Infectious Diseases Course

Selected proceedings of a postgraduate course in infectious diseases conducted by The American College of Physicians in conjunction with Stanford University School of Medicine February 10-14, 1975

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Contents

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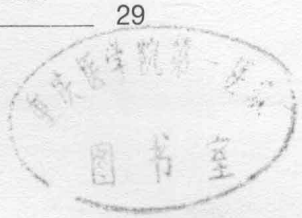
ANTIBIOTICS

BACTERIAL INFECTIONS



Antibiotic Combinations

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There are six situations in which I think the use of antibiotics in combination is very clearly justified. The *first* is when there are two different infections caused by two different organisms in the same patient which are not susceptible to the same antimicrobial agent. A very similar, *second,* situation would be when the organisms in a mixed infection are not all susceptible to a single antimicrobial agent. *Third,* in a fulminating infection, treatment with more than one antibiotic may be indicated until the identity of the causative organism is known. These three situations are so obvious that I will devote no more time to these and now will concentrate on the following three. *Fourth,* when the organism in a fulminating infection is known but the sensitivities are not, it may be highly advisable to use a combination of antibiotics until the facts are in (concurrent use of potentially neurotoxic and/or nephrotoxic drugs should be avoided). The *fifth* reason involves the use of different agents with different toxicities thereby permitting a decrease in dosage and a decrease in toxicity for each of the individual antibiotics. The last and *sixth* reason is that antibiotic combinations have been used for their synergistic effects.

Tuberculosis is one situation where if the organism is known but the sensitivities are not, therapy with a combination of drugs should be instituted until the sensitivities are known and, at that

point, revise therapy, if necessary. There is some controversy about this but in view of the fact that isoniazid and rifampin, the two major drugs available for the treatment of tuberculosis, are likely to have resistance emerge if used alone is reason enough to use them in combination. Because of the recent emergence of ampicillin-resistant *Hemophilus influenzae* strains, it has been suggested that chloramphenicol and ampicillin be used together for initial therapy of meningitis in children. If the infecting strain is susceptible to ampicillin, it can be continued alone at that time.

In fulminating *Staphylococcus aureus* infections one might begin with penicillin-G plus a penicillinase-resistant penicillin or cephalosporin, for approximately 70 to 86% of community acquired staphylococcal infections are resistant to penicillin-G. After the sensitivity report is in, the physician can eliminate the unnecessary anti-staphylococcal agent. Erythromycin is another antibiotic to which resistance may emerge during a single course of therapy for a staphylococcal infection. This does not hold true in the usual situations where erythromycin is used, that is, in streptococcal and pneumococcal infections. In treating staphylococcal infections, however, the physician should use another antibiotic with erythromycin to delay the emergence of resistance.

In fungal infections, resistance develops rapidly to 5-fluorocytosine if it is used alone. To prevent the emergence of resistance, amphotericin-B can be

Situations Justifying Combined Use Of Antibiotics

1. Different infections/organisms in same patient not susceptible to same antimicrobial agent.
2. Mixed infection not susceptible to a single antimicrobial agent.
3. Fulminating infections until the causative organism is identified.
4. Fulminating infections where the causative organism is known, but the sensitivities are not; short-term combination therapy until lab. studies completed.
5. To decrease toxicity of different agents by decreasing dosage of each agent.
6. Increased effectiveness through demonstrated synergistic effects.

used with the 5-fluorocytosine. This way, 5-fluorocytosine can be reserved for situations where amphotericin-B may not be so effective, for instance, in the treatment of fungal meningitis. Although amphotericin-B is used in meningitis, it is often necessary to administer it intrathecally or intraventricularly whereas 5-fluorocytosine readily passes the meninges into the cerebrospinal fluid.

A triple sulfonamide is an example of an antibiotic combination created to reduce toxicity by lowering the dosage of each component. The major toxicity of many single sulfonamides is crystalization within the urinary tract. Because the solubility of each of the three different sulfonamides used in this combination is quite independent of the concentrations of the others, one can use one-third the dose of each and thus reduce the likelihood of having crystalization within the urinary tract and yet have additive therapeutic action equalling a "full" dose of one.

