Is Bacterial Resistance to Antibiotics an Appropriate Example of Evolutionary Change?

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Abstract

Evolutionists frequently point to the development of antibiotic resistance by bacteria as a demonstration of evolutionary change. However, molecular analysis of the genetic events that lead to antibiotic resistance do not support this common assumption. Many bacteria become resistant by acquiring genes from plasmids or transposons via horizontal gene transfer. Horizontal transfer, though, does not account for the origin of resistance genes, only their spread among bacteria. Mutations, on the other hand, can potentially account for the origin of antibiotic resistance within the bacterial world, but involve mutational processes that are contrary to the predictions of evolution. Instead, such mutations consistently reduce or eliminate the function of transport proteins or porins, protein binding affinities, enzyme activities, the proton motive force, or regulatory control systems. While such mutations can be regarded as "beneficial," in that they increase the survival rate of bacteria in the presence of the antibiotic, they involve mutational processes that do not provide a genetic mechanism for common "descent with modification." Also, some "relative fitness" cost is often associated with such mutations, although reversion mutations may eventually recover most, if not all, of this cost for some bacteria. A true biological cost does occur, however, in the loss of pre-existing cellular systems or functions. Such loss of cellular activity cannot legitimately be offered as a genetic means of demonstrating evolution.

Introduction

Because of their rapid rate of replication, ease of laboratory analysis, and the wide diversity of laboratory-generated mutants that can be obtained, bacteria have been described as an excellent model for studying the processes of evolution (Mortlock, 1984). Acquiring resistance to a specific antibiotic provides a clear benefit to the bacterium when exposed to that antibiotic. Thus, the acquisition of antibiotic resistance is commonly cited as an example of "evolutionary change," and has become a popular example of so-called "evolution in a Petri dish." Miller (1999) refers to the development of antibiotic resistance as an example of evolution's "creative force." Barlow and Hall (2002) refer to it as "the unique opportunity to observe evolutionary processes over the course of a few decades instead of the several millennia that are generally required for these processes to occur." (p. 314)

Evolution is often described simply as 'change' or 'change in gene frequency over time' (Dillon, 1978; Johnson, 2000; Patterson, 1978), and evolutionists have almost

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universally maintained that any change in genotype (or even phenotype) is an "evolutionary change." As such, any biological change of an organism, including antibiotic resistance, would fit within this definition. However, mere biological change also fits within a creation model, and thus this "vanilla" definition does not readily distinguish itself from creation. This definition also does not specify the type of change (such as deleterious versus beneficial), thus it fails to offer any predictive value to the theory.

What is more, any change that appears to provide a so-called "beneficial" adaptation is commonly seen as a driving force of evolution. Indeed, some mutations, such as antibiotic resistance, can be beneficial since they may provide the organism an increased ability to survive under very specific environmental conditions. Thus, evolutionists typically conclude that genetic examples of "evolutionary change" are abundant and that creationists are forced to deny this readily observed evidence.

However, the theory of evolution proposes that all life on Earth had a common origin. Hence, all life shares a common evolutionary ancestry from which it has descended, i.e., the "common descent" of life. In a summarizing statement, Darwin (1936) states that "the theory of descent with modification embraces all the members of the same great class or kingdom … all animals and plants are descended from some one prototype." (p. 370). Therefore, through this overall common "descent with modification," the theory of evolution claims to account for the origin and diversity of all biological development on Earth. Thus, common "descent with modification" provides a more appropriate and functional definition of the theory of evolution, and this article will refer to evolution in this context. This definition also entails several "predictions" regarding the types of genetic change necessary for common evolutionary descent (predictions that are in sharp contrast to the "predictions" of a creation model). Such changes must provide more than mere changes in phenotype; they must provide a genetic mechanism that accounts for the origin of cellular functions and activities (i.e., regulatory systems, transport systems, enzyme specificity, protein binding affinity, etc.).

Genetic changes that reduce or eliminate any of these cellular systems provide no genetic mechanism for common "descent with modification." Rather, such changes are actually the antithesis of this descent, reducing or eliminating a pre-existing system of biological complexity (a reversal of "descent with modification"). Therefore, these genetic changes offer no example of a genetic mechanism for the "evolutionary" acquisition of flight by non-flying organisms, cognition by non-cognitive organisms, photosynthesis by non-photosynthesizing organisms, etc. Yet the theory of evolution requires such events to have occurred, and requires mutations capable of such genetic changes. Hence, the predictions of evolution require specific types of changes, not just so-called "beneficial" mutations. Therefore, despite the great claims that have been made, it is imperative to question whether acquisition of antibiotic resistance is a valid example of evolutionary change that supports the predictions of the evolutionary theory (i.e., the theory of common "descent with modification").

Horizontal Gene Transfer

One means by which bacteria can acquire antibiotic resistance is via the horizontal transfer of antibiotic resistant genes. Such transfer of resistance genes is common (Gómez, 1998; Top et al., 2000), accounting for many examples of resistant bacteria. But, horizontal transfer merely involves the transfer of resistance genes already present in the bacterial world.

