Author's response to reviews

Title: Multidrug-resistant Gram-negative Bacteria Colonization of Healthy US Military Personnel in the US and Afghanistan

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Author's response to reviews: see over
Attention:  BMC ID Editor/Reviewers

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Original Manuscript Title: Multidrug-resistant Gram-negative Bacteria and Staphylococcus aureus Colonization of Healthy US Military Personnel in the US and Afghanistan

Modified Manuscript Title: Multidrug-resistant Gram-negative Bacteria Colonization of Healthy US Military Personnel in the US and Afghanistan

Corresponding Author: Clinton K. Murray

We thank the editor for an opportunity to revise our manuscript. Based on the consensus of the two reviewers, along with subsequent email discussion from the editor regarding recommendations for a return to our original submission of separate Gram-negative and Staphylococcus aureus papers, we have removed text, results, discussion, and references that involve Staphylococcus aureus and instead focused on the Gram-negative bacteria (GNB) and multi-drug resistant GNB findings for this manuscript. The manuscript title was also changed accordingly. In response to the detailed, comprehensive reviewer comments, we provide the following responses to specific revision suggestions. As a result of these changes, we believe the revised manuscript is improved and now suitable for publication in BMC Infectious Diseases.

REVIEWER 1: Kyle Petersen

Reviewer's report:
Major Compulsory Revisions:
1. The paper is too long and tries to be all encompassing. I think the data on E coli resistance are compelling and present an important discovery in the field, the MRSA and MSSA data are limited in scope, do not add to the discussion, and are a completely different pathogen with different transmission dynamics so it is difficult to compare the 2. For this reason the authors should strip all the references results and discussion and mention of Staphylococcus out of the paper. This can be submitted as a separate paper at a different time should they choose to do so. The focus of the article should be on E coli and ESBL findings of colonization in troops at home and in the combat theater. This could allow for a little more commentary in the discussion about GNR MDR organisms and their epidemiology, potential problems with peri-operative antibiotic selection, findings of previous investigators of MDRO infections in combat wounded etc. This suggests to me that some MDR is acquired in the hospital, but a fair amount is present and selected for with broad spectrum antibiotics when the patient is critically ill.

The paper was significantly restructured as a GNR/MDR-GNR-only paper. As recommended by the reviewer, all Staphylococcus aureus results, discussion, and references were removed.
2. There are no female participants. This is a major limitation of the study and needs to be commented more in the discussion, does this potentially bias the findings? It certainly limits the applicability to most other normal populations with drug resistance. There is no comment on race variation in the population, was this data collected? Both sex and race could be important modifiers as to host immunity and predisposition to colonization I find it is curious this was not collected.

The US-based population had 31% females, whereas the Afghanistan population was 100% male. A comment about study limitations based on this relatively low female population was inserted in Discussion section. Demographics such as race were not available on all study participants; a comment about this as a potential study limitation was also inserted.

Minor essential Revisions:

3. Page 5 Sentence starting with While MDR-GNB...[6]. is confusing and poorly written, I think you are trying to say drug resistance has been a problem since the 1940s, which is not in the 21st century, or staph has been a problem in casualties? the whole thought should be re-written. If its about staph just drop it anyhow as I think this paper should be about e coli.

Sentence was rewritten to avoid confusion.

5. Page 14 Para that starts with There were 49 US... Combine sentences and Re-write to say there were significant decreases in susceptibility to amp, amp/s, cipro and tmp/smx then give the percentages and p values, this would condense the length of these sentences substantially.

Sentence on antimicrobial susceptibility was condensed based on this suggestion.

6. Page 18 discussion. Comment on significnace of 5 fold increase (sig, highly signific etc.) it strengthens your argument.

Elaborated on significant difference in colonization prevalence between US and Afghanistan populations.

7. Page 20 last sentence of para Additiional studies...Afghanistan. this is speculative. It is doubtful that Afghans have the financial means to give TET to their livestock and no one ate off base by your accounts. I guess you could try to find vendor source and go back and look at use of antimicrobials but this would be extremely hard to do. Might replace with a general statement about global increase in antibiotics in food esp. meats.

Modified sentence in favor of generalized comment.
8. Page 23 Do you have data to suggest MDR is seasonal? A ref here would be helpful. There are reports of Amp/Subb resistance in surgical patients undergoing bowel prophylaxis in civilian trauma surgery. you might comment on this here. Schnuriger et al 2010 add discussion about lack of female subjects here somewhere.

We do not have data to suggest seasonality of MDR, based on the relatively short study period (conducted during summer months). Comments on seasonality, with references to peak incidence rates of GNR infections during summer months, were added. Comment and reference to previously-reported amp-sulbactam resistance in surgical patients was inserted.

9. There is lots of discussion about PFT in the results but little or none about clonality in the discussion, might be good to say something here like there was/was not clonality. This was/was not different than previous studies with gram negative pathogens etc. It looks like a polyclonal phenomenon with no point source to me.

Comment in result section indicates polyclonal nature of the isolates, with only 2 isolates from separate study participants showing clonality. Sentence on polyclonality added to the discussion paper.


These Tables were removed, along with all Staphylococcus aureus data and discussion points.

Discretionary Revisions
11. Methods page 9. Why did you not test for other Beta-lactamases like Amp-C, KPC etc. if CTX TEM and SHV were present but not phenotypically active, these might have been as well, and NDM originated in this part of the world. It would have made a better paper.

Agree that additional Beta-lactamase geno/pheno-type data would improve on the comprehensiveness of the paper. Given the E. coli resistance profiles, it may be unlikely that other genes would be present. We are collaborating presently to look at an exhaustive array of genes and proteins to further characterize our study isolates. Unfortunately, these data are not currently available.

12. Table 2 the last 2 data sets (sites colonized total gram neg isolates) might be better pulled out as a separate bar graph or pie chart as it would be easier to read. Table 2 data were maintained in original format as we believe it reflect the data appropriately with the least amount of space required.


**REVIEWER 2: Richard Murphy**  
**Reviewer’s report:**  
Thanks for the opportunity to review this very interesting paper that improves our understanding of colonization with MDR pathogens in Afghanistan. It is an important new paper.

1. Abstract  
There is an error; it should state that 100 personnel in Afghanistan were enrolled.

**Error was corrected to reflect this, with change from the originally reported “10” personnel to the correct number of “100” personnel.**

2. Introduction  
I think most of the first paragraph could be removed without diminishing the article. I would avoid discussion of pathogens not identified in this manuscript such as the New Delhi strain.

**Introduction paragraph was modified with reviewer’s comments in mind.**

3. Methods  
I am not an authority on microbiology so another reviewer should comment on the microbiology employed.

**No response/specific changes; other modifications made in response to 2nd reviewer’s comments, however.**

4. Results  
Well described. However I would reduce the emphasis on the MSSA and the non MDR gram negative organisms isolated because it is not the focus of the manuscript and does not add much to the importance of the manuscript.

**All results for Staphylococcus aureus and MSSA were removed. Emphasis was placed on the GNR/MDR-GNR findings.**

5. Discussion  
In general the discussion overstates the importance of colonization. I would not infer from the study that you have determine a more appropriate antibiotic for use in prophylaxis (ie that penicillin is inappropriate and moxifloxacin is superior). These recommendations can only come from clinical trials. I think it is somewhat premature to say that these findings should affect choice of empirical antimicrobial therapy by humanitarian medical groups working in the region, as you propose. The discussion should be shortened.
Discussion was shortened significantly and conclusive statements modified to reflect reviewer’s comments.