Manage TB
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Lecture – 11
Phenotypic drug susceptibility testing in Tuberculosis

Hello, welcome to this session on Phenotypic drug susceptibility testing in Tuberculosis and Dr. Gomathi from the National Institute for Research in Tuberculosis Chennai.

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Let us first look at the usefulness of drug susceptibility test, which is in short called as a DST. DST offers guidance to design a suitable regimen at the time of treatment initiation or for change of regimen during treatment, when the patient is failing treatment.

It enables detection of emerging acquired drug resistance in a patient who is on treatment, it provides information on the level of resistance to the drug and level of cross resistance among the same class of drugs and prevalence of resistance in any geographical setting. It enables recalculation of the epidemiological cut off values that may necessitate the need to alter the drug concentrations used in the in vitro tests.

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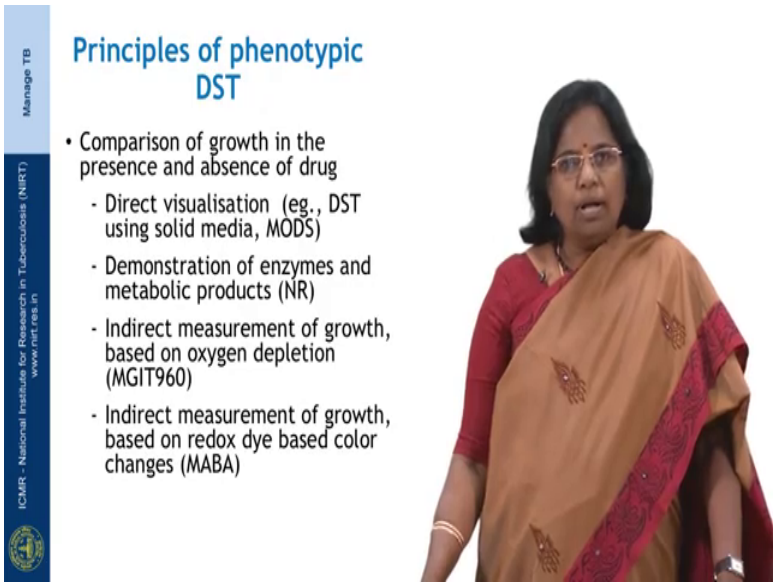

So, any phenotypic DST compares growth in the presence and absence of the drug, which could be by direct visualization of the growth example being the DST using solid media or the microscopically observe drug susceptibility test or by means of demonstration of enzymes and metabolic products, example being the nitrate reductase test or by indirect measurement of growth, based on oxygen depletion in the medium. Example being the MGIT 960 DST or by indirect measurement of growth based on redox dye based colour changes example being the microscopic alamar blue assay.

We will be looking at all these tests in detail in the coming slides.

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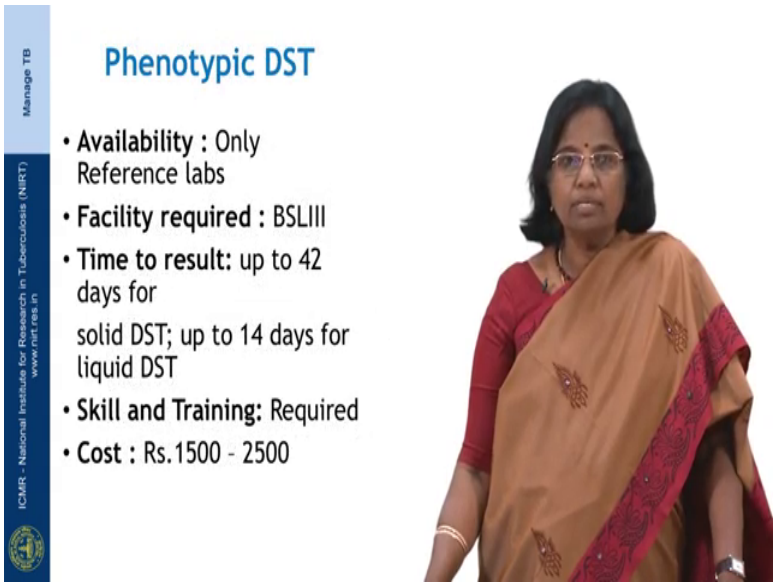

So, where are these phenotypic DST tests available, they are available only in the reference laboratories because of the requirement for the biosafety level 3 facilities?

