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EFFECTS OF ANTIBIOTICS ON GUT MICROBIOTA

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Abstract:

The diversity of the human gut microbiota rapidly develops from birth until three years of age, when it reaches an adult-like stage. After this age, the composition could change as a result of outside influences such antibiotics. Prior research has demonstrated that resilience does not fully develop months after stopping antibiotic use. The short-term consequences of antibiotic consumption on the gut microbial ecology are, however, poorly understood. Here, we looked at the load and makeup of the fecal microbiota in 21 individuals who were treated with broad-spectrum antibiotics such β-lactams and fluoroquinolones right after treatment. For the purposes of microbial load and community composition investigations using quantitative PCR and pyrosequencing of the 16S rRNA gene, respectively, feces samples were taken from each participant one week after treatment and prior to treatment. The core phylogenetic microbiota shrank from 29 to 12 species, and microbial diversity was dramatically reduced by 25% as a result of fluoroquinolones and β-lactam antibiotics. On the other hand, these drugs raised the Bacteroidetes/Firmicutes ratio at the phylum level ($p = 0.0007$, FDR = 0.002). Our results unexpectedly showed that both antibiotic kinds raised the proportion of several unknown taxa that belong to the Gram-negative Bacteroides genus of bacteria at the species level ($p = 0.0003$, FDR<0.016). Moreover, the therapy had an impact on the mean microbial burden. In fact, it was considerably elevated by two times ($p = 0.04$) by the β-lactams.

Key Words: Antibiotics, gut microbiota, broad-spectrum

1. Introduction

Research on the impact of antibiotics on the native gut microorganisms has been thorough since the development of these medications in the 1940s and their subsequent mass manufacture. Most research has focused on the impact of single antibiotics on isolated strains of bacteria in controlled laboratory settings or on particular bacterial species from hosts exposed to antibiotics. Most of these investigations have used high medication concentrations compared to what is typically found in wild microbial communities and have concentrated on harmful bacteria. Our

knowledge on the impacts of antibiotics is mostly focused on how they kill bacteria and the precise genetic and physical traits that make bacteria resistant.

This understanding is limited to a small portion of the gut microbiota and does not consider the broader community. (Modi et al., 2014)

The assumption that broad-spectrum antibiotics have an impact on gut microbiota is contradicted by the maintenance or potential increase in microbial load as well as the preference for Gram-negative over Gram-positive bacteria. This study outlines the impact of antibiotics on the gut microbiota and their potential implications for health and disease.

Methodology:

The study involved 21 hospitalized patients aged 18-80 with non-digestive illnesses, who provided stool samples before and after a seven-day antibiotic treatment. The antibiotic dosage was determined according to the infection's etiology and patient characteristics. Fecal samples were collected before and after the seventh day of antibiotic treatment to study the microbial makeup.

Results:

Twenty-one patients, consisting of 18 men and 3 women with a median age of 69 years, were included in the study after being hospitalized for bronchial infection (N=15), urinary infection (N=1), or other diseases such as pneumonia, bacteremia, or prostatitis (N=5). They were administered antibiotics or antibiotic combinations for seven days and submitted fecal samples right before and one week after initiating the antibiotic treatment, namely on the seventh day.

Healthcare providers frequently recommend antibiotics for treating infections. The selection of antibiotics is clearly outlined in clinical guidelines to target particular diseases, whether they are Gram-positive or Gram-negative bacteria. Yet, there is limited knowledge regarding the impact of antibiotics on the overall structure and quantity of the gut microbiota right after therapy. (Panda et al., 2014)

The human fecal microbiota consists of four primary types of bacteria, known as phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. The first two phyla make up about 80% of the microbiota. Firmicutes consist predominantly of Gram-positive bacteria characterized by low G+C concentration in their DNA, although they also encompass Gram-negative bacteria. Bacteroidetes are Gram-negative bacteria, primarily found in the human gut, with the Bacteroides genus being the predominant representative. Proteobacteria are Gram-negative bacteria that encompass a diverse range of extensively researched diseases. Actinobacteria are a

cluster of Gram-positive bacteria characterized by a DNA composition that contains a high G+C content. (Panda et al., 2014)

