HHS Public Access

Author manuscript

*Ann Epidemiol*. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:

*Ann Epidemiol*. 2016 May ; 26(5): 342–347. doi:10.1016/j.annepidem.2016.02.007.

# The human microbiota: novel targets for hospital-acquired infections and antibiotic resistance

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## Abstract

**Purpose—**Hospital-acquired infections (HAIs) are increasing in frequency due to multi-drug resistant organisms (MDROs) and the spread of MDROs has eroded our ability to treat infections. Healthcare professionals cannot rely solely on traditional infection control measures and antimicrobial stewardship to prevent MDRO transmission. We review research on the microbiota as a target for infection control interventions.

**Methods—**We performed a literature review of key research findings related to the microbiota as a target for infection control interventions. These data are summarized and used to outline challenges, opportunities, and unanswered questions in the field.

**Results—**The healthy microbiota provides protective functions including colonization resistance, which refers to the microbiota's ability to prevent colonization and/or expansion of pathogens. Antibiotic use and other exposures in hospitalized patients are associated with disruptions of the microbiota that may reduce colonization resistance and select for antibiotic resistance. Novel methods to exploit protective mechanisms provided by an intact microbiota may provide the key to preventing the spread of MDROs in the healthcare setting.

**Conclusions—**Research on the microbiota as a target for infection control has been limited. Epidemiologic studies will facilitate progress towards the goal of manipulating the microbiota for control of MDROs in the healthcare setting.

### Keywords

Microbiota; hospital-acquired infection; antibiotic resistance; infection control; antimicrobial stewardship; *Clostridium difficile*; vancomycin resistant enterococcus

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## Introduction

Hospital-acquired infections (HAIs) cause approximately 100,000 deaths per year in the US and the five most common HAIs cost the US healthcare system $9.8 billion annually [1,2]. Approximately 20% of HAIs are due to multi-drug resistant organisms (MDROs) [3]. Colonization of the gastrointestinal tract or other body sites by MDROs is often a first step in the establishment of infection [4]; of patients colonized with MDROs in the intensive care unit (ICU), approximately 20% will develop clinical infection with the same MDRO during their ICU stay [5-7]. Importantly, colonized patients also serve as a reservoir for transmission to others. MDRO's colonize the gastrointestinal tract, respiratory tract, and skin within the context of the microbiota, which is the community of microorganisms that inhabits our bodies. In healthy individuals, the microbiota provides protective functions including prevention of colonization by pathogens and/or expansion of low-density pathogens, (i.e., colonization resistance). Antibiotics and other hospital-associated exposures (e.g., dietary changes) alter the composition, diversity, and density of the microbiota and select for antibiotic resistance [8-10]. Hospitalization-related disruptions of the microbiota may reduce colonization resistance and increase risk for acquisition and expansion of MDRO. Thus, the microbiota may provide a critical target for prevention strategies to limit colonization and spread of MDROs in the healthcare setting.

Research on the potential of the microbiota as a target for infection control has been limited to mechanistic studies in murine models and clinical studies with small numbers of patients and/or highly restricted patient populations. Epidemiologic studies will inform the development of novel interventions to exploit the protective mechanisms provided by an intact microbiota. The goals of this review are to describe limitations of traditional infection control interventions, to summarize key research findings related to the microbiota as a target for infection control interventions, and to outline challenges, opportunities, and unanswered questions in the field.

### Limitations of traditional measures to prevent HAIs with MDROs

Traditional infection control measures include barrier precautions, hand hygiene, decolonization of patients and environmental decontamination [11], and can reduce the incidence of HAI [12-14]. However, various infection control interventions have produced inconsistent results and it is clear that infection control measures alone cannot prevent the spread of MDRO since MDRO rates continue to increase [13, 15, 16]. Antimicrobial stewardship programs are also critical for achieving reductions in the prevalence and spread of MDROs. The successes that are achieved by antimicrobial stewardship programs may be difficult to sustain because increases in the prevalence of MDRO's often lead to increases in empiric prescribing of broad-spectrum antibiotics, which then leads to a cycle of increased antibiotic use and increased resistance [17, 18]. Controlling the spread of MDROs in the healthcare setting will require novel and multifaceted approaches above and beyond the current infection control and antimicrobial stewardship interventions.

