Review

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Hospital-associated microbiota and implications for nosocomial infections

Simon Lax[1,2] **and Jack A Gilbert**[1,2,3,4,5]

[1] Department of Ecology and Evolution, University of Chicago, 1101 E 57th Street, Chicago, IL 60637, USA
[2] Institute for Genomics and Systems Biology, Biosciences Department, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA
[3] Graduate Program in Biophysical Sciences, University of Chicago, Chicago, IL, USA
[4] Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA
[5] College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

The rise of high-throughput sequencing technologies and culture-independent microbial surveys has the potential to revolutionize our understanding of how microbes colonize, move about, and evolve in hospital environments. Genome analysis of individual organisms, characterization of population dynamics, and microbial community ecology are facilitating the identification of novel pathogens, the tracking of disease outbreaks, and the study of the evolution of antibiotic resistance. Here we review the recent applications of these methods to microbial ecology studies in hospitals and discuss their potential to influence hospital management policy and practice and to reduce nosocomial infections and the spread of antibiotic resistance.

Our microbial interaction with built environments

As the global trend toward urbanization has accelerated over the past century, humans have become increasingly tethered to the built environment. From the hospital we are born in to the homes, apartments, and office buildings we live and work in, the indoor environment has become our most intimate ecosystem [1], yet our ignorance of the microorganisms that share this habitat remains profound. The bacteria, fungi, and viruses that colonize these environments may help to shape our own microbiomes (see Glossary) and can fundamentally alter the trajectory of our physiological, immunological, and neurological development. Designing our buildings and city spaces with the microbiome in mind may help to improve our health and mapping the microbial communities of our built environments may help us track biothreats and diseases, develop sophisticated early warning systems, and understand how a changing climate and increasing population density will shape human health. The indoor ecosystem, and the urban environment in particular, are hotspots for reduced microbial diversity and this reduction may be having untold consequences for our health and well-being [2]. This depauperate exposure to a complex microbiome that would normally train a healthy immune system has been linked to the rise in asthma and allergies [2–4].

Our own microbiota is both shaped by and influences the microorganisms that inhabit built environments and recent studies have uncovered the extent to which humans influence the microbiota of the spaces they occupy. This research has furthered our understanding of how microbes interact with and survive and proliferate in built environments and in particular has demonstrated that the human skin microbiome comprises the major microbial source for indoor systems as varied as airplanes, kitchens, offices, and public restrooms [5–9]. The strength of this interaction is such that a built environment can be matched to its occupants based entirely on microbial similarity [5] and that microbial signatures left on objects can be forensically matched to individuals [10].

The microbiology of the built environment has some of its most profound implications for health-care facilities, where hospital-acquired infections (HAIs) have long been among the leading causes of patient deaths [11–14]. Determining how microorganisms colonize, persist, and change in the hospital environment has the potential to elucidate the major sources of these HAIs, but the complexity of the microbial world confounds our attempts to focus on specific pathogens only. It is now essential that we understand the ecology of these frontline medical environments and

Glossary

16S rRna: an rRNA gene common to all prokaryotes, commonly used as a marker gene in amplicon-based microbial surveys.
Antimicrobial-resistance (AMR) gene: a gene conferring resistance to an antimicrobial agent, commonly carried together with other AMR genes on mobile genetic elements.
Lateral gene transfer (LGT): the transfer of genes between unrelated microorganisms rather than through vertical descent.
Metagenomics: the study of genetic material recovered directly from environmental samples, allowing the characterization of microbial communities without depending on culture-based methods.
Methicillin-resistant *Staphylococcus aureus* (MRSA): a Gram-positive bacterium resistant to beta-lactam antibiotics that results in difficult-to-treat infections in hospital environments.
Microbiome: the ecological community of microorganisms within a defined ecosystem (e.g., a soil sample, a human stool sample, a swab from a building surface).
Nosocomial infection: an infection acquired or developed within the hospital environment.

Corresponding author: Lax, S. (simonlax@uchicago.edu).
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elucidate the mechanisms by which the microbiology of a health-care setting can influence patient outcomes. Only then can we hope to engineer solutions to regain control over these outcomes. Traditional studies of health-care-associated infections have relied heavily on cultured isolates, genotyping of known pathogens, and *post hoc* characterization of potential transmission routes. In this review, we discuss how novel sequencing and bioinformatics techniques are revolutionizing our understanding of hospital-associated microbiota, the origin and structure of hospital outbreaks, and the evolution of antibiotic resistance.

