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ANTIMICROBIAL SUSCEPTIBILITY OF URINARY TRACT PATHOGENS OF RESIDENTS IN A LONG- AND MEDIUM-TERM HEALTHCARE FACILITY IN NORTH OF PORTUGAL

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AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA DISSERTAÇÃO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE

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Ricardo André Torres da Silva

“Never stop trying.
Never stop believing.
Never give up. Your day will come!”

Mandy Hale

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Ao concluir esta Dissertação de Mestrado quero expressar o meu reconhecimento a todas as instituições universitárias, pelas quais passei, e que cultivaram em mim a constante necessidade de aperfeiçoamento, aquisição e atualização do conhecimento.

"*Eu descobri que sempre tenho escolhas.*
E muitas vezes, trata-se apenas de uma escolha de atitude."
Judith M. Knowlton

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A minha FAMILIA… Os meus AMIGOS… Tem sido um longo percurso, mas eu sei que estarão sempre comigo.

ABSTRACT

Urinary tract infections (UTIs) are one of the most common healthcare-associated infections (HAIs), including in long and medium-term healthcare facilities (LMTHF), situated at the interface of the hospital and the community settings. In this setting (LMTHF), UTIs are an increasing challenge due to the high occurrence rates, the frequent resident's comorbidities, the diversity of etiologic agents and the diagnostic difficulties. Furthermore, the emergence of resistance to critically important antibiotics restricts the available therapeutic options to treat UTIs and determines the need of monitoring etiologic agents and antibiotic resistance profiles in order to improve diagnosis, treatment and prevention. However, in Portugal, scarce data on the species and antimicrobial susceptibility of bacterial pathogens responsible for UTI in LMTHF is available.

The **aim** of the present study was to evaluate the trends of etiologic agents of UTIs and their antimicrobial resistance patterns in patients of a LMTHF, in the North of Portugal, between november 2010 and august 2015.

A total of 691 urine samples were retrospectively analyzed, from which 471 urine samples (68.2%) were positive for bacterial growth, where 512 uropathogens were identified. *Escherichia coli* was the most common species (38.5%), followed by *Klebsiella pneumoniae* (26.8%), *Proteus* spp. (8.6%) and *Pseudomonas aeruginosa* (7.2%), whilst *Enterococcus faecalis* (4.3%) was the most frequent Gram-positive species. Multi-drug resistance patterns were frequently observed (n=242/512; 47.3%), with the highest rates registered for *K. pneumoniae* (59.1%). The highest antimicrobial resistance rates were observed for penicillins (61.4%-97.5%), followed by fluoroquinolones (43.5%-55.0%) and trimethoprim/sulfamethoxazole (49.6%). On the other hand, nitrofurantoin, cefoxitin, amikacin, piperacillin/tazobactam, carbapenems and glycopeptides were the most active antibiotics (2.0%-10.0%). Amongst the most frequent uropathogens identified (*E. coli* and *K. pneumoniae*), the antibiotic resistance rates to extended-spectrum cephalosporins, trimethoprim/sulfamethoxazole and ciprofloxacin increased substantially, especially after 2011, whereas imipenem, nitrofurantoin and fosfomycin remained stable during the whole period studied (<6.0%). Extended spectrum β-lactamase (ESBL) producers were detected in 24.1% (n=101/419) of *Enterobacteriaceae* isolates, being of note the recent increase of ESBL-producing *K. pneumoniae* (reaching 72.2% in 2015). Two ESBL-producing *K. pneumoniae* strains were simultaneously carbapenemase-producers (KPC-type). Statistically significant differences were not observed between gender and incidence of ESBL-producing strains (*P*=0.524).

The **general conclusion**: This study alerts for the increasing rates of multidrug resistance among urinary pathogens causing UTIs in patients institutionalized in a LMTHF in Portugal, and highlights the need to adapt empirical treatment choices and infection control practices to local data.

KEYWORDS: Antimicrobial Resistance, Extended-spectrum β-lactamases, Multi-drug Resistance, Uropathogens

RESUMO

As infeções do trato urinário (UTIs) são uma das principais infeções associadas aos cuidados de saúde (HAIs), incluindo as unidades de cuidados de saúde de longa e média duração (LMTHF), caracterizadas como a interface entre o ambiente hospitalar e a comunidade. Neste ambiente (LMTHF), as UTIs são um desafio crescente devido às elevadas taxas de ocorrência, às frequentes comorbilidades associadas aos residentes, à diversidade de agentes etiológicos e às dificuldades de diagnóstico. Além disso, a emergência de resistência aos antibióticos criticamente importantes limita as opções terapêuticas disponíveis para o tratamento de UTIs e determina a necessidade de monitorizar os agentes etiológicos e os perfis de resistência aos antibióticos para melhorar o diagnóstico, o tratamento e a prevenção. No entanto, em Portugal, os dados sobre as espécies e suscetibilidade aos antimicrobianos das bactérias responsáveis pelas UTIs em LMTHF é escassa.

O **objetivo** do presente estudo foi avaliar as tendências dos agentes etiológicos das UTIs e seus padrões de resistência aos antimicrobianos em doentes de um LMTHF, no Norte de Portugal, entre novembro de 2010 e agosto de 2015.

Um total de 691 amostras de urina foram analisadas retrospetivamente, das quais 471 (68,2%) foram positivas para crescimento bacteriano, tendo sido identificados 512 patogénicos urinários. *E. coli* foi a espécie mais comum (38,5%), seguida de *K. pneumoniae* (26,8%), *Proteus* spp. (8,6%) e *Pseudomonas aeruginosa* (7,2%), enquanto *Enterococcus faecalis* (4,3%) foi a espécie Gram-positiva mais frequente. Padrões de resistência a múltiplos antibióticos foram observados frequentemente (n=242/512; 47,3%), com elevadas taxas registadas para *K. pneumoniae* (59,1%). As taxas de resistência aos antimicrobianos mais elevadas foram observadas para as penicilinas (61,4%-97,5%), seguidas pelas fluoroquinolonas (43,5%-55,0%) e trimetoprim/sulfametoxazol (49,6%). Por outro lado, a nitrofurantoína, cefoxitina, amicacina, piperacilina/tazobactam, carbapenemos e glicopéptidos foram os antibióticos mais ativos (2,0%-10,0%). Entre os uropatogénicos mais frequentemente identificados (*E. coli* e *K. pneumoniae*), as taxas de resistência aos antibióticos para as cefalosporinas de largo espetro, trimetoprim/sulfametoxazol e ciprofloxacina aumentaram substancialmente, especialmente depois de 2011, ao passo que o imipenem, a nitrofurantoína e a fosfomicina mantiveram-se estáveis durante todo o período estudado (<6,0%). β-lactamases de espetro alargado (ESBL) foram detetadas em 24,1% (n=101/419) dos isolados de *Enterobacteriaceae*, salientando o recente aumento de *K. pneumoniae* produtoras de ESBLs (atingindo 72,2% em 2015). Duas estirpes de *K. pneumoniae*

produtoras de ESBLs foram simultaneamente produtoras de carbapenemases do tipo KPC. Estatisticamente não foram observadas diferenças significativas entre género e incidência de estirpes produtoras de ESBLs (*P*=0,524).

A conclusão geral: Este estudo alerta para o aumento das taxas de multirresistência entre os patogénicos urinários que causam UTIs em doentes institucionalizados de uma LMTHF em Portugal, e destaca a necessidade de ajustar as opções terapêuticas para o tratamento empírico e as práticas de controlo de infeção, de acordo com os dados locais.

PALAVRAS-CHAVE: Resistência aos antimicrobianos, β-lactamases de espetro alargado, Resistência a múltiplos antibióticos, Uropatogénicos

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	DESCRIPTION
AMPs	Antimicrobial peptides
APEC	Avian pathogenic *E. coli*
ASB	Asymptomatic bacteriuria
CaOx	Calcium oxalate
DAEC	Diffusely adherent *E. coli*
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohaemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBL	Extended spectrum β-lactamase
ETEC	Enterotoxigenic *E. coli*
ExPEC	Extraintestinal pathogenic *E. coli*
FDA	Food and Drug Administration
GPs	General practitioners
HAIs	Healthcare-associated infections
HNS	Health national service
IPC	Infection prevention and control
IPSS	Private institutions of social solidarity
LMTHF	Long and medium-term healthcare facilities
LTHFs	Long-term healthcare facilities
MDR	Multi-drug resistance
MBLs	Metallo-β-lactamases
MGEs	Mobile genetic elements
MRSA	Methicillin resistant *Staphylococcus aureus*
NEMEC	Neonatal meningitis-associated *E. coli*
NPV	Negative predictive value
PMQR	Plasmid-mediated quinolone resistance
PPV	Positive predictive value
rUTI	Recurrent Urinay Tract Infections
SEPEC	Sepsis-associated *E. coli*
TLR	*Toll-like* receptor
UPEC	Uropathogenic *Escherichia coli*
UTIs	Urinary tract infections

UUI	Urgency urinary incontinence
VFs	Virulence factors
WBC	White blood cells

CHAPTER 1 - INTRODUCTION

1.1. CHARACTERIZATION OF LONG AND MEDIUM-TERM HEALTHCARE FACILITIES

The long and medium-term healthcare facilities (LMTHF) are an intermediate setting between community and hospital setting (1, 2). These healthcare facilities are part of a national network of continuing healthcare created by legal rule n.º 101/2006 of June 6, with the main aims: rehabilitation, readaptation, social integration and maintenance of life quality, even in irreversible health conditions (3, 4). This national network is coordinated by central (Central Administration of Health System), regional and local team, involving health professionals (doctors, nurses), social assistants and elements of local authorities (5).

In Portugal, the provisions of continuing healthcare facilities ensured by inpatient units have some typologies:

a) Palliative healthcare;
b) Convalescence healthcare: these units have the finality to internment with predictability until thirty consecutive days;
c) Medium-term healthcare: these units have the finality to clinical stabilization, evaluation and integral rehabilitation of the patient, for a period greater than 30 and less than 90 consecutive days;
d) Long-term healthcare: these units were created to provide support to patients with chronic diseases, preventing the deterioration of health status and promoting the life quality, for an internment period higher ninety days (1, 3, 6).

Most countries have separate long-term healthcare facilities (LTHFs) for elderly (e.g. residential homes, nursing homes), physically disabled (sometimes younger population), mentally disabled and psychiatric care. In other countries, mixed LTHFs are the standard (7).

In Portugal, the majority is mixed LTHFs (54.5%), and the remaining is rehabilitation centers (45.5%) (7). The majority of LTHFs are private institutions of social solidarity (IPSS), such as Santa Casa da Misericórdia (54%), other IPSS (23%), private institutions for profit (18%) or health national service (HNS, 5%). The agreements with the HNS decreased 42%. Regarding the demographic characterization, the resident population aged over 65 and 80 year represent 84.5% and 47% of the total, respectively. The female gender corresponded 55.7% of a total resident; 50% of residents were women over the age of 65 and 30.4% of residents were women over the age of 80. The men in this age group represent 16.6% (5).

Medical residential care in national LTHFs are mainly provided by employed medical staff (91.8%), and the remaining by general practitioners (GPs) visiting the LTHFs (1.4%). This trend is not observed in European countries, where 58.5% of medical care are provided by GPs visiting the LTHF (7). According to the last report (first semester of 2015) on national continuing healthcare network, were identified 4222 beds for long-term, 2087 beds in medium-term, 750 beds for convalescence, and 252 beds for palliative care (8). The median size of LTHFs was 23 beds, lower relatively the European median (64 beds). The median percentage of single rooms was 22.2% for a European median of 57.1% (7).

Several LMTHF aspects enhance the possibility of infection acquisition and limit the effectiveness of infection control measures. Among them, are:

a) Continuous flow of residents with several comorbidities in and out of the facility frequently to and from acute care settings increase the risk of infection (1, 2).
b) Residents exposition to high antibiotic pressure with frequent antibiotic prescription without the presence of infection (2).
c) The confluence of a) and b) together with the entrance of residents colonized with multi-drug resistant (MDR) strains acquired in the acute setting (2, 9) turns these settings as important reservoirs of MDR bacteria (10).
d) Social sharing/interaction and close proximity between patients of LMTHF favors the acquisition of microorganisms, thus increasing the risk of infection and admission in acute care hospitals (1, 2).

It is recognized that these factors increase the risk of infection acquisition by LMTHF residents, and particularly for older residents (4, 7, 8).

Comparatively with European crude prevalence of residents with at least one HAI (3.4%), the national LTHFs obtained the greater prevalence (9.5%). The median prevalence in LTHFs ranged from 0.4% in Croatia to 7.1% in Portugal. The European countries with prevalence more close to Portugal were UK-Northern Ireland (6.9%) and Netherlands (5.8%) (7); however, these facilities could be highly diverse, as they can care residents with a broad range of chronic and acute diseases, from different ages, varying in the number and type of invasive procedures which difficult comparisons (7).

From a national point prevalence survey of HAIs and antimicrobial use (2013), conducted in 143 continuing healthcare facilities (49.9% long-term, 29.3% medium-term, 17.4% convalescence and 3.4% palliative healthcare) were identified the resident's most important characteristics, including risk factors for HAIs, such as impaired mobility (73-

78.3%), incontinence (60.4-79.4%), disorientation (40.7-62%), more than 85 years (20.6-27.3%), pressure sores (13.4-21.7%) and urinary catheter (14.6-17.3%) (5).

The continuing healthcare facility of Santa Casa da Misericórdia de Vila do Conde is composed by both, long and medium-term healthcare, which include 15 and 25 beds, respectively, with an overall capacity for 40 patients. The population admitted in both typologies were a majority from the area of Porto district (Porto, Maia, Vila Nova de Gaia, Matosinhos, Vila do Conde, Póvoa de Varzim, Gondomar, Trofa, Vila Nova de Famalicão), and others districts of north (e.g. Viana do Castelo, Braga, Aveiro). However, the units can admit patients of different geographic area of Portugal. These patients were referenced from district/ local hospitals and health centers for these units.

In both, long and medium-term, the patients were mostly female and older, with mean age of 75 ± 12 years (median 78 years). The main comorbidities identified in LTHF were ischemic stroke, following by disuse syndrome, fracture, hemorrhagic stroke, head trauma. In medium-term healthcare the most frequent comorbidities observed were ischemic stroke, following by disuse syndrome, hemorrhagic stroke and tetraparesis.

The LMTHF are composed by infection prevention and control (IPC) committee, and the medical resident care and nursing staff have clinical training in IPC, and access to IPC advice. It was implemented hand hygiene protocol, and the hand disinfection is performed using an alcohol-based solution. It was also, implemented a restrictive list of antimicrobials for prescription, excluding carbapenems, glycopeptides and the intravenously administered antibiotics (exceptional use).

1.2. URINARY TRACT INFECTIONS

Despite the recent description of a normal bladder microbiota and the recognized limitations on the current diagnosis process of urinary pathologies, still there is no new recommendations for UTI diagnosis (11, 12). Future studies will contribute to understand microbiota in relation to urological diseases (e.g. overactive bladder syndrome, urge urinary incontinence (UUI), interstitial cystitis, neurogenic bladder dysfunction, asymptomatic bacteriuria (ASB) and UTI) and may help to clarify on the pathogenesis of these dysfunctions, it might also change how we diagnose, treat and prevent UTI.

1.2.1. CLASSIFICATION OF URINARY TRACT INFECTION

UTI is defined as an infection of the urinary system, and may involve the lower urinary tract or both, the lower and upper urinary tracts (13-16).

Traditionally, UTIs are classified based on clinical symptoms, laboratory data and microbiological findings (17), being subclassified into complicated and uncomplicated, (Table 1) and sepsis. The first requires that the urinary tract has a structural or functional abnormality and includes all upper UTIs (18), but the seconds occurring in a structurally normal urinary tract (17, 19-21).

TABLE 1 - Definition of common terms [readapted from reference (19)].

Pyuria	**> 10 white blood cells (WBC)/mm^3 per high-power field**
ASB	Bacteriuria in the absence of genitourinary signs or symptoms
Symptomatic UTI	Bacteriuria in the presence of genitourinary symptoms (e.g. dysuria, suprapubic pain or tenderness, frequency or urgency)
Uncomplicated UTI	Genitourinary symptoms (e.g. dysuria, suprapubic pain or tenderness, frequency or urgency) with evidence of pyuria plus bacteriuria in a structurally normal urinary tract
Complicated UTI	UTI occurring in a patient with a structural or functional urinary tract abnormality

The guidelines on urological infections of European Association of Urology (2015) cover UTIs and male accessory gland infections because both infections are closely associated in males (Table 2) (17).

TABLE 2 - Classification of urinary tract and male genital infections [readapted from reference (17)].

Uncomplicated lower UTI: Cystitis (acute, sporadic or recurrent)
Uncomplicated upper UTI: Pyelonephritis (acute, sporadic, no risk factor identified)
Complicated UTI with or without pyelonephritis (acute, sporadic, no risk factor identified)
Urosepsis: Systemic inflammatory response syndrome (fever or hypothermia, hyperleukocytosis or leucopenia, tachycardia, tachypnea)
Urethritis: Poorly understood besides sexually transmitted conditions
Male accessory gland infections: Prostatitis, epididymitis, orchitis

Thus, the guidelines of European Association of Urology (2015) classified the UTIs based on anatomical level of infection, grade of severity of infection, underlying risk factors and microbiological findings. The symptoms, signs and laboratory finding focus on the anatomical level of infection, defined the UTIs as:

a) Urethra: urethritis;
b) Bladder: cystitis;
c) Kidney: pyelonephritis;
d) Bloodstream: sepsis (17).

