

The Mechanics of Antibiotic Resistance



Introduction

This document is not designed as yet another scientific review of antimicrobial resistance but rather an explanatory piece outlining the causes of resistance.


It is aimed at the practicing veterinarian, giving an understanding of the mechanisms of resistance in a scientific fashion.

This is reflected in the terminology. The more correct term to use is antimicrobial rather than antibiotic, because the term antibiotic refers to a chemical produced by a microorganism to defend itself against another microorganism; many of the commonly used antimicrobial drugs are synthesized chemically so the strict definition antibiotic does not apply.

In the same vein, as infective disease agents may be other than bacteria, the term microorganism is used.

As the work is not designed to be a specific scientific paper but an instructional document, graphics are used freely in order to aid in understanding of concepts. For the same reason references used are listed at the end but not annotated in the text, for ease of reading.

Copies of references cited can be obtain by e mail to drdenny@ethicalagents.co.nz

A handwritten signature in blue ink. It features a large, stylized 'D' with a checkmark inside, followed by the name 'Dennis Scott' written in a cursive script.

Dennis Scott BVSc MANZCVS AMRLG

Antimicrobial Resistance

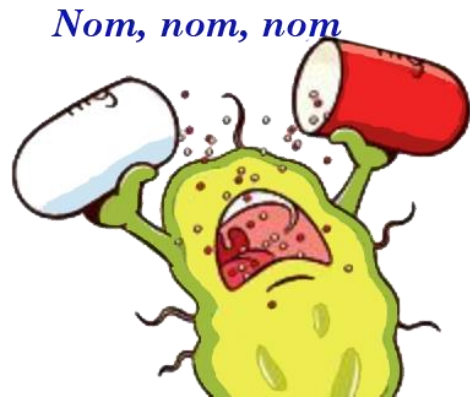
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Resistance is a means whereby a naturally susceptible microorganism acquires ways of not being affected by the drug.

Microbial resistance to antimicrobial agents is not a new phenomenon; it has been going on in soil microorganisms since the dawn of time, as competitive/survival mechanisms by microorganisms against other microorganisms.

Understanding the mechanisms of resistance is important in order to define better ways to keep existing agents useful for a little longer but also to help in the design of better antimicrobial agents that are not affected by the currently known, predicted, or unknown mechanisms of resistance.

Although antimicrobial resistance is a natural biological phenomenon, it often enhanced as a consequence of infectious agents' adaptation to exposure to antimicrobials used in humans or agriculture and the widespread use of disinfectants at the farm and the household levels. It is now accepted that antimicrobial use is the single most important factor responsible for increased antimicrobial resistance.



Clinical versus Microbiological Resistance

From a microbiological point of view, resistance is defined as a state in which an isolate has a resistance mechanism rendering it less susceptible than other members of the same species lacking any resistance mechanism. This definition is valid irrespective of the level of resistance (i.e. low or high level of resistance) and does not necessarily correlate with clinical resistance.

From a clinical point of view, resistance is defined as a state in which a patient, when infected with a specific pathogen, is treated with an adequate antimicrobial dosage and administration schedule, but clinical criteria of cure (at a clinical and/or a microbiological level) are not reached.

Clinical resistance can be due to non-microbial factors such as penetration to the site of infection, walled off abscesses being a prime example. On the other hand there can be microbiological resistance defined in the laboratory but clinical cure despite this. An example is topical therapy in ears or on the skin, where the amount of antimicrobial applied is so great that the infection is controlled anyway.

A clinical breakpoint is an MIC value that correlates with the clinical outcome and that separates those isolates that are considered as clinically susceptible or associated with a high likelihood of therapeutic success from those that are considered as clinically resistant or associated with a high likelihood of therapeutic failure.

Intrinsic versus Acquired Resistance

1) Intrinsic Resistance

Whereby microorganisms naturally do not possess target sites for the drugs and therefore the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to affect their action. With intrinsic resistance the organism possesses properties that make it naturally resistant to certain insults, e.g. the more

complex outer layer of gram negative bacteria makes it much more difficult for certain antimicrobials to penetrate.

A good analogy in people is sun tolerance; darker skinned people have a higher melanin content in the skin that makes them more tolerant of the sun's harsh rays than people with fair skin. This is intrinsic resistance to the sun's rays built up by millennia of genetic selection in hot countries.

Thus intrinsic resistance is considered to be a natural and inherited property with high predictability. Once the identity of the organism is known, the aspects of its anti-microbial resistance are also recognized.

2) Acquired Resistance

Acquired resistance is when a naturally susceptible microorganism acquires ways of not being affected by the drug. Any insult, physical or chemical, has the potential to induce changes in the organism. Again our sun tolerance analogy shows us the fair skinned people, by gradual exposure, (sun tanning) can become more sun tolerant.

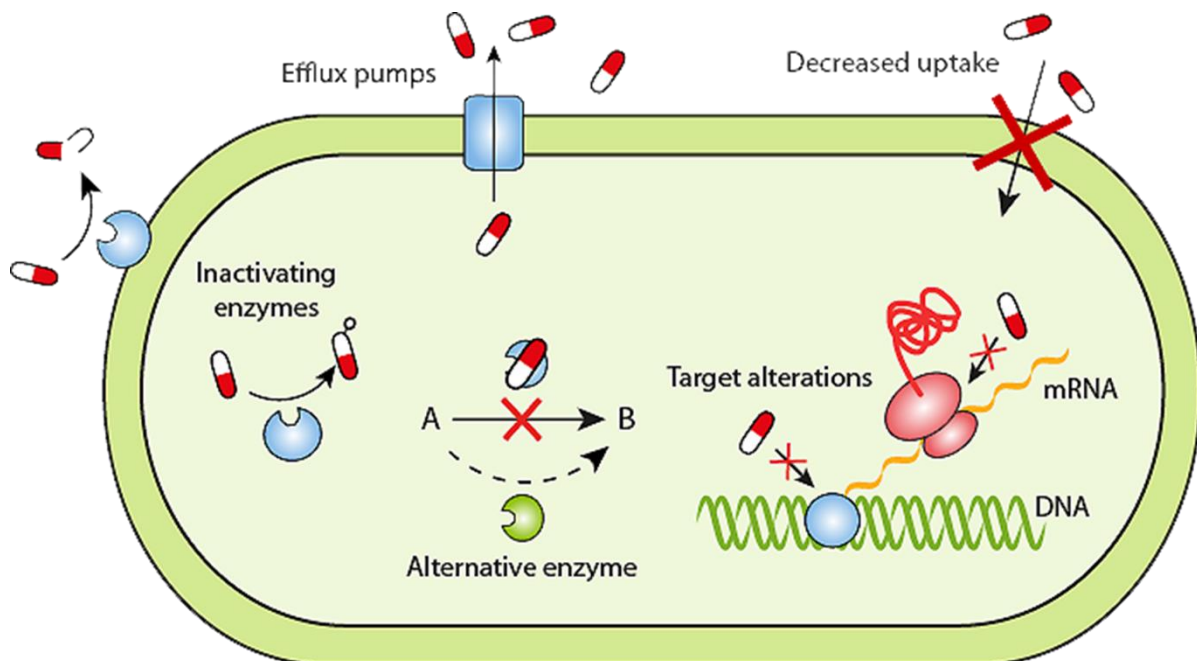
“thus microbes have been performing their own genetic modification for millions of years.”

