Commensals: Underappreciated Reservoir of Antibiotic Resistance

Probing the role of commensals in propagating antibiotic resistance should help preserve the efficacy of these critical drugs

Bonnie M. Marshall, Dorothy J. Ochieng, and Stuart B. Levy

Antibiotic resistance, reported for sulfonamides in the mid-1930s and for penicillins in the 1940s, remains a stubborn quandary. What was once confined mainly to hospitals increasingly involves multidrug resistance that encompasses communities and encircles the globe. Virtually all types of bacterial infections are becoming resistant to antibiotic treatments, according to officials at the Centers for Disease Control and Prevention in Atlanta, Ga. Yet, despite decades of grappling with these issues, we still do not understand fully how genes carrying resistance traits spread, what makes certain species highly promiscuous in transferring those traits, whether there are effective barriers to their spread, and the frequency with which resistance genes move independently or in tandem with other migrating genes.

While evidence points to microorganisms associated with food, animals, and water as the main sources for resistance genes, which of them exerts the most impact is not known. We also do not know how important a role the burgeoning aquaculture industry plays, particularly in those countries where such farms are poorly regulated and may not only abuse antibiotics but sometimes even operate within the confines of wastewater treatment plants. Equally worrisome are the sludge products of urban and rural wastewater treatment plants that are increasingly used for fertilizer—dispersing unknown amounts of resistance genes and antibiotics that withstand standard sewage treatment.

In 2008, the Alliance for the Prudent Use of Antibiotics (APUA) convened microbiologists and other experts to review these and related questions and also to address the role of commensal and other nonpathogenic microorganisms in the overall problem of antibiotic resistance development and dis-

Summary

- Antibiotic resistance, increasingly dominated by multidrug-resistant microorganisms, is a growing threat to public health on a global basis.
- The Alliance for the Prudent Use of Antibiotics (APUA) is developing databases to track commensals and free-living microorganisms that provide a large reservoir for resistance genes that could transfer to pathogens (www.apua.org).
- A move to standardize methods and to improve surveillance systems, with emphasis on gene tracking, will help in analyzing antibiotic resistance.
- Multidisciplinary approaches, including gene-based technologies to study commensal ecology and a focus on aquaculture and wastewater environments, are needed to track resistance.

Bonnie M. Marshall is a Staff Scientist at The Alliance for the Prudent Use of Antibiotics and Research Associate in the Center for Adaptation Genetics and Drug Resistance and the Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Mass. Dorothy J. Ochieng is a Project Manager at The Alliance for the Prudent Use of Antibiotics, and Stuart B. Levy is President of The Alliance for the Prudent Use of Antibiotics and Director of the Center for Adaptation Genetics and Drug Resistance and Distinguished Professor in the Departments of Molecular Biology and Microbiology and of Medicine, Tufts University School of Medicine.

persal. They concluded that a multidisciplinary ecological approach is imperative to tackling these resistance challenges (www.apua.org). Further, research and surveillance should not focus solely on strains and their phenotypic expression of resistance. They also urged investigators to use genetic fingerprinting to gain insights into sources of drug resistance genes and differences in strain behaviors under similar selective pressures. Probing commensals and understanding the role they play in antibiotic resistance should help toward developing effective interventions to control resistance and preserve the efficacy of antibiotics.

Blurred Border between Pathogens and Commensals

A classic example of expanding drug resistance comes from *Staphylococcus aureus,* which emerged with penicillin resistance soon after that drug was introduced. The pathogen later acquired resistance to methicillin and then to third-generation penicillins, and now additional antibiotics, including vancomycin, which is considered the drug treatment of last resort. Similarly, vancomycin-resistant strains of *Enterococcus faecium* are appearing globally. Like many other now-worrisome pathogens, these two bacterial species were once considered relatively harmless residents of the skin and intestinal tract that only sporadically caused problematic infections (Table 1).

