Clinical Microbiology

Antibiotics are medicines used to fight bacterial infections. There are different types of antibiotics. Each type is only effective against certain bacteria. An antibiotic sensitivity test can help find out which antibiotic will be most effective in treating your infection. The test can also be helpful in finding a treatment for antibiotic-resistant infections. Antibiotic resistance happens when standard antibiotics become less effective or ineffective against certain bacteria. Antibiotic resistance can turn once easily treatable diseases into serious, even life-threatening illnesses.

Antibiotic sensitivity testing or **Antibiotic susceptibility testing** is the measurement of the susceptibility of bacteria to antibiotics. It is used because bacteria may have resistance to some antibiotics. Sensitivity testing results can allow a clinician to change the choice of antibiotics from empiric therapy, which is when an antibiotic is selected based on clinical suspicion about the site of an infection and common causative bacteria, to directed therapy, in which the choice of antibiotic is based on knowledge of the organism and its sensitivities. Sensitivity testing usually occurs in a medical laboratory, and may be based on culture methods that expose bacteria to antibiotics, or genetic methods that test to see if bacteria have genes that confer resistance. Culture methods often involve measuring the diameter of areas without bacterial growth, called zones of inhibition, around paper discs containing antibiotics on agar culture dishes that have been evenly inoculated with bacteria. The minimum inhibitory concentration, which is the lowest concentration of the antibiotic that stops the growth of bacteria, can be estimated from the size of the zone of inhibition.

Antibiotic susceptibility testing has occurred since the discovery of the beta-lactam antibiotic penicillin. Initial methods were phenotypic, and involved culture or dilution. The Etest, an antibiotic impregnated strip, has been available since the 1980s, and genetic methods such as polymerase chain reaction (PCR) testing have been available since the early 2000s. Research is ongoing into improving current methods by making them faster or more accurate, as well as developing new methods for testing, such as microfluidics. Antibiotic sensitivity testing, or AST, is a widely-used method of evaluating antibiotic resistance and determining patient treatment plans in clinical settings. There are a number of different methods of AST such as agar dilution, broth dilution and disc diffusion assays. The disc diffusion or ‘Kirby-Bauer’ method involves spreading bacteria on an agar plate and placing paper discs impregnated with antibiotic on the plate. After incubation, the growth of bacteria is observed. Areas around the antibiotic disc where no bacterial growth can be seen are known as ‘zones of inhibition’. These zones show that an antibiotic has been successful in stopping bacterial growth or killing the bacteria. By measuring the diameter of these zones, we can compare the efficacy of antibiotics and monitor antimicrobial resistance.

Sensitivity analysis, also called susceptibility testing, helps your doctor find the most effective antibiotic to kill an infecting microorganism. Infecting microorganisms are organisms such as bacteria or fungi that invade your body and cause an infection. A sensitivity analysis is a test that determines the “sensitivity” of bacteria to an antibiotic. It also determines the ability of the drug to kill the bacteria. The results from the test can help your doctor determine which drugs are likely to be most effective in treating your infection. Doctors use sensitivity testing to determine the right antibiotic treatment for an infection and to monitor changes in bacterial resistance to antibiotics.

Uses

In clinical medicine, antibiotics are most frequently prescribed on the basis of a person's symptoms and medical guidelines. This method of antibiotic selection is called empiric therapy, it is based on knowledge about what bacteria cause an infection, and what antibiotics bacteria may be sensitive or resistant to in a geographical area. For example, a simple urinary tract infection might be treated with trimethoprim/sulfamethoxazole. This is because *Escherichia coli* is the most likely causative bacterium, and may be sensitive to that combination antibiotic. However, bacteria can be resistant to several classes of antibiotics. This resistance might be because a type of bacteria has intrinsic resistance to some antibiotics, because of resistance following past exposure to antibiotics, or because resistance may be transmitted from other sources such as plasmids. Antibiotic sensitivity testing provides information about which antibiotics are more likely to be successful and should therefore be used to treat the infection.

Antibiotic sensitivity testing is also conducted at a population level in some countries as a form of screening. This is to assess the background rates of resistance to antibiotics (for example with methicillin-resistant *Staphylococcus aureus*), and may influence guidelines and public health measures.

The test is done by taking a sample from the infected site. The most common types of tests are listed below.

- **Blood culture**
  A health care professional will take a blood sample from a vein in your arm, using a small needle. After the needle is inserted, a small amount of blood will be collected into a test tube or vial.
- **Urine culture**
  You will provide a sterile sample of urine in a cup, as instructed by your health care provider.
- **Wound culture**
  Your health care provider will use a special swab to collect a sample from the site of your wound.
- **Sputum culture**

You may be asked to cough up sputum into a special cup, or a special swab may be used to take a sample from your nose.