While horizontal acquisition of resistant genes is "beneficial" to those bacteria exposed to a given antibiotic, such gene transfer does not account for the origin or the diverse variety of these genes. As such, it fails to provide a genetic mechanism for the origin of any antibiotic resistance genes in the biological world. Evolution, through the process of common "descent with modification," predicts it can account for the origin and diversity of life on earth; however, the mere shuffling of pre-existing genes between organisms via gene transfer does not provide the necessary genetic mechanism to satisfy this prediction. Nor can it readily account for the simultaneous development of both the antibiotic biosynthesis and resistance genes—an evolutionary enigma (Penrose, 1998). Thus, horizontal transfer of resistant genes cannot be offered as an appropriate example of "evolution in a Petri dish."

Mutations

Mutations, defined as any changes in the DNA sequence (Snyder and Champness, 2003), provide the only known genetic mechanism for producing new genetic activity and function in the biological world. In light of this, only mutations have the potential to provide evolution a mechanism that accounts for the origin of antibiotic resistance. Thus, only that resistance resulting from a mutation is a potential example of "evolution in action" (i.e., common "descent with modification").

In the presence of a particular antibiotic (or other antimicrobial), any mutation that protects the bacterium from the lethality of that compound clearly has a "beneficial" phenotype. Natural selection will strongly and somewhat

precisely select for those resistant mutants, which fits within the framework of an adaptive response. But, molecular analysis of such mutations reveals a large inconsistency between the true nature of the mutation and the requirements for the theory of evolution (Table I).

Table I. Mutation Phenotypes Leading to Resistances of Specific Antibiotics

Antibiotic	Phenotype Providing Resistance
Actinonin	Loss of enzyme activity
Ampicillin	SOS response halting cell division
Azithromycin	Loss of a regulatory protein
Chloramphenicol	Reduced formation of a porin or a regulatory protein
Ciprofloxacin	Loss of a porin or loss of a regulatory protein
Erythromycin	Reduced affinity to 23S rRNA or loss of a regulatory protein
Fluoroquinolones	Loss of affinity to gyrase
Imioenem	Reduced formation of a porin
Kanamycin	Reduced formation of a transport protein
Nalidixic Acid	Loss or inactivation of a regulatory protein
Rifampin	Loss of affinity to RNA polymerase
Streptomycin	Reduced affinity to 16S rRNA or reduction of transport activity
Tetracycline	Reduced formation of a porin or a regulatory protein
Zittermicin A	Loss of proton motive force

Bacterial resistance to the antibiotic, rifampin, can result from a commonly occurring spontaneous mutation. Rifampin inhibits bacterial transcription by interfering with normal RNA polymerase activity (Gale et al., 1981; Levin and Hatfull, 1993). Bacteria can acquire resistance by a point mutation of the β-subunit of RNA polymerase, which is encoded by the *rpo*B gene (Enright et al., 1998; Taniguchi et al., 1996; Wang et al., 2001; Williams et al., 1998). This mutation sufficiently alters the structure of the β-subunit so that it loses specificity for the rifampin molecule. As a result, the RNA polymerase no longer has an affinity for rifampin, and is no longer affected by the inhibitory effect of the antibiotic.

In fact, the level of rifampin resistance that a bacterium can spontaneously acquire can be extremely high. In my laboratory, we routinely obtain mutant strains with a resistance level that is orders of magnitude greater than that of the wild-type strain. When rifampin is present, this mutation provides a decided advantage for survival compared with those cells lacking these specific mutations. But, each of these mutations eliminates binding affinity of RNA polymerase for the rifampin. As such, these mutations do not provide a mechanism accounting for the origin of that binding affinity, only its loss.

Spontaneous resistance to fluoroquinolones (such as ciprofloxacin or norfloxacin) is also a frequent mutation in some bacteria. The primary target of the antibiotic is the enzyme, DNA gyrase, which is comprised of two proteins encoded by the genes, *gyr*A and *gyr*B (Hooper and Wolfson, 1993). Genetic analysis has found that resistance to this class of antibiotics can result from a point mutation in either of these genes (Barnard and Maxwell, 2001; Griggs et al., 1996; Heddle and Maxwell, 2002; Heisig et al., 1993, Willmott and Maxwell, 1993). These mutations of the gyrase subunits apparently cause a sufficient conformational change to the gyrase so that its affinity for the fluoroquinolones is reduced or lost (Figure 1). Again, despite their "beneficial" nature,

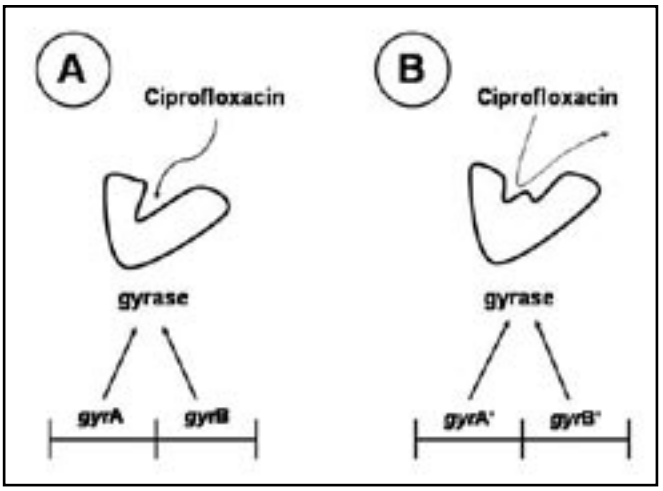


Figure 1. Mechanism of ciprofloxacin resistance. (A) Ciprofloxacin interacts with gyrase, inhibiting its enzymatic activity. (B) A mutation in either of the genes, *gyr*A or *gyr*B, can change the conformational structure of gyrase, and reduce the binding affinity of the enzyme for ciprofloxacin. This results in an inability of the antibiotic to inhibit the gyrase, and the cell becomes resistant to the antibiotic.

these mutations provide no useful model that explains the origin of the gyrase's affinity for the fluoroquinolones.

