Letters to the Editors

Letter: investigating the intestinal mucosa-associated microbiota – relevance and potential pitfalls

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Sirs, We read with great interest the paper from Shanahan *et al.*[1] We completely agree with the authors about the relevant role of an accurate evaluation of the mucosal-associated microbiota, which represents the part of the intestinal flora directly interacting with the host. Most of the studies in the literature, to date, have evaluated the faecal microbiome, since faecal sample collection do not require invasive examination and could be easier to collect for both patients and researchers.

Nonetheless, evidence is mounting about profound differences between faecal bacteria and the mucosal-associated microbiota.[2, 3] Considering that molecular cross-talk between enteric bacteria and intestine takes place at the mucosal surface (i.e. pattern recognition receptors/pathogen-associated molecular patterns interaction), and giving the spatially heterogeneous distribution of different bacterial species throughout the gut, the analysis of only the faecal microbiome could be misleading.[4]

In our institution, we are focusing our research on the evaluation of the mucosal-associated microbiota in different clinical scenarios. We have already demonstrated that the mucosal-associated microbiota is consistently altered in colonic adenomatous polyps' mucosa, compared with adjacent normal mucosa, with a profound reduction in total bacteria concentration that is coupled with an increased production of antibacterial molecules (α-defensins) in adenomas' mucosa.[5] We are currently investigating the mucosal adhesion of probiotic bacteria species in different colonic segments as a target for a possible specific therapeutic utilisation in particular clinical conditions (unpublished data) via real-time-polymerase chain reaction quantification.

Although direct evidence is lacking, it is reasonable to speculate that the issue raised by Shanahan *et al.*, i.e. the possible cross-contamination of bioptic samples with luminal bacteria, may be more relevant for upper gastrointestinal tract biopsies than for ileal and colonic ones.[1] Indeed, for the latter, the bowel-cleansing regimen that precedes the colonoscopy may represent the real confounding factor to take into account. In fact, while bowel preparation can represent, from one side, a negative factor by inducing changes in the mucosal bacteria composition per-se,[6] on the other hand, it may be a positive factor by reducing luminal bacteria concentration and therefore the potential risk of cross-contamination during biopsy collection.[7]

In line with that, the same article by Dave *et al.*, cited by the authors, failed to show significant differences in the microbial diversity of samples obtained using sheathed vs. unsheathed forceps.[8] Nonetheless, the utilisation of the device proposed by Shanahan *et al.*, and the comparison with standard bioptic forceps for ileal and colonic biopsies, could be of interest. Even more remarkable, the better characterisation of bowel cleansing effect on mucosal-adherent bacteria, and the identification and utilisation of cleansing regimens with lower impact on the mucosa-associated microbiome, would help the progress of the research in the intestinal microbiota field.

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REFERENCES

1. Shanahan ER, Zhong L, Talley NJ, Morrison M, Holtmann G. Characterisation of the gastrointestinal mucosa-associated microbiota: a novel technique to prevent cross-contamination during endoscopic procedures. *Aliment Pharmacol Ther* 2016; **43**: 1186–96.
2. Eckburg PB, Bik EM, Bernstein CN, *et al.* Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635–8.
3. Zoetendal EG, von Wright A, Vilpponen-Salmela T, Ben-Amor K, Akkermans AD, de Vos WM. Mucosa-associated bacteria in

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the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl Environ Microbiol* 2002; **68**: 3401–7.
4. Marchesi JR, Adams DH, Fava F, *et al.* The gut microbiota and host health: a new clinical frontier. *Gut* 2016; **65**: 330–9.
5. Pagnini C, Corleto VD, Mangoni ML, *et al.* Alteration of local microflora and alpha-defensins hyper-production in colonic adenoma mucosa. *J Clin Gastroenterol* 2011; **45**: 602–10.
6. Harrell L, Wang Y, Antonopoulos D, *et al.* Standard colonic lavage alters the natural state of mucosal-associated microbiota in the human colon. *PLoS ONE* 2012; **7**: e32545.
7. Jalanka J, Salonen A, Salojarvi J, *et al.* Effects of bowel cleansing on the intestinal microbiota. *Gut* 2015; **64**: 1562–8.
8. Dave M, Johnson LA, Walk ST, *et al.* A randomised trial of sheathed versus standard forceps for obtaining uncontaminated biopsy specimens of microbiota from the terminal ileum. *Gut* 2011; **60**: 1043–9.

Letter: investigating the intestinal mucosa-associated microbiota – relevance and potential pitfalls. Authors' reply

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Sirs, We thank Drs Pagnini and Delle Fave for their thoughtful comments regarding our recent publication investigating the mucosa-associated microbiota.[1, 2] In particular, their comments regarding the spatial heterogeneity of the mucosal microbiota further emphasise the argument that in order to understand host–microbiota interactions, we must investigate niche-specific microbes and communities. It cannot be assumed that a broad luminal snapshot represents the differing niches throughout the intestinal tract.

Our study focussed on the upper gastrointestinal tract, in part due to the unique situation in this region, regarding the presence of microbiota. The duodenum, for example, has historically been considered sterile due to the low bacterial loads present in healthy individuals.[3] In addition, the bacteria that are identified in this region show substantial overlap with the microbiota of the saliva.[4] Thus, contamination during sampling has been considered as an explanation for the identification of these oral-like bacteria in the duodenum. We sought to address this caveat by directly sampling the duodenal mucosa utilising our patented sheathed device. Our results reveal that there is a duodenal mucosa-associated microbiota, and this differs to what is detected when utilising standard biopsy forceps.

In the case of the large intestine, there is no question as to the presence and importance of the mucosa-associated microbiota. Development of bowel preparation techniques that are less impactful on the mucosal microbiota would be an important contribution to our understanding of the microbiota in this niche. The authors mention the use of bowel preparation as a means to removing the luminal contents. However, this does not necessarily negate the use of such a sheathed device as we have described. In fact, the use of bowel preparation is one of the primary reasons for the use of a sheathed device in the large bowel. As any endoscopist would attest, bowel preparation is rarely perfect, and varies based on many factors including patient compliance, motility and possibly the microbiota.[5] Indeed, we are currently investigating these factors, as well as the use of a reward system for patients, as a strategy to improve the quality and consistency of bowel preparations in our Department. The sheathed device also should assist us in overcoming discrepancies in sampling induced by variations in the quality of bowel preparation.

The authors additionally make note of the previous study of the terminal ileum utilising a sheathed-type forceps, which we also cited.[6] This particular study did not identify overall differences in microbiota profiles or diversity when comparing grouped samples obtained with sheathed vs. unsheathed forceps. However, it is important to consider matched samples from individual patients. Subtle changes, in particular bacterial genera, in a single patient, for example, are difficult to assess based on the data in the study by Dave *et al.*[6] The study also did not assess any region of the large bowel, and this represents an area for further investigation regarding luminal contamination.

In conclusion, we concur with Pagnini and Della Fave on the importance of considering sampling methods and bias when investigating the intestinal microbiota. This is particularly relevant when attempting to characterise the mucosal microbiota which is in closest proximity to host tissues. A more detailed understanding of the subtleties of microbiota profiles is essential if we are to move forward