Infection Control in the Multidrug-Resistant Era: Tending the Human Microbiome

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Increasing understanding of the normal commensal microorganisms in humans suggests that restoring and maintaining the microbiome may provide a key to preventing colonization and infection with multidrug-resistant organisms (MDROs). Intact communities of commensals can prevent colonization with MDROs through both competition for space and resources and the complex immunologic and biochemical interactions that have developed between commensal and host over millennia. Current antimicrobials, however, exert tremendous collateral damage to the human microbiome through overuse and broadening spectrum, which has likely been the driving force behind the introduction and proliferation of MDROs. The future direction of infection control and anti-infective therapy will likely capitalize on an expanding understanding of the protective role of the microbiome by (1) developing and using more microbiome-sparing antimicrobial therapy, (2) developing techniques to maintain and restore indigenous microbiota, and (3) discovering and exploiting host protective mechanisms normally afforded by an intact microbiome.

The emergence of the New Delhi metallo-β-lactamase 1 (NDM-1) carbapenemase in the United States follows a number of healthcare-associated, multidrug-resistant organisms (MDROs) posing significant public health challenges including, methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), extended-spectrum β-lactamase (ESBL) producers, specific drug-resistant (eg, clindamycin- or fluoroquinolone-resistant) strains of Clostridium difficile, and Klebsiella pneumoniae carbapenemase (KPC) producers [1–3]. Although microbiologically diverse, each of these MDROs shares several epidemiologic features in common: All are transmitted primarily through direct and indirect contact, colonization commonly precedes infection (with the possible exception of C. difficile), they colonize a large population relative to the subset that they infect, and they colonize persons either at sites of pathologic biofilms (ie, invasive or implanted devices, chronic wounds, renal calculi, devitalized bone, and other biofilms associated with a diseased state) or at body sites normally inhabited by indigenous human microbiota. The collective genome of this indigenous microbiota has been termed the human microbiome and has become the subject of intense study [4]. Although the relevance of some of the early findings from studies of the human microbiome may not be at first readily apparent, an improved understanding of the complex communities of microbial commensals that have coevolved with humans over the millennia will become important for successful infection control in the multidrug-resistant era.

LIMITATIONS OF CURRENT MDRO CONTROL STRATEGIES

Current strategies to address MDROs consist of 3 broad categories: (1) developing new antimicrobial agents, (2) interrupting MDRO cross-transmission, and (3) increasing antimicrobial stewardship efforts. In the case of MDROs, resistance spreads in the human population...
more often by clonal expansion of resistant strains or inter- and intraspecies transfer of preformed resistance elements, such as plasmids or transposons, than by repeated new random genetic mutation or recombination events that are selected de novo (Figure 1A). Thus, how quickly multidrug resistance develops and spreads in vivo depends on (1) how quickly preexisting genetic resistance determinants rearrange or mutate to facilitate cross-species transmission into target pathogens and (2) how quickly resistant pathogen genotypes spread among the human population.

Meanwhile, pathogens exist in complex communities, which through horizontal gene transfer, provide them access to large libraries of resistance determinants that have been millions of years in the making. Venues for this genetic exchange include not only the human microbiome and any physiologic biofilms (ie, biofilms not associated with a disease state) that exist in equilibrium with it [5], but also the aforementioned pathologic biofilms [6]. The microbiomes of animals and the environment also contribute resistance determinants with commensals that can survive in such settings (eg, Clostridium species, Enterococcus species, Enterobacteriaceae), serving as a genetic bridge into the human microbiome. The evolutionary pressure that led to the original selection of a particular resistance determinant may have been directed millions of years ago at organisms whose descendents are now largely unrelated to target pathogens. Because humans entered this race millions of years after our microbial competitors, which vastly outrank us in terms of numbers and access to useful genetic determinants, fighting antimicrobial resistance with more antimicrobials, although a necessary short-term strategy, is a long-term strategy destined to fail.

Interrupting cross-transmission is a proven strategy for reducing the incidence and prevalence of specific MDROs and can slow the emergence of new forms of resistance that have been recently introduced into target pathogens. Broad horizontal approaches, such as hand hygiene and environmental cleaning, form the foundation of modern infection control [7]. Meanwhile, because the number of colonized patients far exceeds the number of those with identified infection, a vertical approach of identifying MDRO-colonized patients to focus isolation precautions (eg, use of gowns and gloves) or decolonization efforts has become an added strategy to prevent cross-transmission. Although controversy lingers over the impact of intensive vertical approaches, the strategy has likely slowed spread of some MDROs in hospitals [8, 9]. Nonetheless, transmission interruption is another tool likely to fail if relied on solely. For example, although aggressive efforts to interrupt transmission have slowed spread or even reduced prevalence of VRE, MRSA, and KPC producers, the effectiveness of such strategies may be more limited where elements that are more transferable across a wide variety of species, such as NDM-1, become prevalent [9–11].