Microbes are more ubiquitous however, and can actually acquire resistance from each other by sharing genetic material. They can pass genetic material from one to another in various ways; thus microbes have been performing their own genetic modification for millions of years.

This is known as horizontal gene transfer (HGT) and can be a much more rapid process than the genetic selection required for intrinsic resistance.

In addition, while our sun tan analogy simply requires more melanin accumulating in skin cells, microbes have several mechanisms they can resort to in order to develop resistance.

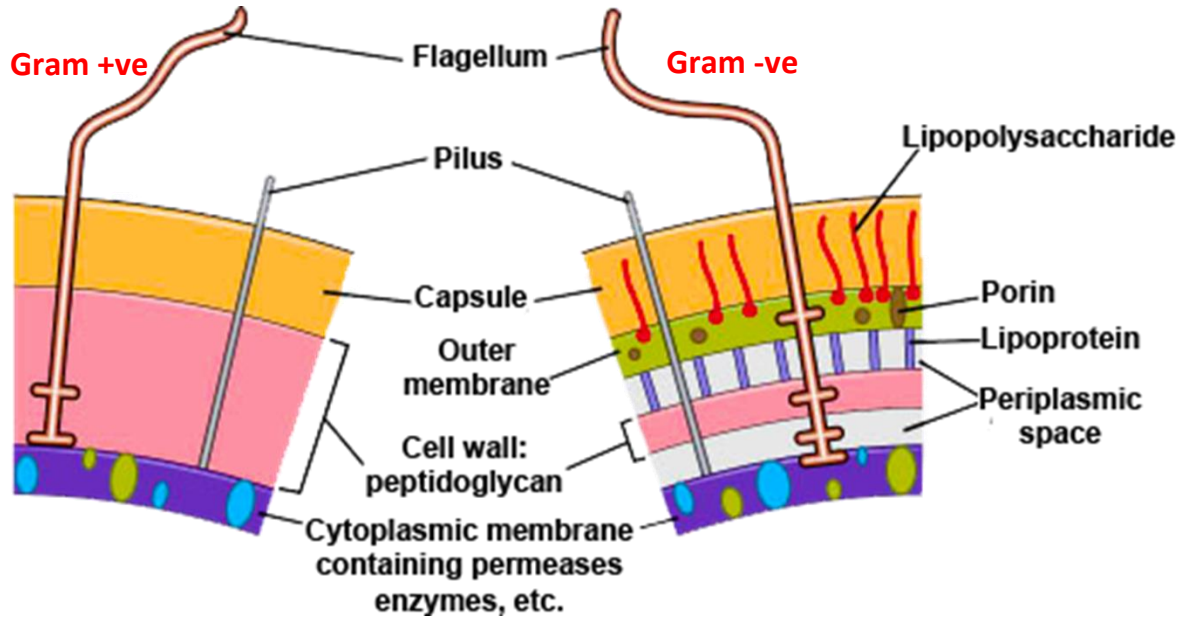
Mechanisms of Resistance



The major resistance mechanisms of microbes are decreased drug uptake, efflux pumps, enzymes that inactivate an antimicrobial chemical and target alterations by mutation. There also are biofilms.

1) Decreased Uptake

As stated above the more complex outer layer of gram negative bacteria makes it much more difficult for certain antimicrobials to penetrate.



Gram positive bacteria have a cell wall composed mostly of peptidoglycan, a very rigid substance. This is a prime target of β lactam antimicrobials such as penicillins and cephalosporins. The antimicrobial locks on to the β lactam structure in the cell wall, preventing expansion, and the cell ruptures as it grows.

Gram negative bacteria have a much thinner cell wall itself and this is protected by a lipopolysaccharide molecule in the capsule, an outer membrane and what is known as the periplasmic space. In short it is a much more heavily armoured vehicle.

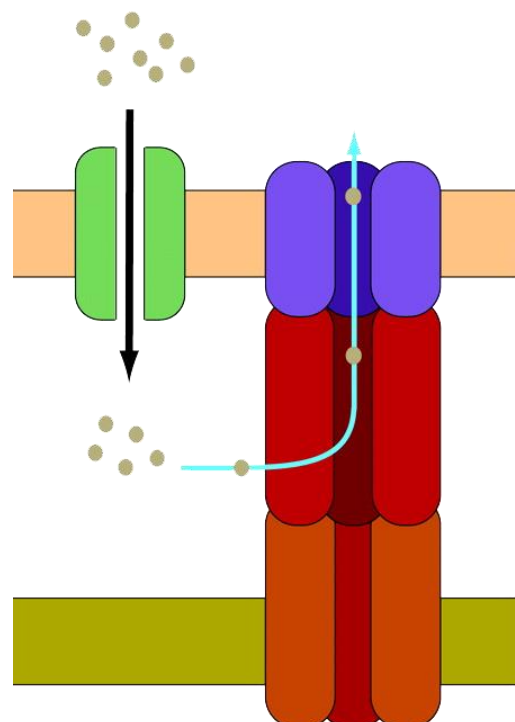
Porins are openings in the cytoplasmic membrane through which antimicrobial agents can gain entry a reduced number of such porins is one means of antimicrobial resistance.

2) Efflux Pumps

Some bacteria, e.g. *Pseudomonas*, have a system called an efflux pump. As its name suggests this is a system whereby the bacterium has a pump to expel ingested chemicals.

Although some of these drug efflux pumps transport specific substrates, many are transporters of multiple substrates.

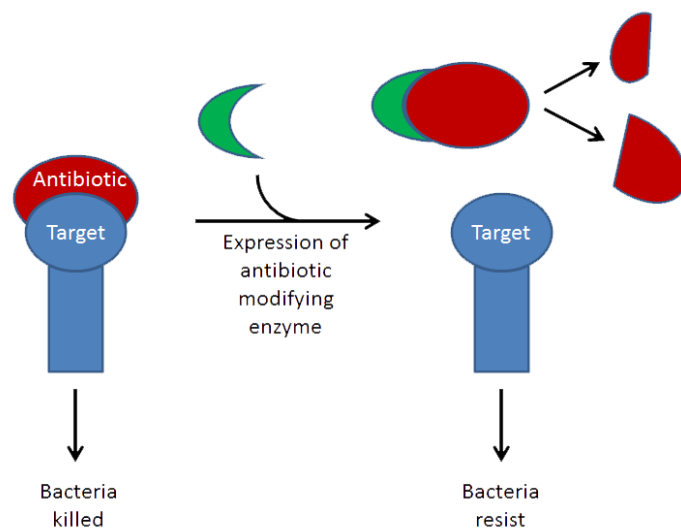
Antimicrobial efflux pumps are believed to contribute significantly to acquired bacterial resistance because of the very broad variety of substrates they recognize, their expression in important pathogens, and their cooperation with other mechanisms of resistance, such



as decreased uptake. Their presence also explains high-level intrinsic resistances found in specific organisms.

