

Author's response to reviews

Title: Air Bacterial Contamination from Hydro debridement of Wounds.

Authors:

Frank L Bowling (frank.bowling@manchester.ac.uk)

Daryl S Stickings (daryl_stickings@hotmail.com)

Valerie Edwards-Jones (v.e.jones@mmu.ac.uk)

David G Armstrong (armstrong@usa.net)

Andrew JM Boulton (ABoulton@med.miami.edu)

Version: 2 **Date:** 12 February 2009

Author's response to reviews: see over

Dear Editor,

Here are our responses to the reviewer comments on our recently submitted paper (MS: 7242410382337594). We have amended the manuscript as per their critique and provided below is a point-by-point detailed account of revisions. The reviewer comments precede our responses, which are designated by italics.

Response to reviewer's report:

Reviewer: Nicoletta Frescos

1. Although this is a low risk project was ethics approval obtained?

1.) *Yes, ethical approval was obtained by the local research ethics group.*

2. The methodology is in some parts is unclear and does not flow, which makes it difficult for the reader to obtain an understanding of the processes undertaken.

2.) *The methodology has been revised.*

2.1 what size was the room and would this impact the aerolization?

2.1) *The room was 5m by 3m, to mimic a typical outpatient community clinic setting.*

2.2 was the air tested prior to the research?

2.2) *Yes it was. See revised methodology.*

2.3 was the room air controlled according to infection control standards for surgical procedures?

2.3) *No, the clinic room did not have controlled air flow. This study refers to a standard clinical outpatient setting. This study was carried out outside of the operating theatre i.e. routine outpatient setting. Similar to conditions in which the Versajet system has been approved.*

2.4 was the air tested prior to each meat being debrided?

2.4) *No, instead a two hour time gap was left between debridements to allow pathogens to disperse. This was to simulate normal clinical practice. See revised method.*

2.5 were all 4 meats debrided consecutively on the one day? would the air samples still have traces of the previous meats contaminants?

2.5) *All samples were debrided consecutively on the same day to standardise incubation time. A two hour time gap was left between debridements of different samples. The clinical room was disinfected after each debridement. It is entirely possible that some pathogens remained suspended in the air. Indeed staph. Aureus was found in air sampled whilst debriding the second sample, which was infected with pseudomonas. This further verifies the findings in respect to air contamination during and after hydrosurgery.*

2.6 was the VJ operator wearing clean sterile clothing for each debridement?

2.6) *Yes, standard surgical scrubs (apron, mask, visor, bonnet, gloves) were worn. Replaced after each debridement.*

2.7 did the VJ operator have prior experience in operating the VJ and was the debridement correct procedure?

2.7) *The operator has had extensive training and clinical use of hydro debridement i.e. Versajet.*

2.8 how close was the air sampler to the operator?

2.8) *The Air sampler was situated at 2.5m from the operator (where, $2.5^2 = 2^2 + 1.5^2$ (horizontal distance of 2m and a vertical height of 1.5m))*

2.9 Figure 1 in the text refers to the set up of the room, however the actual Figure is

not provided. This would provide a clearer understanding of how the room was set up, where the air sampler, settle plates were placed for testing and at what height.

2.9) Now attached. New figure 1 showing diagram schematic.

3. The results section has a few statements that describe methodology ie biopsy procedures and process for EM.

3.0) Now amended into methodology

4. the description of the findings for the swabs requires was unclear to me, it did not seem correct.

4.0) Amended. Our results section is now split into distinct sections to prevent any ambiguity.

5. there are several interesting findings that have not been discussed or some explanation provided ie Table 2: meat 2 shows presence of SA; Table 3 shows an increase in bacteria count in settle plate front right from 45 at 1m to 106 at 2m and 0 at 3m, why would there be such a discrepancy?

5.0) See amended discussion for explanation of table 2. Table 3 showed irregular displacement of pathogens especially front right settles plates. We cannot account for equal or unequal fallout, due to the nature of high pressure spray.

