Author’s response to reviews

Title: Prevalence of carbapenem-resistant Acinetobacter baumannii from 2005 to 2016 in Switzerland

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Prevalence of carbapenem-resistant Acinetobacter baumannii from 2005 to 2016 in Switzerland
Alban Ramette; Andreas Kronenberg BMC Infectious Diseases

Dear Prof. Higgins,

We would like to take this opportunity to thank you and the reviewers for the helpful comments. Originally, we aimed at submitting a shorter account of the study that was performed, but we are happy to be able now to provide more detail about the analyses and about the results in this revised version of the manuscript. We have addressed all points raised, modified the text accordingly and provided new supporting information to further prove the robustness of our analyses and interpretations. The points are detailed in the following sections. We also attached a word document containing the same text pasted below but with the figures and tables accompanying the text.

Jane Turton, Ph.D (Reviewer 1)
This manuscript describes Acinetobacter isolates from 20 participating laboratories across Switzerland during the period 2005 to 2016, concentrating particularly on those that were resistant or had intermediate susceptibility to carbapenems. Data was extracted from the Swiss Antibiotic Resistance Centre (anresis) database. It has the happy message that numbers of carbapenem resistant/intermediate isolates remained consistent over this period. While overall the paper has an interesting and positive message, there are a number of points that need clarification:

1. While I understand that these data were from the anresis database, and were generated from multiple laboratories that may have used a variety of methods, it would be helpful to provide some kind of indication of the methods used for identification and for susceptibility determination and interpretation.

Authors’ answers: Thank you for the recommendation. We now added more text to the Methods section (pages 4 and 5) to better clarify the methodology and the susceptibility determination and interpretation:

"These laboratories send all results from routine testing of clinical bacteriology cultures to the anresis database on a regular basis (weekly or monthly). In contrast to other surveillance systems, all antimicrobial resistance results are sent to the anresis database, not restricting the data either to invasive isolates or to specific microorganisms. To allow for higher comparability with international reports, we therefore used the same approach as in the antibiotic surveillance systems of the ECDC (EARS) and of the WHO-Europe (CASEAR), which restrict their analyses to invasive isolates from blood cultures or cerebrospinal fluid. Duplicate entries were removed and only the first date of occurrence of Acinetobacter spp. isolation in case of re-infected patients was kept for a given year. Isolates from foreign countries were excluded.

We used the interpreted, qualitative data (SIR) as delivered from the participating laboratories, as most microbiological laboratories send only qualitative, interpreted resistance data (SIR). SIR data are not validated by anresis.ch but by the local laboratories sending the data. All laboratories participating in anresis.ch are approved and participate in at least one external quality control program. Isolates were classified as susceptible, non-susceptible or intermediate to at least one of imipenem or meropenem following clinical breakpoints published in the European Committee on
Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org) or, if not available, according to the Clinical and Laboratory Standards Institute document M31-A3 (CLSI).

In addition to SIR data, local laboratories provide accompanying epidemiological information, such as sample location, provider of the sample, patient sex and age, but no clinical data about diagnosis, therapy or outcome."

Could the different methods used explain the greater number of resistant isolates observed in the North East region compared with the others?

=>Authors’ answers: Even if we cannot exclude this hypothesis, it is not very likely in this case: as mentioned above, all laboratories participating in anresis.ch are accredited and participate in at least one external quality control program. SIR classification of isolates follows clinical breakpoints published in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org) or, if not available, in the Clinical and Laboratory Standards Institute document M31-A3 (CLSI).

2. The account focuses on carbapenem resistant isolates without giving an overall impression of total numbers of isolates of Acinetobacter species; it is only from Table 1 that one gets an idea of this.

=>Authors’ answers: The numbers of Acinetobacter isolates were provided in the Abstract (Results section), and in the first paragraph of the Results section. We presented the detailed data in Table 1 including SIR data and taxonomic information for the corresponding isolates over time.

I found Figure 1 confusing- is the frequency of isolation shown in C that of resistant isolates only?
Authors’ answers: Yes, these cases correspond to resistant isolates only. This is now specified in the legend of the figure, as follows: "C) Frequency of resistant Acinetobacter isolates on a daily basis across regions from 2005 to 2016."

Is there an indication from Table 1 that there is a small trend towards greater numbers of Acinetobacter isolations overall, even if the carbapenem resistant numbers are not growing?

