Antibiotics: Mode of action and mechanisms of resistance

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Examining the mechanisms of action of the various antibiotics illustrate how they are effective against pathogenic microorganisms, as they act selectively on vital microbial functions with minimal effects on host functions. Understanding the mechanism of action and the chemical nature of the antimicrobial agents are crucial in the understanding of how resistance develops. Furthermore, understanding the mechanisms of resistance by which bacteria successfully defend themselves against antibiotic assaults could facilitate the development of means to potentiate the efficiency and increase the lifespan of antibiotics. Both the mode of actions and resistances of commonly used antimicrobials will be examined.

Keywords: mode of action; mechanism of resistance; acquired resistance, antibiotics.

Mechanisms of action and resistance of antibiotics

The mechanism of action of antimicrobial agents can be categorised based on the function that is affected by the agents, these generally included the following: inhibition of the cell wall synthesis, or nucleic acid synthesis, inhibition of ribosome function, or cell membrane function and inhibition of folate metabolism. Antimicrobials are one of the most successful forms of therapy in medicine, however the efficiency of antimicrobials is compromised by a growing number of antibiotic resistant pathogens. Resistance can be described in two ways:
A). **Intrinsic resistance** whereby microorganisms naturally do not posses target sites for the antimicrobials and the antimicrobial does not affect them.
B). **Acquired resistance** whereby a naturally susceptible microorganism acquires mechanism to not be affected by the antimicrobial. Mechanisms of acquired resistance include: the presence of an enzyme that inactivates the antimicrobial agent, post- transcriptional or post-translation modification of the antimicrobial agent's target, reduced uptake to the antimicrobial agent and active efflux of the antimicrobial agent. Both the mechanisms of actions and resistances of the mostly commonly used antimicrobial classes will be detailed in Table 1.

Mechanism of action of beta-lactam antibiotics

The beta (β)-lactam antibiotics constitute one of the oldest classes of antibacterial agents. β-lactam antibiotics are a broad class of antibiotics (Figure 1). They consist of all antibiotic agents that contain a β-lactam ring in their molecular structure. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams and carbapenems (1). They act as an irreversible inhibitor of the enzyme transpeptidase, an enzyme used by bacteria to make their cell walls. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidase knows as penicillin binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides, the peptidoglycan precursors to crosslink the peptidoglycan. β-lactam antibiotics mimic the site and competitively inhibit PBP crosslinking of peptidoglycan (2).

Table 1: Mechanism of action and resistance of commonly used antimicrobial agents.

Antimicrobial Family	Mechanism of Action	Resistance Mechanism
Beta-lactam antibiotics	Inhibits cell wall production. Binds enzymes (PBPs) that help form peptidoglycans.	Beta-lactamase production primarily- *bla* genes. Changes cell wall protein enzymes to prevent binding to PBPs.
Cephalosporins		Cephalosporinases
Beta-lactamase inhibitors	Inhibits/binds to beta-lactamase enzymes.	Extended-spectrum beta lactamases (ESBLs). Class A-D.
Aminoglycosides	rRNA- binds to 30S subunit, causing genetic code misread. Inhibits protein production. Effect on cell membrane permeability	Phosphorylation, adenylation and acetylation of aminoglycoside stops them binding.
Fluoroquinolone	Interrupts DNA breakage-reunion step by binding DNA-gyrase or topoisomerase II and topoisomerase IV.	Target modification of DNA gyrase (*gyrA* and *gyrB*). Decreased permeability- outer membrane porins mutations (*ompF*). Efflux pumps.
Folate pathway inhibitors	Purine synthesis for DNA. Interferes folic synthesis.	Chromosomal mutations but more commonly plasmid and integron-mediated resistance. Pathway blocked by resistant dihydrofolate reductase (*dfr* gene).
Tetracycline	rRNA- binds to 30S subunit and interferes with amino acid transfer. Prevents protein production	Inducible efflux *E. coli* etc. (*tetA, tetB, tetC*). Binding site changes (*tetO, tetM* genes)

