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REVIEWER'S COMMENT

Answer to the Letter to the Editor of Manu N. Capoor et al. concerning "Low virulence bacterial infections in cervical intervertebral discs: a prospective case series" by Chen Y, Wang X, Zhang X, et al. (Eur Spine J; 2018: doi: 10.1007/s00586-018-5582-4)

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We appreciate the careful reading and critical evaluation on our work by Capoor et al. The answers to the questions raised in the above letter are listed below.

By the time we started our study, we considered various possibilities of using one of the culture methods previously reported. We did also pay special attention to the method, which included a homogenization step before culture in the studies by Capoor et al. [1, 2]. They found *Propionibacterium acnes* (*P. acnes*) formed biofilm in intervertebral discs by using confocal scanning laser microscopy (CSLM) and fluorescent in situ hybridization (FISH). It might be true that the process of disrupting biofilm could maximize the detection of organisms associated with peri-prosthesis infection, according to Tunney et al. [3]. But in their study, it was ultrasonication, rather than homogenization which served as the control, the key step to increase the detection rate of bacteria around the implants. To what extent homogenization can improve the detection of infection in the setting of degenerative intervertebral discs needs to be verified. On the other hand, the method we used for microorganism culture in our study is simple, widely used and proved to be effective in previous studies of this field [4–7]. In contrast, the method with homogenization is technically more demanding. We had little experience of it, and we were concerned about biased results which might be caused by inappropriate operation such as contamination and inability to maintain anaerobic conditions during the homogenization process. So finally we chose the one that we were familiar with. But we think that Capoor's method is among the very few techniques that can answer the questions not only "yes or no" but also "how much." We will consider using it in the future.

There were many studies concerning low virulence bacteria in the lumbar spine; however, in the field of cervical spine, there were few information that could be referred to. Our study is a preliminary cross-sectional study exclusively conducted in patients with cervical spine diseases. The comparison between our study and other studies which included mainly lumbar spine cases is of less significance than the comparison among those studies. The patients in our study were collected from a single institution and that were all we could acquire for analysis. Although the patient sample was relatively small, the discs samples from them had reached 66. We reported what we observed to the best of our scope based on these samples. Due to the small sample size, however, it was indeed possible to draw a biased conclusion. More observatory researches in this field are needed in the future.

When comparing *P. acnes* with other type of organisms in our results, we referred to the studies of others. Most authors counted bacteria such as *Staphylococcus capitis* and *Staphylococcus epidermis* as a whole group coagulase-negative *Staphylococci* [1, 4, 8], while a few counted them separately [9]. We shall describe the number and proportion of each type of *Staphylococci* we found in a prominent position of our results. However, whether combine these different types of coagulase-negative *Staphylococci* as one group or not would not affect the prevalence of *P. acnes* in the total samples analyzed or the proportion of *P. acnes* in all the organisms detected.

It may be true that enrichment culture is more liable to contamination, compared with direct culture of homogenized

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tissue. In order to assess the possibility of contamination during the enrichment procedure, every time we processed a batch of samples we use a tube of blank broth as negative control which the same operation as processing the samples was applied to except for adding a real sample in. We did not observe microorganism growth in any of the blank broths. Therefore, we thought that the chances of contamination during the enrichment process were quite few. We estimated that it was during the time of surgery when contamination was most likely to take place. The enrichment method frequently appears in studies [4–7]. There is no clear evidence that this method is not suitable for the culture of intervertebral disc tissue.

We stick to strict anaerobic principle throughout the culture process. Our specimens removed from the surgical site were quickly cut into small pieces on the operation table and thrown into the broth as soon as possible. The broths were put into a sealed anaerobic bag immediately and cultured for 14 days. Then, the subculture procedures were carried out in an anaerobic operation cabinet before the blood agar plates were cultured in a sealed anaerobic bag for another 7 days.

Compliance with ethical standards

Conflict of interest We have no conflicts of interest related to the subject of the article.

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