Obviously, the motive when two drugs are used together is for the patient's benefit. As long as the effect of the combination is better than any of the individual components, I believe it is worthwhile. The combination may not be truly synergistic and may be only additive or even mildly antagonistic and still be superior to using a single agent. The best known example in clinical medicine for using a combination for increased effect is endocarditis, especially enterococcal endocarditis. Another situation where concomitant antibiotic therapy has been suggested is in treating Pseudomonas infections. The use of pyrimethamine and sulfamethoxazole has been suggested in the treatment of toxoplasmosis. In norcardiosis it has been shown that ampicillin and erythromycin may be synergistic. The use of tetracycline and streptomycin may be successful in the treatment of brucellosis. Kirby, *et al.,* described a patient with a Pneumocystis infection who was treated with pyrimethamine and sulfadiazine because the drug of

Reasons For Antibiotic Antagonism

1. One drug prevents growth of organisms necessary for activity of the other drug (i.e., chloramphenicol, by inhibiting the growth of organisms, will interfere with the activity of penicillin which needs reproducing bacteria in order to be effective).
2. Chemical inactivation of one drug by another.
3. Potential enzymatic degradation of one agent by another.
4. One drug may block the binding site of the other.

choice, pentamidine, at least at that time, was not immediately available. The patient's fever came down abruptly on this combination but when the patient was put on pentamidine and taken off the pyrimethamine and sulfadiazine, the temperature went up. After being switched back to the combination, the patient continued to improve. In some cases of continuous bacteremias, the use of a combination of a beta-lactam antibiotic and an aminoglycoside has been effective clinically. Although a combination of trimethoprim and sulfamethoxazole is synergistic *in vitro,* Brumfitt observed that the combination was no more effective than trimethoprim alone *in vivo,* in urinary tract infections.

The mechanism of synergism is, of course, dependent on the mechanisms of action of the antibiotics involved. For example, penicillin increases the permeability of the bacteria to the aminoglycoside. Sequential blocking in folic acid synthesis and utilization occurs with the combinations of trimethoprim and sulfamethoxazole and also with pyrimethamine and the sulfonamides.

Antibiotics used in combination can be antagonistic instead of synergistic. Penicillin requires the presence of reproducing organisms in order to exert its bactericidal action. The concomitant administration of a bactericidal agent such as chloramphenicol may prevent growth of the bacteria so that penicillin won't be able to act. Another example of antagonism is the clinical inactivation of one drug by another. A third mechanism of antagonism that has not been observed yet is that one drug may induce enzyme degradation of the other. Also, one drug may block the binding site of the other.

New Antimicrobials

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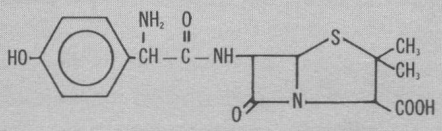
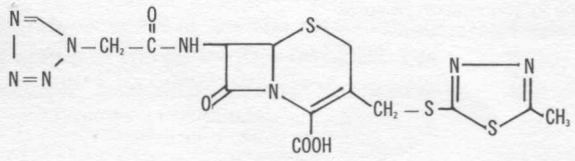
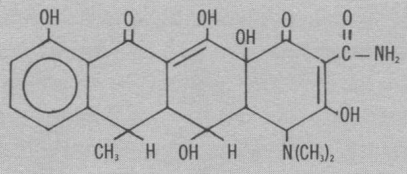
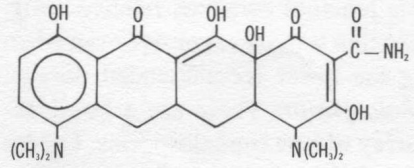
Amoxicillin (Larocin): Modifications of penicillin-G have resulted in a number of antimicrobials possessing various advantages over the parent compound. Amoxicillin is the only widely promoted agent in this class since carbenicillin, and it represents only a minor addition. Amoxicillin has an antibacterial spectrum which is almost identical to that of ampicillin; the principal advantage is that oral absorption of amoxicillin is better, providing blood levels twice as high with comparable doses. Indications are generally similar to those for oral ampicillin such as urinary tract infections, acute otitis media and exacerbations of chronic bronchitis. Amoxicillin has proven to be a good agent for Salmonella infections but it should not be used for Shigellosis. Clinical trials have shown gonococcal infections respond as well to 3 gm amoxicillin as 3.5 gm ampicillin plus probenecid. Compared to ampicillin, the incidence of diarrhea appears to be less with the new agent, although other side-effects (including the incidence of rash) are probably similar. A major question at this juncture concerns relative costs; amoxicillin is more expensive even when using the lower recommended dosage.