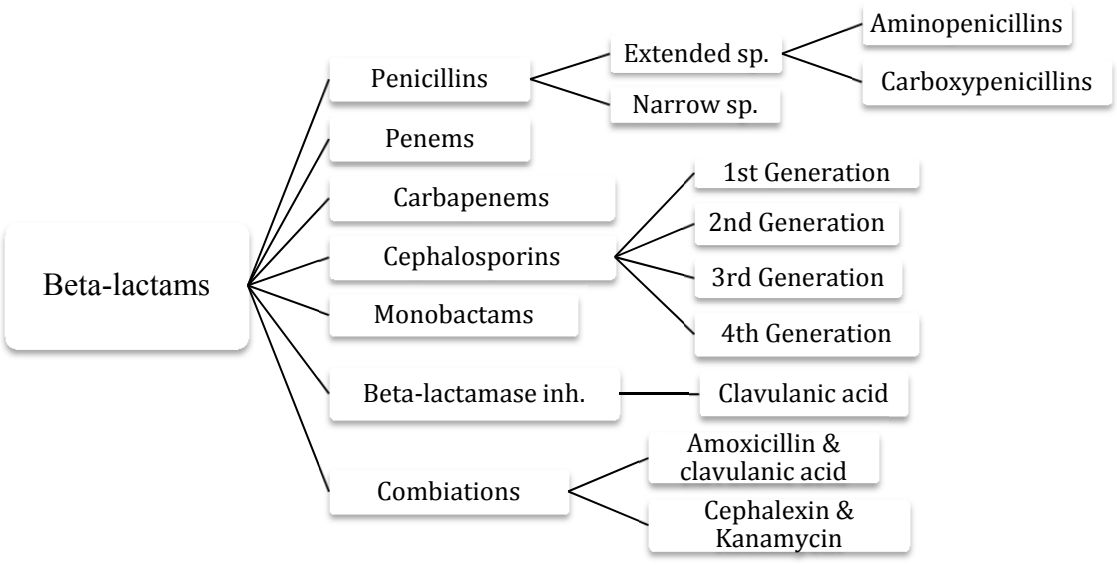

Figure 1: Beta-lactam family of antibiotics.

Mechanism of action of aminopenicillins

The aminopenicillins are a group of antibiotics in the penicillin family. Like other penicillin's this group is characterised by its four-membrane, nitrogen-containing β-lactam ring at the core of its structure, which is crucial to antibacterial activity of this group of antibiotics.

Ampicillin and amoxicillin & clavulanic acid are examples of two aminopenicillins. Amoxicillin is sometimes combined with clavulanic acid, a β-lactamase inhibitor. This combination increases the spectrum of action against microorganisms, and aids in overcoming bacterial antibiotic resistance mediated through β-lactamase production. Clavulanic acid is a suicide inhibitor of bacterial β-lactamase enzyme from *Streptomyces clavuligerus* (3). When administered alone, it only has weak antibacterial activity against most organisms, but when given in combination with β-lactam antibiotics prevents antimicrobial inactivation by microbial lactamase. It does this by binding and irreversibly inhibiting the β-lactamase, this results in a restoration of the antimicrobial activity of β-lactam antibiotics against lactamase-secreting-resistant bacteria. Moreover by inactivating β-lactamase, the accompanying penicillin may be made more potent (4).

Mechanism of action of cephalosporins

Cephalosporins are the second major group of β-lactams and are broad-spectrum. Penicillinase resistance antibiotics derived from an *Acremonium chrysogenum* strain was isolated by Brotzu in 1948 (5). As the cephalosporins are β-lactam antibiotics, they are closely related both structurally and functionally to the aminopenicillins. The mechanism of action, mechanism of resistance and some other properties of cephalosporins are identical to the aminopenicillins. The cephalosporin nucleus can be modified to gain different antimicrobial properties. The semi-synthetic cephalosporins are commonly grouped into four generations based on their antimicrobial activity (6). Each newer generation has significantly greater Gram-negative antimicrobial properties that the preceding generation, in most cases with decreasing activity against Gram-positive organisms. The fourth-generation of cephalosporins, however, has true broad-spectrum activity.

