Commensal Flora May Play Key Role in Spreading Antibiotic Resistance

We need to learn more about commensal flora if we are to better manage this particular window of vulnerability to antibiotic resistance.

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Antibiotic resistance among commensal bacteria represents a major avenue for the development of resistance in bacterial pathogens. Although the concept that commensal flora play a major role in disseminating bacterial resistance was developed at least 30 years ago, it was set aside for several reasons.

First, the more immediate threat to humans from resistant bacteria is due to pathogens, not commensals. Second, it is much easier to study simpler mechanisms of resistance in single species than to follow gene transfers among unrelated species within complex ecosystems. Third, resistance was not considered a major public health problem when new antibiotics were being made available on a regular basis—masking the growing magnitude of this problem and postponing the onset of antibiotic failures in clinical settings.

Strategies for involving commensal bacteria to reduce antibiotic resistance in clinical practice cover only a very small number of situations, meaning that reduced antibiotic use remains the major means for controlling resistance. Even if national and international agencies succeed in curbing unwarranted uses of antibiotics, however, there always will be clinical conditions in which antibiotic treatments are necessary. Hence, we will need to learn more about the impact of antibiotic treatments on resistance in commensal flora if we are to better manage this particular window of vulnerability to antibiotic resistance.

Assessing the role of commensal flora in the development of antibiotic resistance among pathogens is difficult because, typically, resistance increases first in the commensal flora but is transferred to pathogens only afterwards. Thus, patients who become infected with resistant bacteria usually are not those in whom the selecting antibiotics were first used.

For instance, the rise in quinolone resistance of pneumococci in Canada occurred five years after these antibiotics came into use, even though they were used for treating respiratory tract infections, according to Danny K. Chen and coworkers from the University of Toronto. Also, trimethoprim-resistant respiratory tract bacterial pathogens were detected in a San Francisco hospital following a rise in usage of this drug to prevent parasitic Pneumocystis carinii infections in AIDS patients (Fig. 1), according to Jeffrey N. Martin and colleagues from the University of California, San Francisco.

In both these instances, resistance was likely selected in commensal flora and later transferred to pathogens. Indeed, there are two major avenues for the emergence and spread of antibiotic resistance.
resistance in pathogenic bacteria, the first being by direct selection of resistant mutants within the population of pathogenic bacteria at the site of infection. The second is indirect, involving initial selection of resistant bacteria among commensal flora, followed by horizontal transfer of resistance genes to pathogenic species.

Transfer of resistance genes from commensal to pathogenic bacteria was described as early as 1959 by Japanese researchers, who were studying *Escherichia coli*, a commensal found in the colon of humans, and *Shigella dysenteriae*, a pathogen responsible for causing diarrhea that is particularly deadly in developing countries.

More recently, investigators identify several settings as likely sources of antibiotic resistance traits found in intestinal enterobacteria occupying the human gastrointestinal (GI) tract. For instance, close contact between humans and farm animals, particularly those raised in large production facilities, is associated with an increased prevalence of resistance to such drugs. For instance, we recently showed that not only the intestinal ecosystem, but also the nasal and pharyngeal ecosystems among farmers raising pigs are more frequently colonized by drug-resistant commensal bacteria than are those of nonfarmers with whom they are closely matched.

Patients in hospitals are more likely to be colonized by resistant bacteria than are individuals who have not been hospitalized. Moreover, hospital workers have a high rate of carriage of drug-resistant bacteria, according to several studies. However, these studies have tended to explore how resistant bacteria are transmitted, rather than to quantify the risk of increased prevalence of resistant commensal flora among health care personnel in contact with hospitalized patients.

Commensals Could Be Highly Efficient Contributors to Resistance

The indirect mechanism for generating bacterial resistance in pathogens is more efficient than direct selection for several reasons. First, among commensal flora, there are many more targets than within an infectious site in terms of numbers of species—several hundreds versus a single species of pathogen—and in terms of numbers of bacterial cells, around $10^{14}$ commensals versus $10^{8}–9$ cells of a particular pathogen.

