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THE STUDY OF THE PHENOMENON OF ANTIBIOTIC RESISTANCE OF UROPATHOGENIC GERMS IN THE COUNTIES OF SOUTHERN MOLDOVA

PHD THESIS SUMMARY

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Table of contents (of the doctoral thesis):

Published scientific papers:

1. **Rusu A**, Petca A, Mares C, Petca RC, Popescu RI, Negoita S, Danau RA, Chibelean CB, Jinga V. Urinary tract infections in a Romanian population: antimicrobial resistance of uropathogens – a multiregional study. FARMACIA, 2023, Vol. 71, 1. https://doi.org/10.31925/farmacia.2023.1.19 https://farmaciajournal.com/wp-content/uploads/art-19-Rusu_Petca_Jinga-_165-173-re.pdf (ISI-IF1.6)
2. **Rusu A**, Tiliscan C, Adamescu AI, Ganea OA, Arama V, Arama SS, Rascu SA, Jinga V. Carbapenemase-producing uropathogens in real life: epidemiology and treatment at a County Emergency Hospital from Eastern Romania. J Med Life. 2023 May;16(5):707-711. doi: 10.25122/jml-2023-0139. PMID: 37520479; PMCID: PMC10375344. https://pubmed.ncbi.nlm.nih.gov/37520479/ https://medandlife.org/wp-content/uploads/10.-JML-2023-0139.pdf
3. **Rusu A**, Popescu RI, Predoiu G, Petca RC, Ciudin A, Aida Petca A, Aurelian J, Radavoi D, Jinga V. Non-Antibiotic Prevention of Catheter Mechanic and Septic Complications in Patients with Long-Term Indwelling Catheters: a Crossover Prospective Study Involving L-Methionine. MAEDICA – a Journal of Clinical Medicine 2024; 19(2): 212-220 https://doi.org/10.26574/maedica.2024.19.2.212 https://www.maedica.ro/articles/2024/2/2024_19(22)_No2_pg212-220.pdf

INTRODUCTION

Antimicrobial resistance is a worrying and growing phenomenon globally. Probably one of the most cited papers in the medical academic world on infections and bacterial resistance to antibiotics is *Review on Antimicrobial Resistance, Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations.* And we are seeing in certain parts of Europe an increasing number of patients in intensive care units, haematology units and transplant units who have pan-resistant infections, which means that there is no effective treatment available for them. [1]

Multidrug-resistant bacteria pose a major threat to the successful activity in almost all branches of medical practice. For example, cystitis in young women should be very easy to treat with commonly used antibacterial agents, but often the emergence of multiple antibiotic resistance among the organisms associated with these infections means that doctors have to resort to other agents that may not be well tolerated and may even need to be administered intravenously. [2]

There is scientific data from clinical hospitals where research is done on the resistance of bacteria to antibiotic therapy and in addition they are much more likely to participate in programs on the study of antibiotic resistance than smaller hospitals. To generate national estimates for the number of infections, highly cited international reports extrapolate estimates from EARS-Net (*European Antimicrobial Resistance Surveillance Network*) by using the population reported to the EARS-Net. Reports to EARS-Net are mainly from tertiary hospitals. This means that the characteristics of infections in predominantly tertiary hospitals apply as a whole to the estimates that concern the whole country [3]. The assessment of *Review on Antimicrobial Resistance, Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations* acknowledges that the reported numbers are "broad-brush" estimates, that "more detailed and robust work" will be done in the future, and that there is a lack of data especially from the non-academic environment [3]. In this context, in-depth studies that do not come from tertiary centers of medical services are required, and that can improve the overall picture of AMR in Romania.

On the other hand, there are few, or rather almost non-existent, data from the general population and from the area of general medical services in Romania. In medium and small hospitals, in-depth research is not done and the data here are not collected and processed.

A good example of medical health services that address the general population in Romania is a medium-to-small county emergency hospital in a county seat municipality. Such a hospital concentrates the most important part of the medical acts in that county and has the greatest impact at the level of the respective community.

Another aspect is that carbapenem-resistant enterobacteriaceae (CRE), a subset of infection-generating XDR (extended drug resistant) bacteria with modest clinical results on treatment, are most commonly isolated from urine. Patients who have chronic indwelling urinary catheters inserted appear to be at the highest risk [4]. The number of these infections is increasing, placing uropathogens at the forefront of antibiotic resistance. The identification of these aspects and the consecutive correlations are part of the purpose of this research.

The choice of theme is justified with the arguments listed above. The importance and expected impact can be summarized as follows: the conclusions will lead to the development of knowledge in the field of MDR (multi drug resistant) and XDR bacterial infections at the level of non-academic, secondary-level hospital units, where an empty space of scientific data is filled, and will bring medical practice closer to the needs of the community to which it is addressed. The scientific foundations of the implementation of modern concepts of *Antimicrobial Stewardship can be laid*. The impact of urinary catheterization on the dissemination of XDR germs will be evaluated and conclusions will be drawn on measures that can be implemented to limit these phenomena.

Cap.1 Uropathogenic bacteria and antimicrobial resistance

Introduction – epidemiology. The species of bacteria that cause urinary tract infections are mostly known since the dawn of microbiology, and their presence in the external or internal environment has always been regarded as natural, ubiquitous, most of them being commensal and opportunistically pathogenic. The extension of the phenomenon of adaptation of microorganisms to antibiotics initially appeared in the hospital environment, but later expanded beyond its borders. Over time, the ways of spreading resistance have become more complex, involving not only the hospital environment, but also the community environment, through the abuse of antibiotics (which can, for example, unbalance the intestinal flora and facilitate colonization with MDR bacteria), the agriculture, through the use of antibiotics in animal husbandry, or the wastewater field.

Microorganisms involved in the etiology of UTI. Uropathogens (all possessing virulence factors) [5,6]: **Gram-positive bacteria:** I. *Staphylococcus aureus*; II. *Enterococcus*; III. *Streptococcus*. **Gram-negative bacteria:** I. *Escherichia coli*; II. *Klebsiella*; III. *Enterobacter*; IV. *Citrobacter*; V. *Acinetobacter*; VI. *Proteus*; VII. *Morganella*; VIII. *Providencia*; IX. *Pseudomonas*; X. Rare germs (*Myroides odoratimimus, Serratia marcescens,* posibil și *Aerococcus (A. urinae, A. viridans,* și *A. sanguinicola)*, *Corynebacterium urealyticum*, *Actinobaculum schaalii* and even *Gardnerella vaginalis* [7].

A group of these bacteria (ESKAPE) – *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* și *Enterobacter spp* – are the leading cause of hospital-acquired infections globally. There are some variations in the definition of ESKAPE, with the "E" sometimes representing *Escherichia coli*, *Enterobacteriaceae*, or *Enterobacterales*. Clinically, these bacteria are important because they are common in health-care setting, often resistant to multiple drugs and difficult to treat, and last but not least it should be noted that they all have the potential to be uropathogenic. [6]

Phenotypes of natural antibiotic resistance. Two periods in the evolution of antibiotic resistance genes can be distinguished: the first age of four billion years (until the use of antibiotics by humans, encoded chromosomally) and the second age in the evolution of antibiotic resistance with the presence of R factors (resistance plasmids), since the use of antibiotics by humans [8].

Acquired antibiotic resistance. Pathogens can acquire resistance via two major mechanisms: through chromosomal mutations and horizontal gene transfer. Chromosomal mutations can alter some critical enzymes (e.g., mutations in genes encoding gyrase and DNA topoisomerase lead to fluoroquinolone resistance) and regulatory proteins (e.g., mutations in the regulatory protein mgrB in *K. pneumoniae* lead to colistin resistance) that may be involved in the loss, downregulation, or alteration of porins, or may result in an increased expression level of efflux pumps [9]. However, the main mechanisms involved in the spread of AMR in uropathogenic bacteria are represented by horizontal gene transfer.

Production of ESBL. There are 2 classifications widely used for beta-lactamases: the molecular classification based on the amino acid sequence of R. P Ambler and the functional classification scheme of K. Bush and G.A. Jacoby.

Resistance to carbapenems. Resistance to carbapenems occurs through 3 main mechanisms [10]: **1.** the production of carbapenemases, beta-lactamases that are able to inactivate carbapenems together with other beta-lactams; **2.** decrease in antibiotic absorption through a qualitative and/or quantitative change in porin expression and membrane permeability; **3.** Active expulsion of carbapenems from the periplasmic space by means of efflux pumps.

Production of carbapenemazes. Carbapenems are beta-lactamases capable of inactivating carbapenems [11]: I. Class A carbapenems – KPC, SME, IMI, GES; II. Class B carbapenemases, Metallo-β-lactamase (MBL) – are divided into 3 subcategories B1, B2 and B3 (11); III. Class D carbapenemases (also known as oxacillinases) – OXA

Resistance to carbapenems by other mechanisms. Resistance to carbapenems (*P. aeruginosa* can be the ideal example) is mainly due to the change in permeability, and additionally by the contribution of carbapenems and/or the overexpression of efflux pumps.

Colistin resistance. Acquired resistance to colistin occurs through chromosomal mutations (non-transferable by horizontal mechanisms: implicating bacterial outer membrane changes [12], overexpression of efflux pumps [13], polysaccharide capsule overproduction [14]) or plasmidically mediated (horizontal gene transfer) - *mcr* genes (a group that currently has 10 variants) [12].

Fluoroquinolone resistance. Chromosomal resistance to fluoroquinolones has 3 main mechanisms: 1. alteration of cell envelope permeability, 2. expulsion of antibiotic from the cell, and 3. mutations of target enzymes. Plasmid-mediated resistance occurs through genes such as *qnr* (later renamed *qnrA,* along with other discovered alleles *qnrS, qnrB, qnrC, qnrD*, and *qnrVC)* [15].

MRSA as MDR. It involves staphylococcal resistance to penicillin, β-lactams, but also to quinolones, vancomycin, linezolid, daptomycin, sulfamethoxazole, tetracyclines, tigecycline, clindamycin, macrolides or fusidic acid.

Enterococcus VR. Vancomycin-resistant enterococcus (VRE) is one of the exponents of MDR germs. The mechanisms of resistance are complex, usually plasmidically transmitted (frequently VanA and *VanB genes*) [16]. Mobile genetic elements are also involved in the transmission of resistance to other classes of antibiotics, a phenomenon that most often accompanies the transmission of vancomycin resistance.