First-Generation cephalosporins

The first-generation cephalosporins have simple 7β-acylamino side chains. The 3' substituents of the early congeners were derived from the parent 7-aminocephalosporanic acid (7-ACA) in cephalothin, or by simple chemical modification. Cephalexin has activity similar to cephalothin, but somewhat less potent. The combination of cephalexin & kanamycin has the benefit of increasing the coverage of either agent alone against common pathogens and has illustrated good field efficacy against major mastitis pathogens, mainly *Streptococcus uberis, Staphylococcus aureus* and *E. coli* (7). Furthermore there are reports of synergy between these two agents against mastitis-targeted pathogens both by time-kill kinetics and reduction in minimum inhibitory concentration (MIC) of the individual agents in combination relative to each agent alone (8). First-generation cephalosporins typically have good activity against Gram-positive bacteria including Staphylococci, including penicillinase-producing *Staphylococci aureus* and *Staphylococci epidermidis*. Activity is relatively modest against Gram-negative bacteria, but the main organisms that are affected include *E. coli*, *Klebsiella pneumonia, Proteus mirabilis* and *Shigella* species, that do not produce β-lactamases or only produce penicillinases (9).

Second-Generation cephalosporins

In the second-generation cephalosporins semi-synthetic side-chains that were found to be effective were used through the 1970s. The nucleus of cephalosporin offered a distinct advantage over that of penicillin because the 3' substitutes can be varied to modulate the antimicrobial activity and pharmacokinetic properties. This resulted in numerous variants with improved activity against Gram-negative pathogens being synthesized. The typical microbial spectrum of this group includes organisms that are susceptible to first-generation cephalosporins. Moreover second-generation cephalosporins have lower activity against Gram-positive organisms when compared to the first-generation cephalosporins. Expect for penicillinase-producing organisms where the improved β-lactamase stability of the second-generation cephalosporins may give better efficacy (10).

Third-Generation cephalosporins

Third-generation cephalosporins have good efficacy and tolerability. The members of this class are less effective against Gram-positive organisms, compared to first-generation cephalosporins. The third-generation antibiotics are less active against Gram-positive cocci, but there is considerable variability in the activity against Staphylococci and

Streptococci among this group. Cefotaxime has the highest activity against Streptococci, but others have less activity. Moreover third-generation cephalosporins have greater *in vitro* activity against Gram-negative organisms, especially those with β-lactamases when compared with earlier generations. The typical microbial targets of cefpodoxime and ceftiofur include *E. coli, Klebsiella, Acineobacter, Serratia, Enterobacter, Proteus, Providencia, Morganella* and *Neisseria*. Only ceftazidime and cefoperazone have good activity against *Pseudomonas aeruginosa* with ceftazidime having the greatest activity. It is for this reason that ceftazidime has been an important antibiotic for some infections in small animals.

Fourth-Generation cephalosporins

Fourth-generation cephalosporins have increased stability towards hydrolysis by β-lactamases, resulting in less induction of β-lactamase-medicated resistance. This allows the fourth-generation cephalosporins to possess extended Gram-negative coverage when compared to third-generation cephalosporins.

Mechanism of beta-lactam resistance

There are three independent factors which determine the bacterial susceptibility to β-lactam antibiotics: 1) Production of β-lactamase, 2) decreased penetration through the outer cell-membrane to access the cell wall enzymes, 3) the resistance of the target penicillin binding protein (PBP) to binding by β-lactam agents (11). The major mechanism of resistance to β-lactam antimicrobial agents in Gram-negative bacilli is production of β-lactamase hydrolytic enzymes that disrupt the amide bond of the characteristic four-membered β-lactam ring, rendering the antimicrobial ineffective (12; 13). Interestingly the β-lactamases are structurally related to PBP's and it is thought they may have evolved from these β-lactam-binding enzymes of cell wall biosynthesis. These enzymes have been described numerous times in both Gram-negative and Gram-positive organisms and in the Mycobacteria (14; 15). They are variable chromosomally or plasmid encoded and often associated with mobile genetic elements such as transposons and integrons (16). Different bacteria produce β-lactamase that possesses a range of physical, chemical and functional properties (17), some β-lactamases are specific for penicillins (penicillinases), some are specific for cephalosporins (cephalosporinases), and others have affinity for both groups. β-lactamase enzymes have been categorised according to molecular structure and substrate, bacterial type (Gram-negative and Gram-positive), transmission (plasmid coded versus chromosomal coded), and whether they are inducible or constitutive. The Ambler Class classification uses four molecular classes of β-lactamases A-D (Table 2) and includes both metal-dependant (Class B) and metal-independent (Class A, C and D) enzymes (18).

Table 2: β-lactamases molecular Class Classification.