The time to result may be up to 42 days for solid media using DST or up to 14 days for liquid DST. Significant amount of skill and training are required and the DST may cost anything between 1500 to 2500 per test.

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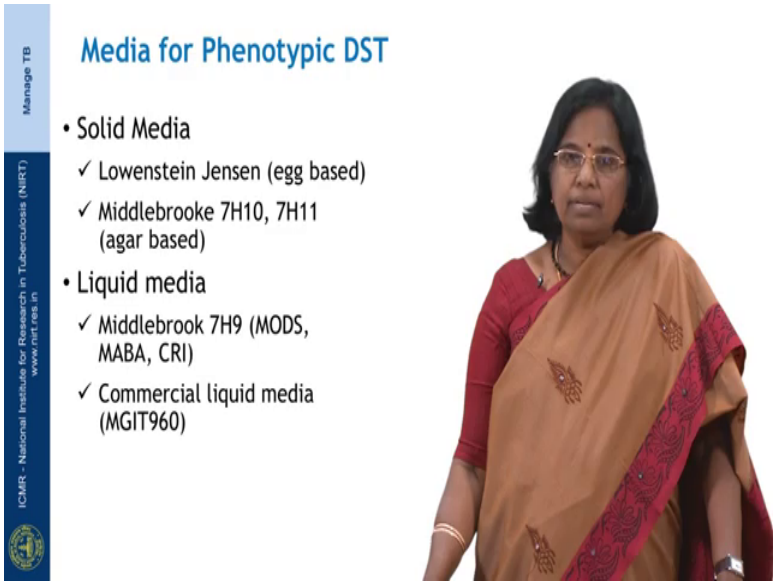

The media that are used for phenotypic DST could be solid media, examples being the Lowenstein Jensen medium or the Middlebrooke 7H10 or 7H11 media or it could be

liquid media, where in house prepared middlebrooke 7H medium can be used or the commercial liquid media can be used in commercial systems.

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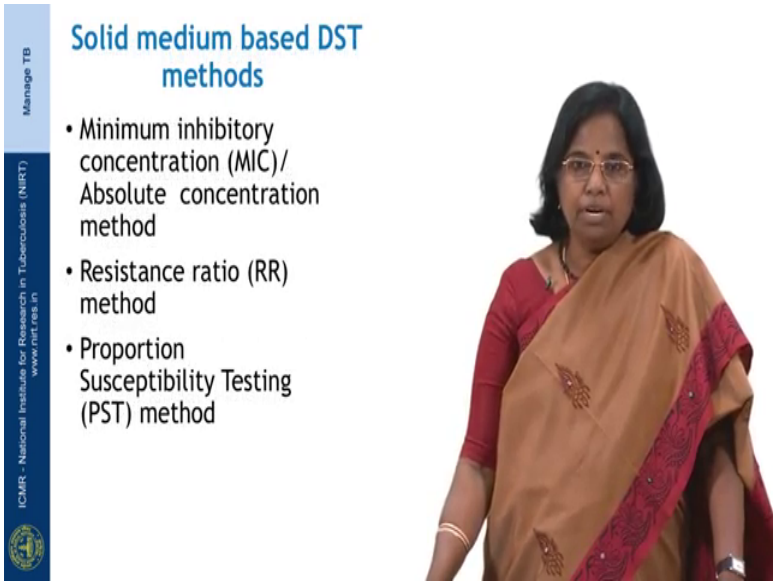

The solid medium based DST methods include, the minimum inhibitory concentration method in short known as the MIC method or the resistance ratio method or the proportion susceptibility testing method, which is in short known as a PST method.