The design of specific, potent efflux pump inhibitors appears to be an important goal for the improved control of infectious diseases in the near future. For example, in ear therapy tris-EDTA has the potential to partially inactivate the efflux pump but this is only a topical specified action not generally available in most situations.

3) Enzyme inactivation



“The most notable example is penicillinase that can inactivate penicillin, but there are others”

Some microorganisms have developed the ability to produce enzymes that are able to inactivate certain antimicrobials. The most notable example is penicillinase that can inactivate penicillin, but there are others.

Clavulanic acid can bind penicillinase leaving the antimicrobial amoxicillin to do its work, and also there are the penicillinase resistant penicillins

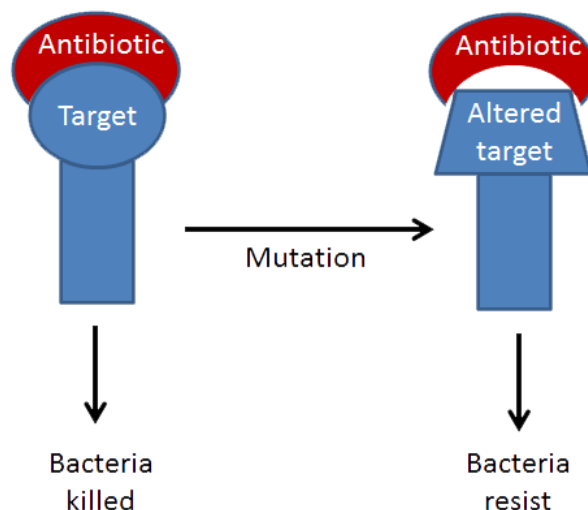
such as methicillin and cloxacillin, but they are still subject to target alterations (see below) making them ineffective over time.

4) Mutation

When an antimicrobial attacks a specific target, whether it be cell wall peptides, ribosomes or nuclear DNA, it locks on to specific receptors on the target.

Bacterial mutation results in the alteration of these receptors so that the antimicrobial can no longer fit and the organism is thus resistant to the effects of the antimicrobial.

Examples of clinical strains showing resistance can be found for every class of antimicrobial, regardless of the mechanism of action. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome and selection in the presence of the antimicrobial.



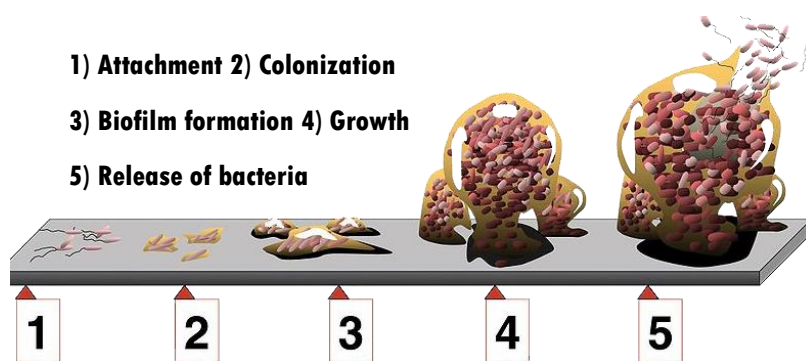
Thus antimicrobials resistant to penicillinase may still be rendered ineffective. This has led to the term methicillin resistant *Staphylococcus aureus* (MRSA) the archetypical multi-resistant organism.

5) Biofilms

Biofilms are complex microbial communities containing bacteria and fungi. The microorganisms synthesise and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface. At the most basic level a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins. The biofilm barrier protects the microorganisms from external threats.

Biofilms have long been known to form on surfaces of medical devices, such as urinary catheters, endotracheal and tympanostomy tubes, orthopaedic and breast implants, contact lenses, intrauterine devices (IUDs) and sutures. They are a major contributor to diseases that

are characterised by an underlying bacterial infection and chronic inflammation, e.g. periodontal disease, cystic fibrosis, chronic acne and osteomyelitis. Biofilms are also found in wounds and are suspected to delay healing in some. Planktonic bacteria attach within minutes and form strongly attached micro colonies within 2–4 hours. They become increasingly tolerant to biocides, e.g. antimicrobials, antiseptics and disinfectants, within 6–12 hours and evolve into fully mature biofilm colonies that are extremely resistant to biocides and shed planktonic bacteria within 2–4 days, depending on the species and growth conditions. They rapidly recover from mechanical disruption and reform mature biofilm within 24 hours.



A unique property of polymicrobial biofilms is the cooperative protective effects that different species of bacteria can provide to each other. For example, antimicrobial resistant bacteria may secrete protective enzymes or antimicrobial binding proteins that can protect neighbouring non-antimicrobial resistant bacteria in a biofilm, as well as transfer genes to other bacteria that confer antimicrobial resistance, even between different species. Horizontal gene exchange is enhanced in biofilms.

The high cell density in biofilms, as compared with that of a planktonic mode of growth, increases the absolute numbers of resistant mutants that can be selectable under antimicrobial pressure.

Another survival strategy that many bacteria in biofilms have developed is for a subpopulation to become metabolically quiescent, i.e. to hibernate. Because bacteria need to be metabolically active for antimicrobials to act, hibernating bacteria in biofilms are unaffected by antimicrobials that would normally kill active bacteria. Research has shown that the lowest concentration required to kill or eliminate bacterial biofilm for many antimicrobials actually exceeds the maximum prescription levels for the antimicrobials. Thus, standard oral doses of those antimicrobials, which effectively kill the normally susceptible bacteria when grown planktonically in a clinical laboratory, may have little or no antimicrobial effect on the same type of bacteria in biofilm form in the patient.

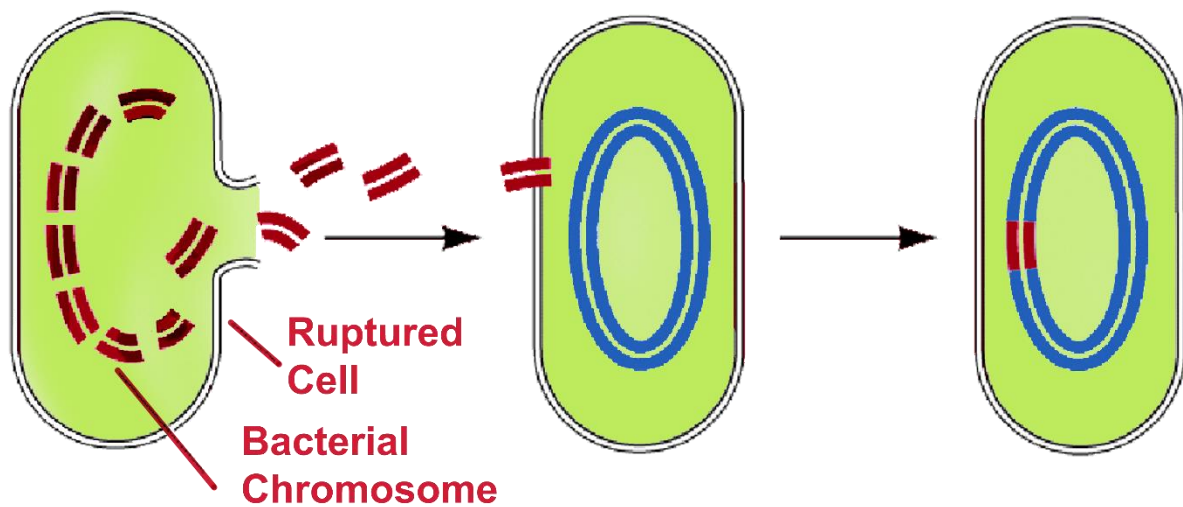
Horizontal Gene Transfer

As stated above HGT is genetic modification by microorganisms themselves and is a very efficient and rapid way of transferring resistance between populations. It is the most relevant mode of resistance emergence and spread in microbial populations.

