11. Antibiotics

INTRODUCTION

The 20th century has been a boom for antimicrobial medication. At the turn of the century infectious diseases were the top three most common causes of death (pneumonia/influenza, tuberculosis, and gastrointestinal infections), now only pneumonia/influenza remain on the top 10 at the #8 spot. **This radical change has been largely due to the discovery and specialization of antimicrobials.** From simple penicillin to the new complex of teixobactin, antimicrobials have certainly undergone a big change since their introduction. New antibiotics and other anti-infective agents will need to be discovered as the great medical struggle of our time, resistance, plays out right before our eyes. It is within that scope, that this chapter aims to cover the basics of antimicrobials so that growing resistance among agents can be countered, for now…

BACTERIA

Antibiotics are a class of **antimicrobials** targeted solely towards the interference with bacterial structures or metabolism (Fig.11.1) resulting in the eradication of bacteria. Bacteria are prokaryotic, ubiquitous organisms that are found everywhere in nature, from the highest peaks to the lowest parts of the ocean. They possess an outer peptidoglycan layer of variable thickness (distinguishes between Gram-positive and Gram-negative bacteria) and an inner membrane where oxidative phosphorylation is performed. Some classes, Gram-negative, also contain an outer membrane, complete with porins and lipopolysaccharide (LPS). Inside the cell the environment is more familiar, with the central dogma of genetics still intact, DNA - > RNA - > Proteins. It is the differences with eukaryotic cells that serve as points of vulnerabilities that antibiotics can exploit. Unfortunately the similarities are a major source of side effects and thus selection of the ideal antibiotic becomes a juggling act of optimal coverage and minimizing of adverse effects.

FIGURE 11.1.

Figure 11.1. *The general bacterial structures targeted during antibiotic therapy. The antibiotic treatments are directed towards structures that are specific for bacterial cells. These structures are either not present in humans, or are considerably different to not be damaged by the antimicrobial treatment. The synthesis of the cell wall (a) can be inhibited by beta-lactams. The outer membrane (b) is targeted by polymyxins. Ribosomes (c) and proteosynthesis can be inhibited by aminoglycosides, tetracyclines, macrolides, lincosamides and chloramphenicol. The synthesis of nucleic acids (d) can be inhibited by quinolones, sulphonamide and trimetoprim.*

Table 11.A.

Table 11.A. ***Antibacterial drugs.*** *The antibiotic drugs can be classified accoring to the structures they are active against.*

General mechanism of antibiotic resistance

Bacteria can overcome antibiotics by a few fundamental mechanisms of resistance. Classification of mechanisms of resistance can be varied and you can find one of them depicted in table 11.B. Because of the resistance to antibiotics *in vitro*, therapy of infection will fail.

TABLE 11.B.

Table 11.B. General mechanism of antibiotic resistance

PENICILLINS

The discovery of **penicillin** and its sister compounds is one of the most important events in the history of the world. For the first time infectious agents could be fought with, on their own turf, and for a long time, without the fear of resistance. Penicillin is thus the archetype of the entire group and, despite its age, still the first line for many important infections. Penicillin is generally used for syphilis, gas gangrene, penicillin-susceptible *Streptococcus pneumoniae*, *Actinomyces* infections and other Gram-positive infections. Penicillin and other members of the group work by binding to **penicillin-binding proteins** (transpeptidases). These transpeptidases act to cross-link the peptidoglycan layer of bacteria, a protective barrier for prokaryotic organisms. By binding at this site penicillins block the cross-linking of the peptidoglycan layer and also activate cell autolysins which further help in the eradication of the bacteria.

Adverse effects: Adverse side effects are shared by all the members of the penicillin group. Although commonly claimed by patients only about 1% of the population are truly allergic to penicillins. Reaction to the drug include a rash and hives. Anaphylactic reactions are estimated to occur in 1-5/10.000 penicillin therapy cases. Cross-reactivity to other β-lactams exists to some extent so care must be taken when administering cephalosporins and other β-lactam antibiotics. Hemolytic anemia is also another side effect which necessitates the removal of the offending penicillin. GI symptoms tend to be less frequent with diarrhea being the most common.

AMINOPENICILLINS

Aminopenicillins are **penicillins that possess a charged R side-group**. This R side-group gives these penicillins a wider spectrum of activity, especially with Gram-negative bacteria like *Haemophilus influenza*, *E. coli*, *Proteus*, *Salmonella* and *Shigella*. It also provides cover of enterococci and it is often the first line of therapy. The main constituents of the group are **ampicillin** and **amoxicillin** (better oral bioavailability).