The clinical and laboratorial criteria for classification of UTIs are listed in Table 3:

TABLE 3 - Criteria for the diagnosis of urinary tract infections, as modified according to Infectious Disease Society of America /European Society of Clinical Microbiology and Infectious Diseases guidelines [readapted from reference (17)].

Category	Description	Clinical features	Laboratory investigations
1	Asymptomatic bacteriuria	No urinary symptoms	> 10 WBC/mm^3 > 10^5 CFU/ml in two consecutive midstream urine cultures in women or one urine culture in men > 24 h apart
2	Acute uncomplicated UTI in women; acute uncomplicated cystitis in women	Dysuria, urgency, frequency, suprapubic pain, no urinary symptoms in 4 weeks before this episode	> 10 WBC/mm3 > 10^3 CFU/ml in midstream urine culture
3	Acute uncomplicated pyelonephritis	Fever, chills, flank pain; other diagnoses exclude; no history or clinical evidence of urological abnormalities (ultrasonography radiography)	> 10 WBC/mm3 > 10^4 CFU/ml in midstream urine culture
4	Complicated UTI	Any combination of symptoms from categories 1 and 2 above; one or more factors associated with a complicated UTI	> 10 WBC/mm3 > 10^5 CFU/ml in women > 10^4 CFU/ml in men, or in straight catheter urine in women
5	Recurrent UTI (antimicrobial prophylaxis)	At least three episodes of uncomplicated infection documented by culture in past 12 months: women only; no structural/functional abnormalities	< 10^3 CFU/ml in midstream urine culture
In a suprapubic bladder puncture specimen, any count of bacteria is relevant			

1.2.2. DIAGNOSIS OF URINARY TRACT INFECTIONS IN LONG AND MEDIUM-TERM HEALTHCARE FACILITIES

The symptoms of UTI in community-dwelling older adults are well defined and include urethritis (hematuria and dysuria), cystitis (urethritis with frequency, urgency and suprapubic pain), and pyelonephritis (fever, nausea/emesis, flank pain preceded or not by

urethritis/cystitis); however, there is no consensus about symptoms that better reflecting an UTI in LMTHF residents (22, 23).

In LMTHF, it is particularly important to differentiate symptomatic UTI from ASB (13), as current guidelines do not recommend screening or treatment of ASB for the following groups of older adults: institutionalized adults, patients with an indwelling urinary catheter, or individuals living in the community (13, 19). Screening and treatment of ASB is only recommended in older adult men undergoing a resection of the prostate and in any urologic procedure in which mucosal bleeding is anticipated or in pregnant (13, 17, 19, 56).

The diagnosis of UTI in LMTHF residents have some challenges such as communication barriers (stroke and dementia), high prevalence of chronic genitourinary symptoms (urgency, frequency, nocturia, incontinence), and absence of a gold-standard laboratory test (bacteriuria with pyuria is common in asymptomatic LMTHF residents) to confirm clinical suspicion of UTI due to the high prevalence of ASB (22).

McGeer *et al.* (1991) issued the first guidelines for diagnosis of UTIs in LMTHF. These criteria are considered as the standard for diagnosis and treatment of UTI, and included fever (≥ 38ºC) or chills, dysuria, frequency or urinary urgency, suprapubic or flank pain, change in urine character, degradation of mental or functional status, or new increased incontinence. These criteria divided the symptoms into major and minor and reinforce the laboratorial confirmation (19, 22).

In 2005, Loeb *et al.* adapted these criteria with aim to reduce the use of antibiotics to treat UTI in LMTHF residents (Table 4) (19, 22).

TABLE 4 - Consensus criteria for diagnosis of urinary tract infections in long-term care residents [reprinted from reference (22)].

Signs and Symptoms	McGeer Criteria[27]	Modified Loeb Criteria[28,29]
1 Fever[a]	**Noncatheterized Residents**	
2 Dysuria 3 Frequency 4 Urgency 5 Flank pain 6 Suprapubic pain 7 Shaking chills 8 Gross hematuria 9 New urinary incontinence 10 Delirium 11 Change in urine character[b] 12 Worsening of mental status 13 Worsening of function status	≥3 signs or symptoms (1 or 7; 2 or 3 or 4; 5 or 6; 11; 9 or 12 or 13)	Dysuria alone *or* Fever *and* ≥1 sign or symptom (2-9) *or* ≥2 signs or symptoms (3-9)
	Catheterized Residents	
	≥2 signs or symptoms (1 or 7; 5 or 6; 11; 12 or 13)	Fever alone *or* ≥1 sign or symptom (5, 7, 10)

[a] Fever for McGeer criteria are defined as ≥100.4°F (≥38°C), and for the modified Loeb criteria as >100°F (>37.9°C) or a 2.4°F (1.5°C) increase above baseline on at least two occasions over the past 12 hours.

[b] Gross hematuria, foul smell, increased sediment, new pyuria, or new microscopic hematuria.

Both guidelines have demonstrated low accuracy in predicting bacteriuria and pyuria, which constitute analytical evidence of UTI. The McGeer criteria had a sensitivity of 30%, a specificity of 82%, a positive predictive value (PPV) of 57%, and a negative predictive value (NPV) of 61%; the accuracy of the modified Loeb criteria was 30%, 79%, 52% and 60%, respectively (22).

Rowe *et al*. proposed an algorithm based on the revised McGeer criteria, with additional suggestions based on adding empirically derived criteria (Figure 1) (19).

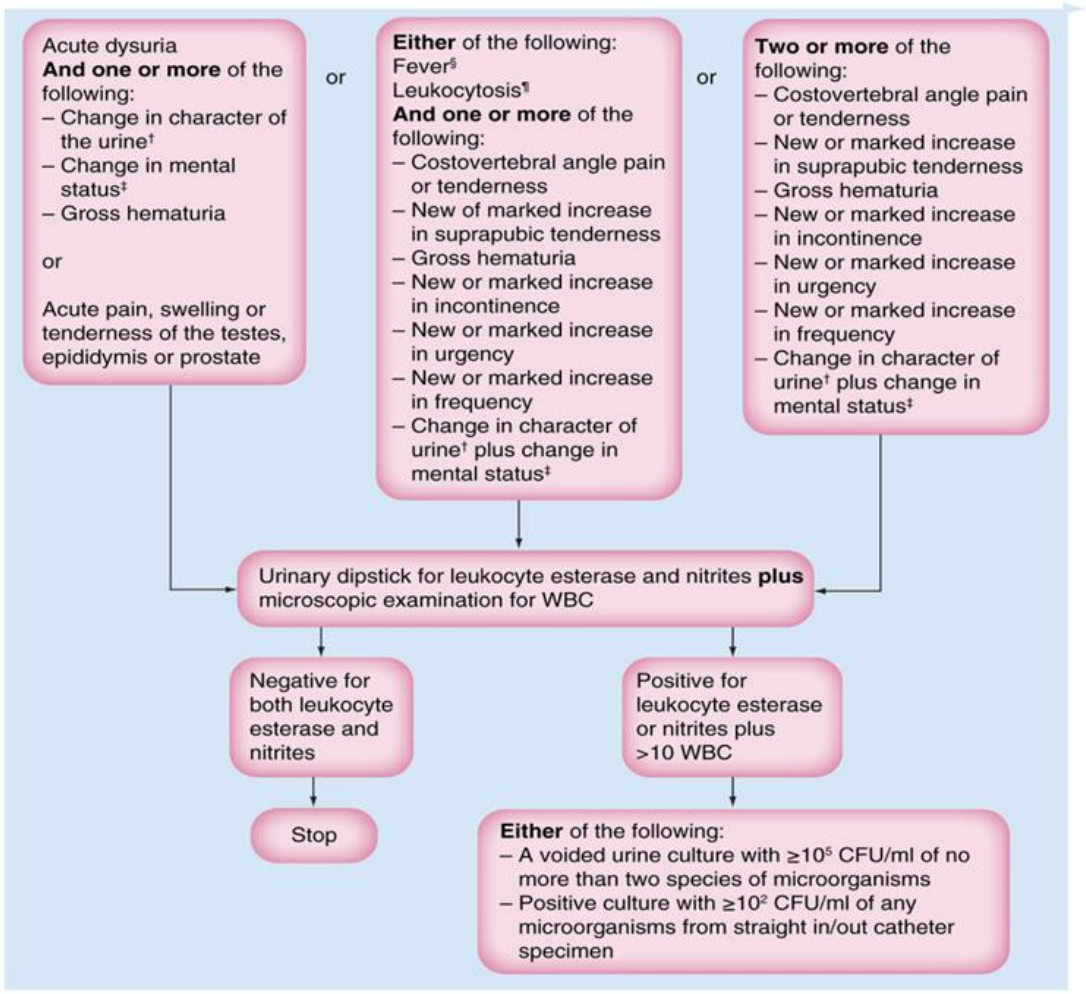

FIGURE 1 - Diagnostic algorithm for urinary tract infection in long-term care facilities in residents without an indwelling catheter [adapted from reference (13)].

† Change in color or odor of urine.

‡ Change in level of consciousness, periods of altered perception, disorganized speech or lethargy.

§ Single temperature ≥37.8ºC (>100ºF) or > 37.2ºC (>99ºF) on repeated occasions, or an increase of > 1.1ºC (>2C) over baseline.

¶ Leukocytosis: > 14,000 cells/mm^3 or left shift >6% or 1500 bands/mm^3. WBC: White blood cell.

1.2.2.1. LABORATORY CONFIRMATION

Bacteriuria and pyuria in urine specimen of LMTHF residents is insufficient to confirm a diagnosis of clinically suspected UTI (13, 22). Both conditions are present in approximately half of residents without a catheter and in most residents with catheter (22).

Based to the Infectious Disease Society of America guidelines, in case of suspected UTI the initial evaluation should include urinalysis for determination of leukocyte esterase and nitrite level by use of dipstick (13). Juthani-Mehta *et al*. demonstrated a NPV of 100% but a PPV of 45% with this method (22). Sundvall and Gunnarsson *et al.* evaluated the

predictive value of combined nitrite and leucocyte esterase dipstick analysis in predicting the presence of urinary pathogen in older nursing home residents, and the NPV obtained was 88% and PPV was 51% (Figure 2) (18, 19).

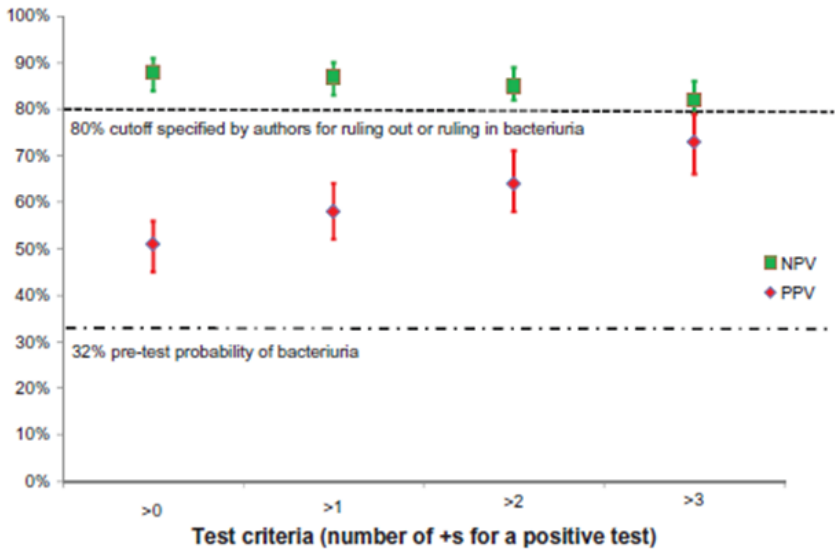

FIGURE 2 - Negative predictive value and positive predictive value for dipstick urine testing for diagnosis of bacteriuria in a nursing home population. Test characteristics of a positive leukocyte esterase and/or a positive nitrite dipstick were compared to urine culture. These results were for visual reading of the dipstick; results with analyzer reading were almost identical [reprinted from reference (18)].

Therefore, if the urinary dipstick is negative for leukocyte esterase and nitrite, the evaluation can be suspended. If pyuria or a positive leukocyte esterase or nitrite results is present, a urine culture should be obtained and, if positive, proceed to antimicrobial susceptibility testing (13, 19, 22).

1.2.3. OCCURRENCE OF URINARY TRACT INFECTIONS IN LONG-TERM HEALTHCARE FACILITIES RESIDENTS

The UTIs are the most common infections in LMTHF (8, 9, 11-17), and the main cause of hospitalization of residents (4, 11), affecting principally the older resident's (8-10). According to a national point prevalence survey in continuing healthcare facilities, during 2013, the UTIs were the most common infections (17.5% confirmed and 20% probable UTIs), followed by skin and soft tissue infections (26.2%) and respiratory tract infections (21.2%) (2), confirming previous findings (2, 10).

However, in a national point prevalence survey on Portuguese hospitals (2013) the respiratory infections (29.3%) were the most frequently, followed by UTIs (21.1%) (24).

These findings support previous studies that claim that in hospitalized patients and community-dwelling adults over the age of 65 years the UTIs are the second most common diagnosed infection (13, 19).

The incidence and prevalence of UTI varies with age and gender, being higher in women than men for all age groups (13). Is frequent in young sexually active women (0.5-0.7 per person-year), while in young men (0.01 per person-year) the incidence in lower. However, increases substantially with age in both genders (13). In a cohort study, the prevalence of UTI in community-dwelling women older than 65 years was approximately 16.5% over a 6-month period. For men aged 65-74 years, the incidence of UTI increases 0.05 per person-year. In both women and men older than 85 years, the incidence of UTI increases significantly (13), with almost 30% of women older than 85 years reporting at least one UTI within a 12-month period (19).

The ASB, a particular entity of UTI (17), is more common between older adults than in younger adults. The prevalence increases significantly with age in both men and women (13, 19). The prevalence of ASB is 1-5% in healthy premenopausal women (17), and is estimated between 6% to 10% in women older than 60 years and 5% in men older than 65 years; however, in institutionalized adults, the incidence of ASB is higher, with estimates ranging from 25% to 50% for women and 15% to 35% for men (19). In spinal cord injury patients a incidence of 23-89% was reported (17).

1.2.4. RISK FACTORS FAVORING URINARY TRACT INFECTIONS IN LONG-TERM HEALTHCARE FACILITIES RESIDENTS

The resident's population of LMTHF feature numerous medical comorbidities such as diabetes, spinal cord injury, stroke, cognitive deficits, urinary/faecal incontinence (13, 25, 26) and risk factors, including advanced age, impaired mobility, chronic illness, immunosuppression (1, 27), urinary catheter (1, 28, 29) and dysbiosis (11), that increases the predisposition to UTIs and ASB (13, 22, 27) and colonization with MDR strains during residence (2, 22).The most relevant of these risk factors are detailed below:

a) **Impaired mobility** in aging adults has been shown to increase the risk of hospitalization in case of UTI. A retrospective cohort study (2008) conducted in adults older than 65 years admitted in a LTHF observed that who were able to walk independently had a 69% reduction in risk of hospitalization for UTI in comparison with older adults who did not walk or required significant assistance.

These results suggest that maintaining or improving mobility in older adults may protect against hospitalization for UTI (19).

b) **Alterations in the urinary microbiota** linked to urologic disease, such as UUI, interstitial cystitis and neurogenic bladder dysfunction were recently suggested with the observation of species strongly associated to UUI, such as *Aerococcus urinae*, *Gardenerella vaginalis* and *Lactobacillus gasseri*, that were absent groups of other ages (11).

c) **Urinary incontinence** is the most common urologic conditions in the older population (30). An estimated 15%-35% of community-dwelling older people and 50% of institutionalized people have severe urinary incontinence. The overall prevalence increases with age in both women and men (30). Consequently, these settings are using more indwelling devices and parenteral antibiotics, exposing the patients to MDR bacteria (22).

d) **Urinary catheterization** is the main risk factor associated with UTI and ASB (13, 31-34) because disrupts defense mechanisms, promoting the easier access of bacteria to the bladder (22), causing bacterial colonization (33), recurrent UTIs (rUTIs) and chronic infections, bladder stones and septicaemia, and the development of antibiotic resistance (35).

The urinary catheter use in acute healthcare facilities is usually for short-term, while long-term catheters are most common for residents of LTHF (19). A sessile bacterial growth (biofilm) occurs frequently in these devices (18, 29), increasing the risk of development of bacteriuria time-dependent of catheterization (22, 29), reaching 100% prevalence at 30 days (17, 22). Catheter encrustations can also obstruct urine flow, promoting urine stagnation and bacterial replication. This process is mediated by urease-producing bacteria, which alkalinize the urine, precipitating hydroxyapatite or struvite crystals (22). Thus, *Proteus* spp., *Providencia* spp., *Morganella morganii* and *Corynebacterium urealyticum* are predominantly found, but *Klebsiella* spp., *P. aeruginosa*, *Serratia* spp. and *Staphylococcus* are too urease producers (in low level) and can mediate this process (17).

e) Any urological condition associated to **obstruction** (exemplified in Figure 3 by hypothetical mechanism of formation of calcium oxalate (CaOx) stones by bacterial growth) increase the risk of UTI (18).