Second, because the commensal genetic pool is so large, it encompasses many more potential means for conferring resistance, including not only single-nucleotide mutations but also complex resistance mechanisms that ordinarily remain more or less silent within subdominant species.

Third, resistant commensal flora may be selected each time an antibiotic is administered, irrespective of the health status of the host, whereas resistant pathogens are subject to selective pressure only when particular patients actually are infected with those bacteria. This point is important because so many patients who receive antibiotics are not actually infected by pathogens but, instead, are being treated prophylactically, or the cause of the infection is a virus that is not susceptible to the drug being administered. In animals, the proportion of noninfected recipients of antibiotic agents is even greater than among humans.

In addition, pharmacodynamics and pharmacokinetics of antibiotics may favor the selection
of resistance amid commensal flora rather than among pathogens. Indeed, we know that low antibiotic doses and prolonged treatments favor the emergence of resistance. Hence, antibiotic regimens take this possibility into account and thus are set to deliver appropriate concentrations of drug to a particular site of infection.

However, these regimens typically do not take into account what happens at various surfaces where commensals may encounter those drugs. For instance, after patients are administered typical doses of the antibiotic ciprofloxacin, its concentrations vary from 2 to 5 mg/liter in the sweat of those patients, according to Niels Hoiby and colleagues at the University of Copenhagen in Denmark. Similarly, this drug ranges from 3 to 5 mg/liter in feces, according to Sophie Pecquet and other colleagues in my laboratory, but only 0.5 to 1.5 mg/liter in the saliva and 0.3 to 0.5 mg/liter in nasal secretions as reported by Rabih Darouiche and colleagues at the Veterans Administration Medical Center (VAMC) in Houston, Tex.

There are three major bacterial ecosystems in humans, including the intestinal, the cutaneous ones, and that of the upper respiratory tract. Variations in drug concentration are a source of differential selective pressures on these commensal ecosystems, thus explaining why resistance occurs so commonly after antibiotic treatments. Each of these commensal ecosystems is affected to some extent during antibiotic treatments.

Commensals in the Intestinal System Provide Insights about Drug Resistance

The intestinal commensal ecosystem is by far the best studied and also the most populous, containing $10^{14}$ colony forming units (CFU) from several hundred species. For many decades, experts said that the intestinal flora is very stable within each individual and among individuals of the same species. The anaerobes at around $10^{11} - 10^{12}$ CFU/g of intestinal content dominate this ecosystem, in which enterobacteria and enterococci are relatively minor players ranging from $10^6 - 10^8$ CFU/g.

How these population balances are maintained is poorly understood, although the process appears to involve anaerobic flora exerting colonization resistance in humans, according to Curtis J. Donskey and colleagues at the VAMC in Cleveland, Ohio. They showed that fecal counts of enterococci that are resistant to glycopeptide antibiotics increase significantly among patients who were treated with antibiotics that are active against anaerobic microorganisms compared to those of patients who were treated with other antibiotics having minimal activity against anaerobes (Fig. 2).

Colonization resistance also prevents the intestinal ecosystem from being colonized by exogenous bacteria, thus stabilizing the intestinal flora against newcomers. However, although a dominant lactobacillus strain can be very stable in some subjects, it can vary greatly in others, according to Anne L. McCartney and colleagues from the University of Otago in New Zealand. What distinguishes these two types of individuals remains to be investigated.
Colonization resistance has been extensively studied in gnotobiotic mice that are inoculated with human fecal flora, providing a relatively convenient means for mimicking and thus investigating relationships among intestinal flora that are typical of humans. For instance, Yvonne Duval-Iflah, Cyrille Tancre`de, and colleagues from Institut National de la Recherche Agronomique in Jouy-en-Josas, France, studied a multiantibiotic-resistant strain of Serratia, an enterobacterial species that does not belong to the commensal flora of humans. In the basal state before being treated with antibiotics, this species was rapidly eliminated from such mice even after repeated inoculations (Fig. 3). Even so, these transient enterobacteria can transfer their drug resistance gene-carrying plasmids to E. coli that are residents of the GI tract, although the resulting transconjugants are also rapidly eliminated.

