Author’s response to reviews

Title: First isolation of Two colistin-resistant Emerging Pathogens, Brevundimonas diminuta and Ochrobactrum anthropi, in a Cystic Fibrosis Patient: a Case Report

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Author’s response to reviews: see over
Dear Editor,

Please find enclosed our revised manuscript entitled « First isolation of Two colistin-resistant Emerging Pathogens, *Brevundimonas diminuta* and *Ochrobactrum anthropi*, in a Cystic Fibrosis Patient : a Case Report ».

All authors have seen and approved the manuscript and the manuscript has not been published and is not under consideration for publication elsewhere. All potential conflicts of interest have been disclosed.

Reviewer comments
Reviewer: Gilbert Greub
Comments to Authors
The paper by Menuet et al reports a case of lower respiratory tract infection in cystic fibrosis patients, which was likely due to two bacteria unusually documented in this setting. This case-report is very interesting since it exemplified that:
- 16S PCR and sequencing is an ideal tool to accurately identify bacteria isolated from clinical samples (i.e. *B. diminuta* could not be identified using API20NE)
- cystic fibrosis patients may be colonized and/or infected with colistin-resistant strains selected through wide use of nebulized colistin in during cystic fibrosis patients
- such isolates will only be documented if selective media are used and if molecular identification of recovered strains will be performed

To determine whether a bacteria isolated from lower respiratory tract is the cause of the infection or is only a colonizer is a difficult task for both clinicians and clinical microbiologists. Here, the fact that the patient did not improved initially with an antibiotic effective on *S. aureus* (ceftazidime) and improved once an antibiotic effective on the two colistin/ceftazidime resistant strains was introduced is the main hint suggesting a role of one or both colistin-resistant strains as an agent of lower respiratory tract infection in the present case. It suggests that cystic fibrosis patients may be colonized by several different bacteria and that the predominant species (i.e here *S. aureus*) may not be the only/main one participating to the pathogenic process. Although, I fully agree with the general conclusions, I would suggest that the authors tune down their conclusions.
regarding the role of S. aureus. Indeed, I think that it is possible that S. aureus also partially participated initially to the pathogenic process. Similarly, since both B. diminuta and O anthropi were present, it is impossible to be sure that one of them was only a colonizer.

We thank the reviewer for the comments on our case report. We agree with the fact that further studies are needed to better understand the importance of such bacteria in pathogenesis in human, especially in immunocompromised patients including cystic fibrosis patients with lung transplant. We believe that such bacteria are rarely reported and probably underestimated because specific media are needed to isolate such organisms. The role of S. Aureus in the participation of the pathogenic process has been added in the discussion (page 5) as suggested.

Minor comments:
page 4: In order to make clear that the 100% homology is with a well documented strain present in a culture collection, I would propose to replace “...as B. diminuta (genbank accession number X87274, 100%)” by “...as B. diminuta (100% homology with B. diminuta strain DSM 1635, genbank accession number X87274)”; and to modify similarly for O. anthropi.

This has been corrected in the revised manuscript (page 4)

a few typing errors need to be corrected:
page 4 : “O. antrop” should read “O. anthropi”

This has been corrected in the revised manuscript (page 4)

page 5: “cellulites” should read “cellulitis”

This has been corrected in the revised manuscript (page 5)

Reviewer: Argyris Michalopoulos

Comments to Authors

In my opinion, the authors should discriminate the detection of these two pathogens from the preceding colistin treatment since these pathogens isolated are usually resistant to polymyxins. Colistin has good activity against Acinetobacter spp. (MIC90 # 2 mg/Lt), Klebsiella pneum. (MIC90 # 1 mg/Lt), E. coli (MIC90 # 2 mg/Lt), P. aeruginosa (MIC90 # 4 mg/Lt), and Enterobacter spp. (MIC50 # 1 mg/Lt). In addition, it may be active against some strains of Shigella spp. (MIC90 # 0.5 mg/Lt), Salmonella spp. (MIC90 # 1 mg/Lt), Citrobacter spp. (MIC90 # 1 mg/Lt), and Escherichia coli (MIC90 # 1 mg/Lt).

Tan TY, Ng SY. The in-vitro activity of colistin in gram-negative bacteria. Singapore Med 2006;47(7):621-4

To clarify whether the isolation of these two colistin-resistant bacteria was linked to a previous colistin treatment, we have changed the title of our case report to “First isolation of Two colistin-resistant Emerging Pathogens, Brevundimonas diminuta and Ochrobacterum anthropi, in a Cystic Fibrosis Patient : a Case Report”. 
I am not sure that these pathogens isolated were the causative agents of pneumonia since apart from them, Staph. aureus was isolated as well (107 CFU/ml, MSSA). In addition, Gram staining showed Gram positive cocci and the patient received tobramycin.

We agree with the fact that *S. aureus* may contribute to the pathogenic process in our case. However, the patient did not improve initially with an effective antibiotic therapy against *S. aureus* and improve using an effective antibiotic treatment on the two colistin/ceftazidime resistant strains suggesting a role of one or both colistin-resistant strains as an agent of lower respiratory tract infection in the present case.

I hope that the revised manuscript is now convenient.

Sincerely yours.

Pr ROLAIN Jean-Marc