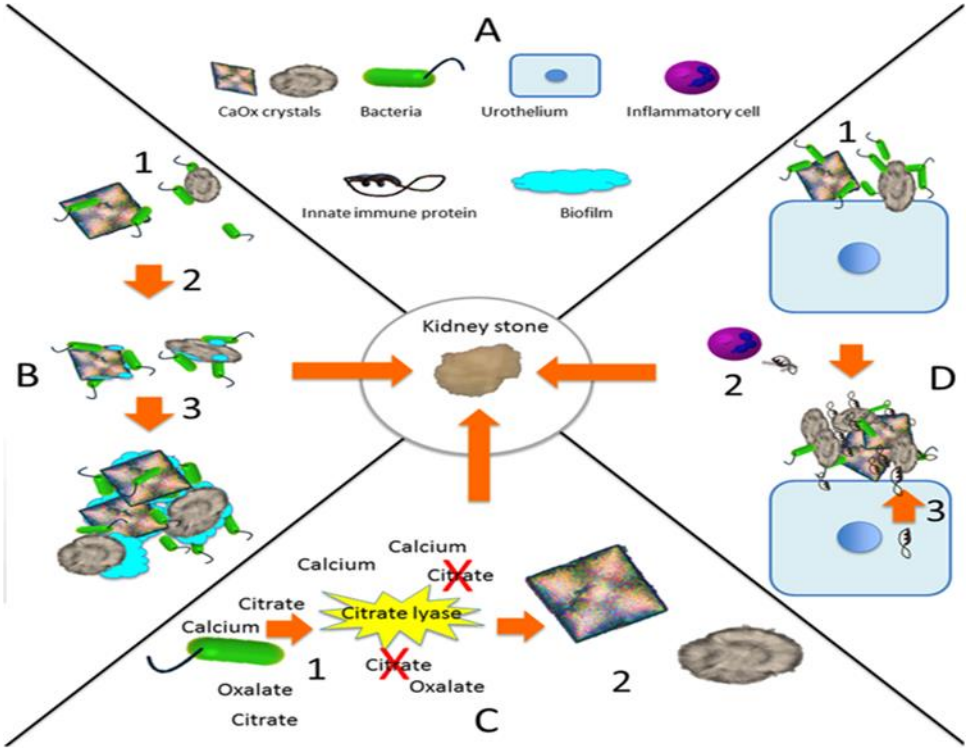

FIGURE 3 - Speculated mechanisms for bacterial contribution to calcium oxalate stones. (**A**) Figure key. (**B**) Bacteria bind to CaOx crystals that may provide a nidus for pyelonephritis or remain persist in a subclinical state (**1**) and bacterial communities form a biofilm (**2**). The biofilm results in crystal aggregation (**3**). (**C**) The bacterial enzymes citrate lyase splits citrate resulting in increased CaOx supersaturation (**1**). CaOx crystals form providing a key element of lithogenesis. (**D**) Bacteria bind to the urothelium (**1**) that results in secretion of innate immune proteins from recruited inflammatory cells (**2**) and urothelium (**3**). The innate immune proteins are incorporated as stone matrix proteins [reprinted from reference (36)].

Most kidney stones are composed primarily of CaOx (80%). *Oxalobacter formigenes* is a Gram-negative, anaerobic bacterium, present in a large proportion of the normal adult population (46-77%), which metabolizes oxalate (derived from both endogenous and exogenous sources) in the intestinal tract, and associated with a reduced risk for CaOx kidney stones (37). The absence of *Oxalobacter formigenes* could lead to increased absorption of dietary oxalate in the colon, and the subsequent increase in urinary oxalate that could favor the development of CaOx stones (37, 38).

Data from previous studies show that patients with kidney stones and hyperoxaluria have a lower prevalence of *Oxalobacter formigenes* in the stool, that can be a potential risk factor for formation of CaOx stones (37, 38).

f) In older men, **hypertrophy prostatic** increases the urinary symptoms and urinary retention, predisposing to chronic prostatitis. This chronic inflammatory process can promote the formation of stones that store bacteria, causing rUTI (22).

g) **Neurological conditions** such as dementia, cerebrovascular disease, Alzheimer´s disease and Parkinson´s disease promote bladder and bowel incontinence and functional decline, affecting the innate defense mechanisms and increase the risk of UTI (18, 22).

h) Patients with **diabetes mellitus** are at a high risk for UTIs, ASB and rUTI, because the hyperglycosuria promote bacterial growth (18, 35). Abnormal glycosylation observed in this pathology can also affect the function of soluble glycoproteins in the urine, favoring the adherence by type-1 fimbriae of Uropathogenic *Escherichia coli* (UPEC) to the urothelial surface. Neutrophil and lymphocyte dysfunction found in diabetics may also compromise the elimination of bacteria from the urinary tract in the later stages of infection (35).

i) In postmenopausal women, the **estrogen deficiency** has been associated with rUTI, cystoceles and urinary incontinence, promoting the ascending of bacteria to the urinary tract (18, 22). Estrogen deficiency impairs the beneficial effects of bacterial colonization of the vagina with *Lactobacillus*, which normally protect against growth of pathogenic bacteria (22).

j) An increased incidence of different comorbidities is associated with aging. Moreover, impaired acquired cellular immunity occurred namely as a result of T-cell dysfunction and blunted cytokine-mediated inflammatory response. Those dysfunctions are accentuated in the setting of diabetes, cancer, and autoimmune disorders (39).

1.2.5. RECURRENT URINARY TRACT INFECTIONS

rUTI are defined as two uncomplicated infections in a 6-month time period or three infections within a year (17, 21, 40). The probability that patients develop a second UTI within 6 months is 25%, and the possibility of recurrence over a 12-month period increases for 46% (40).

Even before the evidence of a resident urinary bacterial community in healthy individuals, it was evident that an independent inoculation of urinary tract would not totally explain the recurrence of UTI episodes (11, 12, 40). In fact, several studies demonstrated that many rUTI episodes (>50%) involved bacterial strains genetically identical, being suggested the involvement of intracellular bacterial reservoirs within the bladder mucosa that would provide persistent urinary pathogens (21, 40).

The UPEC strains, for example, can invade host epithelial cells, including superficial cells (umbrella cells) as well as intermediate and basal cells; enter to cytosol and rapidly multiply, forming a biofilm known as an intracellular bacterial community. The efflux of UPEC and exfoliation of infected superficial cells may promote the dissemination of UPEC within the urinary tract. The UPEC that remain in a dormant state can establish quiescent reservoirs, extremely difficult to eradicate with antibiotic therapy (Figure 4) (40).

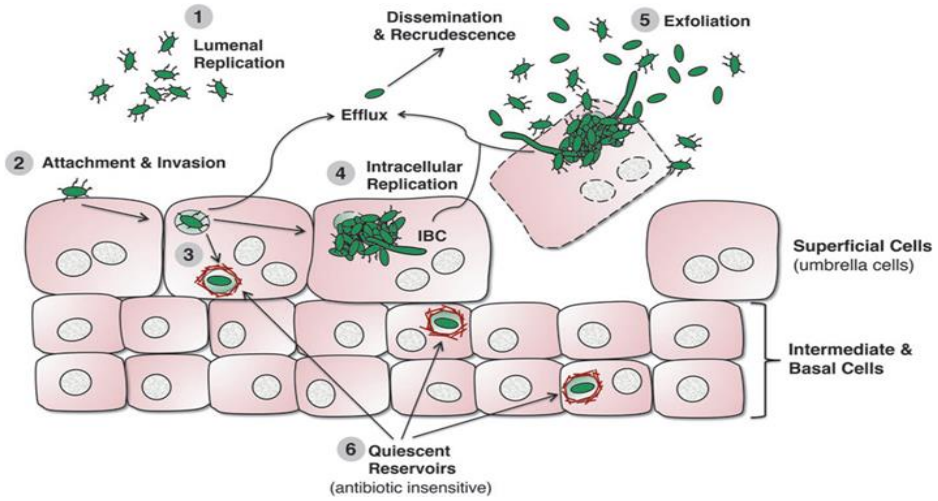

FIGURE 4 - Events that promote the establishment and recurrence of urinary tract infection [reprinted from reference (40)].

Recent findings have suggested that multiple factors, such as gene-gene and gene-environmental interactions and their deregulation may predispose patients to rUTI. It is suspected that some genes may be associated with susceptibility to rUTI, in particular, HSPA1B, CXCR1/2, TLR2, TLR4 and TGF-β1 genes seem to be related with an alteration of the host response to UTIs (41).

1.2.6. NATURAL CONDITIONS PREVENTING URINARY TRACT INFECTION

Several functional mechanisms, such as barrier effect, mucous production, regular bladder emptying, urinary microbiota, urine flow and some chemical characteristics of urine (Table 5) are involved in defense of the urinary tract against UTIs (42).

TABLE 5 - Chemical characteristics of urine influencing urinary tract infections susceptibility [readapted from reference (42)].

Urine parameter	UTI relevance
pH	Optimal bacterial growth in urine occurs between a pH 6-7.
Urea	High urea concentrations are associated with inhibition of bacterial growth
Glucose	Glucosuria can promote bacterial growth.
Calcium	Idiopathic hypercalciuria increases the risk of UTIs.
Iron	Increased iron promotes bacterial growth in urine.
Osmolality	Bacterial growth is inhibited when the urine osmolality is < 200mosm/L or > 1200 mosm/L

Moreover, the cellular and humoral innate immunity have complementary functions in mediating response against UTIs (43).

Cellular innate immunity and cell-associated receptors play an important role in defense against UTIs (41). The chemokines secreted by the infected mucosa promote the recruiting of neutrophils to the urinary tract. The bladder resident macrophages recruit both neutrophils and inflammatory monocytes to the bladder during acute UTI; the inflammatory monocytes induce the transmigration of neutrophils for bladder epithelium (44).

An overgrowth of bacteria also is associated with the induction of a strong innate immune response determined by *Toll-like* receptor (TLR), activation and production of antimicrobial peptides (AMPs) (e.g. cathelicidin, α-defensins, β-defensins, hepcidin, ribonuclease 7), chemokines (e.g. CCL2, CXCL1, CXCL2) and cytokines (e.g. G-CSF or IL-17). The TLR4, TLR5 and TLR6 have a significant role, mediating proinflammatory response and leukocyte recruitment (Figure 5, 6) (42, 43, 45). Moreover, the epithelium and circulating leukocytes increase the production of AMPs (42).

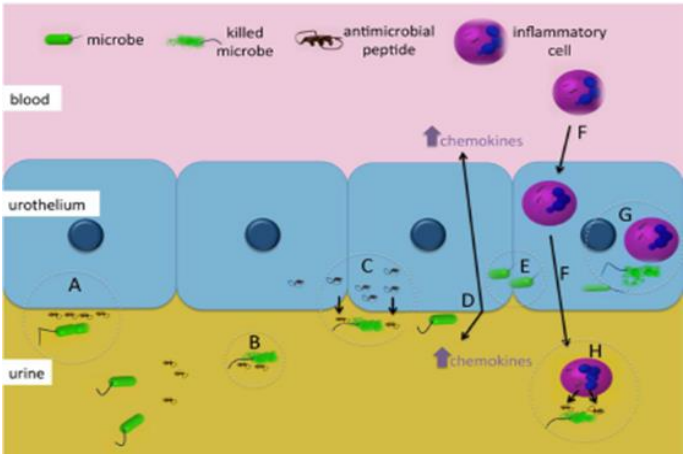

FIGURE 5 - Innate immune mechanisms in the urinary tract. Microbes enter the urinary tract and encounter constitutively expressed AMPs that can inhibit attachment to the urothelium (A) or cause bacterial lysis (B). If bacteria attach to the urothelium, they can induce AMP production, which results in destruction of adherent bacteria (C). When bacteria bind and invade the urothelium, they can elicit the production of chemokines (D/E) that attract inflammatory cells across the urothelium (F). These cells control infection by phagocytosis (G) and secretion of intracellular AMPs (H) [reprinted from reference (32)].

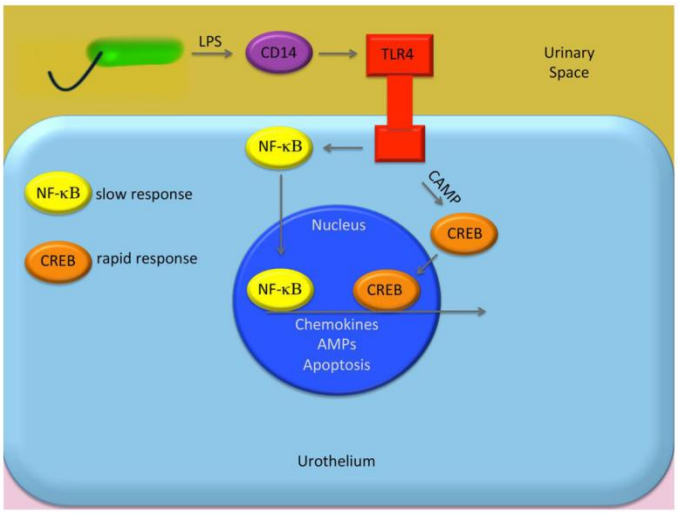

FIGURE 6 - Signaling pathway of *toll-like* receptors in urinary tract infection. Activation of TLRs expressed on the cell membrane by a bacterial ligand sets in motion a series of processes that leads to the release of inflammatory chemokines, cytokines and AMPs [reprinted from reference (32)].

Humoral innate immunity also plays an effective role in UTI. This response is composed by complement cascade and soluble pattern recognition molecules such as collectins, ficolins, and pentraxins (e.g. PTX3). Soluble pattern recognition molecules are essential for elimination of bacteria by their opsonic activity, promoting the phagocytosis and maturation of the phagosome. Polymorphisms in PTX3 were associated with increased susceptibility to UTI in humans (43, 44).

1.2.7. ETIOLOGY OF URINARY TRACT INFECTIONS

The most common organism causing UTI and ASB in both community and healthcare settings are *E. coli*, followed by other *Enterobacteriaceae*, such as *Proteus mirabilis*, *Klebsiella* and *Providencia* species. Gram-positive bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA) and *Enterococcus*, are less commonly isolated, however, are identified with increasing frequency in healthcare settings, associated with chronic indwelling catheters (Figure 7) (13, 19, 20, 26, 46). Nevertheless, the prevalence of bacterial species isolated are different for each LTHF and countries (22, 47). In a cohort of LTHF residents in United States of America, *E. coli* was the predominant urinary pathogen, with a frequency of 53.6%. Other *Enterobacteriaceae* were also common, namely *Proteus* (14.6%), *Klebsiella* (13.9%) and *Providencia* (3.7%) (19, 22). Gram-positive bacteria including *Enterococcus* and *Staphylococcus* accounted for 4.5% and 4.1% of positive urine cultures, respectively. Another study conducted in 32 LTHF from Sweden, the *E. coli* also was the most common urinary pathogen isolated (69%), but the second most common was *Klebsiella* spp. (12%), followed by *E. faecalis* (8%) (48).

In a national point study (2013) of prevalence of infection-acquired in hospital setting and antimicrobial use, covering 18258 patients distributed by 103 hospitals with different typologies (22.3% primary; 40.8% secondary; 22.3% tertiary and 12.6% specialized hospitals), the most common urinary pathogens isolated in UTIs were *E. coli* (31.4%), followed by *Klebsiella* spp. (16.3%), *P. aeruginosa* (11.6%) and *Enterococcus* spp. (10.2%) (24). A previous national point study, from 2012, involving 5150 residents living in 232 continuing healthcare facilities, of which 82.5% corresponding to LMTHF, also demonstrated the dominance of *E. coli*, although in higher frequency (39.5%), followed by *Klebsiella* spp. (10%), *P. mirabilis* (9.5%), *P. aeruginosa* (6.3%) and *S. aureus* (5.8%) (6).

The profile of the urinary pathogens causing UTIs in LMTHF patients, observed in these cohorts studies, resemble hospital-acquired UTIs more than the community-acquired (1, 49, 50). Aforementioned LMTHF residents conditions (please see section 1.1) seem to justify the observed similarity to hospital-UTI pathogens profile (15, 19, 22, 49).

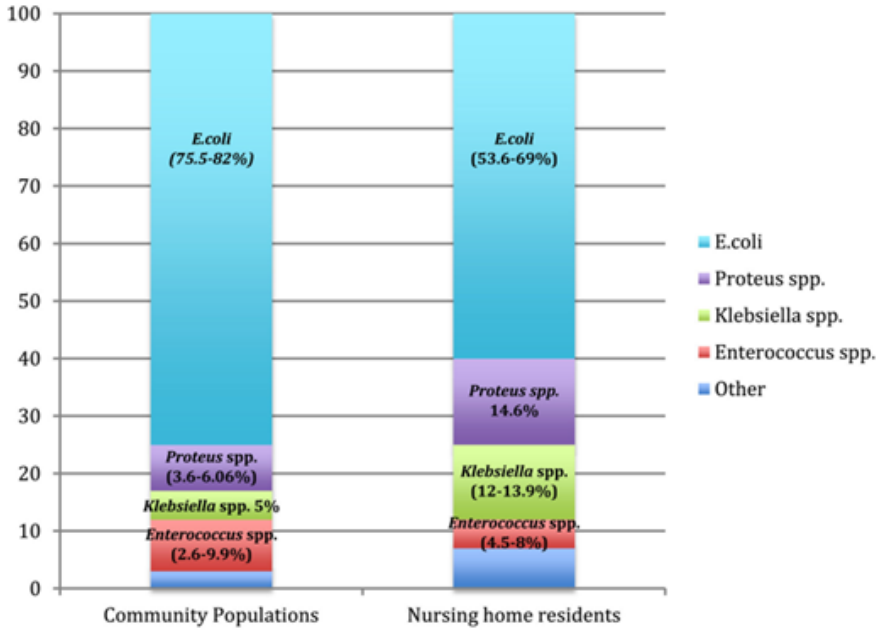

FIGURE 7 - The most common organisms isolated from urinary cultures in older adults [reprinted from reference (19)].

