Author’s response to reviews

Title: Update of incidence and antimicrobial susceptibility trends of Escherichia coli and Klebsiella pneumoniae isolates from Chinese intra-abdominal infection patients

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Version: 1 Date: 28 Aug 2017
Author’s response to reviews:

Editor Comments:

Reviewer reports:
Michael Satlin (Reviewer 1): In this study, broth microdilution antimicrobial susceptibility testing was performed for 12 antimicrobial agents on over 5,000 strains isolated from intra-abdominal infections (IAI) from 21 hospitals in 16 cities in China from 2012-2014. They also performed phenotypic testing for ESBL production based on decreases in MICs with the addition of clavulanate to 3rd-generation cephalosporins. The authors found that 37% of IAI isolates screened ESBL positive, but this percentage decreased over the 3-year period, and that this decrease was largely driven by reductions in the proportion of hospital-acquired K. pneumoniae that were ESBL positive. The authors also found high rates of resistance to 3rd-generation cephalosporins and fluoroquinolones for all isolates. The authors further breakdown their results into community-acquired vs. hospital-acquired by specific organisms, and even specific regions in China.

I have the following recommendations to improve the manuscript:

Major comments:

1) I find that the Discussion is too much repetition of Results and should be truncated to focus on inferences based on the Results. I also found that certain information, such as >80% of ESBL-positive E. coli were susceptible to IPM, ETP, AMK, and TZP were presented 4 times! (Abstract, Results, Discussion, and Summary). There is way too much repetition.
Response: The discussion has been revised completely

Minor comments:

1) Abstract: It would be helpful to provide some sense of the overall #s of isolates that were tested, so that this strength of the paper can be quickly realized by the reader.
Response: We changed the manuscript accordingly
2) Abstract: Instead of merely reporting the antimicrobial agents for which the %susceptibilities are >80% or >70%, I think it would be worth reporting the %susceptible for these key agents (IPM, EPT, AMK, TZP) for E. coli and K. pneumoniae. This would provide the reader with more information.
Response: We changed the manuscript accordingly

3) There are multiple instances of run-on sentences (e.g., lines 134-138, lines 269-273) and some sentences which are not complete sentences (e.g., line 140-141, "Since …"). These should be edited.
Response: Will be done by a native English speaking editor

4) Methods: The data on # of strains and how many were Enterobacteriaceae (lines 165-169) should be reserved for Results and can be removed from Methods section.
Response: We changed the manuscript accordingly

5) Methods: The last sentence in the ESBL detection section (lines 196-199) is already stated (lines 172-175), although this reference can be added to lines 172-175.
Response: We changed the manuscript accordingly

7) Results: I recommend providing a breakdown of the most common organisms, other than just E. coli and K. pneumoniae in the first paragraph (e.g., Enterobacter cloacae, Pseudomonas aeruginosa, and Acinetobacter baumannii).
Response: We changed the manuscript accordingly

8) Results: After reporting the organisms, I then recommend clearly stating the %susceptible for all 5160 isolates to the 12 antimicrobial agents. This information is important to clinicians deciding on empirical therapy for IAIs. I also think one of the main conclusions of the study is that 3rd-generation cephalosporins and fluoroquinolones cannot be relied upon for the treatment of IAIs in these Chinese hospitals. This should be commented on in the Discussion.
Response: We added a new table with this information and emphasized lost susceptibilities to the cephalosporins and fluoroquinolones in the abstract and discussion.
9) Results: Lines 215-220: I think it should be more clearly stated that there was a statistically significant decrease in the proportion of K. pneumoniae isolates that were ESBL producers, but that this decline was not statistically significant for E. coli. I also think the last part of this long sentence ("; which …") can be removed.
Response: We changed the manuscript accordingly

10) Results: Lines 221-225: I think it would be also worth mentioning Proteus mirabilis here.
Response: We added the data of Proteus mirabilis

11) Results: Lines 225-226: I think it makes most sense to start the paragraph with this sentence (ESBL % in all IAIs), then start breaking down by pathogen and eventually explain that the decrease overall in IAI isolates that were ESBL-positive was primarily due to decreased ESBL rates among hospital-acquired K. pneumoniae.
Response: We changed the manuscript accordingly

12) Results: Line 247: Should the time period be 2012-2014?
Response: We changed the manuscript accordingly

13) Table 2: Why is there only a P value comparing %susceptibilities of CA K. pneumoniae to HA K. pneumoniae? (yet there are no P values for E. coli). Also, why are P values reported for some agents and not others?
Response: We recalculated P values for all single agents and did not find single significances. The text has been changed accordingly throughout the manuscript

14) Table 2: On quick inspection, the differences in fluoroquinolone susceptibilities between HA Kp and CA Kp do not seem to be statistically significant. Please recheck this.
Response: See response to 13).