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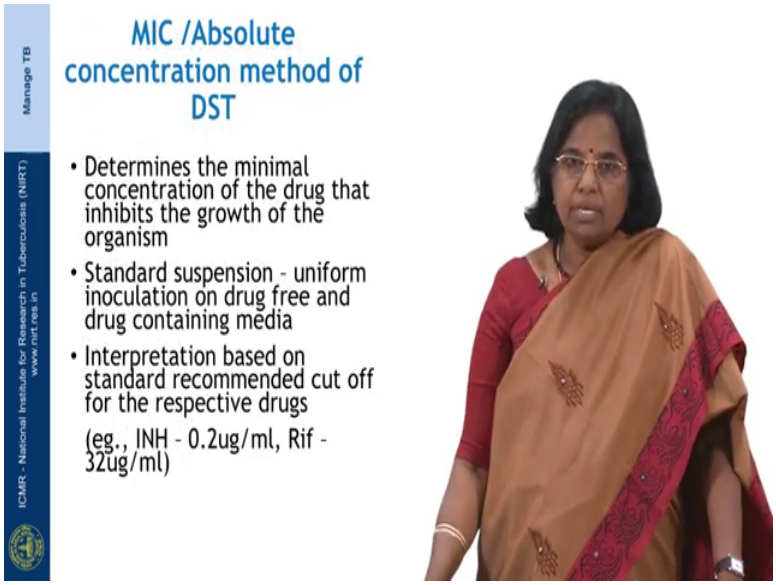

The MIC method determines the minimal concentration of the drug that inhibits the growth of the organism. And standard suspension is taken and uniform volume is

inoculated on the drug free and drug containing media, the slopes are incubated at 37 degrees at 37 degree say for about 28 days.

And growth between the drug medium and the drug free medium is compared and interpretation is based on the standard recommended cut off values for the respective drugs example being for isoniazid, it is 0.2 microgram per ml and for rifampicin it is 32 microgram per ml.

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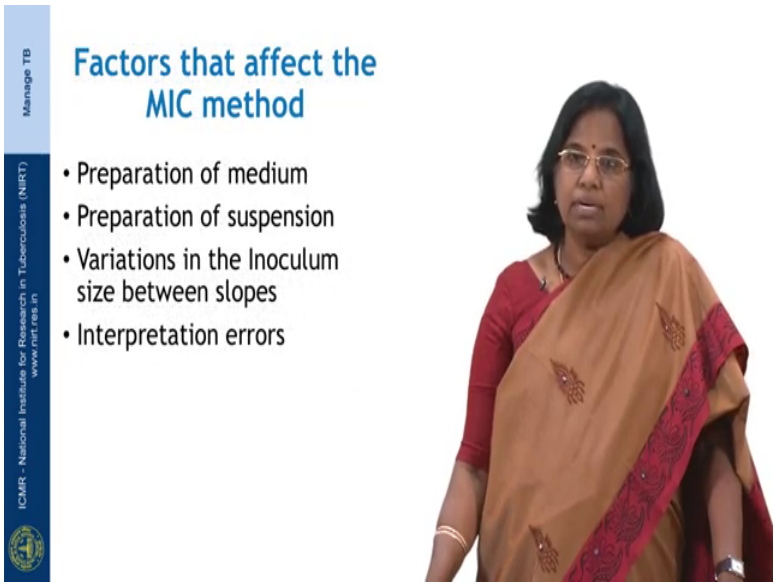

The factors that may affect MIC method are preparation of the medium, because for this test with the medium is being prepared a stock solution of the drug is prepared and dilutions are made to finally, achieve the different concentration of the drug in the slopes. And any errors in the preparation of the medium can result in erroneous results.

Second is the preparation of suspension, we require a uniform suspension here and the presence of even a small clump in the suspension can result to wrong results. And third is the variation in the inoculum size between the slopes, this can also affect the results and finally, the technical errors which could result in interpretation errors.