In noncatheterized patients or with short-term urinary catheter a single bacterium usually causes bacteriuria, and in 10% to 20% of cases is polymicrobial. In contrast, ASB is almost always polymicrobial in patients with chronic urinary catheter use. The etiology in these patients is dynamic because colonization by a specific bacterium is transient, with a mean duration of six weeks. *E. coli* is found in 18% to 35% of cases, followed by *Proteus* in 10% to 60%, *Providencia* in 24% to 60%, *Enterococcus* in 25%, *Pseudomonas* in 6% to 20% and *Enterobacter* in 9% (22).

1.2.7.1. *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* CLONES CAUSING UTI

E. coli and *K. pneumoniae* have been the most frequent species causing UTI in patients from continuing healthcare facilities in Portugal (4, 24).

In the last years several research studies unveiled major groups within these species associated with UTI and to clinically relevant antibiotic resistance mechanisms. The *E. coli* can be classified into four main phylogenetic groups and corresponding subgroups: A (A0, A1), B1, B2 (B22, B23) and D (D1, D2) (51, 52).

Several studies have suggested a relationship between phylogeny and pathogenicity in this specie, with virulent extraintestinal strains belonging mainly to B2 and D phylogroups, and commensal strains to phylogenetic groups A and B1 (51, 53). However, recent

epidemiological data highlights the increasing identification of A and B1 *E. coli* involved in extraintestinal infections (51, 52).

E. coli can be categorized into different pathotypes, depending on the site and type of infection: i) associated with enteric/diarrheal disease, such as enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) and ii) associate with extra-intestinal pathogenesis, such as UPEC, sepsis-associated *E. coli* (SEPEC), neonatal meningitis-associated *E. coli* (NEMEC) and avian pathogenic *E. coli* (APEC), which are generically defined as extra-intestinal pathogenic *E. coli* (ExPEC). UPEC are the mainly cause of community-acquired (70-95%) and nosocomial (50%) UTIs; however, the capacity of colonization and persistence in the urinary tract is depending of the variety of virulence factors (VFs) expressed by UPEC (51).

ExPEC VFs can be grouped in adhesins, toxins, siderophores, capsule, protectins and miscellaneous factors according to their functions. Some of the most important virulence genes of UPEC strains associated with severe UTIs are *aer* that codified for aerobactin, *pap* that codified for P fimbriae, type 1 fimbriae, *afaI* that codified for afimbrial adhesion I, *hly* that codified for hemolysin, *cnf 1* that codified for cytotoxic necrotizing factor 1, *sfa* that codified for S fimbriae, adhesins and fimbriae; however, other virulence genes such as *kpsMT*, *ompT*, *usp*, *iroN*, *iha*, *set 1*, *astA*, group II capsule synthesis; *sfa/foc*, S and *F1C* fimbriae; *iutA*, *traT*, serum resistance; and *fimH*, are known to be involved in pathogenicity of these strains. Previous studies reported that O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83 serogroups are preferentially associated with UPEC strains (51, 54).

Some UPEC are spread worldwide and are found in a variety of niches (human infections, healthy individuals, food and animals), such as the clone ST131 (B2), ST95 (B2), ST69 (D) or ST648 (D), while others are more frequently found in clinical isolates, such as ST393 (D), ST405 (D) or ST38 (D) and ST73 (B2), ST155 (B1) and ST359 (B1), whereas some are frequently identified in animals, such as ST10, ST23 or ST410 clonal complexes (phylogenetic group A) (51).

An example is the O25:H4-ST131 clone distributed by 103 hospitals that initially was associated with the pandemic spread of CTX-M-15 in clinical isolates, and now is highly disseminated in a variety of niches. This clone is responsible for severe extra-intestinal infections and amplification of a variety of ESBL types, such as other CTX-M, SHV, AmpC-types or KPC or NDM (51).

Within phylogenetic group D, clonal group A (CGA) and O15:K52:H1 clonal groups were initially linked with community-associated UTI outbreaks and now are spread worldwide. CGA was initially responsible for up to 50% of trimethoprim/sulfamethoxazole-resistant *E. coli* in women with uncomplicated cystitis and pyelonephritis in several states of USA, and has now spread worldwide. This clone belongs to ST69, and was associated with particular serogroups (O11, O17, O73 and O77) and occasionally produced ESBLs in different countries (TEM-type, CTX-M-15 or CTX-M-14). The O15:K52:H1 clone (belonging to ST393) was mostly associated with UTIs, but also others infections. This clone has been associated with ESBL production (mostly CTX-M-15 or CTX-M-14). Inserted in phylogenetic group D, the ST405 is an MDR clone linked to extra-intestinal infections and frequently associated with CTX-M-15 production, in several geographic regions (51).

Besides B2 and D, *E. coli* clones belonging to phylogenetic groups A (ST10 and ST23) and B1 (ST359 and ST155) have also been increasingly related as ESBL producers (CTX-M-14, CTX-M-15) and linked to extra-intestinal infections, and particularly UTIs (51).

K. pneumoniae is an opportunist nosocomial pathogen, with MDR strains generally associated with UTI and pneumonia and the highly virulent strains linked to invasive infections, such pyogenic liver abscesses, which is an emerging disease particularly frequent in Asian countries (55). *K. pneumoniae* complex suffered recently some restructuration's, with the redesignation of the 3 initially defined phylogroups (KpI, KpII and KpIII) as distinct species, namely, *K. pneumoniae*, *K. quasipneumoniae*, and *K. variicola. K. pneumoniae* is the most frequently detected in clinical isolates (56). Recently, 157 distinct *K. pneumoniae* phylogenetic lineages were depicted based on analysis of the core genome. Moreover, it was demonstrated that the ability to cause invasive community-acquired infections or hospital acquired infections is not determined by lineage per se but may be associated with a specific virulence or antibiotic resistance gene profile acquired horizontally. Nevertheless, some particular lineages are mostly associated with MDR or hypervirulence (56). For instances, some clones have been linked to the worldwide amplification of certain ESBLs or carbapenemases: clone ST15 has been associated with the spread of CTX-M-15 in different European and Asian countries (57), whereas the ST258 clone has been involved in the worldwide spread of KPC enzymes; ST11 clone is also largely distributed in different continents and associated with the production of diverse ESBLs (mainly CTX-M) or carbapenemases (KPC, OXA-48, NDM, VIM) (52).

1.3. MANAGEMENT AND THERAPEUTIC RECOMMENDATIONS FOR URINARY TRACT INFECTIONS IN LONG AND MEDIUM-TERM HEALTHCARE FACILITIES

UTIs treatment represents 30% to 60% of antibiotics used in LTHF (7, 22). In Portugal, 48.5% of antibiotics prescribed in continuing healthcare facilities were used for UTIs treatment (1, 7). This extended use could be explained by application of different diagnostic criteria, the complexity in differ ASB of symptomatic UTI in LMTHF residents and inappropriate antibiotics use (22).

In fact, inappropriate antibiotic use was reported in 40-75% of cases, particularly in nursing homes (13, 22). Moreover, the excessive use of antibiotics are associated with negative consequences, such as development of MDR bacteria (58), side effects (e.g. *Clostridium difficile* infection) and high healthcare costs (13, 18).

Given the difficulty of the differential diagnosis between ASB and symptomatic UTI in LMTHF patients, the increasing resistance to antimicrobials and the high rate of prescription of antibiotics for UTI in this setting it is advisable the use of a facility-adapted protocol to guide diagnosis and antimicrobial selection for suspected UTI (Figure 8). This practice was applied in 22 LTHF in Canada and Idaho, where occurred a decreased by 30% in rate of suspected UTI and the rate of antibiotic use decrease by 20% at 3 months, and these changes persisted at 12 months (22).

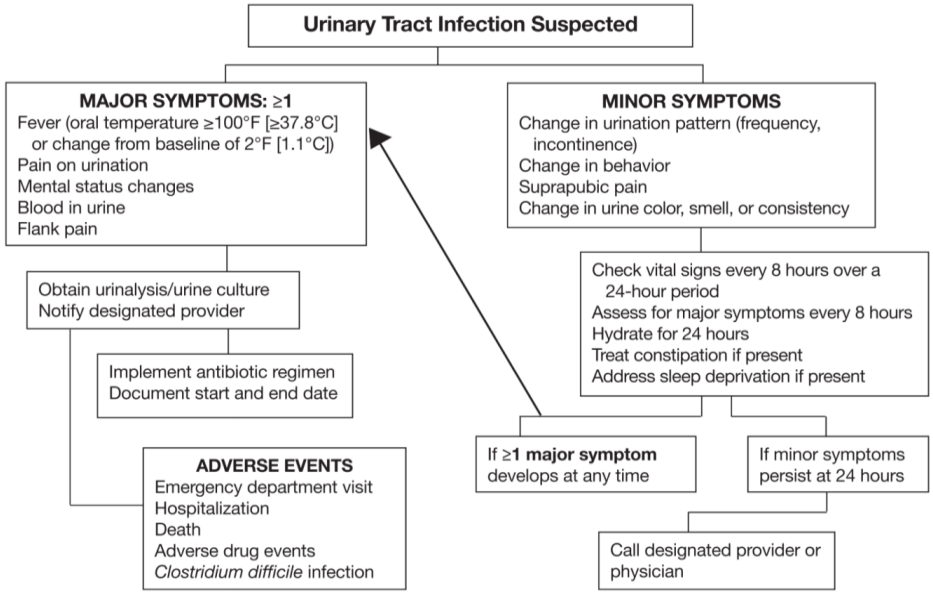

FIGURE 8 - Modified diagnostic algorithm that was used to identify and manage urinary tract infections in our long-term healthcare facility, which had a higher incidence of urinary tract infections than the national average. This algorithm divides symptoms into major and minor according to the strength of evidence of their association with UTI in LTC facilities [reprinted from reference (22)].

Empiric therapeutic of UTI in LMTHF patients should consider the clinical history (e.g. allergy history, renal clearance, comorbidities) of the patient, the local prescriptions patterns, the prevalence of antibiotic resistance in this setting and adverse effect profile associated (14, 22).

The International Clinical Practice Guidelines, updated in 2010 by the Infections Diseases Society of America and the European Society for Microbiology and Infectious Diseases, recommend as first line for empirical therapy for treatment of uncomplicated cystitis the nitrofurantoin monohydrate/macrocrystals 100 mg twice daily for 5 days, or trimethoprim/sulfamethoxazole 160/800 mg twice for 3 days (13); however, antibiotics with local resistance rates greater than 20% should only be used if a urinary culture with antimicrobial susceptibility testing is available (18, 19, 26, 58).

The antibiotic therapy recommended by European Association of Urology (2015) for treatment of uncomplicated cystitis in females is the fosfomycin trometamol 3 g single dose, pivmecillinam 400 mg tid for 3 days, and nitrofurantoin macrocrystal 100 mg bid for 5 days, when available. Most ESBL-producing *E. coli* are still susceptible to fosfomycin; however, in Spain was observed a parallel increase in community use of fosfomycin and resistance to fosfomycin in ESBL-producing *E. coli*. In males it is recommended a treatment with duration of at least 7 days, preferably with cotrimoxazole or a fluoroquinolone and in accordance with the susceptibility testing. In patients with renal insufficiency the choice of antibiotic may be influenced by the decreased renal excretion. Most antibiotics, however, have a wide therapeutic index. No adjustment of dose is necessary until glomerular filtration rate < 20 mL/min, except for antibiotics with nephrotoxic potential (e.g. aminoglycosides). Combination of loop diuretics (e.g. furosemide) and a cephalosporin is nephrotoxic. Nitrofurantoin and tetracyclines are contraindicated (17).

Alternative antibiotics include trimethoprim alone or combined with a sulphonamide, and the fluoroquinolone class. Cotrimoxazole (160/800 mg bid for 3 days) or trimethoprim (200 mg for 5 days).The Food and Drug Administration (FDA) have not recommend the use of fluoroquinolones for uncomplicated cystitis, which should be reserved for when there is no alternative treatment options (59). Fluoroquinolones are associated with several adverse effects including negative ecological effects and selection of resistance strains (17).

Aminopenicillins (amoxicillin) are no longer recommended for empirical therapy because of the worldwide high rates of *E. coli* resistance. Aminopenicillins in combination with a betalactamase inhibitor such as ampicillin/sulbactam or amoxicillin/clavulanic acid and

oral cephalosporins are in general not so effective as short-term therapy and are not recommended for empirical therapy, but can be used in selected cases (17).

In mild and moderate cases of pyelonephritis, the European Association of Urology (2015) recommended oral therapy for 10-14 days. A fluoroquinolone for 7-10 days can be recommended as first-line therapy if the resistance rate of *E. coli* is still < 10%. However, with increasing numbers of fluoroquinolone-resistant *E. coli* in the community the empirical use of fluoroquinolones is restricted. A third-generation oral cephalosporin could be an alternative. As result of increasing *E. coli* resistance rates >10%, the cotrimoxazole is not recommended for empirical therapy, but it can be used in case of sensitivity after confirmation through susceptibility testing. In communities with high rates of fluoroquinolone-resistant and ESBL-producing *E. coli* (> 10%), initial empirical therapy with an aminoglycoside or carbapenem has to be considered until susceptibility testing demonstrates that oral drugs can also be used (17).

In patients with severe pyelonephritis and systemic symptoms (e.g. nausea, vomiting) it is not recommended oral medication, and it has to be treated initially with one of the following parenteral antibiotics, described in the Table 6. The recommendations for others therapeutic indications are also summarized in the Table 6 (17).

TABLE 6 - Summary of recommendations for antimicrobial therapy in urology [reprinted from reference (17)].

Diagnosis	Most frequent pathogens/species	Initial, empirical antimicrobial therapy	Therapy duration
Asymptomatic bacteriuria	*E. coli* (low virulence) Other species can also be found	No treatment Exception: before urological surgery and during pregnancy (under debate)	3 – 5 days prior to surgery according to urine culture[1]
Cystitis, acute, sporadic (uncomplicated), in otherwise healthy women	*E. coli* *Klebsiella sp.* *Proteus sp.* Staphylococci	Fosfomycin trometamol Nitrofurantoin macrocrytsal Pivmecillinam Alternative: Cephalosporin (group 1 or 2) TMP-SMX[2] Fluoroquinolone[3,4]	Single 3 g dose/1day 5 days 3-5 days 3 days 3 days 3 days
Pyelonephritis, acute, sporadic (febrile) (uncomplicated)	*E. coli* *Klebsiella sp.* *Proteus sp.* Other Enterobacteriaceae Staphylococci	Fluoroquinolone[3] Cephalosporin (group 3a) Alternative: Aminopenicillin/BLI Aminoglycoside TMP-SMX[5]	7 – 10 days 10 days After improvement, switch to oral therapy according to sensitivity test
Febrile UTI with urological complicating factors	*E. coli* *Klebsiella sp.* *Proteus sp.* *Enterobacter* *Serratia* Other Enterobacteriaceae *Pseudomonas sp* High risk of multi-resistant strains Enterococci Staphylococci In case of Candida infection	Fluoroquinolone[3] Aminopenicillin/BLI Cephalosporin (group 3a) Aminoglycoside TMP-SMX[5] In case of initial failure (<3 days) Fluoroquinolone (if not initially used) Piperacillin/BLI Cephalosporin (group 3b) Carbapenem + Aminoglycoside Fluconazole Amphotericin B	7-14 days As for Pyelonephritis
Pyelonephritis, acute, severe and complicated			3-5 days after defervescence or control/elimination of complicating factor (drainage, surgery)
Healthcare associated complicated UTI			
Urosepsis			As above Consider combination of two antibiotics in severe infections
Prostatitis, acute bacterial (febrile) Acute Epididymitis (febrile)	*E. coli* Other Enterobacteriaceae *Pseudomonas sp.* *Enterococcus feacalis* Staphylococci	Fluoroquinolone[2] Cephalosporin (group 3a or b) Aminoglycoside TMP-SMX[5]	Initial parenteral After improvement, switch to oral therapy according to sensitivity test 2 (-4) weeks
Prostatitis, chronic bacterial		Fluoroquinolone[2] Alternative to consider based on micro-organism: TMP-SMX Doxycycline Macrolide	Oral 4-6 weeks
Prostatitis, acute/ chronic and Epididymitis caused by	*Chlamydia sp* *Ureaplasma sp*	Doxycycline Fluoroquinolone (e.g. ofloxacin, levofloxacin) Macrolide	7 (-14) days (Follow national guidelines if available)

[1] Bacteriuria is a risk factor, though no clear regimen has been defined in available literature. The given recommendation is a reasonable expert opinion
[2] Only in areas with resistance rate below 20% for E. coli
[3] fluoroquinolones with mainly renal excretion
[4] Avoid fluoroquinolones in acute sporadic cystitis whenever possible
[5] When proven sensitivity
BLI = beta-lactamase inhibitor; SMX = sulphamethoxazole; TMP = trimethoprim.

