Antibiotics and the Human Microbiome: A Survey of Prescribing Clinicians' Knowledge and Opinions Regarding the Link between Antibiotic-Induced Dysbiosis and Immune-Mediated Disease

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ABSTRACT

Altered composition or function of the human microbiome, termed dysbiosis, has been associated with a variety of immune-mediated diseases. Antibiotic use is a well-studied cause of dysbiosis. We conducted an electronic survey of 351 antibiotic-prescribing clinicians in Rhode Island to evaluate antibiotic prescription patterns, knowledge and opinions regarding the importance of the human microbiome and its relation to antibiotics and the immune system. We found that clinicians view the health of the human microbiome as important when prescribing antibiotics; however, they do not feel well-informed or confident in their knowledge about the microbiome or its relevance to patient health. A higher level of self-reported knowledge about the microbiome was associated with increased importance placed on the microbiome and its relevance to medical practice. Our results indicate that clinicians may benefit from continuing medical education on the link between antibiotic-induced dysbiosis and immune-mediated disease.

KEYWORDS: microbiome, antibiotics, dysbiosis, autoimmune, atopy, inflammation

INTRODUCTION

The human body hosts over 100 trillion interdependent commensal, symbiotic, and pathogenic microorganisms collectively termed the human microbiome.[1] These microorganisms regulate critical processes including energy metabolism[2] and immune system homeostasis.[1] Change in the microbiome composition and function, termed dysbiosis,[3] has been associated with many immune-mediated diseases, including disorders that are atopic,[4] inflammatory,[5] and autoimmune.[6,7] Several studies have demonstrated that antibiotics cause dysbiosis in mice and humans. Even short-term antibiotic treatment can decrease microbial biodiversity, alter microbiome metabolic functions, and alter susceptibility of the host gut to colonization by pathogenic microorganisms,[8] which can persist for months after treatment.[9]

The incidence and prevalence of many immune-mediated diseases, such as multiple sclerosis,[10] type I diabetes,[11] and inflammatory bowel disease,[12] have markedly increased over the past several decades.[13] Environmental factors, including antibiotic-induced dysbiosis, may contribute to disease pathogenesis. Indeed, human cohort studies have demonstrated an association between perinatal antibiotic use and risk in the offspring of atopic dermatitis[14] and allergic asthma.[15] Moreover, perinatal and neonatal use of certain antimicrobials is associated with increased risk of type I diabetes in the offspring.[16]

Antibiotics comprise roughly 25% of medications prescribed for children,[17] and an estimated 1 in 4 antibiotic prescriptions is inappropriate.[18] Given the high frequency of inappropriate antibiotic use, the increasing incidence of immune-mediated diseases in developed countries, and growing evidence that links antibiotic-induced dysbiosis with immune-mediated disease, it is important to study this relationship from the perspective of the clinician in practice. Herein, we present the results of an electronic survey of Rhode Island prescribers aimed at evaluating clinicians' antibiotic prescription patterns, their knowledge and opinions of the importance of the human microbiome, and its relation to antibiotics and the immune system.

MATERIALS & METHODS

Survey Development

Survey questions utilized a 5-point Likert scale (1 = Strongly agree, 2 = Agree, 3 = Neither agree nor disagree, 4 = Disagree, 5 = Strongly disagree), ranking options, and multiple choice. Questions were designed to evaluate clinician knowledge and opinions regarding antibiotic prescribing patterns and antibiotic-mediated effects on the human microbiome. Additional demographic questions included years in medical practice, medical specialty, practice location, and prescription patterns.

The survey was pilot-tested with seven Rhode Island physician volunteers who provided feedback on the survey's grammar, clarity, content, and functionality. The Brown University Institutional Review Board (IRB) approved the study. The survey email list was obtained through the Rhode Island Department of Health's publicly available licensee list of all physicians (including MD, DO, and limited practice), nurses (including APRN, LPN, and, RN) and physician assistants. Only those clinicians able to prescribe antibiotics, (MD, DO, PA, APRN) were included.