Authors’ answers: Thank you for pointing this out. Indeed, we found increasingly more Acinetobacter isolations over time. This is now added in the Results section as follows:

"There was a significant increase in the total number of Acinetobacter isolations over time (linear regression, F = 21.559, P< 0.001, R2= 0.65) with 32.1 isolates per year on average (t = 6.39, P< 0.001) increasing at a yearly rate of 3.2 new isolates (t = 4.64, P< 0.001)."

Although the numbers were small, there was evidence of an increase in the numbers of A. ursingii seen over the period - these are generally susceptible to carbapenems but are associated with invasive disease and perhaps it is important to note that - or perhaps the modest increase in numbers of this species is simply because more hospitals are now identifying this species. If you had included isolates from other sources (e.g. wound swabs, sputum, or screening swabs) do you think the results might have been different?

Authors’ answers: We tested those hypotheses by extracting new data for this species in the anresis database. As expected the total number (R+S) of A. ursingii increased over time, and was significantly associated with whether the isolate was invasive or colonizing (Table below), and the specific laboratory involved:

Poisson regression (number of A. ursingii as a function of year and laboratory id)
<table>
<thead>
<tr>
<th></th>
<th>Coeff (exp)</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEAR</td>
<td>1.175</td>
<td>1.53e-08 ***</td>
</tr>
<tr>
<td>Invasive (y)</td>
<td>0.316</td>
<td>&lt; 2e-16 ***</td>
</tr>
<tr>
<td>LAB_ID103</td>
<td>9.041</td>
<td>0.0296 *</td>
</tr>
<tr>
<td>LAB_ID107</td>
<td>4.560</td>
<td>0.1603</td>
</tr>
<tr>
<td>LAB_ID111</td>
<td>4.831</td>
<td>0.1240</td>
</tr>
<tr>
<td>LAB_ID115</td>
<td>2.161</td>
<td>0.4908</td>
</tr>
<tr>
<td>LAB_ID119</td>
<td>1.903</td>
<td>0.5776</td>
</tr>
<tr>
<td>LAB_ID143</td>
<td>10.029</td>
<td>0.0232 *</td>
</tr>
<tr>
<td>LAB_ID144</td>
<td>4.802</td>
<td>0.1244</td>
</tr>
<tr>
<td>LAB_ID145</td>
<td>2.022</td>
<td>0.5105</td>
</tr>
<tr>
<td>LAB_ID167</td>
<td>6.728</td>
<td>0.0627</td>
</tr>
<tr>
<td>LAB_ID168</td>
<td>5.934</td>
<td>0.0803</td>
</tr>
<tr>
<td>LAB_ID172</td>
<td>4.325</td>
<td>0.1524</td>
</tr>
<tr>
<td>LAB_ID175</td>
<td>8.153</td>
<td>0.0385 *</td>
</tr>
<tr>
<td>LAB_ID176</td>
<td>6.825</td>
<td>0.0592</td>
</tr>
<tr>
<td>LAB_ID178</td>
<td>9.941</td>
<td>0.0232 *</td>
</tr>
<tr>
<td>LAB_ID180</td>
<td>1.627</td>
<td>0.6910</td>
</tr>
<tr>
<td>LAB_ID183</td>
<td>9.422</td>
<td>0.0271 *</td>
</tr>
<tr>
<td>LAB_ID184</td>
<td>3.866</td>
<td>0.1956</td>
</tr>
<tr>
<td>LAB_ID258</td>
<td>4.000</td>
<td>0.2150</td>
</tr>
<tr>
<td>LAB_ID83</td>
<td>7.586</td>
<td>0.0457 *</td>
</tr>
</tbody>
</table>

For the comparison in the Poisson regression, LAB_ID297 was chosen as the reference group. Underlined are the laboratories that consistently sent data in the last 7 years.

Therefore, we cannot exclude that over the last 7 years, more laboratories can identify the species, but it is also obvious that there was also a trend towards more isolation over time in the laboratories that consistently sent data.

Yet, although the total number of A. ursingii isolation increased over the last 7 years, there was only a minor increase overall in those that were carbapenem resistant (Poisson regression of
numbers of resistant A. ursingii over time was not significant [residual deviance=0.314, df =2, P= 0.854]):

(see figure in the provided file)

Looking only at invasive isolates, resistant ones were only found from 2014 onwards with only 1 isolate per year. The number of non-invasive isolates increased sharply in the last 10 years.

(see figure in the provided file)

Looking only at colonizing isolates, we observed the same trend, whereby resistant A. ursingii were detected as early as 2011 with one isolate, and the subsequent year the number increased only slightly (below 5-6 isolates yearly).