The main methods are transformation, transduction and conjugation.

1) Transformation

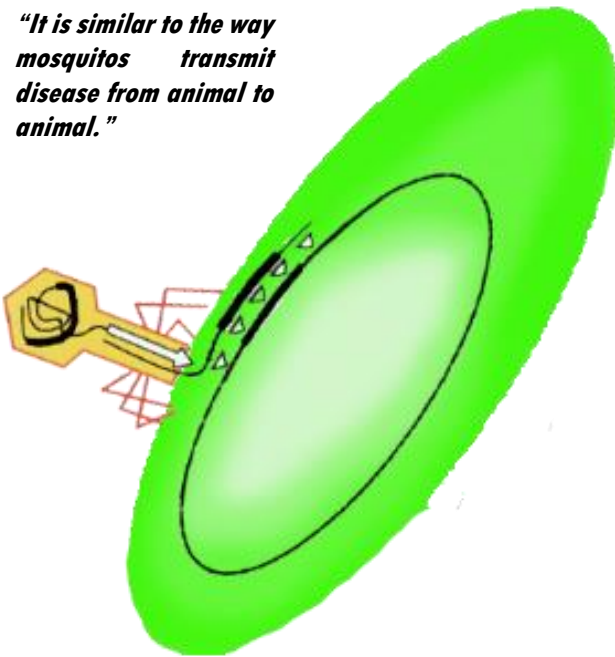
Transformation refers to the ability of microorganisms to utilise snippets of free DNA from their surroundings. DNA from dead cells gets cut into fragments and exits the cell.



The free-floating DNA can then be picked up by competent cells. Exogenous DNA is taken up into the recipient cell from its surroundings through the cell membrane. The exogenous DNA is incorporated into the host cell's chromosome via recombination. Transformation results in the genetic alteration of the recipient cell.

2) Transduction

"It is similar to the way mosquitos transmit disease from animal to animal."



Transduction is the process by which viruses that prey upon bacteria, known as bacteriophages, can transmit genetic material from one organism to another. It is similar to the way mosquitos transmit disease from animal to animal. However, while the mosquito is a passive carrier, bacteriophages are more complicated.

Being viruses themselves they inject their genetic material into a bacterial cell and replicate there to a great degree. Their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat.

The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome. At the same time, some phage genes are left behind in the bacterial chromosome.

When the cell eventually ruptures it emits many more bacteriophages into the surroundings to infect other microorganisms.

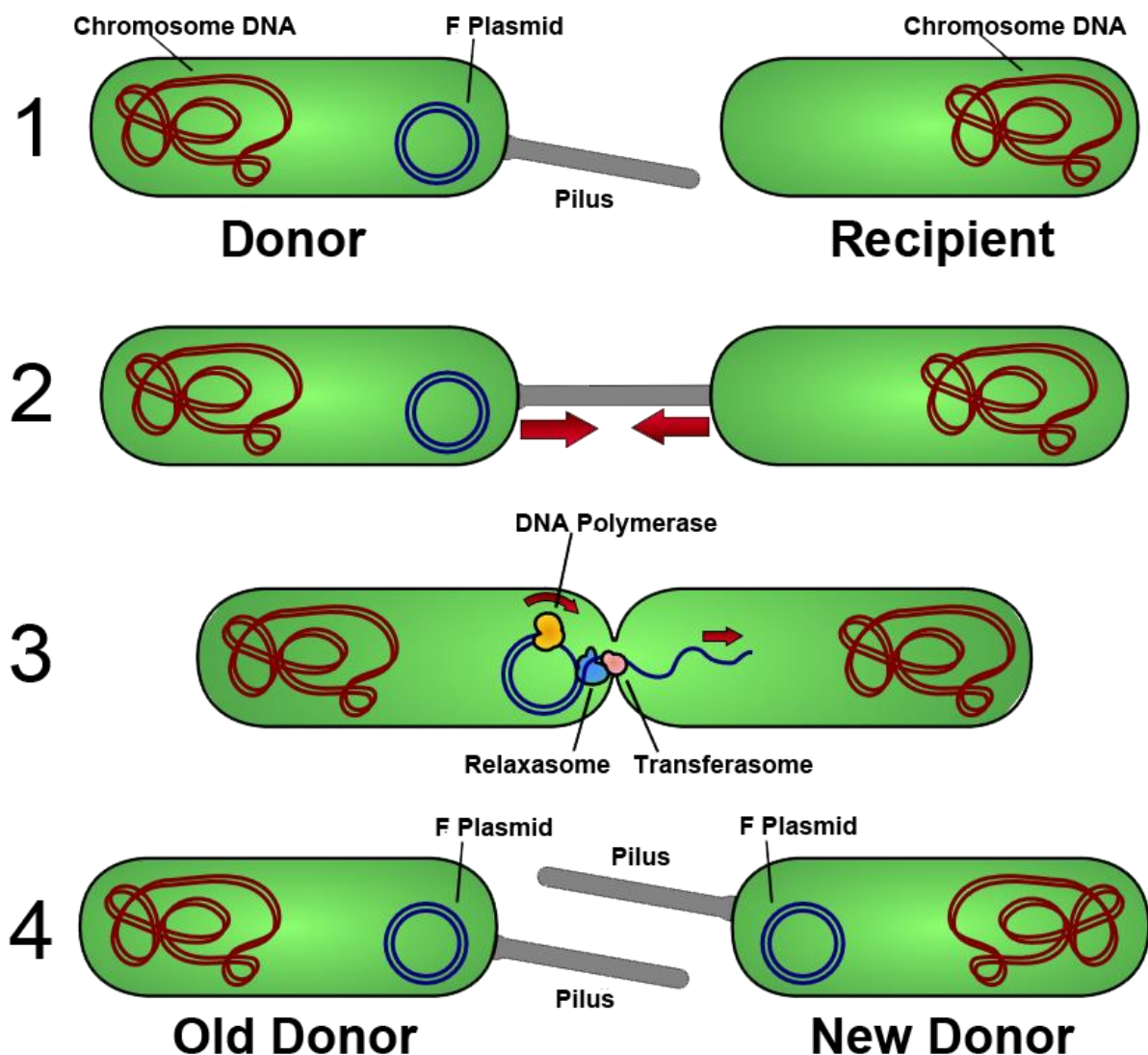
3) Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.

“Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating.”

Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. During conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon.

The fact that this process can occur easily between different species of bacteria makes it especially important. The process is as described in the graphic below:



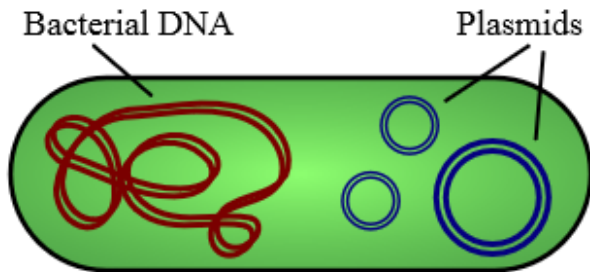
Conjugation diagram 1- Donor cell produces pilus. 2- Pilus attaches to recipient cell and brings the two cells together. 3- The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell. 4- Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor or F-factor.

Plasmids, Transposons and Integrons

These are the nuts and bolts of HGT, how it works.



1) Plasmids

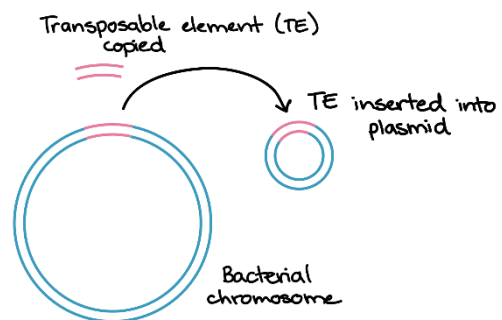


A plasmid is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance. While the chromosomes are big and contain all the essential genetic information for

living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions.

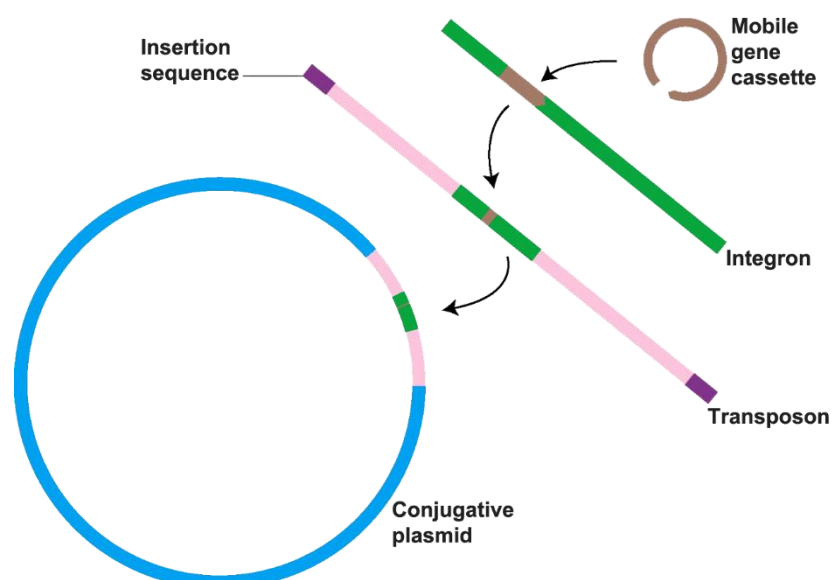
2) Transposons

Plasmids are the 'mothership' of HGT transferring genetic material. A transposable element (TE or transposon) is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genome size. Also known as jumping genes because of their mobility they are the shuttles that also can mobilize genetic material from bacterial chromosome to plasmid and vice versa.



3) Integrons

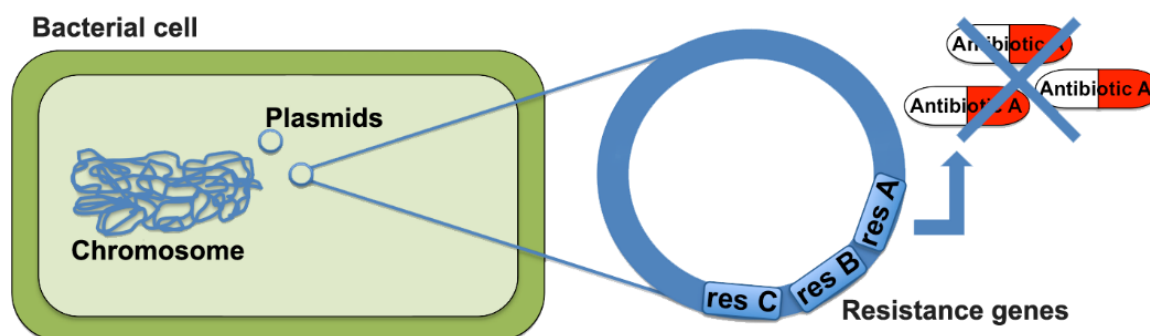
An integron is a genetic element that can catch and carry genes, particularly those responsible for AMR. On their own they are immobile and rely on transposons to carry them around. They are a basic unit and, if plasmids are the mothership and transposons are the shuttles, integrons can be regarded as the cargo boxes that are transferred around.



Integrons are interspecies transferrable meaning that resistance genes can be transferred from one bacterial species to an entirely different one. The graphic above summarizes the process, a mobile gene cassette incorporates itself into a transposon, which carries it to a plasmid that incorporates it into the genome. The plasmid may then be passed to another microbe where the integron, with resistance genes, may possibly be transferred by a transposon into the bacterial DNA.

Co-selection for Resistance

Co-selection for resistance is a vital concept at the heart of our understanding of AMR and comes from knowledge of HGT itself.

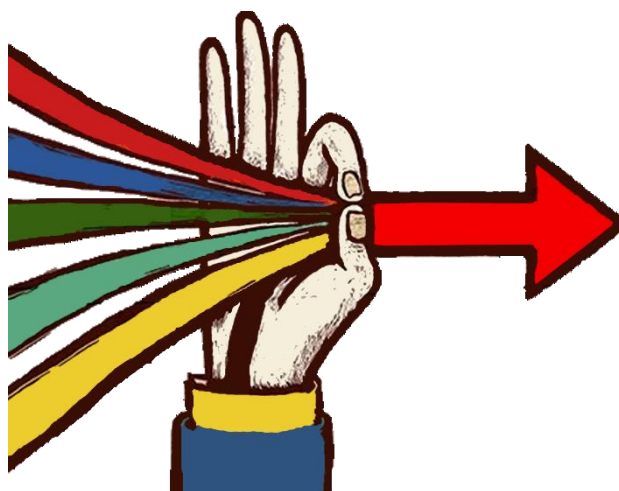


A crucial factor is the fact that integrons often carry the resistance genes for several anti-microbials at the same time. Thus overuse of a less crucial antimicrobial, such as tetracycline may result not only in selection for resistance to tetracyclines but also to other, possibly more critically important, antimicrobials.

This is highly relevant as it means that, while overuse of antimicrobials deemed critically important should always be avoided, it is total antimicrobial use that is the major factor.

