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# PhD THESIS

RISK FACTORS FOR THE ACQUISITION  
OF *PROTEAE* INFECTIONS IN INTENSIVE  
CARE UNITS AND SURGICAL WARDS

## ABSTRACT

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**Keywords:** Carbapenem resistant *Proteeae*, Intensive Care Unit, *P. stuartii*, diabetic, non-diabetic, surgical sections, fatality, antibiotal resistance

# 1. INTRODUCTION

*Proteeae* pathogens are currently a major challenge for clinicians, as they have both a natural resistance to many antibiotics and an increased ability to acquire resistance.

The PhD thesis addresses a priority topic, as the emergence of multidrug-resistant bacteria has become a worrying public health issue. This paper aims to investigate the etiology and patterns of phenotypic and molecular resistance (identification of extended-spectrum beta-lactamases and carbapenemases) in strains from the *Proteeae* group isolated from healthcare associated infections (HCAI) in intensive care unit (ICU) and surgical wards. The emergence of new enzymes and new methods of producing old enzymes threatens the carbapenems (considered reserve antibiotics) currently needed for the treatment of severe HCAI. The rapid increase in resistance to this class of antibiotics has led to a drastic limitation of treatment options.

The subject is all the more essential as the data in the specialized literature are insufficient both regionally and globally.

The results of the study bring novelties in completing the database at national or European level, in terms of antibiotic resistance through the production of MBL. The establishment of a protocol for the detection of MBL-producing strains is of major importance, both in developing therapeutic guidelines and in the prompt recognition of the occurrence of an outbreak of HCAI and taking appropriate measures to control the infections.

## 2. GENERAL PART

*Proteeae* is a group of three genera: *Proteus*, *Providencia* and *Morganella*. Their taxonomy has undergone numerous changes throughout the history and evolution of microbiological science. For a long time, the classification of germs from the genus *Proteus*, *Enterobacteriaceae* family, was debatable, but based on its biochemical properties it was taxonomically split in 1997 into three genera: *Proteus*, *Morganella*, *Providencia*(1). However, in 2016 these opportunistic microorganisms were finally classified as belonging to the family *Morganellaceae* of the order *Enterobacterales*(2)(3). The three genera: *Proteus*, *Morganella*, *Providencia* are opportunistic microorganisms that have produced varying degrees of community and especially healthcare associated infections. There is an increase in the frequency of isolation of these germs due to the increase in the number of immunocompromised patients and the acquisition of

antimicrobial resistance genes of antibiotic resistance or virulence factors that determine their condition as opportunistic pathogens(4).

Interest in these germs has increased recently due to the emergence of infections, especially those associated with healthcare, caused by multi-drug resistant (MDR), as well as the fact that these pathogens have intrinsic resistance to tigecycline, colistin, nitrofurantoin and reduced intrinsic sensitivity to imipenem (5)(6).

Carbapenems play a particularly important role in the fight against pathogenic microorganisms. They are part of the large class of  $\beta$ -lactams, which have the widest spectrum of activity, acting on Gram-positive and Gram-negative bacteria. They are often used as 'state-of-the-art agents' or 'reserve antibiotics' when patients have life-threatening infections or which are thought to be caused by resistant organisms(7). Unfortunately, the dramatic increase in MDR pathogens seriously threatens this class of antimicrobials. Numerous recent studies show very clearly that carbapenem resistance is on the rise worldwide(8)(9)(10).

The widespread use of carbapenems has led to a rapid increase in resistance to this class, becoming a serious threat to global public health. Mechanisms of resistance include the production of carbapenemase-type enzymes, efflux pumps and mutations leading to the absence or reduced expression of two major porins in combination with various beta-lactamases and the modification of penicillin-binding proteins(7).

Carbapenemase production is the main mechanism underlying global carbapenem resistance of pathogens. Carbapenemases are a type of  $\beta$ -lactamase that can hydrolyze these antimicrobials(11).