15) Results: Lines 262-267. I think these inferences should be taken with caution, as for most antimicrobial agents these differences were not statistically significant.
Response: See response to 13).
16) ESBL-producing E. coli and K. pneumoniae are supposed to be susceptible to cefoxitin. Please comment on why you think such a high proportion of these isolates tested cefoxitin-resistant. This would be unusual unless some of these isolates also had AmpC enzymes or carbapenemases.
Response: We agree, that the likely explanation for ESBL-producing E. coli and K. pneumoniae isolates is that they have acquired AmpC beta-lactamases and porin losses.
We added this information together with a new reference into the discussion section.

17) Discussion: Line 333-334. Combining metronidazole to fluoroquinolones as "combination therapy" would not help the problem that there are high rates of resistance to the fluoroquinolones among E. coli and K. pneumoniae in IAI's in this study. Metronidazole only adds anaerobic coverage. Please revise.
Response: We omitted this sentence in the revised manuscript

18) The limitation of not having any genotypic or molecular data for these strains should be noted in the Discussion.
Response: We added this limitations into the discussion section

Brandon Eilertson (Reviewer 2): Major Comments
1. The applicability of this data would be improved by describing the rates of carbapenem susceptibility in more detail and explaining how the trends in carbapenem resistance might effect treatment of these organisms.
2. The very low susceptibility to ETP and IPM among Klebsiella pneumoniae ESBL + isolates is noteworthy and needs to be described further.
3. FQ susceptibility rates are also very dramatic for ESBL positive organisms. The treatment implications of such low susceptibility rates should be discussed.
Response: We addressed and summarized this concerns in the final part of the discussion section
Minor Comments

1. Lines 100-103 This is a confusing way to describe susceptibilities. Give the exact numbers if possible and perhaps just focus on the carbapenems as they are the first-line therapy for treatment of ESBL producing Enterobacteriaceae.
   Response: We changed the manuscript accordingly

2. Line 106 - Change percentages to prevalence
   Response: We changed the manuscript accordingly

3. Line 137 ESBL producing rather than ESBL screen-positive
   Response: We changed the manuscript accordingly

4. Line 171 - Were these pure cultures? Or were other organisms isolated. Can the authors include data on rates of polymicrobial cultures and other species isolated? If the authors are most concerned about resistance rates in the population then inclusion of samples that may reflect colonization rather than infection would be reasonable, however these were excluded.
   Response: We added “GNB strains were collected from consecutive IAI patients between 2012 and 2014 in 21 centers located in 16 Chinese cities. Only gram-negative aerobic and facultative anaerobic bacteria from abdominal infection sites such as the appendix, peritoneum, colon, bile, pelvis and pancreas were included and the strains needed to be pathogenic bacteria associated with clinical infections while gram-positive and anaerobic bacteria were excluded. The specimens were mainly obtained through surgical procedures, but puncture specimens such as intraperitoneal puncture fluid were also included and different gram-negative bacteria that were combined in one sample were also accepted. Exclusion criteria were isolates from drainage liquid or drainage bottles, as well as isolates from feces or crissum abscess environmental samples (not a patient source) or cultures for infection control purposes” to the Materials and Methods, Collection and identification of isolates section.

