UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
DOCTORAL SCHOOL

DOCTORAL THESIS
ABSTRACT

STUDY ON ANTIBIORESISTANCE OF MICROORGANISMS INVOLVED IN HEALTHCARE-ASSOCIATED INFECTIONS

PhD SUPERVISOR:
PROF. UNIV. DR. VALENTIN CÎRLIG

PhD STUDENT:
PETRONELA-CRISTINA GIURGI
(căs. CHIRIAC)

CRAIOVA
2018

CONTENTS

KEY WORDS: ANTIBIOTIC RESISTANCE, HEALTHCARE-ASSOCIATED INFECTION, PEDIATRICS

GENERAL PART

Chapter I. HEALTHCARE-ASSOCIATED INFECTIONS - A MAJOR PROBLEM OF PUBLIC HEALTH

Antimicrobial resistance is the expected result of the interaction of microorganisms with the environment, bacteria developing mechanisms for survival, and some of bacteria are often considered to be resistant to one or more antimicrobials. The development of resistance may be the result of chromosomal gene mutations or the acquisition of external genetic determinants. Clinical sensitivity points (sensitive, intermediate and resistant) are based primarily on the *in vitro* activity of an antibiotic against a bacterial sample combined with certain pharmacological parameters (eg blood concentrations and site of infection). Interpretation of susceptibility patterns may vary depending on the clinical scenario and the availability of treatment options. Healthcare-associated infections result in a long duration of hospitalization, high mortality and increased costs of healthcare. The antibiotic susceptibility profile of a microbial species is the sum of antibiotics to which all or most of the strains are susceptible, a representative, statistically tested sample. The percentage of strains with antibiotic resistance increases in direct proportion to the intensity of their use in a community. That is why we can discuss the initial natural profile and the current profile which is much narrower than the natural one and varies with time and geographical region. In the same geographical area there may be variations in communities, such as multidrugresistance in hospitals as compared to general population. The incidence of resistant strains, amplification and de-amplification of the genetic background of resistance in the indigenous and ambient microbiota varies in time and space under the absolute selective pressure of antibiotics for therapeutic, veterinary or environmental pollutants, but also by the extending of cloning generation time resistant. In 2015, Romania was the country with the second largest antibiotic use for systemic use in Europe, compared to the European Antimicrobial Consumption Network.

Chapter II. MICROORGANISMS INVOLVED IN HEALTHCARE-ASSOCIATED INFECTIONS

Antibiotic susceptibility testing revealed high levels of resistance to antimicrobial agents with the presence of phenotypes of clinical and epidemiological importance, of which the increased incidence of methicillin-resistant strains (MRSA) (41.59%) and the increased frequency of MDR phenotypes (31.11%). In a study carried out also in the north-eastern region of Romania, on the antibiotic resistance of the strains involved in IAAM, in 2012, isolated isolates of MRSA associated a fluoroquinolone resistance of 35.3% (thus far below that of the

rabies by EARS), and in 2013 71.42% (values close to those reported by EARS) and MRSA strains were totally susceptible to fluoroquinolones in both 2012 and 2013. According to the same report, the trend calculated in the 2009- 2012 for 26 countries, has been steadily increasing for 11 countries, including Romania. Resistance to carbapenems increased significantly in Europe during 2005-2012, such strains being found in several countries, such as the Czech Republic and Hungary, but also in Romania. Over the last two decades, antibiotic resistance, especially for Gram negative bacteria, has grown at an alarming rate, requiring a continuing concern for solving and controlling this extremely important therapeutic issue in any medical department, but especially in areas of ICU, neonatology, pediatrics, burns, immunosuppression. Specialists noted a particular concern for the resistance of *Enterobacteriaceae* to third-generation cephalosporins and to aztreonam, with a resistance profile commonly associated with extended spectrum β-lactamase (ESBL) expression. It is known that the *Klebsiella spp*. Strains produce moderate amounts of chromosomal penicillinases, collecting multidrug resistant plasmids. In the early 2000s, these strains quickly spread to hospitals, resulting in a global crisis of isolates resistant to antimicrobials. For patients and health professionals, increasing the antimicrobial resistance of *Klebsiella spp*. Is a threat, especially when discussing carbapenem-resistant isolates. With few therapeutic alternatives remaining as an option, the existence of ESBL-producing *Klebsiella spp* is a challenge for antibiotic management and a problem for hospital guidelines.