Biofilm production. Biofilms, characteristic of all uropathogen species, are communities of microbial cells attached to a surface and embedded in an extracellular polymer matrix produced by themselves. Bacteria in biofilms are more resistant to antibiotics than planktonic cells, due to several mechanisms: limited diffusion of antibiotics through the matrix; transmission of resistance genes within the community; increased expression of efflux pumps and inactivation of antibiotics due to changes in metal ion concentrations or pH values; physiological changes in microbial cells due to nutrient-poor environment (reduced metabolic and growth rates); the presence of metabolically inactive cells known as persistent or dormant bacterial cells, as the bacteria enter a spore-like, undivided, more antibiotic-refractory state; induction of a biofilm phenotype (expression of active mechanisms to combat the harmful effects of antimicrobial agents). [17]

Cap.2 Urinary Tract Infections

General characteristics of urinary tract infections (UTIs) Bacteria that cause urinary tract infections typically enter the bladder through the urethra, with hematogenous dissemination being an exception. The urethral mucosa and epithelial cells can resist the invasion of pathogenic bacteria, maintaining a balance between the urethra and the bacteria, but when the pathogenicity of the bacteria is very strong or the body suffers from external aggressions, this balance is disturbed, and the body's defense function is overcome [18].

Epidemiology

Uropathogenic germs are mostly widespread and commensal, their source being practically everywhere [19,20]. What is particular is the spread of multidrug-resistant germs and the potential sources of infection [9]. It comprises three levels: human (nosocomial and community), veterinary and aquatic (wastewater and surface water).

I. The hospital environment is the possessor of the microbial flora with the highest degree of AMR. The history of the detection of AMR genes can be traced especially in large university centers where complex studies of the resistance profile of pathogens are carried out, but the community environment has also offered the surprise of their presence.

Ia. Horizontally Transmitted Genes Encoding Resistance to Carbapenems and Fluoroquinolones (bla_{qnr}), identified in the hospital environment [9]: $bla_{OXA\text{-}23,24,48,48like}$ – Bucharest, Timișoara, Arad, Reșița, Cluj, Târgu Mureș, Iași, Târgoviște, Râmnicu Vâlcea, Galați; bla_{VIM} - Bucharest, Iași, Cluj-Napoca, Bacău, Târgoviște, Râmnicu Vâlcea, Galați, Timișoara); bla_{IMP} – Cluj-Napoca, Bucharest, Târgoviște, Râmnicu Vâlcea, Iași, Galați, Timişoara; bla_{NDM} - Târgu Mureş, Bucharest, Iași; bla_{KPC} – Bucharest, Galați, Târgoviște; bla_{qnr} – Bucharest, Cluj, Iași, Târgoviște, Galați, Timişoara;

Ib. Genes identified in patients with community-acquired infections [9]: bla_{NDM} – Bucharest; bla_{OXA-48} – Bucharest; bla_{qnr} - Bucharest

II. The veterinary environment has not offered any major surprises regarding AMR, but the amount of data available is also very limited. Thus, no carbapenemaze genes were identified in the field of animal husbandry, but only bla_{qnr} *genes* in 2 locations (the north-east and south-east area in 2017-2018; in Timiș and Arad counties in 2019-2020) and ESBL genes (*blaCTX-M-1, -3, -9, -14, -15* in the north-east and south-east area in 2017-2018; and bla_{TEM} in Cluj 2012-2013) [9].

III. The aquatic environment, however, offered a different picture. Regarding the detection of resistance genes to carbapenems and fluoroquinolones, the following were detected in 2015 – 2020 [9]: bla_{OXA} – Bucharest, Târgoviște, Râmnicu Vâlcea, Galați, Cluj, Timişoara, Iași; bla_{VIM} - Bucharest, Târgoviște, Râmnicu Vâlcea, Galați, Cluj, Timișoara, Iași; bla_{IMP} – Bucharest, Târgoviște, Râmnicu Vâlcea, Galați, Cluj, Timișoara, Iași; Buzău, Brăila, last 2 in Natura 2000 sites; bla_{NDM} – Județul, Bucharest, Târgoviște, Galați, Buzău, Brăila (last 2 in Natura 2000 sites), in the Danube in 2013; bla_{KPC} – Cluj, Bucharest, Târgoviște, Galați counties, in the Danube in 2013; bla_{qnr} - Cluj, Buzău, Brăila, Bucharest, Târgoviște, Galați counties.

Urinary catheters and UTIs. Catheter-associated UTIs refer to UTIs that occur in a person whose urinary tract is currently catheterized or has been catheterized within the last 48 hours. The presence of the urinary catheter creates, through local phenomena, a special environment in the urinary tract and implicitly conditions conducive to colonization, but also diagnostic difficulties [21].

Non-antibiotic treatment of UTIs. There is an urgent need to introduce new solutions in ITU management. Non-antibiotic treatment options are now of great importance, as the extensive use of antibiotics for all types of infections has led to an increase in antibiotic resistance [22].

Cap.3 Urinary tract infections in a general population in Romania – Antibiotic resistance of uropathogens – a multiregional study

3.1 Introduction. An aspect that led here is the lack of large multicenter studies covering the entire territory of Romania, as almost all studies from Romania found in international databases [23], but also in specialized journals, being limited to one center or at most to limited areas of the country. Thus, similar data were searched and collected, from identical periods, from several centers covering all 3 major geographical areas of Romania (Muntenia, Moldova and Transylvania), and also 3 different categories of hospitals, university clinical hospital, county clinical hospital and county emergency hospital. From the data to which I had access, it appears that it is the first national study of uropathogens trying to create an overview as broad and representative as possible for our country.

3.2 Materials and methods. This cross-sectional, retrospective study, placed in four centers, was carried out in the hospitals: "Prof. Dr. Th. Burghele" Clinical Hospital and Elias University Emergency Hospital in Bucharest, Mureș County Clinical Hospital in Târgu Mureș and Vaslui County Emergency Hospital. The database incorporated the results of patients assessed in each hospital for 4 months, between 1 September and 31 December 2018. A total of 15,907 patients were evaluated by urine cultures collected from the mid-stream, of which 3,011 had more than 10^5 CFU/ml. There were identified 2,842 subjects from the previously described cohort who met the criteria for inclusion in the study, representing 1,690 female and 1,152 male patients. The division of patients into centers was as follows: "Prof. Dr. Th. Burghele" Clinical Hospital - 1045, Elias University Emergency Hospital - 981, Vaslui County Emergency Hospital - 553 and Mureș County Clinical Hospital - 263.

3.3 Results and discussion

The presented cohort detected an increased incidence of UTI in female patients compared to the male population, with an increase of almost 50%, representing 1,690 female patients (59.47%), while in men, 1,152 (40.53%) people had clinically manifested UTI.

E. coli is the most common species of bacteria, accounting for 57.31% of the total results, with trends being synchronous in both male and female patients. This is followed by *Klebsiella* spp. (18.64%), *Proteus* spp. (5.48%) and *Pseudomonas* spp. (4.22%), similar data having been previously published by other authors [24–26].

Considering the gram-positive uropathogens, we can say that *Enterococcus spp.* is the main genus, accounting for 11.96%, followed by *Staphylococcus spp.* representing 2.35% of the total samples tested.

Escherichia coli has a small antimicrobial susceptibility to two of the most prescribed antibiotics in UTIs: levofloxacin (R=20.62%) and amoxicillin-clavulanic acid (R=18.17%). The trends are similar for both sexes: fluoroquinolones (men - R = 23.29%, women - R = 19.49%), as well as for aminopenicillins (men - R = 25.36%, women - R = 15.12%). High resistance to fluoroquinolones has also been observed in countries in the region, where resistance rates exceed 20%, results that were published in a recent 2019 study involving European countries, including in the Eastern region [27]. The highest sensitivity to antibiotics tested for *Escherichia coli* was observed for fosfomycin - S=91.03% (males - S=90.72%, females - S=91.17%), followed by ceftazidime - S=77.04% (males - S=59.79%, females - S=84.35%). Promising results were also detected for nitrofurantoin - general sensitivity for the latter S = 65.56%. As such, fosfomycin and nitrofurantoin remain the first-line treatment in uncomplicated UTIs in women, in full accordance with the EAU guidelines, assumed by the ARU (Romanian Urology Association).

Klebsiella spp. it is the second most common gram-negative uropathogen and the present study highlighted the alarming resistance for amoxicillin -clavulanic acid R = 45.66% (men - R = 59.85%, women - R = 31.03%), followed by ceftazidime R = 29.05% (men - R = 43.49%, women - R = 14.17%) and levofloxacin R = 24.71% (men - R = 34.57%, women - R = 14.55%). A predominance of higher resistance rates in the male cohort was observed in all cases with this gram-negative, compared to females, for all antibiotics tested. We also observed significant resistance to amikacin, especially in the male cohort R = 24.53%.

In terms of pathogenicity, *Pseudomonas aeruginosa* is a common causative agent in UTI dynamics and one of the most important microorganisms associated with hospital-acquired and catheter-associated UTIs [28]. Seen as the most common pathogen associated with nosocomial infections, it has a poor prognosis especially due to its XDR profile [29]. The study presented here highlights significant resistance to several classes of antibiotics: levofloxacin R = 48.33%, ceftazidime R = 38.33% and amikacin R = 35.83%. Moreover, alarming resistance was also observed for broad-spectrum classes of antibiotics, such as carbapenems – meropenem and imipenem R = 29.16%.

In this study, *Proteus* was the third most common gram-negative uropathogen, with important rates of resistance to amoxicillin-clavulanic acid, R = 30.12%, followed by trimethoprim-sulfamethoxazole R = 24.35%, levofloxacin R = 17.94%, and ceftazidime R = 15.38%. It should be noted that promising results were observed in this study for amikacin – S = 80.0%.

Enterococcus spp. are the most common gram-positive species involved in UTI pathology. In our study, significant resistance was observed for levofloxacin R = 32.35%, penicillin R = 25.29 and ampicillin R = 16.17%, while the highest sensitivity rates were observed for vancomycin S = 79.11%, followed by nitrofurantoin S = 74.70% and linezolid S = 73.82%. In addition, a relative resistance for fosfomycin S = 62.94% was observed. Linezolid, a second-line treatment for *Enterococcus* spp., has shown good results.

