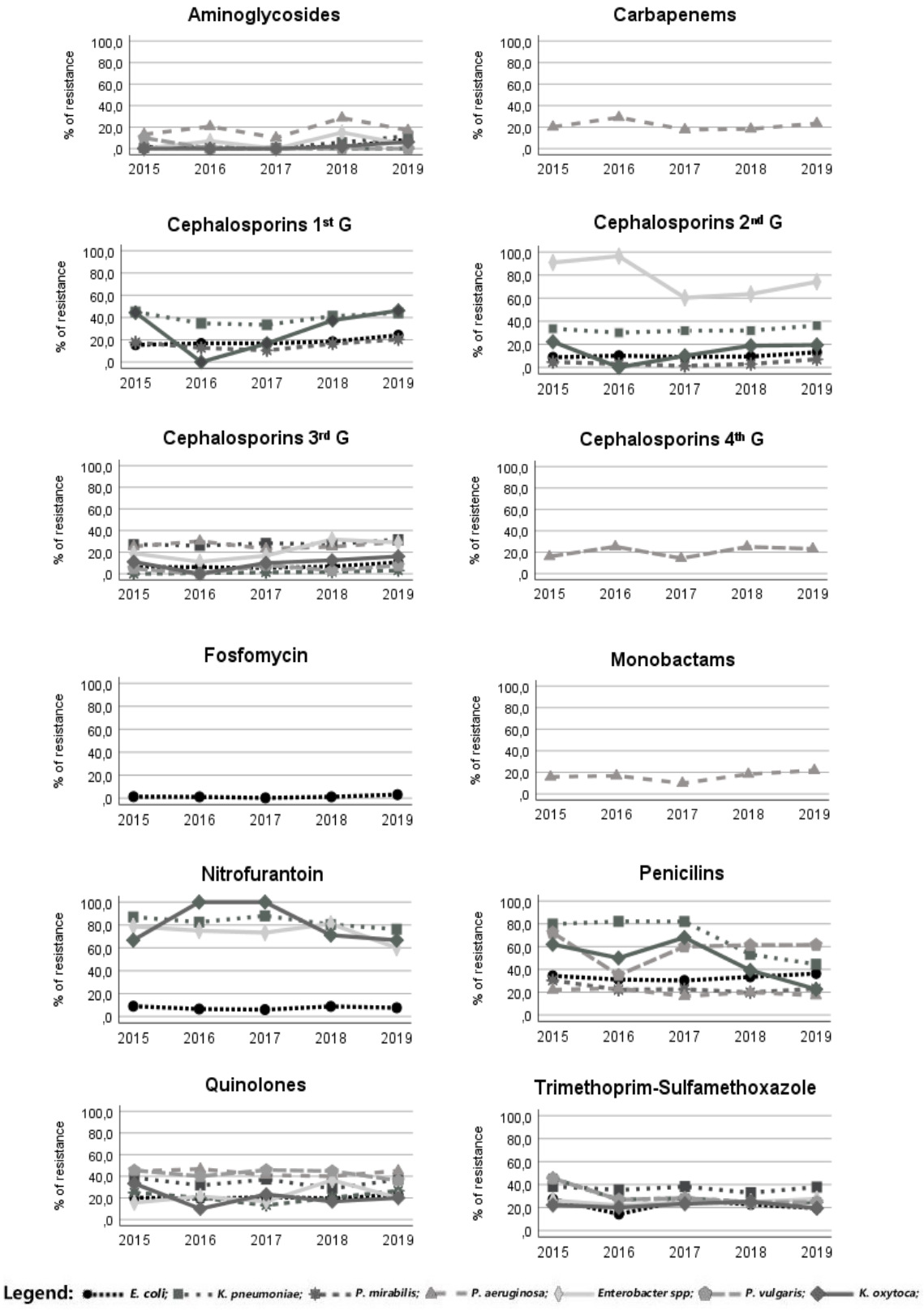

Figure 3 Variation of antimicrobial resistance pattern of Gram-negative bacteria during the study period. For E. coli and K. pneumoniae, in aminoglycosides, cephalosporins 3rd generation and penicilins, only amikacin, cefotaxime, amoxicillin and AMX-CLA are represented.