Chapter III. MECHANISMS OF ANTIMICROBIAL RESISTANCE

Bacteria have developed sophisticated antibiotic resistance mechanisms. Resistance to an antimicrobial class can usually be accomplished by multiple biochemical pathways, and a bacterial cell may be able to use a framework of resistance mechanisms to survive the effect of an antibiotic. The predominant resistance mechanism for Gram-negative bacteria is the production of β-lactamases, while the resistance to these compounds in Gram-positive organisms is largely achieved by changes in their target site, the penicillin-binding proteins. The bacterial cell controls the access of these molecules to the periplasmic space, allowing the production of β-lactamases in sufficient concentrations to bias the pharmacokinetics in favor of destruction of the antibiotic molecule. One of the most successful bacterial strategies to deal with the presence of antibiotics is to produce enzymes that inactivate the drug by adding specific chemical compounds or destroying the molecules themselves, which makes the antibiotic unable to interact with the target. The production of enzymes capable of introducing chemical changes to the antimicrobial molecule is a well-known mechanism of antibiotic resistance acquired in both Gram-negative and Gram-positive bacteria. Most antibiotics affected by these enzymatic changes

exert the mechanism of action by inhibiting protein synthesis in ribosomes. The main mechanism of β-lactamase resistance is based on the destruction of these compounds by β-lactamases. These enzymes destroy the amide of the β-lactam ring, making the antimicrobial ineffective. Many of the antibiotics used in clinical practice have intracellular bacterial targets or, in the case of Gram-negative bacteria, located in the cytoplasmic membrane. Bacteria have developed mechanisms to prevent reaching the intracellular or periplasmic target of the antibiotic by decreasing the absorption of the antimicrobial molecule. This mechanism is particularly important in Gram-negative bacteria, limiting the influx of substances from the external environment. A common strategy for bacteria to develop antimicrobial resistance is to avoid antibiotic action by interference with the target site. To accomplish this, bacteria evolved into different tactics, including target protection (avoiding the antibiotic reaching its binding site) and changes in the target site resulting in low affinity for the antibiotic molecule.

Chapter IV. SOME CLINICAL PECULIARITIES IN HEALTHCARE-ASSOCIATED INFECTIONS

Healthcare-associated urinary infections affect about 1% of patients and account for 40% of all HAIs. Their frequency is higher in burns, urology, ICU and neurology. The GPIU (Global Prevalence of Urology Infections) report shows a prevalence of urinary HAIs in urology departments of 9.7%, although many other studies have reported up to 60% for Europe and especially the southern region. Other European studies reported an antibiotic resistance of *P. aeruginosa* isolates from patients with urinary HAIs about 20% in carbapenems and aminoglycosides. The analysis of risk factors that may be associated with higher rates of antibiotic resistance has shown that in patients undergoing endourological surgery, the prevalence of urinary HAIs with *E. coli* ESBL strains was 62.5%. It is necessary to know the local microbiological model to select appropriate antibiotic treatment and to avoid increased antibiotic resistance. After initiating empirical antibiotherapy based on the local sensitivity model, it should be adjusted according to the results of uroculture and antibiogram. Healthcare-associated pneumonia is one of the most important HAIs, with the mortality can be as high as 30-60% of cases. The most common etiology is caused by *P. aeruginosa, Acinetobacter spp, Klebsiella spp, Enterobacter spp, E. coli*, etc. The American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) recommends obtaining evidence from the lower respiratory tract for culture and a quantitative or qualitative microbiological examination. This guidance also allows the use of tracheal aspirations for their negative predictive value (94% for VAP). Clinical pulmonary infection score (CPIS) takes into account clinical, physiological, microbiological and radiographic evidence that allows a numerical value to predict the presence

or absence of VAP. Catheter-related infections may occur in patients with a venous or arterial, peripheral or central catheter and represent about 15-20% of the total of HAIs. Healthcare-associated bacteriemia and sepsis are responsible for about 10% of the total HAIs wit an etiology of MDR bacteria such as *E. coli, Klebsiella pneumoniae, S. aureus, P. aeruginosa, Enterobacter spp*., etc. Healthcare-associated sepsis is a common complication in neonatal ICUs, where previous studies have reported incidence rates of up to 32%, especially in newborns with very low birth weight (≤1500 g), with gastrointestinal disease and those undergoing major surgical procedures. Although the implementation of the new reduction and control protocols of HAIs can reduce the incidence of sepsis, it remains a leading cause of morbidity and mortality in neonates.

