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GUEST COMMENTARY

(Genome) Size Matters[▽]

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The laws of natural selection dictate that bacteria will eventually develop resistance to practically any antibiotic. Selective pressure exerted by widespread antimicrobial use is a driving force in the development of antibiotic resistance.

—Stuart Levy

The quotation above (http://www.tufts.edu/med/apua/Practitioners/ABRcontrol.html) has led to the commonly held view that all bacteria will eventually become resistant to any antibiotic, given enough time and exposure. However, not all bacteria develop resistance to antibiotics at the same rate (and perhaps some species of bacteria may never develop resistance to certain antibiotics). And some bacteria that have been labeled "resistant" are not, in any clinically meaningful way, actually resistant (i.e., result in a therapeutic failure). Indeed, all antibiotics, even within the same chemical class, are not created equal (in terms of their propensity to select for resistant strains).

Another piece of dubious conventional wisdom is that bacterial resistance to drug therapy was first discovered following the introduction of penicillin. When narrowly parsed, this statement is true (in that one can only find therapeutic failure of a drug when that drug is used therapeutically), but the fact is that the first description of a beta-lactamase-producing (and therefore penicillin-resistant) bacterial strain was published on 28 December 1940 by Abraham and Chain (1) while the first attempt to use penicillin as a therapeutic was in 1941 and the first successful therapeutic use of penicillin was in April of 1942. Rather than blaming the profligate use of antibiotics on the emergence of resistant strains, it should be understood that resistance to antibiotics is a natural thing, at least for bacteria that are freely living in the environment. Indeed, most antibiotics are derived from or based upon the secondary metabolites of other bacteria and these producing bacteria must, obviously, be immune to the action of the antibiotics they produce. Those genes encoding the immunity factors have become, through horizontal gene transfer, the progenitors of many of the resistance determinants we find in pathogenic bacteria. But even when antibiotics are derived from synthetic, rather than natural, organic molecules, resistance can develop rapidly. And the fact that bacteria that are freely living have been selected for survival in harsh environments for 3 billion years (not just since 1942) makes it no surprise that it has been exceedingly difficult to find novel agents active against the problematic multidrug-resistant bacteria.

My opinions should in no way be interpreted to suggest that the inappropriate use of antibiotics does not have serious public health consequences because such inappropriate use and its consequences have been well documented. However, I also believe that we misunderstand resistance in very fundamental ways and there is no better example than the "beta-lactam paradox." Expanded-spectrum cephalosporin antibiotics, when used clinically, appear to rapidly select for resistant strains, especially methicillin-resistant *Staphylococcus aureus*, while penicillins do not appear to exert the same degree of selective pressure (4, 5). This is despite the fact that both are members of the same of class of antibiotics and have similar spectra of antibacterial activity. Should this rather controversial result be valid, none of our current resistance dogma can account for this observation. Are there differential effects on commensal microflora at work here? If so, this may mean that we grossly underestimate the importance of commensal microflora and how they are affected by antibiotic use or even protect their human hosts from infection by pathogenic bacteria. It certainly demonstrates that all antibiotics, even within the same class, are not created equal.

It is observed here that the ability of a given bacterium to evolve toward a multidrug resistance phenotype is a function of genome size. In Table 1, a number of examples are provided, but even an expanded analysis shows that this observation holds true. That is, the larger the genome the greater the propensity of a bacterium to display multidrug resistance phenotypes and the smaller the genome the less likely it is that antibacterial resistance will emerge and disseminate within that species. What is proposed here is that, just as there is a continuum of genome sizes among bacteria, there is a continuum in the ability or propensity of a bacterium to become "multidrug resistant" and that continuum is reflected in the size of the genome. This is not to say that we do not observe resistance to certain agents even in organisms with the smallest genomes (macrolide resistance appears in virtually every pathogen at some level). There is probably a solid biological reason for this observation; organisms with larger genomes are more adaptable to environmental changes because they have more (genetic) information to draw upon. It appears that organisms with smaller genomes have become more "specialized," residing in particular environmental niches (*Treponema pallidum* and the *Chlamydiae* are cases in point), and their lack of versatility in adapting to different environments is also manifest in an inability to develop mechanisms for coping with antibiotics. Indeed, we have learned that virtually each and