Resistance to streptomycin can also result from spontaneous bacterial mutations. In this case, streptomycin blocks bacterial protein synthesis apparently by binding to the 16S rRNA segment of the ribosome and interfering with ribosome activity (Carter et al., 2000; Leclerc et al., 1991). Resistance to the antibiotic can occur by mutations in the 16S rRNA gene, which reduces the affinity of streptomycin for the 16S molecule (Springer et al., 2001). Reduction of specific oligopeptide transport activities also leads to spontaneous resistance of several antibiotics, including streptomycin (Kashiwagi et al., 1998). In these examples, resistance occurred as a result of the loss of a functional component/activity.

Loss of enzymatic activity can result in metronidazole resistance. Interacellular metronidazole must be enzymatically activated before it can serve as an antimicrobial agent. This activation is achieved by the enzyme, NADPH nitroreductase (Figure 2). If the metronidazole is not activated it has no inhibitory effect on the bacterium. Thus, if NADPH nitroreductase activity is absent in the cell metronidazole remains inactive. Loss of the reductase activity can occur by nonsense or deletion mutations in *rdx*A (Debets-Ossenkopp et al., 1999; Goodwin et al., 1998; Tankovic et al., 2000). In addition, NADPH nitroreductase activity can be severely reduced by a single missense mutation (a single amino acid change), which reduces its ability to activate metronidazole (Paul et al., 2001). All these mutations result in loss of the enzyme activity necessary for the drug to be effective in the cell, hence the cell becomes resistant to metronidazole. But, loss of enzymatic activity does not provide a genetic example of how that enzyme originally "evolved." Hence, mutations that provide resistance to metronidazole cannot be offered as true examples of "evolution in a Petri dish."

Several bacteria, including *Escherichia coli*, construct a mulitiple-antibiotic-resistance (MAR) efflux pump that provides the bacterium with resistance to multiple types of antibiotics, including erythromycin, tetracycline, ampicillin, and nalidixic acid. This pump expels the antibiotic from the cell's cytoplasm, helping to maintain the intracellular levels below a lethal concentration (Grkovic et al., 2002; Okusu et al., 1996) (Figure 3). The MAR pump is composed of the proteins MarA and MarB, whose synthesis is inhibited by the regulatory protein, MarR (Alekshun and Levy, 1999; Poole, 2000) (Figure 3). Mutations that reduce or eliminate the repression control of MarR result in overproduction of the MarAB efflux pump, which enables the cell to expel higher concentrations of antibiotics or other antibacterial agents (Oethinger et al., 1998; Poole, 2000; Zarantonelli et al., 1999).

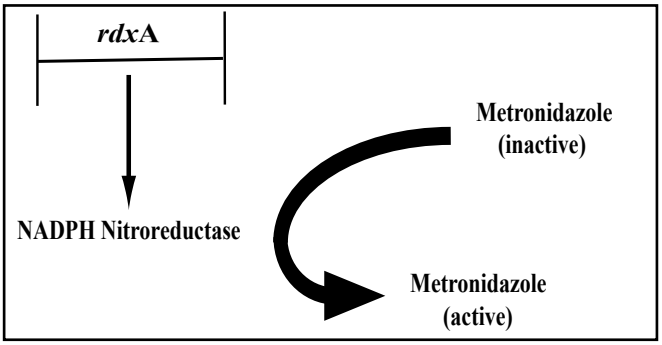


Figure 2. Activation of the antimicrobial agent, metronidazole. After being transported into the cell, metronidazole requires structural modification to obtain its active, antimicrobial form. This activation is achieved by the enzyme, NADPH nitroreductase, which is a product of the *rdx*A gene. Mutations in *rdx*A can prevent synthesis of a functional NADPH nitroreductase activity, which prevents metronidazole from becoming activated.

The protein MarA also acts as a positive regulator by stimulating increased production of both MarA and MarB proteins (Alekshun and Levy, 1999) [Figure 3]. In addition, the MarA protein indirectly inhibits the production of the porin, OmpF, a channel in the membrane that allows entry of some antibiotics into the cell (Cohen et al., 1988). Therefore, increased expression of MarA increases the efflux of antibiotics out of the cell, and reduces the transport of some antibiotics into the cell (Figure 3). Mutations of *mar*R that reduce expression or activity of the MarR protein will thus enable over-expression of the MarAB efflux pump (Linde et al., 2000; Okusu et al., 1996), and provide an increased resistance of the bacterium to various antibiotics (Eaves et al., 2004; Hans-Jorg et al., 2000; Notka et al., 2002) [Figure 3]. MarR defective mutants also possess increased bacterial tolerance to some organic chemical agents, such as cyclohexane (Aono et al., 1998).

Mutations that increase production of this efflux pump enable these bacteria to survive exposure to various antibiotics. As such, this is a beneficial mutation when the antibiotic is present in the environment. However, a mutation that causes loss of regulatory control (in this case the repressor protein, MarR) does not offer a genetic mechanism that can account for the origin of this regulatory control.