The survey was emailed via Qualtrics Experience Management (XM) Survey software to 3,108 unique email addresses in the Fall of 2019. Reminder emails were sent one and two months after the original distribution of the research survey.

Data Analysis

For Likert-type scale questions, responses were numerically coded as above. Mean answer values were compared to calculate statistically significant difference between groups. To quantify self-reported antibiotic knowledge, the average of the numerical values of each participant's responses to the Likert-type questions, "I would feel confident in my knowledge when discussing the human microbiome with patients," and "I consider myself well-informed regarding the significance of the human microbiome with respect to medical practice," were calculated. Individuals were classified as "self-reported knowledgeable" if the average value was greater than 3 and "self-reported unknowledgeable" if the average value was less than or equal to 3.

Prescription frequency was categorized as "Frequently" (daily or weekly), or "Infrequently" (monthly, less than monthly, or never). Regarding practice location, urgent care practitioners were combined with Emergency Department clinicians.

Statistical analyses and figure generation were performed using OriginPro 2018b software. A two-tailed Exact test was used for all statistical comparisons of two groups. Comparisons of more than two groups were performed using one-way ANOVA with Tukey Post-Hoc test.

RESULTS

Characteristics of Respondents

The Survey response rate was 11.3% and a demographic breakdown of the 351 clinician survey respondents is reported in **Table 1 (See Supplement)**. Most respondents were physicians (92%), the majority of whom practiced either internal medicine, family practice, or pediatrics (combined 42.4% of physician respondents) in the outpatient setting (55.7% of all respondents). The majority of respondents have practiced for >20 years and prescribe antibiotics frequently (daily or weekly).

Univariate Analyses

Survey participants viewed clinical reference tools (e.g., UpToDate) as the most important resource when selecting antibiotics (mean rank = 2.26) (**See Supplement, Figure 1**). Infectious disease physician consultation was least important (mean rank = 4.14).

The majority of respondents viewed "prevention of antibiotic resistance" as the most important factor when prescribing antibiotics (mean rank = 1.52; **See Supplement, Figure 2**). However, almost as many respondents ranked "protecting the microbiome" as #1 or #2 (N=132) compared with "avoiding adverse effects" (N=147). Moreover, "protecting the microbiome" (mean rank = 3.04) was viewed more importantly than "reducing costs of care" and "improving patient compliance" (mean ranks = 3.65 and 4.13, respectively).

Most prescribers (89.3%) agreed or strongly agreed that it is important for clinicians to learn about the human microbiome, and 82.1% agreed or strongly agreed that careful selection of antibiotics is important to protect the human microbiome (**See Supplement, Figures 3,4**). However, only 38.7% consider themselves well-informed regarding the clinical relevance of the microbiome, and only 35.8% reported sufficient confidence in their knowledge of the microbiome to discuss its clinical relevance with patients.

Respondents showed the greatest degree of uncertainty when questioned about the connection of the microbiome and the immune system. Approximately 40% of respondents neither agreed nor disagreed with the statement "antibiotic-induced dysbiosis is a feasible predisposing factor for the development of immune-mediated disease," although very few (3.6%) disagreed or strongly disagreed. Most respondents (80.1%) agreed or strongly agreed that they would change their prescribing behaviors given evidence linking antibiotic-induced dysbiosis and development of immune-mediated diseases.

Bivariate Analyses

Emergency Department (ED) providers placed significantly higher mean importance on antibiograms than prescribers from inpatient practice, whereas outpatient prescribers placed highest mean importance on clinical reference tools (**See Supplement, Table 2**). All prescribers placed the least mean importance on infectious disease physician consultation. However, inpatient clinicians placed significantly more importance on infectious disease physician consultation than ED and outpatient prescribers. Outpatient clinicians valued prior clinical experience significantly higher than did inpatient or ED clinicians. Responses to items from Figure 2 did not vary by practice location (data not shown).

