Bacterial Resistance to Antibiotics—A Case of *Un*-Natural Selection

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Abstract

Although the development of bacterial resistance to antibiotics is frequently described as classical Darwinian natural selection in progress, a closer examination reveals no such example. This evaluation is based on a consideration of the basic tenets of "natural selection" in light of our current understanding of the development and spread of resistance mechanisms.

Natural Selection

Natural selection leading to the development of differentiation and subsequent speciation is a process in evolutionary theory which involves three key elements. First, the process requires the existence of genetic variation. This may be introduced to the population through an initial random mutation in one organism. Second, this variation is followed by competition, as a sorting mechanism. During this sorting, organisms expressing a 'preferred' or 'superior' variation are expected to out-populate their inferior counter-parts due to their inherent advantage. Third, the repetition of cycles of variation and competition will, over time, lead to the accumulation of superior traits, i.e. Darwinian gradualism, with the subsequent separation of new species, i.e. 'evolutive speciation'. If natural selection leads to evolutionary improvement in diversity or differentiation, the variations accumulated must in fact not just be 'better adaptations' of existing structures or processing systems, but must eventually introduce new structures or processes which the organism can utilize to its benefit, and selectively pass to its progeny.

Bacterial resistance to antibiotics has been touted in the scientific literature as a verified example of this evolutionary process. For example,

> In many instances of acquired drug resistance natural selection, with its requirement for mutation and biological variation, is the most likely process to explain the resistant phenotype. The essence of this mechanism is the selection of individuals that can withstand the chemical insult and hence outgrow their susceptible counterparts....There are many examples of acquired drug resistance through natural selection. In *E. coli*, structural changes in penicillin-binding proteins can result in resistance to the antibiotics mecillinam or cepholosporin, changes in the structure of the β-subunit of RNA polymerase can confer resistance to rifampicin, and changes in the structure of DNA gyrase can confer resistance to nalidixic acid or novobiocin." (Hayes & Wolf, 1990).

The question to be considered now, is whether the facts related to bacterial resistance mechanisms are consistent with this process of Darwinian natural selection as an explanation for their origin.

Resistance Mechanisms

Although several hundred commercially available antibiotics are now available, most of these compounds are based on just a handful of effective chemical architectures which target only a few critical bacterial structures or processes (see Table I).

In turn, resistance mechanisms can also be subgrouped into 1) those which cause inactivation of the antibacterial agent, such as β-lactamase, 2) those which

Table I. Antibiotic Target Groups and Examples

Cell Wall	ß-lactams, e.g., penicillin family, cephalosporins
Cell Membrane	e.g., polymyxin B, nystatin, magainin
Protein Synthesis	macrolides, e.g., erythromycin tetracyclines aminoglycosides, e.g., streptomycin
DNA Processes	quinolones, e.g., norfloxacin
RNA Processes	rifamycins, e.g., rifampin
Metabolic Analogs	sulfonamides, e.g., sulfanilamide
Other	pro-drugs, e.g., isoniazid (anti-tuberculosis agent) antisense RNA

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modify the target, such as ribosome methylating enzymes or structural mutations, and 3) those that reduce the effective concentration of the antibacterial agent in the cell, such as efflux pumps or overexpression / overproduction of the target (see Figure 1).

The Role of Mutation in the Process of Natural Selection

Mutations appear to play only a minor role in the development of resistance mechanisms since they only act on pre-existing genes.

Families of antibiotics have been developed based on a common structural theme. For example, the penicillins incorporate the β-lactam ring as the common molecular foundation (Figure 2). These molecules are bactericidal due to their interference in the normal synthesis process of the bacterial cell wall. The enzyme, β-lactamase, confers resistance due to its ability to cleave the β-lactam ring, thereby rendering the antibiotic inactive.