For *Staphylococcus spp.* we report the highest resistance of this germ to penicillin with R = 52.23%, followed by trimethoprim-sulfamethoxazole with R = 31.34% and levofloxacin with R = 29.85%. The highest sensitivity was achieved for amikacin with S = 74.62%, linezolid with S = 68.65% and nitrofurantoin with S = 67.71%.

3.4 Conclusions. Alarming resistance rates have been observed for two of the most common and prescribed antibiotics in the management of UTIs: levofloxacin and amoxicillin-clavulanic acid. *Enterococcus* is the most common gram-positive bacteria involved, with top resistance to levofloxacin, ampicillin and penicillin. Thus, this study, which combines results from all three major regions of Romania, may be of the utmost importance in guiding treatment for any clinician managing UTIs, especially in areas where precise data to guide treatment are lacking.

Cap.4 Antimicrobial resistance model of bacteria identified in urine in a county emergency hospital in Romania - SJUVs, a retrospective single center study

4.1 Introduction. The often-cited AMR Review acknowledges that the reported AMR figures are "general estimates" and emphasizes the need for "more detailed and robust work". This lack of data is particularly prevalent in non-academic medical entities, where limited research is conducted, despite its significant impact on healthcare in society [1]. The objective was to evaluate the antimicrobial resistance of bacteria identified in the urinary tract in a medical facility considered to be one of the most common in Romania, a non-academic county emergency hospital. The study took place at the Vaslui County Emergency Hospital.

4.2 Materials and methods. The results of urine cultures covering an entire pre-pandemic year, from January 1, 2018 to December 31, 2018, were studied. All the results of the urine cultures at the Vaslui County Emergency Hospital were taken from the institution's central laboratory, regardless of which department of the hospital the samples came from, including outpatient services.

4.3 Results. Over the course of a year, 1810 positive samples were identified. Of the 1810, 49 were detected with two germs (19 female patients and 30 male patients), totalizeing 1854 germs. The gender distribution was 1234 cultures from females, 576 from males, representing 68.18% for females and 31.82% for males. The age ranged from 0 to 96 years, with a median of 68 and a mean of 62.9731.

Urine cultures were distributed by medical departments and departments as follows: Internal Medicine 400 (21.57%), Infectious Diseases 375 (20.23%), Emergency Unit 222 (11.97%), Neurology 150 (8.09%), Urology 133 (7.17%), Pediatrics 72, Gynecology 62, Cardiology 60, Medical Rehabilitation 53, Diabetes 52, Psychiatry 42, Gastroenterology 38, Obstetrics 28, Intensive Care 27, Dermatovenereology 22, Oncology 17, General Surgery 17, Pulmonology 16, Hematology 16, Delivery Room 15, Laboratory 11, Orthopedics 9, Ophthalmology 5, Pediatric Surgery 5, Nephrology 4 and Rhinolaryngology 3.

Species:*E. coli* 1176 (63.430%); *Klebsiella* 256 (13.808%), fiind *pneumoniae* 139, *spp.*109 și *oxytoca* 8; *Streptococcus* 91 (4.908%); *Enterococcus* 88 (4.747%); *Proteus* 79 (4.261%), din care *mirabilis* 42, *spp.* 34 și *vulgaris* 3; *Enterobacter* 61 (3.290%), cu 60 *spp.* și 1 *cloacae; Pseudomonas* 51 (2.751%), cu *aeruginosa* 50 și *spp.* 1; *Staphylococcus aureus* 27 (1.456%); *Morganella* 7 (0.376%); *Acinetobacter* 7 (0.376%), cu 5 *spp.* și *baumannii* 2; BGNNF 5 (0.270%); *Staphylococcus saprophyticus* 4 (0.216%); *Citrobacter* 1 (0.054%); *Providencia* 1 (0.054%).

Individual sensitivity and resistance calculations were made for *Escherichia coli*, *Klebsiella spp.*, *Streptococcus spp.*, *Enterococcus spp.*, *Proteus spp.*, *Enterobacter spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter spp.*, *Morganella, Staphylococcus saprophyticus, Citrobacter*, *Providencia*.

In an atypical study, the overall sensitivity of germs, meaning all uropathogens, was evaluated without taking into account the species, as empirical antibiotic therapy is administered, without knowing the causative germ.

Fluoroquinolones: Ciprofloxacin (1208 tests) was resistant in 96 situations (R = 5.18%), in 1109 sensitive (S = 59.82%) and in 3 intermediate; the percentages were 7.947% and 91.805%; with a total of 651 samples not tested for ciprofloxacin. Norfloxacin (tested in 1641 samples) was shown to be resistant in 418 cases (R = 22,55%), 1221 Sensitive (S = 65,86%), 2 intermediary (I = 0,11%); the percentages were 25.472%, 74.405% and 0.12%; and 217 cultures were not tested for norfloxacin. Ofloxacina, 1062 probe testate, proved to be in 2 resistant (R = 0.11%), in 1060 sensitive S = 57,17 (percentage 99.81%) and none intermediary, and 797 were not tested. Levofloxacin, 1472 samples tested, appeared in 123 resistant (R = 6,63%), 1346 sensitive (S = 72,60%), and in 3 intermediate; the percentages were 8.356% compared to 91.44%; 387 samples were not tested for levofloxacin.

Aminoglycosides: Gentamicin from 1531 tests occurred in 237 situations with resistance (R = 12.78%), in 1284 with sensitivity (S = 69.26%) and in 10 intermediate (I = 0.54%). The percentages were 15.48% resistant, 83.87% sensitive and 0.65% intermediate. Amikacin, tested 915 times, occurred in 44 cases with resistance (R = 2.37%), in 865 in the sensitive category (S = 46.66%) and in 6 intermediate (I = 0.32%). The percentages were 4.81% resistant, 94.54% sensitive and 0.66% intermediate. Tobramycin was the last aminoglycoside tested, 71 times, detecting 52 resistant germs (R = 2.80%) and 19 sensitive germs (S = 1.02%). The percentages were 73.24% resistant and 26.76% sensitive.

Carbapenems: Imipenem (264 times tested): 40 resistant (R = 2.16%), 222 sensitive (S = 11.97%), 2 intermediate (I = 0.11%); percentage 15.15%, 84.09%, respectively 0.76%. Meropenem (594 times tested): 47 resistant (R = 2.54%), 544 sensitive (S = 29.34%), 3 intermediate (I = 0.16%); percentage 7.91%, 91.58%, respectively 0.51%. Ertapenem (only 6 tests): 3 resistant (R = 0.16%), 3 sensitive (S = 0.16%); 50-50% ratio. As for all germs, there were 47 strains resistant to all carbapenems out of the 1854 identified. This can be translated as 2.54% carbapenem-resistant strains.

Fosfomycin: 1120 strains tested, 23 identified as resistant (R = 1.24%), 1197 as sensitive (S = 64.56%) and none intermediate. Percentage ratio: 2.05% vs. 97.95%.

Nitrofurantoin: 1676 strains tested for nitrofurantoin, of which 241 resistant (R = 13.00%), 1424 sensitive (S = 76.81%) and 11 intermediate (I = 0.59%). Percentage ratio 14.38%, 84.96% vs. 0.66%.

MDR were defined as non-susceptibility to at least one agent in three or more classes of antibiotics [30]. This includes the ESBL-producing strains, MRSA, and resulted in a total of 313 (16,882%).

PDR, defined as strains resistant to all antibiotics tested, were 8 strains that had no sensitivity identified, with the mention that none had been tested for Colistin or Fosfomycin. Out of 8 strains, 4 were only intermediate for only one substance: amikacin or nitrofurantoin.

4.4 Discussions

According to the above data and susceptibility models available in Europe, oral treatment with Fosfomycin 3 g single dose and nitrofurantoin should be considered for first-line treatment [31,32]. In the present study, too, the rates of resistance to fosfomycin are low, and the resistance to nitrofurantoin is less than 15%, which is less than the often-incriminated threshold of 20%. The next antibiotic option, trimethoprim combined with a sulfonamide, appears inadequate, as they should only be considered first-line drugs in areas with known resistance rates for *E. coli* less than 20% [33,34]. R = 21.26%

Ciprofloxacin, ofloxacin and levofloxacin have detected resistance of less than 10%.

In general, resistance to carbapenems is rare, and most of the time there is an alternative for treatment. The incidence of carbapenem-resistance has generally been maintained at the same levels over the years, as subsequent studies have shown [35].

Klebsiella had lower resistance than strains in other studies, for example to amoxicillin - clavulanic acid 34.37% compared to 52.06%, to levofloxacin 8.59% compared to 34.57% (36). Other resistance rates were for ceftazidime 28.51% compared to 29.05%, for amikacin 2.73% compared to 24.53%, and last but not least for meropenem 2.73% compared to 9.24% [36].

Enterococci had lower resistance rates to penicillin, ampicillin and levofloxacin than similar studies in our country. Resistance to linezolid and vancomycin was also at very low levels. Streptococcus has appeared very frequently in cultures and should be mentioned, but as this was a study of the identified germs, no comment will be made on the pathogenicity or clinical implications of this.

Proteus did not follow the same pattern of lower resistance rates. In fact, as an exception, this species of *Enterobacteriaceae* showed higher resistance rates for common oral antibiotics presented in other studies in Romania, such as in amoxicillin - clavulanic acid 43.04% compared to 30.12%, trimethoprim - sulfamethoxazole 48.10% compared to 24.35%. Also, for ceftazidim the resistance detected was higher than in other paperworks, being 26.58% compared to 15.38%, but not the same was for meropenem where no resistance was encountered, compared to 1.28%. In contrast, levofloxacin had lower resistance rates, as all fluoroquinolones in this study found, at 2.53% versus 17.94%. [36]

Unlike all the other species, apart from *Proteus*, *Pseudomonas* is that case in which resistance is significantly higher for all the most important antibiotics, with the exception already commonly encountered for levofloxacin.

Staphylococci followed the same pattern of lower resistance rates than that identified in other studies in our country. Resistance rates are higher for penicillin: 66.67% versus 52.23%; but lower for other antibiotics, such as levofloxacin: 7.41% compared to 29.85%. TMP-SMX also with a better sensitivity in SJUVs, of 70.37% compared to 52.23%.

