Generally Overlooked Fundamentals of Bacterial Genetics and Ecology

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Several important aspects of the antimicrobial resistance problem have not been treated extensively in previous monographs on this subject. This section very briefly updates information on these topics and suggests how this information is of value in assessing the contributions of human and agricultural use of antimicrobial agents on the problem of increasing antimicrobial resistance. The overall themes are (1) that propagation of resistance is an ecological problem, and thus (2) that ameliorating this problem requires recognition of long-established information on the commensal microbiota of mammals, as well as that of recent molecular understanding of the genetic agents involved in the movement of resistance genes.

THE ECOLOGY OF ANTIMICROBIAL RESISTANCE

Although antimicrobial resistance has traditionally been viewed as a problem of the treatment (or treatment failure) of an individual patient in a given clinical setting, it is actually an ecological problem. The science of ecology (the study of how living systems interact with each other and with their nonliving environment) is a relatively new one and has only recently begun to impinge on the practices of Western medicine and agriculture, the settings that have given rise to the antimicrobial resistance problem.

One aspect of ecology, the relationship between a host macroorganism and its commensal microorganisms, is especially significant in this context but has also received only scant attention compared with extensive work on individual pathogenic bacteria. Growing evidence indicates that, with respect to the resistance problem, the 2 most important aspects of the host-commensal ecosystem are (1) that it can serve as a relatively stable reservoir of resistant microorganisms (including potentially pathogenic ones) long after cessation of antimicrobial treatment, and (2) that host macroorganisms are continually being reinoculated by microorganisms from their environments. How do these 2 phenomena impinge on the occurrence and spread of antimicrobial resistance?

THE RESISTANCE RESERVOIR

In general, pathogenic bacteria differ little in their basic cellular biology from commensal bacteria. For example, a pathogen may have the ability to make toxins or invasion factors, but in the fundamental cellular processes targeted by all classical antimicrobials, pathogenic bacteria do not differ significantly from the benign commensal bacteria. This similarity makes sense because a pathogen also needs to be able to grow in or on the host that it invades. The consequences of this are 3-fold: (1) elimination of both benign commensal microbiota and pathogens by antimicrobials, (2) genetic exchange of antimicrobial-resistance genes in the commensal ecosystem, and (3) reinoculation of commensal ecosystems.

Elimination of susceptible commensals and pathogens by broad-spectrum antimicrobials. Administration of an antimicrobial agent may not only kill the pathogen but will also change the composition of the...
commensal ecosystem. Benign commensal bacteria that lack
the relevant resistance gene will die, and those that, by chance,
have the relevant resistance gene will proliferate and expand
into the niche abandoned by the exterminated antimicrobial-
susceptible bacteria. This is true for humans, animals, and
plants, all of which have their own type of commensal micro-
biota (see also Barza, Swartz, this supplement).

For example, a class of antimicrobial agents that can affect
the commensal flora is the ionophores (such as monensin).
Ionophores are deemed acceptable to use in animals because
they are not used in human medicine and because resistance
to them in pathogens of interest has not been observed. Ion-
ophores target almost exclusively gram-positive bacteria, which
constitute a large proportion of the intestinal tract of humans
(table 1) [1] and animals (including ruminants). Administra-
tion of ionophores causes a shift in the microbial populations
colonizing the animal intestinal tract [2, 3] with a concomitant
potential for more facile establishment of pathogens.

It has been assumed that after the antimicrobial treatment
is completed, antimicrobial-resistant commensal bacteria will
not have any selective advantage and will lose out in competi-
tion with the antimicrobial-susceptible commensal bacteria.
Although declines in the incidence of bacteria with resistance
to a specific antimicrobial agent have been noted in individual
hospitals [4] or farms [5] after use of the specific agent has
been restricted or discontinued, a return to the preterm level
of antimicrobial resistance does not usually occur. The wide-
spread dissemination of antimicrobial resistance genes in non-
hospitalized humans, most of whom are not undergoing an-
timicrobial treatment, strongly suggests that resistant strains
can persist in the commensal microbiota in the absence of
selection by any one antimicrobial agent [6–10]. Possible mo-
lecular bases for this phenomenon are considered below.