More than any other antibacterial agent, the β-lactam containing molecules have been extensively modified through the addition of 'side-chains'. Generally speaking, the side chains act physically or chemically to interfere with β-lactamase's function of cleaving the lactam ring, by interfering with the enzyme's ability to productively bind the antibiotic in its active site. However, each new drug variation has typically, in time, been rendered ineffective by the development and spread of a novel β-lactamase, which restores resistance to the bacterium. The novel β-lactamase is usually the result of a mutation acting on the β-lactamase gene which causes a substitution of one or more of the amino acids involved in the process of antibiotic binding and cleavage. The mutant

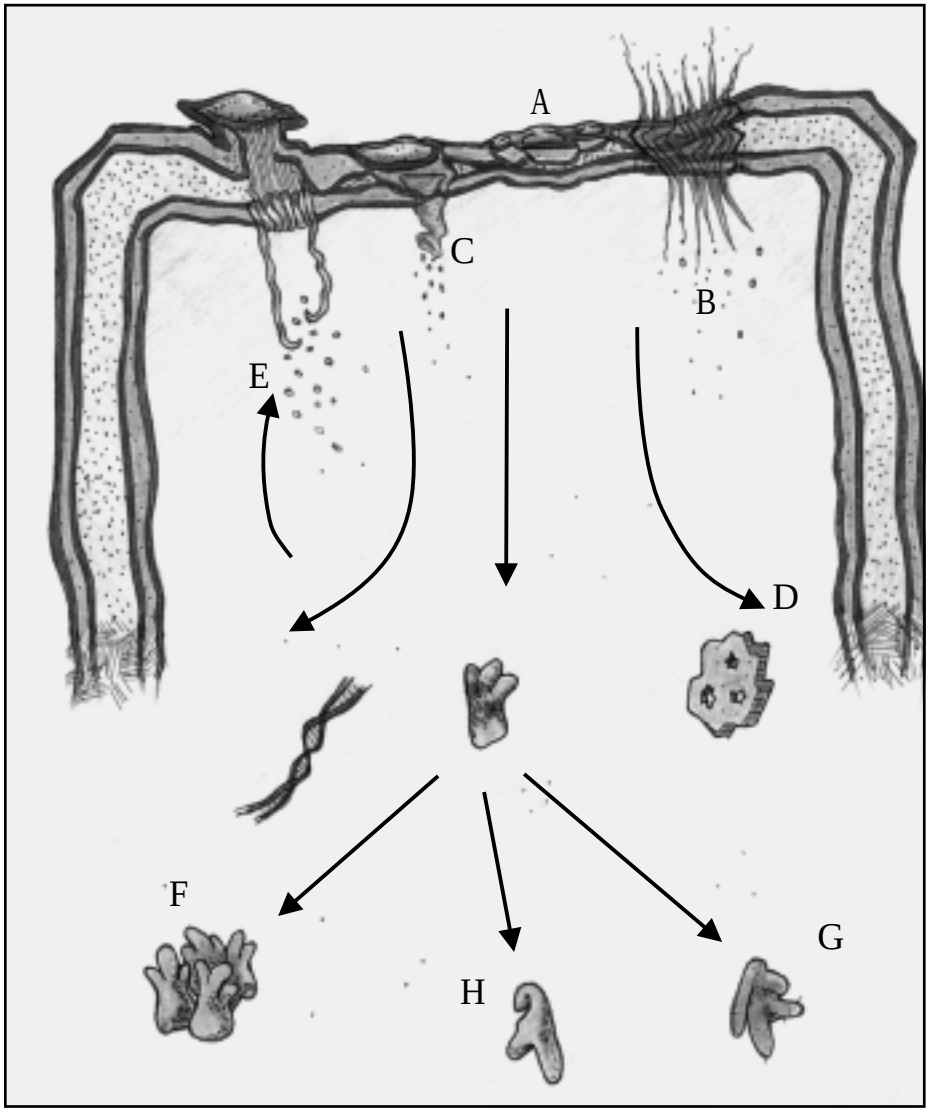

Figure 1. Illustration of mechanisms involved in antibiotic resistance: A) the outer membrane of gram negative bacteria serves as a permeability barrier—however, specific proteins in the outer membrane, called porins, serve as diffusion channels or gateways for some hydrophilic molecules—loss of porins increases the resistance to some antibiotics; B) hydrophobic molecules may diffuse through the membrane itself, but some mutations involving outer membrane biochemistry have an impact on such diffusion rates, thus the mutations potentially increase drug resistance; C) specific trans-membrane transporters serve to import various target molecules such as nutrients, but may also include some antibiotics such as metabolic analogues—mutation or loss of the transporter may increase resistance; D) antibiotics in the periplasm or cytoplasm may be inactivated through modification, isolation, or destruction; E) efflux pumps may apply metabolic energy to push antibiotics from the cytoplasm thereby reducing the effective concentration inside the cell; F) mutations may cause a significant increase in the quantity of the target enzyme, such that the therapeutic concentration of the antibiotic is no longer sufficient to halt that metabolic process; G) mutations may modify the cellular target such that the antibiotic is no longer effective; H) some antibiotics may be effective temporarily, but cellular repair mechanisms, redundant regulatory systems or subsequent protein synthesis later restore vitality to the bacteria.

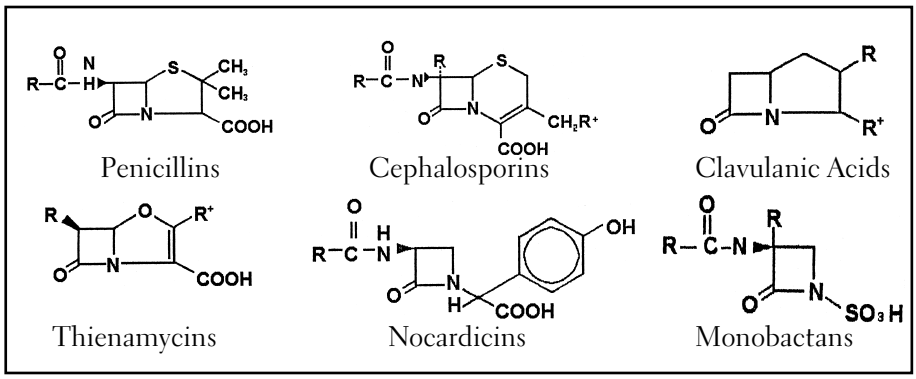

Figure 2. Examples of antibiotics based on the β-lactam structure, which is specifically represented schematically as the square "ring."