In other examples, resistance to erythromycin can also result from the loss of an 11 base pair segment of the 23S rRNA gene (Douthwaite et al., 1985), or a mutation that alters the confirmation of the 23S rRNA—reducing the affinity of the ribosome for the antibiotic (Gregory and Dahlberg, 1999; Vannuffel et al., 1992). Chloramphenicol resistance was obtained by deletion of a 12 base pair region

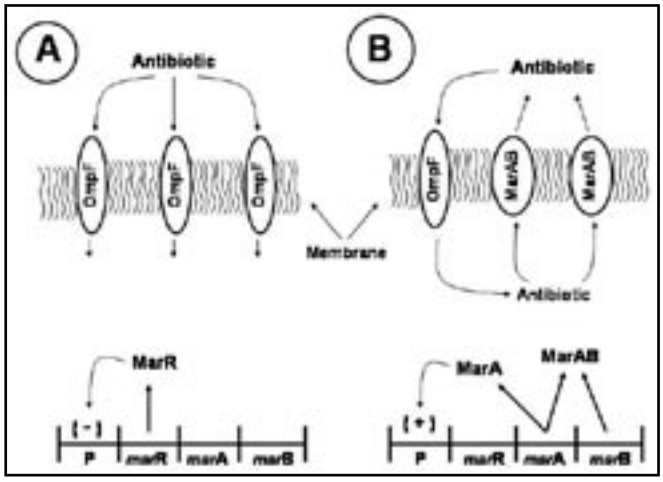


Figure 3. Multidrug resistance efflux pump. (A) Antibiotic sensitive bacterium. The antibiotics enter the cell through various portals, including the OmpF porin. Expression of the *mar*R gene produces the regulatory protein, MarR. This protein binds to the promoter (labeled as P) of the multidrug resistant operon, inhibiting expression of genes *mar*A and *mar*B. (B) Antibiotic resistant bacterium. A mutation of *mar*R that reduces the activity of MarR enables the promoter to function constitutively. Both *mar*A and *mar*B are now expressed. These two proteins form an efflux pump, which transports antibiotic molecules out of the cell's cytoplasm. MarA also binds to the promoter (labeled as P) and increases the transcription rate of the operon, which increases production of both MarA and MarB. In addition, production of MarA indirectly reduces the synthesis of the OmpF porin, thereby reducing the number of these porins in the membrane. The combination of fewer porins for transport of an antibiotic into the cell, and the increased number of MarAB efflux pumps removing the antibiotic out of the cell, provides the bacterium an increased tolerance to several antibiotics.

in domain II of the peptidyltransferase gene (Douthwaite, 1992). Resistance to cephalosporins has been linked to a dramatic alteration of membrane transport kinetics that is similar to porin-deficient strains (Chevalier et al., 1999). Actinonin resistance in *Staphylococcus aureus* results from mutations that eliminate expression of the *fmt* gene (Margolis et al., 2000). Zwittermicin A resistance in *E. coli* is associated with loss of proton motive force (Stabb and Handelsoman, 1998). For *Streptococcus gordonii*, penicillin tolerance may involve loss of regulatory control of the *arc* operon (Caldelari et al., 2000). And, *E. coli* can survive the presence of ß-lactams, such as ampicillin, by halting cell division, making the cell less sensitive to the lethal affect of the antibiotic (Miller et al., 2004).

These resistance mutations described above cause the loss of a pre-existing biological system, including cell division and proton motive force. Even though antibiotic survival is a "beneficial" phenotype, these mutations fail to provide a genetic example of how each of these systems originated. As such, they fail to provide a genetic means to fulfill the predictions of "descent with modification."

Resistance to other antibiotics, such as kanamycin, can result from loss or reduction of synthesis of a transport protein (OppA) [Kashiwagi et al., 1998]. Ciprofloxacin and imipenem resistance can result, at least in part, from the decreased formation of the outer membrane porin, OmpF (Armand-Lefèvre et al., 2003; Hooper et al., 1987; Yigit et al., 2002). An increase in meropenem and cefepime resistance is also associated with loss of OmpF, and another porin, OmpC (Yigit et al., 2002). And, *Enterobacter aerogenes* can become resistant to various antibiotics when a mutation dramatically reduces the conductance of a membrane porin (Dé et al., 2001).

Each of these resistances described in the previous paragraph result from the reduction or loss of a transport system. However, genetic mechanisms necessary for evolution would need to account for the origin of these various transport systems. Thus, these antibiotic resistance mutations do not provide the necessary genetic changes for "common descent." Rather, they are genetically inconsistent with the requirements of evolution, each involving the loss of a pre-existing transport activity.

As a group, the mutations associated with antibiotic resistance involve the loss or reduction of a pre-existing cellular function/activity, i.e., the target molecule lost an affinity for the antibiotic, the antibiotic transport system was reduced or eliminated, a regulatory system or enzyme activity was reduced or eliminated, etc. (Table I). These are not mutations that can account for the origin of those cellular systems and activities. While these mutations would certainly be "beneficial" for bacterial survival when an antibiotic is present in the environment, this benefit is at the expense of a previously existing function. This is analogous to removing an interior wall of a house to make a larger dining room. While this larger dining room may be desirable (i.e., beneficial), the mechanism of removing this wall cannot legitimately be offered as an example of how this interior wall was originally built. Hence, the survival benefit of a mutation is only a portion of the genetic characteristics necessary for mutations to achieve "evolution in a Petri dish." Such mutations must also provide the genetic basis for common "descent with modification." While this directly contradicts the claims made by many proponents

of evolution, the molecular data for antibiotic resistance are very clear.