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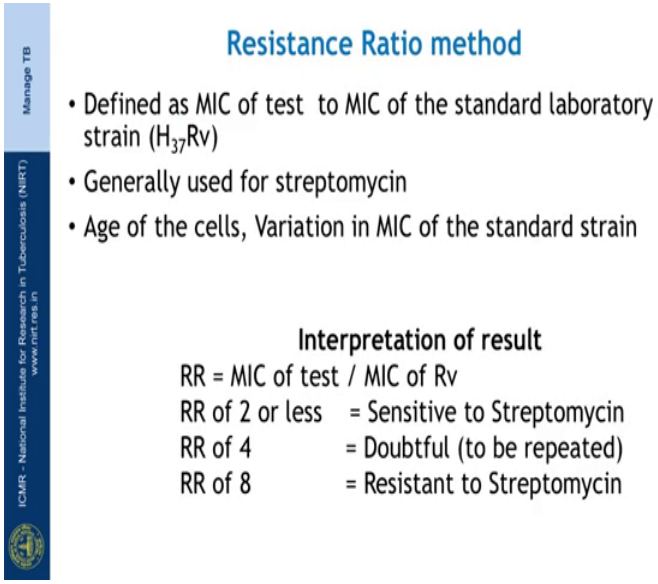

Next we come to the resistance ratio method, which is defined as the MIC of the test to MIC of the standard laboratory strain which is a H 37 R, this is generally used for streptomycin and the factors that could affect this method could be the age of the cells whether it is a test strain or the standard strain and variations in the MIC of the standard strain due to repeated passages of the strain in the laboratory.

The MIC method is setup for both test and the standard strain as usual as itself upon performed in the MIC method. And the MIC of the test the resistance ratio is determined by MIC of the test divided by the MIC of Rv. When the RR is 2 or less, the strain is considered to be sensitive to streptomycin when an RR of 4 it is considered to be doubtful when the test has to be repeated, an RR of 8 or more than that is the strain is considered to be resistant to streptomycin.

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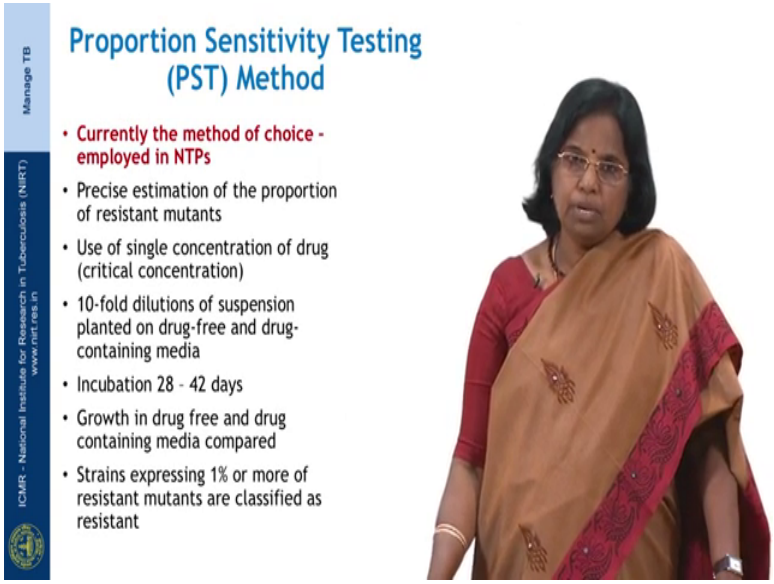

Next we come to the proportion sensitivity testing which is a PST method. This is currently the method of choice employed in NTPs or the National Tuberculosis Programs where the solid method of DST is still being performed.

This method offers precise estimation of the proportion of resistant mutants; the use of a single concentration of a drug is utilized in this methodology, which is usually the critical concentration. 10 for dilutions of the uniform suspension is planted on to drug free and drug containing media, the slopes are incubated up to 42 days and with an interrupt reading being done on the day 28.