Regarding questionnaire items assessing perceived importance of the human microbiome, we found ED clinicians agreed significantly more with the statement: "There is insufficient primary research as a field of study for clinicians to consider how the human microbiome affects patient health," than inpatient or outpatient clinicians (**See Supplement, Table 3**). All other questionnaire items lacked statistically significant differences based on practice location.

Finally, regarding clinicians' opinions on potential effects of antibiotics on the human microbiome, we found clinicians practicing in the ED were significantly more resistant to changing their prescription patterns if presented evidence that antibiotic-induced dysbiosis predisposes patients to the development of immune-mediated disease compared with inpatient or outpatient prescribers (**See Supplement, Table 4**). All other responses by clinicians from each practice location reflected univariate trends.

We found that self-reported knowledge did not influence clinician mean ranking scores for the relative importance of clinical reference tools (data not shown). However, self-reported knowledgeable clinicians agreed significantly more with all statements attesting to the importance of the microbiome and with statements linking the microbiome and immune-mediated disease. (**See Supplement, Tables 5,6**).

To determine whether clinicians' responses to questionnaire items differed by antibiotic prescription frequency, we compared frequently-prescribing (daily or weekly), and infrequently-prescribing (monthly or less frequently) clinicians. We found frequently-prescribing clinicians agreed significantly more with the statement: "It is safer to prescribe narrow-spectrum antibiotics than broad-spectrum antibiotics because more selective killing of pathogenic organisms maintains the diversity of the human microbiome," (data not shown). No other statistically significant differences were found for any other questionnaire item.

DISCUSSION

Perturbation of the normal flora of the microbiome, termed dysbiosis, is associated with a variety of immune-mediated diseases.[4,5,6,7] Based on the established relationship between antibiotic use and dysbiosis,[8] and that recent years have seen increased worldwide incidence and prevalence of immune-mediated diseases[13] and significant inappropriate use of antibiotics,[17,18] antibiotic-induced dysbiosis represents a potential environmental contributor to the development of these diseases. As a preliminary approach to providing a context, we assessed caregiver attitudes and knowledge relevant to this relationship.

Although the microbiome demonstrates marked differences in composition depending on environmental factors like age, geography, ethnicity, and diet,[19,20] consistent changes in microbiome composition have been observed in response to certain antibiotics. For instance, vancomycin and amoxicillin reduce populations of *Enterobacteriaceae* and *Bifidobacterium*, respectively.[2] Similarly, specific changes in the microbiome have been linked with specific disease states.[15,21-23] For instance, atopic dermatitis and asthma have been associated with a decreased total diversity of the human microbiome, whereas inflammatory bowel disease has been linked with decreased proportions of *Clostridia* and *Bacteroides* and increased proportions of *Enterobacteriaceae.*[24,25]

Separated only by intestinal epithelial cells and a thin layer of overlying mucus,[20] the host immune system and intestinal commensal microorganisms interact via well-established molecular mechanisms including toll-like receptor and cluster-of-differentiation signaling.[14,26] For instance, *B. fragilis* use a capsular polysaccharide to induce anti-inflammatory IL-10 expression from dendritic cells via TLR-2 signaling, and *Clostridium* strains can induce TGF-β expression from intestinal epithelial cells.[2] Both mechanisms enhance the function of regulatory T cells (Treg), which are critical for development of immunogenic tolerance. Additionally, microbial fermentation products such as propionate and butyrate may epigenetically enhance Treg function by upregulating the Foxp3 transcription factor.[2]

Germ-free animals have been found to have defects in the development of gastric-associated lymphoid tissue, Peyer's patches, and mesenteric lymph nodes.[17] Perhaps even more importantly, recent studies have shown a dependence of thymic lymphocyte development on the presence of gut microbes in early life.[27] Given these observations, it follows that dysbiosis in early life may disrupt the development of immunogenic tolerance.