enzyme, due to its structural or chemical change, compensates for the changes made to the antibiotic structure, and thereby restores the ability of the enzyme to successfully bind the antibiotic and cleave the ring structure. Through this process, the β-lactamases themselves have become a large family of enzymes which are sub-divided according to their specific antibiotic deactivating profile.

In the above example, the development of resistance to new antibacterial agents is based on the introduction of variation through genetic mutation. Thus, it appears that this may demonstrate the first requirement of the natural selection process. However, the 'functionality' of the resistance mechanism which is the true resistance determinant, i.e., the ability of the enzyme to break the β-lactam ring, did not change even though the specific shapes of the antibiotics the enzyme will de-activate has been slightly modified. Although an existing active catalytic site may tolerate a rare mutational event which modifies the binding pocket of the enzyme, it nonetheless does not represent the development of a distinct new mechanism of resistance. Noting that most antibiotics are, like the β-lactams, merely derivatives based on a particular antibacterial architecture, the statement can be extended to the majority of those in antibiotic families.

A similar form of resistance can occur when a mutation results in gene duplication for the bacterial structure or processing system targeted by a particular antibiotic. In this case, since the mutant bacterium now produces significantly more of the target, it will be more resistant than its normal wild-type relatives to a given dosage level of the antibiotic. In other words, it will take a higher dosage of the antibiotic to overcome the increase in the number of targets in the organism. Again however, it should be recognized that this mutation does not represent the development of a novel functionality, but in effect simply re-establishes the cell's normal operating status quo in the face of the antibiotic insult. The theoretical contribution of gene duplication events toward evolutionary development does not apply in the case of resistance to antibacterial agents, since in this case resistance is conferred because of the stabilizing or restoration of a metabolic process or structure, rather than the introduction of an alternate process or structure with subsequent mutation and diversification.