Since the 1940s when antibiotics were first introduced, the immediate impact of these medications on gut microbiota has primarily been studied using culture methods. Due to the challenge in cultivating cultures for the majority of gut bacteria, the data obtained from this method is inadequate for comprehending the complete antibiotic targets. Recent research have utilized high- throughput sequencing techniques to thoroughly analyze the prolonged impact of antibiotics. The results indicate that therapy leads to a notable change in the gut microbiota composition and a reduction of microbial diversity in the digestive system by around 25% to 33%. The microbiota is quite durable and reverts to its original state a few weeks after stopping the medicine. (Panda et al., 2014)

Recent research on the long-term consequences of antibiotic ingestion have demonstrated that microbiota does not fully recover three months after therapy stops. Differences in resilience seen may be attributed to variations in the approach employed for analyzing microbiota variability: TGGE versus high-throughput sequencing technique (Table S1). (Panda et al., 2014)

A 10-fold decrease in bacterial 16S rDNA was seen in experimental animals following antibiotic treatment, as detected by qPCR. Hill et al. demonstrated that bacterial depletion led to anatomic, histologic, and immunologic alterations indicative of decreased microbial stimulation. The authors demonstrated that the transcript levels of ifng and il17a genes, which encode for IFNγ and IL-17A, were notably decreased in the small intestine of animals treated with antibiotics compared to the control group. This indicates that microbial signals play a role in regulating normal intestinal effector T lymphocyte populations. No data in human adults has been provided on the combination of microbial load and microbial composition analysis before and after using antibiotics. (Panda et al., 2014)

We utilized quantitative real-time PCR (qPCR) and high-throughput sequencing methods to analyze the immediate impact of antibiotics on the composition, structure, and abundance of the gut microbiota in patients undergoing a seven-day antibiotic treatment. Studies have been undertaken for many years to understand how antibiotics affect different strains and species of bacteria in the gut and how drug resistance develops. Recently, attention has shifted towards studying the impact of antibiotics on the taxonomic composition of fecal microbiota, as well as the quantity and variety of antibiotic resistance genes. The impacts on important community-wide characteristics of the gut microbiota and the host response are still not receiving much attention. One of the first observed effects of antibiotics on the stomach was the reduction of colonization resistance, also known as "competitive exclusion." The loss was seen in the increased susceptibility to colonization and illness by Salmonella right after antibiotic treatment. Resource competition and direct interference competition both contribute to the intact microbiota's resistance to pathogen invasion. Indirect factors involve activating several innate

immune response pathways and effector molecules. Antibiotics significantly affect the community structure, leading to major disruptions in resources and interactions between species.

Recent research in mice indicates that antibiotics cause a rise in the amount of host-produced free sialic acid in the intestines. This sialic acid can be used by opportunistic pathogens like Salmonella typhimurium and Clostridium difficile to boost their growth. (Modi et al., 2014)

Research on the impact of antibiotics on gut microbiota has shown reduced bacterial diversity, specific changes in the abundance of certain taxa, partial recovery in most individuals but lasting effects in some, and effects that vary depending on the antibiotic and the individual. Antibiotics with potent and wide-ranging effectiveness against anaerobic bacteria, such as clindamycin, usually have the most significant and enduring impact on the composition of the gut microbiota. Jakobsson researched the effects of a seven-day treatment of clarithromycin, metronidazole, and omeprazole on the pharyngeal and fecal microbial composition. The study revealed significant and long-lasting changes in microbial composition, with certain effects persisting for at least four years following treatment. (Modi et al., 2014)