Other multidrug-resistant bacteria are raising public health concerns. *Campylobacter* spp., which are indigenous to the intestinal tracts of many wild birds, and *Aeromonas* spp., which are native to aquatic environments, are infectious agents that increasingly bear multidrug resistance traits. Additionally, vancomycin resistance is appearing in microorganisms that, until recently, were rarely encountered as pathogens, including *Oerskovia turbata*, *Arcanobacterium haemolyticum*, *Streptococcus bovis*, *Streptococcus gallolyticus*, *Streptococcus lutetiensis*, *Bacillus circulans*, *Paenibacillus* spp., and *Rhodococcus* spp., as well as in the anaerobic genera *Clostridium* and *Eggerthella*. While not yet responsible for large-scale outbreaks, infections attributed to these species signify a worrying trend and pose the threat of disseminating resistance to vancomycin and other antimicrobial drugs to better-adapted pathogens.

People interact nonstop with microbes, most of them harmless or even beneficial. Some 26×10^{28} prokaryotes live in the top 8 m of soil, and another 12×10^{28} in aquatic environments, according to expert estimates. Additionally, 6 billion humans across the globe are colonized with an estimated total of 3.9×10^{23} microbes, of which pathogens constitute only a tiny fraction. Because commensals are widely viewed as harmless, support for their investigation has been scarce. The current Human Microbiome Project deviates sharply from that pattern.

Meanwhile, the heretofore disproportionate focus on antibiotic resistance in "true" and "opportunistic" pathogens was understandable —a consequence of the need to contend with the sequential appearance of resistance in microorganisms that cause illness. However, the associated monovision kept investigators from seriously examining other potential sources of resistance traits. Moreover, some of those other sources, such as anaerobic bacteria, were underappreciated and difficult to study. The blurring of their definition complicates the study of commensal flora. Escalating selective pressures, including those from antibiotic use and immunosuppressive therapies, further obscures boundaries, leading commensals into the realm of pathogens. This crossover phenomenon arises because the physical state of hosts has so much to do with defining which microorganisms are commensals and which are pathogens. A pathogen for one host can be a commensal for another host. Even so, defining a hierarchy for ranking commensals and pathogens helps in alleviating confusion and focusing research efforts (Fig 1).

Commensals Serve as Reservoirs of Resistance Genes

Commensals carry many types of resistance genes, which may be organized within genetic elements called integrons. Integrons carrying genes conferring resistance to older first- and second-generation antimicrobials are being recovered from nearly every type of environment. Moreover, integrons that carry resistance genes for the newer third- and fourth-generation antimicrobials are now appearing in diverse environments, including in commensal microorganisms associated with food animals and with humans (Table 2).

Finding resistance genes in microoganisms

within antibiotic-free environments suggests that those traits occur naturally and that they predate industrial-scale production and distribution of such drugs. Indeed, diverse and widely distributed soil bacteria carry resistance to virtually all antibiotics, including synthetic antimicrobials, some at clinically relevant concentrations, according to recent studies. These soil bacteria are phylogenetically disparate, and some of them are surprisingly close in genetic terms to human pathogens. Thus, on several grounds, these soil-dwelling microorganisms, with their reservoirs of antibiotic resistance traits, could be contributing to the rising levels of multidrug resistance now seen among pathogens that infect humans and other animals.

Escherichia coli and *Enterococcus* spp., which colonize humans and many other mammalian species, also are widely distributed throughout soil and water environments. Ubiquitous and resistant to a host of antibiotics, these species deserve serious attention. However, *E. coli*, which constitute only about 1% of the colonic flora, are outnumbered 20- to 30-fold by anaerobic *Bacteroides* spp. The remaining 70–80% of colonic flora consists of a staggering variety of species, mainly poorly characterized and comparatively ignored anaerobes that merit more scrutiny because they, too, can carry resistance genes. Meanwhile, other environments, particularly aquaculture, wastewater, and sludge, are mixing pots for the exchange of resistance traits and the emergence of novel strains carrying them (Fig. 2).