5.1 The results and Figure 6 and introduce the "removal of dressing" yet this was not mentioned in the methodology.

5.1) See amended methodology regarding dressing removal.

6. the titles of a few of the figures and tables could be more descriptive to get a better understanding of what they represent.

6) Amended

7. there are a few minor spelling mistakes

7) Checked and proof read.

8. interchangeable terminology ie biopsy and curetage

8) Amended interchangeable terminology.

Finally the article could benefit from more discussion on interpreting the results and the implications to clinical practice.

Final) We have significantly increased the content of the discussion.

Reviewer: Cesira Isabella Maria Pasquarella

Is the question posed by the authors new and well defined? The question posed by the authors is original but, according to the text, does not seem well defined. The Authors say: “The purpose of the study was to assess the levels of aerosol contamination with bacteria after hydrosurgical debridement of a simulated infected wounds” (Abstract); “The purpose of this study was therefore to evaluate the potential for aerosolization of microbes using hydro-debridement therapy” (Introduction). Actually, the purpose seems to be also to evaluate the effectiveness of the Versajet in reducing bacterial contamination of the infected wound.

We have amended the abstract and introduction to include, “The effectiveness of hydrosurgery on reduction of bacterial contamination of a wound.”

The methods used to evaluate the air microbial contamination are appropriate. Passive sampling and the active sampling were used to collect micro-organisms from the air; however, it seems that the authors are not familiar enough with the microbiological sampling. The two methods are not well explained in their methodology and they seem to be confused.

For example, in the Abstract, as regard the Methods, they say that “Settle (fallout) culture plates were located at various distances around the clinic room area””The air was sampled using a germ sampler machine”. The first sentence refers to passive sampling whose results are expressed as cfu/plate/time, the second one to active sampling, whose results are expressed as cfu/m³. In the Results the authors report the results obtained by settle plates expressed as cfu/m³ “Analysis of the settle culture plates showed a significant (P<0.001) increase in average microbial counts post-hydrodebridement (n=14) with levels ranging from 950 cfu/m³ to 16780 cfu/m³. Moreover, these figures do not correspond to what is reported in the text [pag. 8 - “analysis of the fallout plates showed a significant (P<0.001) increase in average microbial counts from 50.4 cfu pre-treatment (n.5), to 90.2 cfu post-treatment (n=14)]”.

Methodology has been revised to address the units of measure discrepancy. Ambiguity of clinical set up has been address by including a schematic of the clinical set-up. We have included table 4 with the air sampling results included.

Although the aim of the study is “to evaluate the potential for aerosolization of microbes...” no mention of “air” “micro-organisms” “air sampling” appears in the key words, where some words never mentioned in the text (e.g. diabetes, foot, ulceration) are reported. It is clear to clinicians or nurses that the subject investigated is linked to diabetes, foot, ulceration, however, it might be not so clear for others. The words diabetes, foot and ulcer appear in the references.

We have replaced some of the keywords with more appropriate keywords.

No sufficient details are provided to replicate the work. For example, as regard the passive sampling (fallout), what was the diameter of the plates? How long the exposure time? Where were the settle plates located (height from the floor). As regard the active sampling, which was the instrument used? In the Methods the authors say

“using an air sampler machine (germ sampler)”, in the Results the SAS portable air sampler is mentioned. It is important to give information about the air sampler used, in the perspective of the replication, since the different air samplers give different results. Which medium did the plates contain? The culture media are mentioned with reference to the “determination of the wound flora”.

We have completed an overhaul of our methodology to make it replicable. The diameter of the settle plates was 90mm. A schematic showing clinic set up is now included. The instrument used for air sampling was the SAS Portable SAS Super 90. The medium used on the settle plates was Tryptone soya aga (TSA).

Some information regarding the methods are reported in the results. For example “The biopsies were removed with a 6 mm sterile cutter. The mean weight of the biopsy was 0.058 g.” or “The biopsies taken for EM examination were fixed in 10%.....”