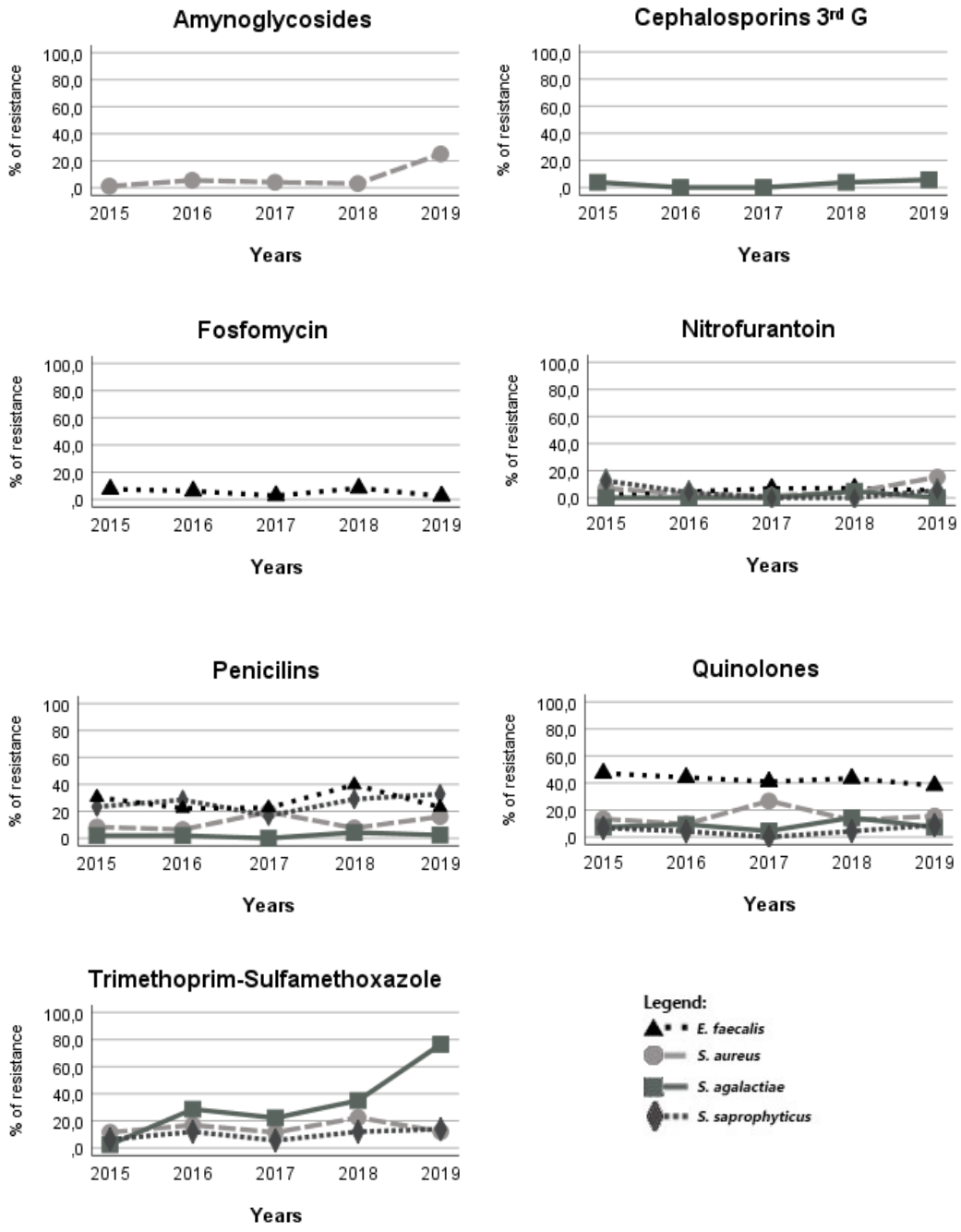

Figure 4 Variation of antimicrobial resistance pattern of the Gram-positive bacteria during the study period.

4.3.1 Pondered bacterial resistance for recommend antimicrobials

Having into account the values of drug resistance of each bacterium and its incidence, it was calculated the pondered resistance patterns according to the

uropathogens incidence (multiplying the bacterium averaged resistance by its incidence) for the two first line antibiotics indicated to treat UTI according to EAU (Table 9). For *E. coli*, the resistance to the first-line therapy antimicrobials tested in this study was low, 1.4% and 7.0% for fosfomycin and nitrofurantoin, respectively. For the other uropathogenic bacteria, resistances were, in most cases (*E. faecalis*, *S. aureus*, *S. agalactiea* and *S. saprophyticus* being the exception), higher, with an average resistance of 5.3% for fosfomycin and 44.3% for nitrofurantoin. The pondered resistance for all bacteria was 1.2% for fosfomycin, and 19.9% for nitrofurantoin (Table 9).

Table 9 Pondered bacterial resistance to the antimicrobials recommended as first line therapy for empirical treatment of urinary tract infection.

		Resistance to first line therapy			
Bacteria	Incidence (%)	FOM (%)	FOM (%)[1]	NIT (%)	NIT (%)[1]
E. coli	70.1	1.4	1.0	7.0	4.9
K. pneumonia	8.9	-	-	81.7	7.3
P. mirabilis	5.5	-	-	100.0	5.4
E. faecalis	3.2	5.3	0.2	3.2	0.1
P. aeruginosa	2.8	-	-	-	-
S. aureus	2.5	-	-	5.9	0.1
S. agalactiae	1.1	-	-	0.7	0.0
Enterobacter spp	0.9	-	-	73.9	0.7
P. vulgaris	0.8	-	-	100.0	0.8
S. saprophyticus	0.8	-	-	4.2	0.0
K. oxytoca	0.8	-	-	77.9	0.6
Average (%)			1.2		19.9

FOM (%) – Average resistance to fosfomycin; FOM (%)[1] – Pondered resistance to fosfomycin; NIT (%) – Average resistance to nitrofurantoin; NIT (%)[1] – Pondered resistance to nitrofurantoin.

The resistance of *E. coli* for the alternative drugs was slightly higher for some antibiotics, being 20.5%, 20.3%, 24.8%, respectively, for the quinolones, AMX-CLA and SXT. Among the tested alternative drugs, *E. coli* showed the lowest resistance to cephalosporins, 14.9%, 10.0% and 7.2% for 1st, 2nd, and 3rd generations, respectively (Table 10). The other bacteria implicated in UTI showed, on average, higher resistance to

these drugs, having an average of 56.1%, 40.3%, 14.3%, 25.6%, 31.1% and 21.7% for cephalosporins 1st, 2nd, and 3rd generation, quinolones, AMX-CLA and SXT, respectively.

The pondered resistance was calculated, and the results were 22.5%, 23.1% and 23.8%, respectively, for quinolones, AMX-CLA and SXT, being those slightly higher than the pondered resistance to first-line antibiotics (Table 10). The isolates showed smaller pondered resistance to cephalosporins from 1st, 2nd, and 3rd generation (16.7%, 12.1%, 7.9%, respectively) than to nitrofurantoin, a first line antibiotic.

Table 10 Pondered bacterial resistance to the antimicrobials recommended as alternative therapy for empirical treatment of urinary tract infection.

		Resistance to alternative therapy											
Bacteria	Incidence (%)	CEP 1st (%)	CEP 1st (%)[1]	CEP 2nd (%)	CEP 2nd (%)[1]	CEP 3rd (%)	CEP 3rd (%)[1]	QUI (%)	QUI (%)[1]	AMX-CLA (%)	AMX-CLA (%)[1]	SXT (%)	SXT (%)[1]
E. coli	70.1	14.9	10.4	10.0	7.0	7.2	5.0	20.5	14.4	20.3	14.2	24.8	17.4
K. pneumonia	8.9	39.3	3.5	33.4	3.3	28.8	2.6	34.6	3.1	69.4	6.2	36.5	3.2
P. mirabilis	5.5	15.2	0.8	4.0	0.2	1.5	0.0	21.6	1.2	9.9	0.5	28.8	1.6
E. faecalis	3.2	-	-	-	-	-	-	42.4	1.4	-	-	-	-
P. aeruginosa	2.8	-	-	-	-	-	-	44.0	1.2	-	-	-	-
S. aureus	2.5	-	-	-	-	-	-	15.7	0.4	11.6	0.3	14.6	0.4
S. agalactiae	1.1	-	-	-	-	2.8	0.0	8.1	0.1	-	-	39.6	0.4
Enterobacter spp	0.9	100.0	0.9	77.8	0.7	21.1	0.2	21.1	0.2	100.0	0.9	22.2	0.2
P. vulgaris	0.8	100.0	0.8	100.0	0.8	4.9	0.0	42.5	0.3	58.9	0.5	41.9	0.3
S. saprophyticus	0.8	-	-	-	-	-	-	5.3	0.0	6.7	0.1	10.8	0.1
K. oxytoca	0.8	32.4	0.3	16.7	0.1	12.5	0.1	20.2	0.2	55.0	0.4	22.5	0.2
Average (%)			16.7		12.1		7.9		22.5		23.1		23.8

CEP 1st, 2nd, and 3rd generation are only represented by cefazolin, cefuroxime and cefotaxime; (%) – Average resistance; (%)[1] – Pondered resistance.