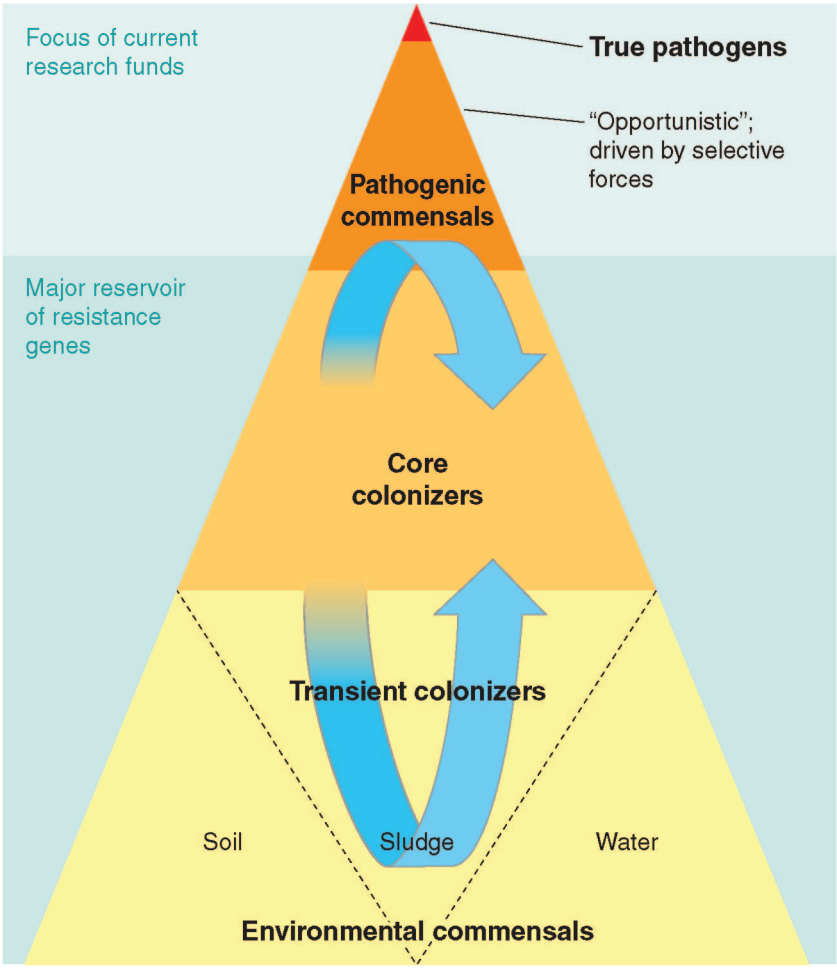
Our current multidrug resistance problem stems largely from horizontal gene transfers—a more efficient mechanism by which microorganisms adapt to environmental changes compared to random mutations. A growing body of evidence indicates that large quantities of genetic material, including antibiotic resistance genes, readily transfer among microbial species. Of the known gene transfer routes, transformation and conjugation appear to occur relatively frequently among densely packed cells of biofilms, such as those found in the intestinal tract and along tooth surfaces. In general, however, conjugation is perhaps the more common mechanism for lateral gene transfers. Little appears to inhibit this means of gene move-

Table 1. Emergence of antibiotic resistance in commensal bacteria causing disease[a]

Species	Disease	Year	Resistance
Acinetobacter baumanii	Nosocomial pneumonia, bacteremia, urinary tract infection, meningitis, septicemia	1985	Imipenem
		1993	Gentamicin
		1998	MDR[b]
		2001	Polymixin
Campylobacter jejuni	Campylobacteriosis	1986	Fluoroquinolone
Clostridium difficile	*Clostridium difficile*-associated diarrhea	1989	Clindamycin
Enterococcus faecalis	Endocarditis, bacteremia, catheter-related infection, intra-abdominal and pelvic infections.	1978	Gentamicin
		1986	Vancomycin
		1986	Gentamicin
		1986	Vancomycin
		1989	Ampicillin
		1989	Penicillin
		2000	Linezolid
Haemophilus influenzae	Bacteremic respiratory tract infections, meningitis, epiglottitis, osteoarthritis	1972	Ampicillin
		1975	Chloramphenicol/ Tetracycline
		1975	Chloramphenicol
		1979	MDR
		1979	Chloramphenicol/ Ampicillin
Klebsiella pneumoniae	Pyogenic liver abcess, bacteremia, RTI[c]	1983	Late Generation Cephalosporins
Pseudomonas aeruginosa	Opportunistic infections in immunocompromised patients	1985	Ceftazidime
		1987	Fluoroquinolone
		1988	Imipenem
Serratia marcescens	Nosocomial infections (UTI)[d]	1986	Fluoroquinolone
		1992	Carbapenem
Staphylococcus epidermidis	Infective endocarditis, IV catheter infections, bacteremia, CSF shunt infections, UTIs, osteomyelitis, vascular graft infections, prosthetic joint infections	1962	Methicillin
		1994	Rifamycin
S. haemolyticus	Septicemia, peritonitis, UTI, infective endocarditis	1984	Vancomycin
		1997	MDR
S. aureus	Boils, styes, furunculosis, pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis and endocarditis.	1942	Penicillin
		1960	Methicillin
		1976	MDR
		2000	Vancomycin
S. pneumoniae	*Streptococcus pneumoniae* infection	1967	Tetracycline
		1977	MDR
		1978	Macrolide