As a conclusion, including A. ursingii isolates from other sources (i.e. non-invasive) would not have changed the results that were reported, which were consistent with relatively few isolations of resistant A. ursingii in Switzerland in the last few years.

We did not report those results in our study, as indicated above, because our study aimed at being comparable with international reports (EARS, CASEAR), which restrict their analyses to invasive isolates from blood cultures or cerebrospinal fluid.

Other comments:

Use of spp. - in many cases you could simply say species. What do you mean by A. baumannii spp.? Are you referring to Acb species? If so, please say so.
=>Authors’ answers: Thank you for raising this point. We used the abbreviation "sp." for the singular and "spp." (standing for "species pluralis", the Latin for multiple species) for the plural in place of the specific name or epithet "species". Generally, authors may also use "spp." as a short way of saying that something applies to many species within a genus, but not to all. We now removed the spp. term throughput and indicate instead "isolates" or "species" accordingly.

We also added additional analysis and sensitivity analysis for both all Acinetobacter isolates and specifically for the ACB isolates. We hope those additions will help clarify our analyses.

Background first sentence - while I agree that these species are the most significant others, including A. ursingii, are important and to infer that only a few Acinetobacter species are clinically significant is misleading. After all, all your isolates were from blood or CSF so you do need to entertain the idea that all the species found can cause serious infections. A. lwoffii is sometimes a contaminant of blood cultures (having been introduced from the skin), but that is not the case for the others.

=>Authors’ answers: We agree with this comment. The sentence now reads:

"In the Gram-negative, strictly aerobic Acinetobacter genus, the species that mostly present a risk as opportunistic human pathogens are A. baumannii, A. nosocomialis, A. pittii and A. calcoaceticus, which belong to the so-called Acinetobacter calcoaceticus-Acinetobacter baumannii (ACB) complex "

Later in the paragraph we also added:

"Yet, uncommon and opportunistic non-baumanii species such as A. ursingii may also cause bloodstream infections (3, 4)"

In the Discussion perhaps you could be clearer on what you mean by national incidence level

=>Authors’ answers: We agree that the term “incidence” was misleading, as we do not know the incidence of the diseases. We replaced the term with “resistance rate”.

Last sentence of Discussion - I'm not sure I understand what you mean when you say you have 'confirmed the implication' of carbapenem resistant Acb strains - what is that implication?

=>Authors’ answers: Thank you. We have clarified the sentence as follows:

"We confirmed the implication of carbapenem-resistant ACB complex strains in the vast majority of clinical infections and nosocomial outbreaks that involved Acinetobacter isolates, as observed previously in Germany over a 5-year period (13)."

Legend to Figure 1 - please make it clearer exactly what is being presented for A, C and D.

=>Authors’ answers: The legend was clarified as follows:

"A) Acinetobacter resistance rates (number of resistant isolates compared to total number of isolates) per region from 2005 to 2016 in Switzerland. Standard deviation bars represent annual fluctuations per region. B) Map of the Swiss regions defined in this study. C) Frequency of isolation of resistant Acinetobacter across Swiss regions from 2005 to 2016 (daily resolution). D) Acinetobacter resistance rates per year and per region in Switzerland."

Spyros Pournaras (Reviewer 2)

This manuscript reports on invasive carbapenem-resistant Acinetobacter spp. isolated from 2005 to 2016 in Switzerland and analyses temporal and regional trends, pointing out that carbapenem-resistant Acinetobacter spp. are much less frequent compared with other European regions and cause limited cases of invasive infections. Although such epidemiological reports are of interest, to my opinion there are some limitations in this manuscript that need to be addressed.

- The actual number of ACB isolates should be 299 and not 244, as can be extracted from the Table 1. So, carbapenem resistance rates for A. baumannii complex isolates (ACB) should be 18.4%, not 22.5%.

=>Authors’ answers: This was corrected in the corresponding sentence page 6. Thank you for identifying this reporting mistake.
- The analysis should include only the 299 A. baumannii complex isolates, which are the most relevant species clinically and not the 333 non-baumannii Acinetobacter spp, which are rarely carbapenem-resistant and only occasionally cause hospital infections. This approach is followed by the ECDC (ref. 6). The other species might be included as hospital pathogens in Switzerland, but not in terms of their carbapenem resistance.