Class	Active site	Examples
A	Inhibitor –susceptible (rare exceptions)	TEM-1, SHV-1, KPC's, OXY, and most ESBL's (including CTX-M)
B	Metallo- β-lactamases	Metalloenzymes: VIM, IMP, SPM, NDM
C	Inhibitor- resistant β- lactamases	AmpC
D	Oxacillin -active β- lactamases that may be inhibitor susceptible	OXA (including rare ESBL phenotypes)

TEM is an abbreviation for Temoneira, named after a Greek patient; SHV-1 -for sulfhydryl variable; KPC's *Klebsiella. pneumoniae* carbapenemases; VIM-1 for "Verona integron-encoded metallo-β-lactamase"; IMP-1 for "active on imipenem", is located on a conjugative plasmid in the *P. aeruginosa* clinical isolate; SPM Metallo enzyme isolated in Sao Paulo; NDM for New Delhi metallo-beta-lactamase (Source: 19; 13)

The β-lactamases enzymes may be named after the substrates that they hydrolyse, the biochemical properties of the β-lactamases, strains of bacteria from which the β-lactamase was detected, a patient or country from which a β-lactamase–producing strain was isolated. For example, TEM is an abbreviation for Temoneira, the first patient from Greece, from whom a TEM β-lactamase–producing strain was reported (19).

Many kinetic, mutagenesis and structural studies have been performed on these enzymes, providing key details of their catalytic mechanisms and substrate specificities. Of particular concern are the enzymes that are capable of targeting the expanded spectrum β-lactams, including the AmpC (Class cephalosporinases) enzyme (20) the extended spectrum β-lactamases (ESBL) (Class A and D) (21) and the carbapenemases that hydrolyse most β-lactams, including the Carbapenems (Class A, B, and D) (22; 23).

Extended spectrum beta lactamases (ESBL)

As there is no precise definition of ESBLs, a commonly used definition is that the ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins and aztreonam (but not the cephamycins or carbapenems). This resistance is conferred by hydrolysis of these antibiotics, β-lactamase inhibitors such as clavulanic acid inhibit the resistance mechanism. This differentiates the ESBLs from the AmpC-type β-lactamases produced by organisms such as *Enterobacter cloacae,* which have third-generation cephalosporins as there substrates but which are not inhibited by clavulanic acid. β-lactamases may be classified as molecular Class A, B, C, or D enzymes, based on amino acid sequences (24).

Mechanism of action aminoglycosides

Aminoglycosides are important treatments against Gram-negative infections. They are particularly active against aerobic, Gram-negative bacteria and act synergistically against certain Gram-positive organisms (25). Aminoglycosides are a therapeutically essential class of antibiotics whose usefulness is often restricted by their toxic potential and residues in food animals. The aminoglycoside antimicrobial compounds are produced from strains of *Streptomyces* spp., *Micromonospora* spp., and *Bacillus* spp.

Neomycin, streptomycin and kanamycin are examples of aminoglycosides. Neomycin is composed of an isomeric mixture of neomycin A and B. Paromomycin and framycetin are other members of the group. Aminoglycosides are aminocyclitols that kill bacteria by inhibiting protein synthesis as they bind to the 16S rRNA and by disrupting the integrity of bacterial cell membrane (26).

Mechanism of aminoglycoside resistance

The primary mechanism for resistance to aminoglycosides of *E. coli* is enzymatic modification involving three families of enzymes: acetyltransferases (AAC), nucleotidyl (adenyl) transferase (ANT) and phosphotransferases (APH) (27; 28). Cross-resistance between aminoglycosides is complex and depends on the gene(s) present (28). Neomycin resistance is conferred by phosphotransferases (APH 3') and acetyltransferase (AAC 2' and 3') (27). Cross-resistance caused by enzymatic modification between streptomycin and other aminoglycosides does not occur except between streptomycin and the aminocyclitol spectinomycin in isolates producing ANT (3')-I (5) (27). Kanamycin cross-resistance is incomplete. Resistance kanamycin is mediated by phosphotransferases (APH 2'' and APH 3'), acetyltransferase (AAC 3' and AAC 6'), and nucleotidyltransferases (ANT 2" and ANT 4') (29; 30). Depending on other gene(s) present cross-resistance with other aminoglycosides occurs. Cross-resistance to kanamycin and fluoroquinolones is encoded on a gene encoding aminoglycoside acetyltransferase *aac (6')-1b-cr* (31).