In both of the above examples, the enzymes or structures involved require the pre-existence of a functional gene, and as a result of mutation, the organism has not gained any new functionality which may be useful to the bacterium for other purposes. Therefore, no 'advancement' or functional diversification has occurred for the organism. Since no new functions or structures have been developed in either of these examples of resistant mutants, these types of mutations are inadequate in themselves as evidence to support Darwinian natural selection leading to speciation.

The origin of resistance genes is difficult to explain.

Mutation of a pre-existing resistance gene as discussed above, does not in fact address the question of how that gene originated in the first place. While some of the bacterial resistance determinants are carried on the bacterial chromosome, the majority of those which have been identified from resistant clinical isolates are carried in the cell as resistance plasmids (R-plasmids or R-factors). R-plasmids are small closed loops of DNA which contain the genetic code for one or more proteins, as well as the DNA promoter sequences necessary for its transcription. Further, for the resistance gene and the plasmid to be reproduced, the DNA must also contain a specific sequence called a replication origin. The replication origin is necessary because plasmid DNA is independent of the bacterial chromosome, and without this the plasmid will not routinely replicate during cell growth and division.

The evolutionary origin and development of R-factors is unknown. It was suggested over 25 years ago that some R-factors found in resistant bacteria may have originated from the genes of organisms that synthesize antibiotics, such as *Streptomyces* (Walker and Walker, 1970). Statements such as, "An ancestral β-lactamase cannot be identified, but *Streptomyces* are ancient and their β-lactamases presumably appeared early in evolution" (Couture et al., 1992) reveal this as a presumptive philosophical hypothesis, rather than an empirically demonstrated one. This concept continues to be promoted as an evolutionary explanation for R-plasmids (Cundliffe, 1989; Wilkins, 1996) despite the fact that, "...we can only speculate on the environmental and evolutionary factors

that play a role in their formation and maintenance" (Benveniste and Davies, 1973).

This explanation however, does not resolve the origin of R-plasmids, but simply defers it. The evolution of antibiotic production through natural selection faces a seemingly insurmountable problem if you consider that as soon as any variation which causes the biosynthesis of a new compound with antibacterial properties is introduced, that organism would in fact be committing suicide. The producing organism would be defenseless to new antibiotic, and the mutation would therefore be immediately lost as no mutant offspring would be produced. Thus, antibiotic production and the production of an applicable resistance mechanism are '**biochemically co-dependent**' in the host. This means that the synthesis of antibiotics cannot occur without an effective resistance mechanism also being in place. It is possible for an organism to synthesize one of the antibiotics that it is not naturally susceptible to, but this is not the common scenario found in many of the wild-type antibiotic producers (Rodriguez et al., 1993). Instead, this problem is addressed for most of the producing organisms by the inclusion of a resistance gene(s) in the antibiotic's biosynthetic gene cluster. This in turn means that the cell must have carried not only a developing system of genes for the antibiotic's biosynthetic enzymes prior to the compound actually being functional as an 'antibiotic', but also the organism must have carried and maintained a system of resistance genes to protect itself, prior to their having any useful functionality. In this scenario, it would appear from an evolutionary perspective that either the fully functional resistance mechanism evolved first, which would be unlikely considering it would have no 'selection value' until perfected, or else the two systems 'co-evolved'. The tight biochemical co-dependency of the two gene systems and their association in biosynthetic gene clusters within the antibiotic producing organisms argues against an evolutionary explanation based on natural selection.

