

## Review Report on Ph.D. Thesis

**Student:** Marc Finianos, M.Sc.

**Thesis Title:** Microbial Genomics for Population Studies of Multidrug-Resistant Gram-Negative Bacteria

**Supervisor:** Asst. Prof. Ibrahim Bitar, Ph.D.

**Workplace:** Faculty of Medicine in Pilsen, Charles University

**Reviewer:** doc. Mgr. Martina Lengerová, Ph.D.

**Workplace:** University Hospital Brno

For his dissertation, Mr. Finianos chose to study a population of multidrug-resistant gram-negative bacteria, primarily from the order Enterobacterales. The main focus of the work is on strains that produce carbapenemases, which currently pose the most serious epidemic threats. Given this context, the topic is highly relevant. The dissertation is structured as a compilation of four articles published at the supervisor's institution, with Mr. Finianos's contributions, primarily involving bioinformatics analyses, acknowledged at the end of each article. The aim of the work was to elucidate the evolution and spread of carbapenem resistance in Enterobacterales within the hospital environment.

A unique collection of bacterial strains obtained from laboratories across the Czech Republic and Lebanon was utilized to achieve the results. Standard microbiological techniques were employed for initial analyses, followed by advanced methods, including next-generation sequencing. Short-read technology provided comprehensive genetic information, while nanopore sequencing allowed for precise reconstruction of bacterial chromosome and plasmid structures. Selected plasmids were experimentally tested for their conjugation abilities. These analyses were complemented by extensive bioinformatic evaluations. The chosen methods are state-of-the-art and entirely suitable for the research topic.

The work contributes new insights, particularly regarding the structure of mobile genetic elements that carry antibiotic resistance genes. It clarifies the spread of these elements within the Czech Republic, utilizing isolates from various microbiological laboratories, and compares findings with sequences from international publications. A key discovery is that the spread of resistance occurs through the sharing of plasmids among different Enterobacterales species, indicating that monitoring should not focus solely on specific phenotypes within individual species during epidemiological investigations. Detailed plasmid structure analysis could assist in identifying sources of these strains, particularly in hospital settings.

Although the research demonstrates a high level of professional quality, I must regrettably highlight several formal shortcomings in the dissertation. These include missing supplementary materials, such as

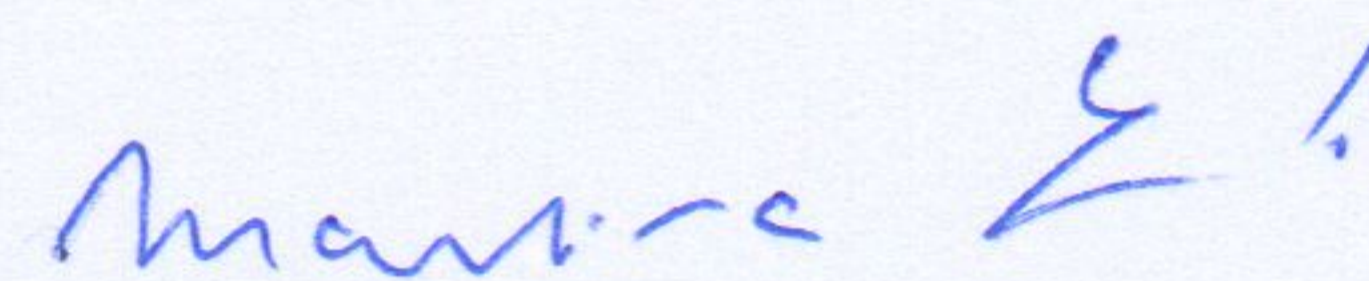
additional images and tables for the embedded publications, incorrect chapter numbering, the absence of page 42, figure legends not clearly distinguished from the text, multiple inconsistent reference lists, inconsistencies in the naming of genera and species, and numerous typographical errors. Additionally, language proofreading would significantly improve the overall quality of the work.

The dissertation successfully fulfilled its stated goals and, by presenting four publications in a journal with an impact factor, exceeds the requirements for completing a Ph.D. program. **Therefore, despite the formal shortcomings, I recommend it for defense.**

#### Dissertation Questions:

1. While the theses focuses on the emergence and spread of carbapenemase-producing strains in the Czech Republic, this is a global issue. Is information about the tracking of such strains collected and shared at the international level?
2. Considering the importance of routine molecular biological analyses for suspected epidemiologically significant strains, how do you envision implementing this in practice? Can we draw on international experiences for guidance?
3. The epidemiological significance of bacterial strains is determined not only by the presence of resistance genes but also by specific virulence factors. Did the studied strains and clones also contain notable virulence factors that may contribute to their epidemiological success?

In Brno, 17<sup>TH</sup> October 2024



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Martina Lengerová