Aminopenicillins are still susceptible to penicillinase enzymes that are produced by *Staphylococcus aureus* and many other formidable bacteria. To counteract this, the aminopenicillins are often paired with penicillinase inhibitors. Ampicillin is given with sulbactam and amoxicillin is given with clavulanic acid.

PENICILLINASE-RESISTANT PENICILLINS

These **semi-synthetic penicillins** do not need to be administered with a β-lactamase (penicillinase) inhibitor, because their bulky R side-group naturally blocks access of the enzyme to the lactam ring. Their main indication are methicillin-sensitive S. *aureus* (MSSA) or which constitutively produces penicillinase and as such is resistant to both garden-variety penicillins and

aminopenicillins. Penicillinase-resistant penicillins are often the first line against *S. aureus* in areas where MRSA is not yet prevalent. The rest of the spectrum follows the narrow uses for penicillin. The main members of the group are **oxacillin**, **nafcillin**, and **dicloxacillin**.

FIGURE 11.2.

Figure 11.2. Transpeptidase PBP2 of S. aureus strains. Beta-lactams are structurally related to the terminal end of the tetrapeptide, bind to PBPs and block interconnection of tetrapetides and pentapeptides. The peptide bridges are necessary for the stability of the cell wall. This prevents cell wall synthesis. PBP2a is a modified target of PBP2 of MRSA strains, which is not able to bind with beta-lactams so synthesis of cell wall is not influenced even if beta-lactam antibiotics are present (TP – transpeptidase enzymatic reaction).

Methicillin was used in the past but it is no longer available due to interstitial nephritis concerns. Resistance to methicillin (and all isoxazollylpenicilins, e.g. oxacillin and all beta-lactams) is caused by the *mecA* gene, which encodes an alternative penicillin-binding protein (PBP2a). The *mecA* gene is part of a 21- to 60-kb staphylococcal chromosome cassette mec (SCCmec) (fig. 11.2), a mobile genetic element that also may contain other genetic structures that may provide further resistance to non-β-lactam antibiotics.

FIGURE 11.3.

Figure 11.3. The origin of the mecA gene remains unknown. It is suspected that the incorporation of the SCCmec mobile genetic element into the S. aureus genome happened only a few separate times in history. This corresponds to the limited clonal lineages of MRSA in existence all over the world.

Resistance to meticillin (beta-lactam ATB) is induced by the mean presence of beta-lactams, hence, induced expression of PBP2a and increases level of resistance to all of beta-lactams (fig. 11.4).

FIGURE 11.4.

Figure 11.4. The genes mecR1 and mecI are located closely to mecA gene on the staphylococcal chromosome. The mecR1 gene encodes the MecR1 signal protein and the mecl gene encodes the mecI regulatory gene (MecI). Promoters and operators are located between mecA and mecR1. MecR1 and MecI have high sequence similarity with BlaR1 and BlaI proteins, which are connected to inducible expression of blaZ gene located on a staphylococcal plasmid. The arrangement of genes for BlaR1 and BlaI proteins is similar to mecA system. This indicates that the mecA regulation genes could have originated from the blaZ system. Topology of the operators seems to be so similar BlaI can regulate expression of PBP2a. BlaR2 is protein suspected for proteolysis of BlaI or MecI. A) Repression of blaZ (mecA) and blaR1-blaI (mecR1-mecI) transcription by BlaI (MecI) protein. B) Induction of beta-lactamase synthesis by presence of beta-lactam antibiotics. Activated MecR1, BlaR or BlaR2 protein degrades MecI a BlaI.

ANTIPSEUDOMONAL PENICILLINS

While the aminopenicillins do have a great spectrum of coverage they do not eradicate a very important organism, *Pseudomonas aeruginosa*. By editing the D side-group on penicillins the antipseudomonal penicillins can cover pseudomonas and have larger coverage of other Gram-negative rods, thus giving them the designation of **extended spectrum penicillins**. The most common representatives of this group are **piperacillin** and **ticarcillin**. As they do lack a bulky R side-group, antipseudomonal penicillins need to be **administered with clavulanic acid for ticarcillin and tazobactam with piperacillin**.