Health-care-associated infections

Health-care-associated infections are an increasingly prevalent threat in the US health-care system. Patients may acquire a pathogenic infection after admittance to a health-care environment although it is often more complicated, as the patient's own microbiome may also harbor certain types of HAI [15]. Exact determination of HAI prevalence is complicated by the lack of a single US surveillance system and the fact that most hospitals limit reporting of HAIs to device-associated and surgical infections, as well as those due to the pathogens *Clostridium difficile* or methicillin-resistant *Staphylococcus aureus* (MRSA). The vast increase in deaths attributed to these two hospital-associated pathogens (HAPs) over the past decade has been shocking, with more than 10 000 deaths per year in the UK attributed to these diseases [16].

A 2011 survey by the Centers for Disease Control found that 4% of patients in acute-care hospitals had at least one health care-associated infection, more than half of which were not associated with devices or operative procedures [17]. They estimate that there were 648 000 patients with 721 800 HAIs in 2011, with a median interval of 6 days between hospital admission and the onset of HAI symptoms. Greater patient age, longer duration of hospital stay, larger hospital size, and the insertion of a central catheter were found to be the greatest risk factors in the contraction of HAIs. A wealth of prior studies have identified dominant HAPs and putative routes of transmission, including physicians' and nursing staff's clothing [18–20], stethoscopes [21], personal phones [22–25], and computer keyboards [26]. What these studies share is a specific focus on known pathogens and a reliance on microbial cultures.

Metagenomic characterization of hospital microbial communities

Historically, studies of hospital microbiota and infection control have relied on culture-dependent methods, taking a 'needle in a haystack' approach to select for specific pathogens rather than assessing the whole microbiome [27]. Such methods are unable to effectively characterize the microbial diversity of abiotic hospital surfaces or the asymptomatic carriage of microbes by hospital staff [27]. An assessment of the full microbial community, by contrast, allows inference of the factors structuring microbial assemblages in hospitals and the effects of building materials and design, cleaning regimens, and abiotic environmental factors on community diversity. For an overview of the high-throughput sequencing-based methods being used in these community analyses, see Box 1.

Box 1. Molecular tools for characterizing hospital-associated microbes

16S rRNA amplicon sequencing
Allows the rapid and inexpensive characterization of a bacterial and archaeal community through the use of universal primers to amplify the highly conserved16S rRNA gene from community members. Although this allows inferences about community structure and diversity, it does not enable the determination of the functional capabilities of the observed microorganisms. As the 16S rRNA gene is so highly conserved, it is possible that pathogenic and nonpathogenic strains of closely related organisms have identical 16S rRNA sequences.

Internal transcribed spacer (ITS) amplicon sequencing
Uses ITS – a piece of nonfunctional RNA situated between structural rRNAs – as a marker gene to characterize fungal communities. As with 16S rRNA, it does not provide information about function.

Population genome sequencing
Allows very fine resolution of an individual microbial strain and has been used to track the spread of infections using isolates that differ by only a single base pair in their genome. Although they provide the best insights into the function and evolution of a specific strain, these methods require the ability to isolate the taxon of interest and view the strain in isolation rather than in a broader ecological context.

Shotgun metagenomic analysis
Randomly shears the entire DNA extracted from a sample into fragments that can be sequenced with high-throughput technology. These raw sequences can be used to assess the relative abundance of gene ontologies in the community and infer its functional potential or to search for target genes, such as those conferring AMR. When sequenced deeply enough, these reads can often be assembled into genomes of individual strains, allowing us to infer LGT and ecological interactions between members of the microbial community.

In many ways, hospitals represent an intriguing model system for the study of built-environment microbial communities, both because of their obvious connection to human welfare and because they allow almost total normalization of building materials, temperature, humidity, air source, and ventilation. This standardization allows us to disentangle microbial interactions between humans and the built environment from the myriad compounding abiotic factors of other systems. With a defined set of microbial sources, patients, staff, water, and air, it should be possible to elucidate patterns of microbial transfer within hospitals and determine how members of these microbial communities persist and grow in response to cleaning regimens and architectural choices.

Nowhere is the threat of HAI more concerning than in neonatal intensive care units (NICUs), where low-birth-weight infants are typically immunocompromised and susceptible to opportunistic pathogens [28]. Infants are born mostly sterile, with acquisition of the gut microbiota being first shaped by delivery method and then by diet and genotype [29–31]. In very-low-birth-weight (VLBW) infants, the development of this microbiome can become disrupted by the common use of broad-spectrum antibiotics, resulting in lower diversity, chaotic fluxes in community composition, and a large number of opportunistic pathogens [32–34]. A 2004 study found that 65% of VLBW infants contracted at least one HAI and 27% of infant

deaths in NICUs included infection as a coded cause of death [35].