The high level of complexity and unknown natural physiological function of some resistance mechanisms further complicates the evolutionary development problem. Many resistance mechanisms are not based just on a single gene, but require the expression of numerous genes to operate. In the case of Gram negative bacteria, the cell has both an inner and outer membrane, with an intervening space called the periplasm (Ferguson, 1992). The outer membrane is a particularly effective barrier which acts to slow the uptake of the antibiotic from the surrounding medium. However, according to the principles of diffusion, under steady state conditions the concentration gradient across the membranes will eventually reach an equilibrium consistent with the chemical nature of the antibiotic and the transmembrane pH gradient (Thanassi et al., 1995). Slow uptake alone therefore does not confer a significant level of resistance. However, it can offer a resistance advantage if it is coupled with a second resistance factor such as an efflux pump system. Such efflux systems selectively collect the antibiotic molecules that do cross the outer membrane barrier, and efflux them back to the outer environment (Paulsen et al., 1996). If efflux is faster than diffusional influx, the internal concentration of the antibiotic can potentially be kept relatively low.

There is a problem however for efflux based resistance if the antibiotic is only pumped from the cytoplasm to the periplasm. In this case it may build up a concentration of molecules in the periplasm. In this situation the outer membrane would act as a diffusional barrier to contain the drug. Increasing the periplasmic concentration of the drug in this way would disturb the concentration gradient across the inner membrane, and thus also increase the rate of diffusion of the antibiotic back through the inner membrane into the cytoplasm. For efflux systems to be an effective resistance mechanism for many types of antibiotics, an additional system must be added. In many cases, such as the chromosomally based *Pseudomonas aeruginosa* MexA-MexB-OprM system (Poole et al., 1996), the efflux pump of the inner membrane (MexB), has been coupled to a protein which provides a bridging conduit across the periplasm (MexA), which in turn is coupled to an outer membrane pore (OprM). The result is the efflux of an antibiotic from the cytoplasm to the external environment in one step, after which the barrier of the outer membrane can then support the resistance gained by the efflux pump system by slowing the inward diffusion of the antibiotic molecules.

The development of an antibiotic efflux pump alone may not confer any practical advantage for the Gram negative bacteria unless it is specifically coupled to the proteins which allow for the complete export of the drug through the outer membrane. In the best known examples, the system is encoded together as a three gene operon, although the Acr systems of *E. coli* apparently share the outer membrane protein TolC. Therefore, even if the gene for one of the components, such as the efflux pump protein, was transferred intact from an antibiotic producer such as *Streptomyces* which is Gram positive and lacks the accessory conduit proteins, it would be ineffective as a significant resistance mechanism. Systems such as these which integrate several additional proteins are themselves biochemically co-dependent for resistance functionality as each single component has limited, if any, resistance activity (Yoneyama et al., 1997). In addition to this high level of complexity, it is interesting to note that the purpose of the family of RND efflux systems, which includes the *Pseudomonas aeruginosa* Mex efflux systems, as well as the *E. coli* Acr efflux systems, is

not known. The natural roles or metabolic substrates suggested to be involved in the efflux systems are at best speculative.

Some bacteria develop resistance due to the 'loss' of structures or genetic information, rather than through a gain of novel processes or structures.

In the case of Gram negative bacteria, the outer membrane contains water filled protein channels called 'porins', which serve as gateways to admit small hydrophilic molecules to the periplasm, including some antibiotics. Several different porins exist, and their channel sizes and shapes vary, which gives some degree of selective permeability for the outer membrane based on the size and shape or electrical charge of the molecule attempting to enter the periplasm (Hancock et al., 1990). In several cases involving resistant strains of bacteria, it has been noted that particular porin types are missing or greatly reduced in number (Medeiros et al., 1987). These mutants may appear to have gained a 'selective advantage' in being more resistant to the antibiotic challenge than their close relatives, but in fact the 'gain' is just the opposite — it is in fact a 'loss' of genetic information and structure. Another example of this type of 'loss' is reflected in the increased resistance conferred due to intrinsic antibiotic efflux systems. In cases such as the *Pseudomonas aeruginosa* MexAB-OprM three-gene operon, the system is transcribed more frequently due to a loss of negative regulatory controls caused by mutation in the negative control gene or genes. What needs to be demonstrated to vindicate the natural selection paradigm is not loss of genes or regulatory controls but evolution of new genes or controls.