HCAI represents a hidden problem that no institution or country can yet completely solve. Surveillance programs for HCAs are present in several developed countries, but are practically absent in most underdeveloped ones (12).

Surveillance of HCAs in the ICU it is all the more necessary as inpatients in these wards have a 5-10 times higher risk of acquiring an infection due to both intrinsic (e.g., immunosuppression) and extrinsic predictive factors (e.g., mechanical ventilation) (13).

Surgical wound infection (SSI) is part of HCAI. SSIs rank second in frequency, accounting for 10 to 40% of total HCAI, after catheter related infections (14)(15). World Health Organization (WHO) estimates that worldwide, 5-12% of hospitalized patients develop HCAs, of which more than 60% are associated with the use of a medical or surgical device(16).

SSIs have a destructive impact on the institution of patient treatment, leading to increased treatment duration, longer hospitalisation and higher costs (17).

### 3. SPECIAL PART

#### 3.1. PURPOSE AND OBJECTIVES

Currently, there is a limited number of studies examining the involvement of *Proteaa* strains in sporadic or interregional HCAI. Increased identification of germs with intrinsic resistance to colistin (*Proteus*, *Morganella*, *Providencia*) has been observed to correlate with an increase in the number of carbapenem resistant *Proteaae* (CRP) strains infections, which requires an increase in the use of colistin in therapy (18)(19). Infections pose a serious danger, especially for the immunocompromised patient, such as the diabetic patient, so that highlighting the growth of MDR pathogens, especially resistant to carbapenems, in these patients, poses particular problems in their treatment (20).

The doctoral research included three studies, which had as objectives:

1. Polymerase Chain Reaction (PCR) investigation of *bla*NDM, *bla*VIM, *bla*TEM and *bla*CTX-M genes in carbapenemase-producing strains, with a focus on *bla*NDM-1,
2. Characterization of strains within the difficult to treat resistance (DTR) phenotype, with identification of factors influencing their acquisition, as well as predictive factors in terms of patient evolution,
3. Impact of *Proteus mirabilis* and *Providencia stuartii* infections on diabetic patients.

#### 3.2. MATERIAL AND METHODS

The current doctoral project was carried out at “Pius Brinzeu” Emergency Clinical County Hospital, Timisoara (SCJUPBT), between July 2017 and April 2019 and included patients hospitalized in the Intensive Care and Surgery wards

Identification, antibiotic susceptibility testing and phenotypic characterization of the strains included in the study were performed in the Microbiology laboratory of SCJUPBT, and genotypic confirmation was performed in the laboratory of the Biochemistry Department of "Victor Babes" University of Medicine and Pharmacy, Timisoara.

**3.2.1.** In the first, observational, cross-sectional study selected bacterial strains were subjected to molecular analysis to identify carbapenemase-encoding genes by the PCR simplex method. Were included meropenem-resistant strains (for detecting the presence of *bla*NDM, *bla*VIM genes) and cefepim-resistant strains for detecting *bla*CTX-M and *bla*TEM genes.

**3.2.2.** The second retrospective study included all the *Proteeae* strains from the samples of patients admitted to ICU and surgery wards, using the database of the first study, with inclusion of strains of interest in the difficult-to-treat resistance phenotype.

**3.2.3.** The third study looked at the correlation and impact of *P.mirabilis* and *P. stuartii* infections in patients with diabetes. This study was observational, retrospective, cross-sectional, over a period of 22 months (2017 - 2019) and targeted inpatients in the ICU and surgery wards of SCJUPBT, including all those from which *P. mirabilis* and *P. stuartii* strains were isolated.

### 3.3. RESULTS

**3.3.1** In the Bacteriology Department of the Clinical Laboratory within SCJUPBT, a number of 8317 samples were processed from inpatients in the ICU and surgical wards for a period of approximately 2 years (July 2017-April 2019). In this study, 400 strains of *Proteeae* were identified, of which 65 CRP were preserved for the identification of resistance genes by the simplex-PCR method.

