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Opinion

Does Person-to-person Contact Confound Microbiota Research? An Important Consideration in the Randomization of Study Arms

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The current paradigm assumes that randomization eliminates all confounding factors.[1] In this opinion, I challenge this view. For instance, changes in parent-child relationships and/or fraternal relationships represent a new phenomenon that did not exist before.[2,3] A curious question arises: Is it possible that changes in person-to-person contact due to pandemics, social distancing, and other factors could alter the microbiota composition in individuals?

A recent paper by Valles-Colomer, published in Nature, on person-to-person transmission of the gut and oral microbiomes has significant implications for medical/biomedical research, medical practice, study design, and data analysis.[4] However, these implications have not received much attention, particularly in contemporary probiotic and antimicrobial research. The study detected astonishing patterns of extensive bacterial strain sharing among individuals, with marked and recognizable intra-household, mother-to-infant, and intra-population transmission patterns. This finding, along with similar studies,[5,6] will likely impact medical and biomedical sciences in many ways. In this opinion, I attempted to connect these pioneering works with recent probiotic supplementation studies conducted during the COVID-19 pandemic,[4–10] just to mention a few. There is no doubt that these studies followed standard procedures. However, I argue that there is a "possibility" of hidden bias that might have arisen due to altered social dynamics, closeness, and person-to-person microbial transmission during the COVID-19 pandemic, particularly in non-randomized clinical trials, and potentially even in small-sample randomized clinical trials.

Compelling evidence shows that changes in parent-child relationships and/or fraternal relationships due to COVID-19-imposed social distancing may introduce bias, leading to inaccurate estimates of results. In particular, publication and expectation biases could lead to significantly higher estimates of efficacy in studies on oral and gut microbiota.[11,12] Here, we must take a closer look at "closeness", defined as the average distance from one node to all others.[13] Recent studies conducted during the COVID-19 pandemic show that the "closeness" between parents and their children/infants was highly dynamic among families.[13] This suggests that behaviors such as kissing and other forms of bodily contact, which can lead to microbiota transmission, varied significantly and were not necessarily consistent across all families.

Methodologically, randomization ensures that potential confounding factors are evenly distributed among treatment groups.[1] However, in short-duration studies, uncertainties may arise from factors such as the nature of oral ecology, microbiome transmissibility, microbial population dynamics, and the varying time courses of interactions and medication effects.[14,15] In such cases, it is unlikely that randomization alone can be considered a reliable method—especially in studies using single-dose interventions. These interventions may preclude the exploration of optimal dose-response relationships for microbiota strains and sub-strains in treatment.

Supporting this argument, there is evidence that even after randomization, significant differences in calorie, carbohydrate, fat, and protein intake may exist between two arms of the clinical trial,[16,17] all of which can significantly influence baseline microbiota levels. Even more interestingly, some randomized clinical trials have shown that participants' baseline gut microbiota (confirmed through beta diversity analyses) differed significantly from controls, though not from each other.[18] Intriguingly, in other medical disciplines like pulmonary medicine, differences in baseline microbiomes have been reported between groups in trials comparing sputum microbiota in adults with cystic fibrosis.[19] This direct and indirect evidence suggests that the possibility of non-normality in baseline microbiota in clinical trials may not be easily dismissed.

The most direct supporting evidence comes from a recent paper by Griffen *et al.*,[5] which enrolled 55 biological and 50 adoptive mother-child dyads to determine the effect of genetic relatedness on the fidelity of oral bacterial transmission. Adoptive mother-child dyads were recruited through adoption agencies. To match the adoptive group by parents' socioeconomic status and children's age, a biological group was also enrolled. To minimize bacterial transmission from biological mothers, only children adopted at birth and unrelated to the adoptive family were included. In the biological group, only genetic birth mothers were included, and fathers and siblings were also sampled when available. To allow for the establishment of an oral bacterial community, children in both the biological and adoptive groups were between three months

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and 12 years of age. Exclusion criteria for all participants included chronic diseases affecting the immune system, oral cavity, or early onset periodontitis. For all three niches sampled—supragingival plaque, saliva/soft tissue, and subgingival plaque—the microbial profiles of adopted and biological children were equally similar to their mothers at both the species and strain levels. No genetic influence was found on the acquisition of oral bacteria. At the strain level, all mothers and their children, regardless of genetic relationship, were significantly more similar to each other than unrelated mother-child pairs. This relationship was less pronounced at the lower resolution species-level approach. Similar results were observed for comparisons between adoptive and biological groups (ISR soft tissue/saliva) when using relative abundance measures instead of presence/absence measures. For instance, one study investigated the effect of fecal microbiomes on mother-infant dyads, especially during the early postpartum period.[6] Based on this study, there is a complex microbial interaction between breastfeeding mothers and their infants, which indirectly supports the idea that changes in the milk microbiome may influence the infant's gastrointestinal microbiome. These two findings provide the most direct evidence for our argument that altered "closeness" during the COVID-19 pandemic has the potential to introduce uncertainties in bacterial transmission.[5,6]