Amikacin, linezolid, and nitrofurantoin (no resistivity identified in both studies for this) remained with the best sensitivity rates and the most promising therapeutic outcomes.

4.5 Conclusions

In general, germ resistance rates to antibiotics appear slightly lower than generally in Romania, but with some peculiarities and exceptions. From some points of view, on the contrary, there is increased resistance to almost all categories of antibiotics, and according to the MDR definition, resistance rates are higher than other centers. A paper published in 2021 in the journal Antibiotics revealed 4.58% MDR strains in 3 hospitals in Romania, compared to almost 17% in this case [37]. On the other hand, the increased rates of resistance in the present study are mostly in first-line treatments (penicillin, ampicillin, nitrofurantoin or trimethoprim), but still having enough alternatives available and less or practically the same resistance to second-line and backup treatments. The general resistance to carbapenems is broadly similar to the overall resistance in Romania, the conclusions of other Romanian authors being about 2% resistance [38,39].

In a common medical facility in Romania, but with high addressability, the antimicrobial susceptibility model is still clinically easily approachable, with apparently lower overall resistance than clinical hospitals, and *antimicrobial stewardship* principles need to be implemented to keep resistance rates as low as possible.

Cap.5 Carbapenemase-producing uropathogens in real life: epidemiology and treatment at a County Emergency Hospital

5.1 Introduction. The study aimed to evaluate patterns of antibiotic resistance, source of contamination, mechanism of contamination (most studies reported that carbapenem-resistant *Enterobacteriaceae* are predominantly isolated from urine samples, and chronic urinary catheters come with the highest risk [4]) and the clinical impact of UTIs caused by carbapenemaze-producing uropathogens in a non-academic clinical facility in Romania. From an epidemiological point of view, this study is a premiere in Romania, not finding in the literature a similar study in our country on difficult-to-treat UTIs.

5.2 Materials and methods. A study conducted at the Vaslui County Emergency Hospital in Romania between October 1, 2021 and September 30, 2022. The study included inpatient or outpatient UTI patients with CRE urine cultures.

5.3 Results

Within a year, 27 patients with CRE were identified and included in the study. The mean age of the cohort was 65.4±18.1 years, with a range of less than one year to a maximum of 85 years. Most patients were men (77.8%), and only 22.2% women (6 women and 21 men).

Microorganisms - the most frequently identified were *Pseudomonas aeruginosa* (8 cases - 29.6%) and *Klebsiella pneumoniae* (8 cases - 29.6%), followed by *Klebsiella terrigena* (5 cases - 18.5%), *Klebsiella oxytoca* (2 cases - 7.4%), *Escherichia coli* (3 cases - 11.1%), *Acinetobacter baumanii* (one - 3.7%). *Klebsiella spp.* accounted for more than half of all cases (55,5%).

According to the inclusion criteria, 96.3% of the bacterial strains tested were resistant to carbapenems, and 3.7% were carbapenem-intermediate.

Table 5.1 CRE sensitivity to antibiotics

	Colistin	Ceftazidim/ Avibactam	Aminoglicoszdes + Fosfomicină			Meropenem	Cefepim	Ceftazidim	Piperacilină - Tazobactam	Levofloxacină	Cefiderocol
			Gentamicină	Amikacină	Fosfomicină						
Susceptibil	*3,70%*	*88,90%*	*44,40%*	*59,30%*	*11,10%*		*3,70%*	*7,40%*	*11,10%*	*3,70%*	*18,50%*
Intermediar						*3,70%*			*3,70%*		
Resistant	*7,40%*	*7,40%*	*40,70%*	*40,70%*	*0*	*96,30%*	*25,90%*	*85,20%*	*77,80%*	*96,30%*	
Ne - testat	*88,90%*	*3,70%*	*14,80%*		*88,90%*		*70,40%*	*7,40%*	*7,40%*		*81,50%*

The designated source of infection for 51.9% of the enrolled patients was the Vaslui County Emergency Hospital. The rest of the cases were associated with four other health units in two other counties (Iasi and Bucharest). The patients in these two cities came from three departments: urology, neurology and oncology. 7 of these inter-hospital cases, coming from the Iasi and Bucharest centers, were discharged from urology services, which suggests that 25.9% of all cases were contaminated in urology departments.

5.4 Discussion

The average age of the cohort exceeded 65 years, in line with the findings of other authors indicating that older age increases the likelihood of acquiring a UTI with multi-resistant antibiotic-resistant germs [40–42].

Colistin, gentamicin, and amikacin play crucial roles in the treatment and clinical outcome of UTIs caused by CRE [43,44]. The lack of colistin testing is a significant limitation in the present study and is a common problem in some hospitals. About half of the patients had aminoglycoside-sensitive CRE strains, highlighting the continued importance of these antibiotics as treatment options and noting that of the strains tested, about 50% showed sensitivity to gentamicin. Ceftazidim, piperacillin-tazobactam and fluoroquinolones, which have been widely used in the past, are no longer suitable options due to the high levels of resistance in this subgroup, which exceed 80%.

Our investigation into the mechanism of infection revealed the involvement of urinary catheters. It was concluded that 66.7% of all cases were probably caused by the insertion/presence of the catheter. The other 9 patients, representing 33.3% of all cases, had no history of catheterization that could have led to contamination. Consequently, we can point out the strong correlation between urinary tract catheterization and healthcare-associated infections, since most of the cases in our study can be attributed to this factor. Furthermore, in addition to bladder drainage, 7.4% of cases involved upper urinary tract instrumentation, consistent with other studies that identified it as a potential risk factor [45].

Although one death cannot provide definitive conclusions, it is important to emphasize that most patients survived, suggesting a favorable prognosis for this disease, as the rest of the patients had a favorable outcome and were discharged.

Our findings indicate a significant circulation of carbapenem-resistant bacteria between medical facilities located at considerable geographical distances, and the attribution of the role of source to another medical facility of the described bacteria was made only after a very rigorous interpretation of all available data [46]. In this study, the source of infection was identified in hospitals in Vaslui, Iasi and Bucharest, so the routes of patients and implicitly of microbes extend over distances of 80 and 350 kilometers. In addition, we can conclude definitively that even medium-sized hospitals harbor dangerous XDR bacteria, including carbapenemase-producing strains. The examination of the distribution of these strains within the hospital showed that the risk of encountering or being contaminated by these dangerous microbes extends in different departments, from the Infectious Diseases department to the Pediatrics department, and moreover, the 12 departments involved (including the ICU) are found on all 6 levels of the main building of the institution, but also in the other two small pavilions that house clinical departments. Each department must be prepared to handle such situations, emphasizing the importance of rigorous catheter handling and adherence to medical practice guidelines, such as the "European and Asian Guidelines for the Management and Prevention of Catheter-Associated Urinary Tract Infections” [47].

What must be emphasized following this study, and may be the main conclusion, is that average hospitals (extrapolating the data from SJUVs), and perhaps not only them, but maybe even the entire national health system, are not really prepared to manage such situations. Following interviews and discussions with both caregivers and medical staff, I understood that the proper management of urethro-bladder catheters is still a goal that will be achieved in the future.

5.5 Conclusions. The current study identified two modes of dissemination of CRE: the inter-hospital route and the intra-hospital route, both of which require increased control measures. A significant number of patients with ERC have been identified, mostly men, with an average age of over 65 years. While most strains were sensitive to ceftazidim-avibactam and about half to aminoglycosides, almost all patients achieved clinical cure after treatment with colistin, aminoglycosides, or ceftazidim-avibactam. The urinary catheter was involved in two-thirds of all patients, appearing to be the main mechanism of dissemination, so rigorous catheter handling is required and the correct procedures must be applied, as stated in the recommendations in medical practice guidelines.

Cap.6 Carbapenem resistance of uropathogens in a county emergency hospital compared to a tertiary urological clinical center

6.1 Introduction. The study aimed to comparatively evaluate the resistance to carbapenems of uropathogens in a county emergency hospital compared to a tertiary urological clinical center in Romania. From an epidemiological point of view, this study represents, in turn, a premiere in Romania.

6.2 Materials and methods. A prospective study was conducted in the "Prof. Dr. Theodor Burghele" Urology Hospital and the Vaslui County Emergency Hospital between June 1, 2021 and December 31, 2022. The study included inpatient or outpatient cases with urinary tract infections (UTIs) with carbapenem-resistant urine cultures.

The microbes were identified by MALDI-ToF-MS and tested for antibiotic susceptibility by the Kirby-Bauer disc-diffusion technique according to EUCAST. Blue Carba, Modified Carbapenem Inactivation Method (mCIM), Rapid Modified Carbapenem Inactivation Method (rmCIM) tests followed. The bacterial DNA was extracted using Qiagen Dneasy kits, then evaluated by fluorimetric method (Qubit 4) and then sequencing "libraries" were prepared using the Nextera XT DNA kit. Sequencing was performed on the Illumina NovaSeq 6000 instrument.

6.3 Results - 6.3.1.1. *Enterobacterales* infections in S.J.U.Vaslui**.** During the study period, 20 strains of *Enterobacterales* were isolated, belonging to two genera: 3 *E. coli* and 17 *Klebsiella* (1 *K. terrigena*, 1 *K. aerogenes* and the remaining 15 *K. pneumoniae*). In some cases, it is about the re-isolation of the strain during successive readmissions and some strains showed phenotypic differences that motivated individual testing.

Blue Carba was positive in 8 cases, and two tests were inconclusive. Of these, one was shown to be positive, and the other was due to lysis by chromosomal AmpC (naturally constitutive) of *K. aerogenes*. The mCIM test was positive in only 6 cases, being negative for both OXA-48-producing *E. coli* strains and three OXA-48-like *K. pneumoniae* strains. The rmCIM test was positive in 11 cases, proving to be reliable in the evaluation of all the examined strains.

Results obtained by next-generation sequencing: A few strains were chosen for next-generation sequencing, identifying 4-7 plasmids in each strain.

Table 6.8 Comparison of plasmids identified in sequenced strains.