The growing recognition that more than one-third of all antimicrobial use in hospitals may be unnecessary has prompted increased attention to antimicrobial stewardship efforts [12, 13]. Unfortunately, such stewardship efforts have been difficult to sustain, in part because the increasing prevalence of MDROs has been met with the need for broader empirical antimicrobial coverage in many clinical situations, resulting in a vicious cycle [14]. Among our established strategies to control MDROs, antimicrobial stewardship may be most in need of reframing in the context of increased understanding of the human microbiome. The antimicrobial effect most epidemiologically important in spreading MDROs in healthcare is probably not the selection of resistant strains from susceptible populations of the same microbial species. Rather, it is the propensity of drugs to perturb the indigenous microbiota, facilitating colonization and expansion of previously selected resistant strains and/or their resistance traits, making the selection pressure of antimicrobial use in spreading MDROs aptly termed “pressure on the human microbiome” (Figure 1A).

THE MICROBIOME AS CONTEXT FOR POTENTIAL NEW CONTROL STRATEGIES

Clearly, given the limitations of each approach used independently, controlling the spread of MDROs will require a multifaceted approach including each of the control strategies discussed, in addition to novel interventions based on an increased understanding of the microbiome in general and, given the challenges of emerging gram-negative resistance, the human intestinal microbiome in particular. The Human Microbiome Project sponsored by the Common Fund at the National Institutes of Health has as its goals (1) determining whether individuals share a core human microbiome, (2) understanding whether changes in the human microbiome can be correlated with changes in human health, (3) developing the new technological and bioinformatic tools needed to support these goals, and (4) addressing the ethical, legal, and social implications raised by human microbiome research [4]. Modern molecular techniques have allowed for deep characterization of the microbiota of the nasal mucosa, oropharynx, skin, urogenital sites, and lower intestine in ways not possible through standard culture techniques. These methods, broadly termed metagenomics, rely largely on polymerase chain reaction amplification of either 16S ribosomal RNA-encoding genes or the entire genomes of the microbiota, followed by high-throughput DNA sequencing.

By applying metagenomics, it has been estimated that the cumulative human lower intestinal microbiota contains at least 1800 genera and 15 000–36 000 species (depending on a conservative vs liberal classification), most of which have never been successfully cultured and the majority of which are contained in
the phyla Firmicutes and Bacteroidetes [15]. One recent study comparing fecal microbiomes of 22 individuals from 4 different countries identified only 3 robust clusters of relative abundances of different phyla, suggesting that the major constituents of the normal human intestinal microbiota are fairly consistent, despite geographic, ethnic, and dietary differences [16]. This human intestinal microbiota develops over the first year of life; emerging evidence suggests that the

Figure 1.  
A, Role of antibiotic pressure and its effect on the human microbiome in the development and spread of multidrug resistance.  
B, Strategies to harness the human microbiome for either preventing or shortening the duration of colonization or reducing the clonal expansion of multidrug-resistant organisms. Abbreviation: MDR, multidrug-resistant.
development of this community is important for the maturation of the immune system, with perturbations in development being associated with atopy in later life [17]. Once established, the intestinal microbiota appears to achieve equilibrium but can be easily perturbed by major changes in diet and exposure to antimicrobials [18–20].

Two seminal studies have provided valuable insight that our current antimicrobials, although very effective anti-infectives, cause tremendous collateral damage to our microbiome. Antonopoulos et al treated inbred mice (interleukin-10 deficient, chosen for their relevance as a model for inflammatory bowel disease) with either ampicillin-metronidazole-bismuth or cefoperazone for 10 days to study effects on their cecal contents for up to 6 weeks [21]. In contrast, Dethlefsen et al enrolled 3 human volunteers given two 5-day courses of ciprofloxacin 6 months apart and studied them intensely, with >50 stool samples per subject collected over a total of 10 months [22]. Through the use of metagenomics, both groups found that the antimicrobials had a rapid, profound, and sustained impact on the lower intestinal flora, with a loss of richness (ie, number of different species surrogates or operational taxonomic units) and diversity (ie, the breadth of phylogenetic diversity represented in the flora).

In the mouse model, there was also a clear shift away from a dominance of Firmicutes and Bacteroidetes to Proteobacteria (including the Enterobacteriaceae) [21]. Whereas the dominance of the Firmicutes and Bacteroidetes returned by 2 weeks of recovery in antimicrobial-treated mice, Proteobacteria richness remained at levels higher than baseline and the diversity of Bacteroidetes remained depressed. A notable exception was observed in mice that, during their 6-week recovery from cefoperazone treatment, were housed with a control mouse that had not been exposed to antimicrobials, allowing these control mice, through natural coprophagic activity, to serve as microbial donors. The antimicrobial-treated mice housed with microbial donors during their recovery period had profiles that appeared to be more similar to control animals than mice who recovered in isolation.