Ciprofloxacin has minimal impact on typical anaerobic bacteria but significantly alters the composition of the gut microbiota. Dethlefsen et al. discovered that after five days of ciprofloxacin treatment, approximately one-third of the bacterial taxa in the gut were affected, leading to a drop in taxonomic richness shortly after exposure. Certain responses in the human patients were consistent, like sudden reductions in bacterial diversity and loss of many Ruminococcaceae. Other responses were unique, such as the extent or timing of community composition recovery after each ciprofloxacin administration. Most individuals showed almost full recovery within four weeks following exposure, while certain compositional effects persisted for up to six months. The absence of symptoms or indicators in these individuals highlights the significance of functional redundancy within the microbiome. A second exposure to ciprofloxacin, equivalent to the first one, resulted in comparable acute effects but led to incomplete recovery in some instances. Ecologists have observed that combined stressors, such as antibiotics, can result in unexpected ecological outcomes. (Modi et al., 2014)

Research is also being conducted to investigate the impact of antibiotics on additional characteristics of the host that are connected with microbiota. Cho et al. demonstrated that exposing mice to low doses of antibiotics early in life not only changed the composition of their intestinal microbiota but also led to increased body fat mass, bone density, and production of short-chain fatty acids by the intestinal microbiota. Additionally, it affected hepatic fatty acid metabolism. The extent to which these effects manifest in children and their dependency on other microbial, host, and environmental factors is still unknown. (Modi et al., 2014)

Methodology:

The study involved 21 hospitalized patients aged 18-80 with non-digestive illnesses, who provided stool samples before and after a seven-day antibiotic treatment. The antibiotic dosage

was determined according to the infection's etiology and patient characteristics. Fecal samples were collected before and after the seventh day of antibiotic treatment to study the microbial makeup.

Genomic DNA extraction was performed using a mixture of guanidine thiocyanate, 0.1 M Tris (pH 7.5), and 10% N-lauryl sarcosine. The 16S rRNA gene V4 variable region was amplify using PCR. The sequences were analyzed with QIIME and stored in the NIH Short Read Archive. After removal of low-quality reads, 159,536 high-quality sequences were obtained from 42 samples by pyrosequencing, with an average of 3945 sequences per sample.

Sample: samples were subjected to rarefaction analysis with 10 repetitions at 100 intervals, ranging from 100 to 2000 sequences. Microbial community changes were analyzed by β diversity studies using 2091 sequences for each sample, except for one sample which had 1331 sequences. A rarefaction curve was generated to evaluate sequencing depth, and an average value of 98.26% was determined.

Instrument: Microbiological burden assessment was performed using quantitative real-time PCR (qPCR) with primers V4F_517_17 and V4R_805_19 to measure the microbial quantity. The data were analyzed using Applied Biosystems Sequence Detection Software 1.4.

Statistical Analysis: Statistical analysis was performed using the D'Agostino-Pearson omnibus normality test, paired t-test, Wilcoxon matched-pairs signed-rank test, and ANOVA. Taxa associated with antibiotic use were identified using the otu_category_significance.py function in the QIIME pipeline and ANOVA. (Panda et al., 2014)

Results

Changing microbiota following treatment:

Based on 16S rRNA sequence analysis and all antibiotic types, we found that seven days of treatment caused a global change in microbial community structure, as shown by the separate clustering of samples before and after antibiotic intake with weighted and unweighted UniFrac methods (Figure 1 and Figure S2).

These methods compare microbial communities by how much their species share branch length on a bacterial tree of life. This suggests antibiotics changed microbial numbers and composition. Weighted (38%) UniFrac clustered more than unweighted (15%), suggesting antibiotics altered both abundance and composition.