	Plasmid	**Identity (%)**	**Plasmid**	**Identity (%)**	**Plasmid**	**Identity (%)**
	Col440II	96.81	ColRNAI	100	Col440II	96.81
	IncFIA(HI1)	98.2	ColpVC	96.89	IncFIA(HI1)	98.2
	-	-	IncFIB(K)	98.93	IncFIB(K)	100
	-	-	IncL	100	IncL	100
	IncR	100	-	-	IncR	100
	repB(R1701)	99.2	-	-	repB(R1701)	99.2
	-	-	-	-	IncFII(K)	97.3
	Table 6.7		Table 6.5		Table 6.6	
Species	*K. pneumoniae*		*K. terrigena*		*K. pneumoniae*	
ß-lactamase			OXA-48		OXA-48	
ST	101		307		101	

6.3.1.2. Infections with non-fermentative microorganisms (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*) in S.J.U.Vaslui. During the study period, 10 strains of *P. aeruginosa* and one strain of *A. baumannii* resistant to carbapenems were isolated, of which 4 isolates came from a single patient and 2 from another patient.

Increased impermeability associated with porin mutation - For these strains, carbapenemase production tests were negative. Of the 10 strains of *Pseudomonas aeruginosa* and one strain of *A. baumannii*, 4 were positive using the Blue Carba protocol, and two were positive using the mCIM protocol.

The carbapenemase type was identified using NG Carba immunochromatographic tests, which were positive in 2 out of 3 cases. In both cases, type VIM carbapenemase-producing strains were identified.

In the case of the *A. baumannii* strain, the carbapenemase type was identified using Coris Resist Acineto immunochromatographic tests, being OXA40/OXA58.

In the case of a strain of *P. aeruginosa*, there were phenotypic arguments for the production of carbapenemase, requiring genome-wide sequencing to determine it. It was performed by the Illumina paired-end sequencing technique. The data was analyzed using online ResFinder, hosted by The Center for Genomic Epidemiology within the Technical University of Denmark: https://cge.food.dtu.dk/ [48]. The results indicate the production of multiple genes that confer resistance to beta-lactams (*bla*$_{OXA-488}$, *bla*$_{PAO}$, ***bla***$_{GES-5}$), but also to aminoglycosides (*aph(3')-IIb, aadA6, aph(3')-XV*), quinolones (*crpP*), cyclins (*tetG*), fosfomycin (*fosA*), chloramphenicol (*catB7*), sulphonamides (*sul1*).

The experimental rmCIM test yielded positive results for all strains shown to be carbapenemamase-producing.

6.3.2. Carbapenems resistance of uropathogens in a tertiary urological clinical center. During the study period, 200 strains of *Enterobacterales* were isolated. Isolates were identified by MALDI-ToF-MS. After the elimination of Gram-positive microorganisms (*Enterococcus*, n = 2), those producing chromosomal carbapenemases (*Myroides*, n = 2) and Gram-negative microorganisms that are not part of the *Enterobacterales* or non-fermentative families (*Alcaligenes*, *Chrysiobacter*, n = 2), 273 microorganisms were included in the study. Of these, 73 strains were nonfermenting gram-negative bacilli - NFGNB (66 *Pseudomonas aeruginosa* and 7 *Acinetobacter spp).*

Resistance profile and carbapenemase production of *Escherichia coli* strains isolated from infections from the "Prof Dr. Th. Burghele" Clinical Hospital. Within the strains, only one probe was identified as carbapenemase producing, being positive in both the BlueCarba assay, mCIM and rmCIM. The carbapenemase type was identified by NG Carba immunochromatographic assays and was determined to be NDM. As for resistance to complementary antibiotics, resistance to aminoglycosides between 33% and 63% (amikacin, gentamicin, tobramycin) is noted. The level of resistance to fluoroquinolones was particularly high (~90%). Tigecycline, a last resort antibiotic, has retained its effectiveness in most cases. Antibiotics with urinary visa, Nitrofurantoin (10% resistant), Fosfomycin (12% resistant) and Trimethoprim-Sulfamethoxazole (45% resistant) are also evaluated.

Resistance profile and carbapenemaze production of *Klebsiella* spp. strains isolated from infections from the "Prof Dr. Th. Burghele" Clinical Hospital. Unlike *E. coli*, in the case of isolated *K. pneumoniae* strains, only 25% have an ESBL profile with susceptibility to carbapenems. Phenotypic carbapenemase production tests showed 73% positivity for BlueCarba (plus 5% inconclusive tests – uninterpretable), 71% positivity for mCIM and 77% positivity for rmCIM. Immunochromatographic tests demonstrated the presence of strains producing KPC (n=1), NDM (n=17), VIM (n=2), OXA-48 like (n=45), NDM +

OXA-48-like (n=38) meaning 2 types of carbapenemase in co-production. As for resistance to complementary antibiotics, there is a high resistance to aminoglycosides between 80% and 90% (amikacin, gentamicin, tobramycin). The level of resistance to fluoroquinolones was particularly high (~85-95%). The level of resistance to trimethoprim-sulfamethoxazole was 80%. A significant number of *K. pneumoniae* strains showed resistance to all antibiotics tested and were evaluated by immunochromatographic tests to determine the profile of carbapenemases produced, demonstrating the co-production of NDM carbapenemases and OXA-48-like. The evaluation of the group profile by MLST (multilocus sequence typing) and plasmids indicates that there is a convergent evolution of the strains towards a phenotype producing two carbapenemases, but there are at least two separate genetic profiles.

Table 6.19 Comparison of plasmids identified in the 2 sequenced *Klebsiella* strains.

	Plasmid	**Identity (%)**	**Plasmid**	**Identity (%)**
	IncFIA(HI1)	98.45	IncHI1B (pNDM-MAR)	99.47
	IncFIB(K)	100	IncFIB (pNDM-MAR)	99.54
	IncFII(Yp)	99.13	-	
	IncL	100	IncL	100
	IncR	100	-	-
	repB(R1701)	99.2	-	-
Species	*K. pneumoniae*		*K. pneumoniae*	
b-lactamase	NDM-1 + OXA-48		NDM-5 + OXA-48	
ST	101		383	

Regarding the other *Enterobacteriaceae* (n = 35) except *E. coli* and *Klebsiella* spp., the evaluation of the carbapenemase production profile revealed that 10 strains were producing carbapenemaze type NDM. Thus, 8 strains of *Enterobacter cloacae* and 2 strains of *Providencia stuartii were identified as carbapenemaze producers*.

Pseudomonas aeruginosa - testing of the mechanisms leading to beta-lactam resistance led to the identification by immunochromatographic tests of a number of 29 (representing 44%) carbapenemaze-producing strains, all of which are VIM type. In addition, another 16 strains show complex changes in the expression and function of natural chromosomal cephalosporinase, associated with hyperexpression of efflux pumps, leading to resistance to ceftazidim-avibactam. Imipenem-relebactam has proven to be the best method of screening carbapenem-producing strains. In terms of resistance to aminoglycosides, tobramycin showed 100% resistance, gentamicin showed 100% susceptibility to the use of adapted doses (intermediate status), and amikacin showed a susceptibility level of about 60%. Resistance to fluoroquinolones was high, towards 90%, against both ciprofloxacin and levofloxacin.

Acinetobacter spp. - 4 were *Acinetobacter baumannii*, 2 were *A. pitii* and one was *A. calcoaceticus*. Carbapenemasi-producing were 3 of the 4 strains of *Acinetobacter baumannii*, and the type of carbapenemase produced was evaluated using the Coris RESIST Acineto assay, two demonstrating OXA-23 production, and one OXA-40/58.

6.4 Discussions

The first aspect that stands out is the disproportionate number between the 2 centers, being almost 9 times higher in the tertiary urology center. This relative rate of multiplication is also maintained with regard to the proportion of genera, indicating a certain similarity in the percentages of the identified XDR-type species. Regarding the carbapenem-producing germs, we can say that they are about half of the carbapenem-resistant germs (15 out of 31 in Vaslui - 48.39%; and 147 out of 273 in Bucharest - 53.85%). The proportion by gender of carbapenemase-producing uropathogens again shows similarity by gender, *Klebsiella* 60% in Vaslui and 70% in Bucharest, *Pseudomonas* 20% in Vaslui and 19.86% in Bucharest.

6.4.1.1 *Carbapenemase-producing Escherichia coli* is still relatively rare in the urinary tract, the present study identifying only 3 strains, 2 in SJUVs and one in the "Prof. Dr. Th. Burghele" Hospital, each possessing the carbapenemaze most frequently found in *Klebsiella* in that institution (OXA-48 in Vaslui and NDM in Bucharest), suggesting a process of inter-species dissemination.

6.4.1.2 The genus *Klebsiella* is at the forefront of the discussion about carbapenems resistance, being the best represented, and accounting for more than half of all the germs involved.

IncL plasmids carrying *blaOXA-48 genes have been identified in strains of Klebsiella* spp. both in SJUVs and in the "Prof. Dr. Th. Burghele" Clinical Hospital Bucharest, these plasmids being incriminated in the spread of antibiotic resistance even at the intercontinental level [49]. K. *terrigena* was also identified in the study, although less frequent encountered, but still producing OXA-48.

From the point of view of the distribution of carbapenemases produced, there is a notable difference between those of the County Hospital and the Tertiary Center of Urology. In the case of *Enterobacteriaceae*, in the county emergency hospital all strains were producing OXA-48, while in the tertiary urology center there was a predominance of NDM-type enzymes. Notably, co-producing strains of carbapenemases type NDM and

OXA-48-like have been encountered in the clinic. New generation sequencing demonstrated the presence of both NDM-1 and NDM-5, thus demonstrating that the evolution of the strains is not homogeneous [50,51]. NDM-5 is a variant characterized by the modification of some key amino acids that give the enzyme stability and activity in the presence of low amounts of Zn2+ [52].

In these situations, with NDM present in the clinical theater, therapeutic options are very limited. Cefiderocol is a new generation cephalosporin, which forms an extracellular free ferric iron complex, with siderophore action, which allows it to be transported through the outer membrane of Gram-negative bacteria, in order to exert its bactericidal activity by inhibiting cell wall synthesis [53]. This pharmacological property made cefiderocol active against several clinically relevant Gram-negative bacteria. However, there is emerging resistance, especially in the case of NDM-type enzyme-producing strains [54-56].

