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Epidemiology and the microbiome

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Until recently,the great successes of hygiene and sanitation in preventing disease obscured the evidence that some microbes found inside the healthy body were supposed to be there.Clinicians, pubic health workers and scientists were blinded to the importance of their existence. But now, because of the exquisite sensitivity of the polymerase chain reaction combined with fast and accurate genetic sequencing, there is indisputable evidence that microbes are found in healthy body sites previously considered sterile: the lung [1], the uterus [2] and even the blood [3].Further, there is strong evidence that we need microbes to grow and maintain a healthy digestion system andprime the immune system [4].

If microbial communities can be 'good' then a dysfunctional microbial community can be 'bad.' Dysfunctional communities are hypothesized to be the cause of several conditions characterized by inflammation, such as periodontitis [5], inflammatory bowel disease [6]and bacterial vaginosis [7] where no single infectious agent has been identified as a causal factor. 'Bad' microbial communities are also associated with obesity [8], cancer [9], and a variety of other conditions. Whether 'good' or 'bad,' inhabitants of microbial communities communicate with each other and their environment (the human host), fight battles, and share food webs. Community structures can enhance or inhibit invasion by newcomers and resist attack by the human host and therapies. Despite having a word for this since 1877 (symbiosis), until recently we have overlookedthis critical factor in disease pathogenesis and transmission.

That microbiota contribute to human health as well as disease is a great insight, but we have a long way to go before we can evaluate the relative contribution of microbiota to disease pathways [10]. There are technical hurdles to conducting population-based studies of the microbiome and comparing results across studies:

significant variation can be attributed to differences in sample collection, storage, and processing, as noted by Fu et al. [11], Brook et al. [12], Robinson et al. [13], and Van de Wijgert et al [14] in this issue. Furthermore, microbiome data are highly dimensional and compositional in nature. This poses significant challenges in analysis, as discussed in the articles by Fodor et al [15], Gloor et al [16] and Van der Wijgertet al [14]. The field has yet to settle on the optimal strategy for finding the signal in this complex compositional data, integrating epidemiologic variables, or developing an analog to Koch's postulates for the microbiome. In particular, determining the extent that microbiota are a necessary versus a sufficient cause of a particular outcome needs further exploration.

Although we await results of large, population-based assessments of the microbiome, it is already clear that there are interactions among environmental exposures, human behavior and the microbiota which may confound or modify disease-microbiome associations as reported by Singh and Manning [17], Foxman et al. [18], and Mai [10]. However, it is difficult to evaluate the importance of reports of variation (despite statistical significance) in the microbiome with health and disease, because we do not yet know how much variation is required to affect human health. This poses challenges for estimating sample size and power [19].

Nonetheless, microbiome studies are already generating important new insights that will affect every subspecialty of epidemiology. Because of their role in colonization resistance it is plausible that the microbiota array could be a target for infection control in hospital settings [20]. Less obvious is evidence that lung microbiota may mediate or moderate human response to air pollution [21] that alteration of the gut microbiota may

be co-factors in the development of psychiatric disorders [22] or that microbiota may hold the key to the etiology of chronic inflammatory conditions like inflammatory bowel disease and periodontitis. This is exciting stuff, and has captured the imagination of the public as well as much of the scientific community.

It also offers an opportunity and challenge for epidemiologists. The opportunity is in characterizing the role of the microbiome in health and disease byconducting studies that address the many criticisms of the design, conduct and analysis ofmicrobiome studies. The challenge is in evaluating if and when results of these exciting new studies should be implemented [23]. Epidemiologists are very aware that we often need to make decisions to protect the public's health based on limited information [24] and that those decisions requiresa thorough evaluation of the risks and benefits.

Currently, we have limited insight into the magnitude of risks or benefits associated with disruptions of the microbiome. For example, a disruption of the microbiome occurs following a short course of antibiotics [25]. Multiple courses of antibiotics can lead to infection by *Clostridium difficile,* which – most of the time – is treated effectively with an antibiotic. It is only the most severe cases that are resistant to antibiotic therapy that are candidates for fecal transplant. Microbiome research was helpful in establishing the mechanism underlying the risk of *Clostridium difficile* infection associated with antibiotic use, but has made no contribution to our understanding of how to prevent transmission and acquisition: good infection control and antibiotic stewardship [26]. How much does a single course of antibiotics increasea hospitalized individual's risk of*Clostridium difficile*infection by disrupting their

microbiota? And how much does that risk increase following each course of antibiotics? We have no idea.

Similarly, there is substantial evidence that the microbiota of babies delivered by cesarean section is different from those delivered vaginally [27]. Babies born via cesarean section have increased risk of celiac disease, asthma, and type 1 diabetes [28, 29]. Estimated increases in risks range from 20%, to 40%, for diseases that occur from 2.28/1000 (type 1 diabetes) to 8/100 (asthma) children [30, 31]. One hypothesized mechanism is that because an infant born by cesarean has a different microbiota from one born vaginally, the resulting changes in the microbiota –immune system interactions lead to disease. Is this true? We don't know. The supporting evidence is much more tenuous than that regarding antibiotics and *C. difficile*. And even if we could point to specific changes in the microbiota as the underlying mechanism, how much of theincreased risk of these adverse health outcomes (which are multi-factorial) might be attributed to lack of exposure to mother's vaginal microbiota during birth?

A recent study suggests that feeding infants born via cesarean their mother's vaginal microbiome might somewhat restore the microbiome to that of a vaginally borne infant [32]. This also risks exposing the infant to known potential infection, e.g., Group B streptococcus. Are the potential benefits of this intervention worth the risks? A great thing about being an epidemiologist is we know how to design the right studies to answer these questions. We hope that this special issue has inspired you to jump into the fray, but with eyes wide open.

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