- **Throat culture**
  Your health care provider will insert a special swab into your mouth to take a sample from the back of the throat and tonsils.

Testing procedure:

Sensitivity analysis starts with a bacterial sample. The doctor gets this sample by sampling the infected area. The doctor can sample any area that has an infection.

Samples may be taken from:

- blood
- urine
- sputum (spit)
- inside the cervix
- a pus-containing wound

The doctor will send the sample to a laboratory, where it'll be spread on a special growing surface. The grown bacteria is known as a culture, and bacteria in the culture will grow and multiply. During the culture process, pathogens are isolated (separated out from any other microbes present). Each pathogen, if present, is identified using biochemical, enzymatic, or molecular tests. Once the pathogens have been identified, it is possible to determine whether susceptibility testing is required. Susceptibility testing is not performed on every pathogen; there are some that respond to established standard treatments. For example, strep throat, an infection caused by *Streptococcus pyogenes* (also known as group A streptococcus), can be treated with ampicillin and does not require a test to predict susceptibility to this class of antibiotics. The bacteria will form colonies, or large groups of bacteria, that will each be exposed to different antibiotics.

These colonies can be susceptible, resistant, or intermediate in response to the antibiotics:

- **Susceptible** means they can’t grow if the drug is present. This means the antibiotic is effective against the bacteria.
- **Resistant** means the bacteria can grow even if the drug is present. This is a sign of an ineffective antibiotic.
- **Intermediate** means a higher dose of the antibiotic is needed to prevent growth.

Susceptibility testing is performed on each type of bacteria or fungi that may be relevant to the individual's treatment and whose susceptibility to treatment may not be known. Each pathogen is tested individually to determine the ability of antimicrobials to inhibit its growth. This is can be measured directly by bringing the pathogen and the antibiotic together in a growing environment, such as nutrient media in a test tube or agar plate, to observe the effect of the antibiotic on the growth of the bacteria. Resistance can also be determined by detection of a gene that is known to cause resistance to specific antibiotics.

Methods:

Testing for antibiotic sensitivity usually occurs in a laboratory. Once a bacterium has been identified following microbiological culture, antibiotics are selected for susceptibility testing. Susceptibility testing methods are based on exposing bacteria to antibiotics and observing the response (phenotypic testing), or specific genetic tests (genetic testing).

Methods used may be qualitative, meaning a result indicates resistance is or is not present; or quantitative, using a minimum inhibitory concentration (MIC) to describe the concentration of antibiotic to which a bacterium is sensitive. There are nearly a dozen factors that can affect the results of antibiotic sensitivity testing, including failure of the instrument, temperature, moisture, and potency of the antimicrobial agent. Quality control (QC) testing helps to ensure the accuracy of test results; QC guidelines are available from the Clinical and Laboratory Standards Institute (CLSI).

Phenotypic methods

Testing based on exposing bacteria to antibiotics uses agar plates or dilution in agar or broth. The selection of antibiotics will depend on the organism grown, and the antibiotics that are available locally. To ensure that the results are accurate, the concentration of bacteria that is added to the agar or broth (the inoculum) must be standardized. This is accomplished by comparing the turbidity of bacteria suspended in saline or broth to McFarland standards—solutions whose turbidity is equivalent to that of a suspension containing a given concentration of bacteria. Once an appropriate concentration has been reached, which can be determined by visual inspection or by photometry, the inoculum is added to the growth medium.

The disc diffusion method involves selecting a strain of bacteria, placing it on an agar plate, and observing bacterial growth near antibiotic-impregnated discs. This is also called the Kirby-Bauer method, although modified methods are also used. Small paper discs containing antibiotics are placed onto a plate upon which bacteria are growing. If the antibiotic inhibits microbial growth, a clear ring, or zone of inhibition, is seen around the disc. The bacteria are classified as sensitive, intermediate, or resistant to an antibiotic by comparing the diameter of the zone of inhibition to defined thresholds which correlate with MICs. Mueller-Hinton agar is frequently used in this antibiotic susceptibility test. Standards exist as to how the testing is performed, and how the test results are interpreted. The CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide standards for the type and depth of agar, temperature of incubation, and method of analysing results. Disc diffusion is considered the cheapest and most simple of the methods used to test for susceptibility, and is easily adapted to testing newly available antibiotics or formulations. Some slow-growing and fastidious bacteria cannot be accurately tested by this method, while others, such as *Streptococcus* species and *Haemophilus influenzae*, can be tested but require specialized growth media and incubation conditions.

Gradient methods, such as Etest, use a plastic strip placed on agar. A plastic strip impregnated with different concentrations of antibiotics is placed on a growth medium, and the growth medium is viewed after a period of incubation. The minimum inhibitory concentration can be identified based on the intersection of the teardrop-shaped zone of inhibition with the marking on the strip. Multiple strips for different antibiotics may be used. This type of test is considered a diffusion test. In agar and broth dilution methods, bacteria are placed in multiple small tubes with different concentrations of antibiotics. Whether a bacterium is sensitive or not is determined by visual inspection or automatic optical methods, after a period of incubation. Broth dilution is considered the gold standard for phenotypic testing. The lowest concentration of antibiotics that inhibits growth is considered the MIC.

Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) is another method of phenotypic susceptibility testing. This is a form of time-of-flight mass spectrometry, in which the molecules of a bacterium are subject to matrix-assisted laser desorption. The ionised particles are then accelerated, and spectral peaks recorded, producing an expression profile, which is capable of differentiating specific bacterial strains after being compared to known profiles. This includes, in the context of antibiotic susceptibility testing, strains such as beta-lactamase producing *E coli*. MALDI-TOF is rapid and automated. There are limitations to testing in this format however; results may not match the results of phenotypic testing, and acquisition and maintenance is expensive. Automated systems exist that replicate manual processes, for example, by using pictures and software analysis to report the zone of inhibition in diffusion testing, or dispensing samples and determining results in dilutional testing.

Genetic methods

Genetic testing, such as via polymerase chain reaction (PCR), DNA microarray, DNA chips, and loop-mediated isothermal amplification, may be used to detect whether bacteria possess genes which confer antibiotic resistance. An example is the use of PCR to detect the mecA gene for beta-lactam resistant *Staphylococcus aureus*. Other examples include assays for testing vancomycin resistance genes vanA and vanB in *Enteroccocus* species, and antibiotic resistance in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. These tests have the benefit of being direct and rapid, as compared with observable methods, and have a high likelihood of detecting a finding when there is one to detect. However, whether resistance genes are detected does not always match the resistance profile seen with phenotypic method. The tests are also expensive and require specifically trained personnel.

Polymerase chain reaction is a method of identifying genes related to antibiotic susceptibility. In the PCR process, a bacterium's genome is denatured. Primers specific to a sought-after gene are added to a solution containing the DNA, and a DNA polymerase is added alongside a mixture containing molecules that will be needed (for example, nucleotides and ions). If the relevant gene is present, every time this process runs, the quantity of the target gene will be doubled. After this process, the presence of the genes is demonstrated through a variety of methods including electrophoresis, southern blotting, and other DNA sequencing analysis methods. DNA microarrays and chips use the binding of complementary DNA to a target gene or nucleic acid sequence. The benefit of this is that multiple genes can be assessed simultaneously.

Reporting

The results of the testing are reported as a table, sometimes called an antibiogram. Bacteria might be marked as sensitive, resistant, or having intermediate resistance to an antibiotic. Specific patterns of drug resistance or multi drug resistance may be noted, such as the presence of an extended-spectrum beta lactamase.

The sensitive, resistant or intermediate resistance to antibiotics is reported based on the minimum inhibitory concentration. It is compared to known values for a given bacterium and antibiotic. For example, the CLSI defines *Streptococcus pneumoniae* as sensitive to penicillin if MICs are ≤0.06 μg/ml, intermediate if MICs are 0.12 to 1 μg/ml, and resistant if MICs are ≥2 μg/ml. Such information may be useful to the clinician, who can change the empiric treatment, to a tailored treatment that is directed only at the causative bacterium. Sometimes, whether an antibiotic is marked as resistant is also based on bacterial characteristics that are associated with known methods of resistance such as the potential for beta-lactamase production.

Results for a sensitivity analysis:

Once the bacterial cultures have been grown and tested with antibiotics, the doctor can analyze the results. These results can help determine the best antibiotic to treat your infection.

Susceptible

The doctor will usually choose an appropriate drug from the report that was listed as “susceptible,” meaning it can fight the bacteria.

Intermediate

The patient be prescribed a drug from the “intermediate” group if there are no known drugs available in the susceptible group. The patient will likely have to take a higher dosage and for a longer time period if the patient is taking a drug from the intermediate group. The patient may also experience medication side effects.

Resistant

An antibiotic that bacteria have shown resistance to shouldn’t be used to treat your infection. The doctor will decide which drug is best if several antibiotics are shown to be effective in killing the microorganism causing the infection. The patient may be prescribed a combination of antibiotics if a bacterium is “resistant” to all of the drugs that are usually used to treat an infection. This combination of drugs is meant to work together to fight the bacteria. Drugs in this category can be more expensive and may have to be given intravenously (through a needle in the vein). The patient may also have to take the combination of drugs for an extended time period.

Further testing

Some infections may require further testing because it’s known that the drugs normally used to treat the bacteria or fungi causing the infection aren’t always effective. It is also possible for the sample taken from the infection to have more than one microorganism. Susceptibility testing may be used to figure out which antibiotic or combination of antibiotics will be most effective in treating the different types of bacteria causing the infection.