In addition to carbapenemaze-mediated resistance, the acquisition of other genes that confer resistance to many other classes of antibiotics, and which in turn were identified in our study, should also be highlighted. Thus, the following genes were detected at both study sites: *aac(6')-Ib-cr, blaOXA-1, blaOXA-9, blaCTX-M-15, fosA, OqxA, OqxB, tet(A), dfrA14, aph(6)-Id, aac(6')-Ib, aadA1, aph(3'')-Ib, sul2*. Some genes were found only in Vaslui (*blaSHV-28*, *fosA6*, *qnrB1*), but they had correspondence in Bucharest (*blaSHV-100, blaSHV-148, fosA5, qnrS1*). Most genes that were not found in the other center were identified in Bucharest (*rmtC, msr(E), mph(A), mph(E), catA1*). These data, to which are added the *NDM bla genes*, suggest a greater genetic variability in the center of Bucharest, therefore at the same time a greater potential of AMR.

Looking at the identified plasmids, from 4 to 7 per strain in Vaslui and from 4 to 6 in Bucharest, it can be concluded that mobile genetic elements are ubiquitous and generate the risk of dissemination of antibiotic resistance.

6.4.2 Carbapenemase producers NFGNB.

Carbapenemase-producing *Acinetobacter* is still relatively rare as an uropathogen, the present study identifying only 4 strains, one in SJUVs and 3 in the "Prof. Dr. Th. Burghele" Hospital. The strains, all being *A. baumanii*, were detected as having OXA-40/58 (n=2) or OXA 23 (n=2).

Pseudomonas aeruginosa is the second most representative exponent of carbapenemaze uropathogens, accounting for about one-fifth of all strains. All of these were identified as VIM, with one exception represented by a strain of S.J.U.Vs positive to GES-5.

On the other hand, the tertiary urology center has a national addressability, the "Prof. Dr. Th. Burghele" Clinical Hospital Bucharest having a great notoriety in Romania. As a result, the degree of difficulty of the cases addressed is much higher and the area of collection of cases is much higher. At the same time, the risk of prior colonization of treated patients is higher, and the complexity of the case sometimes goes hand in hand with the acquisition of AMR.

6.5 Conclusions

An in-depth analysis of the strains profile of carbapenem-producing Gram-negative bacilli shows a one-race picture in which tertiary medical centers hold an undesirable detached first place, both in terms of the number of strains and in terms of the types of carbapenemases encountered, but also of other resistance genes encountered.

Cap.7 Non-Antibiotic Prevention of Mechanical and Septic Complications of Urinary Catheter in Long-Term Indwelling Catheters Patients: A Prospective Crossover Study Involving L-Methionine

7.1 Introduction. The burden of bacterial resistance in Europe is already high. CRE, a subset of MDR and XDR bacteria that pose great difficulties for treatment, are most commonly isolated from urine in most studies, and patients who have chronic internal urinary catheters appear to be at the highest risk [4]. As the number of these infections is increasing both globally [57] and in our country [58], an area that requires immediate action is looming. In this context, we can emphasize the strong bond between urinary catheters, bacteriuria, UTI, *antimicrobial stewardship* and AMR.

The main mechanism underlying this binding is the production of biofilm and its crystallization, which leads to two main outcomes: mechanical catheter blockage and the inability to achieve sterility of urine by administering antibiotics, while promoting antibiotic resistance [59]. To control this crisis of antimicrobial resistance, non-antibiotic approaches are essential to provide the means of reducing symptoms without resorting to the use of antibiotics.

The agent *L-methionine*, which has been on the market for many years, has a special mode of action, achieving its effect by acidifying the urine [60]. This study seeks to establish the reduction of mechanical and septic complications of long-term urinary catheters when *L-methionine* is administered.

7.2 Materials and methods. This study was conducted in an academic clinical unit, *Prof. Dr. Theodor Burghele Clinical Hospital*, in Bucharest, Romania. The target population was long-term bladder urinary catheter carriers.

Sterile controlled manipulation and sectioning of the old catheter were used, and 3 sections were utilized for evaluation. The fragment at the top of the probe, labeled with VfC, was placed in fluid thioglycolate medium and it was sent for microbiological analysis following known protocols [61]. Sections FC1 and FC2, located above and below the balloon segment, were photographed next to a measuring scale. After obtaining images of the catheter sections, the initial internal free area (IIFA) and the actual internal free area (AIFA, permeability) of the probe were measured using SketchAndCalc™. The difference between the two areas was expressed in percentages. This was done for both the FC1 and FC2 sections, and the most relevant data between FC1 and FC2 were used for each patient enrolled in the study.

L-methionine 500 mg was administered to the patient after initial evaluation, while a new Foley catheter was placed. Participants were instructed to take 2 capsules every day, at 12-hour intervals, for the first three days and then use pH bands to measure the values. Subsequently, *L-methionine* 500 mg was administered according to the measured urinary pH: 2 per day at pH < 6.5 and 3 per day at pH >6.5.

The recommendation was to change the catheter every one-month interval. The second visit was prepared for about a month later, when a new catheter change should have taken place, or when the Foley probe was not draining properly, as the patient had been instructed. The probe was again handled appropriately and with the same procedure. A urinalysis was collected for pH measurement.

The third visit was scheduled after another 2 months, about 3 months after the first visit, when, again, a new catheter change should have taken place. The probe was again sterile handled, with the tip segment used for culture. Subsequently, all enrolled patients were evaluated for catheter exchange interval 3 months after discontinuation of treatment, based on data from their presentations. The results were compared with those obtained in first evaluations.

7.3 Results. A total of 22 patients with chronic indwelling urinary catheters were enrolled. Of the enrolled study population, demographics show that there were 6 (27.27%) female patients and 16 (72.73%) male patients. The mean age of the study population was 79.41 ± 6.26 years.

Patients had a mean catheter exchange interval of 7.32 days, with a minimum of 3 days and a maximum of 13 days. None of these patients reached the recommended exchange interval of 1 month. All reasons for catheter exchange were grouped into acute urinary retention, gross hematuria, and symptomatic urinary tract infection. The main reason for premature catheter exchange was urinary retention due to catheter blockage secondary to fouling (in 63.64% of cases), and symptomatic urinary tract infection is the second most common reason (in 27%).

At the time of the first visit, the urinary pH was recorded and the average was 7.49±0.47. The measurement of the areas resulted in a median IIFA of 3.42 mm2 and an AIFA area of 1.21 mm2. The average value of the free inner area of the catheter was 34.56%, which means that only 34% of the internal surface of the catheters was permeable for urine flow. The main pathogen identified at study enrollment was *Proteus mirabilis, which* was most associated with symptomatic urinary tract infections as a reason for early catheter exchange.

At the time of inclusion, the catheter-related events that were recorded were mainly acute urinary retention due to catheter blockage in 63.6% of cases, followed by symptomatic urinary tract infection in 27.3%, and after one month of treatment with *L-methionine 500 mg*, the number of catheter-related events decreased significantly for episodes of acute urinary retention at 18.2%. At three months, fewer catheter-related adverse events were observed, being present in only 9.1% of patients, with a total of 2 cases requiring early Foley catheter exchange for acute urinary retention.

To demonstrate that medical intervention would lead to a reduction in the number of problems associated with catheters, such as acute urine retention and fewer bouts of symptomatic infections, we resorted to the null hypothesis, which holds that the results will not be affected by medical intervention. We did a "t pair" test using pH levels both before and after medical intervention. On analysis, it was shown that the average urinary pH after treatment was substantially lower (M = [5,67], SD = [0,44]) compared to pH before treatment (M = [7,49], SD = [0,47]), resulting in a statistically significant difference (t(21) = 12,87, p<0,001).

The option was for a non-parametric "chi-square" test. By testing the differences between initial catheter-related events and those recorded after 1 month of treatment, we obtained a statistically significant result for the data observed, χ^2([2], [22])=19,72, p<0,001. This test shows that the observed reduction in catheter-related events after treatment for one month with *L-methionine 500 mg* is statistically significant.

Performing a similar statistical analysis of catheter-related events that occurred after one month of treatment until the end of the study at three months showed, χ^2([1], [22]) = 14,72, p<0,001.

Microorganisms identified on a case-by-case basis, at first, at one month and then at three months show that treatment with *L-methionine* will change the bacterial type.

Patients who were enrolled in the study were reassessed in terms of catheter exchange interval after 3 months of cessation of *L-methionine*. The approximate exchange interval that was recorded was compared with the first data obtained, and the statistical analysis was done by the "paired t" test. The results show t(19)= -1,836, CI (-3,1 – 0,2), p=0,082.

7.4 Discussions

The urine had an alkaline pH prior to treatment, which is recognized to be the ideal environment for bacterial colonization and biofilm development [62], especially for urease-producing bacteria [63]. About one-third of the IIFA was the effective internal free zone (AIFA), indicating a high degree of fouling and a corresponding decrease in permeability.

The frequency of incidents, AIFA and pH value decreased dramatically since the second visit. AIFA and IIFA were almost two-thirds apart, and the pH was acidic. When patency increased, the number of urinary catheter-related incidents dropped to five over the course of a month, down from 22 at the beginning – a decrease of 77.3%. In addition, there were fewer catheter-related events after three months of treatment compared to one month of treatment, indicating a total decrease of 90.9%, considering from the beginning.

An interesting aspect of the present study is the comparison between the catheter exchange interval at enrollment and that after 3 months of cessation of treatment. The results show that patients with chronic catheters tend to return to a state similar to that before starting *L-methionine*.

Sterilization of bladder contents was not the goal of the study, and urine cultures were consistently positive. Patients with long-term catheters have colonized bladders [64]. Many species are present at low concentrations [65,66]. Consequently, the bacterial flora in the catheterized urinary tract varies over time, with different species predominating at different times [67]. The first, second and third cultures are entered into a table that accurately describes the bacterial changes with the change of species at each result. The main goal of this study was to minimize septic complications, eventually achieving zero occurrences in the study series. The absence of CA-UTI is probably not statistically significant due to the small sample size, but it could indicate remarkable infection control.

Current results indicate that treating patients with chronic urinary catheters with L-methionine effectively manages mechanical and septic complications at a reasonable cost and achieves appropriate antibiotic use and administration goals, reducing the pressure that leads to the emergence of antibiotic resistance (AMR). This method also fully meets all recommendations for the care of indwelling urinary catheters [47]. Reducing the frequency of doctor visits, specialist treatments, and hospital admissions can reduce the overall cost of medical care by preventing specific complications.