These mutations also cannot provide a mechanism that continues to "evolve" the level of protein specificity or protein activity that is necessary for normal cellular function. While such mutations are excellent examples of bacterial adaptation, they are actually the antithesis of the mutational change necessary for evolution. Yet, these are the very examples evolutionists offer as verifiable demonstrations of "evolutionary change." Ironically, these mutations are, in fact, verifiable examples of a creation model—initial complexity being mutated to a level of greater simplicity.

The spontaneous acquisition of antibiotic resistance is often referred to as "gaining" resistance, but it is more appropriately identified as a loss of sensitivity. Thus, antibiotic resistance results from the loss of pre-existing systems in the bacterial cell. Such changes clearly provide no genetic mechanism for the origin of such cellular features as enzyme specificity, transport activity, regulatory activity, or protein binding affinity. Yet, evolutionists consistently claim that mutations do provide a genetic mechanism for the origin of biological activity and common "descent with modification," and consistently offer the types of mutations described above as examples.

Fitness Cost of Antibiotic Resistance

While mutations that provide resistance to an antibiotic can be considered "beneficial," they often come with a physiological cost (Andersson and Levin, 1999; Maisnier-Patin et al., 2002). In fact, Björkman et al. (2000) conclude that most types of antibiotic resistance will impart some biological cost to the organism. For example, rifampin resistance in *Mycobacterium tuberculosis* (Billington et al., 1999), *E. coli* (Reynolds, 2000), and *Staphylococcus aureus* (Wichelhaus et al., 2002) resulted from mutations to the RNA polymerase that also reduced the relative fitness of most of the mutant strains. Although the biological cost reported by these researchers was generally not severe, it was measurable.

Mutations resulting in clarithromycin resistance in *Helicobacter pylori* reduce the relative fitness of the organism (Björkholm et al., 2001). Resistance to high levels of fluroquinolone by *Salmonella enterica* involves mutations that impart a high fitness cost to the organism (Giraud et al., 2003). And, *fus*A mutations that provide fusidic acid resistance to *Staphylococcus* sp. impose a significant loss of "relative fitness" (Gustafsson et al., 2003; MacVanin et al., 2000). Resistance to actinonin by *S. aureus* also accompanies a dramatic loss of "fitness" resulting in significant growth impairment (Margolis et al., 2000). *E. coli* resistance to streptomycin may dramatically reduce the rate of protein biosynthesis (Zengel et al., 1977). And, some bacteria suspend cell division to minimize their sensitivity to ampicillin (Miller et al., 2004), which clearly reduces the overall fitness of the organism.

This cost of "relative fitness" appears to vary considerably depending on both the organism and the antibiotic. Many of the resistant mutants that have been studied, however, including some of those mentioned above, can subsequently eliminate some or much of the fitness cost by reversion or suppression mutations, which also stabilizes the mutation (Andersson and Levin, 1999; Lenski, 1998; Massey et al., 2001). The degree that a reversion mutation restores fitness probably depends on the location of the mutation and whether a single mutation is able to restore some or all of the wild-type "fitness."

Clearly the fitness of some mutant strains is permanently reduced (sometimes dramatically), and evolutionists have typically ignored such affects in their rush to promote antibiotic resistance as "evolution in the Petri dish." In fact, they often test relative fitness of these mutants under very narrow cultivation parameters, which minimizes the detectable loss of fitness for a given mutation. On the other hand, the fitness loss of some mutants is negligible (esp. following reversion mutations). So, the effect of spontaneous resistance on bacterial fitness appears to vary from mutant to mutant. Thus, creationists have probably tended to over-stress the significance of reduced "fitness" in antibiotic resistant bacteria by applying the concept to all such mutants.

Resistant mutations do impose a biological cost, though, in the loss of pre-existing biological systems and activities. Such biological cost is not compensated by reversion or suppression mutations. Even though such mutations may not always result in detectable levels of reduced "fitness," they stand as the antithesis of common "descent with modification."

Summary

Resistance to antibiotics and other antimicrobials is often claimed to be a clear demonstration of "evolution in a Petri dish." However, analysis of the genetic events causing this resistance reveals that they are not consistent with the genetic events necessary for evolution (defined as common "descent with modification"). Rather, resistance resulting from horizontal gene transfer merely provides a mechanism for transferring pre-existing resistance genes. Horizontal transfer does not provide a mechanism for the origin of those genes. Spontaneous mutation does provide a potential genetic mechanism for the origin of these genes,

but such an origin has never been demonstrated. Instead, all known examples of antibiotic resistance via mutation are inconsistent with the genetic requirements of evolution. These mutations result in the loss of pre-existing cellular systems/activities, such as porins and other transport systems, regulatory systems, enzyme activity, and protein binding. Antibiotic resistance may also impart some decrease of "relative fitness" (severe in a few cases), although for many mutants this is compensated by reversion. The real biological cost, though, is loss of pre-existing systems and activities. Such losses are never compensated, unless resistance is lost, and cannot validly be offered as examples of true evolutionary change.

References

Andersson, D.I., and B.R. Levin. 1999. The biological cost of antibiotic resistance. *Current Opinion in Microbiology* 2:489–493.

Aono, R., N. Tsukagoshi, and M. Yamamoto. 1998. Involvement of outer membrane protein TolC, a possible member of the *mar-sox* regulon, in maintenance and improvement of organic solvent tolerance of *Escherichia coli* K-12. *Journal of Bacteriology* 180:938–944.