Some of the greatest efforts to characterize hospital-associated microbiota with high-throughput culture-independent techniques have focused on NICUs. The use of 16S rRNA gene sequencing enables the characterization of low-abundance and unculturable microorganisms. Although these methods cannot determine which microorganisms are viable and metabolically active, they can help track the spread of a microbial taxon through the hospital environment, which is of critical importance in determining the role of hospital surfaces and equipment in vectoring microorganisms.

A recent study of two San Diego NICUs found far more microbial diversity than revealed by earlier culture-based studies of NICU surfaces [36]. Interestingly, comparison with other built environment surfaces, such as those in offices and restrooms, revealed similar taxonomic profiles dominated by the genera *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Enterobacter*, and *Neisseria*. Despite similarity in abundant genera, some NICU samples were differentiated by a large number of Enterobacteriaceae sequences, including the taxa *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Salmonella*, which commonly inhabit the digestive tract and are well known ICU pathogens that can easily proliferate in hospitals. Using a Bayesian source-tracking algorithm, the authors showed that the dominant source of NICU microorganisms was human skin.

A second NICU study used metagenomic analysis to determine the extent to which hospital surfaces are the source of colonizing microbes in the gastrointestinal (GI) tract of premature infants [37]. The authors collected fecal samples from two infants every third day for the first month of life, as well as samples from NICU surfaces. Dominant gut taxa were similar to those found in the nursing room, especially those on feeding and intubation tubing. Numerous antibiotic-resistance, biofilm-formation, and starvation-resistance genes were detected in the genomes of microbes in the fecal samples of both infants, potentially explaining how certain organisms are able to persist in such a regularly sterilized environment.

Few other studies have made use of the increasingly high-throughput sequencing pipelines that have reshaped the field of microbial ecology in recent years. The first ICU survey to make use of high-throughput sequencing characterized the microbial communities on inanimate surfaces of the ICU wards of a Spanish hospital [38]. The study also took samples from the entrance hall to the hospital, to test how the extent of cleaning and the number of people occupying a space influence hospital microbial communities. Although the study found substantially less microbial diversity in the ICU compared with the entrance hall, the 1145 taxa detected in the ICU demonstrate the surprisingly great diversity that can inhabit a space that is constantly being sanitized and treated with antibiotics. The advent of culture-independent microbial identification has upended our ideas about sterility in general. Even surveys of NASA clean rooms used for spacecraft assembly, which are rigidly sealed and sterilized, have revealed that these rooms harbor hundreds of microbial taxa [39]. If it is indeed impossible to completely sterilize a built environment, that leaves open the question of which taxa are best able to survive intensive cleaning regimens and what potentially deleterious consequences may arise from this selective pressure placed on microorganisms.

The spread of antimicrobial resistance in hospital environments

The widespread use and accumulation of antibiotics in the environment over past decades has resulted in a worldwide crisis of antibiotic-resistant bacteria. This rapid rise in microbial resistance is largely driven by transfer of antimicrobial-resistance (AMR) genes between taxa through lateral gene transfer (LGT), which represents one of the most dramatic and detrimental consequences of anthropogenic impacts on the evolution of other species [40]. The saturation of the environment with antimicrobial compounds has placed strong selective pressure on the uptake of AMR genes, which are often contained on complex DNA vectors also carrying resistance to disinfectants and heavy metals [41,42].

Most antibiotics were originally isolated from soil-dwelling bacteria that also carried AMR genes protecting them from their own metabolites. The AMR genes and DNA vectors found in modern pathogens can often be traced back to their environmental roots [43–45], suggesting that natural microbial ecosystems contain a vast reservoir of AMRs that can become acquired by pathogens. The human microbial ecosystem may be the most important of all for AMR transfer, which traditional clinical strategies have done much to exacerbate [40]. Historically, antibiotics were designed to treat illness without the identification of a specific pathogen through broad-spectrum activity, which resulted in strong selection pressure on a correspondingly broad group of microbial taxa. This indiscriminate selective pressure can drive LGT events in groups of previously commensal organisms that were not the target of the antibiotics and can promote the growth of resistant pathogenic strains at the expense of commensal and beneficial ones [40].

