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Major articles

Intestinal microbiome disruption in patients in a long-term acute care hospital: A case for development of microbiome disruption indices to improve infection prevention

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Background: Composition and diversity of intestinal microbial communities (microbiota) are generally accepted as a risk factor for poor outcomes; however, we cannot yet use this information to prevent adverse outcomes.

Methods: Stool was collected from 8 long-term acute care hospital patients experiencing diarrhea and 2 fecal microbiota transplant donors; 16S rDNA V1-V2 hypervariable regions were sequenced. Composition and diversity of each sample were described. Stool was also tested for *Clostridium difficile*, vancomycin-resistant enterococci (VRE), and carbapenem-resistant *Enterobacteriaceae*. Associations between microbiota diversity and demographic and clinical characteristics, including antibiotic use, were analyzed.

Results: Antibiotic exposure and Charlson Comorbidity Index were inversely correlated with diversity (Spearman = -0.7). Two patients were positive for VRE; both had microbiomes dominated by *Enterococcus faecium*, accounting for 67%-84% of their microbiome.

Conclusions: Antibiotic exposure correlated with diversity; however, other environmental and host factors not easily obtainable in a clinical setting are also known to impact the microbiota. Therefore, direct measurement of microbiome disruption by sequencing, rather than reliance on surrogate markers, might be most predictive of adverse outcomes. If and when microbiome characterization becomes a standard diagnostic test, improving our understanding of microbiome dynamics will allow for interpretation of results to improve patient outcomes.

In recent years, research on the collective genome of microbial communities, known as the microbiome, living in or on humans has accelerated.[1,2] A healthy intestinal microbiota assists in digestion and metabolism and protects against pathogen invasion and overgrowth of pathobionts, which are commensal bacteria that can intermittently reside as minor members of the microbiota and also can act as pathogens when that microbiota becomes disrupted.[3] Loss of microbial diversity or protective species and overgrowth or dominance by a single organism are characteristic of microbiome disruption.

From birth, environment and host factors impact a person's microbiota. However, capturing a lifetime of exposures is not feasible. Microbiome disruption is generally accepted as a risk factor for poor outcomes, such as infection and, as recently suggested, sepsis.[4] However, we cannot yet use microbiome status to predict

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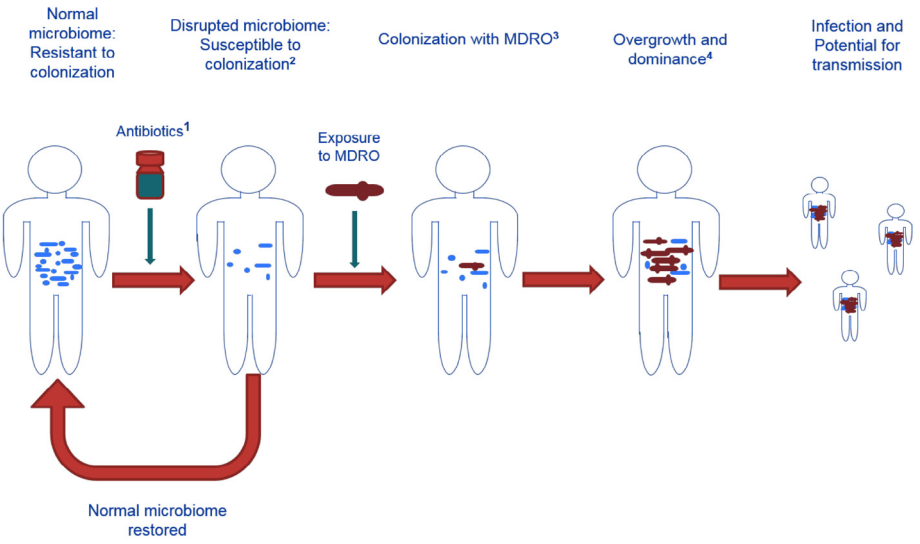