Alekshun, M.N., and S.B. Levy. 1999. The *mar* regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends in Microbiology* 7:410–412.

Armand-Lefèvre, L., V. Leflon-Guibout, J. Bredin, F. Barguellil, A. Amor, J.M. Pagès, and M.-H. Nicolas-Chanoine. 2003. Imipenem resistance in *Salmonella enterica* serovar wien related to porin loss and CMY-4 ß-lactamase production. *Antimicrobial Agents and Chemotherapy* 47:1165–1168.

Barlow, M., and B.G. Hall. 2002. Phylogentic analysis shows that the OXA β-lactamase genes have been on plasmids for millions of years. *Journal of Molecular Evolution* 55:314–321.

Barnard, F.M., and A. Maxwell. 2001. Interaction between DNA gyrase and quinolones: effects of alanine mutations at GryA subunit residues Ser^{83} and Asp^{87}. *Antimicrobial Agents and Chemotherapy* 45:1994–2000.

Billington, O.J., T.D. McHugh, and S.H. Gillespie. 1999. Physiological cost of rifampin induced in vitro in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 43:1866–1869.

Björkholm, B., I. Nagaev, O.G. Berg, D. Hughes, and D.I. Andersson. 2000. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* 287:1479–1482.

Björkholm, B., M. Sjölund, P G. Falk, O.G. Berg, L. Engstrand, and D.I. Andersson. 2001. Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*. *Proceedings of the National Academy of Science* 98:14607–14612.

Caldelari, I., B. Loeliger, H. Langen, M .P. Glauser, and P. Moreillon. 2000. Deregulation of the arginine deiminase (*arc*) operon in penicillin-tolerant mutants of *Streptococcus gordonii*. *Antimicrobial Agents and Chemotherapy* 44:2802–2810.

Carter, A.P., W. M. Clemmons, D.E. Brodersen, R.J. Morgan-Warren, B.T. Wimberly, and V. Ramakrishnan. 2000. Functional insights from the structure of the 30S ribosomal subunit and its interaction with antibiotics. *Nature* 407:340–348.

Chevalier, J., J.-M. Pagès, and M. Malléa. 1999. *In vivo* modification of porin activity conferring antibiotic resistance to *Enterobacter aerogenes*. *Biochemical and Biophysical Research Communications* 266:248–251.

Cohen, S.P., L. M. McMurry, and S.B. Levy. 1988. *mar*A locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli*. *Journal of Bacteriology* 170:5416–5422.

Darwin, C. 1936. *Origin of Species and The Descent of Man* (Modern Library Reprint Edition) Random House, New York.

De, E., A. Baslé, M. Jaquinod, N. Saint, M. Malléa, G. Molle, and J.-M Pagès. 2001. A new mechanism of antibiotic resistance in Enterobacteriaceae induced by a structural modification of the major porin. *Molecular Microbiology* 41:189–198.

Debets-Ossenkopp, Y.J., R..G.J. Pot, D.J. van Westerloo, A. Goddwin, C.M.J.E. Vandenbroucke-Grauls, D.E. Berg, P.S. Hoffman, and J.G. Kusters. 1999. Insertion of mini-IS605 and deletion of adjacent sequences in the nitroreductase (*rdx*A) gene causes metronidazole resistance in *Helicobacter pylori* NCTC11637. *Antimicrobial Agents and Chemotherapy* 43:2657–2662.

Dillon, L.S. 1978. *Evolution: concepts and consequences*, C.V. Mosby, St. Louis, MO.

Douthwaite, S. 1992. Functional interactions within 23S rRNA involving the peptidyltransferase center. *Journal of Bacteriology* 174:1333–1338.

Douthwaite, S., J.B. Prince, and H.F. Noller. 1985. Evidence for functional interaction between domains II and V of 23S ribosomal RNA from an erythromycin-resistant mutant. *Proceedings of the National Academy of Science* 82:8330–8334.

Enright, M., P. Zawadski, P. Pickerill, and C.G. Dowson. 1998. Molecular evolution of rifampicin resistance in *Streptococcus pneumoniae*. *Microbial Drug Resistance* 4:65–70.

Eaves, D.J., V. Ricci, and L.J.V. Piddock. 2004. Expression of *acr*B, *acr*F, *acr*D, *mar*A, and *sox*S in *Salmonella enterica* serovar typhimurium: role in multiple antibiotic resistance. *Antimicrobial Agents and Chemotherapy* 48:1145–1150.

Gale, E.F., E. Cundliffe, P.E. Reynolds, M.H. Richmond, and M.J. Waring. 1981. *The Molecular Basis of Antibiotic Action*. John Wiley & Sons, New York.

Giraud, E., A. Cloeckaert, S. Baucheron, C. Mouline, and E. Chaslus-Dancla. 2003. Fitness cost of fluoroquinolone resis-

tance in *Salmonella enterica* serovar *Typhimurium*. *Journal of Medical Microbiology* 52:697–703.

Gómez, L.R. 1998. Evolution of bacterial resistance to antibiotics during the last three decades. *International Microbiology* 1:279–284.

Goodwin, A., D. Kersulyte, G. Sisson, S.J.O.V. van Zanten, D.E. Berg, and P.S. Hoffman. 1998. Metronidzole resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdx*A) that encodes an oxygen-insensitive NADPH nitroreductase. *Molecular Microbiology* 28:383–393.

