

Many microbiome studies flawed by contamination

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Many published microbiome studies are likely to have been contaminated and may incorrectly report the presence of microorganisms unintentionally introduced from the laboratory environment, says a study published in the open access journal *BMC Biology*. The findings could explain why unexpected bacteria have been previously identified in clinical samples and suggests that studies may have prematurely proposed links to disease.

The ecosystem of more than 100 trillion microorganisms that live in our bodies - the human [microbiome](#) - is essential for a range of vital functions, including the digestion of food, synthesizing nutrients, and preventing disease. Even in areas of the body which normally contain relatively small numbers of microorganisms, such as the lungs, changes in microbial communities are thought to play a role in the development of disorders, including chronic [obstructive pulmonary disease](#) and asthma.

New DNA sequencing technologies have been contributing to the rapid expansion of microbiome research. However, a new study led by the Wellcome Trust Sanger Institute systematically shows that contaminating bacteria are easily introduced from DNA extraction kits, chemical reagents and the laboratory environment, and can affect the results of microbiome analyses.

The researchers found in some cases that 270 different varieties of bacteria were present in control samples, when only one variety would

have been observed if there was no contamination at all. Samples containing low amounts of material or 'biomass', such as those taken from the blood or lungs, were far more likely to be prone to contamination than high-biomass samples from faeces.

Dr Alan Walker, who led the study at the Sanger Institute and is now based at the University of Aberdeen, said: "Recent advances in DNA sequencing technology - which allow extraordinary depth of sequencing - are being used by researchers to analyse extremely sparse microbial populations. What we have now shown is that these types of samples are susceptible to contaminant DNA from any source, whether it is at the time of collection, from handling samples, or during the extraction or amplification process. This can critically impact study results, and we're now advising caution to researchers studying microbiota in low biomass environments."

While the issue of contamination has been reported in the past, for the first time the researchers systematically assessed its impact on samples of varying biomass. To do this, they took a pure culture of *Salmonella bongori* bacteria, and investigated the communities of bacteria which were detected following different dilutions of the culture. Not only did they see an increase in the abundance of contaminating bacterial DNA with increasing dilutions, but at the highest dilutions, the amount of contaminating DNA even exceeded the original *Salmonella* DNA.

The study was repeated across different laboratories, and the contaminating bacterial varieties were found to differ between locations, due to the contaminants present in different chemical reagents and the wider environment. Many of the contaminating varieties of bacteria were those normally found in soil and water, and on the human skin.

The researchers also found a great deal of overlap between the varieties of contaminating bacteria found in their study, and those that have been

reported as noteworthy in previous low biomass microbiome studies. The authors say that while these varieties may have genuinely been present in previous study samples, in many cases they were biologically unexpected, for example, rhizosphere-associated bacteria (naturally found near the roots of plants) that had been implicated in human disease.

To show how contamination could have further impacts on studies, the team also investigated nasal swab samples from a Thai-Burmese refugee camp, which have previously been used to study the carriage of pneumococcal disease. The researchers initially observed patterns of nasal microbiota variation but later realised that these were simply due to different types of contamination from the various batches of DNA extraction kits. The authors say this serves as a reminder of the significant, and potentially misleading, impact that contaminants can have on microbiome studies and their conclusions.

To address these contamination issues, the authors suggest a number of steps which researchers can take when studying low biomass samples. These include using negative controls to identify varieties of [bacteria](#) that might be contaminating samples, maximizing the biomass of the starting sample through filtration or enrichment, and minimizing the risk of contamination during [sample](#) collection.

More information: *BMC Biology*,
www.biomedcentral.com/1741-7007/12/87

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