Mechanism of action of sulphonamides and potentiated sulphonamides

Sulphonamides are one of the oldest groups of antimicrobial compounds still in use today. Sulphonamides have been in clinical use for fifty years and resistance is common, the addition of trimethoprim (trimethoprim-sulphonamide) or ormetoprim (ormetoprim-sulfadimethoxine) is used to broaden the spectrum and increase antibacterial activity against bacteria that would have been resistant to either drug used alone. Trimethoprim and ormetoprim are chemically called diaminopyrimidines.

As sulphonamides are structurally similar to para-aminobenzoic acid (PABA), sulphonamide action is dependent on the chemical similarity with PABA. Sulphonamides act as a false substrate and compete with PABA for the enzyme dihydrofolate synthase and block the synthesis of dihydrofolic acid (DHFA), and in turn trimethoprim inhibits the synthesis of tetrahydrofolic acid (THFA) and folate cofactor is inhibited (Figure 2). The folate cofactor acts as a 1-carbon donor for the synthesis of nucleic acid (DNA). The result of blocking the biosynthesis of folate coenzyme in bacteria is that the growth and division are stopped. Since mammalian cells use preformed folates from the diet and bacteria cannot use preformed folates and must synthesise their own folic acid, the sulphonamides demonstrate selective bacteriostatic toxicity (32).

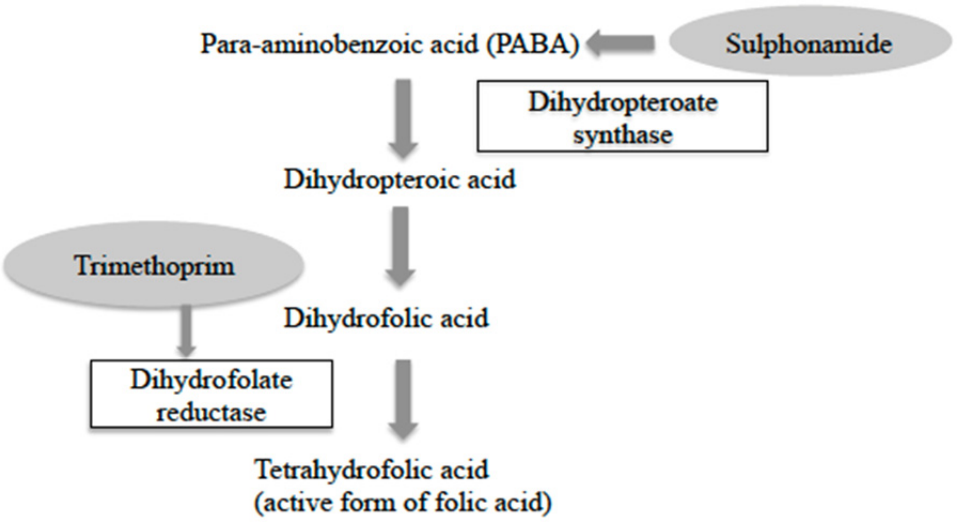

Figure 2: Simplified pathway of the sulphonamide and trimethoprim combinations.
Sulphonamides compete with para-aminobenzoic acid for the enzyme dihydropteroate synthase; this inhibits synthesis of dihydropteroic acid, a precursor of dihydrofolic acid and tetrahydrofolic acid. Trimethoprim inhibits the enzyme dihydrofolate reductase, an enzyme critical to the synthesis of tetrahydrofolic acid.

Sulfamethoxazole-trimethoprim combination is active against Gram-negative and Gram-positive organisms including *E. coli*, Streptococci and Staphylococci. The antibacterial spectrum does not include *Pseudomonas* or *Mycobacterium* spp.

Mechanisms of sulphonamides and potentiated sulphonamides resistance

Trimethoprim resistance develops rapidly in bacteria, however resistance to sulphonamides and trimethoprim in combinations occur far slower. Resistance has become widespread due to the extensive use of sulphonamides over many years. Resistance occurs via chromosomal and plasmid-mediated mechanisms.

Chromosomal trimethoprim resistance. Chromosomal resistance to trimethoprim (TMP) could be one of three theoretical types. The first includes the chromosomal location of transposon *Tn*7 (33). The second, was from a mutational loss in bacteria of their ability to methylate deoxyuridylic acid to thymidylic acid. This makes them dependent on an external supply of thymidylic (34). The result of this mutation relieves the cellular dihydrofolate reductase (DHFR) from its major task of regenerating the tetrahydrofolate. Which is used in the deooxyuridylate methylation process. This results in the cell being able to have a relatively large fraction of its DHFR inactivated by TMP. The third mechanism could be due to the gene for DHFR. These changes could be combined with regulatory mutations, leading to the cellular overproduction of the enzyme and high levels of TMP resistance (35).