Gregory, S.T., and A.E. Dahlberg. 1999. Erythromycin resistance mutations in ribosomal proteins L22 and L4 perturb the higher order structure of 23 S ribosomal RNA. *Journal of Molecular Biology* 289:827–834.

Griggs, D.J., K. Gensberg, and L.J. Piddock. 1996. Mutations in *gyr*A gene of quinolone-resistant Salmonella serotypes isolated from humans and animals. *Antimicrobial Agents and Chemotherapy* 40:1009–1013.

Grkovic, S., M.H. Brown, and R.A. Skurray. 2002. Regulation of bacterial drug export systems. *Microbiology and Molecular Biology Reviews* 66:671–701.

Gustafsson, I., O. Cars, and D.I. Andersson. 2003. Fitness of antibiotic resistant *Staphylococcus epidermidis* assessed by competition on the skin of human volunteers. *Journal of Antimicrobials and Chemotherapy* 52;258–263.

Hans-Jörg, L., F. Notka, M. Metze, B. Kochanowski, P. Heisig, and N. Lehn. 2000. *Antimicrobial Agents and Chemotherapy* 44:1865–1868.

Heddle, J., and A. Maxwell. 2002. Quinolone-binding pocket of DNAgyrase: role of GryB. *Antimicrobial Agents and Chemotherapy* 46:1805–1815.

Heisig, P., H. Schedletzky, and H. Falkenstein-Paul. 1993. Mutations in the *gyr*A gene of a highly fluoroquinolone-resistant clinical isolate of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* 37:696–701.

Hooper, D.C., and J.S. Wolfson. 1993. *Quinoline Antimicrobial Agents*. ASM Press, Washington, DC.

Hooper, D.C., J.S. Wolfson, E. Y. Ng, and M. N. Swartz. 1987. Mechanisms of action and resistance to ciprofloxacin. *American Journal of Medicine* 82:4A:12–20.

Johnson, G.B. 2000. *The Living World*. McGraw-Hill, New York.

Kashiwagi, K., M.H. Tsuhako, K. Sakata, T. Saisho, A. Igarashi, S.O.P. daCosta, and K. Igarashi. 1998. Relationship between spontaneous aminoglycoside resistance in *Escherichia coli* and a decrease in oligopeptide binding protein. *Journal of Bacteriology* 180:5484–5488.

Leclerc, D., P. Melancon, and L. Brakier-Gingras. 1991. Mutations in the 915 region of *Escherichia coli* 16S ribosomal RNA reduce the binding of streptomycin to the ribosome. *Nucleic Acid Research* 19:3973–3977.

Linde, H.-J., F. Notka, M. Metz, B. Kochanowski, P. Heisig, and N. Lehn. 2000. In vivo increased resistance to Ciprofloxacin in *Escherichia coli* associated with deletion of the C-terminal part of MarR. *Antimicrobial Agents and Chemotherapy* 44:1865–1868.

Lenski, R.E. 1998. Bacterial evolution and the cost of antibiotic resistance. *International Microbiology* 1:265–270.

Levin, M.E., and G. F. Hatfull. 1993. *Mycobacterium smegmatis* RNA polymerase: DNA supercoiling, action of rifampicin and mechanism of rifampicin resistance. *Molecular Microbiology* 8:277–285.

MacVanin, M., U. Johanson, M. Ehrenberg, and D. Hughes. 2000. Fusidic acid-resistant EF-G perturbs the accumulation of ppGpp. *Molecular Microbiology* 37:98–107.

Maisnier-Patin, S., O.G. Berg, L. Liljas, and D.I. Andersson. 2002. Compensatory adaptation to the deleterious effects of antibiotic resistance in *Salmonella typhimurium*. *Molecular Microbiology* 46:355–366.

Margolis, P.S., C.J. Hackbarth, D.C. Young, W. Wang, D. Chen, Z. Yuan, R. White, and J. Trias. 2000. Peptide deformylase in *Staphylococcus aureus*: resistance to inhibition is mediated by mutations in the formyltransferase gene. *Antimicrobial Agents and Chemotherapy* 44:1825–1831.

Massey, R.C., A. Buckling, and S.J. Peacock. 2001. Phenotypic switching of antibiotic resistance circumvents permanent costs in *Staphylococcus aureus*. *Current Biology* 11:1810–1814.

Miller, C., L.E. Thomsen, C. Gaggero, R. Mosseri, H. Ingmer, S.N. Cohen. 2004. SOS response induction by ß-lactams and bacterial defense against antibiotic lethality. *Science* 305:1629–1631.

Miller, K.R. 1999. *Finding Darwin's God*. Harper Collins, New York.

Mortlock, R.P. 1984. *Microorganisms as Model Systems for Studying Evolution*. Plenum Press, New York.

Notka, F., H.-J. Linde, A. Dankesreiter, H.-H. Niller, and N. Lehn. 2002. A C-terminal 18 amino acid deletion in MarR in a clinical isolate of *Escherichia coli* reduces MarR binding properties and increases the MIC of ciprofloxacin. *Journal of Antimicrobial Chemotherapy* 49:41–47.

Oethinger, M., I. Podglajen, W.V. Kern, and S.T. Levy. 1998. Overexpression of the *mar*A and *sox*S regulatory gene in clinical topoisomerase mutants of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* 42:2089–2094.