Growth in drug free and containing media is compared and strains expressing 1 percent or more of the resistant mutants are classified as resistant.

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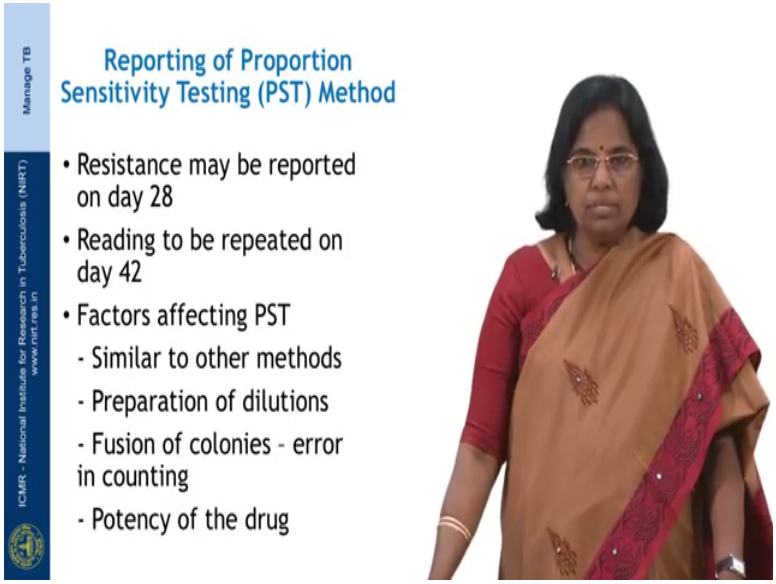

Here the resistance any resistance observed may be reported on day 28, and the reading has to be repeated on day 42 in the case of drug susceptible strains. The factors that are affecting the PST could be similar to other methods and since we are preparing dilutions of the suspension here, any small clump in the suspension can lead to erroneous results and wrong results.

So, since we accounting the colonies in the slopes here, fusion of colonies could lead to errors in counting and also the potency and the expiry period of the drug can also affect the PST method results.

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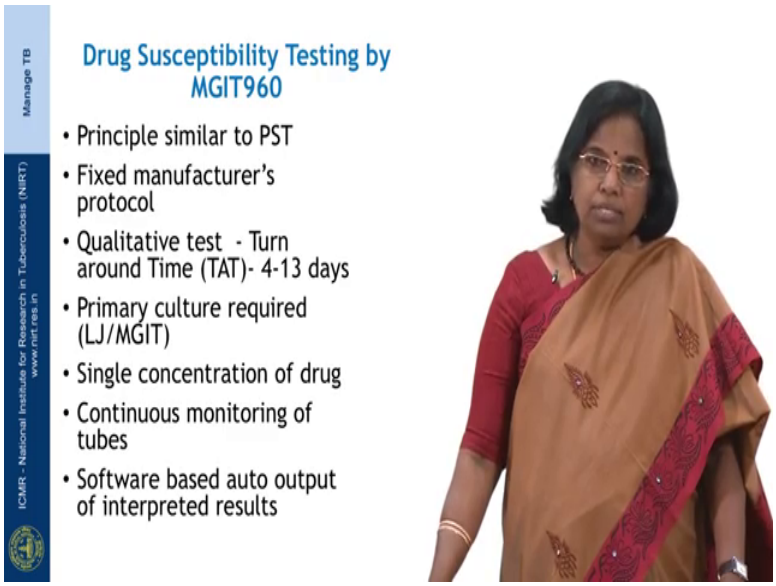

We move onto drug susceptibility testing by MGIT 960. The principal is similar to PST, where a fixed manufacturers protocol has to be followed. It is a qualitative test with a turnaround time of between 4 to 13 days.

A primary culture is required this could be either from LJ or from MGIT; a single concentration of the drug is used. The continuous monitoring of the tubes is equipped with software based auto output of interpreted results.