**Genetic exchange in commensal ecosystems.** Here again,
there is limited information on the rates and extent of such
exchanges, although existing data do show that it can occur
[11–14]. The high numbers of bacteria and the rich nutritional
resources of most commensal niches make them ideal settings
for gene exchange. Thus, even without colonizing, an entering
pathogen might obtain resistance genes from the commensal
microbiota. Alternatively, a transient benign microbe carrying
a resistance plasmid might transfer the plasmid to another com-
mensal bacterium during passage through the human intestinal
tract or while the bacteria reside on the skin or the mucosal
surfaces of the upper respiratory tract or vagina.

**Reinoculation of commensal ecosystems.** Although ani-
mals and plants are most obviously reinoculated by environ-
mental sources, humans are also continuously exposed to ex-
ogenous bacteria, benign and pathogenic, from other humans,
animals, fomites, and food [15–17]. Nonspecific host defenses,
including secreted lytic enzymes and stomach acidity in animals
(including humans), limit colonization by these exogenous bac-
teria. However, one of the most potent factors involved in
preventing colonization by pathogens is the competitive effect
of the autochthonous microbiota (see also Barza, this supple-
ment). For example, the intestinal tracts of most mammals are
colonized by ~20 or so different genera, with the majority of
commensal bacteria belonging to as few as 6–8 genera of bac-
teria [18, 19]. However, there may be as many as 400 different
species that colonize this niche even in humans; many strains

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**Table 1. Typical numbers of prokaryotes in human feces.**

<table>
<thead>
<tr>
<th>Genus or group</th>
<th>Gram stain group</th>
<th>Log_{10} no. of cultivatable organisms per gram of feces (dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>Negative</td>
<td>11.3, 9.2–13.5</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>Positive, low G + C</td>
<td>10.7, 5.0–13.3</td>
</tr>
<tr>
<td>Anaerobic cocci</td>
<td>Positive, low G + C</td>
<td>10.7, 4.0–13.4</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>Positive, high G + C</td>
<td>10.2, 4.9–13.4</td>
</tr>
<tr>
<td>Clostridium</td>
<td>Positive, low G + C</td>
<td>9.8, 3.8–13.1</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Positive, low G + C</td>
<td>9.6, 3.6–12.5</td>
</tr>
<tr>
<td>Actinomyces</td>
<td>Positive, high G + C</td>
<td>9.2, 5.7–11.1</td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>Positive, high G + C</td>
<td>8.9, 4.3–12.0</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Positive, low G + C</td>
<td>8.9, 3.9–12.9</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>Negative</td>
<td>8.7, 4.0–12.4</td>
</tr>
<tr>
<td>Fusobacterium</td>
<td>Negative</td>
<td>8.4, 5.1–11.0</td>
</tr>
<tr>
<td>Other facultative anaerobes</td>
<td>Negative</td>
<td>6.8, 0.7–12.7</td>
</tr>
</tbody>
</table>

**NOTE.** From Finegold et al. [1]. C + G, content of guanine and cytosine in microbe’s chromosome.
of these species are distinct from those of the same genus that colonize nonhuman mammals. Moreover, in any individual, the ensemble of strains can be quite idiosyncratic [20].

With such extensive and specific barriers to colonization by exogenous bacteria, it is hard to see how colonization by transient benign or pathogenic microbes could take place at all. Recent research indicates that, for unknown reasons, the intestinal microbiota may be quite dynamic and, in some people, readily subject to turnover of species and strains [20]. Such individuals, as well as those whose commensal bacteria had been recently eliminated by antimicrobial treatment [21], might be especially subject to recolonization with allochthonous commensals or even pathogens.