We have removed the paragraph on Biopsies from the results.

It should be explained why samples of meat were taken for histology and scanning electron in the light of the purpose of the study.

To assess bacterial load of a wound, samples were taken for SEM and Histology to look for pathogens deep to the superficial tissue. This is illustrated in figure 5.

Nothing in the Methods is said about statistical analysis.

For statistical analysis we used Minitab v15. For significance testing we used 2-sample T tests on our parametric data. (See method)

The methods should have been divided into different parts, for example:

- evaluation of bacterial contamination of the meat
- evaluation of air bacterial contamination by active sampling
- evaluation of air bacterial contamination by passive sampling

We have amended our methodology and results into three distinct parts for clarity.

The author say that the pork skin was sterilized using 90% alcohol. It is not appropriate to use the term “sterilization” by using alcohol which is a disinfectant.

We have changed the term sterilization to disinfected.

Are the data sound and well controlled? The collection of data is not well explained and, consequently, the data reporting is not clear.

For some results reported in the text or in the tables there is not correspondence in the Methods. The methods should be consistent with the results, and vice versa the results with the methods. As the Methods, also the Results should be divided into different parts corresponding to the Methods.

We have revised our method with respect to our results to improve reading flow.

In Table 2 are reported the results obtained by using the active sampling; however, it is not clear where the results reported in the abstract and at page 9 (950 cfu/m³ and 16780 cfu/m³) come from.

We have included a new table (Table 4) with the results of air load measured across all four samples.

The data reported at page 8 (585 cfu/m³ and 5335 cfu/m³) need to be explained in the way they were collected (in the Methods). The legend of Figure 6, for example, is matter of methods.

Figure 6 legend has been transferred to the methodology and an explanation given on how the results were obtained.

How do the authors explain some increase in bacterial (*Pseudomonas*) contamination of meat Post Versajet? Any comments regarding the bacterial count on the three different sites of the meat?

We cannot take into account the irregularity of wound surfaces. The site which saw an increase in bacterial count post-versajet was a wound designed to simulate a sinus. It is possible that taking a wound swab from a sinus does not accurately report bacterial load.

In Table 2, how was it possible to find *Staphylococcus aureus* in the air during the debridement process of Meat 2 which was contaminated with *Pseudomonas aeruginosa*?

All meats were debrided consecutively on the same day, to standardise incubation time. A two hour time gap was left between debridements of different meats. The clinical room was disinfected after each debridement. It is entirely possible that some pathogens remained suspended in the air and on hard surfaces following cleaning. One of the objectives of the study was to mimic a clinical situation, thus evaluating bacterial fallout after consecutive treatments. This further verifies the findings in respect to air contamination during and after hydrosurgery. This has been amended in the methods.

The 16780 cfu/m³ contamination actually seems to be very high to be counted. It means that a number of about 1000 cfu were counted on a plate 55 mm in diameter (if the SAS was used) after a collection of 100 L.

As one visible cfu may have arisen from more than one cell, when the plate becomes crowded the actual number of visible cfus does not represent a true figure. Therefore a statistical calculation is applied to give a more probable count.

Do the title and abstract accurately convey what has been found? No. The title "Microbiological fallout...." refers only to one of the two sampling methods (the passive method). A more appropriate title would have been "Bacterial contamination from...." or "Air bacterial contamination.....". The Abstract is not clear in the methods,

but in particular in the results. The two sampling methods are confused, as explained previously.

We concur that the title of the study refers only to passive sampling and have therefore amended it to, "Air Bacterial contamination from Hydrodebridement of Wounds."

We hope this meets with your requirements for publication in the Journal of Foot and Ankle research.

Please do not hesitate to contact me if you have any further queries.

Yours sincerely,

Frank L. Bowling
University of Manchester,
Manchester Royal Infirmary.

Frank.bowling@manchester.ac.uk

+44 0161 276 8691
+44 07515 683358