PERSONAL PART

AIM AND OBJECTIVES. The purpose of the research theme is to evaluate the antibiotic resistance of the identified microorganisms in the isolates of patients with healthcare-associated infections in a pediatric hospital in our country. **The specific objectives** of the doctoral research were as follows:

1. Evaluation of healthcare-associated infections in terms of prevalence and demographic data. To accomplish this aim, a descriptive retrospective study was carried out in a pediatric clinical emergency hospital in the Northeastern region of Romania. The studied wards were: ICU, pediatric surgery and orthopedics, pediatric oncology, burns department, general pediatric wards. The study group was a number of cases diagnosed with healthcare-associated infections and reported over a 2-year interval (2016-2017). The descriptive study included demographic data on patients, admission diagnosis, associated pathology etc.

2. Study of the antibiotic resistance of isolated microorganisms from patients with healthcare-associated infections in a pediatric hospital from a region of Northeastern Romania. I intended to conduct a comparative study of the antibiotic resistance of various pathogenic microorganisms isolated from pediatric patients in ICU, surgery, oncology wards etc. The study group was represented by hospitalized patients diagnosed with healthcare-associated infections (sepsis, urinary tract infection, wound infection, cutaneous infection, conjunctival infection, catheter-associated infection, ventilator-associated pneumonia etc.). The study took into consideration microorganisms more frequently isolated and with and recognised antibiotic resistance such as: *Klebsiella spp, Pseudomonas spp, Escherichia coli, Enterobacter spp*. etc.

METHODS AND STUDY DESIGN AND STAGES. Place of study: ”Sf. Maria” Clinical Emergency Hospital for Children, Iasi, Romania. The institutional acceptance for the conduct of studies is signed by the Hospital Medical Ethics Committee and Director Committee.

Chapter V. HEALTHCARE-ASSOCIATED INFECTIONS IN PEDIATRICS. DESCRIPTIVE STUDY ON A GROUP OF PEDIATRIC PATIENTS WITH ACUTE BACTERIAL GASTROENTERITES

The study aim was to describe the cases of healthcare-associated acute gastroenteritis reported in a pediatric hospital of Northeastern Romania. We conducted a descriptive study of a group of 615 cases admitted between 2012 and 2016. Gastroenteritis with *Campylobacter spp.* was most commonly reported in pediatric patients, especially in infants aged 0-1 years and children aged 2-6 years. Cases of infections with *Salmonella spp* were also common. A competent management of the prevention and control of HAIs, especially acute gastroenteritis, in a children's emergency hospital in a region that includes rural areas in development should be the most important issue for professionals involved in management of surveillance and control of HAIs, as well as clinicians, epidemiologists and microbiologists, in order to prevent and avoid antimicrobial resistance.

Chapter VI. ANTIBIORESISTANCE OF SOME BACTERIA INVOLVED IN THE ETIOLOGY OF HEALTHCARE-ASSOCIATED INFECTIONS IN PEDIATRICS

The descriptive study was carried out on a group of 411 patients admitted to the hospital between January 1 - March 31, 2016. We found that *Staphylococcus aureus* was resistant to more than a third of penicillin G and oxacillin isolates, *Streptococcus pneumoniae* was resistant to penicillin G and erythromycin, and *Klebsiella pneumoniae* to amoxicillin with clavulanic acid and cephalosporins of II nd, III rd and IV th generations, *Haemophilus spp* to cefaclor, and *E.coli* strains to ampicillin and cephalosporins of II nd, III rd and IV th generations.

