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Tests for identification of bacteria& Susceptibility testing

BACTERIAL IDENTIFICATION

The isolated bacteria are further processed through one or few of the procedures mentioned below so as to identify the bacteria

* Staining of the isolated bacteria
* Motility testing
* Biochemical testing
* Serological tests
* Phage typing
* Identification disc testing
* Semiautomated and Automated identification systems
* Molecular techniques

Staining of the isolated bacteria: The isolated bacteria are stained by various methods depending upon the bacteria in focus. Various staining techniques are as follow:

1. Gram staining: differentiates bacteria into two types
Gram positive pathogenic bacteria are staphylococci, streptococci, pneumococci, etc
Gram negative bacteria can be either cocci or bacilli. Gram negative
pathogenic bacteria commonly encountered are E.coli, Klebsiella, Salmonella spp, shigella, etc
2. Albert staining: is performed in case if one suspects a Corynebacterium spp.
3. Acid fast staining: is performed in cases suspected of Mycobacterial
infection. Eg. Tuberculosis, leprosy, etc.
4. Special staining is necessary in case of spirochetes and other organisms.
(ii) **Motility testing**:Motility testing is performed and is then observed under the microscope.
(iii) Biochemical tests: many biochemical tests available for bacterial identification. used biochemical tests are:

A. Catalase test

The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive Micrococcaceae from catalas enegative Streptococcaceae. It is also valuable in speciation of certain gram positives such as Aerococcus urinae (positive) from Aerococcus viridans (negative) and gram negative.

B. Coagulase test

The coagulase test differentiates strains of Staphylococcus aureus from other coagulase-negative species,clumping or clots of any size indicate a positive response. there are several other species of Staphylococcus (produce clumps of cells in the slide test) which are positive for coagulase activity. S.schleiferi and S. lugdunensis may give positive results.

C. Oxidase test

is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called

indophenol oxidase.In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product.

D. Indole test

the ability of an organism to degrade the amino acid tryptophan and produce indole. It is designed to distinguish among members of the family Enterobacteriaceae.

E. Citrate Test

the ability of bacterial isolate to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the PH of the medium is demonstrated by the color change of a pH indicator. The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates.

F. Urease test

identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide.

G. Serology:

It detection of antigens by enzyme or fluorescence immunoassays. it is also used to confirm identification obtained by other methods.

H. Phage typing

is a method used for detecting single strains of bacteria. It is used to trace the source of outbreaks of infections. The viruses that infect bacteria are called bacteriophages ("phages" for short) and some of these can only infect a single strain of bacteria. These phages are used to identify different strains of bacteria within a single species. Molecular techniques.

I. DNA-DNA hybridisation and DNA base sequencing. These methods are Amplification techniques like Polymerase chain reaction, ligase chain reaction, strand displacement amplification, and nucleic acid sequence based amplification are being used in clinical laboratories for direct detection of bacteria.

Susceptibility testing:

* is used to determine which antimicrobials will inhibit the growth of the bacteria or fungi causing a specific infection. The results from this test will help a healthcare practitioner determine which drugs are likely to be most effective in treating a person's infection.
* Ordered at the same time as a culture of a potentially infected site, such as a wound, urine, or blood culture.

- Some types of infections may require testing because the bacteria or fungi isolated from an infection site are known to have unpredictable susceptibility to the drugs usually used to treat them. Some examples include staphylococci ("staph") and Pseudomonas aeruginosa.
- to determine which antibiotic or antibiotic combinations will be most effective in treating all the different types of bacteria causing the infection.

* when an infection does not respond to treatment to see if the pathogen has developed resistance and to determine which antimicrobial drug would be more effective.

- A sample for culture and susceptibility testing should be collected before the start of any treatment with an antimicrobial drug, unless the test is used to monitor the effectiveness of treatment.

* Results of the testing are usually reported as:

* Susceptible — likely, may be an appropriate choice for treatment

- Intermediate — may be effective at a higher dosage, or more frequent dosage, or effective only in specific body sites where the antibiotic penetrates to provide adequate concentrations
- Resistant — not effective at inhibiting the growth of the organism in a laboratory test; may not be an appropriate choice for treatment

Bacterial cultures usually require 24-48 hours to grow the pathogen and obtain a pure culture for further testing. Cultures for fungus and tuberculosis may take much longer — up to 6 to 8 weeks since these microbes grow more slowly.