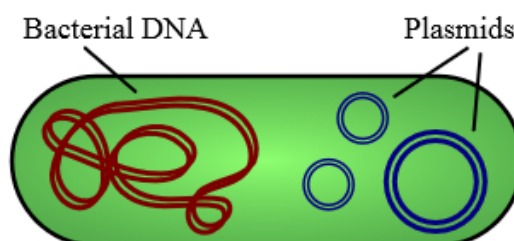
Horizontal Gene Transfer – The Gist

- 1) Horizontal gene transfer (HGT) is a major mechanism for the growth of antimicrobial resistance (AMR).
- 2) HGT is more rapid than simple mutation.
- 3) HGT is mediated by three mechanisms.
 - a. Transformation
 - b. Transduction
 - c. Conjugation
- 4) Conjugation is a major means of HGT
- 5) Integrons contain the basic genetic material and they are picked up by transposons which insert them into plasmids or chromosomes
- 6) Plasmids may be shared with different bacterial species meaning that commensals (non-target organisms) are also important in the spread of AMR.
- 7) Integrons (hence plasmids and transposons) generally carry resistance genes to more than one antimicrobial, resulting in co-selection for resistance to several antimicrobials at the one time.



While overuse of antimicrobials deemed critically important should always be avoided, it is total antimicrobial use that is the major factor.

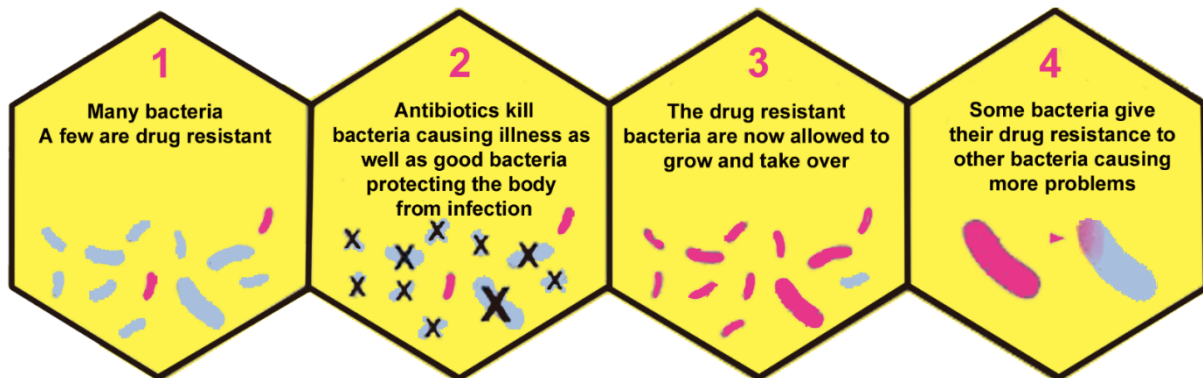
Plasmids may be shared with different bacterial species meaning that commensals (non-target organisms) are also important in the spread of AMR.



Selection for Resistance

This is reasonably straightforward. Susceptible organisms are eliminated leaving resistant ones to multiply and become the population. Organisms may be partially resistant and susceptible to higher levels of antimicrobials. Think of a tsunami, getting to higher ground will ensure safety but the higher the wave the higher the sanctuary has to be. This has led to the mutant selection window concept.

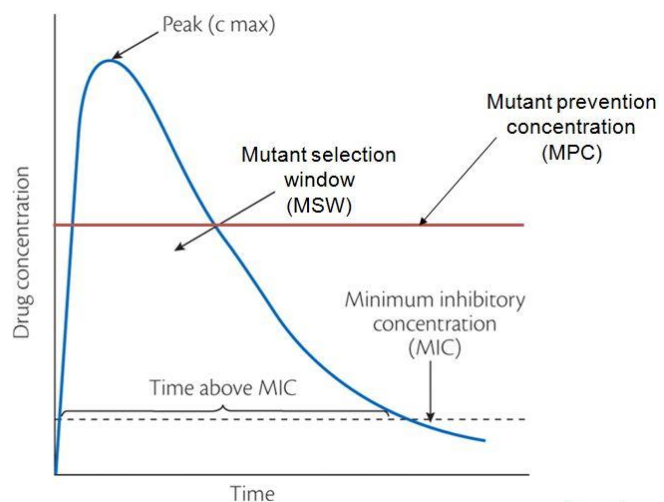
In some, rare, instances the gene encoding for resistance may put the organism at a genetic disadvantage hence resistance may reduce when the insult is removed.



Mutant Selection Window

This is the period on an antimicrobial decay curve where the greatest risk of selection for resistance takes place. We have the term minimum inhibitory concentration (MIC) being extremely relevant. This is the lowest level an antimicrobial will inhibit growth of microorganisms and varies between antimicrobial and also target microorganism, i.e. the MIC for enrofloxacin for E coli differs from that of penicillin, and also differs from the enrofloxacin MIC for Streptococcus spp. For an antimicrobial to select for resistant organisms it must first of all have an inhibitory action on susceptible organisms, if not there is no selection pressure. Therefore at or just above the MIC is the period when there is most selection pressure for resistance genes.

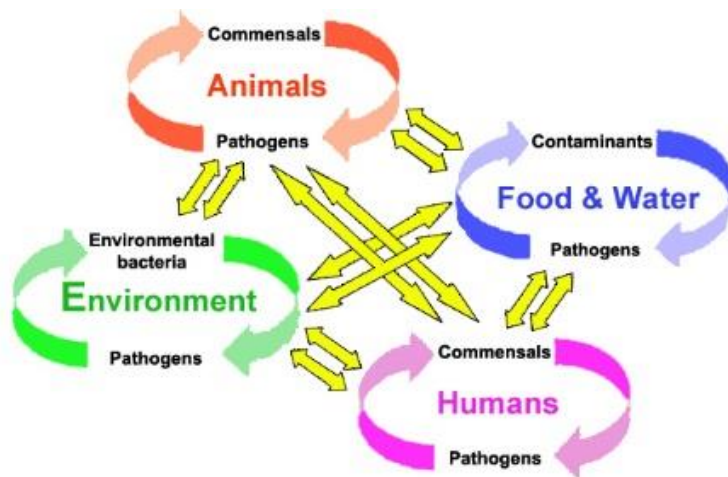
This brings in the term mutant prevention concentration (MPC), which is the level above which the antimicrobial inhibits the development of single step mutants. Above this concentration cell growth requires the existence of two or more resistance mutations. Since two concurrent mutations are expected to arise rarely few mutants will be amplified selectively when a susceptible population is exposed to drug concentrations above the MPC.



The area between the MIC and the MPC is the dangerous concentration range in which mutants are selected most frequently. Hence this area is designated as the mutant selection window (MSW).

While this hypothesis was originally postulated for concentration dependent antimicrobials (see below) MPCs have been established for many time dependent antimicrobials also.

Spread of Resistance



Spread of both resistant bacteria and the potential for horizontal gene transfer means that resistance can spread rapidly not only within a population but to a wider arena. The close interplay between humans, animals and their environment can be seen in this graphic.

This led to the formation of the One Health approach to antimicrobial resistance whereby humans, animals and the environment are all

taken into consideration when dealing with issues of antimicrobial resistance; concentrating on only one sector will not be very successful and control of all must be co-ordinated to be effective.