Chromosomal sulphonamide resistance. As sulphonamide (SUL) antimicrobials act as competitive inhibitors of dihydropteroate synthase (DHPS) they block folate biosynthesis in the bacterial cell wall (36). Chromosomal mutations in the *dhps* gene can be isolated in the laboratory (37).

Plasmid-borne trimethoprim resistance. Plasmid-mediated trimethoprim (TMP) resistance is caused by non-allelic and drug-insusceptible variants of chromosomal DHFR (38).

Plasmid-borne dihydrofolate reductase (DHFR). There are 16 different types of plasmid-borne mediating resistance to TMP found in Gram-negative bacteria (39), with amino acid sequence analysis on nucleotide sequencing defining the majority of them (40).

Plasmid-borne sulfonaminide (SUL) resistance. SUL resistance in Gram-negative enteric bacteria is largely plasmid borne. It is due to the presence of alternative drug-resistant variants of the dihydropteroate systhase (DHPS) enzymes (41). Two plasmid-encoded enzymes have been characterised by nucleotide sequence and two respective genes were determined (42), the two types of DHPS encoded by *sul1* and *sul11* show 57% amino acid identity. In a study by Shin et al., they found that *E. coli* and *K. pneumonia, sul1* and *dfr* genes were highly prevalent in relation to integron1, illustrating that the resistance genes are often found linked to genes coding for resistance to other unrelated antimicrobials (43).

Mechanism of action of fluoroquinolones

The fluoroquinolones are synthetic broad-spectrum antibacterial drugs. There has been a great deal of research on this class of drugs, to better understand their mechanism of action, the antimicrobial spectrum and pharmacokinetics for clinical use in a diverse range of animal species has been carried out.

Fluoroquinolones interfere with bacterial DNA metabolism by the inhibition of two enzymes, Topoisomerase II (DNA gyrase) and Topoisomerase IV. DNA gyrase is the primary target in Gram-negative organisms, whereas Topoisomerase IV is more affective in Gram-positive.

DNA gyrase and Topoisomerase IV, have a very similar protein structure, both being composed of four subunits (two A and two B) encoded by *gyrA* and *gyrB*, and *parC* and *parE* respectively. The principal function of DNA gyrase is to introduce negative supercoils into the linear DNA double helix, which results in the highly condensed 3-demensional structure of DNA (44). The general function of Topoisomerase is less understood, however it is know that the enzyme plays a fundamental role in the splitting process of the DNA daughter chains after chromosomal duplication (45).

Fluoroquinolones mechanism of action is based on the DNA presented as single strands, forming a bubble-shaped binding pocket. Two quinolone molecules self-assemble to form a dimer structure inside the gyrase-induced DNA enzyme pocket; they bind to the gyrase complex by electrostatic forces (44). By inhibiting DNA gyrase, permanent gaps in the DNA strands induce synthesis of repair enzymes (endonucleases), which initiate uncoordinated repair, leading to irreversible damage and cell death.

All currently available fluoroquinolones have the same core quinolone structure, with various chemical substitutions and side group's accounting for the different physical characteristics of each drug. These differences account for variations in lipophilicity, oral absorption, volume distribution (Vd), and elimination rate. However, they do not affect the antimicrobial spectrum, for example enrofloxacin has one fluorine substitution, difloxacin has two and orbifloxacin has three fluorine substitutions, but the presence of more than one fluorine does not increase antibacterial effects (46). The quinolones are divided into four generations based on their antibacterial spectrum, earlier-generation agents are, in general, more narrow-spectrum than the later ones (47). Fluoroquinolones are used in human and veterinary medicine and are listed as 'Critically Important Antimicrobials' by WHO (48).

Mechanisms of fluoroquinolone resistance

Fluoroquinolone resistance is usually chromosomally mediated (49). Plasmid mediated, transferable fluoroquinolone resistance has been described (50; 51). Fluoroquinolone resistance isolates usually contain one or more mutations in a small section of *gryA* or *parC,* mutations in *gyrB* and *parE* are rare (52). Where mutations in bacteria have given rise to a resistant DNA gyrase, mutations then occur in the topoisomerase IV genes (and vice versa for Gram-positive bacteria) resulting in a highly resistant bacterium. Active efflux pump can be overexpressed to enhance the excretion of quinolones from the cell. This enhanced efflux in turn causes increased minimum inhibitory concentrations of several drugs, including fluoroquinolones, tetracycline, chloramphenicol, and ampicillin (53, 54; 55). Mutations that enhance efflux occur as a primary step to allow the bacteria to survive.