CEPHALOSPORINS

Cephalosporins also belong to the β-lactam family alongside penicillins. They act by **binding to penicillin-binding protein** and **inhibiting its transpeptidation action**. Due to a change in the molecular structure the cephalosporins are **less susceptible to penicillinases** than the penicillin group. The cephalosporins are divided into **five generations**, each successive generation tends to lose Gram-positive coverage in exchange for extension of Gram-negative efficacy.

First generation (cefazolin, cephalexin) tend to have penicillin Gram-positive coverage (staphylococci, betahemolytic streptococci and *S. pneumoniae*) alongside with *Proteus*, *Klebsiella* and *Escherichia coli*.

Second generation (cefaclor, cefuroxime, cefoxitin) lose *Staphylococcus epidermidis* cover but gain *Haemophilus*, *Neisseria*, and *Enterobacter* activity.

Third generation is a very popular and widely used set of cephalosporins (cefotaxime, ceftazidime, ceftriaxone). Ceftriaxone especially has many indications as empiric and even as definitive cover of a multitude of bacteria. It covers bacterial meningitis, Lyme disease and even the pesky HACEK group (including *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* species. Ceftazidime is also special in that it is the first cephalosporin to have a real chance against *Pseudomonas aeruginosa*. Due to the cephalosporins natural resistance to penicillinases, a lactamase inhibitor is not required.

Fourth generation (cefepime) is a newer generation and also features pseudomonal cover, alongside the return of great Gram-positive activity – staphylococci, streptococci and enterococci. Unfortunately it does not fight against MRSA, which was a major driving force for the development of fifth generation cephalosporins.

Fifth generation (ceftaroline) are the newest cephalosporins on the market. They have broad Gram-positive and Gram-negative cover and have the ability to kill MRSA but not as a pneumonia agent. There is a tradeoff in the end though, as pseudomonal cover is mostly lost, making them largely ineffective in settings such as burn wound infections and ventilation-associated pneumonia.

Adverse effects: Cephalosporins do tend to share some of the same problems as penicillins and as such can also be cross-reactive with penicillins. Hypersensitivity reactions are still a real possibility as is autoimmune hemolytic anemia. Their broad cover can eradicate vitamin K producing bacteria and cause bleeding sequelae. A very important side effect is the increase of nephrotoxicity with the concomitant use of aminoglycosides, a side effect that can be mitigated by not using them alongside one another.

OTHER BETALACTAM DRUGS

MONOBACTAMS

Aztreonam has an antimicrobial spectrum similar to that of gentamicin and tobramycin, aminoglycoside antibiotics. Approved indications for its use include infections of the urinary tract or lower respiratory tract, intra-abdominal and gynecologic infections, septicemia, and cutaneous infections caused by susceptible organisms.

CARBAPENEMS

Among the β-lactams currently available, carbapenems are unique because **they are relatively resistant to hydrolysis by most β-lactamases**, in some cases act as "slow substrates" or inhibitors of β-lactamases, and still target penicillin binding proteins. Of the many hundreds of different β-lactams, carbapenems possess the **broadest spectrum of activity** and greatest potency **against Gram-positive and Gram-negative bacteria** also including **anaerobes** e.g. *Bacteroides fragilis*. As a result, they are often used as "last-line agents" or "antibiotics of last resort" when patients with infections become gravely ill or are suspected of harboring resistant bacteria. They are effective against serious nosocomial infections, septicemia, pneumonia, osteomyelitis, gynecological infections and complicated urinary tract infections.

VANCOMYCIN

Vancomycin was and is a great addition in the fight **against MRSA**. Not only does it cover the aforementioned super-bug, but it also covers other serious Gram-positive infections that are often resistant to other antibiotics such as ***Clostridium difficile*** and ***S. epidermidis***. It is drug of choice to treat **Gram-positive endocarditis**, **sepsis**, **endophthalmitis** and **shunt infections**. The mechanism of action is similar to penicillins but it is not quite the same. Instead of binding to the transpeptidase enzyme, vancomycin **binds to the D-ala, D-ala portion of the growing peptidoglycan wall**. By binding to these precursors, the antibiotic essentially precludes their addition to the peptidoglycan layer, thus **weakening it and eventually killing the bacterial cell**. This change of mechanism of action is attributed to its change in structure (it is not a lactam antibiotic), which is also the reason that vancomycin is not susceptible to lactamases produced by many Gram-positive bacteria. While the antibiotic does in fact eradicate MRSA, it has not all been good news. Due to an incorporation form enterococci DNA, the *VanA* gene has "jumped" to create a super-bug known as VRSA or vancomycin resistant *S. aureus*. Thankfully there are still antibiotics to which this bacteria is susceptible, ceftaroline, linezolid, daptomycin, dalfopristin-quinupristin, but it does not take a large leap of faith to realize that, as the resistance window widens the susceptibility window shrinks, making this an important battleground for next-generation antibiotics.