An antibiotic should have the following characteristics:

- It should be toxic to the infecting organism while harmless to the host cells.
- It should stay in toxic form for a sufficient amount of time to affect the infecting microorganism.
- Sufficient amounts of it should reach the site of infection to kill the infecting agent.
- The infecting agent should be sensitive to it.

To determine the sensitivity of microorganisms to antibiotics. Two such procedures are :

1- The Kirby-Bauer Disc Method:

This method is also called the agar diffusion method or the disk diffusion method. The procedure followed is simply that a filter disk impregnated with an antibiotic is applied to the

surface of an agar plate containing the organism to be tested and the plate is incubated at 37°C for 24-48 hours. As the substance diffuses from the filter paper into the agar, the concentration decreases as a function of the square of the distance of diffusion. At some particular distance from each disk, the antibiotic is diluted to the point that it no longer inhibits microbial growth.

The effectiveness of a particular antibiotic is shown by the presence of growth-inhibition zones. These zones of inhibition (ZOIs) appear as clear areas surrounding the disk from which the substances with antimicrobial activity diffused.

The diameter of the ZOI can be measured with a ruler and the results of such an experiment constitute an antibiogram.
The agar diffusion method uses commercially available filter paper disks, each containing a defined concentration of a specific antibiotic. The relative effectiveness of different antibiotics provides the basis for a sensitivity spectrum of the organism. This information, together with various pharmacological considerations, is used in the selection of an antibiotic for treatment.
The size of the zone may be affected by:
1-the density or viscosity of the culture medium,
2- the rate of diffusion of the antibiotic,
3- the concentration of the antibiotic on the filter disc,
4- the sensitivity of the organism to the antibiotic,
5- the interaction between the antibiotic and the medium.

Interpretation of zones of inhibition (in mm) for Kirby-Bauer antibiotic susceptibility test.

Antibiotic	Disk Conc.	Diameter of zone of inhibition (ZOI)		
		Resistant	Intermediate	Susceptible
Amikacin	10 μg	≤11	12-13	≥14
Ampicillin	10 μg	≤11	12-13	≥14
Bacitracin	10 units	≤8	9-11	≥13
Cephalothin	30 μg	≤14	15-17	≥18
Chloramphenicol	30 μg	≤12	13-17	≥18
Clindamycin	2 μg	≤14	15-16	≥17
Erythromycin	15 μg	≤13	14-17	≥18
Gentamicin	10 μg	≤12	13-14	≥15
Kanamycin	30 μg	≤13	14-17	≥18
Lincomycin	2 μg	≤9	10-14	≥15
Methicillin	5 μg	≤9	10-13	≥14
Nalidixic acid	30 μg	≤13	14-18	≥19
Neomycin	30 μg	≤12	13-16	≥17
Nitrofurantoin	0.3 mg	≤14	15-16	≥17
Penicillin				
vs. staphylococci	10 units	≤20	21-28	≥29
vs. other organisms	10 units	≤11	12-21	≥22
Polymyxin	300 units	≤8	9-11	≥12
Streptomycin	10 μg	≤11	12-14	≥15
Sulfonamides	0.3 mg	≤12	13-16	≥17
Tetracycline	30 μg	≤14	15-18	≥19
Vancomycin	30 μg	≤9	10-11	≥12

2- The Minimum Inhibitory Concentration (MIC)

is used to determine the lowest concentration that is needed to kill the pathogen at the site of infection and still inhibits the growth of a pathogen, can be determined using serial dilution methods and gives an indication of the dosage of that antibiotic that should be effective in controlling the infection in the patient. A standardized microbial inoculum is added to the tubes containing serial dilutions of an antibiotic, and the growth of the microorganism is monitored as a change in turbidity.

MIC can even be performed on body fluids without isolating and identifying the pathogenic microorganisms. For example, blood or cerebrospinal fluid containing an infecting microorganism can be added to tubes containing various dilutions of an antibiotic and a suitable growth medium. An increase in turbidity would indicate that the microorganism is growing and that the antibiotic at that concentration was ineffective in inhibiting microbial growth.

Conversely, a lack of growth would indicate that the pathogenic microorganisms were susceptible to the antibiotic at the given concentration. **The Minimum Inhibitory Concentration (MIC) Method:**

there are three main reagents necessary to run this assay: the media, an antimicrobial agent, and the microbe being tested. The most commonly used media is cation-adjusted Mueller Hinton Broth, due to its ability to support the growth of most pathogens and its lack of inhibitors towards common antibiotics.

Calculate concentration of antibiotic per ml through:

MIC= lowest concentration of A.B inhibits growth + highest concentration of A.B allow growth of M.O
2