## Role of the microbiota in preventing acquisition and/or expansion of potential HAI-associated pathogens

In healthy individuals, the gastrointestinal microbiota provides several important immunologic, metabolic, and protective functions including colonization resistance [19, 20]. Colonization resistance was first described in a series of seminal papers dating back to the 1970s [21, 22]. These studies examined the impact of antibiotics on the gastrointestinal flora and showed that 1) a lower infectious dose of pathogens (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*) was needed to establish colonization in mice who had received antibiotics, 2) antibiotic treatment resulted in overgrowth/expansion of exogenous pathogens, and 3) the extent of colonization resistance differed by the antibiotic used [21, 22]. Colonization resistance was attributed to anaerobic bacteria that were abolished by antimicrobial treatment [21]. Colonization resistance may be preserved in the presence of some antibiotics, for example, β-lactamase producing anaerobes help preserve colonization resistance in the gut by inactivating β-lactam antibiotics [23, 24]. Murine studies also indicate that in addition to colonization resistance, an intact microbiota can mediate clearance of gastrointestinal pathogens once infection has been established [25].

Mechanisms that mediate colonization resistance include competition for nutrients and adherence sites, direct inhibition of pathogens via substances excreted by members of the microbiota, and innate and adaptive immune responses induced by commensals [26, 27]. Antibiotic induced changes in the composition and diversity of the gastrointestinal microbiome may also lead to the disruption of carbohydrate and bile metabolism and the loss of anaerobes that produce short chain fatty acids [28, 29]. Short chain fatty acids help regulate regulatory T cells and promote homeostasis within the gastrointestinal tract and may play a critical role in colonization resistance [30] although some studies were unable to correlate short chain fatty acid production with lower levels of pathogen colonization [31, 32].

## Impact of antibiotics on the microbiota

Antibiotics have a profound impact on the gastrointestinal and upper respiratory tract microbiota. Specific antibiotics favor survival of non-pathogenic and pathogenic species within the microbiota if they are resistant to the prescribed antibiotic. Antibiotics can alter bacterial loads, relative abundance of commensals, and lead to lower levels of diversity in the microbiota [8, 9, 33]. Dethlefsen et al. studied three healthy volunteers receiving two courses of ciprofloxacin separated by six months and followed them for over ten months [33]. They observed a rapid loss of diversity within 3-4 days of ciprofloxacin exposure. Rebounds in bacterial diversity were observed after approximately one week but the microbiota did not return to its original composition. Other authors have shown that the impact of antibiotics can persist for years after the original treatment [9].

Antibiotics exert a powerful selective pressure on the microbiota that extends beyond altering the prevalence of specific taxa. The microbiota provides a reservoir of resistance genes, termed the antibiotic resistome, which can be horizontally transferred within and across species and lead to the emergence of antibiotic resistance in pathogens [34, 35]. Sommer et al. characterized the reservoir of antibiotic resistance genes in the oral and gut

microbiota in two healthy individuals who had no previous antibiotic exposure in the prior year [36]. Of concern, close to half of the resistance genes from 572 cultured gastrointestinal bacteria strains were identical at the nucleotide level to antibiotic resistance genes previously identified in human pathogens. Hu et al. examined the gut microbiota of 162 individuals from Spain, China, and the Netherlands and identified a total of 1,093 antibiotic resistance genes [37]. The high level of diverse antibiotic resistance determinants represents a substantial reservoir of genes that could potentially be transferred to, and emerge in, human pathogens. The antibiotic resistome also contains cryptic resistance genes that may evolve into resistance genes under appropriate conditions [34]. Some genes within the antibiotic resistome may have sequence matches to known resistance genes but may not be functional [38]. Moreover, there may be barriers to gene exchange with human pathogens in the case of certain resistance genes. Epidemiologic studies, including randomized controlled trials, can address questions regarding the differential impact of different classes of antibiotics on the antibiotic resistome and track how resistance determinants spread in the healthcare setting.