Adverse Effects:

Vancomycin has some classical reactions and side effects associated with it. Thrombophlebitis is prevalent, especially at the site of injection. The antibiotic can cause both nephrotoxicity and ototoxicity, making it a poor choice in patients with decreased renal function. If so, vancomycin serum concentration should be monitored. Of particular note is the non-IgE mediated reaction known as red man syndrome, a condition in which the patient presents with diffuse flushing of the trunk and extremities.

AMINOGLYCOSIDES

Aminoglycosides are a very important **group of protein synthesis antibiotics**. They are often the last line in **severe Gram-negative infections** and are regularly a part of combined treatment when the agent of infection has not been uncovered yet. They work by **irreversible binding of the 30S subunit of bacterial ribosomes**. This prevents the correct translocation of a growing peptide and also causes misreading of incoming mRNA sequences. The most often encountered members of the aminoglycoside group are **streptomycin**, **gentamicin**, **neomycin**, **tobramycin** and **amikacin**. Streptomycin is a common agent and is used in *Yersinia pestis* infections as well as in tularemia and in TB as an antibacterial of 2nd line. The rest of the drugs are used in prolonged or life-threatening Gram-negative infections such as infective endocarditis and severe sepsis.

Adverse Effects:

Aminoglycosides have several adverse effects, which can limit their use in everyday practice. They are very ototoxic and are contraindicated in the hard of hearing and especially in the elderly unless no alternative agent is available. They are also nephrotoxic and as such are to be used with caution in anyone with decreased renal function or end stage renal disease. Lastly they are teratogenic and are not to be used by pregnant women. Gentamicin can be used, if no other options are immediately available as it is the least teratogenic. If so drug serum concentration should be monitored.

MACROLIDES

Macrolides are the **drug of choice in penicillin allergic patients with Gram-positive infections**. They work by **blocking translocation of a growing peptide** due to their **binding to the 50S subunit of the bacterial ribosome**. Macrolides are also used for ***Bordetella*** infections and a multitude of atypical pneumonia infections such as ***Legionella***, ***Mycoplasma*** and ***Chlamydia*** **infections**. The group is made up of three popular members, **erythromycin**, **clarithromycin** and **azithromycin**. Gram-positive cover is greatest with clarithromycin and weakest with azithromycin. To the contrary it is with azithromycin that Gram-negative cover is greatest and weakest with clarithromycin. Erythromycin is the middle of Gram-positive and Gram-negative coverage and tends to be less used today due to its stronger adverse effect profile.

Adverse Effects:

Macrolides are universally known and sometimes used as motility agents. Through their activation of motilin in the gastrointestinal tract they help speed up bowel transit time. A skin rash is possible after macrolide use but thankfully the effect is usually only evident in the first couple of administrations. Another rarer but potentially life-threatening effect is arrhythmia formation. Great caution should be used in patients with a prolonged QT interval and in fact macrolides are contraindicated in patients with a congenital QT prolongation.

TETRACYCLINES

Tetracyclines are a very diverse group of antibiotics, both for their range of bacterial coverage and their numerous side effects. Throughout the years there have been many formulations of tetracyclines that have been pushed through by pharmaceutical companies but **tetracycline**, **doxycycline** remain some of the most well-known representatives. Their main mechanism of action is **preventing the attaching of tRNA to the 30S subunit** and thus **inhibiting protein synthesis**. The **coverage of this group of antibiotics is quite large** indeed. They are often used in

Lyme disease, rickettsial and chlamydial infections. They can be used in *Legionella*, *Ureaplasma* and *Helicobacter pylori* infections as well. **Tigecyklin** is first drug of glycylciclines. It has a broad spectrum of activity against **many Gram-positive, Gram-negative** (enterobacteriae, *Pseudomonas aeruginosa* and *Acinetobacter* spp.), and **anaerobic organisms**. Coverage includes many multidrug-resistant strains of Gram-positive organisms, such as MRSA and MRSE, penicillin-resistant *Streptococcus pneumoniae*, and VRE species. Tigecycline has also shown activity against organisms that have developed resistance to tetracycline via various mechanisms.