Although the human microbiome has been a prominent topic of research in recent years, few studies have been aimed at evaluating the consequences of antibiotic-induced dysbiosis. Nonetheless, the aforementioned evidence supports a connection between antibiotic-induced dysbiosis and subsequent immune-mediated disease. It follows that a clinician's antibiotic prescribing patterns might influence patients' microbiomes towards a dysbiotic state. To our knowledge, our study is the first survey of medical providers evaluating prescriber awareness and opinions regarding the link between antibiotics, microbiome dysbiosis, and immune-mediated disease.

When asked to rank five commonly utilized clinical resources when prescribing antibiotics most clinicians placed first- or second-most importance on the use of antibiograms, prior clinical experience, and clinical reference tools, whereas antimicrobial stewardship programs and infectious disease physician consultations were considered less important. Given the emerging nature of information on antibiotic use, dysbiosis and immune-mediated disease, antimicrobial stewardship programs assume a unique role.

The observation that clinicians overwhelmingly agreed that prescription of specific antibiotics is important in preventing antibiotic resistance was expected. In contrast, we were surprised to learn that prescribers viewed protection of the human microbiome to be almost as important as avoiding adverse effects when selecting antibiotics. Moreover, almost 90% of clinicians agreed or strongly agreed that it is important for clinicians to acquire knowledge regarding the human microbiome and that alterations to the human microbiome have important implications for human health. Fewer than 40% of respondents considered themselves knowledgeable in this area.

Finally, we found a high degree of uncertainty with statements about the link between dysbiosis and autoimmune diseases, with many respondents neither agreeing nor disagreeing. Overall, our data suggest prescribers recognize the importance of the microbiome when prescribing antibiotics but do not feel confident in their relevant knowledge.

Opinions regarding the human microbiome appear to

differ across practice settings. This may, at least in part, be due to differences in time and resource constraints or patient populations treated. ED providers valued consultation of other providers less than inpatient providers. However, ED providers more highly valued quicker clinical references (e.g., UpToDate) than did inpatient providers. Additionally, ED providers agreed more than inpatient and outpatient providers that the human microbiome is insufficiently well understood to consider during medical practice. ED providers were also more resistant to modifying their prescribing practices given evidence linking antibiotic-induced dysbiosis and immune-mediated disease. Inpatient providers more equally utilized all mentioned clinical resources, perhaps highlighting the need for additional expertise when caring for patients with multiple complex medical issues in the inpatient setting.

As our study findings are limited to Rhode Island and our response rate was low, our results may have limited generalizability. Additionally, our use of "self-reported knowledge (of the human microbiome)" is a limitation of the study because we were unable to assess actual knowledge of the human microbiome. Nonetheless, as expected, we found respondents with a higher self-reported knowledge thought the microbiome was more important and considered it more highly when prescribing antibiotics. However, self-reported knowledge was not associated with a difference in the resources utilized or factors considered when prescribing antibiotics. Future studies assessing concrete knowledge of the pathophysiologic effects of antibiotics on the human microbiome would reveal whether the above is indicative of a Dunning-Kruger effect.

More frequent antibiotic prescribers have a larger impact on patients' microbiomes. However, we found almost no significant differences in any survey responses based on prescription frequency. The lack of an overall trend suggests frequently-prescribing clinicians do so for reasons unrelated to knowledge or opinions regarding the microbiome (e.g., patient pathology, patient volume, or low confidence in clinical knowledge).

Given our findings, investigation into clinicians' actual knowledge regarding the human microbiome seems warranted. The low confidence in reported knowledge of the human microbiome indicates clinicians might benefit from continuing medical education regarding antibiotic-induced dysbiosis and immune-mediated disease, particularly given the growing research in this field and growing clinical importance for individuals and populations. Additionally, further primary research investigating the link between dysbiosis and development of specific immune-mediated diseases could highlight appropriate narrow-spectrum pathogen-specific antimicrobials to promote microbiome health.