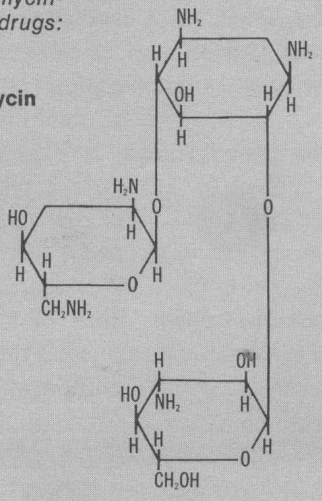
Cephalosporins: There are a bewildering array of new cephalosporins. Unlike many of the semisynthetic penicillins, the new cephalosporins have had little

The Newer Antibiotics, Their Precursors, And Clinical Comments

ANTIBIOTIC AND CONFIGURATION	(IMMEDIATE PRECURSORS)	CLINICAL COMMENTS
Penicillins:		
amoxicillin 	(ampicillin)	• Essentially same spectrum of activity, except for diminished activity against *Shigella* for amoxicillin • Possible decreased incidence of diarrhea with amoxicillin
Cephalosporins:		
cefazolin 	(cephalosporin C)	• Good IM tolerance; less nephrotoxic than cephaloridine • High and prolonged blood levels; high bile levels
Tetracyclines:		
doxycycline 	(tetracycline)	• Well absorbed orally and slowly excreted; prolonged half-life makes b.i.d. administration possible • Can be used safely in patients with renal failure • More active than parent compound against *B. fragilis*
minocycline 	(tetracycline)	• Long acting with prolonged half-life • May cause vertigo in a large proportion of patients • More active than parent compound against *B. fragilis*

Streptomycin-related drugs:

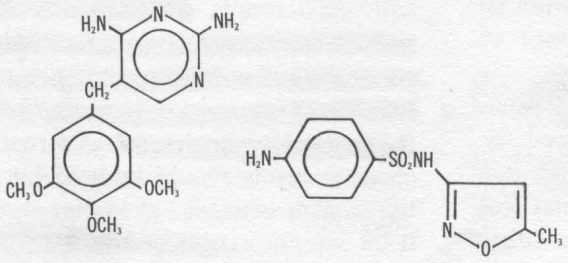
tobramycin



- Somewhat more active than gentamicin against *P. aeruginosa in vitro*
- Used primarily in chronic urinary tract infections, chronic prostatitis, and prophylaxis for recurrent urinary tract infections

Combination Drugs:

trimethoprim + sulfamethoxazole



- Both agents block folic acid biosynthesis at different points
- Used primarily in chronic urinary tract infections, chronic prostatitis, and prophylaxis for recurrent urinary tract infections

effect on antibacterial spectrum of the parent compound, cephalothin. This does not apply to two experimental cephalosporins (cefamandole and cefoxitin) which have not been released yet. The main differences between the currently available agents relate to oral absorption and pharmacokinetics:

Agent	*Peak Serum Level**	*Half Life*
1. Short-acting		
Cephalothin (Keflin)	15-20	40
Cephradine (Velosef, Anspor)	10-20	40
Cepharipin (Cefadyl)	10-15	40

Agent	Peak Serum Level*	Half Life
2. Long-acting		
Cephaloridine (Loridine)	35-40	80
Cefazolin (Ancef, Kefzol)	60-80	100
3. Oral agents		
Cephalexin (Keflex)	15-20	
Cephradine (Velosef, Anspor)	15-20	

**1 gm IM for parenteral agents; 500 mg p.o. for oral agents*

Cephalosporins are greatly overused. Principal indications are for 1) infections involving Gram-positive cocci in patients allergic to penicillin, 2) infections involving aerobic Gram-negative bacilli when the pathogen has been identified and susceptibility tests show efficacy and 3) in combination with an aminoglycoside for selected cases of severe sepsis.