[a] Excerpted from K. E. Jones, N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, and J. L. Gittleman, Nature **451:**990-994, 2008. Suppl. information at www.nature.com/nature.
[b] Ampicillin-chloramphenicol-trimethoprim/sulfamethoxazole.
[c] RTI, respiratory tract infections.
[d] UTI, urinary tract infections.

FIGURE 1



Hierarchy of commensals (not to scale). This group of microbes comprises only a tiny fraction of the total microbial environment. Greatly underated and understudied are the multitudes of "core" and transient colonizers, i.e., commensals that constitute the major reservoirs of resistance genes. To some degree, commensals can be distinguished by their place in the environment and the relationships with their hosts. Some colonizers of the skin, oropharynx, and intestinal tract rarely if ever cause disease (e.g., the lactic acid bacteria). Yet another group is considered generally nonpathogenic, but when imbalances or shifts occur in the selective pressures on their microbial niches, these species can be propelled to the status of pathogens, made more problematic if they have acquired resistance or virulence genes from neighboring commensals. These constitute the group of opportunistic or "pathogenic commensals." *Staphylococcus aureus*, which regularly or transiently colonizes about 80% of humans, now frequently crosses this border. *S. epidermidis* and other traditionally commensal coagulase-negative staphylococci only occasionally appear as nosocomial infections and only under extreme selective pressures, such as indwelling catheters and depressed immunologic states. Species that commonly harbor native or "constitutive" resistance in their chromosomes (e.g., *Pseudomonas, Stenotrophomonas,* and *Acinetobacter*), also may emerge under these conditions. Most commensals, however, exist as environmental residents of soil and water habitats, many of which may become transient colonizers of humans and animals through the food chain and other routes of exposure. The accumulated evidence suggests widespread gene exchange among these groups.

ment across dissimilar genera (Fig. 3). Transfer of antibiotic resistance genes from commensals to pathogens depends on the density of donor and recipient cells, the availability of a transfer mechanism, nutrition, and selective pressures. In this regard, the intestinal environment is considered optimal.

Gene cassettes within integrons, found in plasmids and chromosomes, are a major means for transporting and incorporating antibiotic resistance genes. Recently class 1 integrons, lacking antibiotic resistance genes, but bearing the phylogenetic signature of lateral gene transfer, were found on the chromosomes of nonpathogenic soil and freshwater *Betaproteobacteria*. Their close resemblance to class 1 integrons that are common among pathogens suggests that environmental *Betaproteobacteria* were an original source of these genetic elements.

ROAR Project Is Evaluating Commensals

While the medical community scrambles to handle emerging, drug-resistant superbugs, some investigators are pondering the origins of this resistance. The idea that resistance genes exist and appear clinically under selective pressures is a 30-year-old concept that was fueled by discovery of rare resistance genes within bacteria isolated from undisturbed environments. The gradual appearance of resistance in pathogens and subsequent demonstrations of widespread intergeneric gene migrations lent further credence to the idea that free-living microorganisms or commensal flora could be harboring transferable resistance genes. Another hypothesis is that genes converge, giving rise under special circumstances to de novo resistance genes.

During the 1990s, APUA began to examine commensals as possible carriers of antibiotic resistance genes through its Reservoirs of Antibiotic Resistance (ROAR) project, a collaborative effort that also involved Abigail Salyers of the University of Illinois in Champaign-Urbana. She and one of us (S.B.L.) along

with other microbiologists suspected that commensal microorganisms were silently feeding drug resistance genes to species of clinical interest, making those commensals reservoirs of resistance genes.