References

1. Toor D, Wsson MK, Kumar P, et al. Dysbiosis Disrupts Gut Immune Homeostasis and Promotes Gastric Diseases. *Int J Mol Sci.* 2019;20(10).
2. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology.* 2014;16(7):1024-1033.
3. Francino MP. Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances. *Frontiers in microbiology.* 2015;6:1543.
4. Lee SY, Lee E, Park YM, Hong SJ. Microbiome in the Gut-Skin Axis in Atopic Dermatitis. *Allergy Asthma Immunol Res.* 2018;10(4):354-362.
5. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A.* 2007;104(34):13780-13785.
6. Abdellatif AM, Sarvetnick NE. Current understanding of the role of gut dysbiosis in type 1 diabetes. *Journal of Diabetes.* 2019;11(8):632-644.
7. Girbovan A, Sur G, Samasca G, Lupan I. Dysbiosis a risk factor for celiac disease. *Med Microbiol Immunol.* 2017;206(2):83-91.
8. Lange K, Buerger M, Stallmach A, Bruns T. Effects of Antibiotics on Gut Microbiota. *Digestive diseases (Basel, Switzerland).* 2016;34(3):260-268.
9. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS biology.* 2008;6(11):e280.
10. Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol.* 2010;9(5):520-532.
11. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabet Med.* 2006;23(8):857-866.
12. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology.* 2012;142(1):46-54.e42; quiz e30.
13. Lerner A, Patricia W, Matthias T. The World Incidence and Prevalence of Autoimmune Diseases is Increasing. *International Journal of Celiac Disease.* 2015;3:151-155.
14. Lee SY, Yu J, Ahn KM, et al. Additive effect between IL-13 polymorphism and cesarean section delivery/prenatal antibiotics use on atopic dermatitis: a birth cohort study (COCOA). *PloS one.* 2014;9(5):e96603.
15. Metsala J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clinical and experimental allergy : Journal of the British Society for Allergy and Clinical Immunology.* 2015;45(1):137-145.
16. Kilkkinen A, Virtanen SM, Klaukka T, et al. Use of antimicrobials and risk of type 1 diabetes in a population-based mother–child cohort. *Diabetologia.* 2006;49(1):66-70.
17. Vangay P, Ward T, Jeffrey, Knights D. Antibiotics, Pediatric Dysbiosis, and Disease. *Cell Host & Microbe.* 2015;17(5):553-564.
18. Fleming-Dutra KE, Hersh AL, Shapiro DJ, et al. Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010-2011. *JAMA.* 2016;315(17):1864.
19. Gupta VK, Paul S, Dutta C. Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. *Frontiers in microbiology.* 2017;8:1162-1162.
20. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. *Nature Reviews Immunology.* 2017;17(4):219-232.
21. Goulet O. Potential role of the intestinal microbiota in programming health and disease. *Nutrition reviews.* 2015;73 Suppl 1:32-40.

22. Ipci K, Altintoprak N, Muluk NB, Senturk M, Cingi C. The possible mechanisms of the human microbiome in allergic diseases. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery.* 2017;274(2):617-626.
23. Miyoshi J, Bobe AM, Miyoshi S, et al. Peripartum Antibiotics Promote Gut Dysbiosis, Loss of Immune Tolerance, and Inflammatory Bowel Disease in Genetically Prone Offspring. *Cell reports.* 2017;20(2):491-504.
24. Li M, Wang M, Donovan SM. Early development of the gut microbiome and immune-mediated childhood disorders. *Seminars in reproductive medicine.* 2014;32(1):74-86.
25. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clinical & Experimental Allergy.* 2014;44(6):842-850.
26. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell.* 2004;118(2):229-241.
27. Ennamorati M, Vasudevan C, Clerkin K, et al. Intestinal microbes influence development of thymic lymphocytes in early life. *Proc Natl Acad Sci U S A.* 2020;117(5):2570-2578.

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