There have been numerous studies documenting the LGT of AMR *in vivo* in hospital environments [46–50]. Many of these studies have focused on the acquisition of beta-lactam antibiotic resistance by *S. aureus* (MRSA). Methicillin resistance is conferred by the *mecA* gene, which is carried on a mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). SCC*mec* is widespread in coagulase-negative *Staphylococcus* (CoNS), a group not traditionally regarded as pathogenic but which shares the same ecological niche as *S. aureus* in the human anterior nares. There is evidence for frequent horizontal transfer of SCC*mec* between CoNS and *S. aureus*, which will be of fundamental importance in combating and understanding MRSA epidemiology [47,48]. Phylogenetic studies of *S. aureus* isolates from the lungs of a chronically infected cystic fibrosis patient taking heavy doses of antibiotics found strong evidence for local adaptation of a single isolating strain that became heterogeneously insensitive to antibiotic treatment [51]. These types of longitudinal studies are critical for documenting the emergence of AMR *in vivo* so that

antibiotic-treatment failure can be more specifically explained [52].

Although Gram-positive pathogens have dominated research into AMR evolution, *in vivo* AMR transfer has also been documented in Gram-negative strains such as *Klebsiella pneumoniae* and *E. coli* [46]. It is extremely important that our understanding of nosocomial infections and antibiotic resistance in health-care environments is not limited to our experience but that we are able to reach beyond what we expect to find.

Discovery and characterization of new pathogens

Determining which microbial taxa are potentially disease causing can be difficult, especially because antibiotic-resistance genes are widespread and found even in the remotest of environments [53]. Advanced molecular analytical techniques, including whole-genome sequencing, allow the identification of nosocomial pathogens beyond the most studied diseases (e.g., MRSA, *C. difficile*) and into emerging threats such as Gram-negative multidrug-resistant bacteria.

Researchers were able to trace a 2011 outbreak of antibiotic-resistant *K. pneumoniae* at the US National Institutes of Health (NIH) Clinical Center through whole-genome sequencing of patient isolates, although only 41 nucleotides in a genome of 6 million base pairs were variable between isolates [54]. This very high level of resolution enabled the formation of a transmission network pointing to three independent transmission events from a single index case; these transmissions ultimately led to hospital-wide dissemination of the outbreak strain.

The *K. pneumonia* strains from the NIH study were carbapenem-resistant Enterobacteriaceae (CREs), which, in recent years, have become a particularly formidable threat, with some investigations reporting a mortality rate as high as 80% [55]. Carbapenems are an antibiotic class of last resort, making CREs a class of HAI that is almost impossible to effectively treat. They are easily transferred in health-care settings from patients and staff with asymptomatic colonization and have the potential to spread carbapenem resistance through plasmid transfer to other human gut microbiota [54]. Although such transfer is well documented in model organisms and laboratory strains, much less is understood about how antibiotic resistance may transfer in hospital settings [55]. This ability of microbial taxa to exchange genetic material requires us to think beyond whole-genome epidemiology and to think of pathogenic taxa within the context of the full microbial community.

Tracking pathogen movement with high-throughput sequencing has an impact beyond single hospitals and can be used to explore interhospital and even global routes of pathogen transmission. Using phylogenetic models, researchers were able to track the historical spread of a specific MRSA strain from London and Glasgow to smaller regional hospitals where local endemic strains began to circulate [56]. On an even larger scale, phylogenetic models based on high-throughput sequencing have been used to compare globally distributed MRSA isolates and to track the worldwide spread of AMR over the course of four decades [57].

The rapid advances in sequencing technology used to characterize the full genomes of cultured isolates also allow us to detect AMR genes across the full microbial community, including those in unculturable microorganisms. Although reducing our ability to characterize a specific strain, shotgun metagenomics enables us to embrace the full phylogenetic diversity of each microbial sample, including microorganisms of potential benefit.

Effect of cleaning regimens and abiotic factors

Despite the obvious public health interest in reducing nosocomial infection rates, there remains very little understood about the sources of most infections, including the extent of airborne transmission. Research on airborne microbes in built environments has looked at their relationship to airflow in hospital rooms and found that indoor air passed through mechanical ventilation was less diverse but more enriched in organisms closely related to human pathogens [58]. By contrast, opening the windows in patient rooms has been found to significantly reduce the percentage of potentially pathogenic airborne bacteria [59], a realization that goes back as far as Florence Nightingale's demonstration that opening windows on wards of Crimean War casualties led to an improvement in patient outcomes.

