Author’s response to reviews

Title: Microorganisms involved in Deep Neck Infection (DNIs) in Greece: detection, identification and susceptibility to antimicrobials

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Author’s response to reviews:

Dear Editor,

Thank you very much for your evaluation of our manuscript entitled “Microorganisms involved in Deep Neck Infections (DNIs) in Greece: detection, identification and susceptibility to antimicrobials”. In this revised version the suggestions of the reviewers were taken under consideration.

In more details:

Reviewer 1: Thank you very much for your useful comments.

2) Why did only 462 out of 610 (76%) patients have cultures and 16S performed? An explanation is required.
Clinical samples were not taken from 24% of our patients mainly due to the insufficient pural material. In these cases, empirical therapy was immediately applied according to local guidelines (see lines 167-168).

Are the microbiology of the remaining 24% of patients expected to be similar to those who did have cultures obtained?

We believe that the microbiological data of these patients would be similar.

3) What transport medium, if any, was used for the aerobic and anaerobic cultures?

We used the BD ESwab™ collection and transport system which helps the collection and the transport of clinical specimens containing aerobic, anaerobic and fastidious bacteria from the infected site to the testing laboratory (see lines 116-117).

4) How was anaerobiosis established in the anaerobic cultures (GasPak? Glove box?)? Please clarify.

We used BD BBL™ GasPak™ anaerobic for anaerobic incubation (see line 125).

5) The effect of prior antibiotics on culture positivity is an important question. This can be answered statistically by chi-squared or Fisher's exact test. Spearman correlation by rank order is not an appropriate statistical test for this question. How do you "rank order" use of antibiotics? How do you "rank order" culture positive or negative? Please re-analyse your data. The same applies to the question of age and cultures. You can rank order age, but not rank order culture positivity.

Statistical analysis of the data was analyzed by using chi-squared and Fisher's exact test according to your suggestion (see lines 144-145, 172-173, 177-178).

Page 7, line 31.

The sentence “All descriptive data … potential correlations.” was changed as follows:

“All descriptive data were reported in percentages. Pearson Chi-Square was used to assess potential correlations.” (see lines 144-145)
Page 8, line 39.
The phrase “(Spearman’s Rho: -0.008; P: 0.865 > 0.05)” was changed as follows:
“(Pearson Chi-Square: 0.029; P: 0.864 > 0.05)
Page 8, line 53.
The phrase “(Spearman’s Rho: -0.087; P: 0.062 > 0.05)” was changed as follows:
“(Pearson Chi-Square: 2.301; P: 0.129 > 0.05)

6) In Table 1, please provide information on which deep neck spaces were involved in single vs.
multiple space involvement. Suggest providing the data in 3 columns: first column for name of
spaces; second column for no. with single space involvement, 3rd column for no. with multiple
space involvement.
The Table 1 was changed.

7) In Table 2, provide all 16S results, not just those which were negative by culture.
The Table 2 was changed.
It is surprising that only 40% of samples were positive for 16S but 55% were positive by culture.
One would have expected that 16S would give a higher yield since it is less likely to be
influenced by prior antimicrobial therapy. Can the authors speculate why this was the case?
The sensitivity of the 16S rRNA PCR, when it is applied directly to the clinical samples, is
depending on the microbial load. So, it is possible to obtain negative results for clinical samples
with a very low microbial load. The traditional culture, which enhances the growth of the
bacteria after incubation, can give more positive results than the molecular method (see lines
247-252).

8) P. 10, line 26-31: Imipenem and meropenem are highly active against anaerobes and would
not require addition of metronidazole or clindamycin for anaerobic coverage. Furthermore,
metronidazole is only active against anaerobic gram negative bacteria but less active against
certain anaerobic gram-positive bacilli.
We agree with your comment and the sentence was changed (see lines 210-211)
9) One third of culture negative samples (11/33) had positive 16S but no bacterial identification. The authors suggested that this might have been due to the presence of multiple organisms. However, sequencing should have provided results for individual components.

In the present study, analysis of the PCR products was based on Sanger sequencing, which is not able to distinguish more than one microorganism per sample. Probably in the future, the implementation of the next generation sequencing technology could solve this limitation (see lines 252-255).

Reviewer 2: Thank you very much for your useful comments.

P9 Line 39 "sequencing analysis failed to identify the microorganisms involved, given that a polymicrobial genetic pattern was obtained". Would you add some sentence about this in the discussion part and give as a limitation.

In the present study, analysis of the PCR products was based on Sanger sequencing, which is not able to distinguish more than one microorganism per sample. Probably in the future, the implementation of the next generation sequencing technology could solve this limitation (see lines 252-255).

Table 2: Fusobacterium necrophorum and Actinomyces israelii were the predominant pathogens not identified by conventional culture method. Would you discuss more this about the reason? Is this related to anaerobic bacteria killed by antibiotic ? or related to culture method ?

Reasons related to the failure of the conventional culture to isolate these microorganisms could be the inappropriate sample collection, the fragility of them and the short incubation of the anaerobic culture (5 days) (see lines 240-243)

Is any underlying disease related to these two pathogen?

Underlying disease related to these two pathogens were not found.

And what is the clinical implication after getting 16S rRNA PCR result? Did this change the empirical antibiotic choice?

In our clinical setting the antimicrobials used for DNIs empirical therapy includes often IV of ampicillin-sulbactam combined or not with metronidazole or clindamycin. These antimicrobials are effective for both pathogens.

Sincerely yours,
Efi Petinaki, MD