Chapter VII. ASPECTS REGARDING PEDIATRIC INFECTIONS WITH *KLEBSIELLA PNEUMONIAE* ESBL

Out of the study group performed between 2016 and 2017, with 59 cases of healthcare-associated infections with *K. pneumoniae*, we selected a subgroup of 24 admitted patients. Various pathological products have been collected from the subgroup individuals, in order to detect extented- spectrum betalactamases producing *K. pneumoniae* strains (ESBL). The antibiotic resistance of *K. pneumoniae* strains demonstrated that all 24 isolates were found to be ampicillin-resistant and amoxicillin + clavulanic acid resistant too. 18 strains of *K. pneumoniae* were also resistant to cephalosporins such as cefuroxime, ceftazidime, ceftriaxone, cefepime.

Chapter VIII. *PSEUDOMONAS AERUGINOSA* IN THE ETIOLOGY OF HEALTHCARE-ASSOCIATED INFECTIONS IN PEDIATRICS

The descriptive study was performed on a group of 28 patients with various healthcare-associated infections with *Pseudomonas aeruginosa*, out of a total group of 214 cases of healthcare-associated infections associated registered in 2016. Our study provided some indications regarding the prevalence of *P. aeruginosa*-associated infections in pediatric patients and guidelines for antibiotic therapy. The sensitivity of circulating strains was increased for amikacin and tobramycin. Antibiotic resistance of isolated strains was important for imipenem and meropenem, suggesting that circulating strains in the hospital / or regional community would present genetic elements responsible for the presence of carbapenemases and the importance of further studies to identify them.

Chapter IX. ANTIBIORESISTANCE OF *ESCHERICHIA COLI* STRAINS INVOLVED IN HEALTHCARE-ASSOCIATED INFECTIONS IN PEDIATRICS

The retrospective study was conducted between 2016 and 2017 on a group of 26 patients (13 patients in each year of study), in which various pathological products were collected for the detection of *E. coli* strains involved in healthcare-associated infections with various clinical manifestations. The profile of the pediatric patient most commonly involved in a HAI with *E. coli* corresponds to a 6-year female patient, with a length of stay up to 25 days until the diagnosis of HAI. We expect the isolated *E. coli* strain from such a patient not to be ESBL but to have an antimicrobial resistance of more than 50%, especially to penicillins and cephalosporins of II nd generation, but most likely to be sensitive to amikacin.

Chapter X. ANTIBIORESISTANCE OF *ENTEROBACTER SPP.* STRAINS INVOLVED IN HEALTHCARE-ASSOCIATED INFECTIONS IN PEDIATRICS

The retrospective descriptive study was conducted between 2016 and 2017 on a group of 13 patients with various clinical symptoms of healthcare-associated infections due to *Enterobacter spp* strains. Taken into consideration that some of these isolates were ESBL strains, in the general context of microbial multidrugresistance, this study is of great importance for the hospital management on prevention and control of HAIs. Our study provides some useful indications of the prevalence of HAIs with *Enterobacter spp* in pediatric patients and guidelines for antibiotic therapy. The isolated *Enterobacter spp.* strains were totally resistant to ampicillin and amoxicillin + clavulanic acid. The resistance was lower for cephalosporins of II nd and III rd generation. These findings highlighted the fact that isolates from pediatric patients, particularly

the neonatology ICU department, have an important antibiotic resistance and should be considered for the management of HAIs and hospital guidelines.

Chapter XI. FINAL CONCLUSIONS. DIRECTIONS OPENED BY THE DOCTORAL STUDY. LIMITS OF THE RESEARCH

The management of healthcare-associated infections in a pediatric hospital requires further studies in order to continue to identify the microorganisms and their susceptibility to antibiotics as well as associated risk factors, in order to organize effective prevention measures and appropriate treatment for reduce the cost of hospitalization and last but not least to improve the quality of life in pediatric patients. Detailed analysis of the etiological spectrum of healthcare-associated infections in pediatrics and antibiotic resistance of circulating strains provides sufficient data to suggest the need to implement a comprehensive research on such infections in a pediatric hospital with the final goal of decreasing their incidence, being essential for both the hospital and the community of pediatric patients and their families.