In Portugal, the recommendations adopted for treatment of UTIs are defined in guidelines elaborated by general direction of health, and were adjusted based in the recommendations of the International Clinical Practice Guidelines, updated in 2010 by the Infections Diseases Society of America and the European Society for Microbiology and Infectious Diseases for community-acquired UTIs (Table 7) (60). However, this guideline

does not consider that etiology and antimicrobial resistance pattern of urinary pathogens in LMTHF patients may differ from community-acquired UTIs, compromising the efficacy of antimicrobial therapeutic response. Moreover, we lack studies reporting the antibiotic susceptibility of isolates causing UTI in Portuguese LMTHF residents in order to appropriately support empirical therapy.

TABLE 7 - Treatment recommended for therapeutic indication [adapted from reference (60)].

Population	Antibiotics	Dosage	Duration
Uncomplicated acute cystitis in female	Nitrofurantoin	100 mg 6/6 hours	5 – 7 days[#]
	Fosfomycin	3000 mg/day	1 day
	Amoxicillin/clavulanic acid *	625 mg (500 + 125 mg) 8/8 hours	5 – 7 days
Pyelonephritis – mild to moderate cases	Ceftriaxone followed by Cefuroxime	1 gr IV or IM (1 take) 500 mg 12/12 hours	7 – 14 days
Pyelonephritis – mild to moderate cases in patients intolerant to beta-lactams	Levofloxacin	750 mg/day	5 days
Pyelonephritis – severe cases (sepsis)	Ceftriaxone	2 gr/day IV or IM	Decision in hospital setting
Pyelonephritis – severe cases in patients intolerant to beta-lactams	Gentamycin followed by antibiotic therapy directed by susceptibility testing	5 mg/Kg/day IV	Decision in hospital setting
Asymptomatic bacteriuria in candidates to resection of the prostate	Ceftriaxone	1 gr IV, 12/12 hours (start 24-48 hours before surgery)	3 – 6 days

* Alternative antibiotic therapy, if antibiotics above are unavailable or contraindicated

[#] In males, in absence of prostatitis, the therapy should have a duration of 7-10 days

1.4. β-LACTAMASES WITH CLINICAL RELEVANCE IN THE MAIN ETIOLOGIC AGENTS OF URINARY TRACT INFECTIONS

ESBLs and carbapenemase producers are the MDR pathogens causing UTI of special concerns in healthcare institutions. In this section, ESBLs and carbapenemase features will be addressed, unveiling their epidemiology and clinical relevance (61, 62).

β-lactamase production constitutes the most important mechanism of resistance to β-lactam antibiotics among *Enterobacteriaceae* (63).

The classification of β-lactamase enzymes have been based on the chemical constitution of their active site (serine or zinc) or their hydrolytic profiles over distinct β-lactams (penicillins, cephalosporins, monobactams, carbapenems) and their inactivation by classical β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) (64, 65). They are: i) the Ambler molecular classification and ii) the Bush-Jacoby-Medeiros functional classification:

i. **Ambler classification:** divides β-lactamases into four major classes (A, B, C and D) based on amino acid sequence homology. In this classification, β-lactamases of class A, C and D are serine-β-lactamases (serine residue in the active site), whereas class B enzymes are metallo-β-lactamases (MBLs) (zinc atom in the active site) (64).

ii. **Bush-Jacoby-Medeiros classification**: divides β-lactamases into four main groups, based on functional similarities (substrate and inhibitor profile) (65):
   a) **Group 1/Ambler class C:** includes cephalosporinases that are encoded in the chromosome and/or in plasmids, and that are not inhibited by the classical β-lactamase inhibitors (65).

   b) **Group 2/Ambler classes A and D:** represent a heterogeneous group of β-lactamases that includes penicillinases, cephalosporinases, oxacillinases and carbapenemases, which are inhibited by the classical β-lactamase inhibitors. Different subgroups are further considered, one of which includes ESBLs (2be subgroup) (65).

   c) **Group 3/ Ambler class B:** includes MBLs, which are enzymes with a high hydrolytic activity over carbapenems and that are inhibited by chelating agents, such as ethylenediamine tetraacetic acid. They are not inhibited by classical β-lactamase inhibitors and do not hydrolyze monobactams (65).

   d) **Group 4:** includes enzymes that are not inhibited by classical β-lactamase inhibitors and that cannot be classified in the other groups (65).

β-lactamases are intrinsically produced by particular species (e.g. AmpC in *Citrobacter freundii* or SHV-1 in *K. pneumoniae*) or they could be acquired throughout horizontal transfer, commonly associated with plasmids. ESBLs, qAmpCs and carbapenemases are β-lactamases that have been acquired by several species with ability to inactivate several

β-lactam antibiotics relevant in the treatment of human infections. The emergence and dissemination of *Enterobacteriaceae* producing ESBLs, qAmpCs and/or carbapenemases in different settings represent a major concern in many countries (66-70), with serious implications for both public health and infection control practices (71).

The molecular epidemiology of ESBL- or carbapenemase-producing *Enterobacteriaceae* has been worldwide described in detail, but there are few recent data about epidemiological features of these β-lactamases-producing *Enterobacteriaceae* in Portuguese LMTHF. Moreover, few data is available for qAmpC epidemiology in clinical setting (66-69, 71).

1.4.1. EXTENDED-SPECTRUM β-LACTAMASES-PRODUCING *ENTEROBACTERIACEAE*

ESBLs have the ability to hydrolyze penicillins, first-, second-, third- and four-generation cephalosporins, and monobactams, but not cephamycins and carbapenems, and are inhibited by β-lactamase classical inhibitors such as clavulanic acid, tazobactam or sulbactam (72-74). Most ESBLs belong to the Amber class A of β-lactamases. Certain class D OXA-derived enzymes are also included within ESBLs (73).

The genes coding for ESBLs (bla_{ESBL})$_s$ are plasmid-mediated and the most commonly mechanism of resistance described among *Enterobacteriaceae* species (63). Clonal and/or plasmid dissemination are frequently associated with the evolution of *bla* genes, resulting in new ESBL variants (50).

ESBLs can be classified into different families, according to their amino acid sequence. ESBLs belonging to the TEM, SHV or CTX-M types are the most frequently reported among *Enterobacteriaceae* and are worldwide disseminated, whereas OXA, PER, GES, IBC and VEB seem to be confined to specific geographic areas (75).

TEM and SHV ESBLs were the most frequently identified in nosocomial *K. pneumoniae* and *Enterobacter* spp. isolates (75, 76). To date, more than 200 TEM and 150 SHV variants have been identified (77), reflecting the rapid emergence and evolution of these enzymes under the selective pressure of antibiotic (78).

Whereas most of variants TEM and SHV have been only sporadically described and/or confined to specific geographic areas, others have a global distribution throughout different settings (76, 78). For example, TEM-24 has been mainly associated with nosocomial outbreaks in different Europeans countries (Spain, Portugal, Belgium and

France), while SHV-12 and TEM-52 are widely disseminated in the different settings. SHV-2, SHV-5 and TEM-10 are also widespread but mainly in the hospital setting (72).

More recently, CTX-M enzymes have become the most prevalent ESBL family in both the nosocomial and community settings, being mainly identified in *E. coli* isolates (72). They evolved from genes that have been captured by mobile genetic elements (MGE), such as IS*Ecp1* or IS*CR1*, from the chromosome of different species of *Kluyvera* spp. (53, 79).

These enzymes hydrolysis preferentially the cefotaxime than ceftazidime. However, a few variants have afterwards been described with ability to hydrolyze both cephalosporins (53, 79, 80).

Nowadays, CTX-M β-lactamases include more than 130 different enzymes (77) that are clustered into six groups according to their amino acid identities, namely the CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 and CTX-M-45 groups (53, 79). CTX-M-14 and CTX-M-15 are worldwide disseminated in both community and hospital settings (Figure 9) (74).

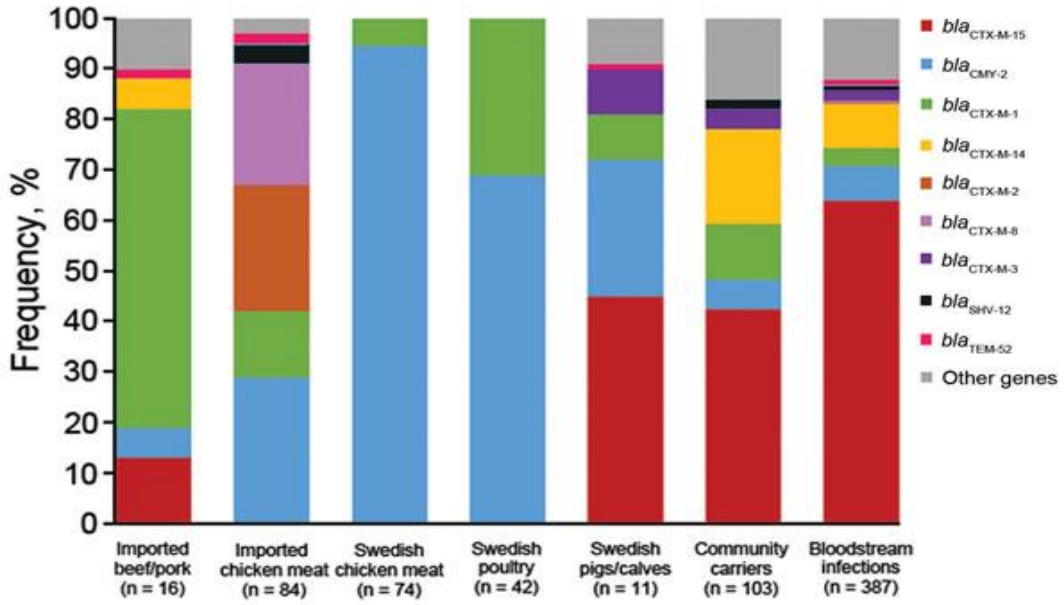

FIGURE 9 - Frequency of overlapping extended-spectrum β-lactamase– and plasmid-encoded AmpC genes in *Escherichia coli* isolates from various sources, Sweden. Data for leafy greens were excluded because there were only two isolates (both $bla_{CTX\text{-}M\text{-}1}$) from this source [reprinted from reference (74)].

Other CTX-M enzymes are confined to particular geographic regions, such as CTX-M-2 (Canada, South America, Israel and Japan), CTX-M-3 (Eastern Europe, South Africa and

China), and CTX-M-9 (Spain, United Kingdom and Japan). CTX-M-1 and CTX-M-32 are frequently identified among nosocomial isolates, mostly in Mediterranean countries, but also among animals and environmental bacteria (72). In Portugal the CTX-M-1, CTX-M-15 and CTX-M-32 are frequently observed (Figure 10) (81).

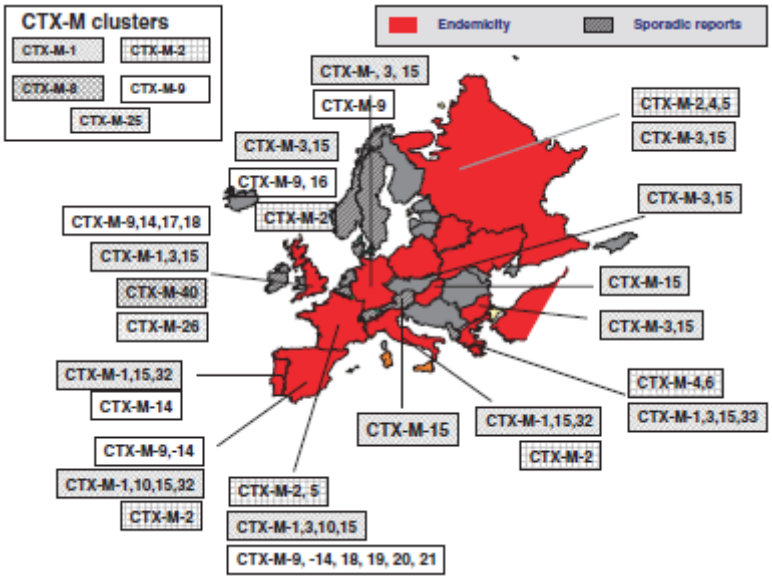

FIGURE 10 - Distribution of CTX-M enzymes in Europe [reprinted from reference (81)].

The major clone associated to expansion of ESBL-producing *Enterobacteriaceae* is the ST131 in *E. coli*, which has been responsible for the worldwide spread of CTX-M-15 (75, 82), however, this clone has also been detected encoding other ESBLs (CTX-M-1, -2, -3, -9, -14; SHV-12) or carbapenemases (NDM-1), highlighting its potential for diversification. Other *E. coli* clones belonging to group D (ST69, ST393, ST405) are also disseminated among different hosts, often causing urinary tract infections and producing ESBLs (mainly CTX-M), cephamycinases (qAmpCs), carbapenemases (NDM) and/or methylases (AmrA, RmtB). In addition, A and B1 *E. coli* (clonal complexes such as CC10 or CC23) are also identified among ESBL-producing isolates in nosocomial setting (72).

The ST15 *K. pneumoniae* clone has been associated with the spread of CTX-M-15 in different European and Asian countries (57, 72). The ST11 *K. pneumoniae* clone are also extensively distributed in different continents and linked to the production of diverse ESBLs (mainly CTX-M) or carbapenemases (KPC, OXA-48, NDM, VIM) (72). Outbreaks of ESBL-producing *Enterobacteriaceae* other than *E. coli* or *K. pneumoniae* isolates have also been described, but in most cases they seem to be of local significance. An epidemic *E. aerogenes* strain encoding TEM-24 has been detected in different European countries

(80), although it was able to acquire other ESBLs (SHV-12, SHV-5, TEM-20) (83). Other reports included outbreaks of *E. cloacae* (CTX-M-9), *P. mirabilis* (CTX-M-2), *S. marcescens* (CTX-M-3) and *K. oxytoca* (TEM-7) in different countries (72, 80).

ESBL production constitutes one of the most relevant antibiotic resistance mechanism (84) and has been observed mostly in *Enterobacteriaceae*, mainly in *E. coli* and *K. pneumoniae* (85); however, all other clinically-relevant *Enterobacteriaceae* species could produce these β-lactamases (73, 86). The level of expression and properties of β-lactamases, and the co-presence of other resistance mechanisms (others β-lactamases, increased activity of efflux pumps, modifications in membrane permeability) can result in the large variety of resistance phenotypes observed among ESBL-producer isolates (73).

ESBL-producers isolates are commonly resistant to different antimicrobial category besides β-lactamases, including fluoroquinolones, trimetoprim/sulfametoxazol and aminoglycosides, which contribute to the selection and persistence of MDR ESBL strains and plasmids in both clinical and community settings (75).

Fluoroquinolone resistance is being increasingly reported among *Enterobacteriaceae* (mainly in *E. coli* and *K. pneumoniae*) (87). It has been associated with mutations at DNA gyrase (GyrA) and/or topoisomerase IV (ParC) chromosomal genes and/or with the emergence of plasmid-mediated quinolone resistance (PMQRs) genes (75, 87-90). PMQRs encode Qnr proteins (e.g. QnrA, QnrB, QnrC, QnrD and QnrS) protecting target enzymes, acetylases that affect the activity of some fluoroquinolones and aminoglycosides [AAC(6')-Ib-cr], and/or efflux systems that pump fluoroquinolones out of the bacterial cell (QepA and OqxAB) (87, 90). Some PMQR mechanisms have been associated with the production of particular ESBL-types, such as AAC(6')-Ib-cr and CTX-M-15, QnrA and CTX-M-9 group enzymes, or QnrS1 and VIM-1 (72).

Portugal is one of the European countries with higher rates of ESBL-producers in clinical setting (84, 91), leading frequently to therapeutic failures (92). Long-term hospitalizations leading to nosocomial infections and exposure to antibiotics, increase the risk of acquisition of ESBL-producing isolates (93).

In Portugal, it is unknown the extent of these enzymes in urinary isolates from LMTHF. A national point study (2012) conducted in continuing healthcare (integrating different typologies) demonstrated that resistance rate to third-generation cephalosporin was 24.7%, not differing between ESBL or AmpC β-lactamases for all type of infections (1).

1.4.2. ACQUIRED AMPC-β-LACTAMASES-PRODUCING *ENTEROBACTERIACEAE*

Chromosomal-encoded AmpC β-lactamases were initially identified in some *Enterobacteriaceae* species (e.g. *E. coli*, *Shigella* spp., *Citrobacter* spp., *Serratia* spp., *Hafnia alvei*, *Morganella* spp., *Enterobacter* spp.), *Acinetobacter* spp., *Pseudomonas* spp., and other genus (94), however, AmpC β-lactamases encoded by plasmid-mediated genes were also observed in different species, including some that naturally lack the chromosomal bla_{AmpC} genes, such as *Klebsiella* spp., *P. mirabilis* and *Salmonella* spp. (94, 95). Eight AmpC-types are recognized: ACC, ACT, CFE, DHA, FOX, LAT, MIR, MOX (96).