Adverse Effects:

Perhaps even more famous than the group members themselves are the multitude of side effects that tetracycline can cause. Discoloration of teeth is a particularly worrying one, and as such causes tetracyclines to be contraindicated in children in age less than 8 years. Tetracyclines are also contraindicated in pregnancy due to their effect on the growing skeleton of the fetus. Ototoxicity and recurring tinnitus are more rare adverse effects. Of particular note is the phototoxicity that comes with this group, a clear warning to patients who use the antibiotic for acne management, to avoid sunlight.

FLUOROQUINOLONES

Another group with a **wide range of microbial coverage** is fluoroquinolones. This group of antibiotics works by **inhibition of bacterial enzymes** known as **topoisomerases**. Topoisomerases act to uncoil DNA when it becomes supercoiled, a state which can cause DNA damage and subsequent cell death. Much like cephalosporins, fluoroquinolones also have generations but unlike cephalosporins, fluoroquinolones gain Gram-positive coverage as the generation increases.

First generation: Nalidixic acid (not fluorinated and thus not a true fluoroquinolones) is no longer used due to its narrow spectrum of activity.

Second generation: Ciprofloxacin, ofloxacin and norfloxacin. A widely used group that counters *Pseudomonas* infections, *Bacillus anthracis*, *E. coli*, *Salmonella* and other Gram-negative systemic infections.

Third generation: Levofloxacin, ofloxacin are alternatives in Streptococcus *pneumoniae* and other community-acquired pneumonias due to their infrequent dosing, preferable on an outpatient basis.

Fourth generation: Moxifloxacin (which along with levofloxacin) is known as a respiratory quinolone. It is a popular choice with respiratory infections, especially if the potential for anaerobic seeding is a real threat.

Adverse Effects:

A wide spectrum of coverage does not come without a hefty fine in the adverse effect department. Fluoroquinolones are known for their propensity to cause GI upset and debilitating myalgias. Like tetracyclines they are also potentially teratogenic by targeting bones and cartilage in a growing fetus. In fact the potential damage is not ameliorated later on in childhood and thus fluoroquinolones are contraindicated in patients under the age of 18-20. Care should also be taken in patients taking prednisone concurrently, as reports of tendinitis and even tendon rupture are very prevalent not only in literature but the clinic as well.

FOLATE ANTAGONISTS

This section deals with folate antagonists as an over-encompassing summary. **Bacteria require folate for DNA synthesis** and without it cannot replicate. The first agents are **sulfonamides** which **block the conversion of PABA** (para-aminobenzoic acid) **into dihydropteroic acid by inhibiting dihydropteroate synthase**. The most often used is sulfamethoxazole but sulfasalazine (for inflammatory bowel diseases) and sulfadiazine (with silver for topical use) are prescribed as well. The other agent of the folate antagonists is **trimethoprim**. It **inhibits the conversion of dihydrofolic acid** (a product after the above dihydropteroic acid) **to tetrahydrofolic acid via blockage of dihydrofolate reductase**. These drugs are bacteriostatic when given alone but given in concert they block folate synthesis at multiple steps, thus making them bactericidal. When combined (they are very rarely given alone due to quick resistance buildup) they destroy a multitude of bacterial agents. They kill **UTI bacteria, *Salmonella*, *Shigella*, *Pneumocystis jirovecii* and act as a prophylaxis and treatment of toxoplasmosis infections**. They often find other, more off-label, uses as antibiotics against skin MRSA, *Proteus* and even ***Stenotrophomonas maltophilia*** *infections*.

Adverse Effect:

Folate is not only required by bacteria, but also by their descendants, eukaryotes. As such they cause a host of adverse effects in patients. Nephrotoxicity is a major problem in those with reduced renal capacity and those in end stage renal disease. Like tetracyclines they are also phototoxic and can cause hypersensitivity reactions. An important adverse effect is the triad of pancytopenia (megaloblastic anemia, leukocytopenia and thrombocytopenia). This is a direct side effect of the bone marrow suppression caused by trimethoprin, as folic acid is extremely important in hematopoiesis.