REFERENCES (SELECTION)

1. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol. 2010; 1:134.
2. Amin H, Zafar A, Ejaz H, Jameel N-A. Phenotypic characterization of ESBL producing *Enterobacter cloacae* among children. Pakistan J Med Sci 2013;29(1):144-147.
3. Chen HL, Lu JH, Wang HH, Chen SJ, Chen CJ, Wu KG, Tang RB. Clinical analysis of Enterobacter bacteremia in pediatric patients: a 10-year study. J Microbiol Immunol Infect. 2014;47(5):381-6.
4. Dantes R, et al. *National burden of invasive methicillin-resistant Staphylococcus aureus infections, United States, 2011*. JAMA Intern. Med. 2013; 173, 1970–1978.
5. Gardete S, Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. The Journal of Clinical Investigation. 2014;124(7):2836-2840.
6. Iredell J, Brown J, Tagg K. Antibiotic resistance in *Enterobacteriaceae*: mechanisms and clinical implications. BMJ. 2016; 352:h6420.
7. Kaspar T, Schweiger A, Droz S, Marschall J. Colonization with resistant microorganisms in patients transferred from abroad: who needs to be screened? Antimicrob Resist Infect Control. 2015; 4:31.
8. Lai CC, Ji DD, Wu FT, et al. Etiology and Risk Factors of Acute Gastroenteritis in a Taipei Emergency Department: Clinical Features for Bacterial Gastroenteritis. J Epidemiology. 2016; 26(4):216-223.

9. Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, Harbarth S. Methicillin-resistant *Staphylococcus aureus*. Nat Rev Dis Primers. 2018 31;4:18033.
10. Logan LK, Gandra S, Mandal S, et al. Multidrug- and Carbapenem-Resistant Pseudomonas aeruginosa in Children, United States, 1999-2012. J Pediatric Infect Dis Soc. 2017, 24;6(4):352-359.
11. Medernach RL, Logan LK. The Growing Threat of Antibiotic Resistance in Children. Infect Dis Clin North Am. 2018;32(1):1-17.
12. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. *Microbiology spectrum*. 2016;4(2):10.
13. National Healthcare Safety Network (NHSN) July 2013 CDC/NHSN Protocol Clarifications. 2013.
14. Paczosa MK, Mecsas J. *Klebsiella pneumoniae*: Going on the Offense with a Strong defense. Microbiol Molec Biol Rev: MMBR. 2016; 80(3):629-661.
15. Page DB, Donnelly JP, Wang HE. Community-, Healthcare- and Hospital-Acquired Severe Sepsis Hospitalizations in the University HealthSystem Consortium. Critical care medicine. 2015;43(9):1945-1951.
16. Paalanne N, Husso A, Salo J, et al. Intestinal microbiome as a risk factor for urinary tract infections in children. Eur J Clin Microbiol Infect Dis. 2018; 37 (10): 1881-1891.
17. Parcell BJ, Oravcova K, Pinheiro M, et al. *Pseudomonas aeruginosa* intensive care unit outbreak: winnowing of transmissions with molecular and genomic typing. J Hospital Infection. 2018;98(3):282-288.
18. Paulsson M, Granrot A, Ahl J, et al. Antimicrobial combination treatment including ciprofloxacin decreased the mortality rate of *Pseudomonas aeruginosa* bacteraemia: a retrospective cohort study. Eur J Clin Microbiol Infect Dis. 2017;36(7):1187-1196.
19. Puentes SS, Dunstan M. Escherichia coli Complications in Pediatric Critical Care. Crit Care Nurs Clin North Am. 2018; 30(1):149-156.
20. Tsao LH, Hsin CY, Liu HY, et al. Risk factors for healthcare-associated infection caused by carbapenem-resistant *Pseudomonas aeruginosa*. J Microbiol Immunol Infect 2018; 51(3):359-366.
21. Verstraete EH, Mahieu L, De Coen K, Vogelaers D, Blot S. Impact of healthcare-associated sepsis on mortality in critically ill infants. Eur J Pediatr. 2016;175(7):943-52.
22. Willson DF, Hoot M, Khemani R, et al. Pediatric Ventilator-Associated Infections: The Ventilator-Associated Infection Study. Pediatr Crit Care Med. 2017;18(1):e24-e34.
23. Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A Review on Antibiotic Resistance: Alarm Bells are Ringing. Muacevic A, Adler JR, eds. *Cureus*. 2017; 9(6):e1403.