*Proteeae* strains were most frequently isolated from wound swabs (39.50%), bronchial aspirates (20%), followed by urine cultures (15.25%), blood cultures (8.5%) and catheter tips (7%).

The 65 strains resistant to at least one carbapenem (CRP) belong to the *Providencia* and *Proteus* genera, with an overall incidence of 16.25% - 6.25% for the genus *Proteus* and 45.79% for the genus *Providencia*. Most CRP strains came from ICU patients - 86.15% (95% CI: 19.9-43.4), 63% being isolated especially in bronchial aspirates (32.31%, 95% CI: 21.2-45.1) and blood cultures (30.7%, 95% CI: 19.9-43.4).

After PCR testing of the 65 strains, MBL-type carbapenemase was detected in 56 (86.15%; 95% CI: 75.3-93.5) of the CRP strains, with the predominant gene substrate being the *bla*NDM gene in 55 strains (84.62%). 95% CI: 73.5-92.4). The prevalence of CRP strains was increased in *P. stuartii*, most strains being MBL – *bla*NDM (61.53% of CRP), followed by *P. mirabilis* MBL-*bla*NDM (20% of CRP). In one isolate of *P. mirabilis* and one of *P. stuartii* that were phenotypically resistant to carbapenems, no carbapenemase production was identified, and in the case of *Providencia* strain the presence of the *bla*CTX-M gene was noticed. The simultaneous presence of two resistance genes (*bla*VIM, *bla*NDM) was noticed in one *P.stuartii* isolate. It is also worth mentioning that all the 5 isolates in which genes encoding KPC carbapenemase production were detected came from the Neurosurgery ward. Of the strains included in the study, 66.15% were resistant to cefepim (95% CI: 53.4-77.4), and PCR investigation led to the identification of *bla*CTX-M resistance gene in 7 (10.76%) CRP strains.

The fatality of the cases in which CRP was isolated was 49.23% compared to 33.43% in the global sample ( $p = 0.014$ ) and 22.07% among those with carbapenem-sensitive strains ( $p < 0.001$ ).

**3.3.2.** Most *Proteeae* strains came from wound secretions (43.50%), bronchial aspirates (20%), urine (15.25%) and blood cultures (8.5%) being poorly represented in catheter tips (7%), peritoneal fluid (1.75%) and sputum (0.50%) cultures.

The numerical dominance of the species *P. mirabilis* and *P. stuartii* was noted, distinguished in the samples of patients from the ICU and the surgery wards.

*P. mirabilis* strains showed resistance to fluoroquinolones (32.65%), extended spectrum beta-lactamase producers (ESBL) phenotype (22.86%), carbapenems (13.06%) and aminoglycosides (12.34%). The behavior of *P. stuartii* strains was manifested by resistance to fluoroochionolones (85.11%), carbapenems (75.53%), ESBL phenotype (64.98%), aminoglycosides (54.26%).

Observing the resistance to antibiotics of the first line, DTR was highlighted in a percentage of 21% (95% CI 17.2-25.4). Monitoring of antimicrobial resistance and inclusion in resistance phenotypes, it was observed that 61.25% (95% CI 56.3-66.0) of the *Proteeae* were of the MDR type, of which 29.25% (95% CI 24 , 9-34.0) eXtensive Drug Resistance (XDR) type, and 2.75% (95% CI 1.3-4.7) were Pan Drug Resistance (PDR). Of the XDR strains, 71.79% were XDR-DTR and 28.21% XDR were susceptible to at least one class of first-line antibiotics. The *Proteeae* DTR strains were represented in a percentage of 84.21%, noting the species *P. stuartii*, *P. mirabilis*, *M. morganii* and *P. rettgeri*

*P. mirabilis* strains were predominantly of MDR type (51.02%), 14.28% of the strains were of XDR type and 1.63% of PDR type. 25.71% of the strains were susceptible to all the antimicrobials tested (WT) and 9.79% were DTR. Of the strains of *P. stuartii* (N=94), 92.55% were of the MDR type, 74.46% XDR type and 5.31% PDR type. 3.19% of strains were susceptible to all antimicrobials tested (WT) and 58.51% were found to be DTR type.

