• Comment and Response •

Comment on: "Conjunctival microbiota variations in a subset of middle-aged and elderly individuals from Beijing, China"

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Dear Editor,

We read with great interest the article by Zhao *et al*[1] titled "Conjunctival microbiota variations in a subset of middle-aged and elderly individuals from Beijing, China". The authors highlighted an increased isolation rate of *Corynebacterium spp.* and *Micrococcus spp.,* emphasizing the need for greater attention and research from the ophthalmology community. Notably, in recent literature, *Corynebacterium* species are frequently identified[2]. One possible explanation for the underrepresentation of organisms such as *Corynebacterium spp.* and *Micrococcus spp.* in earlier studies may be the limitations inherent to traditional culture methods. In this study, Zhao *et al*[1] applied 16S rDNA gene sequencing, which enhances the detection range and sensitivity compared to conventional culture-based diagnostics.

Traditional microbiological methods typically employ media such as chocolate agar, 5% sheep blood agar, or MacConkey agar. However, certain pathogens—such as *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Legionella pneumophila*, and various fungi—require specialized media (*e.g.*, Lowenstein-Jensen medium, Skirrow agar, BCYE agar, or Sabouraud agar) and extended incubation periods, sometimes up to 3wk, for optimal detection.

In our own investigation of conjunctival microbiota in patients undergoing corneal tattooing, we found that *Corynebacterium spp.* was particularly challenging to isolate using chocolate agar, whereas 5% sheep blood agar proved significantly more effective[3]. This finding was statistically supported[4]. Similarly, in our ongoing clinical studies, *Corynebacterium spp*. consistently shows a higher affinity for 5% sheep blood agar. Furthermore, in a contact lens wearer presenting with keratitis, *Micrococcus luteus* was detected—along with Brucella broth medium and Thioglycollate broth isolates—after 7d in enriched liquid media[5]. These findings underscore the value of advanced molecular techniques such as 16S rDNA sequencing, which not only broaden the detectable spectrum of microorganisms but also reduce diagnostic timeframes.

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Author Reply to the Editor:

Dear Editor,

We are very grateful to Omer Faruk Yilmaz and colleagues for their attention to and comments on our paper titled "Conjunctival microbiota variations in a subset of middle-aged and elderly individuals from Beijing, China". We

are also appreciative of the editor for providing the opportunity to delve deeper into the discussion of our article.

As mentioned by the reviewer, the high detection rates of *Corynebacterium spp.* and *Micrococcus spp.* in our study highlight the potential variations in the conjunctival sac microbiota distribution among middle-aged and elderly individuals, and also underscore the advantages of employing 16S rDNA gene sequencing methods. 16S rDNA sequencing is a molecular biological technique based on the 16S ribosomal RNA (rRNA) gene, widely used for the analysis of microbial community structure and the identification of microorganisms. Compared with traditional methods, this approach has indeed expanded the scope of microbial detection and enhanced the sensitivity of detection[1].

Corynebacterium is a genus of Gram-positive rods with high nutritional requirements for culture. In this study, *Corynebacterium maecii* (*C. maecii*), a lipophilic species, was the most common. Due to its incomplete fatty acid synthesis genes, it relies on exogenous fatty acids for growth. Common culture media are BHI agar with Tween-80 or blood agar. It's strictly aerobic, forming minute colonies after 48–72h at 35℃–37℃[2-3]. Our prior research indicated that the detection rate of *Corynebacterium* showed a positive correlation with the severity of meibomian gland dysfunction (MGD). Notably, *C. maecii* was exclusively detected in patients with moderate to severe MGD, suggesting a close link to MGD severity. Given the lipid-rich nature of meibomian gland secretions, which provide the nutrients required for its growth, this finding is of particular interest[4].

When employing conventional culture methods, *Micrococcus luteus* can only be detected after being cultured on blood agar at 30℃–35℃ for 48–72h[5]. To ensure the accuracy of detection results, particularly when bacterial numbers in the sample are low or the bacteria exhibit slow growth, it is advisable to extend the incubation period to 7d. These findings further emphasize the value of advanced technologies like 16S rDNA sequencing, which not only expands the scope of microbial detection but also shortens the diagnostic timeline.

We fully agree with the reviewer's insights on microbial detection methods. In our study, we chose 16S rDNA gene sequencing to better understand the conjunctival microbiota, especially hard-to-culture microbes. This method helps us deeply explore the relationships between the conjunctival microbiota and various factors, thus offering stronger support for relevant ophthalmic research and clinical practice.

Thanks again to the reviewer for the attention to and valuable comments on our study.

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