## Factors affecting the microbiota and risk of colonization and disease in hospitalized patients

To this point, studies of the impact of antibiotics on the microbiota have most often focused on healthy individuals [9, 33]. Exposures are quite different in hospitalized patients. In addition to receiving multiple courses of antibiotics, hospitalized patients are exposed to other factors such as stress, intravenous nutrition, and other medications, which all may impact the microbiota. Zaborin et al. examined fecal samples from 14 ICU patients and five healthy controls [39]. The flora of healthy volunteers contained approximately 40 genera and high relative abundances of Firmicutes and Bacteroidetes. In contrast, 36% of the ICU patients had extremely low levels of diversity, defined as having >90% abundance of one bacterial taxon. These patients also experienced rapid fluctuations in the flora and replacement of one dominant taxon by another. The microbiota of hospitalized patients was dominated by *Enterococcus*, *Staphylococcus* and Enterobacteriacea, phylogenetic groups that include potential HAI-associated pathogens and MDROs [39]. These data provide important insights into the potential differences in the microbiota between healthy individuals and hospitalized patients. However, the study contained a small number of subjects presenting with a diverse range of medical conditions and length of hospital stay. Individuals respond differently to antibiotic treatment [9, 33]. Factors such as age, presence of comorbid conditions, types of medical interventions, and length of stay can also impact the risk of MDRO acquisition [3, 40–43]. Thus, it is reasonable to assume that patient demographics and clinical characteristics will have differing impacts on the microbiota.

Taur and colleagues characterized the fecal microbiota of 94 patients undergoing allogenic hematopoietic stem cell transplant using 16S rRNA gene sequencing [44]. The authors evaluated temporal trends in microbiota diversity and intestinal domination by a particular taxon to determine whether the composition of the microbiota was related to clinical outcomes. A given taxon was considered dominant if they were the most abundant taxon and comprised ≥ 30% of the sequence reads. *Enterococcus* was the most frequent dominating taxon followed by *Streptococcus* and *Proteobacteria* in 40.4%, 37.2%, and 12.8% of patients, respectively. Intestinal domination by members of the genus *Enterococcus* was

associated with an increased risk of vancomycin-resistant *Enterococcus* (VRE) bacteremia (Hazard Ratio (HR) 9.35, 95% confidence interval (CI)= 2.43-45.44) and *Proteobacteria* domination was associated with an increased risk of gram-negative bacteremia (HR 5.46, 95% CI=1.03-19.91) [44]. This important study lends credence to the idea that microbiota related methods to prevent initial colonization and expansion by VRE would provide an additional tool to prevent HAI due to VRE.

The aforementioned studies by Zaborin et al. [39] and Taur et al. [44] greatly advance our understanding of the impact of hospitalization on the microbiota. The stem cell transplant patients studied by Taur et al. are a very special patient population that undergoes chemotherapy, radiation, and high levels of antibiotic use. The study by Zaborin et al., while based on a limited number of patients, suggests that extreme shifts in the flora may also be observed in ICU patients with other underlying medical conditions. In order to accurately understand how hospital associated risk factors impact on the microbiota, future studies must include sufficient sample sizes, detailed patient demographic and clinical data, and appropriate comparison groups.

## Potential reduction of risk of HAI associated with reconstituted/transplanted microbiota

The potential translational value of microbiome studies is exemplified by the use of fecal microbiota transplants (FMT) to treat *Clostridium difficile* infections, and several studies of the microbiota and HAI have focused on this pathogen. *C. difficile* is an anaerobic spore-forming bacterium that is the leading cause of hospital-acquired diarrhea in the United States [45]. In 2011, *C. difficile* was associated with an estimated 453,000 infections [46]. Of these, 293,300 (65.8%) were associated with healthcare and 104,400 (24.2%) had an onset in the hospital. A randomized controlled trial showed that 94% of patients who received FMT resolved infection without relapse compared to 31% who received vancomycin alone ($P<0.001$) [47].

