Clinical identification of bacteria in human chronic wound infections: culturing vs. deep 16S ribosomal DNA sequencing

Significance
The paper by Rhoads et al. describes the analysis of 51 chronic wound samples obtained from patients with various types of non-healing wounds for comparison of culture and 16S rDNA sequencing methodology for identification of bacteria in the wound.

The topic of chronic wounds (CW) is very important. CW already affect millions of patients yearly at enormous cost but further represent an increasing clinical problem as lifestyle-associated diseases such as obesity, diabetes and cardiovascular disease continues to rise in the global population. An underlying problem with the treatment of CW has been the accurate diagnosis of the microbial etiology of the persistent infection. Several papers, including seminal work by the authors, have now shown that routine culture underestimates the presence of bacteria in the wound and that molecular methods give a more accurate depiction of the microbial burden. This is key to more accurate and precise diagnosis which will result in more rapid and targeted treatment. This manuscript compares culture methods and 16S deep rDNA sequencing.

Methods:
Chronic wounds from 51 subjects were sampled from a variety of different types of wounds following debridement of the wound as part of standard care and duplicate samples were tested in parallel. One half was analyzed by routine culture in a hospital-based microbiology laboratory and the other half was analyzed following DNA extraction from the sample, amplification of the 16S portion of ribosomal DNA pyrosequencing of 16S amplicons using Roche’s FLX Titanium platform. Samples were then analyzed for determining relative abundance of the bacteria taxa identified.

Strengths: The problem of molecular identification of so many types of bacteria in clinical samples where few bacteria were previously detected by culture raises difficulty for clinicians trying to interpret these results. This study tries to address this difficulty by demonstrating that deep sequencing better resolved the bacteria present by showing that of the most abundant bacteria (dominant), only half were cultured, and far fewer proportions of bacteria were identified by culture in the
major and minor classifications. The paper also discusses discrepancies between the 2 methods.

Weaknesses: The authors need to more clearly address how the current paper differs from other publications (including their own) indicating that molecular methods find more organisms and do a better job at identifying them than culture. If the goal of the paper is to help clinicians become more comfortable with interpreting molecular diagnostic data then the manuscript needs to clarify the results for clinicians who may not have a very deep understanding of microbiology, particularly in terms of interpreting statistical data reporting bacterial abundance. The limitations of the study should also briefly be addressed/acknowledged. For example, the expertise and cost of the Roche FLX Titanium system is beyond many clinical microbiology labs’ resources and capabilities. Finally the message regarding how high throughput sequencing data improve the therapeutic approach to wound care is somewhat lost.

Accept with major revision.

Major revisions:
The results and discussion should be tightened up and shortened. Specifically how the data support each conclusion should be stated clearly.

Several parts of the study need to be clarified:
Clarify inclusion and exclusion criteria for patients.
Better clarify type of wounds (this may be relevant to the type of infection/microbial burden).
Why was culture for anaerobic organisms not undertaken? Clinical microbiology labs generally do this if requested. Is this not routinely done for chronic wounds, especially in light of several papers in the literature showing that anaerobes are present in CW?
Results should consistently indicate percent followed by (the sample number/total number of samples) as in the abstract.
The statistical analysis needs to be better outlined and explained if the target audience is to be clinicians in the field. Better clarify software and analysis showing relative abundance and particularly regression analysis used. What is OR trying to show? If it is showing the difference in effect of diagnostic power of Roche platform over culture is this the best way to indicate effect size?
Figure legends should be more explanatory. Include titles for figure legends. E.g. Figure 4: Increased relative abundance of a bacterial genus increases the likelihood that the genus will be cultured. (This may help clarify the point each figure is trying to make.)
Should figs 1 and 2 be combined to show that molecular methods although in bacteria which are much less abundant? Same for Figs 4 and 5?
Citations need to be added to discussion to complement how research is supported by other published work or how it differs. If it differs, discuss why it might differ, e.g. different methodology, etc. Specifically relevant work by
Kirketerp-Moller et al. 2008; Fazli et al. 2009; Thompsen et al. 2010 could be briefly discussed.

Minor suggested revisions:

Page 7: “These results can be unsettling for clinicians because ever since Robert Koch… Change to: “These results can be unsettling for clinicians who view culture as the gold-standard for bacterial identification. Our results suggest that culture testing may be insufficiently sensitive in some cases (or overly sensitive in other cases) and therefore require carefully interpretation.”

Page 9: Regardless of the cause of the bacteria’s aversion to growth in culture (change to …”failure of bacteria to grow”)

Page 10: “Because genetic detection of antibiotic resistance requires prior knowledge of the gene(s) (e.g. mecA, VanA, extended spectrum beta lactamases) …However, phenotypic testing of antibiotic resistance has a couple of shortcomings that are not often considered. “ It is not clear how the present molecular approach would overcome these shortcomings. Clarify how the present technology could improve over culture or remove from discussion.

Page 14: FLX Titanium technology is mispelt

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.