After adequate scientific research, it can be said that the present study is one of the few research projects that evaluates the long-term effects of *L-methionine* on urinary catheters.

7.5 Conclusions. Treatment with *L-methionine* in patients with chronic indwelling urinary catheters indicated good control of mechanical and septic complications at reasonable costs with minimal complications. However, future research on numerically superior cohorts and placebo control groups should be conducted to confirm the current results.

FINAL CONCLUSIONS

1. UTIs are a major health problem in which there is still no wide overview of the whole of Romania. Further studies and continuous updating of the data are needed. Thus, this study, detailed in Chapter 3, which combines results from all three major regions of Romania, may be of the utmost importance in guiding treatment for any clinician managing UTIs, especially in areas where precise data for treatment guidance are lacking. The data generated here confirmed that *Escherichia coli* is the ubiquitous pathogen involved, followed by *Klebsiella spp*. as the second most common gram-negative agent. Alarming rates of resistance have been observed in Romania for two of the most common and prescribed antibiotics in the management of UTIs: levofloxacin and amoxicillin-clavulanic acid. *Enterococcus* is the most common gram-positive bacteria involved, with top national resistance to levofloxacin, ampicillin and penicillin.

2. Doing a separate analysis on male and female cohorts on a population in Romania, we showed that there is a difference in antibiotic sensitivity between the two, with a higher resistance rate in the male population, to which thus does not apply the same rates of microbial resistance as in the general population of the UTI, which is dominated by women.

3. In a common medical unit in Romania, such as the Vaslui County Emergency Hospital, but with high addressability, the antimicrobial susceptibility model is still relatively easily approachable from a clinical point of view, with a lower overall resistance than tertiary clinical hospitals, but with some particularities such as *Pseudomonas aeruginosa* (possibly the first GES carbapenemase-producing strain identified in Romania).

4. *Antimicrobial stewardship* principles must be implemented to keep resistance rates as low as possible, mentioning in particular the exceptional sensitivity to fluoroquinolones. The existence of general guidelines is not always satisfactory, and it is necessary to adapt antibiotic treatment to local patterns of resistance, which must be known, and additionally such managing provides better results. It is necessary to adhere to the guidelines of medical practice and to become aware of local particularities.

5. The dissemination of carbapenem-resistant *Enterobacteriaceae* has two ways: the inter-hospital route and the intra-hospital route, both of which require increased control measures. Urinary catheterization has been identified to be the main mechanism of their dissemination, so rigorous catheter handling is required and the correct procedures must be applied, as mentioned in the recommendations in the medical practice guidelines.

6. In the dissemination of multi-resistant germs over long inter-hospital distances, many contaminated cases in departments of urology are involved, this medical specialty being in our study on the first place.

7. The examination of the distribution of carbapenem-resistant strains showed that the risk of encountering or being contaminated by these dangerous microbes extends in many departments, from the Infectious Diseases department to the Pediatrics department, identifying most of them in the institution studied (including the ICU).

8. Any medical specialty must be prepared to manage UTI with CRE, either directly or by calling on the hospital’s specialized personnel in these infections, emphasizing the importance of knowing the rigorous handling of the urinary catheter in accordance with medical practice guidelines, such as the "European and Asian Guidelines for the Management and Prevention of Catheter-Associated Urinary Tract Infections".

9. There are several specialties in which there is an increased risk of contamination with multi-resistant uropathogens, as they hold the first places in the series identified in the above studies: Infectious diseases, Internal medicine, Neurology, Urology.

10. Tertiary clinical medical centers hold an undesirable detached first place, both in terms of the number of strains and in terms of the types of carbapenemases encountered (the tendency of associating two types of carbapenemases - NDM with OXA - is worrying). It should be noted that in the county hospital studied, the frequency of MDR/XDR germs with resistance to carbapenems is rarer and the genetic variability of resistance to other antibiotics is lower.

11. The presence of *blaNDM* genes in the institution restricts therapeutic options even to cefiderocol to which emerging resistance occurs (especially in the case of NDM-type enzyme-producing strains).

12. Early detection and immediate control measures are essential to control the spreading. From a public health perspective, I see the need for an active national surveillance system for multidrug-resistant organisms that would allow early warnings and, subsequently, a rapid assessment of the extent of the problem at national level. National programs based on molecular surveillance (technologies apparently and at the moment inaccessible to medium and small medical institutions) in infection control are needed in our country's hospitals, in order to detect emerging resistance genes, as well as high-risk clones and stop them. By virtue of these elements, I consider it essential to have an institution at national level adequately equipped and efficiently built to fight this epidemic.

13. There is a medical approach for the control of bacteriuria and complications of urinary catheterization without the use of antibiotics, thus in full accordance with the principles of *antimicrobial stewardship*, as the use of antimicrobials inevitably leads to the selection of MDR bacteria and subsequently XDR or even PDR.

14. *L-methionine* treatment in patients with chronic urinary catheters provides good control of mechanical and septic complications of indwelling device, at reasonable costs, safely and in compliance with the principles of *antimicrobial stewardship*.

Bibliography

1. AMR Review Paper - Tackling a crisis for the health and wealth of nations_1.pdf [Internet]. [citat 27 ianuarie 2024]. Disponibil la: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

2. European Centre for Disease Prevention and Control, European Medicines Agency. The bacterial challenge : time to react : a call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new antibacterial agents [Internet]. LU: Publications Office; 2009 [citat 14 februarie 2023]. Disponibil la: https://data.europa.eu/doi/10.2900/2518

3. de Kraker MEA, Stewardson AJ, Harbarth S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLOS Med. 29 noiembrie 2016;13(11):e1002184.

4. van Duin D, Kaye KS, Neuner EA, Bonomo RA. Carbapenem-resistant Enterobacteriaceae: a review of treatment and outcomes. Diagn Microbiol Infect Dis. februarie 2013;75(2):115–20.

5. Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, et al. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database. 1 ianuarie 2020;2020:baaa062.

6. The Global Antibiotic Research & Development Partnership (GARDP). ESKAPE [Internet]. REVIVE. [citat 18 februarie 2024]. Disponibil la: https://revive.gardp.org/resource/eskape/?cf=encyclopaedia

7. Kline KA, Lewis AL. Gram-Positive Uropathogens, Polymicrobial Urinary Tract Infection, and the Emerging Microbiota of the Urinary Tract. Microbiol Spectr. aprilie 2016;4(2):10.1128/microbiolspec.UTI-0012–2012.

8. Martínez JL. Natural Antibiotic Resistance and Contamination by Antibiotic Resistance Determinants: The Two Ages in the Evolution of Resistance to Antimicrobials. Front Microbiol. 13 ianuarie 2012;3:1.

9. Barbu IC, Gheorghe-Barbu I, Grigore GA, Vrancianu CO, Chifiriuc MC. Antimicrobial Resistance in Romania: Updates on Gram-Negative ESCAPE Pathogens in the Clinical, Veterinary, and Aquatic Sectors. Int J Mol Sci. 26 aprilie 2023;24(9):7892.

10. Codjoe FS, Donkor ES. Carbapenem Resistance: A Review. Med Sci. 21 decembrie 2017;6(1):1.

11. Boyd SE, Livermore DM, Hooper DC, Hope WW. Metallo-β-Lactamases: Structure, Function, Epidemiology, Treatment Options, and the Development Pipeline. Antimicrob Agents Chemother. 21 septembrie 2020;64(10):e00397-20.

12. Sharma J, Sharma D, Singh A, Sunita K. Colistin Resistance and Management of Drug Resistant Infections. Can J Infect Dis Med Microbiol J Can Mal Infect Microbiol Médicale. 10 decembrie 2022;2022:4315030.

13. Aghapour Z, Gholizadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, et al. Molecular mechanisms related to colistin resistance in Enterobacteriaceae. Infect Drug Resist. 24 aprilie 2019;12:965–75.

14. Gogry FA, Siddiqui MT, Sultan I, Haq QMohdR. Current Update on Intrinsic and Acquired Colistin Resistance Mechanisms in Bacteria. Front Med [Internet]. 2021 [citat 26 februarie 2024];8. Disponibil la: https://www.frontiersin.org/articles/10.3389/fmed.2021.677720

15. Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. Ann N Y Acad Sci. septembrie 2015;1354(1):12–31.

16. Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 mai 2001;32 Suppl 2:S133-145.

17. Delcaru C, Alexandru I, Podgoreanu P, Grosu M, Stavropoulos E, Chifiriuc MC, et al. Microbial Biofilms in Urinary Tract Infections and Prostatitis: Etiology, Pathogenicity, and Combating strategies. Pathogens. 30 noiembrie 2016;5(4):65.

18. Zhou Y, Zhou Z, Zheng L, Gong Z, Li Y, Jin Y, et al. Urinary Tract Infections Caused by Uropathogenic Escherichia coli: Mechanisms of Infection and Treatment Options. Int J Mol Sci. 23 iunie 2023;24(13):10537.

19. Uroweb - European Association of Urology [Internet]. [citat 1 martie 2024]. EAU Guidelines on Urological Infections - THE GUIDELINE - Uroweb. Disponibil la: https://uroweb.org/guidelines/urological-infections/chapter/the-guideline

20. Rowe TA, Juthani-Mehta M. Diagnosis and Management of Urinary Tract Infection in Older Adults. Infect Dis Clin North Am. martie 2014;28(1):75–89.

21. Neal DE, Steele R, Sloane B. Ultrasonography in the differentiation of complicated and uncomplicated acute pyelonephritis. Am J Kidney Dis Off J Natl Kidney Found. noiembrie 1990;16(5):478–80.

22. Wawrysiuk S, Naber K, Rechberger T, Miotla P. Prevention and treatment of uncomplicated lower urinary tract infections in the era of increasing antimicrobial resistance—non-antibiotic approaches: a systemic review. Arch Gynecol Obstet. 2019;300(4):821–8.

23. PubMed [Internet]. [citat 9 martie 2024]. PubMed. Disponibil la: https://pubmed.ncbi.nlm.nih.gov/

24. Pricop C. COMPARATIVE RESEARCH BETWEEN SOLIFENACINTAMSULOSIN VERSUS TAMSULOSIN ALONE IN EXPULSION OF JUXTAVESICAL URETERAL STONES. FARMACIA. 26 iunie 2022;70(4):652–7.