4.3.2 Bacterial resistance pattern by age

The bacterial resistance grew with the patient age. The bacterial isolates from elderly patients showed more resistances, in general, than the other age groups (Chi-Square test, $p < 0.05$), presenting in average resistance to approximately 2 antibiotics while the others age groups showed on average resistance to one antibiotic (Table 11).

E. coli, *S. aureus*, *K. pneumoniae* were the uropathogens that showed more differences in antibiotic resistance for the different age groups (Table 12 and 13). These bacteria were generally more resistant in the elderly.

Table 11 Average number of antimicrobials to which the main uropathogens were resistant, by age group.

Age group	Nº	Average and Standard deviation
Children	282	1.15±1.50
Adolescents	157	1.02±1.40
Young adults	1277	1.01±1.45
Adults	4753	1.35±1.80
Elderly	8556	2.33±2.38

For *E. coli* and *K. pneumoniae*, in aminoglycosides, cephalosporins 3rd generation, and penicilins, only amikacin, cefotaxime, amoxicillin and AMX-CLA were taken into account.

For the uropathogens *S. agalactiea*, *P. aeruginosa* and *S. saprophyticus,* the difference between age groups was not so evident. *K. oxytoca* did not show any significant differences between age group. Ciprofloxacin was the antimicrobial for which the difference between age groups was more notorious, being significant in six, *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, S. aureus, S. agalactiea* and *Enterobacter* spp, of the eleven bacteria (Tables 12, 13 and 14). The difference was also high for amoxicillin, which showed significant differences in 4 of the 8 bacteria tested, in *E. coli*, *P. mirabilis*, *E. faecalis*, *S. saprophyticus* (Tables 12, 13 and 15).

Table 12 Antimicrobial resistance pattern by age group for *Escherichia coli* and *Klebsiella pneumoniae*.

Antimicrobial group		Antimicrobials	Children (0-12 years)		Adolescents (13-18 years)		Young Adults (19-34 years)		Adults (35-64 years)		Elderly (>65 years)	
			N	%	N	%	N	%	N	%	N	%
Escherichia coli												
Aminoglycosides		Amikacin*	36	5.6	20	0.0	614	0.2	2530	0.7	4046	2.4
β-lactam	Cephalosporins 1st G	Cefazolin*	191	8.9	84	8.3	851	8.1	3265	11.8	5135	23.7
β-lactam	Cephalosporins 2nd G	Cefuroxime*	211	4.3	99	3.0	961	3.5	3666	5.9	5877	14.2
β-lactam	Cephalosporins 3rd G	Cefotaxime*	211	3.3	99	2.0	959	2.4	3659	4.5	5868	10.0
β-lactam	Penicilins	Amoxicillin*	211	40.8	99	42.4	961	35.5	3666	40.0	5879	53.4
β-lactam	Penicilins	AMX-CLA*	211	14.2	99	21.2	961	13.2	3663	14.9	5874	25.2
Quinolones		Ciprofloxacin*	13	7.7	21	9.5	924	7.1	3661	13.2	5870	27.5
Miscellaneous agents		Nitrofurantoin*	210	5.7	98	7.1	960	5.2	3665	5.5	5872	9.5
Miscellaneous agents		Fosfomycin*	195	0.5	88	1.1	873	0.6	3357	0.8	5316	1.5
Miscellaneous agents		SXT*	210	20.0	99	12.1	961	17.7	3666	19.1	5875	30.4
Klebsiella pneumoniae												
Aminoglycosides		Amikacin	1	0.0	2	0.0	37	2.7	185	4.9	727	3.7
β-lactam	Cephalosporins 1st G	Cefazolin*	4	25.0	8	12.5	48	18.8	234	29.1	811	44.4
β-lactam	Cephalosporins 2nd G	Cefuroxime*	5	20.0	10	10.0	57	12.3	296	21.3	1000	38.5
β-lactam	Cephalosporins 3rd G	Cefotaxime*	5	0.0	10	10.0	57	10.5	296	18.6	992	33.3
β-lactam	Penicilins	AMX-CLA*	5	80.0	10	70.0	57	47.4	296	54.1	997	65.2
Quinolones		Ciprofloxacin*	0	0.0	1	0.0	57	12.3	296	23.6	997	39.1
Miscellaneous agents		Nitrofurantoin	4	25.0	10	70.0	54	70.4	275	81.5	930	82.5
Miscellaneous agents		SXT*	5	20.0	10	10.0	57	17.5	296	76.4	997	58.3

* Statistically significant differences (p-value < 0.05) of antimicrobial resistance between age groups.

Table 13 Antimicrobial resistance pattern by age group for *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antimicrobial group		Antimicrobials	Children (0-12 years)		Adolescents (13-18 years)		Young Adults (19-34 years)		Adults (35-64 years)		Elderly (>65 years)	
			N	%	N	%	N	%	N	%	N	%
Proteus mirabilis												
Aminoglycosides		Amikacin	10	0.0	1	0.0	49	0.0	180	0.0	333	0.9
β-lactam	Cephalosporins 1st G	Cefazolin	46	13.0	13	0.0	56	10.7	214	14.5	408	16.9
	Cephalosporins 2nd G	Cefuroxime	45	6.7	16	0.0	68	0.0	246	3.7	471	4.7
	Cephalosporins 3rd G	Cefotaxime	45	4.4	16	0.0	67	0.0	246	1.2	470	1.7
	Penicilins	Amoxicillin*	46	63.0	16	0.0	68	25.0	246	28.9	471	43.5
		AMX-CLA	45	13.3	16	0.0	68	2.9	246	8.5	471	11.7
Quinolones		Ciprofloxacin*	4	25.0	2	0.0	65	4.6	246	8.1	471	31.0
Miscellaneous agents		SXT*	46	17.4	16	12.5	68	20.6	246	21.1	470	35.7
Staphylococcus aureus												
Aminoglycosides		Gentamicin*	1	0.0	8	0.0	62	0.0	121	1.7	78	11.5
Penicilins		AMX-CLA*	3	0.0	1	0.0	30	0.0	90	2.2	319	13.8
Quinolones		Ciprofloxacin*	2	50.0	0	0.0	31	29.0	100	45.0	353	49.3
Miscellaneous agents		Nitrofurantoin*	3	0.0	1	0.0	32	0.0	93	3.2	343	5.8
		SXT	5	0.0	22	4.5	90	12.2	165	8.5	101	29.7
Pseudomonas aeruginosa												
Aminoglycosides		Amikacin	3	0.0	0	0.0	3	33.3	64	20.3	354	16.7
		Gentamicin	2	0.0	0	0.0	3	33.3	64	31.3	359	30.9
		Tobramycin	1	0.0	0	0.0	3	33.3	61	21.3	353	21.2
β-lactam	Carbapenems	Imipenem	6	0.0	0	0.0	3	33.3	64	29.7	360	21.1
	Cephalosporins 3rd G	Ceftazidime	6	0.0	0	0.0	3	33.3	64	26.6	361	27.1
	Cephalosporins 4th G	Cefepime	6	0.0	0	0.0	3	0.0	63	23.8	362	20.4
	Monobactams	Aztreonam	6	0.0	0	0.0	3	0.0	63	15.9	359	16.7
	Penicilins	PIP-TAZ	6	0.0	0	0.0	3	33.3	64	18.8	362	20.4
Quinolones		Ciprofloxacin*	2	0.0	0	0.0	3	66.7	64	59.4	362	41.2