To explore this possibility, ROAR began to focus on tracking the genes within commensal microorganisms, rather than the organisms themselves. If resistance was flowing from commensals to pathogens, tracking resistance genes could help to anticipate what might emerge among clinically important strains. The premise was that commensal flora could serve as barometers of resistance. However, the absence of global surveillance and standardized systems for phenotypic and genotypic tracking posed immediate challenges, which ROAR began to address by supporting research to fill some of these gaps. ROAR also began to support efforts to monitor resistance patterns in commensal bacteria (www.roarproject.org) and to envision other Web-based bioinformatics systems.

These efforts led directly to other challenges. For example, published information on commensals is scattered and difficult to track in part because microbiologists are not consistent in their use of the term. To remedy this problem, ROAR collected articles describing resistance in these populations, based on the following definition for commensals: "bacterial strains deemed not actively responsible for a pathogenic process and derived from humans, animals, or plants, or recovered from environmental sources such as air, water, soil, sludge, etc."

The ROAR collection of reports on commensals now includes more than 1,100 articles (published between 1969 and 2008), extracted from more than 260 journals. The reports describe isolates from 200 countries on all six continents that were collected between 1916 and 2008. They also describe more than 300 resistance genes and 144 virulence genes in species representing 66 different bacterial genera. The database is readily searchable and includes variables such as date, specific sources and sites where species were isolated, antibiotic exposure status, antibiotic susceptibility and multidrug resistance traits, and resistance transfer evaluations. Each annotation in the database can be linked directly to its PubMed citation.

Table 2. Detection of late-generation β-lactam resistance in commensal *E. coli* from different environments[a]

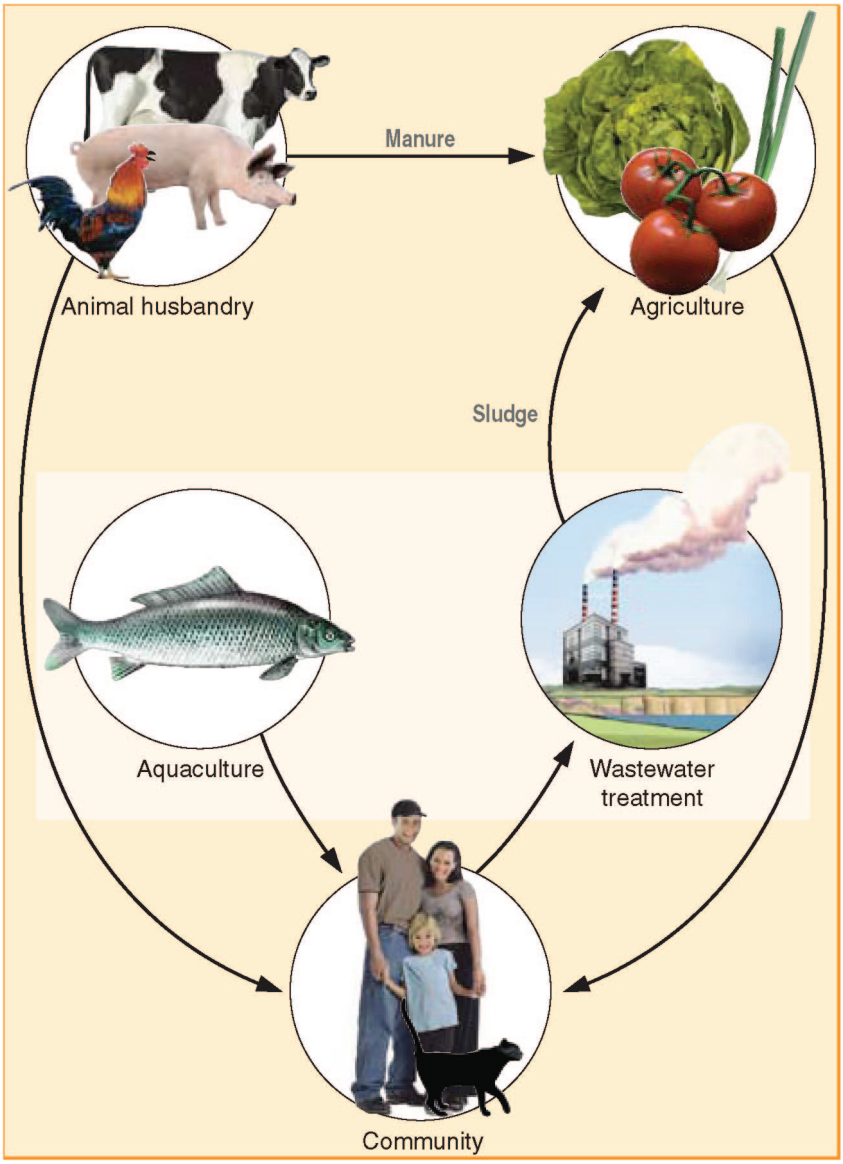
Resistance	Geographic site	Source	Frequency (%)
Bovines			
Amoxicillin-clavulanate	Canada	beef calves	3.9
		feedlot beef	1.2
	U.S. (Wisconsin)	Dairy cows	0.2/1.2[b]
Cefoxitin	Canada	beef calves	3.2
	U.S.	Dairy cows	0.7/0[b]
Ceftiofur	Canada	beef calves	2.9
		feedlot beef	0.7
	U.S.	Dairy cows	0.7/0[b]
	U.S. (Pennsylvania)	Dairy cows	11[c]
Cefoperazone	Great Britain	Abbatoirs	0.1
Swine			
Amox/clav	U.S. (8 states)	finishing farm	0.2/0.52[b]
	U.S. (Texas)	all/piglets only	2.2/6.8[b]
	Canada	Finishing farm	0.4–0.7
	Great Britain	Abbatoirs	0.2
	Thailand		5.8
Cefoxitin	U.S. (Texas)	all/piglets only	2.0/6.8[b]
	Canada	grow-finish farms	0.6–0.7
	Thailand		8.7
Ceftiofur	U.S. (8 states)	finishing farm	0.0/0.3[b]
	U.S. (Texas)	all/piglets only	2.4/7.1
	Canada	grow-finish farms	0.1
Ceftriaxone	U.S. (Texas)	all/piglets only	0.06/0.5
Ceftazidime	Great Britain	Abbatoirs	0.1
Cefoperazone	Great Britain	Abbatoirs	0.1
Sheep			
Amox/clav	Great Britain	Abbatoirs	0.2
Poultry			
Cefazolin	Korea		0.6
Cefoxitin	Korea		0.6
Humans			
Cefuroxime	Jordan		32
Cefazolin	Korea	Healthy students	0.6
Potable water			
Cefuroxime	Jordan		4