Two logistic regression models were developed to identify the risk and protection factors that influenced the acquisition of the strains included in the DTR phenotype. The excess mortality in the DTR sample versus the non-DTR subsample was 16,37%.

**3.3.3.** Between July 2017 and April 2019, 339 patients from the ICU and surgery wards were identified, from which 245 unduplicated strains of *P. mirabilis* were isolated, respectively 94 strains of *P. stuartii*. Of the total number of diabetic patients admitted to the ICU and surgery wards, 35.77% had infections with the two species mentioned above, while infections with these strains in non-diabetic patients were only 4.04%.

The peak of the number of infections coincides for both diabetic and non-diabetic patients, being recorded in July 2018, there is a correlation related to environmental conditions, such as high temperature.

The Spearman correlation coefficient between the monthly distribution of infections with strains of interest among diabetic and non-diabetic patients was 0.519, statistically significant ( $p=0.011$ ).

One third of the diabetic group, especially the patients admitted to the surgery wards, were infected with *Proteeae*, the majority being sensitive strains of *P. mirabilis*, as opposed to the non-diabetic group, predominant from ICU, usually with HCAI produced by MDR *P. stuartii* strains.

Among diabetic patients, those infected with *P. stuartii* had a significantly longer duration of central venous and urinary catheterization, mechanical ventilation and a higher proportion of bacterial resistance, also explained by the fact that 72.22% of infections were HCAI. 66.67% of *P. stuartii* infections were bloodstream infections, while 64.00% of *P. mirabilis* infections were wound infections, 2/3 of which being community-acquired

Factors influencing the probability of dying during hospitalization, identified by Cox regression, were: age, vasopressor therapy, species, duration of antibiotic therapy, DTR strains, naso-gastric nutrition, ESBL strains, duration of urinary catheterization.

### 3.4. CONCLUSIONS

**3.4.1.** The increase in prevalence was observed especially in *P. stuartii* and *P. mirabilis*. The most common carbapenemase identified was NDM-1, which provides bacteria with a high level of resistance. The spread of these NDM-1 carbapenemase-producing isolates is drawing attention to growing problems, with rapid spread to hospital units, raising questions about the epidemiological significance.

These strains have shown various other mechanisms of resistance, often being extremely difficult to treat and have led to an excess of fatality of 27,16%. Mechanical ventilation lasting more than 96 hours, tracheostomy and prolonged antibiotic therapy were the three independent risk factors identified.

**3.4.2.** The correlation of the presence of DTR strains with treatment difficulties was indirectly concluded in the literature in research on *P. stuartii* strains of MDR type. *P. stuartii* has an increased risk of healthcare associated infections being a risk factor for contracting an infection with the DTR strain, and the combination of invasive procedures such as bladder catheterization and mechanical ventilation significantly decreases patients' chances of survival.

From the point of view of the DTR phenotype, in the case of *Proteeae* species it can be appreciated that the members of this group become XDR / PDR type pathogens, with strong implications related to the treatment options and prognosis of patients, hospitalized mainly in high risk wards.

**3.4.3.** The study presents preliminary data on the importance of infectious pathology produced by *Proteeae* species with a focus on *P. stuartii* and the risk factors involved in diabetic patients, data that need to be confirmed by a more significant population group and require new research directions.

Although diabetic patients had fewer infectious risk factors and antimicrobial resistance was significantly lower, survival did not differ from that of patients without diabetes, which draws attention to the implications of diabetes as comorbidity. Identification of *P. stuartii* strains of the DTR / ESBL phenotype determined an increase of at least 2 times the probability of dying, and the presence of nasogastric nutrition tripled it.

The thesis contains 25 figures (all, original), 19 tables and 217 references. During the elaboration of the doctoral thesis, 5 ISI articles were published (two as main author and one as corresponding author) and papers were communicated 8 in the form of oral presentation or poster at national and international conferences.