There are several natural lineages of qAmpCs described in species or genus, namely the *Enterobacter* group (MIR-1, ACT-1), the *C. freundii* group (LAT and most of the CMY types), the *M. morganii* group (DHA-1 and DHA-2), the *H. alvei* group (ACC-1), the *Aeromonas* group (CYM-1, CMY-8, CMY-9, FOX, MOX) and the *A. baumannii* Group (ABA) (52, 95). More recently was identified a new qAmpC (CDA-1) in a *Cedecea davisae* strain. Phylogenetic analysis demonstrated a high similarity to the chromosome-encoded β-lactamase from *Enterobacter* (52).

AmpC β-lactamases were positioned in the Ambler C class. These enzymes are resistant to β-lactamase inhibitors (clavulanic acid) and hydrolyze penicillins, first-generation cephalosporins, and depending on the enzyme, second-generation cephalosporins including cephamycins (e.g. cefoxitin, cefotetan) and third-generation cephalosporins to a lesser extent (73, 97). The AmpC-type (ACC-1) exceptionally not confer resistance to cephamycins and is inhibited by cefoxitin (94).

Enterobacteriaceae are divided in different groups, according with resistance phenotypes conferred by AmpC:

1- **Group 0** (*Enterobacteriaceae* lacking AmpC): *Salmonella* spp. and *P. mirabilis* lack natural AmpC β-lactamase genes, being intrinsically susceptible to penicillins, aztreonam, cephalosporins and carbapenems (94).

2- **Group 1** [producing intrinsic ("wild-type")]: exhibit also susceptibility phenotype (e.g. *E. coli* and *Shigella* spp.) (98), despite they produce very low levels of a non-inducible (lacks an ampR gene) chromosomal AmpC-type cephalosporinase (94).

3- **Group 2** (low-level cephalosporinases): In many *Enterobacteriaceae* species (e.g. *E. cloacae*, *E. aerogenes*, *C. freundii*, *Providencia* spp., *M. morganii*, *H. alvei*,

Serratia spp., *Pantoeae agglomerans*), the chromosomal AmpC expression is inducible by β-lactams (strong inducers include cefoxitin, imipenem, and clavulanic acid) (94, 98). The low-level cephalosporinases comprises resistance to penicillins/β-lactamase inhibitor combinations, and first-generation cephalosporins. They can be divided in sub-groups according with their behavior to cefuroxime and cefoxitin: (i) species usually susceptible both antibiotics (*H. alvei*, *P. rettgeri*, *P. stuartii*, and *P. agglomerans*); (ii) species more resistant to cefoxitin than cefuroxime (*E. cloacae*, *E. aerogenes*, and C. *freundii*); and (iii) species more resistant to cefuroxime than cefoxitin (*S. marcescens* and *M. morganii*) (98).

4- **Group 3** (high-level cephalosporinases): characterized by more or less resistance to penicillins (except to amdinocillin or temocillin), first-, second-, at least one third-generation cephalosporins and aztreonam. Fourth-generation cephalosporins are usually more active. The synergy test (phenotypic method) to detect ESBL expression with clavulanic acid and third-, fourth-generation cephalosporins or aztreonam is negative. Cephamycins are inactive except for ACC-1 in the specie *H. alvei*. Resistance conferred by AmpCs to different β-lactams can be partially or totally restored in the presence of cloxacillin (AmpC β-lactamase inhibitor) (98). The hyperproduction of AmpC in species with inducible expression of this enzyme (e.g. *Enterobacter* spp., *C. freundii*, *Providencia* spp., and *S. marcescens*) is thought to be derived from the action of the LysR-type transcriptional regulator AmpR, whereas in *E. coli* the high-level cephalosporinases is mainly due to mutations in the promoter or attenuator region and/or gene duplication (52, 98).

5- **Group 4** (acquired AmpC β-lactamases): in this group, the high-level cephalosporinase phenotype can result from acquisition of a plasmid encoded *bla*AmpC gene (qAmpC) (52).

The qAmpC most dominant and globally widespread is CMY-2, reported not only in *E. coli* isolates but also among *Salmonella* spp. from different clinical settings (52, 69). DHA-1 is the second most prevalent qAmpC, and has been particularly detected among *K. pneumoniae* recovered from hospitals. Other qAmpC-types have been only sporadically reported. Most of qAmpC producers other than *Salmonella* have been associated with urinary tract infections. The relevance of clonal expansion, plasmid dissemination or both mechanisms in the spread of qAmpCs varies with the bacterial species and/or the qAmpC-type (52).

Studies involving multiple clinical settings, revealed CMY-2 as the dominant qAmpC, with exception of a longitudinal Portuguese study (2002-2013) performed in isolates of different settings [community (53%), hospital (47%)] reporting the dominance of DHA-1 among *Enterobacteriaceae* isolates in different clinical settings, mostly in *K. pneumoniae* isolates, representing 96% between 2002 and 2008 *versus* 55% in 2009 to 2013, while CMY-2 was mostly recovered in *E. coli* isolates, representing 4% between 2002 and 2008 *versus* 45% in 2009 to 2013 (99).

The characterization of CMY-2 producing *E. coli* population revealed a high clonal diversity and the absence of a predominant clone, however, have been detected some widespread lineages of *E. coli* belonging to phylogenetic group B2 (ST95, ST131), D (ST69, ST393, ST405, ST648), and A e B1 clonal complexes (ST10, ST155, ST168, ST448) (52).

The dissemination of DHA-1, in Portugal, was associated with ST11-*K. pneumoniae* clone (belonging to clonal group CG258), despite it had been more commonly linked to the worldwide spread of different carbapenemases (KPC-2/-3; VIM-1/-4; NDM-1; OXA-48/-245) or ESBLs (SHV-5/-12; CTX-M-3/-15; SFO-1) (52, 67). In Portugal, 48% of DHA-producing *K. pneumoniae* isolates belonged to ST11, obtained from several clinical institutions (hospital and community) at different regions (99).

Caniça *et al*. (2014) evaluated a group of 124 *Enterobacteriaceae* isolates resistant to third generation cephalosporins, collected in distinct healthcare facilities of different Portuguese regions, and identified 86.3% (107/124) ESBLs producers, revealing a diversity of class A β-lactamases from different families, like TEM, SHV and GES, as well as detected class C enzymes like plasmid-mediated AmpC β-lactamases (PMAβs, DHA-1, and CMY-2) and chromosomal AmpCs in *Enterobacter* and *Citrobacter* spp. (100).

Although qAmpCs are normally encoded by genes located in plasmids, the dissemination of bla_{qAmpC} between the same or different *Enterobacteriaceae* species are also associated with the horizontal transmission of other MGEs. The plasmids more frequently identified were IncI1 and IncA/C; these have been associated with spread of bla_{CMY-2} among members of the family *Enterobacteriaceae* (mostly clonally unrelated *E. coli* or *Salmonella* spp.) recovered from clinical or non-clinical settings (52). Recently, IncA/C plasmids carrying bla_{CMY-4} or bla_{CMY-16} have acquired other antibiotic resistance genes, such as genes coding for carbapenemases (bla_{NDM-1}, bla_{VIM-4}, bla_{OXA-48}, $bla_{OXA-204}$) and/or quinolone resistance proteins (*qnrA6*), ESBLs ($bla_{CTX-M-15}$, bla_{PER-1}), 16S rRNA methylases (*armA*), and fosfomycin resistance (*fosA3*). Diverse plasmid families associated to bla_{DHA-1} were

identified in *K. pneumoniae*, such as IncL/M, IncHI2, IncR and IncFIA groups. Plasmids carrying qAmpCs genes (*bla*$_{\text{CMY-2-}like}$, *bla*$_{\text{DHA-1-}like}$, *bla*$_{\text{FOX-}like}$, *bla*$_{\text{ACT-1}}$, *bla*$_{\text{ACT-2}}$, *bla*$_{\text{ACT-7}}$, *bla*$_{\text{ACT-16}}$) often carry multiple other β-lactam resistance genes, including genes coding for ESBLs (TEM-type, SHV-type, CTX-M-type, PER-1, OXA-1) and/or carbapenemases (OXA-48/-162/-204, VIM-1/-4, IMP-1/-4, NDM-1, KPC-2, CARB-2/PSE-1) (52, 101, 102), as well as resistance elements to non-β-lactam antibiotics, such as aminoglycosides (e.g. *armA*, *rmtB*, *rmtC*), fluoroquinolones [e.g. *qnrA*, *qnrB*, *qnrS*, *aac(6')-Ib-cr*], and sulfonamides (e.g. *sul1*), amongst others (52, 99).

1.4.3. CARBAPENEMASE-PRODUCING *ENTEROBACTERIACEAE*

Carbapenemases are β-lactamases that have the ability to hydrolyze almost all β-lactams (including carbapenems), and most of them are not inhibited by the classical β-lactamase inhibitors (79). However, the decrease susceptibility to carbapenems in *Enterobacteriaceae* can be caused by ESBLs or AmpC enzymes combined with decreased permeability due to alteration or down-regulation of porins (e.g. inactivation of genes coding for outer membrane proteins, with decrease production of OmpK35 and OmpK36 in *K. pneumoniae*) (44, 58, 60, 63). Ertapenem is the carbapenem most affected by this mechanism (58).

The carbapenemases are distributed in all Ambler classes, although Ambler classes A, B and D includes the most epidemiologically relevant (73, 103-105). Class A and B enzymes usually confer higher resistance levels to carbapenems; variants have been described with low-level resistance profiles, which hinder their identification by susceptibility tests in the laboratory (106, 107).

These enzymes have emerged as a consequence of the increasing use of carbapenems in infections caused by ESBL-producing *Enterobateriaceae* (76, 108). The most frequent belong to class A (KPCs), class B (MBLs) and class D (OXAs) β-lactamases (73).

The KPCs are clinically and epidemiologically the most important enzyme among *Enterobacteriaceae* species, associated with prolonged in-hospital stay (109), conferring resistance to penicillins, to first-, second- and third-generation cephalosporins, carbapenems and monobactams, and being inhibited by clavulanic acid (106). KPCs have been considered endemic in the United States of America, Israel, Greece and Italy, and outbreaks have also been reported in China, Brazil and several European countries. These enzymes have also been identified in other *Enterobacteriaceae* species such as *E.*

coli, *Enterobacter* spp. and *K. oxytoca*. The ST258 clone has been involved in the worldwide spread of KPC enzymes (72).

The MBLs hydrolyze all β- lactams except aztreonam, and are inhibited by chelator agents such as ethylenediamine tetraacetic acid, but not by clavulanic acid. These enzymes are encoded by genes located at the chromosome of several Gram-positive and Gram-negative species (e.g. *Bacillus* spp., *P. aeruginosa, Stenotrophomonas maltophilia*, *Aeromonas* spp.), or by plasmid-encoded genes acquired by horizontal gene transfer (mainly in *K. pneumoniae* and *E. coli*) (110). The most common plasmid-mediated MBLs detected among *Enterobacteriaceae* species are VIM, IMP and NDM-1 types (79, 106, 110). MBL-producing *Enterobacteriaceae* have been described worldwide, with VIM-1 and VIM-2 being the most disseminated variants, mainly in *K. pneumoniae* (79, 106, 108). VIM-producing *Enterobacteriaceae* are endemic in Greece (Spain, Italy, Denmark, Hungary, Brazil, Argentina), whereas IMP-types are more prevalent in Asian countries (72). NDM-1 was identified for the first time in a *K. pneumoniae* isolate from an Indian patient previously hospitalized in New Delhi Sweden in 2008 (111), and have been imported into Europe, Asia, North America and Australia by those who have travelled or were hospitalized in the India (112). These β-lactamases have been identified in different *Enterobacteriaceae* species (mainly *K. pneumoniae* and *E. coli*, but also *Enterobacter* spp., *C. freundii*, *M. morganii* and *Providencia* spp.) (72).

The OXAs are a heterogeneous group of β-lactamases with activity over amino- and ureido-penicillins, oxacillin, cloxacillin and carbapenems, which are inhibited by sodium chloride (113). These enzymes are frequently reported among *A. baumanni* and *P. aeruginosa* (OXA-23, OXA-24, OXA-51, OXA-58), but members of this group (most frequently OXA-48) are increasingly being reported among *Enterobacteriaceae* species (113). OXA-48 was firstly identified in a *K. pneumoniae* isolate in Turkey (72), and more recently have disseminated in the Middle East and North Africa, with several outbreaks occurring in Europe (United Kingdom, Belgium, France, Spain and The Netherlands) (79, 106, 114). Some OXA-48-like variants (e.g. OXA-163, OXA-181) have also been identified, differing from OXA-48 by a few amino acid substitutions or deletions (114).

Carbapenems are considered first-line therapy for infection by MDR *Enterobacteriaceae*; however, the increasing emergence of carbapenemases have demonstrated higher mortality rates in patients infected with carbapenem-resistant *Enterobacteriaceae*, due to reduced therapeutic options (115).

In Portugal, is unknown the extent of carbapenemases in urinary isolates from LMTHF, but a national point study (2012) conducted in continuing healthcare (integrating different typologies) revealed that resistance rate to third-generation cephalosporin and carbapenemes was 6.5% for all type of infections (1).

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CHAPTER 2 – OBJECTIVES AND OUTLINE OF THE STUDY

2.1. STATEMENT OF OBJECTIVES

UTIs are known to be one of the most common bacterial infections in both community and hospital settings.

In recent years with the ageing of the population and the expansion of LMTHF, placed at an interface between these settings, a new ecological niche with particular conditions for transmission of bacterial pathogens and/or MDR bacteria emerged.

UTIs are an increasing challenge in LMTHF due to the high occurrence rates, the frequent resident's comorbidities, the diversity of etiologic agents, the diagnostic difficulties, as well as the growing antibiotic resistance rates.

The rapid emergence or increase of resistance to critically important antibiotics, together with the dynamics of species and/or clones with variable antibiotic resistance profiles, determine the need of a continuous monitoring of etiologic agents and antibiotic resistance profiles in this setting in order to appropriately assist empiric treatment of UTI.

The general ***aim*** of the present study was to provide comprehensive and longitudinal data on the etiologic agents of UTI in LMTHF patients and their corresponding antimicrobial susceptibility rates, in order to contribute to improve infection control practices and appropriately support empirical treatment.

This study focused on the following ***specific aims***:

1. To evaluate in LMTHF residents with UTI, the occurrence and trends of etiologic agents.
2. To determine the antimicrobial susceptibility rates of the different uropathogens identified and the frequency of MDR pathogens.
3. To analyze the trends in antibiotic resistance rates for the most common uropathogens.

4. To determine the frequency of clinically important antibiotic resistance mechanisms (ESBLs and carbapenemases) in major pathogens responsible for UTI.

2.2. OUTLINE OF THE DISSERTATION

The findings answering the *specifics aims* of the dissertation are presented in a research article format, and will be subject to subsequent submission to a scientific journal:

I. Silva R, Novais Â, Peixe L. TRENDS IN ETIOLOGY AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA CAUSING URINARY TRACT INFECTIONS IN A LONG- AND MEDIUM-TERM HEALTHCARE FACILITY UNIT IN NORTH OF PORTUGAL. **Manuscript Final Draft**

CHAPTER 3 – RESULTS AND DISCUSSION

TRENDS IN ETIOLOGY AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA CAUSING URINARY TRACT INFECTIONS IN A LONG- AND MEDIUM-TERM HEALTHCARE FACILITY UNIT IN NORTH OF PORTUGAL

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Keywords: Antimicrobial resistance, Extended-spectrum β-lactamases, Multidrug-resistance, Uropathogens

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MANUSCRIPT FINAL DRAFT

TRENDS IN ETIOLOGY AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIA CAUSING URINARY TRACT INFECTIONS IN A LONG- AND MEDIUM-TERM HEALTHCARE FACILITY UNIT IN NORTH OF PORTUGAL

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OBJECTIVE: The goal of this study is to evaluate the etiology of urinary tract infections (UTIs) and the antimicrobial resistance patterns of uropathogens identified among patients from a Portuguese long and medium-term healthcare facility unit.

MATERIALS AND METHODS: A retrospective analysis of bacterial pathogens responsible for UTIs and their corresponding antimicrobial resistance patterns identified among a total of six hundred and ninety-one urine samples from patients institutionalized in Santa Casa da Misericórdia de Vila do Conde was conducted between November 2010 and August 2015. Antimicrobial susceptibility rates and antibiotic resistance mechanisms were detected using antibiotic panels of the commercially available automated system MicroScan® (autoSCAN® System; BECKMAN COULTER).