The core phylogenetic microbiota dropped from 29 to 12 taxa, accounting for 36% of sequences and changing from Faecalibacterium to Bacteroides as the leading genus. Two Bacteroides taxa were novel to the core's 12 microbial species before treatment (Table 2). Bacteroides genus increased 2.5-fold ($p=0.0003$, FDR=0.016). Antibiotics (β-lactams and fluoroquinolones) decreased Firmicutes and increased Bacteroidetes at the phylum level ($p<0.001$; FDR=0.002) (Figure 2). Figure S3 shows that antibiotics alone or in combination increased the

Bacteroidetes/Firmicutes ratio, except for piperacilin/tazobactam and levofloxacin/metronidazole. This medication combination inhibits peptidoglycan subunit synthesis (piperacilin) and β-lactamase (tazobactam). Both levofloxacin and metronidazole block nucleic acid production enzymes. (Panda et al., 2014) nurse satisfaction, workload, and documentation ease among 719 nurses [24]. These improvements were observed at three and six months after implementation, in addition to a baseline. The study identified the nurses' impression of interacting with the pharmacy as a barrier. The results indicated that the tendencies in communication remained constant over time. The adoption of novel technologies can be impacted by a multitude of factors, including the age of the customer, prior experience with paper-based systems, and the perspectives of nurses regarding the implementation's potential negative impact on patient safety. Notwithstanding the obstacles associated with the integration of novel technologies, nurses consistently demonstrate enhanced performance as they gain proficiency in the novel systems.

Individuals worldwide are increasingly becoming informed and involved in their own healthcare by utilizing social media resources and online health applications and tools. 64% of patients desire new technologies that allow their doctor to remotely track their health via vital signs and treatment responses from a device in their own residence, in accordance to the Dell Officer and Patient

Survey [29]. Furthermore, a significant majority of patients (61%), request access to their personal health records via an Internet portal or a private website. Already, a significant number of individuals are utilizing wellness monitoring technologies. 59 percent of individuals already utilize a home well-being surveillance device, such as a glucose monitor, blood pressure tester, or another device, and 56% of patients have electronic information exchange with their physician or hospital, according to this survey. Patients have the capacity to significantly influence the enhancement of their own health by utilizing technology that facilitates self-care [30].

The nursing profession stands to gain significantly from the implementation of ubiquitous technology. Nevertheless, the participation of nurses in the development and assessment of these items is critical in order to instill confidence in their data and applicability. Wearable technology can deliver information in a manner that is more dependable and less intrusive. The patient's use of digital-reporting apparatus constitutes an overt act that promotes active engagement in self-care. The implementation of wearable devices during patient care provides healthcare providers and nurses with the ability to tailor treatment to the specific issues and requirements that have been identified through data analysis.

In contrast to earlier technologies that were confined to collecting data within a laboratory or office setting throughout patient visits, the advanced technologies represent a paradigm shift toward greater patient care customization and responsiveness. Clinical decision support that leverages the wealth of information offered by wearable technology permits the formulation of

judgments on the basis of an infinite number of data points, including subjective as well as objective information.

It has been demonstrated repeatedly that the well-being and recuperation of patients are positively impacted by the support of family and acquaintances [31]. By utilizing peripheral gadgets, relatives can be promptly notified of any concerns or potential emergencies, enabling them to take appropriate action. More nursing-focused, evidence-based research utilizing ubiquitous technology is required. In addition to engaging with patients throughout the adjustment and learning phases, identifying potential emergencies, and deciphering the data obtained, nurses are at the forefront of device education. Nevertheless, it is indisputable that the vast majority of research conducted on medically significant peripheral devices has originated from disciplines apart from nursing. It is essential for the nursing profession to initiate in-depth research utilizing these emerging technologies in order to improve and advance the profession. Simply put, wearable technology is an additional instrument that nurses must participate in the development and implementation of in order to deliver improved nursing care.