[a] Source: extracted from http://www.roarproject.org/
[b] Antibiotic-free/conventional.
[c] Ceftiofur used on 18% of farms surveyed.

Mining the ROAR Database

The ROAR review of commensal microorganisms is stimulating a wide range of questions regarding antibiotic resistance. However, the data needed to answer such questions are fragmentary at best. A key question is whether antibiotic use in agriculture and aquaculture is driving resistance genes from animal-associated bacteria into strains that cause human disease. Substantial but indirect evidence suggests that resistance genes pass from bacteria that colonize animals to bacteria associated with humans.

FIGURE 2



The interconnectedness of microbial communities and routes of transfer. Each arrow represents a route by which the simultaneous flow of commensals and pathogens can occur. Antibiotics are reported in varying concentrations in all these niches and act as selective agents on both pathogens and commensals. However, the complexities of these impacts have not yet been modelled. Note: In some countries, aquaculture occurs within the confines of sewage treatment facilities, creating a close link between these two environments.

Similar resistance genes are being identified in dissimilar microbial species, implying that migrations and other changes occurred (Fig. 3). These migrations appear to be relatively recent because similar strains that were isolated earlier than 1970, which was before antibiotic use grew to be so extensive, do not carry the same antibiotic resistance genes.

Strong evidence for direct transfers of antibiotic resistance traits from animal- to human-associated microorganisms comes from analysis of genera such as *Salmonella, Vibrio, Campylobacter, Yersinia,* and *Listeria*. They reside as commensals in many animal species, behaving as pathogens when humans consume raw, smoked, fermented, or undercooked foods. In such cases entire bacteria along with their resistance genes are transported from one host species to another. For example, when fluoroquinolones are used in poultry operations, humans exposed to those poultry food products pick up fluoroquinolone-resistant *C. jejuni* and become infected. However, there is still little direct evidence to show transfer of resistance genes from animal-associated *E. coli, Bacteroides,* or *Enterococcus* species into comparable human flora that subsequently cause antibiotic-resistant infections. The most persuasive evidence for this link remains a 20-year-old study in which the growth-promoting antibiotic nourseothricin (a streptothricin antimicrobial not used in human medicine) was used for two years as a feed additive for pigs on several neighboring farms. Plasmid-mediated streptothricin resistance was found in 33% of coliforms from pigs with diarrhea and also in 18% of pig farm employees and farm families, in 16% of healthy outpatients in that vicinity, and in 1% of urinary tract isolates from outpatients from surrounding communities.