Among the available parenteral cephalosporins, cefazolin is considered the intramuscular agent of choice since it is less painful than cephalothin and less nephrotoxic than cephaloridine. Other possible advantages of cefazolin include higher and more prolonged blood levels; high bile levels; no deacetylation to a less active metabolite, and possible cost benefit. Oral cephradine and cephalexin are judged equal, but both suffer from the ready availability of alternative, cheaper oral agents for most infections. The principal indication for these oral cephalosporins is Klebsiella urinary tract infection which can be treated with an oral agent.

Tetracyclines: Two new tetracycline derivatives are minocycline (Minocin) and doxycycline (Vibramycin). Both are long acting with half lives of 16-18 hours. Advantages of doxycycline are ease of oral administration (twice daily) and relative safety in renal failure. The main disadvantage is excessive cost compared to tetracycline with essentially the same antibacterial spectrum. Minocycline may cause vertigo in a large proportion of patients and this limits usefulness. Both of these new tetracyclines are somewhat more active against *B. fragilis* compared with the parent compound.

Trimethoprim-sulfamethoxazole: Cotrimoxazole (Bactrin, Septra) is a combination of two antagonists of folic acid metabolism which are synergistic against a wide variety of bacteria. This combination has been used in Europe for numerous types of infections since 1969 with good results. The only FDA approved indications at present in this country are for "chronic urinary tract infections", *Pneumocystis carinii* pneumonia, and typhoid fever. Alternative, cheaper agents should be used for initial oral treatment of most urinary tract infections. Trimethoprim-sulfamethoxazole is preferred for chronic prostatitis and for prophylaxis in patients with recurrent symptomatic urinary tract infections.

Tobramycin (Nebcin): This is a new aminoglycoside with limited advantages over gentamicin. Pharmacokinetic properties of these two agents are identical. Tobramycin is somewhat more active against *Pseudomonas aeruginosa in vitro;* most other strains of gram-negative bacilli which are resistant to gentamicin are also resistant to tobramycin.

Antibiotics: Mechanisms Of Action

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Antibiotics that are clinically useful may be divided into five distinct groups on the basis of their mechanism of action. They achieve their antimicrobial effects by: (1) interfering with cell wall biosynthesis, (2) altering cytoplasmic membrane function, (3) inhibiting protein synthesis, (4) affecting DNA metabolism, and/or (5) disrupting intermediary metabolism.

Inhibitors Of Cell Wall Biosynthesis

The most important members of the class of drugs that interfere with cell wall synthesis are the penicillins, cephalosporins, vancomycin, bacitracin, cycloserine and ristocetin.

One of the most significant differences between bacterial and mammalian cells is the rigid supporting cell wall of bacteria which protects the protoplasmic membrane of the organism from osmotic and mechanical trauma. Bacteria are capable of maintaining an intracellular environment at 10-30 atmospheres greater than the osmotic pressure of the external environment. Substances that interfere with cell wall biosynthesis will produce an osmotically sensitive organism that will rupture and die when it undergoes division and growth.

The rigidity of the cell wall is caused in large measure by the peptidoglycan, a cross-linked latticework structure. It consists of two components – a polysaccharide chain composed of strands of alternating units of N-acetylglucos-

Mechanisms Of Antibiotic Action

I **Drugs that interfere with cell wall synthesis:**

- All penicillins
- All cephalosporins
- Vancomycin
- Bacitracin
- Cycloserine
- Ristocetin

II **Drugs that interfere with cytoplasmic membrane function:**

- Polymyxin-B
- Colistin
- Amphotericin-B
- Nystatin

III **Drugs that inhibit protein synthesis, either transcription or translation:**

- Aminoglycosides, such as:
  - Gentamicin
  - Kanamycin
  - Streptomycin
- Tetracyclines
- Chloramphenicol
- Rifampin
- Macrolides
- Lincomycin

IV **Drugs that affect DNA metabolism:**

- Nalidixic acid
- Griseofulvin

V **Drugs that affect intermediary metabolism:**

- Sulfonamides
- Trimethoprim
- 5-fluorocytosine

amine and N-acetylmuramic acid which are cross-linked to one another by means of short peptide chains which are to some extent genus specific. In the cell wall of *Staphylococcus aureus,* tetrapeptide units (alanine-glutamic acid-lysine-alanine) are bonded to the acetylmuramic acid residues, and pentaglycine chains bridge between the tetrapeptide moieties on adjacent chains.