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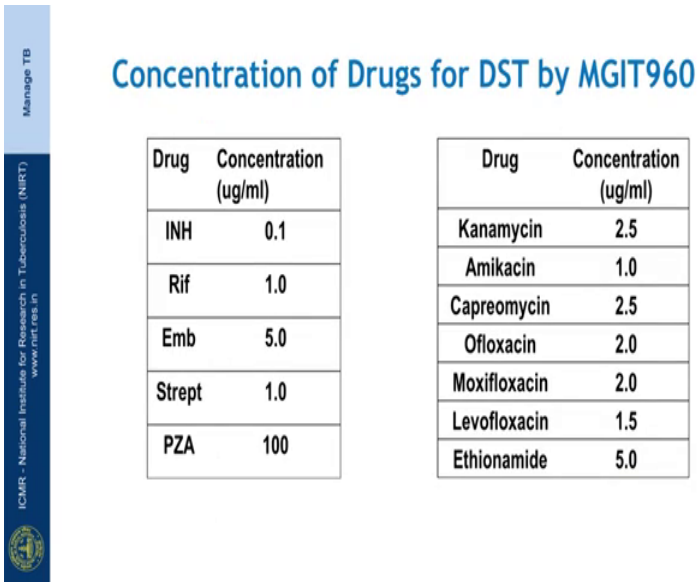

These are the concentrations that are employed in MGIT 960 DST.

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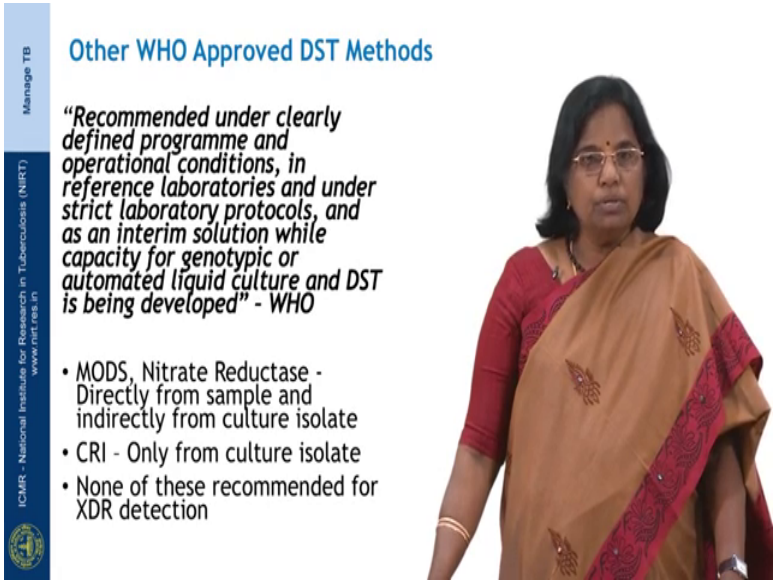

In addition to these recommended methods, WHO has approved of other DST methods these are non commercial methods. These methods are recommended under clearly defined program and operational conditions in reference laboratories and industry laboratory protocols, and as an interim solution while capacity for genotyping or automated liquid culture and DST is being developed.

Examples of these essays are the modes, for the nitrate reductase assay, which can be used for direct detection of DST from the sampled or indirectly from culture isolate.

The colorimetric redox indicator dye based tests are to be used only from culture isolate, but none of these methods are to be employed for XDR detection.

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So, direct DST by nitrate reductase test. This is based on the ability of M tb to reduce nitrate to nitrite, the substrate nitrate is included in the medium which is prepared with and without drugs.

Reduction to nitrite is indicated by colour changes using specific reagents and the colour changes indicate growth, in addition to visual detection of colonies single concentration of drug is used in this methodology, the results are available in 7 to 14 days, the technique is inexpensive and high levels of sensitivity and specificity with cultures is observed for first line anti-TB drugs.

The tests can be done on sputum directly for isoniazid and rifampicin DST.