**GENETIC LINKAGE OF ANTIMICROBIAL RESISTANCES**

**Most antimicrobial-resistance genes in bacteria occur in genetically linked arrays.** Largely overlooked in the epidemiological literature on the spread of antimicrobial resistance is explicit consideration of the well-established fact that the multiple antimicrobial resistances in most clinically isolated Enterobacteriaceae are not the result of single, sequential, chance spontaneous mutations of the target genes of the antimicrobial agents occurring in all of these strains. Rather, in most cases, especially in the last 2 decades, such multiresistant strains result from acquisition of tandem arrays of genetically linked resistance genes borne by integrons or other transposons that can reside in the chromosome and on conjugative or mobilizable plasmids [22]. Although single point mutations in target genes can give rise to the resistance genes [23, 24], mutations are rare events (10^-7 to 10^-6 per cell per generation) [25] in comparison with the transfer of resistance genes (previously derived from such mutant chromosomal genes) now carried by mobile plasmids and transposons in many bacteria in humans, animals, plants, and the environment (10^-3 to 10^-4 per cell per generation). Indeed, in the case of the conjugative transposons that can operate in both gram-positive and gram-negative bacteria, transfer of the resistance element from cell to cell can be induced by antimicrobial exposure [26].

Genetically linked transmissible resistance was first reported in hospital isolates in the early 1960s, just 20 or so years after the widespread introduction of antimicrobial therapy for infectious diseases [27–31]. Transmissible resistance has been reported with increasing frequency in clinical isolates and in agricultural and environmental isolates [32–41] from the mid-1970s to the present. There was ample evidence as early as 1978 that such plasmids were distributed worldwide in hospital and environmental settings [42]. In many cases, the plasmids carrying the multidrug-resistant gene cassettes have been extensively documented and even sequenced [43].

**Implications of genetic linkage for spread of multiresistance.** Each gene cassette can provide resistance to a chemically distinct class of antimicrobial agents. The first ominous aspect of multiple-resistance transfer agents, such as plasmids, transposons, and integrons, is that they can collect and recombine extant resistance gene cassettes in almost any combination [44, 45]. Consequently, treatment with any given antimicrobial agent can result in selection for bacteria resistant not only to that specific agent, but by genetic linkage of resistance genes, to other unrelated antimicrobial agents. The consequence of this “mix and match” gene cassette transfer is not realized by most clinicians, who, in treating with an aminoglycoside, assume they will only be selecting for strains resistant to that antimicrobial agent (or to a narrow cross-resistant set of related aminoglycosides). This would be true if the above-noted assumption about spontaneous point mutations were actually the basis for most nosocomial or community-acquired resistance; indeed, 60 years ago, this might have been true. However, multiresistant isolates can now be readily isolated from all populations of contemporary human-associated bacteria (commensals or pathogens), as well as many related bacteria associated with domestic animals or with commercial horticultural crops, and in bodies of water experiencing any human, urban, or agricultural effects [35, 37, 38, 40, 46]. Selecting (by treating) with one antimicrobial agent will enrich the population for strains resistant to all antimicrobial agents whose resistance genes are genetically linked to the one for the antimicrobial agent used.

Moreover, nonantimicrobial environmental toxins such as heavy metals can also select for multidrug-resistant plasmids. Copper is frequently used in horticulture [47], and arsenicals are frequently used in animal feed [48, 49] (McEwen and Fedorka-Cray, this supplement) and horticulture [50] (W. K. Vencill, personal communication; T. Murphy, personal communication). Humans have used heavy metals, including arsenic, silver, and mercury, as medications from ancient times and now continuously ingest mercury from dental restorations [51]. Resistances to all these metals and many others that occur in the environment, including cadmium, lead, cobalt, nickel, and tin, are found on plasmids of both clinical and environmental gram-positive and gram-negative bacteria [47, 52, 53], in many cases along with antimicrobial-resistance genes [54, 55].