* Statistically significant differences (p-value < 0.05) of antimicrobial resistance between age groups.

Table 14 Antimicrobial resistance pattern by age group for *Enterococcus faecalis*, *Streptococcus agalactiea*, and *Enterobacter* spp.

Antimicrobial group	Antimicrobials	Children (0-12 years)		Adolescents (13-18 years)		Young Adults (19-34 years)		Adults (35-64 years)		Elderly (>65 years)	
		N	%	N	%	N	%	N	%	N	%
Enterococcus faecalis											
Miscellaneous agents	Fosfomycin	2	0.0	1	0.0	30	6.7	93	4.3	324	5.9
	Nitrofurantoin	3	0.0	1	0.0	32	0.0	93	3.2	343	5.8
Penicilins	Amoxicillin*	3	0.0	1	0.0	30	0.0	90	2.2	319	13.8
	Ampicillin	3	0.0	0	0.0	32	0.0	92	7.6	338	8.3
Quinolones	Ciprofloxacin	2	50.0	0	0.0	31	29.0	100	45.0	353	49.3
	Levofloxacin*	1	0.0	0	0.0	29	17.2	97	28.9	345	42.9
Streptococcus agalactiea											
β-lactam: Cephalosporins 3rd G	Cefotaxime	0	0.0	1	0.0	16	12.5	73	1.4	86	2.3
β-lactam: Penicilins	Amoxicillin	0	0.0	1	0.0	15	6.7	66	0.0	84	3.6
	Ampicillin	0	0.0	1	0.0	15	0.0	74	0.0	85	2.4
Miscellaneous agents	Nitrofurantoin	0	0.0	1	0.0	13	0.0	61	1.6	71	0.0
	SXT	0	0.0	0	0.0	14	42.9	54	29.6	66	47.0
Quinolones	Ciprofloxacin*	0	0.0	1	0.0	10	50.0	49	2.0	56	7.1
	Levofloxacin	0	0.0	0	0.0	15	6.7	72	2.8	86	11.6
	Ofloxacin	0	0.0	0	0.0	11	9.1	68	4.4	65	12.3
Enterobacter spp											
Aminoglycosides	Amikacin	0	0.0	2	0.0	5	0.0	34	2.9	98	5.1
Cephalosporins 2nd G	Cefuroxime*	0	0.0	2	50.0	5	60.0	36	83.3	101	77.2
Cephalosporins 3rd G	Cefotaxime	0	0.0	2	0.0	5	0.0	36	0.0	99	28.3
Quinolones	Ciprofloxacin*	0	0.0	0	0.0	5	0.0	36	5.6	101	27.7
Miscellaneous agents	SXT	0	0.0	2	0.0	36	11.1	101	27.7	144	22.2

* Statistically significant differences (p-value < 0.05) of antimicrobial resistance between age groups.

Table 15 Antimicrobial resistance pattern by age group for *Proteus vulgaris*, *Staphylococcus saprophyticus* and *Klebsiella oxytoca*.

Antimicrobial group		Antimicrobials	Children (0-12 years)		Adolescents (13-18 years)		Young Adults (19-34 years)		Adults (35-64 years)		Elderly (>65 years)	
			N	%	N	%	N	%	N	%	N	%
Proteus vulgaris												
Aminoglycosides		Amikacin*	1	0.0	0	0.0	1	0.0	13	15.4	85	0.0
β-lactam	Cephalosporins 3rd G	Cefotaxime	3	0.0	1	0.0	1	0.0	16	6.3	102	4.9
β-lactam	Penicilins	AMX-CLA	3	0.0	1	0.0	1	0.0	17	64.7	102	60.8
Quinolones		Ciprofloxacin	0	0.0	0	0.0	1	0.0	17	41.2	102	43.1
Miscellaneous agents		SXT	3	0.0	1	100.0	1	0.0	17	47.1	102	42.2
Staphylococcus saprophyticus												
Quinolones		Ciprofloxacin	0	0.0	2	0.0	39	5.1	63	4.8	10	10.0
Miscellaneous agents		Nitrofurantoin	2	0.0	5	0.0	40	2.5	63	4.8	10	10.0
Miscellaneous agents		SXT	2	0.0	5	0.0	40	7.5	63	11.1	10	30.0
Penicilins		Amoxicillin*	2	0.0	4	25.0	32	56.3	56	46.4	9	55.6
Penicilins		AMX-CLA	2	0.0	5	0.0	40	10.0	63	3.2	10	20.0
Klebsiella oxytoca												
Aminoglycosides		Amikacin	0	0.0	0	0.0	3	0.0	21	0.0	80	2.5
β-lactam	Cephalosporins 1st G	Cefazolin	0	0.0	0	0.0	3	0.0	22	31.8	77	33.8
β-lactam	Cephalosporins 2nd G	Cefuroxime	1	0.0	0	0.0	4	0.0	25	8.0	90	20.0
β-lactam	Cephalosporins 3rd G	Cefotaxime	1	0.0	0	0.0	4	0.0	25	4.0	90	15.6
β-lactam	Penicilins	AMX-CLA	1	0.0	0	0.0	4	0.0	25	48.0	90	60.0
Quinolones		Ciprofloxacin	0	0.0	0	0.0	4	0.0	25	8.0	90	24.4
Miscellaneous agents		Nitrofurantoin	1	0.0	0	0.0	3	100.0	24	79.2	85	77.6
Miscellaneous agents		SXT	1	0.0	0	0.0	4	0.0	25	20.0	90	24.4

* Statistically significant differences (p-value < 0.05) of antimicrobial resistance between age groups.