In the study by Dethlefsen et al, the human intestinal microbiota began its return toward baseline 1 week after completion of the first course of ciprofloxacin [22]. Whereas the return in richness and diversity appeared to be nearly complete in 2 of the 3 human subjects by the end of 6 months of follow-up, the third subject had a much flatter, depressed trajectory in these 2 indices. All 3 subjects had clearly depressed indices over the 2–4 months following their second course of ciprofloxacin, with much flatter trajectories of recovery, relative to that observed following their first course. Thus, the response of the human intestinal microbiota to perturbation by ciprofloxacin was different across subjects and, in the same subjects, between the first and second course of drug, with evidence suggesting the community may be very long in recovery, if ever achieved, following repeated insults.

These perturbations caused by antimicrobials reduce the colonization resistance (ability of the microbiota to resist colonization with a new organism) and resilience (ability of the microbiota to recover from alterations in its composition), allowing for displacement of mutualist organisms with non-mutualist pathogens, including MDROs and C. difficile, that would otherwise not be able to take hold [23]. This has been demonstrated in studies with humans showing that antimicrobial exposure is needed for colonization with MDR gram-negative bacteria and C. difficile and in mouse models showing that antimicrobials are required for colonization with VRE and MDR gram-negative bacteria [23–29]. This is true of both colonic microbiota and skin, because antimicrobial exposure contributes to colonization with MDR Acinetobacter [30].

There are several possible mechanisms by which an intact microbiome prevents colonization with MDROs. The most obvious means is by direct competition for food sources and sites for cellular adherence. However, there is a growing body of knowledge about the complex immunologic and biochemical interactions between the microbiota and their human host that have developed through our coevolution [31]. Mouse models have shown that bacterial antigens such as lipopolysaccharide and flagellin can stimulate Toll-like receptors, resulting in the release of endogenous antimicrobial peptides, which appear to be vital to the dynamic equilibrium of the human microbiota and can prevent colonization with MDROs such as VRE [26, 32]. This discovery illuminates a potential avenue to help restore or maintain normal microbiota through careful manipulation of these interactions.

**EXPLOITING THE MICROBIOME TO PREVENT OR SHORTEN DURATION OF MDRO COLONIZATION**

There are several translational research objectives and strategies that could harness our growing understanding of the human microbiome to combat multidrug resistance (Table 1 and Figure 1B). The most apparent of these is preserving the microbiota through antimicrobial stewardship that is refocused with the microbiome in mind. To achieve this objective, models should be developed and refined that reliably predict the impact of an antimicrobial or an antimicrobial combination on the microbiota, specifically those shifts in the microbiome that promote colonization and spread of MDROs. Results could better direct stewardship efforts, with the goal of reducing MDRO prevalence; these models could also be used in the preclinical approval process of a new antimicrobial, promoting development of drugs that have market advantage specifically because of a favorable profile in this realm [28]. In addition
to refocusing how we approach stewardship and assessing the impact of specific drugs, added means, such as methods to break down or bind antimicrobials, should be pursued to preserve indigenous microbiota [33, 34].

In addition to preserving the microbiome, methods should be developed to restore the microbiome after it is damaged. There has been growing interest in both the scientific and the lay literature on the use of probiotics to prevent *C. difficile* infection and diarrhea in general and to promote overall human health. Although data suggest efficacy for current probiotics in achieving some but not all of these ends, the current approach of administering a single microorganism to restore equilibrium to such complex communities is very limited. To date, the most successful method for restoring the mammalian intestinal flora has been through fecal microbiota transplantation (FMT), for example, either from a donor mouse placed in the same cage, as in the study by Antonopoulos et al, or the growing number of successful human FMTs performed to treat recurrent *C. difficile* infection [21, 35, 36]. Just as metagenomics have been used to demonstrate successful engraftment of a donor microbiome in the mouse, successful engraftment and durable alteration of microbiota have been demonstrated with human FMTs [21, 37, 38].