A similar phenomena may occur with antibiotics that function as substrate analogues to produce competitive inhibition. An analogue resembles the natural substrate of an enzyme, but it causes metabolic interference when it competes with the natural substrate in binding to that enzyme, blocking its activity. Such types of antibiotics may tie up the enzyme in competitive but non-productive interactions, or may even disable it, thereby adversely affecting the growth of the bacterium. One form of resistance to these antibiotic analogues occurs when a mutation in the enzyme reduces the affinity for its natural metabolic substrate, and thus also for the substrate analogue antibiotic. Such a mutation may permit the bacteria to better tolerate a transient antibiotic insult due to its much slower metabolic rate in the targeted process, whereas its more robust relatives may be killed or severely damaged by the antibiotic. From the perspective of natural selection, not only has no new function been developed in this example, but worse, this mutation has taken a 'superior' form of an enzyme, i.e. one of higher affinity and processing capacity, and substituted through mutation an 'inferior' form of the enzyme, which lacks its predecessors more efficient structure. This cannot be considered an evolutionary advancement.

Competition

R-plasmids are promiscuously shared across species, but may be lost to progeny.

One of the issues which makes the spread of antibiotic resistance in bacteria a significant medical problem is the fact that the R-plasmids are often spread from one cell to many others. The spread occurs not only to these species, but also potentially across genus levels, and in some examples even beyond this taxonomic rank. This is accomplished through fertility factors which are often included on the R-plasmid itself, and which promote the transfer of the DNA from cell to cell. The end result of this promiscuous behavior is that the resistance mechanisms can be spread broadly, even in the absence of an ongoing antibiotic threat. The paradigm of evolutionary natural selection stipulates that a superior trait will more likely be passed to an offspring. A phenotypic change in the population occurs through out-populating the competition with numerous 'superior' progeny. The behavior of freely giving away ones 'advantage' would appear to be in direct conflict with natural selection. In this situation, the advantage of the resistance mechanism is lost as a superior trait over those other genetic lineages with which a bacterium is expected to be in close competition. The progeny of the resistant strain, therefore, have gained no competitive advantage.

An additional issue related to R-plasmids is the fact that their replication is not directly tied to the replication of the bacterial chromosome. When the bacterium reproduces, it divides its cytoplasm between the two daughter cells. If the replication of the R-plasmid is slower than that of the bacterial chromosome, it may in fact become diluted and lost to some of the progeny during the process of cellular fission. One of the challenges of biotechnology is establishing installed plasmids as 'stable' plasmids, so that they will not be lost or unfavorably over-replicated during cellular growth.

Thus, since an advantage carried on an R-plasmid is typically shared with those of a different genetic heritage, and further, since it may easily be lost to some of the progeny, it appears that R-plasmids are in direct conflict with the expectations of the natural selection process.

Selection is based on catastrophe, not competition.

When faced with an antibiotic challenge, possessing or not possessing the resistance mechanism is not simply a matter of 'competition'—it is routinely the difference between life and death for the organism. While survival is logically a benefit to the individual, it does not specifically explain how evolutionary advancement occurs. Prior to the antibiotic challenge the resistance mechanism has no real selection advantage. Since there is therefore little control over which of the bacterial cells, or more specifically, which 'genetic lineage' passes along the resistance mechanism to its off-spring, in a given wild-type population the more 'robust' bacterial strains may be wiped out entirely, while the 'inferior', i.e. less advanced, may become the sole survivors. Thus, the determination of which genetic lineage will 'out-populate' is based on a single determinant which is completely independent of the organisms overall wild type robustness or developmental advancement. Many 'superior' traits in metabolism and physiology can therefore be completely erased from the population gene pool based on the organisms lack of a single determinant, i.e. the resistance mechanism, which had previously offered no fitness benefit. This is counterproductive to the natural selection process.