4.4 Multidrug resistant bacteria implicated in UTI

The percentage of multidrug resistant bacteria was calculated and MDR isolates differed between 6% and 40%. The most common MDR bacteria implicated in UTI were *K. pneumoniae* (40.4%) and *P. aeruginosa* (34.7%) (Table 5). The biggest responsible bacteria for UTI, *E. coli*, showed a multidrug resistance of 23.3%. *S. saprophyticus* and *S. agalactiea* presented the lowest multidrug resistance (5.8% and 6.8%) respectively (Table 5). No significant differences (Chi-Square test, $p < 0.05$) in the incidence of MDR bacteria were observed during the period studied.

The incidence of MDR bacteria was higher in the elderly (Chi-Square test, $p < 0.05$).

Chapter 5

5. Discussion

As already observed in other studies, *E. coli* was the most common bacteria implicated in UTI, being responsible for more than half of the infections (70.1%). *E coli* is part of the intestinal flora and therefore easily colonizes the urinary tract, causing more frequently cystitis. However, this bacterium can ascend through the ureters to the kidneys, causing more serious infections such as pyelonephritis (Flores-Mireles et al., 2015). *K. pneumoniae* (8.9%) and *P. mirabilis* (5.5%) were, respectively, the second and third uropathogenic more implicated in UTI, such as observed in other studies at community level (Costa et al., 2018; Curto et al., 2019; Passadouro et al., 2014).

Even though *E. coli* was the most common uropathogen in both sexes, its incidence was significantly higher in women (76.2%) than in men (55.6%), likely due to anatomic and physiological reasons, since the length of the urethra is smaller for women, allowing the enterobacteria to rise the bladder easier. As observed in other studies (Amna et al., 2013; Costa et al., 2018; Linhares et al., 2013), *E. faecalis* and *P. aeruginosa* were the bacteria that most contributed to the differences between males and females. Both bacteria were more frequently associated with male infections (8.8% and 8.1% for *E. faecalis* and *P. aeruginosa,* respectively, in males, against 1.8% and 1.6% in females). In males, UTIs are frequently more complicated, due to anatomic abnormalities, requiring surgical intervention (e.g. catheterization) (Sabih & Leslie, 2020). Therefore, non-*E. coli* uropathogens (such as *E. faecalis* and *P. aeruginosa*) are more likely implicated in UTI in males, since these bacteria are frequently related to complicated UTIs (Amna et al., 2013; McLellan & Hunstad, 2016). In fact, *Enterococcus* and *Pseudomonas* have been associated with infections related to catheters in the upper urinary tract (Cole et al., 2014; Lara-Isla et al., 2017; Tien et al., 2017).

As it has been documented before (Costa et al., 2018; Foxman, 2014; Linhares et al., 2013; Sanchez et al., 2016), urinary tract infections increased with the patient age. The elderly was responsible for more than half of the infections (56.9%). A variety of reasons can explain this increase, since the elderly are more prone to frequent hospitalizations which exposes them to nosocomial pathogens, the residence in care

facilities, the frequent use of antimicrobials, the frequent use of urogenital catheters, the decrease in adaptive and innate immunity, and also previous cases of UTI (Costa et al., 2018; Rowe & Juthani-Mehta, 2014). Moreover, post-menopause women go through changes in vaginal flora because of the declining of estrogen levels, decreasing production of glycogen, and diminish of the presence of *Lactobacillus*, which decreases the defences against UTI. For older man, prostatic hypertrophy may cause urinary retention and high postvoid residuals, which may also help developing UTI (Rowe & Juthani-Mehta, 2014).

Even though one of the risks and causes for UTI in adolescents to rise is the beginning of sexual activity (Becknell et al., 2015), children in this study had more cases of UTI than adolescents. The biggest number of infections in children may be related to first few months of life, when infants have their susceptibility heightened due to an incomplete developed immune system, or uncircumcised boys during first years of life (Becknell et al., 2015). Another explanation might be because at young age, *Lactobacillus* is absent, so the female vagina is pH neutral and does not produce glycogen (Madigan et al., 2018), allowing the easier colonization of uropathogens.

The occurrence of *P. mirabilis* was higher in children and adolescents, as seen before in other studies (Costa et al., 2018; Linhares et al., 2013). This incidence may be explained by the presence of this bacterium in the preputial sac of young boys, has it has been isolated in 13.7% of uncircumcised males up to 8 years of age (Laway et al., 2012). However, for young girls this uropathogen also represents an important role, despite of its low frequency in the young female genital tract flora, being most likely from fecal flora due to an inadequate hygiene (Beyitler & Kavukcu, 2017). The frequency of *S. saprophyticus* implicated in the UTI was more notorious in young women than in men in general. It has been reported that UTI caused by *S. saprophyticus* in young females is associated with recent sexual intercourse and the use of vaginal spermicides (interfering with the normal vaginal flora), but can still be found in men with UTI occasionally (Raz et al., 2005).

Comparing our results to Linhares et al., (2013), the incidence of some of the main bacteria responsible for UTI changed. *E. coli* remained the principal uropathogen for the infections, but *S. aureus* decreased, passing from second pathogen responsible (6.0%) to

sixth (2.5%) in the current study. This may be related to changes in the detection method, as *Staphylococcus* represented changes in the incident bacteria in 10 years difference. Another difference is *Klebsiella,* as 10 years ago only represented 4.3% of the urinary infections, but *K. pneumoniae* was the second most found microorganism in our study, 8.9%, which may be related to the increase in resistance rates detected. The bacteria least found in both studies varied, but since these uropathogens had very small and similar percentages and it is easy to find variations.

The incidence of *E. coli*, the most important uropathogen implicated in UTI, was constant during the period of the study. However, *K. oxytoca* and *K. pneumonia* incidence in UTI showed an increase over the course of the years. With average life expectancy increasing, its expectable for elderly patients' hospitalization to increase as well, inducing a rise in the transmission of bacteria strains between hospital and the community. Being *K. pneumonia* and *K. oxytoca* commonly found in hospital environment, this may explain the increase of both, since there was in fact an increase of these bacteria for the elderly group during the study period.

Gram-negative bacteria were in general more resistant than Gram-positive bacteria. The outer membrane of Gram-negative bacteria is the main reason for their resistance to a large range of antibiotics. Gram-negative outer membrane alterations such as changing hydrophobic properties or mutations in porins and other factors, can create resistance (Breijyeh et al., 2020). Gram-positive bacteria lack this important layer, making them less resistant to antibiotics than Gram-negative ones.