Fig 1. Causal pathway from health to disease: MDI. *MDI*, microbiome disruption indice; *MDRO*, multidrug-resistant organism. [1]Antibiotic MDI indicates the potential an antibiotic has for disrupting the intestinal microbiome. [2]Disrupted microbiome status MDI characterizes the degree and type of disruption in the intestinal microbiome and the susceptibility to colonization by a MDRO. [3]MDRO colonization MDI indicates susceptibility to overgrowth and dominance by a MDRO. [4]MDI characterizing overgrowth and dominance by a MDRO indicates susceptibility for infection with a MDRO and the potential for transmission to others through skin-environment contamination.

or prevent poor outcomes. One way to translate increasing understanding of the microbiome to the field of infection control is via the development and use of microbiome disruption indices (MDIs) (Fig 1). Such indices could become standardized criteria for not only characterizing the status of a patient's microbiome but also evaluating and communicating the disruptive potential of various drugs, including antibiotics. Applications for MDIs range from improving antibiotic stewardship, infection control, and clinical management of patients, to assigning a risk index to antibiotics and other microbiota disruptive drugs during the drug approval process.

Among the host and environmental factors that lead to microbiota shifts,[5-8] antibiotic exposures cause dramatic disruptions, lasting ≥6 months.[9] Not only do antibiotic-induced disruptions lead to a loss of colonization resistance to multidrug-resistant organisms (MDROs), but once colonization does occur, further disruptions can lead to dominance (defined as a single MDRO constituting ≥30% of the microbiota), which is associated with the occurrence of invasive infection and increased transmission risk through skin and environmental contamination.[10,11] MDROs, such as *Clostridium difficile*, vancomycin-resistant enterococci (VRE), and carbapenem-resistant *Enterobacteriaceae* (CRE), are major public health concerns in health care settings, where they are transmitted between patients and can colonize the lower intestine in more individuals than they infect.[12,13]

Long-term acute care hospital (LTACH) patients are a population with high antibiotic consumption,[14] likely leading to severe intestinal microbiota disruption. In an effort to make a case for the potential impact of MDIs in improving infection control, we describe and compare the microbiomes from LTACH patients with prior antibiotic exposure, when individuals are most susceptible to MDRO colonization, with those of fecal microbiota transplant donors from a small pilot study, subsequently described. We also examine associations between intestinal microbiome diversity and clinical and demographic characteristics.

METHODS

Study design and participants

The study was a cross-sectional pilot evaluation of the clinical characteristics and intestinal microbiome from a convenience sample of 8 LTACH patients with new onset diarrhea and 2 healthy fecal microbiota transplant donors.[15,16] The donors had no history of antibiotics in at least the previous 90 days and were not taking any other medications. Neither the LTACH patients nor the donors had history of Crohn disease, ulcerative colitis, or other inflammatory bowel disease.

Patients were enrolled sequentially at first diarrheal episode during December 2013 through February 2014 when stool was being collected for *C difficile* diagnostic polymerase chain reaction (PCR) testing (GenExpert; Cepheid, Sunnyvale, CA). Providers (S.L. and J.M.) consented patients (or a family member for patients unable to consent) to have stool collected for microbiome analysis.

Data collected on each patient during retrospective chart review (A.W.C.) included demographics, proton pump inhibitor use, previous *C difficile* infection, comorbidities, and antibiotic use in both the LTACH and acute care settings. Each antibiotic was classified by when it was administered in reference to the date of stool collection: day of or day before, during the 7 days before, and during the 30 days before stool collection. Data were used to calculate cumulative antibiotic days[17] and the number of days exposed to any (ie, ≥1) antibiotic. Antibiotics were categorized into the following classes: carbapenems, cephalosporins (first-generation), cephalosporins (third- and fourth-generation), β-lactam/β-lactamase inhibitor combinations, fluoroquinolones, glycopeptides (vancomycin), metronidazole, or other antibiotics. For vancomycin, route of administration was documented.

The Emory University Institutional Review Board approved this study protocol. No incentives were provided for participation.

MDRO colonization status

In addition to *C difficile* diagnostic PCR testing, patient stool was cultured for VRE using Spectra VRE chromogenic agar (Remel, Lenexa, KS) and screened for CRE using a selective medium containing ertapenem.[18] At the time of collection, stool specimens were deidentified; linkage to the clinical specimen was known only to one author (C.S.K.).