METRONIDAZOL

Originally introduced for treatment of ***Trichomonanas vaginalis*** **infections**, however, it is also effective in treatment **amebiasis**, **giardiasis** and **serious anaerobic infections** including also the infection caused by ***C. difficile***.

ANTITUBERCULOTICS

Antituberculotics are a **heterogeneous group of drugs** specifically designed to work against mycobacteria, and ever more specifically, against *Mycobacterium tuberculosis*. Tuberculosis is often treated with four major drugs which make up the nice mnemonic **RIPE (Rifampin, Isoniazid, Pyrazinamide, Ethambutol)**. The standard treatment runs for **two months with all four agent** and then **four months with two**, slight modifications can be made for multidrug resistant and extensively drug resistant TB but a complete discussion is beyond the scope of this book.

Rifampin is the first drug to consider in the fight against tuberculosis. It acts by **inhibiting the DNA-dependent RNA polymerase in mycobacterial cells**. This prevents protein transcription and thus prevents the ability for the bacteria to correctly produce proteins. The drug also has activity in *Haemophilus* and *Neisseria meningitidis* prophylaxis where it can be used on close contacts of infected patients. While a very widely used and important drug, rifampin is perhaps best known for its numerous side effects. The drug is a cytochrome P450 inducer, which causes many interactions with other drugs by decreasing their concentration in plasma. It is also hepatotoxic

but perhaps the most famous of all are the orange secretions. Body fluids such as tears, urine, sweat, and even saliva are tinged with a nonhazardous orange hue.

Isoniazid is another very important antituberculotic and is part of the two-for-four month regimen after the initial use of all four drugs for two months (the other being rifampin). It works by decreasing the synthesis of mycolic acids. While the correct target has not been completely elucidated, inhibition of fatty acid synthase II has been shown to be directly affected. Isoniazid is also the only drug that can be used for solo prophylaxis against TB (other agents tend to build a resistance in tuberculosis strains). The drug has been known to cause neurotoxicity in patients by inhibiting vitamin B6 pathway enzymes and thus concomitant administration of vitamin B6 can be used to mitigate this toxicity. The other important toxicity is hepatotoxicity, which is crucial to consider when the patient is an alcoholic, a common predisposing condition, and also when the drug is administered with other hepatotoxic substances.

Pyrazinamide is the next agent in the armamentarium against tuberculosis. It acts through an uncertain mechanism but a hypothesis of fatty acid synthase I inhibition has been put forward by the scientific community. Notable toxicities are hepatotoxicity (a pattern should be emerging by now), GIT discomfort and exacerbation of acute intermittent porphyria.

Ethambutol is the last drug in the tetrad of RIPE. It blocks arabinosyltransferase and thus inhibits the polymerization of carbohydrates in the mycobacterial cell wall. Common side effects include decreased uric acid excretion, can help precipitate gout, and retrobulbar optic neuropathy that can manifest as decreased visual acuity and central scotomas.

A multitude of second line agents are present for **multidrug resistant strains** of tuberculosis and are only listed here for the sake of completion. Many of them are in fact already covered earlier in the chapter, thus obviating the need for a meticulous search.

2nd line agents: streptomycin, capreomycin, moxifloxacin, azithromycin, cycloserine, ethionamide.

SUSCEPTIBILITY TESTING TO ANTIMICROBIALS

The main purpose is to test sensitivity (susceptibility) of a given microorganism to a known concentration of the drugs and to select the most appropriate drug for treatment. The European Committee for Antimicrobial Susceptibility Testing (EUCAST) published guidelines for performance and interpretation of antibiotic susceptibility testing.

BASIC METHODS

The qualitative method is also known as the **disk diffusion method** (fig. 11.2, B1, C1, D1). Antibiotic molecules diffuse out from a disk into the agar creating a concentration gradient of antibiotics. A particular concentration of the antibiotic inhibits bacterial growth (overnight, 37˚C) so an **inhibition zone (IZ)** around the antibiotic disk is detected. (fig. 11.3 and 11.4). Depending on the diameter (mm) of the inhibition zone the results are categorized as **sensitive** or **resistant**. The zones can be read visually or automatically. The results can be influenced by numerous factors (e.g. bacterial inoculum size, thickness of agar) so all the parameters should be standardized.