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TABLE 1. The propensity for organisms to become multiply resistant appears to be a function of genome size

Organism	Genome size (Mb)	Comment
Pseudomonas aeruginosa	6.3	Can be and often is "panresistant"
Klebsiella pneumoniae	5.9	Panresistance described
Salmonella enterica serovar Typhi	4.8	
Yersinia pestis	4.8	
Escherichia coli	4.6	
Mycobacterium tuberculosis	4.4	"Extreme" MDR[a] strains have emerged
Vibrio cholerae	4.0	
Listeria monocytogenes	2.9	
Staphylococcus aureus	2.8	
Staphylococcus epidermidis	2.6	Multidrug resistance but no panresistance
Neisseria gonorrhoeae	2.2	
Neisseria meningitidis	2.2	
Streptococcus pneumoniae	2.1	
Streptococcus pyogenes	1.9	Some resistance but none to beta-lactams
Haemophilus influenzae	1.8	
Helicobacter pylori	1.7	
Borrelia burgdorferi	1.4	
Treponema pallidum	1.1	Very little resistance observed
Rickettsia prowazekii	1.1	
Chlamydia trachomatis	1.0	
Ureaplasma urealyticum	0.75	
Mycoplasma genitalium	0.58	

[a] MDR, multidrug resistant.

every time a bacterium either acquires a novel resistance determinant or a mutant strain arises with decreased susceptibility to an antibacterial drug, the bacterium experiences a "fitness burden." With time, compensatory mutations are selected in which the bacterium accumulates mutations that allow for something like wild-type growth in a strain that is now phenotypically resistant (e.g., *topA* mutations in *gyrB* mutant strains) (2, 3). Bacteria with larger genomes simply have a greater opportunity to develop these compensatory mutations. It must be emphasized that it does not matter whether we are discussing the acquisition of a novel resistance gene as opposed to a mutation that alters the target or results in up-regulation of an efflux pump. The accumulating evidence tells us that all require some form of adaptation. Another consequence of this phenomenon is that antibiotic cycling in health care settings is unlikely to result in a reversion of the local microflora to susceptibility as the compensatory mutations "lock in" the resistance phenotype.

Consistent with the "size matters" hypothesis is the common observation that it is very difficult to develop genetic systems in organisms with small genomes. Anyone who has ever tried to introduce a plasmid into a group A streptococcus knows that this is no easy undertaking, and what about experimental genetic systems for organisms with even smaller genomes (e.g., *Chlamydia trachomatis*)? Almost none exist, despite intense effort, and even when we do see examples of horizontal gene exchange in the genomic midgets, the resistance phenotypes expressed are not especially profound.

I and several of those I have discussed this observation with were perplexed that it had not previously been articulated. Although to be fair, others have suggested it is a trivial, if not nonsensical, observation and worthy only of cocktail party conversation . . . in fact, I believe that this is an important guide as to where and which organisms we actually need novel antibacterial agents for. It suggests that new drugs for *Streptococcus pyogenes*, *Streptococcus pneumoniae*, or *Haemophilus influenzae* are not of the highest priority and should not be the prime focus of drug discovery efforts (in that regard, I do find some agreement among infectious disease clinicians). However, for organisms like *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Mycobacterium tuberculosis*, and the 800-pound gorilla of resistant, pathogenic bacteria, *Pseudomonas aeruginosa*, the unmet need is clear and again a consensus is reached that these are appropriate target organisms for drug discovery efforts.

So, if "size matters" really is a profound observation, why was it missed? Perhaps the simple reason is that most academic investigators are driven by their funding agencies to "focus." By working on individual organisms (such as *H. influenzae*), one is living in a 1.8-Mb world and mainly focusing on (small?) changes in in vitro MICs which may have little to no clinical relevance (although may well reveal very interesting biology). Indeed, the grant review panels ("study sections") have worked long and hard to maintain the amateur status of researchers who propose more global studies on bacterial drug resistance and microbial ecology, with "lack of focus" (rather than quality of science or public health significance) being the principal criticism. Such laser-like focus precludes the big picture to the point of obscuring the obvious.

And in the face of so much misleading conventional wisdom, I ask how can we control resistance to antibacterial agents when we do not understand it?

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