Mechanism of action of tetracyclines

Tetracycline antibiotics were isolated from various species of *Streptomyces* in the late 1940s and early 1950s. Since the 1950s many semisynthetic structural modifications have been made on the tetracycline molecule to yield other tetracycline molecules with different pharmacokinetic properties and antimicrobial activities. Tetracycline encapsulates the related compounds oxy-, and chlortetracycline, doxycycline and minocycline. Oxytetracycline and chlortetracycline were discovered in 1948, tetracycline in 1953 and doxycycline in 1967, and minocycline in 1972 (56). Of these chlortetracycline and oxytetracycline are natural products while the others are semisynthetic.

Tetracyclines possess antibacterial activity by binding to the 30S ribosomal subunit of a susceptible organism. Following ribosomal binding the tetracycline interferes with the binding of aminoacyl-tRNA to the messenger RNA molecule/ribosome complex; this disrupts the bacterial protein synthesis (57). Tetracycline binds with the 70S ribosomes found in mitochondria and can also inhibit protein synthesis in mitochondria (58). Tetracyclines are bacteriostatic and illustrate great affectivity against multiplying bacteria. Tetracycline is a broad-spectrum antimicrobial and used for a wide variety of Gram-positive and Gram-negative bacterial infections.

Mechanisms of tetracycline resistance

The mechanisms of acquired resistance include: 1) energy dependent efflux of antibiotic (membrane efflux proteins), or 2) altered target whereby the ribosome is protected from binding of tetracyclines. A third, less common mechanism was discovered where bacterial enzymes attack tetracycline (57).

The efflux system was mediated by different membrane-associated protein called Tet proteins from the major facilitator superfamily (MFS) (59). The export proteins function by virtue of their capacity to translocate a complex of tetracycline and a divalent metal ion. The metal ion bears a positive charge, with CO^{2+} being the most effective in supporting transport, Mg^{2+}, Mn^{2+}, Cd^{2+} and Ca^{2+} can also complex with tetracycline to form a substrate (60). By exporting tetracycline out of the cell, it reduces the intercellular concentration of tetracycline and protects most of the ribosome from the action of the tetracycline.

The second type of tetracycline resistance reported was a protein-based ribosomal protection mechanism, first found in Streptococci (61). Tet (M) and Tet (O) are the best-studied determinants of ribosomal protection proteins and are widely distributed. When tetracycline binds to ribosomes they normally stop the elongating of synthesising proteins. These proteins interact with the ribosome causing the tetracycline to dislodge from the ribosome. This protects the bacteria from tetracycline's inhibitory activity and allows cellular growth (56). Efflux pumps or protection of ribosomal target are the main types of resistance mediation.

Finally, the third mechanism involves cytoplasmic enzymes that are capable of inactivating tetracycline. One enzyme, Tet(X) has been confirmed for activity in vitro (62). Tet(X) is a flavoprotein monooxygenase that inactivates tetracycline antibiotics by monohydroxylation coupled with spontaneous, non-enzymatic breakdown (63). The only report of a human pathogens with the potential to inactivate tetracycline occurred in 2013, from a urinary tract infections in Sierra Leone, when 11 isolates where positive for Tet(X) (64).

The resistance mechanisms are encoded by *tet*-genes that can be located on transferable elements. Antimicrobial potency are different between tetracyclines, cross-resistance is complete (65).

Conclusion

As illustrated different antimicrobial agents have distinctive modes of action against various microorganisms. The potency of antimicrobial agents is largely influenced by the nature of their structure and degree of affinity to certain targets sites within microbial cells. Understanding the mode of action of antimicrobial agents is fundamental in the understanding of how resistance develops against them. The efficiency of antimicrobial agents is being compromised by a growing number of antibiotic resistance pathogens due to their overuse and inappropriate use (66). By understanding the mechanism of resistance that bacteria use to defend themselves against antimicrobial agents should aid us in producing innovative antimicrobials with novel modes of action.

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