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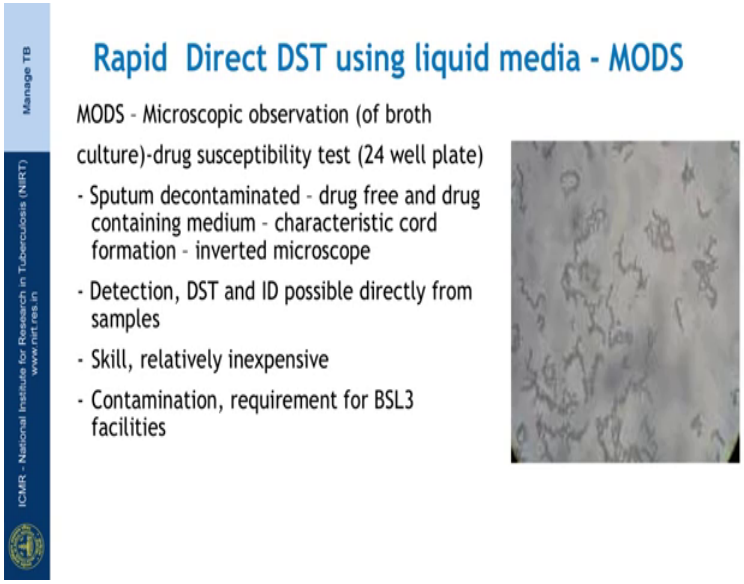

Next we come to MODS methodology which is the Microscopic Observation of Drug Susceptibility employed using in house prepared 7H medium, 7H 9 medium in a 24 well plate format. Sputum is decontaminated and the uniform volume is added to drug free and drug containing medium in the wells.

And growth is exhibited by characteristic code formation, which is observed under inverted microscope. This methodology can be used for direct detection, drug susceptibility and identification of MTB directly from samples. This require skill, but it is relatively inexpensive. This is highly prone for contamination and bio safety issues hence BSL3 facilities are required for this.

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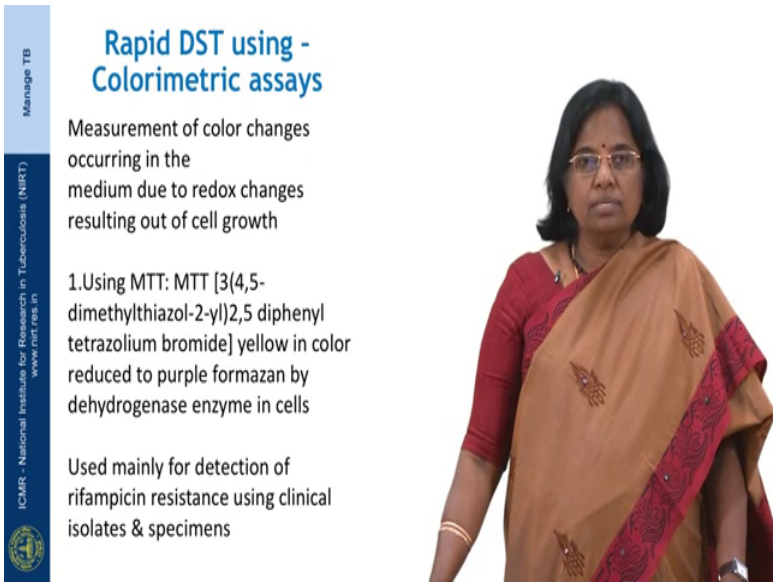

Next is rapid DST using colorimetric assays? Here this is based on measurement of colour changes occurring in the medium, due to redox changes resulting out of microbial growth.

The first one is using MTT, which is dimethylthiazole diphenyl tetrazolium bromide which is originally yellow in colour but gets reduced to purple formation by the dehydrogenase enzyme in the cell. This methodology is used mainly for detection of rifampicin resistance using clinical isolates and specimens.