The complex series of reactions that involve formation of the uncross-linked peptide and glycan polymers can proceed without interference by penicillins or cephalosporins. The beta-lactam antibiotics act during the completion of the cross-link. Part of the penicillin molecule is structurally similar to a portion of the tetrapeptide. Normally, during cross-linking, a transpeptidation reaction occurs which links the terminal glycine of the pentaglycine to the fourth residue of the pentapeptide [D-alanine], releasing the fifth residue, also D-alanine. Thus, this reaction forms a tetrapeptide from a pentapeptide. The active portion of penicillin is structurally similar to D-alanyl-D-alanine and competes with it during the transpeptidase reaction. This interferes with cross-linking and produces an osmotically fragile cell wall which eventually ruptures resulting in bacterial death.

Gram-positive organisms are much more sensitive to penicillin than are gram-negative organisms. The latter have an outer membrane which makes them relatively impermeable to penicillin G and certain other penicillins. However, ampicillin and carbenicillin are able to penetrate this outer membrane more easily than penicillin G.

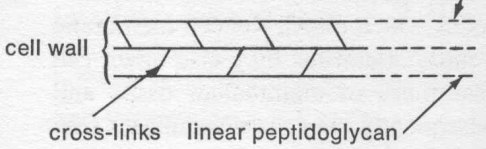
The mechanism of action of the

cephalosporins is very similar to that for the penicillins. Vancomycin, a glycopeptide antibiotic prevents the incorporation of cell wall amino acids into the peptidoglycan fraction. Therefore, it acts at a site proximal to that of the penicillins. Bacitracin acts by inhibiting a dephosphorylation reaction required for regeneration of a lipid carrier required for cyclic synthesis of peptidoglycan. Its action is not limited to cell wall synthesis since it can also affect bacterial protoplasts. Cycloserine interferes with the synthesis of the pentapeptide of the peptidoglycan by inhibiting conversion of L-alanine to D-alanine and by blocking synthesis of the dipeptide D-alanyl-D-alanine from D-alanine.

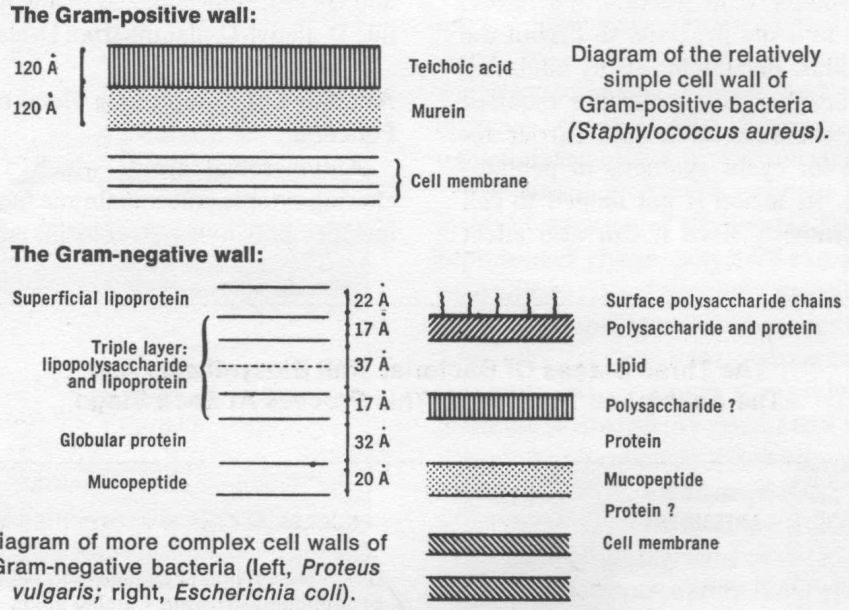
Alteration of Cytoplasmic Membrane Function

Antimicrobial agents which act by altering cytoplasmic membrane function include: polymyxin-B, colistin, ampho-

The Three Stages Of Bacterial Wall Biosynthesis And The Antibiotics That Inhibit This Process At Each Stage