=>Authors’ answers: We now have thoroughly revised the Results section by analyzing the number of resistant isolates and resistance rates at the genus level (ie. Acinetobacter isolates including non-ACB species) and for the ACB species only. We have added a new supplementary Table (Supp. Table 1), where both the main analysis and sensitivity analyses (laboratories that always provided data) are presented for all isolates and for ACB isolates separately.

- In regions with low prevalence of ACB infections, possible outbreak occurrence should be taken into consideration and may affect the overall resistance rates, as typically occurred with the increase in 2010 in the North East region due to outbreak in the same intensive care burn unit.

=>Authors’ answers: We completely agree on this point, and discuss this in detail. Although at least one possible outbreak was identified in our study, there was no overall increase in carbapenem-resistant Acinetobacter isolates in Switzerland as observed in other European countries.

- Some data about the overall antibiotic resistance phenotype of the 299 ACB isolates and particularly of the 55 carbapenem-resistant ones should be presented so that the reader is aware of available treatment alternatives. Was there any colistin resistance (by broth microdilution) issue?

=>Authors’ answers: We added co-resistance data of the carbapenem-resistant Acinetobacter isolates in the first paragraph of the result section.
"Co-resistance to other antibiotics as aminoglycosides (47/55, 86%), trimethoprim-sulfamethoxazole (42/54, 78%) and fluoroquinolones (47/55, 86%) was high, whereas no colistin-resistance was reported for 23 isolates tested."

Colistin resistance was tested on 23 isolates only. As we do not have the isolates, it is not possible to retest all isolates. As resistance data for all invasive Acinetobacter isolates are reported annually in the CAESAR report, we did not provide these data in this manuscript (http://www.euro.who.int/__data/assets/pdf_file/0005/354434/WHO_CAESAR_AnnualReport_2017.pdf?ua=1).

- The carbapenemase-encoding genes of the 55 carbapenem-resistant ACB and also their clonal composition would be of interest. Are there any of these isolates available for testing?

=>Authors’ answers: We agree with the reviewer that this would be interesting. Unfortunately, as indicated in the methodology section, we only had access to resistance data and not to isolates directly. Therefore we cannot perform these analyses.

David Wareham (Reviewer 3)

The authors have produced a very short manuscript on the prevalence of carbapenem-resistant Acinetobacter spp in Switzerland. This uses laboratory reporting data collected as part of a surveillance scheme - anresis - for which no specific details are provided.

Further knowledge surrounding the epidemiology of MDR Acinetobacter infections in Switzerland is of interest but the manuscript requires far more in depth analysis of the data and the implications. The following major points should be addressed:

- There is no adequate description of the criteria to define an invasive isolate - Blood or CSF is suggested - how many from each - how many bloodstream isolates were likely catheter associated or contaminants.
Four out of 58 carbapenem-resistant isolates were isolated from cerebrospinal fluid, the rest from blood cultures. However we are not able to definitively exclude contaminants. More than one positive blood culture were observed in 18/54 cases, but as mentioned in the manuscript, clinical data were not available for this study. However, Acinetobacter of the ACB complex – which represent the vast majority of isolates in our study – are not typical contaminants. Although higher mortality has been described in patients with multiple positive blood cultures for extensively drug-resistant A. baumannii, even a single positive blood culture is usually judged clinically significant and prompts therapy (https://doi.org/ 10.1371/journal.pone.0180967).

There is no accompanying clinical data on whether these isolates were though significant.

- No description of the methods to undertake susceptibility testing, what drugs were tested and how.

- No molecular analysis of any strains were undertaken for either resistance or identification
It is suggested that the higher rate in the North East region may be associated with a nosocomial outbreak but no evidence or analysis is provided to support this.

Authors’ answers: This outbreak was not published. Nevertheless being able to trace all 5 out of 5 carbapenem-resistant Acinetobacter bacteremia of a whole region and a whole year to one single intensive care unit within six weeks is, in our view, a very strong indicator of an outbreak situation. To be clear we added

"…highly suggestive of an outbreak because 5 resistant Acinetobacter isolations, all belonging to ACB, occurred for the whole region within six weeks in the same intensive care unit of burn patients."

to the corresponding sentence.

The discussion is minimal and ultimate conclusion only for more surveillance to be carried out.

Authors’ answers: We have amended the discussion section with some text about strengths and limitations of our study.

Overall the data available has not been sufficiently analysed or presented in a meaningful way for publication without major revision.

Authors’ answers: We hope that, with the addition of new information and of supplementary analyses to our revised manuscript, the study is now more clearly described and worth publishing.