A mathematical model was developed to evaluate the impact of FMT on transmission of *C. difficile* in the ICU [48]. Lofgren et al. modeled a series of scenarios including widespread use of FMT after *C. difficile* and prophylactic use of FMT in high-risk patients (e.g., patients who received antibiotics) to prevent incident *C. difficile* infections. FMT after *C. difficile* was not associated with a reduction in incident HAIs. Prophylactically treating patients had a statistically significant but minimal impact on incident infections. The model necessarily used simplifying assumptions that may not be correct [49]. However, this model provides a framework for analyzing microbiota-based interventions for control of HAIs.

## Critical protective taxa in the gastrointestinal microbiota

Concerns regarding the unpleasant nature of FMT and risks of pathogen transfer, which are especially acute in immune compromised patients, have led researchers to focus on identification of mechanisms and key species to prevent acquisition and infection with *C. difficile*. Successful treatment of *C. difficile* infection by FMT is associated with shifts in the microbiota from a low-diversity diseased state characterized by high abundance of Proteobacteria and Bacilli, to a more diverse microbial community with higher levels of Bacteroidetes and Clostridium [50, 51]. A study of 338 individuals examined the microbiota

in hospitalized patients with and without *C. difficile* associated diarrhea and healthy controls [52]. Low abundance or absence of Bacteroides, Porphyromonadaceae, Lachnospiraceae, and Ruminococcaceae was associated with *C. difficile* diarrhea. Buffie et al. used a combination of human, murine and mathematical modeling studies to identify individual taxa associated with protection from *C.difficile* infection and the mechanisms involved [53]. These investigators compared the microbiota in mice and hospitalized patients and identified four bacteria species, *Clostridium scindens*, *Barnesiella intestihominis*, *Pseudoflavonifractor capillosus* and *Blautia hansenii*, that were potentially protective for *C. difficile* infection. Adoptive fecal transfer experiments in mice showed that *C. scindens* alone and a cocktail of the four bacterial species inhibited growth of *C. difficile* and toxin production. *C. scindens* is a 7 α-dehydroxylating intestinal bacterium that can convert stimulatory combinations of bile acids into inhibitory combinations of bile acids. Specific bile acids in the gut promote spore germination, and secondary bile acids inhibit the growth of vegetative *C. difficile* [54]. The adoptive fecal transfer results need to be confirmed in humans but are an important step in identifying multi-species probiotics that could be used to treat infections.

The microbiota may provide an optimal target for control of other MDROs that are common causes of HAI. Research has also focused on identifying taxa associated with colonization and expansion of VRE. Murine studies have correlated VRE clearance with intestinal recolonization by bacteria of the genus *Barnesiella* [55]. *Barnesiella* was also associated with lower levels of VRE in allogenic hematopoietic stem cell transplant patients; patients with VRE domination had significantly lower levels of *Barnesiella* compared to patients without VRE domination [55].

Stiefel et al. developed a potential strategy to prevent colonization and expansion by VRE and *C. difficile* [56]. Mice were colonized with a cephalosporinase-producing strain of *Bacteroides thetaiotaomicron,* which prevented overgrowth of VRE and *C. difficile* in the presence of systemic therapy with ceftriaxone [56]. While these data are promising, the investigators had to pretreat mice with piperacillin-tazobactam to create a favorable environment for *B. thetaiotaomicron* to establish colonization. It is not clear that single species transfers, with bacteria such as *B. thetaiotaomicron,* would effectively establish in the human gastrointestinal tract. Moreover, such a strategy would only work for patients receiving β-lactam therapy and raises ethical concerns regarding introduction of a bacteria species that carries a horizontally transmissible resistance determinant.

## The microbiota and prevention of acquisition and spread of MDROs at non-gastrointestinal sites

Many microbiome-related studies focus on the gastrointestinal tract although pneumonia is the most common HAI, representing approximately 20% of all infections [43]. The microbiota differs across body sites and data from one anatomic site likely cannot be generalized to another site [57]. Several hundred bacterial taxa colonize the upper respiratory tract [58, 59]. The upper and lower respiratory tracts are a complex and connected ecosystem and there is growing recognition that pneumonia may occur as a consequence of disruptions in homeostasis in the respiratory tract microbiota [19, 60, 61]. A few culture-independent studies have examined the respiratory tract microbiota in

