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ABSTRACT OF THE DOCTORAL THESIS

**ASPECTS REGARDING THE EVOLUTION OF CARBAPENEMASE-PRODUCING ENTEROBACTERALES IN
ROMANIA**

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Antibiotic resistance among members of *Enterobacteriales*, especially the plasmid-mediated resistance, is subject to continuous evolution. At present, infections caused by carbapenem-resistant enterobacteria are associated with most therapeutic failures. Carbapenemase-producing *Enterobacteriales* (CPE) were first described in the early 2000s and interest in their study is growing due to the risk they pose globally. In Romania, the presence of CPE was first documented between 2010 and 2012, the strains being positive for *bla*_{NDM} or *bla*_{OXA-48-like} genes. Subsequently, CPE strains with *bla*_{KPC} and *bla*_{VIM} genes were identified in different regions of the country, the carbapenemases detected most frequently were OXA-48, followed by NDM-1 and KPC. In our country, the production of the OXA-48 enzyme is associated almost exclusively with the species *Klebsiella pneumoniae*, in contrast, the *bla*_{NDM-1} gene is found on different species of *Enterobacteriales*. Similarly to the *bla*_{OXA-48-like} gene, *bla*_{NDM-1} usually presents plasmidic localization but the interspecies transferability of this gene appears to be much higher. In recent years, several Romanian hospitals have begun to report an increased incidence of extraordinary infections caused by *Providencia stuartii* strains, with an alarming rate of carbapenem resistance. The increase in the prevalence of infections due to bacteria with intrinsic resistance to colistin, such as *Providencia spp.*, is the consequence of the excessive use of this antibiotic, which in turn is driven by the spread of resistance to carbapenems.

The research studies were based on two hypotheses:

1. The dissemination of carbapenemase genes is due to a small number of successful plasmids
2. In parallel with the increasing prevalence of carbapenemase genes, due to the selective pressure exerted on the hospital flora by excessive use of colistin, the prevalence of *Enterobacteriales* with intrinsic resistance to colistin (*Proteus spp.*, *Serratia spp.*, *Providencia spp.*, *Morganella spp.*) increases as well and shows a high rate of acquisition of these genes.

The main purpose of this research was the molecular characterization of CPE strains: confirmation of the presence of carbapenemase-encoding genes, study of the plasmid content of CPE strains, and an overview of circulating carbapenemases in our region, focusing on NDM-1-producing strains.

In the first study we analyzed the CPE strains isolated in Tîrgu-Mureș County Emergency Clinical Hospital between November 2012 and October 2013, a period of major importance, which followed immediately after the first signaling of these strains in the country. The aim of the study was to check whether during this period the prevalence of NDM-1 and OXA-48-producing enterobacteria has already undergone significant changes or not, respectively whether other types of carbapenemases have emerged. A second objective was the comparative assessment of carbapenem resistance levels depending on the produced carbapenemases. At the same time we wanted to identify the plasmids harboured by these strains. The central question this study sought to answer: how important the role of plasmids was in the interspecies dissemination of the *bla*_{NDM-1} gene and what was the epidemiological impact of the spread of NDM-1-positive CPE strains.



The main objective of the second study was the analysis of the genetic background and the establishment of the phylogeneticity of *P. stuartii* strains isolated from patients hospitalized in different hospitals in the country. In the context of the spread of carbapenemase genes among enterobacteria, this study was based on the hypothesis that the carbapenem resistance of *P. stuartii* strains was due to the production of carbapenemases and not due to other resistance mechanisms.

The goal of the third study was to assess the prevalence of intrinsically colistin-resistant *Enterobacterales* in 2017-2019 and to verify whether this particular subgroup of pathogens and opportunistic pathogens acquired plasmids and carbapenemase genes from other CPE strains. Based on the results of the study performed on *P. stuartii* strains and data from the literature, we hypothesized that the prevalence of these species showed an increasing trend during the study period and at the same time carbapenemase-producing strains emerged due to the acquisition by conjugation of various broad-host plasmids already identified in the circulating CPE strains.

Of the two hypotheses underlying this research, one was refuted and the other was only partially confirmed: the spread of CPE in Romania was not due to the dissemination of successful plasmids, and in the case of enterobacteria with intrinsic resistance to colistin the increase in prevalence was observed only in the case of two species, *P. stuartii* and *S. marcescens*. The results of this research are not enough to explain the events that led to the spread of CPE in Romania.

P. stuartii represents a high nosocomial risk, and not only as a urinary pathogen, the incidence of extraordinary infections are increasing. The spread of NDM-1 carbapenemase-producing *P. stuartii* was due to the clonal dissemination of a successful strain and not due to the repeated acquisition of carbapenemase-encoding genes by susceptible strains.

In *Enterobacterales*, the bla_{NDM-1} gene is harbored by a wide range of plasmids, generally broad-host (IncA / C, IncL, IncM) or intermediate-host (IncH) plasmids, but different bacterial species exhibit an increased affinity for certain types of plasmids. Our results suggest that the spread of the bla_{NDM-1} gene among *Enterobacterales* is not due to the interspecies dissemination of specific successful plasmids.

The spread of CPE in Romania cannot be explained only through the possible conjugative transfers of certain plasmids between the different *Enterobacterales* species.