5. Lines 199 - Is this definition of HA vs CA still accepted?
   Response: Yes, it is still valid.
Response: The section has been revised completely

7. Lines 222-225 - the authors initially narrow the focus of the paper to Klebsiella pneumoniae and E. coli but now mention Klebsiella oxytoca. The denominator for the percentages quoted here is unclear.
Response: The section has been revised completely

8. Lines 225-226 - "IAI strains" is vague and would suggest these numbers include Pseudomonas and Acinetobacter isolates. If this is incorrect specify which species are included here.
Response: We added the information from Table 2 uppermost line (all IAIs included E. coli., K. pneumonia, P. mirabilis and K. oxytoca)

9. Lines 235-236 - Abscesses must occur in an organ or space and are generally from another organ source. Are these abscesses where the sample is from a potential intraperitoneal space? Or is this a case where the source may be unspecified?
Response: I am sorry, but we do not have that level of detail in the isolate “demographics” provided by the study sites. All the forms include are descriptors like “abscess” or “peritoneal fluid” or “liver”, etc.

10. Lines 248-255 - need to specify the individual rates and trends for AMK, ETP and IPM here. >70 or >80 is very vague should highlight whether that means 71 or 99%.
Response: We added all susceptibilities from Figure 1 into this section

11. Line 257 - susceptibility rates
Response: We changed the manuscript accordingly

12. Lines 257-8 - specify is this for both organisms or just E. coli
Response: We changed the manuscript and mentioned both organisms.

13. Line 264 specify for which drugs, again this wording is unclear.
Response: We changed the complete section and the table

14. Lines 269-271 - this is just a restatement of results - just state that the rates are similar to 2002-2009.
Response: We changed the manuscript accordingly

15. Line 276 - remove the word constant
Response: We removed constant

16. Lines 280-283 - again this is a restatement of results. Why did the decrease happen?
Response: The decrease of ESBL+ GNBs might be explained by new restrictions for the clinical application of antimicrobial agents, which has been introduced by the Chinese ministry of health in 2012

17. Lines 292-293 - was this test performed? This may be true need to cite prevalence of this clone in China to justify
Response: Though we did not include this kind of bacterial analyses, we cited a recent reference, which supports our assumption

18. Line 297, 300 - These references are not numbered, need to be included in the bibliography if not already
Response: We changed the manuscript accordingly

19. Line 300 - cite other studies showing high rates of carbapenem resistance in Eastern China
Response: We added a new reference

20. Line 307 - were these changes statistically significant, if so include p values.
Response: No significance, only a trend

21. Lines 311-313 - again awkward wording, the dramatic change is the drop from 2013 to 2014, say so
Response: We changed the text accordingly

22. Lines 317-381 - may be worth describing the molecular epidemiology of the ESBL epidemic in China here, which enzymes? Do they vary between the hospitals and the community.
Response: Again, as was stated in the new “limitations of our study” section, molecular characterization of these isolates is not yet available, so we cannot comment on the molecular epidemiology as requested. That said, however, it is something we are working to do, and such data will be published as soon as available.
However, we added some information about ESBL genes in China derived from the literature into the discussion section

23. Lines 319-321 - This statement suggests that administration of IV antibiotics in Chinese hospitals was inconsistent in the past but is now improving. Is this correct? The more logical causes would be improved antibiotic restrictions in the inpatient setting paired with improved infection control.
Response: see response to 16)

24. Lines 326-327 - You need to describe your IPM and ETP susceptibility rates in the Results and then put them in perspective here, especially note the decreased susceptibility to ETP and IPM in Eastern China compared to other regions.
Response: We changed the manuscript accordingly

25. Line 333 - The decreased susceptibility to fluoroquinolones is notable here. Likely related to outpatient over prescribing. Combination therapy of an E.coli of KP with FQ and metronidazole will still not kill a FQ resistant organism, another class of antibiotic is needed
Response: We omitted the former statement

26. Table 2 - Are these values for all years pooled?
Response: Yes, they are pooled.
If improvements to the English language within your manuscript have been requested, you should have your manuscript reviewed by someone who is fluent in English. If you would like professional help in revising this manuscript, you can use any reputable English language editing service. We can recommend our affiliates Nature Research Editing Service (http://bit.ly/NRES_BS) and American Journal Experts (http://bit.ly/AJE_BS) for help with English usage. Please note that use of an editing service is neither a requirement nor a guarantee of publication. Free assistance is available from our English language tutorial (https://www.springer.com/gb/authors-editors/authorandreviewertutorials/writinginenglish) and our Writing resources (http://www.biomedcentral.com/getpublished/writing-resources). These cover common mistakes that occur when writing in English.

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