Thus, use of any antimicrobial or other selective agent selects for all the resistance genes in these arrays as well as for the plasmids where they reside. Regardless of the fact that they confer resistance to distinct chemical classes of antimicrobial agents, these genes are coselected because they are physically linked to each other. The reality of this genetic relatedness (i.e., actual physical linkage of genes) trumps control efforts that are based only on the more limited concept of chemical relatedness. This may be why, despite periodic cycling of antimicrobial
agents in hospitals or agricultural settings (see below), the prevalence of multiresistant bacteria not only does not diminish but even continues to increase [6, 56]. Unfortunately, recognition of the importance of physical linkage of resistance genes still escapes policy makers, who continue to promulgate guidelines for animal use solely on the basis of whether an antimicrobial agent is used (or is similar to one used) in human medicine [57].

**Implications of linked resistance for the usable “lifetime” of newly introduced antimicrobial agents.** The second ominous aspect of these multiple resistance transfer agents is that they appear to be quite ancient, widely distributed among human-, animal-, and plant-associated eu- bacteria, capable of moving readily among all members of the eu- bacteria, and capable of accumulating and disseminating new resistance genes as they arise by spontaneous point mutations under strong selection or as they occur in antimicrobial-producing bacteria [57, 58]. Such mobile elements are the engines of the ubiquitous horizontal transfer of all kinds of genes; they antedated by millennia the widespread use of antimicrobials by humans. The resistance-bearing versions of these mobile elements were probably originally selected by exposure to antimicrobial agents or heavy metals in the environment. As noted above, the origin of some of the genes currently spread by plasmids and transposons was a spontaneous mutation in the chromosomally encoded target gene. Where did the other resistance genes come from originally? As Julian Davies postulated [57], and as has since been proven in many cases, many were recruited from the chromosomal genes of antibiotic-producing soil bacteria (e.g., the aminoglycoside resistances). Others are adapted versions of the ubiquitous efflux pumps present in the genomes of most eu- bacteria (e.g., the quaternary ammonium resistance gene, qac) [59, 60]. Regardless of their sources, the key concept here is that these genes were “recruited” from their original chromosomes and assembled into tandem arrays on transmissible genetic elements. The “recruiting agent” may be one or more other genes carried by the mobile elements (e.g., the integrases and insertion sites of the integrons) or other genes yet to be identified.

Although as yet there are no biochemical data on the agents of initial recruitment, it is worth noting that variant integrase genes are found in every eu- bacterial genomic sequence yet reported [61, 62], often in huge arrays [63]. All sequenced eu- bacterial chromosomes also show evidence of considerable horizontal gene exchange, suggesting that when a spontaneous point mutation to resistance arises in the target gene of a novel antimicrobial agent, there is a finite (but unknown) chance for it to be picked up by ubiquitous transmissible plasmids, transposons, and bacteriophages and spread to other bacteria.

Although the existence of linked multiple resistance genes on bacterial plasmids and transposons has been known for >2 decades, most of the extant literature on antimicrobial resistance is based on epidemiologic prevalence studies, usually reporting assessment of a single resistance gene in a single organism of interest to the investigator (or to those supporting the study) [23, 64–70]. Thus, information on the degree to which linked resistances allow for selection (and thus persistence) of unrelated resistance genes is difficult to discern. However, some recognition of these well-established, underlying molecular processes is beginning to appear in studies of antimicrobial resistance [9, 71].

**BASIS FOR THE PERSISTENCE OF ANTIMICROBIAL RESISTANCE**

Early studies of plasmids were plagued by high spontaneous losses of these elements from laboratory strains unless selective pressure was exerted. This gave rise to a reasonable assumption that later became unexamined dogma: only selective pressure by antimicrobials kept plasmids in a population; lacking selective pressure, bacteria carrying these genes would be at a disadvantage and would be lost from any ecosystem. However, during the last 40 years, many studies have demonstrated the persistence of antimicrobial-resistant bacteria even after antimicrobial use was discontinued in hospital [72, 73], community [74], and agricultural settings [5, 56, 75]. More recent studies have also demonstrated the presence of antimicrobial-resistant bacteria in contexts where antimicrobial agents have not been used [6, 76]. Likely this persistence is based in part on the phenomenon of linkage described above; periodic exposure to any antimicrobial will maintain a multiresistance array in a bacterial population. However, there are several additional reasons why early assumptions about ready loss of resistance in the absence of selection are not correct.