Other studies support the idea that microorganisms associated with humans accumulate antibiotic-resistance genes from animal-associated microbes. For example, farmers and ranchers began using avoparcin, which is related to vancomycin, to supplement feed for livestock and poultry more than 20 years ago. Meanwhile, more and more vancomycin resistance has accumulated among enterococci, a commensal of the human gut, via Tn1546-like transfer elements. Slight differences in nucleotides

from these mobile elements make it possible to determine whether the antibiotic-resistance genes in enteroccoci derive from commensals associated with swine or poultry. Again, the findings strongly implicate pork and poultry consumption as sources of these antibiotic resistance traits now common in human commensals.

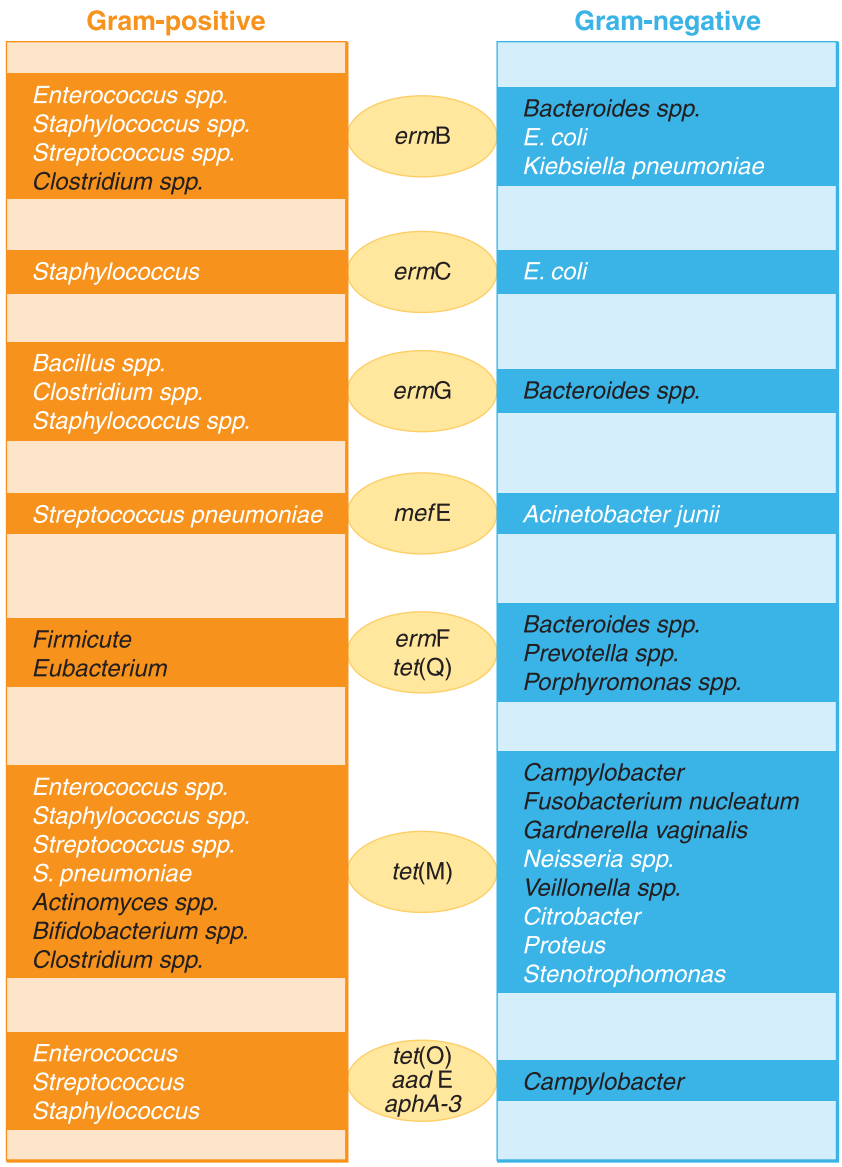
Such evidence is not the same as direct tracking to prove the commensal connection. Direct approaches prove highly impractical, both ethically and because of requirements for long-term monitoring.

