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:

Larissa, 8-9-2019

Dear Editor,

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

Reviewer 1: Thank you very much for your useful comments and for your time.
2. Line 118: what is meant by "pural material"? Do you mean "purulent material?"

Page 6, line 120: It was corrected.

3. Line 124: change "being testing" to "being tested"

Page 6, line 126: It was corrected

4. Line 173: the question whether prior antimicrobial therapy influenced the culture results is an important one. Please show the available data from your studies. You can probably combine the data for adults and children (to give you more numbers). I'm not sure if Pearson Chi Square is the appropriate statistic to use. You should consider a 2x2 contingency table (i.e. column headings prior antibiotics Yes or No; row headings culture positive and culture negative). What you wish to test is whether the distribution of culture results in your study was a random effect or not, using Chi-square statistics (null hypothesis). Use Yates' correction if any cell contains 5 or less. If p value is significant, then the difference in culture results is not a random event but rather is related to prior antibiotic therapy. Also, not finding a significant difference does not mean no difference, but rather a significant difference has not been demonstrated, possible due to insufficient power.

Table 4 was added which shows our data regarding prior antimicrobial therapy and culture results of children and adults combined. It should be noted that in our previous analysis also, data of adult and children were already combined.

Page 7, line 146

The sentence “Pearson Chi-Square was used to assess potential correlations.” was changed to “2x2 contingency tables and Chi-Square tests were used to assess potential correlations.”
The phrase “while 45,6% (210/462) of them had taken antibiotics before admission (46,3% of adults and 35,3% of children).” was changed to “while 45,6% (210/462) of them (46,3% of adults and 35,3% of children) had taken antibiotics before admission (Table 4).”

The sentence “No significant correlation was noted between antibiotics uptake before culture and positivity or negativity of culture results (Pearson Chi-Square: 0,029; P: 0,864 > 0.05).” was changed to “Chi-Square tests did not demonstrate any significant difference (p>0,05) between prior antibiotics uptake and positive or negative culture results (Table 5), maybe due to insufficient power.”

Table 5 was added which shows the results of chi square tests regarding prior antimicrobial therapy and culture.

5. Line 178: how do you assess correlation between age and culture results using Pearson Chi-Square? The chi-square statistic utilize categorical data. By age difference, are you simply comparing those over the age of 18 with those under the age of 18? Please clarify. If you are asking is there a difference in culture positivity from adults vs. children, then, a 2x2 contingency table would again be appropriate, with column headings of no. of adults and no of children, and row headings of culture positive and culture negative.

The phrase “No significant correlation was noted among age and culture results (Pearson Chi-Square: 2,301; P: 0,129 > 0.05).” was changed to “No significant correlation was noted in culture positivity from adults vs. children (Pearson Chi-Square: 2,301; P: 0,129 > 0.05 / Yate’s Continuity Correction: 1,790; P: 0,181>0.05 / Fisher's Exact Test: P: 0,153>0.05).”
6. Line 185: "All anaerobic bacteria were susceptible to .....metronidazole". Not true. Actinomyces spp. are almost universally resistant to metronidazole. You are probable referring to all anaerobes cultured were sensitive to metronidazole. Please clarify.

Page 9, line 188
It was changed as ‘’all anaerobic bacteria which were isolated by culture…."

7. Line 211: What is your recommendation for empirical antimicrobial therapy for patients from central Greece based on your antimicrobial susceptibility data? Please make a clear recommendation. Is ampicillin-sulbactam plus metronidazole or clindamycin appropriate? Would you recommend the use of clarithromycin? The references you provided (17, 18, 19) were very old (2001-2008) and not current.

According to our epidemiology, we propose as empirical therapy the intravenous use of a beta-lactam /beta-lactamase inhibitor; metronidazole or clindamycin can be added only in specific cases such as in immunocompromised patients. Although clindamycin has a broad range of activity against aerobic Gram-positive cocci and anaerobic organisms, clinicians must be prudent with its usage due to the increased rate of resistance. On the other hand, metronidazole has excellent in vitro activity against most obligate anaerobic bacteria (Bacteroides, Fusobacterium, etc) but is inactive for Propionibacterium acnes and Actinomyces israelii.

We have replaced the reference Ann Otol Rhinol Laryngol 2001 by a newer, Ann Otol Rhinol Laryngol 2010 and we have also added three recent references( 20-21-22).

8. Line 215: "The DNI microbiology is characterized by generally being polymicrobial ....". Please provide data from your own studies: how many were polymicrobial, unimicrobial, and culture negative?

Page 10, line 226: In the present study, cultures have obtained from 462 patients; 252 were unimicrobial, 3 were polymicrobial and 207 were culture-negative.
9. The statement that "the most frequent pathogens in bacterial cultures were Gram-negative ..." is misleading. The reference cited (22) is atypical in that only 12.5% of intra-operative cultures were positive. The authors of this paper also explained that the predominance of gram-negative bacteria was likely due to hospital-acquired infection in their ICU patients. I would leave out this reference altogether.

The reference was deleted

10. Line 232: For reference 27 cited, please clarify that the authors of this paper were comparing the bacteriology between those less than 1 years of age and those above 1 year old.

Page 11, line 238: it was clarified

11. Table 1: Ludwig's angina does not occupy a single space, but in fact involves 2 spaces, the submental space and the sublingual space.

Table 1 was changed.

12. Table 2: please show totals for the column "Culture+16SrRNA and row headings of "Gram-positive", "Gram-negative" and "Gram-negative" under Anaerobic bacteria.

Table 2 was changed.

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

For the treatment recommendation esp. in the conclusion ,you said "we suggest intravenous infusion of "amoxicillin-clavulanic acid combined with metronidazole"as the first-line treatment.", suggestion revision .Because other B-lactam/BL inhibitor: e.g ampicillin/sulbactam or pip/tazo may also be considered. And some experts recommended 3rd generation cephem + metronidazole. And if you consider the result " Finally, all anaerobic bacteria isolates were susceptible to clindamycin and to metronidazole." Why not use clindamycin? And what is the
rationale that we can not use only B-lactam/Bactam inhibitor as empirical therapy? What is the disadvantage if you did not use metronidazole? No data or guidelines support the use of two anti-anaerobic drugs in clinical setting.

We agree with your comment and a paragraph was added:

Page 10, line 218:

According to our epidemiology, we propose as empirical therapy the intravenous use of a beta-lactam/beta-lactamase inhibitor; metronidazole or clindamycin can be added only in specific cases such as in immunocompromised patients. Although clindamycin has a broad range of activity against aerobic Gram-positive cocci and anaerobic organisms, clinicians must be prudent with its usage due to the increased rate of resistance. On the other hand, metronidazole has excellent in vitro activity against most obligate anaerobic bacteria (Bacteroides, Fusobacterium, etc) but is inactive for Propionibacterium acnes and Actinomyces israelii.

Sincerely yours,

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