**PLASMID-ADDITION SYSTEMS**

Bacterial plasmids carry genes that kill a daughter cell if it fails to get a copy of the plasmid upon division. These genes, referred to as “plasmid addiction” [77] or “postsegregational killing” [78] systems, come in various forms, but all result in a kind of bacterial apoptosis, destroying any daughter cell that chances to “give up” its plasmid. Such addiction systems are ubiquitous among large transmissible plasmids of gram-negative bacteria. At equilibrium, the concentration (prevalence, in this context) of any component in a system (e.g., a resistant bacterium) is determined equally by its rate of synthesis (acquisition of resistance genes) and its rate of decay (loss of resistance genes). Plasmids can not only control their own acquisition via conjugation or mobilization but can also prevent their loss. As a result, periodic selection for any plasmidborne resistance gene
allows that plasmid and all the genes it carries to become a fixed component of the population.

ADAPTIVE MUTATIONS IN CHROMOSOMAL GENES

Drug efflux pumps. Among the largest class of functions found in many prokaryotic genomes are membrane-mounted protein pumps for ridding the cell of toxic substances ranging from metal ions to disinfectants to antimicrobials [60, 79]. Often similar in structure, and usually dependent on ATP or the proton motive force as an energy source, these ubiquitous P- or ABC-type transporters are homologs to the multidrug transporters of higher eukaryotes. Most of these pumps are indiscriminate in their substrate range, frequently handling a wide variety of either hydrophobic or ionic substrates. The resistance they provide to any particular antimicrobial agent is less than the resistance provided by a mutation in the target gene, conferring intrinsic resistance, or even the resistances provided by the various plasmidborne genes. They can also readily mutate to provide slightly higher resistance, often as a result of increased expression [79]. Occurrence of such variants among clinical isolates suggests that these pumps do play a real-world role in multidrug resistance. Several such pumps, including those providing resistance to tetracycline, quaternary ammonium compounds, and a variety of toxic metal ions often used as disinfectants, have moved from the chromosome to plasmids and enjoy worldwide distribution in human-, animal-, and plant-associated bacteria as well as in bacteria in fresh and estuarine waters.

Compensatory chromosomal mutations. Because there is often a cost of any alteration in the target gene as it becomes resistant to an antimicrobial agent, cells have ways of adapting to offset this evolutionary cost. The periodic selection of chromosomal mutations has been shown in laboratory strains to compensate [80] for loss of fitness engendered either by plasmid carriage or spontaneous chromosomal resistance mutations. It is not known to what degree such spontaneous adaptations contribute to persistence of resistance in field isolates, although Pseudomonas strains colonizing patients with cystic fibrosis often have an enhanced rate of mutation [81].

EFFORTS AT CONTROL BASED ON SWITCHING THE ANTIMICROBIAL AGENT USED

The strategy of changing the antimicrobial agent used is most often referred to as “antibiotic cycling” and involves the replacement in a human medical or agricultural setting of an antimicrobial agent to which many bacteria in the community exhibit resistance with a different antimicrobial for which there is less resistance in the particular environment at the moment [4]. In the United States, when this is done in human medicine, it is limited to hospital rather than community practice; in countries such as Denmark [82], managed care also allows such regimens to be implemented via outpatient therapy. In the United States, cycling regimes in clinical practice demonstrate highly varied, but generally limited, success [73].