Predicting Emergence of Antibiotic-Resistant Pathogens

Antibiotic-resistance genes will transfer from commensals to pathogens in vitro with remarkable fluidity. For example, multiple resistance loci can transfer via conjugal means among commensal *E. coli*, pathogenic *E. coli*, and *Salmonella* strains in a simulated porcine ileum fermenter. However, predicting resistance trait transfers is difficult for many reasons, including vast differences in when they appear. For instance, resistance to penicillin appeared within a few years of its clinical use, whereas it took more than 30 years before vancomycin resistance began to appear in clinical isolates. These disparities undermine attempts to model antibiotic resistance on a general basis. Further, resistance is studied in depth in only a few microbial species, mainly *E. coli* and *Enterococcus*, adding to the difficulty in predicting how others might contribute, particularly anaerobic genera such as *Bacteroides* and *Clostridia*.

Meanwhile, it remains a formidable challenge to identify appropriate resistance genes to track in microorganisms in natural and agricultural settings and then to monitor for transfer into microbes associated with humans, including pathogens in clinical settings. In the absence of such direct experimental data, we plan to continue developing the ROAR database for monitoring commensal flora as an alternative means for analyzing how they contribute to the antibiotic resistance problem.

FIGURE 3



Evidence for transfer of resistance genes between gram-positive and gram-negative commensal bacteria and between aerobic (white typeface) and anaerobic (black typeface) species. This indirect evidence derives from the finding of virtually identical resistance genes (ovals) in the different species (blocks) isolated from the mammalian colon and from other environmental sites (not a complete listing). The resistances encoded are as follows: *erm, mef* = macrolides; tet = tetracyclines; aad, *aphA-3* = aminoglycosides. (Adapted from: A. Salyers et al., Trends Microbiol. **12:**412- 416, 2004; P. Courvalin, Antimicrob. Agents Chemother. **38:**1447–1451, 1994; K. K. Ojo et al., J. Antimicrob Chemother. **57:**1065–1069, 2006).

ACKNOWLEDGMENTS

This article is based on a meeting, coordinated by APUA, held in Boston on 2 June 2008 and supported by the National Biodefense Analysis and Countermeasures Center (NBACC) and the National Institute of Allergy and Infectious Disease through R24 grant AI50139.

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the National Biodefense Analysis and Countermeasures Center (NBACC), Department of Homeland Security (DHS), or Battelle National Biodefense Institute (BNBI).

SUGGESTED READING

Alekshun M. N., and S. B. Levy. 2006. Commensals upon us. Biochem. Pharm. **71**:893–900.

Andremont, A. 2003. Commensal flora may play key role in spreading antibiotic resistance. ASM News **69**:601–607.

Barza, M., and S. L. Gorbach (ed.). 2002. The need to improve antimicrobial use in agriculture: ecological and human health consequences. Clin. Inf. Dis. Suppl. 3.

Blake, D. 2003. Transfer of antibiotic resistance between commensal and pathogenic members of the *Enterobacteriaceae* under ileal conditions. J. Appl. Microbiol. **95**:428–436.

Dantas, G., M. O. A. Sommer, R. D. Oluwasegun, and G. M. Church. 2008. Bacteria subsisting on antibiotics. Science **320:**100–103.

D'Costa, V. M., K. M. McGrann, D. W. Hughes, and G. D. Wright. 2006. Sampling the antibiotic resistome. Science **311:**374–377.

Hummell, R., H. Tschape, and W. Witte. 1986. Spread of plasmid-mediated nourseothricin resistance in connection with antibiotic use in animal husbandry. J. Basic Microbiol. **26**:461–466.

Levy, S. B. 2002. The antibiotic paradox: how the misuse of antibiotics destroys their curative powers, 2nd ed. Plenum Press, New York.

Martinez, J. L. 2008. Antibiotic and antibiotic resistance genes in natural environments Science **321:**365–367.

White, D. G., M. N. Alekshun, and P. F. McDermott (ed.). 2005. Frontiers in antimicrobial resistance: a tribute to Stuart Levy. ASM Press, Washington, D.C.