Quantitative methods are usually performed through the **broth microdulition method** (fig. 11.2, B.2, C.2) or **agar dilution method**. These are valuable procedures to conduct if we need to determine the **minimum inhibitory concentration (MIC).** The MIC is the lowest concentration [μg/ml] of antibiotic that inhibits visible growth of a microorganism and is the required

concentration for treatment of a critical infection. Depending on the pharmacokinetic and pharmacodynamic data of the antibiotic and organ affected, the therapy is suitably customized.

FIGURE 11.5.

Figure 11.5. *Disk diffusion and broth microdilution methods. To perform the disk diffusion method, a bacterial inoculum is prepared in nutrient broth (A) and streaked over the agar surface with a cotton swab (B1). Inoculum is dried for 15 minutes and the disks are positioned and cultivated overnight at 37˚C (C1). Size of IZs are read and interpreted. To perform the broth microdilution method microplate wells are inoculated with a bacterial inoculum (B2). After overnight cultivation the MIC is read. The MIC corresponds to the wells with lowest concentration of antibiotic without visible growth. (C2). The darker wells symbolize growth of culture.*

INTERPRETATIONS OF RESULTS

Organisms are categorized as **susceptible** or **resistant** to a drug depending on size of the IZ on the agar or the MIC in the microplate wells. The interpretation is based on a globally standardized consensus. For example, if the IZ for a particular antibiotic is 25 mm and the breaking point for the antibiotic is 22mm, then the zone is larger than the breaking point for the antibiotic and thus the bacterial isolate is susceptible to the antibiotic. However, if the breaking point for MIC of a susceptible bacteria is 8 µg/ml and the bacterial culture growth is visible in the first well of the second microplate row with concentration of the antibiotic 256 µg/ml, then the bacteria is considered to be resistant to the antibiotic.

FIGURE 11.6.

Figure 11.6. *Disk diffusion method. Isolate of Staphylococcus aureus susceptible to all antibiotics tested (A). IZ of the isolate around a co-trimoxazol disk (B). Pseudomonas aeruginosa is naturally resistant to ampicillin (at position 9 o'clock) and co-trimoxazol (at position 11 o'clock)(C). The isolate is susceptible to the rest of antibiotics.*

FIGURE 11.7.

Figure 11.7. *Klebsiella pneumoniae isolate susceptible to all the antibiotics tested except ampicillin to which it has natural resistance (plate on the left, disk at position 12 o'clock).*

5.5. OTHER METHODS; PROCEDURES & INTERPRETATION

E-test: combination of the quantitative and qualitative methods. A concentration gradient of an antibiotic on a polymer strip is laid on the surface of an inoculated agar plate creating an elliptical zone of inhibition. The narrowest end shows the lowest amount of antibiotic needed to inhibit growth i.e. MIC (figure 11.5).

FIGURE 11.8.

Figure 11.8. *Diagram of E-test indicated with the arrow where should be read the result (A). MIC to imipenem in Klebsiella pneumonia isolate (8 ml/l) indicated resistance to the antibiotics (B).*

Other methods are depicted in figure 11.6.

FIGURE 11.9.

Figure 11.9. *The amplicon of mecA gene in S. aureus isolates from patient 1 and 2 (A), detection of K. pneumoniae isolate producing extended spectrum beta lactamases (ESBL) - an inhibitor of beta lactamases contained in the central disk show distorsions of the IZ around the betalactams (B),*

erythromycin disk on the right induce the resistance of clindamycin (chopped IZ on the side of erythromycin).

Automated antibiotic systems
Fully automated systems which reduce workload and facilitate efficient workflow for incubating and reading susceptibility plates or MIC determination (e.g. VITEK).

MALDI-TOFF technology
Besides microbe identification, it is useful tool especially for the detection of enzymatic resistance (e.g. confirmation production of carbapenemases within 3 hours in clinical isolates of *Acinetobacter baumannii*, *Pseudomomas* spp. and *Enterobacteriaceae*).

Drug susceptibility of *Mycobacterium tuberculosis* can be determined either by observation of growth or metabolic inhibition in a medium containing anti-tuberculosis drug, or by detection, at the molecular level, of mutations in the genes related to drug action. From a technical standpoint, drug susceptibility is determined on the basis of growth (or metabolic) inhibition induced by the drug by the means of: 1) macroscopic observation of growth in drug-free and drug-containing media; 2) detection or measurement of the metabolic activity or products; 3) lysis with mycobacteriophage; and 4) detection of genetic mutations using molecular techniques.