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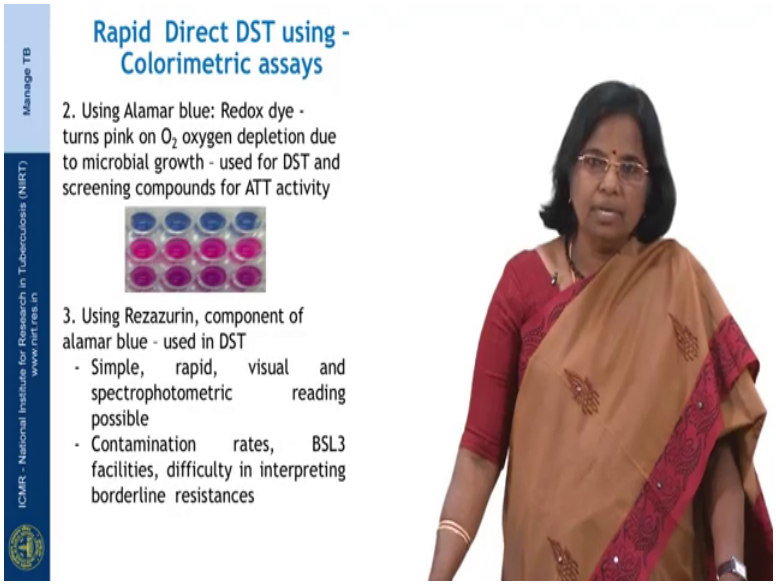

The next one is the alamar blue assay, which is again a redox dye this turns pink due to oxygen depletion in the medium resulting out of microbial growth. This is used for DST as well as for screening compounds for anti TB activities. The third one is rezazurin which is a component of alamar blue the principal is the same.

Now, all these methodologies are simple rapid and both visual and spectrophotometric readings are possible. All these methodologies are highly prone for contamination and require BSL 3 facilities; they also have difficulty in interpreting borderline resistances.

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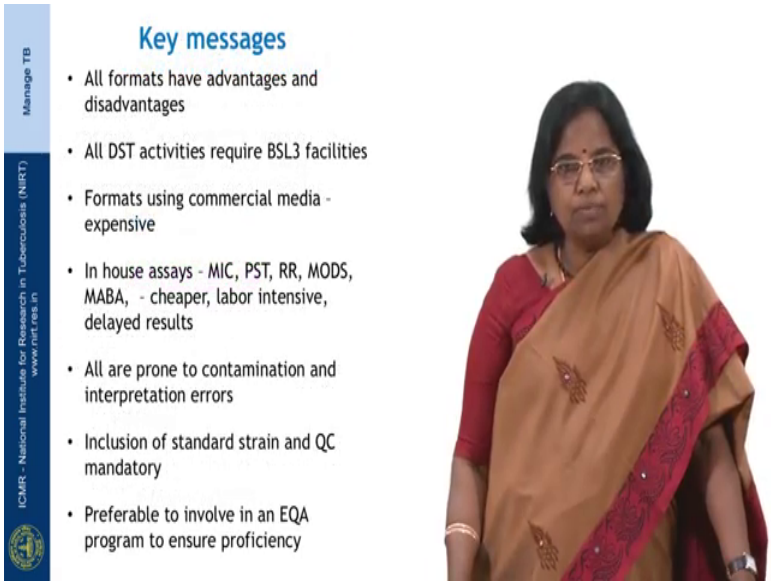

The key messages from this session are that, all formats have advantages and disadvantages. All DST activities required BSL3 facilities, format using commercial media are expensive, as a such as MIC PST resistance ratio MODS and MABA which make use of in house prepared media are cheaper, but they are labour intensive and offer delayed results.

All are prone to contamination and interpretation errors and a inclusion of a standard strain and quality control is mandatory for all these techniques and it is preferable for the lab to get involved in an external quality assurance program to ensure proficiency. With this we come to the end of the session on phenotypic DST methodologies.

Thank you for your attention.