Technological advances in our ability to control air circulation have lead to increased isolation between the indoor and outdoor environments, so that in many hotels, offices, and hospitals it is not possible to open windows. This serves two core purposes. First, it reduces the likelihood of negative air exchange with the outside, reducing the potential escape or ingress of pathogens or pollutants. Second, it makes control of the building environment's temperature and humidity significantly more efficient, increasing energy efficiency and reducing the cost of running these facilities. In many ways, almost inadvertently the built environment has been developed over the past 120 years to become increasingly inhospitable to microbial life. Environments are kept dry and surfaces are often covered with antimicrobial materials. While the built environment and control of its parameters has undoubtedly reduced the spread of communicable diseases, it has also changed our microbial relationship to the environment. In the hospital environment, where patients are often immunosuppressed or microbially dysbiotic, being exposed to an ecosystem with very limited microbial diversity may be a good thing. However, it is also possible that lack of exposure to a rich, diverse microbiome may exacerbate certain conditions and therefore negatively influence patient outcomes.

In the absence of a diverse microbial community, surface environments could play host to communicable pathogens that would otherwise be outcompeted by a diverse microbiota, as has recently been demonstrated in a study of post-cleaning microbial succession in public restrooms [9]. This study found that, after full decontamination with bleach, a late-stage successional community developed after 8 h that comprised mostly skin-associated taxa and that remained stable over the course of weeks. By contrast, early successional communities were unstable and more

dominated by gut-associated taxa related to potential pathogens.

Understanding the ecological competition that could shape the persistence and resistance phenotype of HAPs is an obvious priority for studies such as the Hospital Microbiome Project (http://www.hospitalmicrobiome.com), which will use metagenomic analyses to characterize hospital-associated microbiota, including those in understudied environments such as hospital water systems. This initiative is exploring a hospital in Chicago as if it were an ecosystem, from a microbial perspective, to quantify the ecological processes that shape the pathogenic, commensal, and benign populations within the community. Linking this information to the rest of the ecosystem, including the microbiota associated with patients and staff, and the contextual data that define the environment such as building-science properties (light, temperature, humidity, and occupation density [60]), patient treatments, medical and building operation policy, and disease outbreaks will enable this study to characterize the metaparameters that shape the microbial ecology and hence infer the nosocomial infection state of this health-care environment.

Concluding remarks

Built environments comprise chemical and physical habitats unprecedented in the natural world that may have untold consequences for the selection and growth of microorganisms. The hospital environment, despite the exquisite control imposed on its biological matrix, remains home to a bewildering diversity of microorganisms. Understanding the ecology of these complex communities will be likely to pay considerable dividends in the control of health-care associated infections and the spread of antibiotic resistance. However, we still have a considerable way to go in understanding and manipulating this environment so that we can control the ecological succession, structure, and pathogenicity of the indoor microbial world. Continued research is needed to quantify this environment, to explicitly examine how the metabolism of individual organisms and metabolic interactions between these organisms and with their environment structure the community dynamics that have been observed (Box 2). If we can learn how to capture these processes in metabolic models, we may develop the potential to forecast how these communities will respond to changes in hospital management practice, patient treatment, and even architectural modifications. The complexity of these interactions is extraordinary and requires concerted, coordinated efforts with multidisciplinary teams to appropriately parameterize, quantify, and model these ecosystems. Instead of trying to enforce our will on a great unknown, we will be able to tweak our environment to influence the microbial mechanism that has such a profound influence on our health and well-being.

Box 2. Suggested priorities for future research

Controlled clinical studies to determine biomarkers associated with disease onset and intrahospital transmission events

Large-scale surveys of human-associated microbiota, such as the NIH Human Microbiome Project, have made clear how variable a 'healthy' microbiome can be between individuals. This makes longitudinal studies from the same individuals, pre- and post-hospitalization, imperative for the characterization of taxa associated with disease and hospital transmission.

Determining the sources and reservoirs of nosocomial infections

There is still no systematic analysis of the source and development of microbial reservoirs in hospital environments. Especially little is known about the contribution of hospital air and water to infection development.

Greater study of cleaning strategies and selective decontamination protocols

Cleaning practices and antibiotic treatments are usually targeted to specific microorganisms without considering the effect on the ecology of the full microbial community. There targeted perturbations of the microbiome can unintentionally reduce competition against pathogenic organisms and result in their increased proliferation and virulence.

Characterizing the effect of architectural and technological choices on the microbial ecology of hospital environments

Choices in building and device materials may have critical implications for the differential survival and proliferation of microbial taxa. Interactions of surface type, touch frequency, materials, and cleaning regimens need to be better mapped to clinical symptoms in patients.

Greater study of antibiotic-resistant Gram-negative bacterial infections

Despite the recent identification of many multidrug-resistant Gram-negative bacteria and their increasing contribution to nosocomial infections, we know very little about the origin and transmission of Gram-negative pathogens, which are especially understudied relative to MRSA and *Clostridium difficile*.

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