Reviewer’s report

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

Version: 0 Date: 21 Jun 2019

Reviewer: Anthony Chow

Reviewer's report:

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Synopsis: 610 consecutive patients with DNI admitted to a tertiary hospital in Southern Greece were enrolled. Aerobic and anaerobic cultures as well as 16S molecular techniques were performed to identify microbial etiology prior to empiric antimicrobial therapy. 16S identified fastidious organisms including anaerobes from two-thirds of patients (22/33) that had negative bacterial cultures.

Comments & Questions:

1) Generally a well executed study and well written manuscript.

2) Why did only 462 out of 610 (76%) patients have cultures and 16S performed? An explanation is required. Are the microbiology of the remaining 24% of patients expected to be similar to those who did have cultures obtained?

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

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

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.
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.

7) In Table 2, provide all 16S results, not just those which were negative by culture. 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?

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.

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.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**

If not, please explain in your comments to the authors.

Unable to assess

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**

If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

**Quality of written English**

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