The 'fitness cost' associated with competition and sorting based on resistance factors has been recognized (Lammerts, 1967; Medeiros et al., 1987; Pan and Spratt, 1994) and is currently being studied (Schrag and Perrot, 1996). After the antibiotic insult, the population appears to 'recover' some of its lost genetic potential over a number of subsequent generations. The fact remains however, that the surviving bacteria are not as robust as the unchallenged wild-type. For example, in the case of *E. coli tonB* mutations, the bacterium is resistant to some antibiotics, but is typically avirulent as a result of its inability to take up iron in the host environment (Nikaido, 1994). Another example of the inferior nature of the resistant mutants can be seen from studies of the RND family of drug efflux systems. Difficulties in culturing laboratory strains have been encountered due to an undefined deleterious effect caused by overproduction of these proteins or their activity. This may be due to unfavorable changes in the membrane structures of the strains, or may be the result of the efflux pumps extruding needed metabolic intermediates from the cell. Similarly, Levy commented, "The resistant strain has no real advantage without the presence of an antibiotic...The strain would die out due to natural competition." (Levy, 1997).

Obviously the mere survival of a few fortunate bacteria must be a better evolutionary outcome than a complete elimination of the species altogether. In this light, even if the species survival does occur through just the inferior strains, the possession of resistance mechanisms would appear to be a bearable 'fitness penalty' for the forthcoming generations. However, resistance alone subordinates all the other aspects of 'fitness' in the face of a catastrophic antibiotic assault, thereby dealing a set-back to gene pool expansion and the speciation process. This pushes such resistance characteristics outside the normal expectations of the natural selection paradigm and Darwinian gradualism, into a separate theoretical discussion (Wilson, 1997).

The extensive application of commercial antibiotics has created an 'un-natural' environment for the development of resistant strains.

The following comments serve to emphasize just how 'un-natural' the antibiotic resistance environment really is. Firstly, the chemical architecture of the active agent, and the microbial targets for commercial antibiotic preparations are selected purposefully and by design, not by 'random mutation' letting nature run its course. Secondly, the compounds are often semi-synthetic, meaning they may be compounds that for practical reasons will never be synthesized by micro-organisms in the wild, i.e. they are not 'natural' molecules. Thirdly, in order to enhance their therapeutic effect, commercial antibiotic preparations are typically purified, concentrated and super-potent, i.e. they have been made to a very high strength, well beyond that normally found in a natural environment. Fourth, these designed, artificial, superpotent drugs are not only being used in therapeutic treatment of bacterial infections, but are being broadcast at large, for example in animal feed supplements. For example, in 1994 Denmark used approximately 24 kg of vancomycin for human therapy, while 24,000 kg of avoparcin, a glycopeptide antibiotic known to induce cross resistance to vancomycin, was used in animal feed. Similarly, from 1992 to 1996 Australia averaged annually 582 kg of vancomycin for human use, while an additional 62,642 kg per year of avoparcin was imported for agricultural applications (Witte, 1998). This is not the equivalent of a natural environment of close quarter competition in a tight ecological niche, between the antibiotic-producer and its so-called competitor. As a result, the resistance mechanisms are being found in a broad range of non-clinical / non-competitive settings where selection would not normally be expected to operate. Fifth, the ecology involving clinical pathogens is not necessarily the natural host / pathogen relationship. For example, a medical treatment including antibiotics may wipe out the microbial flora which are normal and helpful to the body. As a consequence, bacteria which happen to possess resistance mechanisms gain a free range to grow and cause clinical problems, even though they are normally not a problem. Resistance as expressed in its clinical set-

ting is therefore not being expressed in the natural environment, but in an ecological niche which lacks the normal competitive checks and balances of the wild-type population. Webb and Davies (1993) note,

> Analysis of bacterial collections from the preantibiotic era indicates that although plasmids were present in some of the strains, they did not harbor antibiotic resistance genes. The conclusion is that the development of antibiotic-resistant microbial populations occurred after the introduction of antibiotics into clinical use.