STAGE	ANTIBIOTIC	SITE OF ACTION IN CELL	PROCESS IN CELL WALL SYNTHESIS
I	Cycloserine	Cell interior	UTP + N-acetylglucosamine + phosphoenolpyruvate + amino acids Precursor formation UDP-acetylmuramyl pentapeptide
II	Ristocetin Bacitracin Vancomycin	Bound to cell membrane	Attachment to membrane phospholipid, additional chemical modification, transport of basic repeating unit through the membrane, and attachment to preexisting cell wall
III	Penicillins Cephalosporins	Cell exterior	 Cross-linking of linear peptidoglycan strands—the final event in cell wall formation

Cell Wall Structure: Gram-Positive vs. Gram-Negative Organisms



tericin-B, and nystatin. The cytoplasmic membrane provides an osmotic barrier preventing free diffusion of substances between the external and internal environments of the bacterial cell wall. The cytoplasmic membrane has a counterpart in mammalian cells. Thus, antibiotics which attack the cell membrane of microorganisms may also affect cell membranes of mammalian tissue and consequently may show significant toxicity when used in man.

Antibiotics of the polymyxin group, including colistin, are cyclic peptides with molecular weights of about 1,200. They are produced by various Bacillus species. Polymyxins and colistin have the ability to produce a disorientation of the cytoplasmic membrane so that it no longer functions as an effective osmotic barrier. The addition of polymyxin at bactericidal concentrations to sensitive cells results in rapid release of nucleic acids and other cell contents. Polymyxin is taken up to a greater extent by the cell walls of sensitive as opposed to resistant bacteria.

Polyene antibiotics such as amphotericin B and nystatin act by binding to a sterol moiety present in the cytoplasmic membrane of sensitive cells. This causes an alteration in membrane permeability resulting in the loss of cell contents. Because polyenes require the presence of the sterol moiety, they are active against yeast, a wide variety of

fungi and other eukaryotic cells but have no action against bacteria.

Inhibitors Of Protein Synthesis

Antimicrobial agents which affect protein synthesis include rifampin; the aminoglycosides such as streptomycin, kanamycin and gentamicin; the tetracyclines; chloramphenicol; macrolides and lincomycin. Protein synthesis can be divided into two major processes: transcription which involves DNA-dependent RNA polymerase production of messenger and transfer RNA's and translation which involves the use of messenger and transfer RNA's to make protein. An antibiotic which inhibits either translation or transcription will inhibit protein synthesis. Rifampin is currently the only clinically useful antibiotic which interferes with transcription. It interacts with RNA polymerase and the DNA complex, producing conformational changes that inhibit initiation of the production of RNA. This action is bactericidal. It is of some interest that rifampin has very little effect on mammalian RNA polymerase systems.

The other antibiotics of clinical importance which inhibit protein synthesis do so at the translation stage. Streptomycin acts principally by interacting with a 30S ribosomal subunit protein. However, it also interferes with codon recognition, has modest effects of transpeptidation, and has major effects on translocation which is the process of moving along the messenger RNA. The other aminoglycoside antibiotics of clinical importance—neomycin, kanamycin and gentamicin—act in a fashion very similar to streptomycin. Spectinomycin, an aminocyclitol antibiotic that is structurally similar to the aminoglycosides, interferes with protein synthesis by binding to a 30S ribosomal subunit protein. However, unlike the aminoglycosides, it is bacteriostatic rather than bactericidal and its major effect is on translocation.

Tetracycline, like the aminoglycosides and the aminocyclitols, interacts with the 30S ribosomal subunits but it does so in a different fashion. Tetracycline inhibits binding of the aminoacyl t-RNA to 30S ribosomal subunits and also inhibits termination of protein synthesis.

Chloramphenicol interferes with translation by interacting with 50S ribosomal subunits. Its principal effect is on transpeptidation required for peptide bond formation. It also directly inhibits peptidyl transferase. The complete mechanism by which erythromycin, the most frequently used macrolide antibiotic, inhibits protein synthesis has not been fully established. However, it has been shown to bind to 50S ribosomal subunits producing conformational changes resulting in interference with peptide bond formation. Erythromycin inhibits transpeptidation and indirectly interferes with translocation. Lincomycin interferes with protein synthesis by binding to 50S ribosomal subunits. It inhibits transpeptidation, translocation and termination.

Interference With DNA

Nalidixic acid and griseofulvin are the only two clinically useful antimicrobial agents that interfere with DNA. Nalidixic acid interacts with the stacked bases of DNA and interferes with the