Although FMT is currently the most effective means to restore the human intestinal microbiome, it suffers from repulsive esthetics and inherent risk of pathogen transmission. To overcome the latter and, perhaps, the former shortcomings, studies could use human microbiome “auto-banking” as a means for studying the impact of restoration on MDRO control. For example, a sample of a patient’s indigenous microbiota (in this case stool) could be obtained at admission to an inpatient facility or in the ambulatory setting (before antimicrobial therapy is initiated). These samples could be frozen for later reimplantation of a patient’s native microbiota after completion of antimicrobial therapy. After patients have completed their therapeutic course of antimicrobials and are ready for reimplantation, they may already have become colonized with an MDRO; whether microbiome restoration alone could ameliorate this colonization is unknown. Topical or nonabsorbable antimicrobial therapy may be needed to suppress the MDRO prior to reimplantation, much as a course of vancomycin is commonly used to treat active infection (and possibly suppress colonization) before FMT for recurrent *C. difficile* infection. There may be formidable methodological and logistical challenges to extending such human microbiome banking to sites other than the intestine (ie, optimizing recovery methods and maintaining viability) or expanding use to routine practice. Moreover, although the Food and Drug Administration’s Division of Vaccines and Related Product Applications has general guidance that encompasses clinical trials for the therapeutic and prophylactic use of probiotics [39], a regulatory framework informed by early translational findings will need to be established to study human microbiota banking for reimplantation and transplantation. Despite these challenges, demonstrating the ability of a restored microbiome to shorten the incidence, duration, or magnitude of human intestinal colonization with other MDROs in addition to *C. difficile* would offer important proof of concept for increasing focus on preserving and restoring the microbiome in infection control.

As more is learned about the complex immunologic and biochemical interactions that underlie the dynamic equilibrium of the human microbiome, more sophisticated tools may become available. This is illustrated in the recent report by Mohamadzadeh et al that describes successful downregulation of intestinal inflammation in a mouse model of inflammatory bowel disease through administration of *Lactobacillus acidophilus* that had been genetically manipulated to inhibit its lipoteichoic acid synthesis [40]. This highlights how an advanced understanding of the complex cross-talk between bacteria and,
in this case, the dendritic cells of the mouse intestine led to development of a genetically engineered, advanced probiotic. Similarly, as host-microbiome interactions relating to MDRO colonization are better understood, colonization may be reduced through advanced probiotics and possibly without the need for live bacteria, instead achieved through manipulation of these immunologic and biochemical interactions to trick the host to act as though the microbiome is intact. The careful use of bacterial antigens or a balanced mix of endogenous antimi-

can be prevented in mice receiving broad-spectrum anti-

MDROs. Mouse models have given proof of concept for these potential interventions by showing that colonization with VRE store the microbiome and/or prevent colonization with MDROs. Mouse models have given proof of concept for these potential interventions by showing that colonization with VRE can be prevented in mice receiving broad-spectrum anti-

microbial peptides may be all that is needed to maintain or re-

store the microbiome and/or prevent colonization with MDROs. Mouse models have given proof of concept for these potential interventions by showing that colonization with VRE may be achieved through manipulation of these bacterial antigens lipopolysaccharide or flagellin [26, 32].

This alternative perspective on how to view the struggle with MDROs faces several major challenge areas and unknowns. It is not known how long in the emergence of new resistance phenotypes before decreased fitness cost or other evolutionary hurdles are overcome that allow an MDRO to outcompete native members of the microbiota or otherwise circumvent the colonization resistance afforded by an intact microbiome. For example, it is encouraging that, despite its emergence almost 40 years following the emergence of MRSA as a healthcare-

adapted MDRO, colonization and infection with community-

adapted strains of MRSA still appear to be at least somewhat associated with prior antimicrobial exposure, suggesting that indigenous microbiota may still offer some protection from colonization [41, 42]. Nonetheless, it has been suggested that, owing to a convergence of specific virulence properties and resistance, community-adapted MRSA infection may be less associated with preceding colonization than infection caused by healthcare-adapted strains [43].

There are also insults other than antimicrobials that naturally perturb the microbiome. For example, host viral infections may be an important natural means by which host micro-

organisms are perturbed. As such, there may be relationships between norovirus infection and C. difficile carriage or disease or viral upper respiratory illness and MRSA carriage or pneu-

monia that deserve further study. Moreover, these possibilities raise the question of whether these naturally occurring perturbations should also be tended to through microbiome-

restoration techniques. Although the development of bacterial vaccines and immunologic therapies could be viewed as a fourth major category of current MDRO control strategies that offers promise in reducing overall reliance on antimicrobials, concerns have been raised that these may also alter the normal human microbiome and thereby promote MDRO colonization. For example, recent evidence indicates that there is a temporary increase in S. aureus carriage following vaccination with the 7-valent pneumococcal vaccine [44]. However, other evidence suggests no overall association between a history of vaccina-

tion and colonization prevalence with either S. aureus or, specifically, MRSA [45]. There is also the uncertain role of human genetics in carriage of specific pathogens. Although early work is being done to determine genetic determinates of MRSA colonization, much more remains unknown [46]. Finally there are the persistent problems posed by pathologic biofilms that presently appear beyond the reach of the homeo-

static effects of the indigenous microbiota and may therefore offer safe harbor sites for MDRO colonization [6, 47]. Thus, advances in the understanding and amelioration of pathologic biofilms will likely be necessary to realize all the potential benefits to infection control afforded by tending the human microbiome.

Notes
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