However, when such mice are being actively treated with antibiotics, the resistant strains rapidly establish residence in the GI tract, while drug-susceptible strains disappear. Also of note, the counts of drug-resistant enterobacteria are higher than those of susceptible E. coli before treatment. After antibiotic treatments cease, total counts of enterobacteria return to baseline levels, suggesting colonization resistance is restored. Moreover, the multiresistant strain of Serratia is eliminated but the remaining and predominant E. coli population consists of transconjugants.

Altogether, this study suggests that inoculating exogenous resistant bacteria while administering antibiotics could lead to prolonged modifications in bacterial resistance within intestinal commensal bacteria. Such a sequence of events probably also can occur among humans. Moreover, even in developed countries, industrialized foods can be a source of drug-resistant gram-negative and gram-positive bacteria. Maintaining a sterile diet strongly reduces intestinal colonization by drug-resistant bacteria.

Antibiotic treatments can significantly affect the prevalence of bacterial resistance among intestinal enterobacteria. For instance, within two weeks of taking trimethoprim, drug-resistant strains take over the enterobacterial intestinal population in subjects taking this drug, and this effect lasts several weeks after drug treatment ends. Colonization by enterobacteria resistant to the antibiotic being administered seems to increase linearly with the extent of treatment. The prevalence of carriage of resistant E. coli varies from one country to another, with resistance often more pronounced in developing than in developed countries, most probably because of the wider availability of drugs without prescription among the former.

Resistant commensal enterobacteria can circulate between individuals. Travelers to developing countries may be colonized by resistant enterobacteria in the absence of antibiotic treatment. Even in developed countries with high hygienic standards, resistant enterobacteria bacteria may disseminate within households. Several additional factors appear to influence the extent of colonization by antibiotic-resistance enterobacteria. For instance, drug-resistant bacteria colonizing Nepalese people decrease with population density, access to allopathic medical care, and distance from the capital of the country, according to Judd L. Walson and colleagues.

**FIGURE 3**

Antibiotics promote colonization by resistant bacteria. In gnotobiotic mice harboring microflora, a susceptible strain of E. coli (open squares) within a complex human, multiresistant strains of Serratia liquefaciens (open circles) are rapidly eliminated just as resistant E. coli transconjugant (black squares). During treatment with ampicillin both resistant strains are eliminated while the susceptible E. coli disappeared (downward arrows indicate the lower limit of detection of a given strains) (see Duval-Iflah et al., Infect. Immun. 28:981–990).
from Tufts University Medical School in Boston, Mass.

In general, there is little doubt that intestinal enterobacteria constitute a pool of antibiotic-resistant microorganisms. Moreover, there is evidence pointing to a correlation between the resistance among commensal enterobacteria and that found among enterobacterial pathogens, not only at the individual level but also at the population level in a given country.

**Dissemination of Resistance within Human Ecosystems**

Antibiotic resistance genes spread among enterobacteria in the GI tracts of humans as well as several other animal species. Resistance genes, such as *tetQ* that confers resistance to tetracycline and *erm* that confers resistance to erythromycin, can exchange among *Bacteroides* spp. and among *Bacteroides* and other anaerobic genera that populate the human colon in high densities. These genes are homologous to those that confer resistance to these antibiotics in enterobacteria, suggesting that multiple exchanges can occur among enterobacteria and anaerobes.

Gene exchanges probably also occur in other human ecosystems, particularly in the oropharynx between *Streptococcus pneumoniae* and other non-groupable streptococci. For instance, *S. pneumoniae* become resistant to penicillin after genes encoding similar penicillin-binding proteins (PBPs), which serve as target proteins of β-lactam antibiotics, recombine to form mosaic PBPs. Often, parts of those recombined genes derive from non-groupable streptococci, which tend to be more resistant to antibiotics.