Okusu, H., D. Ma, and H. Nikaido. 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-resistance (Mar) mutants. *Journal of Bacteriology* 178:306–308.

Patterson, C. 1978. *Evolution*. Cornell University Press, Ithaca, NY.

Paul, R., S. Postius, K. Melchers, and K.P. Schäfer. 2001. Mutations of the *Helicobacter pylori* genes *rdx*A and *pbp*1 cause

resistance against metronidazole and amoxicillin. *Antimicrobial Agents and Chemotherapy* 45:962–965.

Penrose, E. 1998. Bacterial resistance to antibiotics—a case of un-natural selection. *Creation Research Society Quarterly* 35:76–83.

Poole, K. 2000. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. *Antimicrobial Agents and Chemotherapy* 44:2233–2241.

Reynolds, M.G. 2000. Compensatory evolution in rifampin-resistant *Escherichia coli*. *Genetics* 156:1471–1481.

Springer, B., Y.G. Kidan, T. Prammananan, K. Ellrott, E. C. Böttger, and P. Sander. 2001. Mechanisms of streptomycin resistance: selection of mutations in the 16S rRNA gene conferring resistance. *Antimicrobial Agents and Chemotherapy* 45:2877–2884.

Stabb, E.V., and J. Handelsman. 1998. Genetic analysis of zwittermicin A resistance in *Escherichia coli*: effects on membrane potential and RNA polymerase. *Molecular Microbiology* 27:311–322.

Snyder, L., and W. Champness. 2003. *Molecular Genetics of Bacteria*. ASM Press, Washington, DC.

Taniguchi, H. H. Aramaki, Y. Nikaido, Y. Mizuguchi, M. Nakamura, T. Koga, and S. Yoshida. 1996. Rifampicin resistance and mutation of the *rpo*B gene in *Mycobacterium tuberculosis*. *FEMS Microbiological Letters* 144:103–108.

Tankovic, J., D. Lamarque, J.-C. Delchier, C.-J. Soussy, A. Labigne, and P.J. Jenks. 2000. Frequent association between alteration of the *rdx*A gene and metronidazole resistance in French and North African isolates *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy* 44:608–613.

Top, E.M., Y. Moënne-Loccoz, T. Pembroke, and C.M. Thomas. 2000. Phenotypic traits conferred by plasmids. In Thomas, C.M. (editor), *The Horizontal Gene Pool*, pp. 249–285. Harwood Academic, Amsterdam, The Netherlands.

Vannuffel, P., M. Di Giambattista, E.A. Morgan, and C. Cocito. 1992. Identification of a single base change in ribosomal RNA leading to erythromycin resistance. *Journal of Biological Chemistry* 267:8377–8382.

Wang, G., T. J. M. Wilson, Q. Jiang, and D.E. Taylor. 2001. Spontaneous mutations that confer antibiotic resistance in *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy* 45:727–733.

Wichelhaus, T.A., B. Böddinghaus, S. Besier, V. Schäfer, V. Brade, and A. Ludwig. 2002. Biological cost of rifampin from the perspective of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 46:3381–3385.

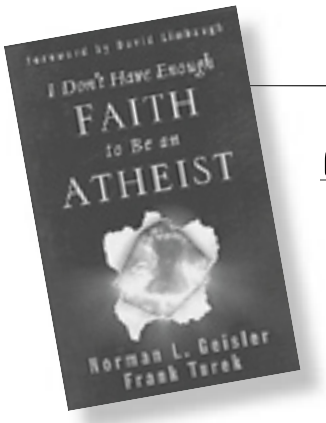
Williams, D.L., L. Spring, L. Collins, L.P. Miller, L.B. Heifets, P.R.J. Gangadharam, and T.P. Gillis. 1998. Contribution of *rho*B mutations to development of reifamycin cross-resistance in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 42:1853–1857.

Willmott, C.J.R., and A. Maxwell. 1993. A single point mutation in the DNA gyrase A protein greatly reduces the binding of fluoroquinolones to the gyrase-DNA complex. *Antimicrobial Agents and Chemotherapy* 37:126–127.

Yigit, H., G. J. Anderson, J. W. Biddle, C. D. Steward, J. K. Rasheed, L.L. Valera, J.E. McGowan, and F.C. Tenover. 2002. Carbapenem resistance in a clinical isolate of *Enterobacter aerogenes* is associated with decreased expression of OmpF and OmpC porin analogs. *Antimicrobial Agents and Chemotherapy* 46:3187–3822.

Zarantonelli, L., G. Borthagaray, E.-H. Lee, and W. M. Shafer. 1999. Decreased azithromycin susceptibility of *Neisseria gonorrhoeae* due to *mtr*R mutations. *Antimicrobial Agents and Chemotherapy* 43:2468–2472.

Zengel, J.M., R. Young, P.P. Dennis, and M. Nomura. 1977. Role of ribosomal protein S12 in peptide chain elongation: analysis of pleitropic, streptomycin-resistant mutants of *Escherichia coli*. *Journal of Bacteriology* 129:1320–1329.



Book Review

I Don't Have Enough Faith to Be an Atheist
by N.L. Geisler and F. Turek
Crossway Books, Wheaton, IL. 2004, 447 pages, $16.00.

Which requires the believer to have more faith, Christianity or atheism? This is the intriguing question asked by the authors. We often think it takes great faith to believe that the Bible is the true Word of God, that Jesus is God and rose from the dead, and that we are products of intentional design. But authors Geisler and Turek assure us