The interplay between humans and animals is well documented. The risk of resistance developing in food animals and resistant organisms being transferred to humans by other close animal contact or via the table is real enough but hygiene, either at slaughter or food preparation, and cooking can dramatically reduce this risk. There is also the very close contact between many people and their pets that can result in resistance transfer; this can be pet to human or the other way, human to pet.

The environment is vitally important; we are all part of it, humans and animals. In addition it is clear that the resistance risk is much higher where there are concentrated populations, such as in hospitals and intense animal rearing situations (poultry>pigs>cattle feedlots). Antimicrobial use is higher in monogastric species (poultry and pigs), compared to other food producing animals. Use is much lower in the extensive pasture based farming systems that predominate in New Zealand.

The high amount of air travel in the human population can disseminate resistant infections and also breeding stock importation can propose a great risk. The latter may be mitigated in the future by using semen transport instead of live animals.

Antimicrobial residues, resistance genes and microorganisms can spread for some distance via airborne particulate matter from large cattle feedlots and effluent from drug manufacturing has been found to contain extremely high concentrations of antimicrobial residues. As 70% of emerging zoonotic diseases originate in wildlife the presence in wildlife of resistance to critically important antimicrobials is a significant public health concern.

Antimicrobials differ in how efficiently they are processed in animals and how long residues remain bioavailable in the environment. The development of antimicrobials that rapidly biodegrade in the environment would be a positive step in minimising the spread of antimicrobial resistance. Although it is attractive to think that concentration dependent antimicrobials are more likely to exert selection pressure in soil or water, before they are diluted, in comparison to time dependent antimicrobials which require sustained high concentrations in order to have an effect on microbial viability, adsorbed antibiotics are not biologically available. For that reason fluoroquinolones, aminoglycosides and tetracyclines are more easily neutralized by soil than are β lactams and so actually may pose less risk.

Appropriate/Optimal Antimicrobial Use

Antimicrobial use and emergence of resistance are undoubtedly linked. However, to some extent, emergence of resistance can be avoided or at least diminished with appropriate antimicrobial regimens.

We hear a lot of the term 'appropriate antimicrobial use' and 'optimal antimicrobial use' (although 'suboptimal' is the word mostly used). What do these terms actually mean?

1) Bactericidal versus bacteriostatic

Broadly speaking, antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system. Bactericidal agents kill the microorganisms and therefore with or without a competent immune system of the host, the bacteria will be dead in most instances.

successful therapy depends on reducing the load to a level whereby the body itself can eliminate the infection.

So, as the vast majority of patients treated have a competent immune system, it is not really essential to kill absolutely all of the microorganisms; successful therapy depends on reducing the load to a level whereby the body itself can eliminate the infection.

2) Concentration versus time

The other major mechanism of classifying antimicrobial agents is whether they are concentration dependent or time dependent antimicrobials.

Concentration dependent antimicrobials target protein synthesis or nuclear material and cause rapid bacterial kill. The greater the antimicrobial concentration the more efficient the kill. Fluoroquinolones and aminoglycosides are typical concentration dependent antimicrobials and the limit to their concentrations is toxicity.

On the other hand some antimicrobials have targets such as the cell wall structure so rely upon on active growing microbes. These then need to be above MIC, preferably MPC, for a considerable period of time. These are known as time dependent antimicrobials and include bacteriostatic antimicrobials as well as β lactams.

3) Sub lethal dosing

Low concentrations of certain antibiotics, including fluoroquinolones and β lactams, have been reported to fuel mutagenesis and to increase the risk for emergence of resistance. It is speculated that antibiotic-resistant mutants might present a fitness gain in the presence of sublethal antibiotic concentrations.

Dose regimens avoiding subinhibitory concentrations should be ensured, particularly during the first part of the antimicrobial treatment.



This can justify a high loading dose in the case of those antimicrobials for which distribution into the infection site may be decreased by serum protein binding or because of the physicochemical characteristics of the compound.

4) Bacterial load and MPC

Bacterial load can be vital. With a low bacterial load there is a low probability of many resistance organisms being present. Dosing to above MIC will eliminate almost all the organisms and the body's defence mechanisms should be able to take care of the odd, if any, organism not killed by the antibacterial.

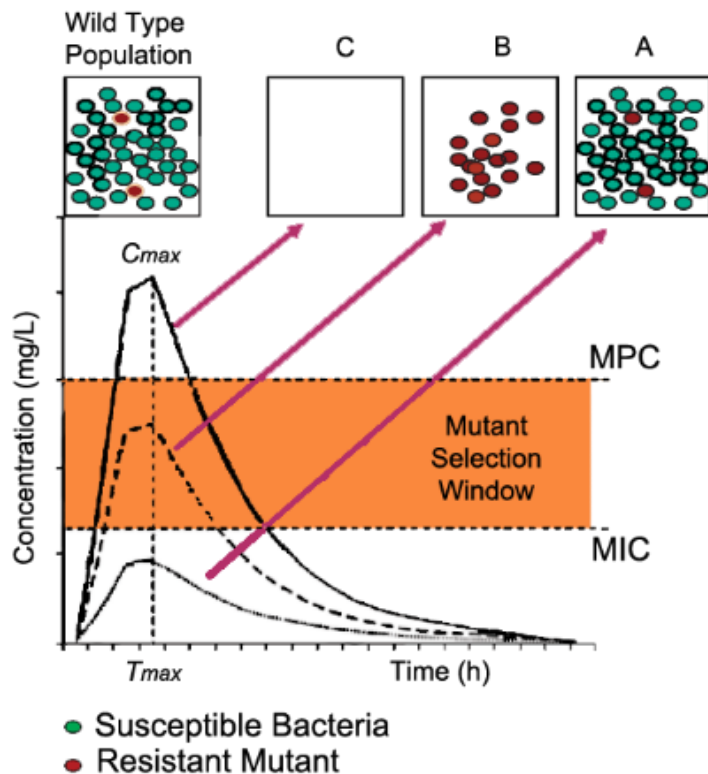
If there is a high bacterial load then the chances are greater that there are resistant organisms so dosing to MIC selects for their survival and persistence. (Group B in the accompanying graphic).

Therefore the higher the bacterial load the more important it is to be able to dose above the MPC.

If there is a high bacterial load then the chances are greater that there are resistant organisms

The limits to the clinical application of the MPC concept are imposed by a potential toxicity encountered at high antimicrobial doses.

Furthermore, horizontal gene transfer can be a significant confounding factor. As different microbial species have a different MPC level to each antibacterial then the antibacterial concentration may well be in the mutant selection window of non-target, or commensal bacteria.