RESULTS: A total of 471 urine samples (68.2%) were positive for bacterial growth, where 512 uropathogens were identified. *Escherichia coli* was the most prevalent species (38.5%), followed by *Klebsiella pneumoniae* (26.8%), *Proteus* spp. (8.6%) and *Pseudomonas aeruginosa* (7.2%), whilst *Enterococcus faecalis* (4.3%) were the most frequent Gram-positive bacteria. Multidrug resistance patterns were frequently observed (n=242/512; 47.3%), with the high rates registered for *K. pneumoniae* (59.1%). The highest antimicrobial resistance rates were observed for penicillins (61.4%-97.5%), followed by fluoroquinolones (43.5%-55.0%) and trimethoprim/sulfamethoxazole (49.6%). On the other hand, nitrofurantoin, cefoxitin, amikacin, piperacillin/tazobactam, carbapenems and glycopeptides were the most active antibiotics (2.0%-10.0%). Amongst the most frequent uropathogens identified (*E. coli* and *K. pneumoniae*), the antibiotic resistance rates to extended-spectrum cephalosporins, trimethoprim/sulfamethoxazole and ciprofloxacin increased substantially, especially after 2011, whereas imipenem, nitrofurantoin and fosfomycin remained stable during the whole period studied (<6.0%). ESBL producers were detected in 24.1% (n=101/419) of *Enterobacteriaceae* isolates, being of note the recent increase of ESBL-producing *K. pneumoniae* (reaching 72.2% in 2015). Two ESBL-producing *K. pneumoniae* strains were simultaneously carbapenemase-producers. Statistically significant differences were as not observed between gender and incidence of ESBL-producing strains (*P*=0.524).

CONCLUSIONS: We found alarming rates and trends of MDR pathogens causing UTIs in the period studied, alerting for the need of further surveillance in these settings. Whilst some antibiotics commonly used for empirical treatment proved to be of little utility. Nitrofurantoin and fosfomycin were considered the most appropriate antimicrobials. Thus, empirical antibiotic prescription in long and medium-term healthcare facilities should be guided by and adjusted to local data.

KEYWORDS
Antimicrobial resistance, Extended-spectrum β-lactamases, Multidrug-resistance, Uropathogens

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the most common healthcare-associated infections and the incidence of multidrug-resistant (MDR) bacteria causing UTIs is an increasing Public Health problem in different settings such as hospitals, the community and long-term care facilities, including in Portugal (1-7).

The long and medium-term healthcare facilities (LMTHF) are an intermediate setting between the community and the hospital settings, where the frequent flow to acute care hospitals and the close proximity between residents increase the risk of infections and favors the acquisition and transmission of MDR bacteria (8, 9). In fact, the resident's population of LMTHF is generally old, with frequent comorbidities (e.g. diabetes, spinal cord injury, stroke, dementia, urinary or faecal incontinence) and present several risk factors for developing UTIs, including advanced age, impaired mobility, chronic illness, immunosuppression, use of urinary catheters, and inappropriate antimicrobial use favoring colonization with MDR bacteria during hospitalization (9-12).

In Portugal, the etiology and antibiotic resistance patterns of the most common pathogens causing UTIs in either hospital and community settings is well known (4, 5, 7, 13,14) but the information regarding the epidemiology in LMTHF is scarce and based mostly on a point-prevalence study (15, 16), where data on antimicrobial resistance rates of urinary pathogens from LMTHF is missing.

The aim of the present study was to evaluate the etiology of UTIs and the antimicrobial resistance patterns of the corresponding uropathogens in patients from a Portuguese LMTHF unit, between November 2010 and August 2015.

2. MATERIALS AND METHODS

2.1. STUDY DESIGN

A retrospective analysis of the etiology and antimicrobial resistance patterns of bacterial pathogens causing UTIs in the Santa Casa da Misericórdia de Vila do Conde was performed between November 2010 and August 2015. This unit consists on a LMTHF of 40 beds, located in the North of Portugal, and data was collected at the Clinical Analysis Laboratory.

For UTIs patients, demographic data including age and sex of the resident and date of sample collection was recorded.

2.2. COLLECTION OF SAMPLES AND ISOLATES IDENTIFICATION

Six hundred ninety-one urine samples were analyzed in the study period (2010-2015). Only samples with pyuria (5–10 white blood cells per high power field or more) and bacteriuria (≥ 10^5 CFU/mL), following clinical symptoms (mild fever, dysuria, pollakiuria, frequency and urgency, hematuria, cloudy urine or strong odor, suprapubic pain, new or worsening of incontinence, or deterioration in functional or mental status) were considered as positive.

Urine samples with polymorphic bacterial growth (growth of three or more bacterial species) were discarded. Worked up clean-catch midstream morning urine specimens were collected using sterile containers, according to standard procedures. Urine samples were inoculated aseptically on CLED and MacConkey agar using calibrated wire loops (1µl) and then incubated aerobically at 37ºC for 24 to 48 hours. Uropathogens identified in positive cultures, were identified by Gram-staining and standard biochemical procedures using commercial systems MicroScan® (autoSCAN® System; BECKMAN COULTER).

2.3. ANTIMICROBIAL SUSCEPTIBILITY TESTING AND ANTIBIOTIC RESISTANCE MECHANISMS

Antimicrobial susceptibility patterns and antibiotic resistance mechanisms were inferred using antibiotic panels of the commercial automated system MicroScan® (autoSCAN® System; BECKMAN COULTER) specific for *Enterobacteriaceae* (Urine Combo 69), Gram-negative non-*Enterobacteriaceae* (Neg Non Entero Combo 71) and Gram-positive cocci (Pos Combo 42). Antibiotic susceptibility tests were determined and interpreted using guidelines and clinical breakpoints established by the Clinical and Laboratory Standards Institute (CLSI) (17) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/).

The antibiotics tested for *Enterobacteriaceae* were ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, cefoxitin, cefuroxime, cefotaxime, ceftazidime, cefepime, ertapenem, imipenem, meropenem, norfloxacin, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, fosfomycin, nitrofurantoin and trimethoprim/sulfamethoxazole.

Gram-negative bacteria non-*Enterobacteriaceae* were tested against piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, aztreonam, norfloxacin, ciprofloxacin, levofloxacin, imipenem, meropenem, doripenem, amikacin, gentamicin, tobramycin, colistin, fosfomycin and trimethoprim/sulfamethoxazole.

For Gram-positive bacteria, the panel of antibiotics comprises penicillin G, ampicillin, amoxicillin/clavulanic acid, ciprofloxacin, levofloxacin, gentamicin, tobramycin, teicoplanin, vancomycin, rifampin, trimethoprim/sulfamethoxazole, fosfomycin and nitrofurantoin.

For all *Enterobacteriaceae* isolates, the production of extended-spectrum β-lactamase (ESBL) was confirmed by using ceftazidime and ceftazidime/clavulanic acid and the cefotaxime and cefotaxime/clavulanic acid combinations, in the automated system MicroScan® (autoSCAN® System; BECKMAN COULTER), according to criteria established by the EUCAST.

Carbapenemase production was confirmed by the Blue-Carba test (18).

2.4. STATISTICAL ANALYSIS

Demographic data, urinary pathogens and antibiotics resistance patterns obtained were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0. Frequencies and percentages were generated for categorical variables, such as sex, age, urinary pathogens genus or/species and rate of isolation.

The genus or/species of bacteria more frequently isolated were introduced in multiple correspondence analyses to detect possible correlations with antimicrobial resistance patterns.

Chi-square test was applied to detect association between ESBL and sex. A *P*-value of ≤0.05 was considered statistically significant.

2.5. ETHICAL CONSIDERATIONS

Ethical approval was obtained by the Ethics Committee of Santa Casa da Misericórdia de Vila do Conde. Resident's privacy was protected by de-identification of records. All the data obtained during the study were kept confidential, and used only for the purpose of this study.

3. RESULTS

3.1. ETIOLOGY OF BACTERIAL UROPATHOGENS

A total of 471 cultures were positive (n=471/691; 68.2%), 430 of them (91.3%) had monomorphic bacterial growth, while 41 (8.7%) presented two different bacterial species, corresponding to a total of 512 uropathogens isolated and analyzed.

The patient's age ranged from 28 years to 98 years, with mean age of 75±12 years (median 78 years). A total of 414 (n=414/691; 59.9%) urine samples were from female patients whereas 277 (n=277/691; 40.1%) were from males, corresponding to a male to female ratio of 1:1.49. The rate of positive samples was similar between females and males (Table 1).

Table 1 Gender distribution of patients with positive samples with UTI.

Demographic characteristics		Positive No. (%)	Negative No. (%)	Total No. (%)
Gender	Male	184 (66.4)	93 (33.6)	277 (100.0)
	Female	287 (69.3)	127 (30.7)	414 (100.0)
	Total	471 (68.2)	220 (31.8)	691 (100.0)

Most of the UTIs were caused by Gram-negative bacteria (92.2%), while Gram-positive bacteria were much less identified (7.8%). *Escherichia coli* was the most common uropathogen isolated, followed by *Klebsiella pneumoniae*, *Proteus* spp. and *Pseudomonas aeruginosa,* whilst *Enterococcus faecalis* was the most frequent Gram-positive cocci. *E. coli* and *K. pneumoniae* were isolated more frequently in female (29.5-42.3%) than male's patients (22.5%-32.5%), while the frequency of isolation of *Acinetobacter* spp., *P. aeruginosa* and *Staphylococci* was higher in males (4.0%-9.0%) than females (0.3%-6.1%) (Table 2).

Table 2 Uropathogens from urine samples of patients with UTI.

Microorganism	Female		Males		Total	
	No.	%	No.	%	No.	%
Escherichia coli	132	42.3	65	32.5	197	38.5
Klebsiella pneumoniae	92	29.5	45	22.5	137	26.8
***Proteus* spp.**	25	8.0	19	9.5	44	8.6
Providencia stuartii	7	2.2	7	3.5	14	2.7
***Citrobacter* spp.**	6	1.9	3	1.5	9	1.8
Morganella morganii	1	0.3	7	3.5	8	1.6
Other *Enterobacteriaceae*	4	1.3	6	3.0	10	2.0
***Acinetobacter* spp.**	4	1.3	11	5.5	15	2.9
Pseudomonas aeruginosa	19	6.1	18	9.0	37	7.2
Other no fermentative gram-negative	0	0.0	1	0.5	1	0.2
Staphylococcus aureus	0	0.0	5	2.5	5	1.0
Coagulase negative *Staphylococcus*	1	0.3	3	1.5	4	0.8
Enterococcus faecalis	15	4.8	7	3.5	22	4.3
Enterococcus faecium	6	1.9	3	1.5	9	1.8
Total	312	100	200	100	512	100

In general, *E. coli* was more frequently involved in UTIs than *K. pneumoniae*, although similar rates were observed in 2012, 2013 and 2015 (Figure 1).

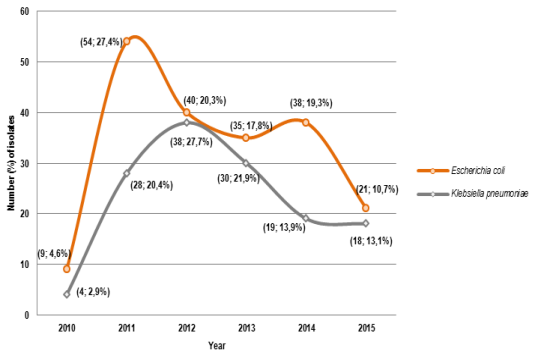

Figure 1 Incidence of *Escherichia coli* and *Klebsiella pneumoniae* causing UTI (November 2010 - August 2015.

3.2. OVERALL ANTIBIOTIC RESISTANCE PATTERNS

Multidrug resistance patterns were frequently observed among urinary pathogens (n=242/512; 47.3%), especially for *Providencia* spp. (92.9%), *Acinetobacter* spp. (86.7%), *Morganella morganii* (75.0%) and *P. aeruginosa* (62.2%). It is of note that the incidence of MDR *E. coli* (39.1%) was lower compared to that of *K. pneumoniae* (59.1%).

When the overall antimicrobial resistance rates of all bacterial uropathogens are considered, the highest scores were obtained for penicillins (61.4%-97.5%), followed by fluoroquinolones (43.5%-55.0%) and trimethoprim/sulfamethoxazole (49.6%). Nitrofurantoin, cefoxitin, amikacin, piperacillin/tazobactam, carbapenems and glycopeptides showed lower resistance rates (2.0%-10.0%) (Table 3). Nevertheless, these patterns vary according to the particular bacterial families, genera or species, as described below.

Almost all *Enterobacteriaceae* isolates demonstrated high resistance rates to fluoroquinolones, ampicillin and trimethoprim/sulfamethoxazole (0.0%-71.4%) whilst the opposite was observed for amikacin, piperacillin/tazobactam, nitrofurantoin and carbapenems (0.0%-12.5%). *Acinetobacter* spp. isolates showed high resistance rates to all antibiotics (≥ 40%), including to carbapenems. *P. aeruginosa* was frequently resistant to fluoroquinolones, 3rd and 4th generation cephalosporins (36.1%-90.5%) whereas the most active antibiotics were meropenem, followed by doripenem, colistin, amikacin, aztreonam and imipenem (5.3%-18.9%).

All Gram-positive cocci, with exception of *E. faecalis*, were resistant to fluoroquinolones and ampicillin (100%). On the other hand, *Staphylococci* and *E. faecalis* demonstrated high susceptibility to glycopeptides (0.0%-7.1%) and *E. faecalis* to nitrofurantoin (0.0%). Despite the uncertainty on its clinical efficacy, the trimethoprim/sulfamethoxazole was tested against *Enterococci* revealing a high resistance rate (100%). All *S. aureus* were susceptible to fosfomycin and 80.0% were susceptible for nitrofurantoin.

3.3. TRENDS IN ANTIMICROBIAL RESISTANCE RATES FOR THE MOST COMMON UROPATHOGENS

The resistance rates to the most frequent therapeutic options observed for *E. coli* and *K. pneumoniae* in the study period are represented in Figure 2A and Figure 2B, respectively.

In *E. coli*, resistance rates to cefuroxime and cefotaxime have been increasing since 2011, whereas resistance to trimethoprim/sulfamethoxazole and ciprofloxacin both peaked during 2011 (>50%) and lowered subsequently (especially ciprofloxacin) till 2015. Resistance to imipenem, nitrofurantoin and fosfomycin remained stable during the whole period studied (< 6.0%).

Regarding *K. pneumoniae*, resistance rates to almost all antibiotics increased after 2011, with the exception of imipenem, which remained at <6.0% in 2015.

Resistance to carbapenems was observed in three *K. pneumoniae* isolates from 2014 to 2015; two of them were concomitantly producers of ESBL and carbapenemase (KPC-type, data not shown), whereas the other was negative for carbapenemase production.

Table 3 Antimicrobial resistance rates of isolates causing UTI

Antimicrobial category [a]	Antimicrobial agent [a]	*E. coli*		*K. pneumoniae*		*Proteus* spp.		*P. stuartii*		*Citrobacter* spp.		*M. morganii*		Other *Enterobacteriaceae*		*Acinetobacter* spp.		*P. aeruginosa*		Other no fermentative gram-negative		*S. aureus*		CNS		*E. faecalis*		*E. faecium*		Total antimicrobial resistance rate
		T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	T	R %	R (%)
Aminoglycosides	Amikacin [b]	63	3.2	38	2.6	11	0.0	5	0.0	1	0.0	3	0.0	3	0.0	5	40.0	33	15.2	1	100.0	-	-	-	-	-	-	-	-	11 (6.7%)
	Gentamicin	196	17.9	137	46.7	44	11.4	-	-	9	22.2	8	37.5	10	20.0	15	80.0	37	37.8	1	100.0	5	20.0	4	50.0	-	-	-	-	141 (30.2%)
	Tobramycin	196	18.9	137	52.6	44	2.3	-	-	9	22.2	8	25.0	10	20.0	15	73.3	37	21.6	1	0.0	1	100.0	1	100.0	-	-	-	-	137 (29.8%)
Ansamycins	Rifampicin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	6.7	8	75.0	7 (30.4%)
Antipseudomonal penicillins + β-lactamase inibitors	Piperacillin/ tazobactam	196	3.6	137	6.6	44	0.0	14	0.0	9	0.0	8	0.0	10	0.0	-	-	37	27.0	1	0.0	-	-	-	-	-	-	-	-	26 (5.7%)
Carbapenems	Ertapenem [b]	62	0.0	38[a]	7.9	11	0.0	5	0.0	1	0.0	3	0.0	3	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3 (2.4%)
	Imipenem	196	0.0	137	1.4	44	0.0	14	0.0	9	0.0	8	12.5	10	0.0	5	100.0	37	18.9	1	0.0	-	-	-	-	-	-	-	-	15 (3.3%)
	Meropenem [b]	61	0.0	38[a]	5.3	11	0.0	5	0.0	1	0.0	3	0.0	3	0.0	3	66.7	19	5.3	-	-	-	-	-	-	-	-	-	-	5 (3.5%)
	Doripenem	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	6.3	-	-	-	-	-	-	-	-	-	-	1 (6.3%)
1st and 2nd generation cephalosporins	Cefazolin [c]	134	20.9	99	50.5	30	6.7	-	-	-	-	-	-	1	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80 (30.3%)
	Cefuroxime	195	19.0	137	51.1	41	48.8	9	77.8	8	100.0	-	-	9	55.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	128 (32.1%)
3rd and 4th generation cephalosporins	Cefotaxime	195	16.4	137	47.4	44	6.8	14	7.1	9	11.1	5	10.0	10	20.0	15	93.3	21	90.5	-	-	-	-	-	-	-	-	-	-	138 (30.7%)
	Ceftazidime	196	16.3	137	48.9	44	4.5	14	7.1	9	22.2	8	50.0	10	40.0	15	66.7	37	40.5	1	0.0	-	-	-	-	-	-	-	-	137 (29.1%)
	Cefepime	135	14.8	99	43.4	33	3.0	9	0.0	8	0.0	5	20.0	7	0.0	12	91.7	36	36.1	1	0.0	-	-	-	-	-	-	-	-	89 (25.8%)
Cephamycins	Cefoxitin	195	1.0	137	6.6	44	4.5	14	0.0	-	-	8	37.5	2	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16 (4.0%)
Fluoroquinolones	Ciprofloxacin	196	38.8	137	47.4	44	34.1	14	92.9	9	22.2	8	62.5	10	10.0	15	86.7	37	59.5	1	100.0	5	100.0	4	100.0	22	50.0	9	100.0	242 (47.4%)
	Levofloxacin [b]	62	33.9	38	63.2	11	45.5	5	100.0	1	100.0	3	66.7	3	0.0	3	66.7	33	60.6	1	100.0	5	100.0	4	100.0	22	50.0	9	100.0	110 (55.0%)
	Norfloxacin	194	40.7	137	48.2	44	31.8	14	85.7	9	22.2	8	75.0	10	10.0	-	-	5	60.0	-	-	-	-	-	-	-	-	-	-	183 (43.5%)
Folate pathway inhibitors	Trimethoprim/ Sulfamethoxazole	196	42.3	137	62.8	44	47.7	14	71.4	9	22.2	8	50.0	10	10.0	15	46.7	-	-	-	-	5	0.0	4	25.0	6	100.0	2	100.0	223 (49.6%)
Glycopeptides	Teicoplanin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0.0	4	0.0	21	4.5	9	22.2	3 (7.5%)
	Vancomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0.0	4	0.0	14	7.1	7	28.6	3 (10.0%)
Monobactams	Aztreonam	2	50.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	18.8	1	0.0	-	-	-	-	-	-	-	-	7 (20.0%)
Nitrofurans	Nitrofurantoin	194	0.5	-	-	-	-	-	-	9	11.1	-	-	8	0.0	-	-	-	-	-	-	5	20.0	4	0.0	22	0.0	9	22.2	5 (2.0%)
Penicillins	Ampicillin	195	68.2	-	-	41	31.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	100.0	4	100.0	8	0.0	2	100.0	156 (61.4%)
	Penicillin G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	100.0	4	100.0	22	95.5	9	100.0	39 (97.5%)
Penicillins + β-lactamase inibitors	Amoxicillin/ clavulanic acid	195	14.4	137	29.2	44	9.1	-	-	-	-	-	-	2	0.0	-	-	-	-	-	-	1	100.0	1	100.0	-	-	-	-	74 (19.5%)
Phosphonic acids	Fosfomycin	195	2.6	137	17.5	44	38.6	14	92.9	9	11.1	8	87.5	10	20.0	-	-	20	85.0	-	-	5	0.0	4	25.0	-	-	-	-	87 (19.5%)
Polymyxins	Colistin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	12.5	-	-	-	-	-	-	-	-	-	-	4 (12.5%)