Missing in reports of such cycling practices is any ecological perspective—that is, long-term data that might reveal any general trends in the background level even in a single treatment unit or throughout the entire hospital or farm after repeated rounds of such cycling. Is it ever possible to achieve a “pre-antibiotic” prevalence of susceptibility, even for a single antimicrobial agent? Does subsequent reintroduction of the replaced antimicrobial result in a faster increase in resistance during the second “cycle” of its use? Also generally missing are data on the effect of replacement on the prevalence of resistances to other antimicrobial agents, especially those that might be genetically linked to the resistance locus whose reduction is desired. Moreover, owing to the stochastic occurrence of the need as well as ethical requirements to implement such interventions, carefully matched replications of such experiments are lacking [73]. Metastudies, as well as encouragement to report unsuccessful cycling attempts, would contribute considerably to evaluating the actual utility of this practice, the basis of which is questionable in light of linkage of unrelated resistance genes.

Finally, the implicit assumption that a decline in the prevalence of a resistant bacterium in a single hospital or farm unit as a result of antimicrobial withdrawal can be mapped precisely onto the behavior of these bacteria in the individual human or animal treated is erroneous. Notable reduction in resistance prevalence often takes weeks or months to occur (if it does so at all) [6, 56, 83], and the effect of antimicrobial withdrawal is rarely deconvoluted from replacement by antimicrobial-naive patients or animals (who simply lack the resistance locus in question) entering the hospital or farm. Reports of even modestly “successful” (e.g., 2-fold) reduction in the numbers of bacteria resistant to a single antimicrobial agent in a single hospital have thus given rise to the narrow view among physicians that it is possible, by these practices, to control the spread of resistance. This perspective overlooks the fact that discharged patients spread their resistant microbes to their home communities, as do outpatients.

ANTIMICROBIAL RESIDUES IN SURFACE WATERS

A final ecological issue that is gaining prominence is the presence and possible effect of myriad pharmaceutical residues, including antimicrobials, in sources of potable water [35, 84–87]. When detected, most antimicrobial agents appear intact, rather than as metabolites. The exception is erythromycin,
several metabolites of which are often found. Most antimicrobials are below detectable limits; only erythromycin (and its derivatives) are routinely found at detectable levels. Various sulfa derivatives are also more frequently found, whereas tetracycline and penicillin are almost never observed. In all cases, the amounts observed are in the parts per billion range, ~1000-fold below what would select for enrichment of resistant bacteria.

Thus, it is reasonable to conclude that resistant bacteria found in surface waters have not been selected by the vanishingly small amounts of antimicrobial agents in those waters but have traveled there via animal or insect vectors, in airborne dusts [88, 89], or simply in the flow of the waters after being released from some antimicrobial-rich setting. However, as noted above, given the stability of plasmids and other resistance replicons, thousands of bacterial generations may have taken place since that selective exposure.

CONCLUSION

The continuous exchange of bacteria between humans and their environment and among the genetic elements of these bacteria means that imposition of selection on any microbial ecosystem will result in proliferation of highly resistant bacteria. Long-established molecular mechanisms inherent in the bacteria themselves ensure that, once acquired, these resistance genes will be lost very slowly (and maybe not at all) from their large and ubiquitous populations. Discovering new antibiotics will buy us time, but the same ancient molecular mechanisms will ensure their eventual loss of efficacy as well. All sectors that use antibiotics—human medical, veterinary, and horticultural—need to cooperate in devising novel methods to minimize proliferation of resistant bacteria while meeting their respective therapeutic and economic needs. Such cooperative efforts must be based on a thorough grasp of the position of the bifidobacterial and Lactobacillus microbiota of the human colon. Antimicrobial resistance gene on an epidemic plasmid. Science 1995; 267:1206–7.

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27. Guinee P. Transfer of multiple drug resistance from animal and human antibiotics—human medical, veterinary, and horticultural—need to cooperate in devising novel methods to minimize proliferation of resistant bacteria while meeting their respective therapeutic and economic needs. Such cooperative efforts must be based on a thorough grasp of the population biology of commensal as well as pathogenic bacteria, the mechanisms of gene exchange among them, and the simple ecological principle that everything is connected to everything else.