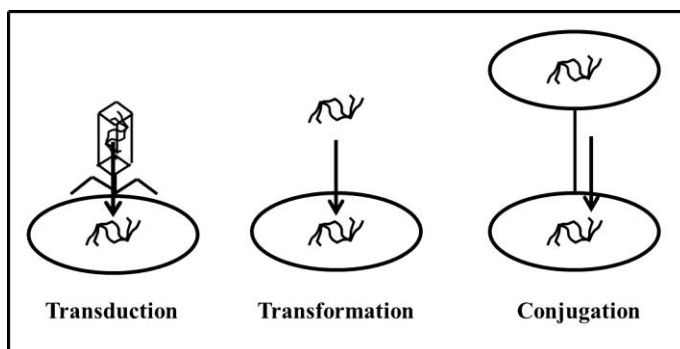


The three different situations where an antibiotic is administered. Curves represent the pharmacokinetics (concentration over time) of an antimicrobial agent and squared boxes represent the bacterial population.

(A) The pharmacokinetic curve is below the MIC; thus, no selection of a resistant mutant subpopulation within the wild-type population is expected.

(B) The pharmacokinetic curve is mainly within the MSW; therefore, the resistant mutant subpopulation within the wild-type population can be selectable.

(C) The pharmacokinetic curve surpasses the MPC; thus, the susceptible bacteria are inhibited and selection of a resistant mutant subpopulation is potentially avoided.



Therefore it is difficult to avoid the selection of resistance in commensal bacteria, which can subsequently transfer resistance traits to pathogens.

This underlines the importance of horizontal gene transfer and also the fact that, when dealing with nature, there are many grey areas.

5) Pk/pd

The MIC and the MPC are measures of the concentration of antimicrobial required at the biophase (site of infection). This is known as the pharmacodynamics. The other important parameter is the concentration of antimicrobial that can be attained at the biophase; this is the pharmacokinetic aspect. The pharmacokinetic/pharmacodynamic ratio (pk/pd) gives a measure of drug efficacy.

The kinetics often varies between different tissues, e.g. for an antimicrobial that is excreted unchanged and concentrates in urine, the dose for a urinary tract infection may be totally inappropriate for a lung infection.

This is generally the domain of the pharmaceutical industry and regulatory bodies for label recommendations but clinicians should be aware of the tissues they are treating and antimicrobial access to them.

6) Frequency and duration of dosing

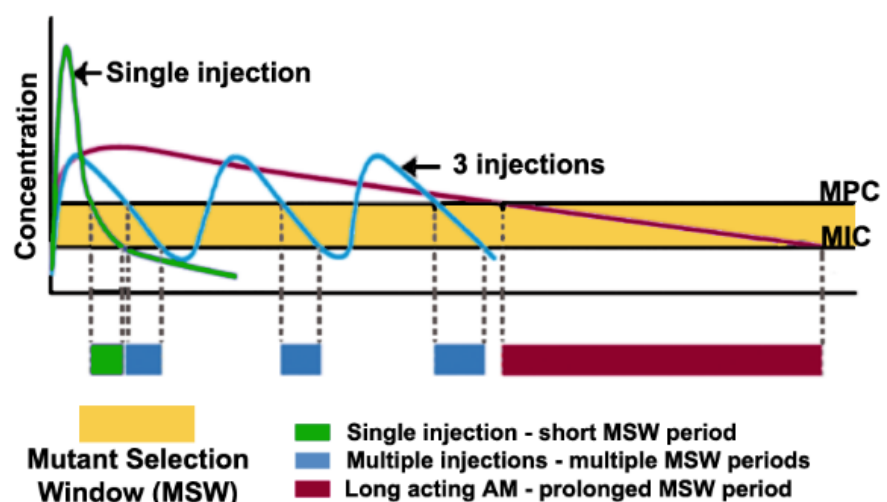
As stated above the amount of total antimicrobial administered is the relevant factor for concentration dependent antimicrobials so many are administered once per day. With time dependent antimicrobials needing to be above MIC or MPC for the time they are in the body then more continuous infusion is required.

Although penicillin/streptomycin combinations are no longer used they did, at the time follow this concept. The procaine penicillin would have a 24 hour duration and the streptomycin an 8 hour duration. The combination was given once per day; continuous infusion for penicillin and pulse dosing for streptomycin.

The important thing, from an antimicrobial resistance point of view is the duration of therapy. The basic recommendation has always been for as long as necessary. The buzz word phrase of the '90s was "Go to the end of the bottle, even if the disease disappears."

Now the emphasis has changed to as short as necessary to effect clinical cure. If the disease is under control, and there is no risk of relapse, then antimicrobial therapy should cease.

At some stage of the decay curve the concentration will be within the mutant selection window; the more doses the more time within the MSW increasing risk of resistance.



7) Long Acting Antimicrobials

It has become fashionable to utilize longer acting antimicrobial preparations for matters of convenience and owner compliance.

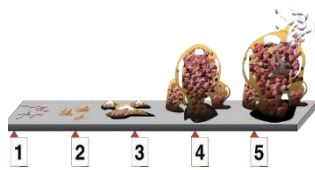
The longer acting product has a more drawn out decay curve and so spends a considerably longer period of time within the mutant selection window. Therefore, from an antimicrobial resistance point of view, choosing longer acting products is not a great option.

Conclusion

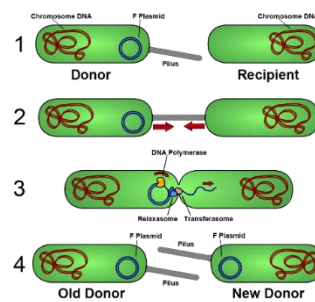
Understanding how antimicrobial resistance develops, the principles of horizontal gene transfer, selection for resistance and the interaction between humans, animals and the environment is crucial to developing means of minimising resistance to antimicrobial therapy.

The MPC hypothesis and an understanding of sublethal dosing and pk/pd may present means of more rational therapy resulting in optimal antimicrobial use, which encompasses successful therapy with the minimisation of antimicrobial resistance.

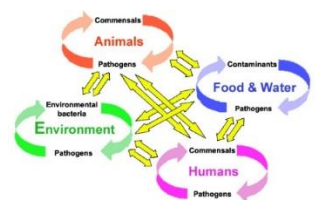
Graphic Acknowledgments



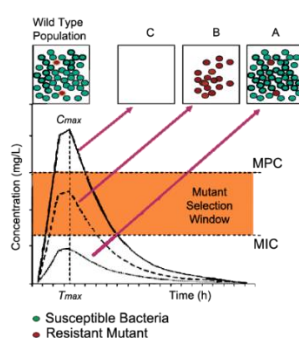
Biofilms: adapted from "Looking for Chinks in the Armor of Bacterial Biofilms" Monroe D PLoS Biology Vol. 5, No. 11, doi:10.1371/journal.pbio.0050307 via Wikimedia commons.



Conjugation: adapted from graphic created by Adenosine (Own work) [CC BY-SA 3.0 (<http://creativecommons.org/licenses/by-sa/3.0>)], via Wikimedia Commons)



Spread of resistance: adapted from Boerlin and White. Antimicrobial Resistance and its Epidemiology. Antimicrobial Therapy 4th Edition (Giguère *et al*) Blackwell Publishing



Mutant selection window: adapted from Cantón, Horcajada, Oliver, Garbajosa and Vila. Inappropriate use of antibiotics in hospitals: The complex relationship between antibiotic use and antimicrobial resistance. *Enferm Infecc Microbiol Clin*. 2013; 31 (Supl 4):3-11

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