The uropathogen responsible for more than half of the UTI, *E. coli*, was the bacteria that presented the lowest antimicrobial resistance. Its resistance to first line antimicrobials recommended by EAU for uncomplicated cystitis UTI, fosfomycin (1.4%) and nitrofurantoin (7%), was considerably low, therefore, these drugs can be considered suitable for the empirical treatment of *E. coli* UTI in the studied community. However, *K. pneumonia,* the second most frequent bacteria, presented resistance to this antimicrobial in 81.7% of the cases, a value higher than the usual. Zanichelli et al. (2019) also observed high resistance of *K. pneumonia* to nitrofurantoin in an 8-year study (2008-2016). Similar results were also obtained in a UTI community study in Russia (Rafalskiy et al., 2020).

Relatively to the alternative antimicrobials recommended by EAU, some of the studied antibiotics were adequate to treat UTI empirically in the community. The cephalosporins were effective against *E. coli*, only less than 20% of the isolates were resistant to this drug. Cefotaxime was the most effective against *E. coli*, with 7.2% of the isolates resistant, showing a resistance close to nitrofurantoin, a first-line antimicrobial. This antibiotic can be also a good option against other bacteria since their resistance to this drug was not high. Taking into consideration the pondered resistance results, it can be suggested that cephalosporins may be a better option than nitrofurantoin (suggested as a first line therapy) to empirical treatment since they proved to have a smaller pondered resistance.

According to the Bonkat et al. (2018), antibiotics can be used empirically against bacteria to treat cystitis when the resistance to these drugs is lower than 20%. In this study, the resistance of *E. coli* to SXT was 24.8%, being even higher in bacteria isolated from men (33.1%), so this antimicrobial should not be an option in this area. The resistance of *E. coli* to ciprofloxacin and AMX-CLA was 20.5% and 20.3%, respectively. Similar bacterial resistance values, close to 20% to these antibiotics has been observed in other studies (Costa et al., 2018; Curto et al., 2019; Passadouro et al., 2014).

The results of this study support the idea that the choice of empirical antimicrobial therapy should consider the sex of the patient. On average, uropathogens isolated from male patients registered a higher resistance to antimicrobials. Linhares et al. (2013) in a study done in the same area, also found significant differences in susceptibility pattern between women and men. The average number of antimicrobials resistant to the uropathogens according to the sex of the patients was calculated, and males proved to be resistant to a higher number of antimicrobials. This may be due to men being more associated to other uropathogens than *E. coli,* since *E. coli* proved to be one of the bacteria less resistant to the antimicrobials. In fact, males were more related to *P. aeruginosa,* a bacterium usually found in hospital environment and therefore more prone to resistances.

For the empirical treatment of pyelonephritis, it is recommended by EAU, antimicrobials that can reach adequate renal tissue levels, such as quinolones and

cephalosporins, if the resistance local rates are lower than 10%. This was not the case of this study for quinolones, as most uropathogens showed a resistance higher than 20%, indicating that this drug should not be prescribed empirically in cases of pyelonephritis. The cephalosporins can be a good option, but it should be taken into account that oral cephalosporins achieve significantly lower concentrations than intravenous cephalosporines (Bonkat et al., 2018).

In the treatment of complicated UTI, if an intravenous therapy is to be applied, an amoxicillin with the addition of an aminoglycoside or a cephalosporin $2^{nd}/3^{rd}$ generation with an aminoglycoside are recommended by EAU (Bonkat et al., 2018). For this case, the second option is a safer choice since amoxicillin revealed higher resistance rates than cephalosporins. The aminoglycoside selected can be amikacin, as its resistance levels were low. The ciprofloxacin in these cases is recommended as an oral option, but the local rates should be lower than 10%, which is not the case for the region of this study.

The antimicrobial resistance, in general, as already observed in other studies increased with age (Passadouro et al., 2014; Sanchez et al., 2016). The main bacteria isolated in this study showed significant differences between the different age groups, probably because older patients are most likely to have recurring infections due to frequent hospitalizations, which allows the transmission of bacterial resistance between hospital and community. However, resistance increases varies by antibiotic class, possibly reflecting variation in the rates of the prescribed antibiotics (Cortes-Penfield et al., 2017).

Multidrug resistance is a risk factor for inappropriate empirical treatment, and it is associated with an increased mortality. This study detected high numbers of bacteria resistant to 3 or more antimicrobial classes. The bacteria most responsible for UTI, *E. coli* and *K. pneumoniae*, reached 23% and 40%, respectively, while *P. aeruginosa* showed 35% of MDR bacteria. Comparing these results to a study done 10 years ago, in the same area (Linhares et al., 2013), it was noticed an increase in MDR bacteria. Linhares et al. (2013) observed a rate of MDR of 17% for *E. coli*, 35% for *Klebsiella spp,* and 25% for *P. aeruginosa*. These results reinforce the problem of resistant bacteria in the community, as a growing problem in our society. This increase in resistance is probably due to the

misuse and/or overuse of antibiotics and transmission of resistance between community and hospitals.

For the treatment of UTI caused by multidrug resistant bacteria, imipenem and amikacin are good options as last resort antimicrobials, such as observed before (Cho et al., 2016; Costa et al., 2018; Linhares et al., 2013).

Even though *E. coli* did not show huge changes in antibiotic resistance when comparing our results to results from 10 years ago (Linhares et al., 2013), we can see increases of resistance bigger than 10% for cefazolin, cefuroxime, AMX-CLA, nitrofurantoin, and ciprofloxacin for *K. pneumoniae* when compared to *Klebsiella* spp. Also, *P. aeruginosa* showed an increase higher than 10% in resistance to gentamicin, imipenem, ceftazidime, cefepime and aztreonam. *P. mirabilis* exhibited a rise in resistance bigger than 10% for amoxicillin and SXT, but other tested antimicrobials showed a decrease in resistance. *P. vulgaris* registered resistances rates at least 18% higher when compared to 10 years ago for AMX-CLA and ciprofloxacin, although it decreased resistance 15% for SXT.

Comparing our results to Linhares et al., (2013), it was registered an increase in age for the tested samples. The mean age of our data was 64 years old while for Linhares was 54 years old. Patients from the current study were more than half (56.9%) composed by elderly, while for Linhares et al., only 38.6% of the patients were from that age group. This may help explain the increase in resistance, as older patients are associated to higher resistance to antimicrobials for the reasons described above, as elderly are most likely to have frequent hospitalizations, transmitting bacterial resistance between hospital and community.

Chapter 6

6. Conclusion

The results obtained in this study indicated *E. coli* as the most prevalent uropathogen, being responsible for more than half of the urinary tract infections. As age increased, differences between female and male increased as well.