hospitalized patients [62–65]. Reductions in microbial diversity have been associated with *P. aeruginosa* colonization in cystic fibrosis patients, which indicates that the respiratory tract flora also plays a role in mediating colonization resistance [66]. In contrast, murine studies indicate that a normal, healthy nasal microbiota does not prevent colonization by *Klebsiella pneumoniae* while a normal, healthy gastrointestinal microbiota does [67]. Bousbia et al. described the microbiota in bronchoalveolar lavage samples from pneumonia patients in the ICU and controls. The microbiota was unexpectedly complex; the investigators identified 160 different bacteria species and up to 16 different microorganisms in a single sample. Pathogens such as *P. aeruginosa* and *Streptococcus* species were commonly detected in both patients and controls [67]. Further studies, especially longitudinal studies, are required to evaluate the role of the respiratory microbiota in facilitating colonization resistance and to determine causality. These data could then be used to design interventions to prevent HAIs in the respiratory tract and other non-gastrointestinal sites.

## Challenges, opportunities, and unanswered questions

With recent technologic advances, the ability to characterize the microbiota has increased dramatically. Model animal systems have provided great insights and the influence of genetics and environmental factors can be studied under controlled conditions [27, 68, 69]. However, the microbiota and immune systems of mice and humans differ, and although murine models can show proof of concept, they are not a substitute for epidemiologic human studies [53, 70, 71].

The composition, diversity and taxonomy of the microbiota varies greatly between individuals [57]. The diversity and composition of microbiota samples can represent the normal variation between people or temporal fluctuations that are independent of the disease state [72, 73]. Adequately powered longitudinal studies are needed to capture the transition from homeostatic to dysbiotic states [73–75]. Such studies may require complex study designs and large datasets. Analyses of the microbiota can be added to ongoing epidemiologic studies and may be feasible as sample processing and high-throughput sequencing costs go down. However, researchers are increasingly recognizing the potential for contamination with environmental bacterial DNA despite the use of molecular biology grade water and commercial kits [76]. The problem is especially acute when low biomass samples are examined using platforms with high depth of coverage [76, 77]. Recommendations to mitigate contamination include collecting and sequencing controls from each batch of storage medium, DNA extraction, and PCR kits along with the study samples. The need to sequence reagent controls may present challenges when pre-existing collections are used for microbiota and metagenomic analyses in epidemiologic studies.

Several important questions can be addressed by epidemiologic studies and include: What are the key protective taxa that facilitate colonization resistance for a given MDRO? How do antibiotic-induced changes in microbiota facilitate or prevent acquisition of potential pathogens? Which antibiotics have the greatest negative impact on the microbiota? Which bacterial taxa and antibiotic resistance genes represent the greatest threat? What are the clinically relevant genes within the antibiotic resistome? What other factors (e.g., stress, steroids, opiates) negatively impact the microbiota?

An improved understanding of the role of the microbiota in preventing or facilitating the spread of MDROs in the healthcare setting may well yield novel approaches towards prevention of HAIs. For example, strategies for selectively introducing commensals (e.g., probiotics) to promote optimal levels of biodiversity in the gastrointestinal tract and other sites may emerge as effective methods for preventing HAIs. Trials of the effectiveness of probiotics for infection control have produced mixed results [78, 79]. Probiotics are highly strain and species specific, and effective probiotics for prevention of HAI would likely have to contain mixtures of multiple species or be targeted towards specific pathogens. Auto-banking of microbiota samples and FMT may also provide viable options for restoration of a healthy microbiota [80]. Greater understanding of colonization resistance and the taxa that play critical roles within the healthy microbiota is needed. Epidemiologists can and should play an important role in creating well designed and adequately powered epidemiologic studies to reach the translational goal of manipulating the microbiota for controlling the spread of MDRO in the healthcare setting. Epidemiologist can also play an important role in translating findings into clinical interventions. Over the long term, such studies will allow investigators to develop preventive measures in the areas of infection control, antibiotic stewardship, and probiotics.

## Acknowledgements

The authors gratefully acknowledge Janneane Gent for critical review of the manuscript. This research was supported by NIH5K24AI079040-07 to A.D.H.

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