(T) Total number of bacterial isolates tested against each antimicrobial agent; **(R %)** Percentage of bacterial isolates resistant to antimicrobial agent; **(-)** Antimicrobial not tested

[a] Exclusion of species with intrinsic resistance to antimicrobial agents or categories

[b] Amikacin, ertapenem, meropenem and levofloxacin were tested from January 2014

[c] Cefazolin was tested between November 2010 and January 2014

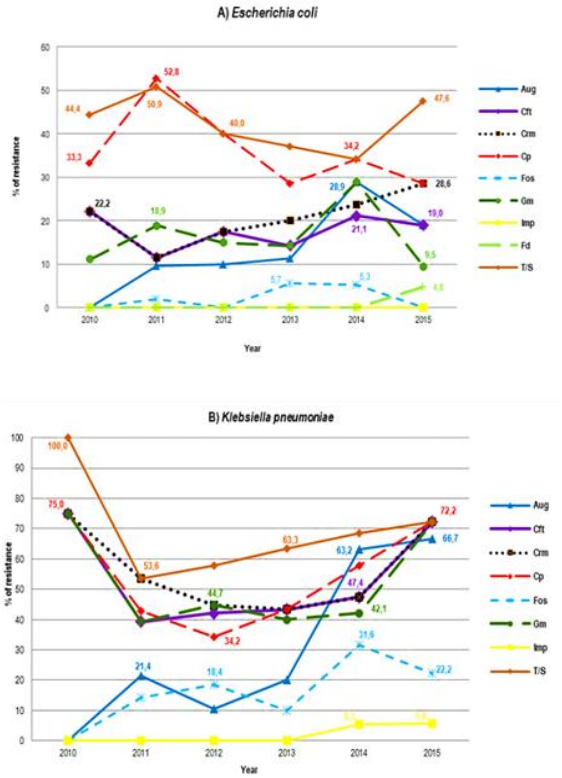

Figure 2 Temporal patterns of resistance to selected antibiotics for A) *Escherichia coli* and B) *Klebsiella pneumoniae*, between November 2010 and August 2015. Y-axis represents resistance in percentage of bacterial isolates for year. Aug (amoxicillin/ clavulanic acid); Cft (cefotaxime); Crm (cefuroxime); Cp (ciprofloxacin); Fos (fosfomycin); Gm (gentamicin); Imp (imipenem); Fd (nitrofurantoin); T/S (trimethoprim/sulfamethoxazole).

The presence of ESBLs was detected frequently among *Enterobacteriaceae* isolates (n=101/419; 24.1%), corresponding mostly to *K. pneumoniae* (n=64), *E. coli* (n=32), *M. morganii* (n=3), *P. vulgaris* (n=1) and *E. cloacae* (n=1). It is of interest to highlight that the rate of ESBL among *K. pneumoniae* isolates is higher than that of *E. coli* during the whole period analyzed (Figure 3). Moreover, a notable increase in the rate of ESBL-producing *K. pneumoniae* was observed more recently (from 43.3% in 2013 to 72.2% in 2015) whereas the rates of ESBL-producing *E. coli* remained between 11.2% and 22.2% during the study period. Statistically significant differences were not observed between the incidence of ESBL-producing strains and gender (*P*=0.524).

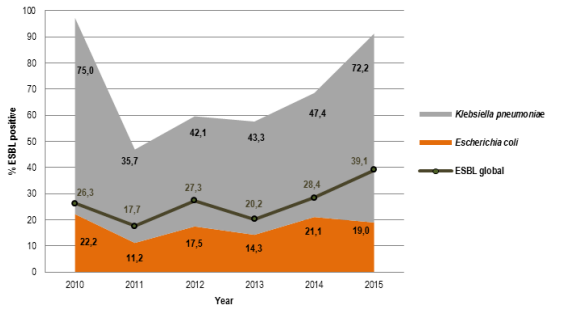

Figure 3 Distribution of the two main ESBL-producing species (%): evolution from November 2010 to August 2015.

All ESBL-producing *E. coli* and *K. pneumoniae* were resistant to ampicillin, cefazolin, cefuroxime, cefotaxime, ceftazidime and cefepime. Most of these isolates have high resistance rates to gentamicin, tobramycin, trimethoprim/sulfamethoxazole fluoroquinolones, and amoxicillin/clavulanic acid (34.4%-100%). These isolates showed the highest susceptibility rates to carbapenems, nitrofurantoin, fosfomycin, cefoxitin, amikacin and piperacillin/tazobactam (0.0%-14.1%) (Table 4).

Table 4 Antimicrobial resistance rates of the two main ESBL-producing species.

Antimicrobial category	Antimicrobial agent	*E. coli*		*K. pneumoniae*	
		T	R %	T	R %
Aminoglycosides	Amikacin [a]	13	7.7	22	4.5
	Gentamicin	32	53.1	64	89.1
	Tobramycin	32	62.5	64	92.2
Antipseudomonal penicillins + β-lactamase inibitors	Piperacillin/ tazobactam	32	9.4	64	9.4
Carbapenems	Ertapenem [a]	13	0.0	22	13.6
	Imipenem	32	0.0	64	3.1
	Meropenem [a]	12	0.0	22	9.1
Cephamycins	Cefoxitin	32	0.0	64	6.3
Fluoroquinolones	Ciprofloxacin	32	90.6	64	79.7
	Levofloxacin [a]	12	100.0	22	95.5
	Norfloxacin	31	90.3	64	78.1
Folate pathway inhibitors	Trimethoprim/ sulfamethoxazole	32	65.6	64	96.9
Nitrofurans	Nitrofurantoin	31	0.0	-	-
Penicillins + β-lactamase inibitors	Amoxicillin/ clavulanic acid	32	34.4	64	46.9
Phosphonic acids	Fosfomycin	31	3.2	64	14.1

(T) Total number of bacterial isolates tested against each antimicrobial agent; **(R %)** Percentage of bacterial isolates resistant to antimicrobial agent; **(-)** Antimicrobial not tested

[a] Amikacin, ertapenem, meropenem and levofloxacin were tested from January 2014

4. DISCUSSION

This study provides systematic data about the urinary bacterial pathogens responsible for UTIs and their antibiotic resistance patterns during a five-year period in a Portuguese LMTHF unit, providing a framework for appropriate guidance and prescription.

The species distribution of our sample (the most common bacteria identified were *E. coli* and *K. pneumoniae*) are in accordance with data from surveillance reports available in the country in this setting (15, 16). Nevertheless, it is of interest to highlight the higher incidence of *K. pneumoniae* in our population (26.8%) than that reported in previous studies (9, 15), which is in accordance with the increasing involvement of *K. pneumoniae* isolates in nosocomial infections (19).

In our study, 47.3% of clinical isolates exhibited MDR patterns. This rate is similar to that observed in bacteria responsible for hospital- and long-term care facilities-acquired UTIs and higher than that commonly observed among pathogens causing community acquired UTIs (9, 10, 20). Indeed, the high-level of consumption of antibiotics may be responsible for this resistance (15, 16), particularly for fluoroquinolones (3, 6). The resistance rates observed for penicillins, first, second and third generation cephalosporins, fluoroquinolones, gentamicin, tobramycin and trimethoprim/sulfamethoxazole in this study is remarkable, particularly among *Enterobacteriaceae*.

In our study, the incidence of ESBL producers (24.1%) is similar to that obtained in the national surveillance report considering long-term care facilities (16). Considering the age of the patients in this setting (median 78 years), the high

incidence of ESBL-producing isolates is in accordance with previous studies showing that age over 65 years old is a risk factor for β-lactamase-mediated resistance to oxyimino-β-lactams in patients infected with enterobacteria (9, 21, 22). In accordance with previous studies in Portugal or other European countries, *K. pneumoniae* (63.4%) was the main species identified, followed by *E. coli* (31.7%) (4, 5, 10, 21, 23, 24). In these isolates, besides extended-spectrum β-lactams resistance, the co-resistance profiles observed for other antibiotic classes (fluoroquinolones, trimethoprim/sulfamethoxazole, gentamicin, tobramycin and amoxicillin/clavulanic acid) is particularly problematic in UTI and a long-term care setting since it limits therapeutic options and empirical antibiotic choices (20). In fact, the use of antibiotics with antibiotic resistance rates exceeding 10-20% is associated with an increased risk of treatment failure and selection of antibiotic resistant strains (25, 26).

Conversely, the highest susceptibility rates were found for carbapenems, nitrofurantoin, fosfomycin, cefoxitin, amikacin and piperacillin/tazobactam. However, it is of interest to highlight the increased resistance to fosfomycin observed for ESBL-producing *K. pneumoniae* (14.1%) comparatively with *E. coli* (3.2%). This is worrisome because this antibiotic is increasingly used to treat UTIs caused by ESBL-producing strains (25, 27), and an acquired fosfomycin resistance gene (*fosA3*) was already detected in a clinical isolate in Portugal, alerting for the risk of further transmission (28).

In Portugal, the treatment of UTIs in long-term care settings units is based in guidelines issued by the General Direction of Health for community-acquired urinary infections that do not take into account the differences in the epidemiology and susceptibility patterns of urinary pathogens in the different contexts. For this reason, it is necessary to adequate UTI treatment recommendations in these long-term care settings to the evolving epidemiology and changing bacterial susceptibility in order to optimize antibiotic prescription and subsequently clinical outcomes in institutionalized patients (20, 29).

Considering the high prevalence of MDR isolates in this study, (and particularly of ESBL-producers), and since the efficacy of amoxicillin/clavulanic acid in the treatment of UTIs caused by these isolates has not been well-established (25), the nitrofurantoin and fosfomycin seem to be reasonable alternatives for the treatment of uncomplicated UTIs. While the nitrofurantoin requires longer courses of therapy and its therapeutic efficacy decreases in patients with compromised renal function, the fosfomycin is a satisfactory alternative because of its single-dose treatment, prolonged post-antibiotic effect and reduced secondary effects (25). Both antibiotics are not recommended for complicated or upper UTI (25, 30). The fluoroquinolones should be reserved for other than acute cystitis or as an alternative when these agents cannot be used (2, 31).

As control the transmission of MDR isolates it is important to: i) notify the identification of MDR isolates by the laboratory to the healthcare facilities units; ii) to identify reservoirs of MDR bacteria in different healthcare settings; iii) to sign and notify MDR carriers especially when they are transferred to other healthcare institutions as well as when they are admitted in our unit; iv) to adopt barrier precautions for colonized and infected patients (antiseptic hand-washing, wearing gloves and gown) and isolation or cohorting when possible.

5. CONCLUSION

In conclusion, we found alarming rates and trends of MDR pathogens implicated in UTIs among patients from a long-term care facility in Portugal, alerting for the need of further surveillance in these settings. The antibiotic resistance patterns observed (nitrofurantoin and fosfomycin were considered the most appropriate antimicrobials) suggest that empirical antibiotic prescription needs to be adjusted to local data.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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CHAPTER 4 – CONCLUSIONS

The LMTHF are an intermediate setting between the community and the hospital settings, with a marked expansion in recent years. Different LMTHF aspects and resident's conditions enhance the possibility of infection acquisition namely by MDR bacteria. Although UTIs are common infections in LMTHF, information regarding the uropathogens epidemiology in Portuguese LMTHF is scarce and corresponding data on antimicrobial resistance rates is missing, highlighting the opportunity and relevance of the present study.

Overall, the results of this dissertation conducted to the following **general CONCLUSION**:

This study alerts for the increasing rates of MDR among etiologic agents causing UTIs in patients institutionalized in a LMTHF in Portugal, and highlights the need to adapt empirical treatment choices and infection control practices to local data.

Particular conclusions are summarized as follows:

- Gram-negative bacteria (92.2%, especially *E. coli* and *K. pneumoniae*) were the most prevalent uropathogens identified all over the period analyzed, which is in line with the species distribution observed among urinary pathogens from the hospital setting;

- High antibiotic resistance rates were observed for β-lactams (penicillins, first-, second- and third-generation cephalosporins), fluoroquinolones and trimethoprim/sulfamethoxazole, whereas nitrofurantoin, fosfomycin, cefoxitin, amikacin, piperacillin/tazobactam, carbapenems and glycopeptides were the most active antibiotics;

- The MDR patterns frequently observed (47.3%, especially among *Enterobacteriaceae*, *Acinetobacter* spp., and *P. aeruginosa*) reduce significantly the treatment options for these urinary pathogens and suggest that these settings seem to act as reservoirs of MDR bacteria and need to be further surveyed;

- The increasing antibiotic resistance rates observed amongst the most common uropathogens (*E. coli* and *K. pneumoniae*) is worrying, where the recent extraordinary increase of MDR *K. pneumoniae* isolates deserves to be highlighted;

- The high and increasing occurrence of ESBLs among *Enterobacteriaceae* is alarming (especially for *K. pneumoniae*) and compromises almost all therapeutic options available for empirical treatment, being imipenem, nitrofurantoin or fosfomycin the most viable therapeutic options;

- From this point of view, it is necessary that the empirical antibiotics regimen of UTIs-acquired in long and medium-term healthcare facilities be adjusted according to local data.

ANEXES

ETHICS COMMITTEE DECLARATION

SANTA CASA DA MISERICÓRDIA DE VILA DO CONDE

Declaração

A Santa Casa da Misericórdia de Vila do Conde com sede na Rua Rainha Dona Leonor, n.º 123 em Vila do Conde, contribuinte n.º 501382356, representada pela Diretora Geral – Maria da Conceição de Castro Antunes, declara para os devidos efeitos que autoriza o seu colaborador – Dr. Ricardo André Torres Silva – a fazer o levantamento de dados e respetivo tratamento estatístico, referente a utentes da Unidade de Cuidados Continuados Integrados, entre o período de Novembro de 2010 e Agosto de 2015, no âmbito da elaboração da tese de dissertação "Antimicrobial Susceptibility of Urinary Tract Pathogens of Residents in a Long- and Medium-Term Healthcare Facility in North of Portugal".

Para o efeito, deverá:

a) Respeitar as imposições legais na matéria de proteção de dados, nomeadamente a Lei de Proteção de Dados 67/98 de 26 de Outubro, a lei 12/2005 de 26 de Janeiro de informação genética e informação na saúde.

b) Realizar o acesso à informação de forma anonimizada e somente para os fins a que se propõe, supra identificados.

Por ser verdade e me ter sido solicitada, assino e carimbo a presente declaração, aos catorze dias do mês de Novembro do ano dois mil e dezasseis.

A Diretora Geral

- Maria Conceição Antunes, Dr.ª -

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