Effect of β-lactam:

β-lactam antibiotics interfere with bacterial cell wall formation by attaching to penicillin-binding proteins, causing cell death. Gram-negative and Gram-positive bacteria are targeted by them. Amoxiclav, a medication that combines amoxicillin and clavulanic acid, lowered microbial diversity by 20% and increased Bacteroidetes/Firmicutes. It also enhanced Gram-negative Bacteroidia and Bacteroidales groups and 20-fold increased an unknown Bacteroides taxon. (Panda et al., 2014)

Effect of Fluoroquinolones:

Fluoroquinolones are potent antibacterial drugs used to treat hospital-acquired bacterial infections caused by organisms including Legionella pneumophila and Mycoplasma pneumoniae. They block bacterial DNA gyrase and topoisomerase IV. Fluoroquinolones elevate the proportion of Bacteroidetes in patients without affecting the overall microbial quantity. Levofloxacin is prescribed for respiratory, urinary tract, gastrointestinal, and abdominal infections. It impacts 14 bacterial taxa, resulting in a rise of 10 unknown Bacteroides and 1 unknown Coproccocus, and a decrease of 1 unknown Blautia.

Statements varied in their level of relevance, but most expressed a high degree of agreement with favorable opinions on the care and communication between nurses and patients. (Panda et al., 2014)

Discussion:

Antibiotic resistance in the gut microbiota has been mostly researched by genomic and metagenomic methods, or by observing the characteristics of certain species. The dynamics of resistance evolution at the community level and the mechanisms that support the microbiota

under antibiotic stress are still not well understood. Pinpointing the origin of this resistance is crucial for comprehending the development of resistance. Resistance can occur spontaneously due to the variability of bacterial genomes or through the transfer of genetic information across cells. Drug resistance has a natural and essential function in microbial ecosystems, as evidenced by the human microbiome acting as a significant source of antibiotic resistance genes. Intestinal colonization by resistant bacteria can happen within three days after birth. Resistance traits have also been observed in isolated human populations with minimal antibiotic exposure.

We utilized qPCR and 454 pyrosequencing of the 16S rRNA gene to examine the immediate impact of fluoroquinolone and β-lactam antibiotics on gut microbiota. The data indicate that after seven days of therapy, there was a significant and widespread disruption in the composition and organization of the gut microbial population. Our findings demonstrated that a reduction of microbial diversity by around 25% was linked to an increase in Bacteroidetes groups, which are Gram-negative bacteria, irrespective of the type of antibiotic used. Previous studies indicated that species from the Bacteroides genus, like B. fragilis, were sensitive to amoxiclav and levofloxacin. However, our research found that both drugs notably increased various taxa within this genus.

Our results align with earlier studies on the decrease in gut microbial diversity. This decline seems to be a prevalent characteristic, regardless of the specific type or amounts of antibiotics, or the experimental paradigm employed (human/animals).

Our data oppose the common belief about the impact of broad-spectrum antibiotics on gut flora. These medications do not lower both Gram-positive and Gram-negative bacteria as previously thought. Instead, they cause a notable rise in Gram-negative bacteria.

Our surprising findings, indicating a potential increase rather than a decrease in microbial burden, contradict recent research utilizing qPCR, or culture techniques). The scientists observed a notable reduction in microbial load after taking antibiotics for 3 to 7 days, as anticipated. As far as we know, there are no known research employing qPCR to evaluate microbial load in relation to antibiotic investigations in human adults.

The disparity in microbial diversity or load between our human studies and animal models may result from variations in the type and dosage of antibiotics used. utilized significantly higher concentrations of antibiotics (amoxicillin/metronidazole/bismuth) in a mouse model compared to the current investigation. In a prior study utilizing a rat model, we utilized antibiotics (vancomycin/imipenem) that were 17 times more concentrated.

Our work, albeit employing a high-throughput approach to analyze the impact of β-lactams and fluoroquinolones on the human microbiota, has various constraints. Given that this study required individuals who met specified recruitment requirements, we utilized a rather small group. In addition, future studies should incorporate a questionnaire on food or probiotic consumption to eliminate any external influencing variables. Furthermore, our study did not

involve a longitudinal analysis as we only examined two samples per participant, a methodology that has been previously used and published by other research groups. In the future, our study could differentiate between viable and non-viable bacteria by utilizing a PCR-based approach with propidium monoazide.

The maintenance or potential increase in microbial burden linked to a decline in variety implies that removing microbes sensitive to these antibiotics creates room for resistant strains to proliferate and take over the environment. The varying susceptibility of microbes to antibiotics may be the reason why full recovery is not achieved long after therapy has stopped.

