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PHARMACOLOGY
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Antimicrobial resistance
List and explain 4 mechanisms of antimicrobial resistance
- **Limiting uptake of drugs(Drug uptake)**
- **Modification of drug target**
- **Enzyme inactivation**
- **Mutations**
- **Biofilms**

◆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.

◆AcquiredResistance

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.

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 antimicrobial resistance

Bacteria develop antimicrobial resistance by several mechanisms. Because of differences in structure, there is variation in the types of mechanisms used by Gram positive bacteria.

Gram negative bacteria make use of all four main mechanisms, whereas Gram positive bacteria less commonly use limiting the uptake of a drug h(don't have an LPS outer membrane), and don't have the capacity for certain types of drug efflux mechanisms.

•Limiting uptake of drugs(Drug uptake)

There is a natural difference in the ability of bacteria to limit the uptake of antimicrobial agents. The structure and function of the LPS layer in G-bacteria provides a barrier to certain types of molecules.

Certain bacteria modify their cell membranes prom channels;thereby preventing the antimicrobials from entering into the cell.

There are two main ways in which the porin channels can limit drug uptake: a decrease in the number of porins present and mutations that change the selectivity of the porin channel.

This strategy has been observed in many Gram bacteria such as pseudomonas, Enterobacter and klesbsiella species against drugs such as imipenem,ahminoglycosides and quinolones.

Gram positive bacteria,Staphylococcus aureus, recently has developed resistance to vancomycin. Of the two mechanisms that S.aureus uses against vancomycin.

S.aureus produced a thickened cell wall which makes it difficult for the drug to enter the cell and provides an intermediate resistance to vancomycin. These strains are designated as VISA strains.

•Modification of a drug target

There are multiple components in the bacterial cell that may be targets of antimicrobial agents and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs.

Alteration of the cell wall or cytoplasmic membranes

One of the mechanism of resistance to beta-lactate drugs used almost exclusively by G+ bacteria is via alterations in the structure and/or number of PBPs(penicillin-binding proteins)

PBPs are transpeptidases involved in the construction of peptidoglycan in the cell wall

A change in the number (increase in PBPs that have a decrease in drug binding ability, or decrease in PBPs with normal drug binding) of PBPs impacts the amount of drug they can bind to that target.

A change in the structure of S.aureus by acquisition of mecA gene) May decrease the ability of the drug to bind, or totally inhibit drug binding.

The glycopeptides(eg vancomycin) also works by inhibiting cell wall synthesis and lipopeptides(e.g daptomycin) work by depolarizing the cell membrane. Resistance to vancomycin has become a major issue in the enterococci(VRE- Vancomycin resistant enterococci) and in Staphylococcus aureus (MRSA- Methicillin-resistant Staphylococcus aureus).

Resistance is mediated through acquisition of van genes which result in changes in the structure of peptidoglycan precursors that cause a decrease in the binding ability of vancomycin. Daptomycin requires the presence of calcium for binding. Mutations in genes (e.g MprF) change the charge of the cell membrane surface to positive, inhibiting the binding of calcium, and therefore daptomycin .

Resistance to drugs that target the Ribosomal subunits May occur via Ribosomal mutation(aminoglycosides), ribosomal subunits methylation most commonly involved erm genes or ribosomal protection (tetracyclines)

These mechanisms interfere with the ability of the drug to bind to the ribosome.

Selective toxicity(antibiotics), only affect the bacterium not human, due to affect the specific genes

that only bacteria.

For drugs that target Nucleic acid synthesis(fluoroquinolones), resistance is via modifications in DNA gyrase (G-bacteria e.g gyrA) or topoisomerase IV(G+ bacteria e.g grlA).

These mutations cause changes in the structure of gyrase and topoisomerase which decrease or eliminate the ability of the drug to bind to these components.

•Inactivation of a drug

There are two main ways in which bacteria inactivate drugs by

•Actual degradation of the drug or by

•Transfer of a chemical group to the drug

Actual degradation of the drug

The Beta-lactamases are a very large group of drugs hydrolyzing enzymes. Another drug that can be inactivated by hydrolization is tetracycline, via the tetX gene.

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 making them ineffective over time.

•Actual efflux of drug

Bacteria possesses chromosomally encoded genes for efflux pumps. Some are expressed constitutively, and other are induced or over-expressed (high level resistance is usually via a mutation that modifies the transport channel) under certain environmental stimuli or when a suitable substrate is present. The efflux pumps function primarily to rid the bacterial cell of toxic substances, and many of these pumps will transport a large variety of compounds.

Most bacteria possess many different types of efflux pumps. There are five main families of efflux pumps in bacteria classified based on structure and energy source:

•The ATP-binding cassette (ABC) family

•The multidrug and toxic compound extrusion

•The small multidrug resistance(SMR) family

•The major facilitator superfamily(MFS)

•The resistance-nodulation cell division(RND)family

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.

•Mutations

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.

•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, orthopaedic and implants, contact intrauterine devices (IUDs) and sutures.

1) Attachment
2) Colonization
3) Biofilm formation
4) Growth
5) Release of bacteria

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.