Thesis review Lenka Křížová
Genetic Basis of Multidrug Resistance in Acinetobacter baumannii
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Reviewer:
Dr Paul G. Higgins
University of Cologne
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Section 1.
This is a very well written thesis that has investigated various aspects regarding the biology of *Acinetobacter baumannii*. The thesis comprises six papers that follow the theme of antimicrobial resistance mechanisms and molecular epidemiology of *A. baumannii*. These papers have been published in internationally renowned peer-reviewed journals and have been well-cited. The candidate has produced original work that has been performed and written to a high degree. In addition, I have had the pleasure to have heard some of these results in oral presentations at different congress and symposia. The aims of the thesis are clear and have been met. **Based on this, I hereby state that I consider this thesis suitable for an award of Ph.D.**

Specific comments:

The introduction is fairly comprehensive and covers the main topics surrounding Acinetobacter, including taxonomy, epidemiology and antimicrobial resistance mechanisms.

The first three papers have identified different mechanisms of antibiotic resistance, particularly carbapenem-resistance, and the molecular epidemiology of multidrug resistant *A. baumannii* isolated from the Czech Republic. It was found that the majority of isolates were European clone II and these were the only ones that were carbapenem-resistant. Prior to this, European clone I was considered the dominant clone (both in the Czech Republic and throughout Europe) and this paper represents one of the first reports of the displacement of clone I. In addition this study investigated other antimicrobial resistance mechanisms and remains one of the most complete characterisations of MDR *A. baumannii* to be published. Two further papers characterise in detail a carbapenem resistant strain mediated by at least three different mechanisms: NDM-1, OXA-23 and OXA-51.

One of the most interesting pieces of work to come from this thesis is the mechanism of sulbactam resistance. It has long been known that sulbactam has activity against Acinetobacters which is unusual because this compound is a beta-lactamase inhibitor. Because of this activity, sulbactam can be prescribed, often in combination with ampicillin, to treat Acinetobacter infections. However, resistance to sulbactam has been described but the molecular mechanism was not known. In the 4th publication presented in this thesis, this mechanism was investigated. Using several methods, for example gene copy number, expression of TEM-1 and transfer of resistance, it was shown that sulbactam resistance is mediated through TEM-1.

The final two studies have investigated resistance islands. These are large genetic structures that have integrated in the chromosome, and encode multiple antibiotic resistance genes. Study 5 can be considered one of the more important papers from this thesis. Effectively it is a historical study with an MDR isolate that dates to 1977 and was found to be European clone I (currently the

oldest described). By using this archived strain the authors were able for the first time to demonstrate that these genomic islands are not a recent phenomenon. Because they encode multiple resistance genes, it was speculated (not unreasonably) that their possession has a selective advantage, and this may account for the successful clonal expansion of European clone I during the 1970-1990's. The second paper on this theme was a very ambitious piece of work that investigated resistance islands from 26 MDR European clone I strains. Perhaps the main finding was that there seems to be a reduction in the size of these resistance islands over time. This probably reflects that many of the antibiotics that were available in the 1970's and 1980's are no longer in use.

These 6 papers are tied together at the end in the discussion, and as outlined above, have contributed greatly to our understanding of this organism. Throughout this work, the candidate has shown her scientific merit and originality, experimental design, choice of and mastering of the methods.

Section 2:
This body of work has previously been subject to peer review, published and is well-cited. There is therefore little to be critical about, and any criticism from me reflects my own subject matter prejudices. However, the introduction only discusses one MLST scheme when there is in fact a second MLST scheme. The introduction also states that in the 1970's Acinetobacter was recognised as a significant pathogen; I have been under the impression that the organism was relatively unknown at this time. One further point that could have been more prominent in the discussion is the role of insertion elements.

Section 3.
Some general questions for the candidate:

1. What do you consider are the most important methods to identify species of Acinetobacter?
2. What in your opinion is the natural habitat/reservoir of *A. baumannii*?
3. What are the important virulence factors of *A. baumannii*?
4. Could you give a working definition of multidrug resistance (MDR)? Throughout this thesis MDR is mentioned, but at no time do I recall seeing it defined.
5. How would you treat an MDR Acinetobacter?
6. Given that many antibiotic resistance genes are acquired and do not appear to be linked to a particular epidemiological lineage, what makes EUII strains so successful and widespread?
7. European clone or International clone? Should the nomenclature change?
8. Colistin resistance, how much of a problem do you think it will be?