Sixth, when antibiotics are produced from live cultures, the purification process will remove the living organisms from the final product. However, it has been demonstrated that not all of the cellular 'debris' or components from dead cells in the culture are removed. As a result, many antibiotic preparations have been contaminated with the DNA of the antibiotic producing organism, including their internal resistance genes (Webb and Davies, 1993). Clinical administration of antibiotic therapy therefore seems more like a domestic breeding program than a natural competition.

In considering the 'un-natural' development and spread of resistance mechanisms with respect to biotechnology, molecular biologists design plasmids or chromosomes to include drug resistance in addition to the gene under study as a tool for screening or isolating transformed mutants from the non-transformed population. While this concept might be thought of as an example of a 'gain in function' for the organism, i.e. picking up a new gene along with the resistance gene, it is still merely an example of horizontal gene transfer. Thus, in addition to being the result of 'intelligent design' in the laboratory as opposed to a natural selection process, biotechnology, as noted with the origin of antibiotic resistance mechanisms above, still does not answer the question of the actual 'origin' of the information content for the gene, i.e. nothing new has been developed — only transferred.

Evolutive Speciation

In addition to failing the requirements for the first two steps in Darwinian natural selection, i.e. mutation / variation followed by a sorting of the new resistant phenotype through competition, the acquisition and spread of resistance genes also fails the third requirement, i.e., the formation of new 'species'. Resistant mutants share the species specific genome and therefore a basic physiology with their related non-resistant wild-type population. A novel mutation which confers resistance to an organism and subsequently to the population, in itself therefore, lacks any true 'advancement' on the species level. Identifying some minor variation or adaptation does not overrule the fact that the species genome is at best 'stable', or at worst 'degenerative' (Moore, 1974). Natural selection requires the accumulation of superior traits leading to a distinct species, however, even the collection of numerous resistance mechanisms in an organism will not alter the basic physiology of that organism. Further, it has been long recognized that a resistant mutant strain may actually have a loss of rather than an advancement of genetic information, or may be otherwise less robust than the wild-type non-resistant organisms (Lammerts, 1967). Thus, even if mutation and competition could be shown to be tools for working the natural selection process, the resulting population has not graduated to a new more complex form, but will simply continue to survive, at best only consistent with the current species genome and phenotype. An antibiotic challenge may therefore be an agent to force a 'selection' within the population, but that selection is not the kind that leads to the differentiation of new more advanced organisms or species. In other words, no 'evolutive speciation' has been demonstrated due to the addition of resistance genes.

Summary and Conclusions

Natural selection does not provide a satisfactory explanation for the origin of even one gene for a functional resistance mechanism, further, it offers no means through which such an acquisition can make a population distinct enough to be considered a new species. If the development and spread of antibiotic resistance mechanisms were truly the result of Darwinian natural selection, there should be ample evidence of the introduction of 'mutation and variation', followed by 'competition' which favors the superior organism, resulting speciation. Instead however, we see that for each of these expectations, the evidence stands against natural selection. Mutation plays only a minor role, merely causing minor modifications of a pre-existing gene whose origin cannot be explained naturally. Further, some aspects of resistance are based on a loss of structures or processes, not the gain or addition of genetic information. Resistance mechanisms are often shared promiscuously amongst a species or genus, rather than conferring an advantage to one genetic lineage. Resistance is not a matter of gradualism through competition, but of survival in catastrophe. Finally, the environment for the development and spread of antibiotic resistance is 'un-natural'.

While embellished Darwinian stories, specific survival scenarios, or odd exceptions may offer interesting theoretical discussion, the facts confirm that antibiotic resistance is not an example of Darwinian natural selection. Bacterial resistance to antibiotics appears to be a case of "un-natural selection."

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Erratum

In CRSQ volume 34, page 233, there is an error in the sentence:

> "Although the Deluge appears to be the only reasonable explanation for the geologic conditions necessary to form the deposit, greater precision in historical placement of the deposit than that presented here does not appear justified by the scientific data is is probably unwise." The word "is" is incorrectly repeated. Replace the first "is" by "and."