Even though *E. coli* was the most prevalent uropathogen, this bacterium was between the most susceptible to antibiotics. *E. coli* was susceptible to nitrofurantoin and fosfomycin, the first-line drugs indicated to treat uncomplicated UTI according to EAU, but the same was not observed for nitrofurantoin in other Gram-negative uropathogens. Since *E. coli* is by far, the uropathogen responsible for more cases of UTI (70%), these antibiotics can still be considered good choices for empirical therapy. Although, when the clinical history of the patient indicates recent hospitalizations or previous cases of UTI by other Gram-negative isolates, nitrofurantoin should not be a first option. *E. coli* presented higher resistance to the alternative antibiotics SXT, ciprofloxacin, and AMX-CLA when compared to first line antibiotics, but cephalosporins, such as cefuroxime and cefotaxime (cephalosporins from 2nd and 3rd generation), can be a good alternative treatment. According to the EUA, SXT, AMX-CLA, and ciprofloxacin should not be suitable to treat UTI empirically patients of the studied region. For the empirical treatment of pyelonephritis, cephalosporins are the better option in terms of resistance, even though oral cephalosporins reach lower concentrations than intravenous cephalosporins. To treat severe cases of UTI, such as multi-drug resistant bacteria, imipenem and amikacin can be considered as good last resource antimicrobials.

As urinary tract infection is a very common illness, its diagnosis and treatment have significant implications for patient's health and growth of antibiotic resistance. In summary, it can be stated that monitoring periodically the microbial resistance of each region is essential in order to perform the best empirical antibiotic therapy against these infections and prevent or decrease the resistance among uropathogens strains.

Chapter 7

7. References

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Annexes

Table 16 Evolution of resistance of *Escherichia coli* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	1290	1.7	1961	0.7	1895	0.7	1689	2.0	411	8.5
		Gentamicin	69	39.1	21	57.1	39	61.5	52	48.1	147	52.4
		Tobramycin	102	48.0	22	63.6	41	78.0	52	67.3	82	73.2
β-lactam	Carbapenems	Imipenem	61	4.9	23	17.4	42	7.1	52	11.5	135	4.0
	Cephalosporins 1st G	Cefazolin	2044	15.4	2038	16.8	1963	16.8	2308	18.4	2073	24.2
	Cephalosporins 2nd G	Cefuroxime	2044	8.7	2038	10.1	1963	9.0	2310	9.4	2459	13.1
	Cephalosporins 3rd G	Cefotaxime	2041	6.3	2038	6.2	1962	5.5	2307	6.8	2449	10.6
		Ceftazidime	58	69.0	23	60.9	45	68.9	52	55.8	62	73.5
	Cephalosporins 4th G	Cefepime	58	55.2	22	50.0	42	57.1	52	55.8	57	72.6
	Monobactams	Aztreonam	61	63.9	23	65.2	42	76.2	51	68.6	82	68.3
	Penicilins	Amoxicillin	2044	48.4	2038	46.0	1963	46.7	2310	45.9	2461	47.7
		AMX-CLA	2043	20.3	2038	16.2	1960	18.3	2306	20.9	2461	25.1
		PIP-TAZ	62	41.9	22	36.4	41	22.0	51	43.1	160	37.2
Quinolones		Ciprofloxacin	1999	20.3	1961	19.4	1898	20.7	2240	19.7	2391	23.0
Miscellaneous agents		Nitrofurantoin	2040	9.0	2037	6.5	1963	6.0	2309	8.8	2456	7.6
		Fosfomycin	1129	1.3	2025	1.1	1946	0.2	2292	1.2	2437	3.1
		SXT	2042	26.2	2037	21.9	1962	24.4	2310	24.9	2460	27.4

Table 17 Evolution of resistance of *Klebsiella pneumoniae* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	149	0.7	171	0.6	229	0.9	243	5.8	160	11.9
		Gentamicin	42	52.4	33	75.8	62	67.7	73	54.8	147	55.1
		Tobramycin	95	33.7	30	86.7	61	77.0	73	65.8	96	74.0
β-lactam	Carbapenems	Imipenem	45	2.2	33	6.1	63	3.2	74	6.8	140	10.7
	Cephalosporins 1st G	Cefazolin	173	45.1	173	34.7	233	33.5	313	41.2	213	44.1
	Cephalosporins 2nd G	Cefuroxime	173	33.5	173	30.1	233	31.8	313	31.9	476	36.3
	Cephalosporins 3rd G	Cefotaxime	173	27.2	173	26.0	233	28.3	313	27.2	468	28.8
		Ceftazidime	43	72.1	32	75.0	63	87.3	74	90.5	148	83.8
	Cephalosporins 4th G	Cefepime	47	51.1	33	66.7	63	60.3	74	77.0	144	80.6
	Monobactams	Aztreonam	46	80.4	33	81.8	63	88.9	74	89.2	96	83.3
	Penicilins	AMX-CLA	173	79.8	173	82.1	233	82.0	312	53.2	474	44.5
		PIP-TAZ	44	31.8	33	30.3	61	42.6	73	50.7	146	54.1
Quinolones		Ciprofloxacin	170	38.8	171	31.6	229	37.1	309	28.8	472	36.9
Miscellaneous agents		Nitrofurantoin	169	87.0	171	82.5	233	88.0	311	80.4	389	76.3
		SXT	172	38.4	173	35.3	233	38.2	313	32.9	474	37.8

Table 18 Evolution of resistance of *Proteus mirabilis* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	106	0.9	149	0.7	136	0.7	158	0.0	24	0.0
β-lactam	Cephalosporins 1st G	Cefazolin	130	17.7	165	12.7	153	10.5	171	16.4	118	20.3
	Cephalosporins 2nd G	Cefuroxime	130	4.6	165	3.0	152	1.3	171	2.9	228	7.0
	Cephalosporins 3rd G	Cefotaxime	130	0.0	164	0.6	153	1.3	171	1.8	226	3.1
	Penicillins	Amoxicillin	130	47.7	165	35.2	153	37.3	171	29.8	228	36.0
		AMX-CLA	130	13.1	165	9.1	152	7.9	171	9.9	228	10.1
Quinolones		Ciprofloxacin	124	25.0	149	20.1	136	13.2	158	20.3	221	26.7
Miscellaneous agents		SXT	130	45.4	164	26.8	153	28.1	171	24.6	228	24.6

Table 19 Evolution of resistance of *Proteus vulgaris* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	20	10.0	20	0.0	24	0.0	25	0.0	11	0.0
β-lactam	Cephalosporins 3rd G	Cefotaxime	21	4.8	20	0.0	25	8.0	31	3.2	26	7.7
	Penicilins	AMX-CLA	22	72.2	20	35.0	25	60.0	31	61.3	26	61.5
Quinolones		Ciprofloxacin	22	45.5	20	40.0	24	45.8	29	44.8	25	36.0
Miscellaneous agents		SXT	22	50.0	20	40.0	25	36.0	31	48.4	26	34.6