Moreover, genes encoding quinolone resistance can be transformed and expressed in *S. pneumoniae*, with the rate being higher when donor species are phylogenetically close to *S. pneumoniae*, according to Laurent Gutmann and his group at the University of Paris VI in France. These investigators showed that carriage of quinolone-resistant non-groupable streptococci is just as frequent in hospitalized patients that received quinolone as in those that had not, but this rate is much higher than in individuals who had not been hospitalized. This finding suggests that there is a high rate of antibiotic resistance gene transfer and dissemination within the pharyngeal flora of hospitalized patients.

Antibiotic resistance among staphylococci is a major public health problem in hospitals worldwide. Excretion of antibiotics, such as ciprofloxacin in sweat during treatments, is associated with increased skin colonization by ciprofloxacin-resistant *S. epidermidis*. Also, it is assumed that the *mecA* gene that confers resistance to all β-lactams in *S. aureus* originates in coagulase-negative staphylococci. For example, in *S. sciuri*, a widely distributed coagulase-negative staphylococci, the *mecA* gene is present, resistance is expressed only in mutants that are derepressed for expression of the effector PBP2a protein, according to Shang Wei Wu and colleagues from the Rockefeller University in New York, N.Y. Furthermore, resistance can transfer from derepressed *S. sciuri* to *S. aureus*.

**Means for Reducing Antibiotic Resistance Include Role for Commensal Flora**

Reducing antibiotic use seems to be the best means for reducing overall resistance. For short-
term studies, such as those performed in hospital settings, the temporal relationship between antibiotic use and resistance may appear complex, perhaps requiring sophisticated statistical analysis to account for delays between changes in antibiotic use and in resistance rates, according to Dominique Monnet from the Staten Serum Institute in Copenhagen.

Nonetheless, some examples are being documented for pathogenic strains, particularly *S. pneumoniae*. In Iceland, for instance, reducing antibiotic use by 13% led to an estimated 10% decrease in resistance to penicillin in that species, according to Daren J. Austin and colleagues from the University of Oxford in England. Similarly in France, decreased antibiotic resistance of *S. pneumoniae* followed reductions in antibiotic use in a population-based intervention, according to Didier Guillemot from the Pasteur Institute in Paris.

More generally in Europe, this relationship between reduced antibiotic use in a particular population segment and lowered levels of antibiotic resistance among pathogenic species circulating within that population is well demonstrated—for instance, consider a country-by-country comparison of susceptibility-to-penicillin rates among *S. pneumoniae* isolates (Fig. 4). Such findings led some public health experts to suggest that reducing the carriage of serotypes associated with antibiotic resistance by use of pneumococcal conjugate vaccine may have a greater short-term impact than would decreasing antibiotic use because decreasing antibiotic use will take a long time before being widely accepted.

In another specific case, the prevalence of glycopeptide-resistant enterococci colonizing residents from Germany decreased markedly following a progressive reduction of the agricultural use of avoparcin (also a glycopeptide antibiotic) throughout Western Europe (Fig. 5), according to Ingo Klare and colleagues at the Robert Koch Institute in Germany. Here again, there was some delay after antibiotic use was reduced before resistance carriage rates came down. The role of exchanges of resistance genes among bacteria within the commensal flora as such ecosystems adapt to new environmental conditions remains to be investigated.

It seems also that differences exist between antibiotic regimens in terms of selection of resistant bacteria in the commensal flora, but data are sparse. Indeed, we showed in my laboratory that when antibiotics from different classes—for instance, amoxiclav versus ofloxacin or telithromycin—are compared, strains resistant to the antibiotic absorbed by particular patients are preferentially selected. Moreover, among newborn patients in intensive care units, usage of penicillin-tobramycin instead of ampicillin-cefotaxime as first-line empiric treatment is associated with dramatic reductions in colonization of such newborns by drug-resistant gram-negative rods, according to Peter de Man and colleagues from Erasmus University in the Netherlands. Furthermore, there was also a borderline trend towards reducing secondary infection rates.

Altogether, it appears that the need to curb antibiotic resistance will have to take into account the role of the commensal flora in the development of resistance. This may result in profound changes, certainly quantitative but also qualitative, in the ways we currently use antibiotics.
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