25. Kline KA, Schwartz DJ, Lewis WG, Hultgren SJ, Lewis AL. Immune Activation and Suppression by Group B Streptococcus in a Murine Model of Urinary Tract Infection ▿. Infect Immun. septembrie 2011;79(9):3588–95.

26. Nielubowicz GR, Mobley HLT. Host-pathogen interactions in urinary tract infection. Nat Rev Urol. august 2010;7(8):430–41.

27. Ny S, Edquist P, Dumpis U, Gröndahl-Yli-Hannuksela K, Hermes J, Kling AM, et al. Antimicrobial resistance of *Escherichia coli* isolates from outpatient urinary tract infections in women in six European countries including Russia. J Glob Antimicrob Resist. 1 iunie 2019;17:25–34.

28. Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K. Urinary tract infections caused by *Pseudomonas aeruginosa*: A minireview. J Infect Public Health. 1 ianuarie 2009;2(3):101–11.

29. Paz-Zarza VM, Mangwani-Mordani S, Martínez-Maldonado A, Álvarez-Hernández D, Solano-Gálvez SG, Vázquez-López R. [Pseudomonas aeruginosa: Pathogenicity and antimicrobial resistance in urinary tract infection]. Rev Chil Infectologia Organo Of Soc Chil Infectologia. aprilie 2019;36(2):180–9.

30. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. martie 2012;18(3):268–81.

31. Gupta K, Hooton TM, Roberts PL, Stamm WE. Short-course nitrofurantoin for the treatment of acute uncomplicated cystitis in women. Arch Intern Med. 12 noiembrie 2007;167(20):2207–12.

32. Lecomte F, Allaert FA. Le traitement monodose de la cystite par fosfomycine trométamol (Monuril®) : analyse de 15 essais comparatifs portant sur 2048 malades. Médecine Mal Infect. 1 martie 1996;26(3):338–43.

33. Gupta K, Stamm WE. Outcomes associated with trimethoprim/sulphamethoxazole (TMP/SMX) therapy in TMP/SMX resistant community-acquired UTI. Int J Antimicrob Agents. iunie 2002;19(6):554–6.

34. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin Infect Dis Off Publ Infect Dis Soc Am. octombrie 1999;29(4):745–58.

35. Rusu A, Tiliscan C, Adamescu AI, Ganea OA, Arama V, Arama SS, et al. Carbapenemase-producing uropathogens in real life: epidemiology and treatment at a County Emergency Hospital from Eastern Romania. J Med Life. mai 2023;16(5):707–11.

36. Rusu A. URINARY TRACT INFECTIONS IN A ROMANIAN POPULATION: ANTIMICROBIAL RESISTANCE OF UROPATHOGENS – A MULTIREGIONAL STUDY. FARMACIA. 26 februarie 2023;71(1):165–73.

37. Petca RC, Negoiță S, Mareș C, Petca A, Popescu RI, Chibelean CB. Heterogeneity of Antibiotics Multidrug-Resistance Profile of Uropathogens in Romanian Population. Antibiotics. 2 mai 2021;10(5):523.

38. Chibelean CB, Petca RC, Mareș C, Popescu RI, Enikő B, Mehedințu C, et al. A Clinical Perspective on the Antimicrobial Resistance Spectrum of Uropathogens in a Romanian Male Population. Microorganisms. 5 iunie 2020;8(6):848.

39. Petca RC, Mareș C, Petca A, Negoiță S, Popescu RI, Boț M, et al. Spectrum and Antibiotic Resistance of Uropathogens in Romanian Females. Antibiotics. 1 august 2020;9(8):472.

40. Lee DS, Choe HS, Kim HY, Yoo JM, Bae WJ, Cho YH, et al. Role of age and sex in determining antibiotic resistance in febrile urinary tract infections. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. octombrie 2016;51:89–96.

41. McGregor JC, Elman MR, Bearden DT, Smith DH. Sex- and age-specific trends in antibiotic resistance patterns of Escherichia coli urinary isolates from outpatients. BMC Fam Pract. 2013;14:25.

42. Lagacé-Wiens PRS, Simner PJ, Forward KR, Tailor F, Adam HJ, Decorby M, et al. Analysis of 3789 in- and outpatient Escherichia coli isolates from across Canada--results of the CANWARD 2007-2009 study. Diagn Microbiol Infect Dis. martie 2011;69(3):314–9.

43. Bader MS, Loeb M, Brooks AA. An update on the management of urinary tract infections in the era of antimicrobial resistance. Postgrad Med. martie 2017;129(2):242–58.

44. Aslan AT, Akova M. The Role of Colistin in the Era of New β-Lactam/β-Lactamase Inhibitor Combinations. Antibiotics. 20 februarie 2022;11(2):277.

45. Kaliyeva S, Simokhina N, Lavrinenko A, Zhussupova G, Zhunusov S, Semenikhina P, et al. Bacterial Colonization Incidence before and after Indwelling Double-J Ureteral Stents. Antibiotics. 24 iunie 2022;11(7):850.

46. Trevino SE, Babcock HM, Henderson JP, Lane MA, Beekmann SE, Polgreen PM, et al. Perceptions and behaviours of infectious diseases physicians when managing urinary tract

infections due to MDR organisms. J Antimicrob Chemother. decembrie 2015;70(12):3397–400.

47. Tenke P, Kovacs B, Bjerklund Johansen TE, Matsumoto T, Tambyah PA, Naber KG. European and Asian guidelines on management and prevention of catheter-associated urinary tract infections. Int J Antimicrob Agents. februarie 2008;31 Suppl 1:S68-78.

48. Center for Genomic Epidemiology [Internet]. Disponibil la: www.genomicepidemiology.org

49. Villacís JE, Reyes JA, Castelán-Sánchez HG, Dávila-Ramos S, Lazo MA, Wali A, et al. OXA-48 Carbapenemase in Klebsiella pneumoniae Sequence Type 307 in Ecuador. Microorganisms. 19 martie 2020;8(3):435.

50. Hornsey M, Phee L, Wareham DW. A Novel Variant, NDM-5, of the New Delhi Metallo-β-Lactamase in a Multidrug-Resistant Escherichia coli ST648 Isolate Recovered from a Patient in the United Kingdom ▿ . Antimicrob Agents Chemother. decembrie 2011;55(12):5952–4.

51. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a New Metallo-β-Lactamase Gene, blaNDM-1, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in Klebsiella pneumoniae Sequence Type 14 from India. Antimicrob Agents Chemother. decembrie 2009;53(12):5046–54.

52. Cheng Z, Thomas PW, Ju L, Bergstrom A, Mason K, Clayton D, et al. Evolution of New Delhi metallo-β-lactamase (NDM) in the clinic: Effects of NDM mutations on stability, zinc affinity, and mono-zinc activity. J Biol Chem. 10 august 2018;293(32):12606–18.

53. Isler B, Vatansever C, Özer B, Çınar G, Aslan AT, Falconer C, et al. Higher rates of cefiderocol resistance among NDM producing Klebsiella bloodstream isolates applying EUCAST over CLSI breakpoints. Infect Dis Lond Engl. septembrie 2023;55(9):607–13.

54. Isler B, Aslan AT, Akova M, Harris P, Paterson DL. Treatment strategies for OXA-48-like and NDM producing Klebsiella pneumoniae infections. Expert Rev Anti Infect Ther. noiembrie 2022;20(11):1389–400.

55. Karakonstantis S, Rousaki M, Kritsotakis EI. Cefiderocol: Systematic Review of Mechanisms of Resistance, Heteroresistance and In Vivo Emergence of Resistance. Antibiotics. 27 mai 2022;11(6):723.

56. Simner PJ, Bergman Y, Conzemius R, Jacobs E, Tekle T, Beisken S, et al. An NDM-Producing Escherichia coli Clinical Isolate Exhibiting Resistance to Cefiderocol and the Combination of Ceftazidime-Avibactam and Aztreonam: Another Step Toward Pan-β-Lactam Resistance. Open Forum Infect Dis. 1 iulie 2023;10(7):ofad276.

57. Temkin E, Fallach N, Almagor J, Gladstone BP, Tacconelli E, Carmeli Y, et al. Estimating the number of infections caused by antibiotic-resistant Escherichia coli and Klebsiella pneumoniae in 2014: a modelling study. Lancet Glob Health. septembrie 2018;6(9):e969–79.

58. Miftode IL, Pasare MA, Miftode RS, Nastase E, Plesca CE, Lunca C, et al. What Doesn't Kill Them Makes Them Stronger: The Impact of the Resistance Patterns of Urinary Enterobacterales Isolates in Patients from a Tertiary Hospital in Eastern Europe. Antibiotics. 20 aprilie 2022;11(5):548.

59. Trautner BW, Darouiche RO. Role of biofilm in catheter-associated urinary tract infection. Am J Infect Control. mai 2004;32(3):177–83.

60. L-methionine in patients with neurogenic bladder disorders: Executive summary of final report A04-02, Version 1.0. În: Institute for Quality and Efficiency in Health Care: Executive Summaries [Internet]. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2005 [citat 31 martie 2024]. Disponibil la: http://www.ncbi.nlm.nih.gov/books/NBK84132/

61. Clinical & Laboratory Standards Institute [Internet]. [citat 9 martie 2024]. Clinical & Laboratory Standards Institute: CLSI Guidelines. Disponibil la: https://clsi.org/

62. Stickler DJ. Clinical complications of urinary catheters caused by crystalline biofilms: something needs to be done. J Intern Med. august 2014;276(2):120–9.

63. Mobley HL, Warren JW. Urease-positive bacteriuria and obstruction of long-term urinary catheters. J Clin Microbiol. noiembrie 1987;25(11):2216–7.

64. Feneley RCL, Hopley IB, Wells PNT. Urinary catheters: history, current status, adverse events and research agenda. J Med Eng Technol. 17 noiembrie 2015;39(8):459–70.

65. Tenney JH, Warren JW. Long-term catheter-associated bacteriuria: species at low concentration. Urology. noiembrie 1987;30(5):444–6.

66. Azevedo AS, Almeida C, Melo LF, Azevedo NF. Impact of polymicrobial biofilms in catheter-associated urinary tract infections. Crit Rev Microbiol. august 2017;43(4):423–39.

67. Breitenbucher RB. Bacterial changes in the urine samples of patients with long-term indwelling catheters. Arch Intern Med. august 1984;144(8):1585–8.