Table 20 Evolution of resistance of *Enterobacter spp* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	33	0.0	28	7.1	30	0.0	20	15.0	28	3.6
β-lactam	Cephalosporins 2nd G	Cefuroxime	33	90.9	28	96.4	30	60.3	22	63.6	31	74.2
	Cephalosporins 3rd G	Cefotaxime	32	18.8	27	11.1	30	16.7	22	31.8	31	29.0
Quinolones		Ciprofloxacin	32	15.6	28	21.4	30	16.7	22	36.4	30	20.0
Miscellaneous agents		Nitrofurantoin	33	78.8	28	75.0	30	73.3	22	81.8	25	60.0
		SXT	33	27.3	28	14.3	30	26.7	22	22.7	31	19.4

Table 21 Evolution of resistance of *Klebsiella oxytoca* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	8	0.0	2	0.0	30	0.0	47	2.1	15	6.3
β-lactam	Cephalosporins 1st G	Cefazolin	9	44.4	2	0.0	30	16.7	48	37.5	13	46.2
	Cephalosporins 2nd G	Cefuroxime	9	22.2	2	0.0	30	10.0	48	18.8	31	19.4
	Cephalosporins 3rd G	Cefotaxime	9	11.1	2	0.0	30	10.0	48	12.5	31	16.1
	Penicilins	AMX-CLA	9	100.0	2	100.0	30	96.7	48	39.6	31	22.6
Quinolones		Ciprofloxacin	9	33.3	2	0.0	30	23.3	48	16.7	30	20.0
Miscellaneous agents		Nitrofurantoin	9	66.7	2	100.0	30	100.0	45	71.1	27	66.7
		SXT	9	22.2	2	0.0	30	23.3	48	25.0	31	19.4

Table 22 Evolution of resistance of *Pseudomonas aeruginosa* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
Aminoglycosides		Amikacin	90	13.3	107	20.6	89	10.1	56	28.6	82	17.1
		Gentamicin	91	30.8	107	36.4	90	20.0	58	41.4	82	28.0
		Tobramycin	91	25.3	100	27.0	90	18.9	57	19.3	80	13.8
β-lactam	Carbapenems	Imipenem	94	20.2	107	29.0	91	17.6	60	18.3	81	23.5
	Cephalosporins 3rd G	Ceftazidime	95	25.3	106	30.2	91	23.1	60	25.0	82	29.3
	Cephalosporins 4th G	Cefepime	94	16.0	107	25.2	91	14.3	60	25.0	82	23.2
	Monobactams	Aztreonam	95	15.8	107	16.8	91	9.9	60	18.3	78	21.8
	Penicillins	PIP-TAZ	95	22.1	107	23.4	91	16.5	60	20.0	82	17.1
Quinolones		Ciprofloxacin	92	44.6	107	46.7	90	41.1	60	40.0	82	45.1

Table 23 Evolution of resistance of *Enterococcus faecalis* to antimicrobials during the study period.

Antimicrobial groups	Antimicrobial	2015		2016		2017		2018		2019	
		N	%	N	%	N	%	N	%	N	%
Penicilins	Ampicillin	109	6.4	114	3.5	74	5.4	70	8.6	98	14.3
	Amoxicillin	108	6.5	111	8.1	73	12.3	53	13.2	98	14.3
Miscellaneous agents	Nitrofurantoin	105	1.9	111	4.5	72	6.9	70	7.1	114	5.3
	Fosfomycin	107	7.5	115	6.1	74	2.7	72	8.3	82	2.4
Quinolones	Ciprofloxacin	108	33.3	115	24.3	75	36.0	73	41.1	102	56.9
	Levofloxacin	106	42.5	113	38.1	75	36.0	72	37.5	106	36.8

Table 24 Evolution of resistance of *Staphylococcus aureus* to antimicrobials during the study period.

Antimicrobial groups	Antimicrobial	2015		2016		2017		2018		2019	
		N	%	N	%	N	%	N	%	N	%
Aminoglycosides	Gentamicin	83	1.2	73	5.5	74	4.1	32	3.1	8	25.0
Penicilins	AMX-CLA	84	8.3	77	6.5	77	19.5	66	7.6	75	16.0
Quinolones	Ciprofloxacin	82	13.4	73	9.6	75	26.7	64	12.5	71	15.5
Miscellaneous agents	Nitrofurantoin	86	7.0	77	2.6	78	1.3	67	4.5	66	15.2
	SXT	86	11.6	77	16.9	78	11.5	67	22.4	75	12.0

Table 25 Evolution of resistance of *Staphylococcus saprophyticus* to antimicrobials during the study period.

Antimicrobial groups	Antimicrobial	2015		2016		2017		2018		2019	
		N	%	N	%	N	%	N	%	N	%
Penicilins	Amoxicillin	15	46.7	22	40.9	12	33.3	24	50.0	30	60.0
	AMX-CLA	16	0.0	25	16.0	18	0.0	25	8.0	36	5.6
Quinolones	Ciprofloxacin	15	6.7	24	4.2	18	0.0	23	4.3	34	8.8
Miscellaneous agents	Nitrofurantoin	16	12.5	25	4.0	18	0.0	25	0.0	36	5.6
	SXT	16	6.3	25	12.0	18	5.6	25	12.0	36	13.9

Table 26 Evolution of resistance of *Streptococcus agalactiea* to antimicrobials during the study period.

Antimicrobial groups		Antimicrobial	2015		2016		2017		2018		2019	
			N	%	N	%	N	%	N	%	N	%
β-lactam	Cephalosporins 3rd G	Cefotaxime	52	3.8	27	0.0	36	0.0	26	3.8	35	5.7
β-lactam	Penicilins	Ampicillin	53	0.0	27	3.7	35	0.0	26	0.0	34	2.9
β-lactam	Penicilins	Amoxicillin	51	3.9	22	0.0	35	0.0	24	8.3	34	1.9
Miscellaneous agents		SXT	32	25.0	21	28.6	27	22.2	20	35.0	34	76.5
Miscellaneous agents		Nitrofurantoin	48	0.0	21	0.0	21	0.0	21	4.8	35	0.0
Quinolones		Ciprofloxacin	19	10.5	20	5.0	29	6.9	18	11.1	30	10.0
Quinolones		Levofloxacin	53	5.7	26	11.5	35	2.9	25	8.0	34	11.8
Quinolones		Ofloxacin	53	5.7	26	11.5	28	3.6	21	23.8	16	0.0