Hence, the consistent utilization of these antibiotics may alter the microbiota to support resistant bacterial strains over time. Future research using different types of antibiotics could determine if the findings of this study can be applied to other drugs. (Panda et al., 2014)

Conclusion

In summary, given that antibiotics are our primary defense against bacterial infections, it is crucial to utilize knowledge gained from the development of resistance in living organisms to create new approaches to combat the spread of this resistance. The gut microbiome contains bacteria that have resistance functions capable of crossing ecological borders. The situation is made more complex by the extended duration of resistance presence and the tendency for several resistance components to be activated together, which has significant consequences for the uncontrolled development of cross-resistance. It is uncertain what controls the spread of resistance in clinical settings and whether there are certain characteristics that can isolate the resistome from pathogen exposure. This information flow has negative effects on the gut microbiota, as resistant pathogens become more formidable competitors for colonizing the host. An analysis of evolutionarily beneficial, context- specific control of horizontal gene transfer could limit the spread of powerful genetic material and reduce harmful effects on the human host. To maintain functional capability after stress, it is crucial to study how beneficial gene transfer happens in microbiota through horizontal gene transfer (HGT) and how it may be sustained and encouraged. Studying the effects of environmental disruptions on the gut microbiome is important for maintaining the balance of beneficial microorganisms, which is crucial for human health and fostering better interactions with our microbial partners.

References

1. Lange, K., Buerger, M., Stallmach, A., & Bruns, T. (2016). Effects of Antibiotics on Gut Microbiota. Digestive Diseases (Basel, Switzerland), 34(3), 260–268. https://doi.org/10.1159/000443360

2. Modi, S. R., Collins, J. J., & Relman, D. A. (2014). Antibiotics and the gut microbiota. Journal of Clinical Investigation, 124(10), 4212–4218. https://doi.org/10.1172/jci72333

3. Panda, S., El khader, I., Casellas, F., López Vivancos, J., García Cors, M., Santiago, A., Cuenca, S., Guarner, F., & Manichanh, C. (2014). Short-Term Effect of Antibiotics on Human Gut Microbiota. PLoS ONE, 9(4), e95476. https://doi.org/10.1371/journal.pone.0095476

4. Human Microbiome Project Consortium: Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-214.

5. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al: Diversity of the human intestinal microbial flora. Science 2005;308:1635-1638.

6. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al: A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464:59-65.

7. Dethlefsen L, Huse S, Sogin ML, Relman DA (2008) The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol 6: e280.

8. Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, et al. (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5: e9836.

9. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, et al. (2012) High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother 56: 5811–5820.

10. Manichanh C, Reeder J, Gibert P, Varela E, Llopis M, et al. (2010) Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. Genome Res 20: 1411– 1419.

11. Blandino G, Milazzo I, Fazio D, Puglisi S, Pisano M, et al. (2007) Antimicrobial susceptibility and beta-lactamase production of anaerobic and aerobic bacteria isolated from pus specimens from orofacial infections. J Chemother 19: 495–499.

12. Stein GE (2008) Schooley S, Tyrrell KL, Citron DM, Nicolau DP, et al (2008) Serum bactericidal activities of moxifloxacin and levofloxacin against aerobic and anaerobic intra-abdominal pathogens. Anaerobe 14: 8–12.

13. Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, et al. (2009) Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect Immun 77: 2367–2375.

14. Umenai T, Hirai H, Shime N, Nakaya T, Asahara T, et al. (2010) Eradication of the commensal intestinal microflora by oral antimicrobials interferes with the host response to lipopolysaccharide. Eur J Clin Microbiol Infect Dis 29: 633–641.

15. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Foureau DM, et al. (2009) Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol 183: 6041–6050.

16. Jernberg C, Löfmark S, Edlund C, Jansson JK: Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J 2007;1:56-66.

17. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol. 2013;13(11):790–801.