Considering the complex network of correlations between parent-infant relationships and microbiome transmission, the results of probiotic supplementation studies would inevitably be affected, especially when sample sizes are small. It would be prudent to consider these issues when designing future studies.[20–22]

Altered oral and gut microbiota are implicated in the development and progression of many medical conditions.[23] On the other hand, clinical trials typically enroll a minimum sample size based on alpha statistics.[24,25] With these considerations, it seems highly unlikely that randomization alone accounts for the confounding effect of inter-individual microbiota variation and differences in closeness. This implies that many clinical trials conducted during the COVID-19 pandemic may have been subject to hidden bias. This bias is not confined to clinical trials but spans a wide range of diseases influenced by differential oral and gut microbiota. It also affects daily clinical practice. Heterogeneous results in clinical trials might be partially explained by the lack of standardized methodologies to match participants (i.e., cases and controls) in terms of oral and gut microbiota dynamics at each step of the study process, highlighting the need for clear guidelines.[26–29]

Moreover, the perspective of these novel studies on person-to-person microbial transmission creates a unique opportunity to test a myriad of hypotheses.[4–6] For instance, if microbiome composition contributes to a particular disease or condition, sharing a household with someone who has a distinct gut or oral microbiota pattern could influence study results and potentially predict the outcome of interest, at least to some extent.

Consider this hypothesis: if microbiome composition contributes to glucose intolerance, sharing a household with a shift worker who is already known to have a higher risk of metabolic disturbances would theoretically increase the risk of metabolic disturbances, again, at least to some extent.[30] Most readers would agree that conducting a clinical trial under such conditions would be methodologically, practically, and economically challenging. However, a researcher could easily test this hypothesis by co-housing host mice with a mouse exposed to the variable of interest and then measuring the microbiota and glucose homeostasis of the host to gather preliminary data. Similar experiments based on studies of person-to-person microbial transmission would represent a major advance in microbiota research.[4–6]

This argument can be criticized in several ways:

- Firstly, clinical trials should account for this bias in future research. However, there is currently very little insight or perspective on how to address this issue in real clinical trial settings. Many factors, some of which are still unknown, can impact microbiome composition and, consequently, disease outcomes. The real question is how to incorporate this consideration. I call for suggestions on the best methods to account for this potential bias. One lesson for the next pandemic is the need to develop tools to measure "closeness" as a confounding factor, both qualitatively and quantitatively.
- Secondly, it might be argued that the level of closeness between parents and children was different during the pandemic, such as through behaviors like kissing. Do we know for certain if parents' behavior changed during the pandemic? Yes, we spent more time with our families, but outside the pandemic, children would have been exposed to other kids at school or kindergarten, which would have introduced them to a broader array of people and children, potentially affecting their microbiomes differently.
- Thirdly, applying proper randomization and using an appropriate sample size should balance out any effects that social distancing might have had on the microbiome. Additionally, the period of social distancing was relatively short, and normal life has resumed since the end of the pandemic. It could be argued that we are uncertain whether there will be any lasting effects.
- Lastly, many other factors, such as genetics, nutrition, lifestyle, and access to healthcare systems, would likely play a more significant role in this context.

In conclusion, if this argument proves valid, we could extrapolate that all prognostic, diagnostic, cross-sectional, and interventional studies should account for the potential confounding effect of closeness differences when designing studies that involve body microbiota. This possible confounding variable would also impact allocation methods and sample size determination formulas used in clinical trials. This opinion has important implications for pharmacological, microbial, and infection studies, both clinically and epidemiologically. Furthermore, it underscores the need to develop practical tools for measuring closeness as a confounding factor, both qualitatively and quantitatively, for future preparedness.

Aesthetically, I like to refer to this confounding phenomenon as "French Kiss Bias", even though we know oral and gut microbiota are transmitted via multiple routes.

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Conflict of interest

The author declares no competing interests.

Author contributions

RR is the sole author of this manuscript.

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