Manage TB
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Lecture - 14
Molecular Diagnosis of Tuberculosis
Session 02

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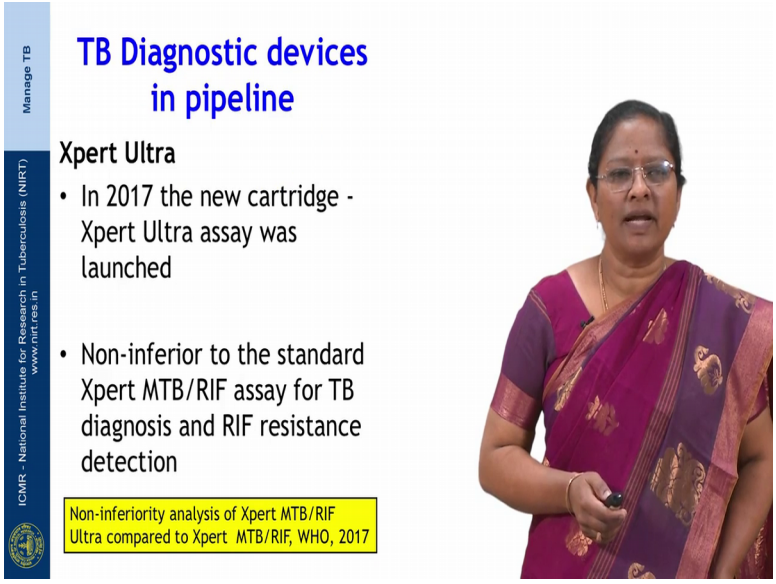

Now, I would like to discuss about the TB diagnostic devices, which are in pipeline. The first one is the expert ultra. The expert ultra was launched in 2017 and this is a new cartridge which can be used in the expert platform instead of the MTB RIF cartridges that is currently being used.

An initial study using this expert ultra have shown that it is not inferior to the standard x MTB RIF assay, for TB diagnosis and rifampicin reduction.

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So, the next device that is in the pipeline is called as the GeneXpert Omni. So, this is still the developmental process as a point of care test, and it uses the same cartridge. The advantages that, it does not require a specialized laboratory facility for usage of this device, it is a portable device and it has its own inbuilt battery, but will be able to perform only one test at a time and the time duration is a same as that of a gene expert.

But the results of this could be sent as an SMS to the patients and it can work in a room temperature and the cartridge and the devices can be operated in a field.

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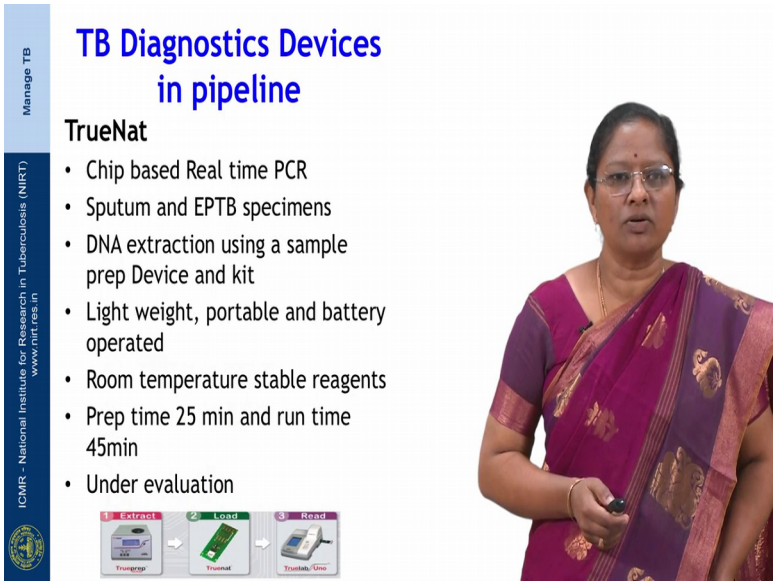

And other diagnostic device which is in pipeline, which is called as a TrueNat device, this is an indigenous device that has been developed in India. This is a chip based real time PCR method and the state that detects rifampicin resistance as well as diagnosis for tuberclosis in sputum as well as an extra pulmonary specimens.

The DNA extraction for this usage in this device is based on a simple sample prep device and the kit that comes along with this device. This machine as such is a light weight machine portable and battery operated and this again decide for a point of care technique, and the reagents is stable at room temperatures. The preparation time for the biological specimens is 25 minutes and run time is 45 minutes.

This device is right now under evaluation by the central TB division; right now this device is under evaluation by rntcp for looking at its performance before it could be rolled out.

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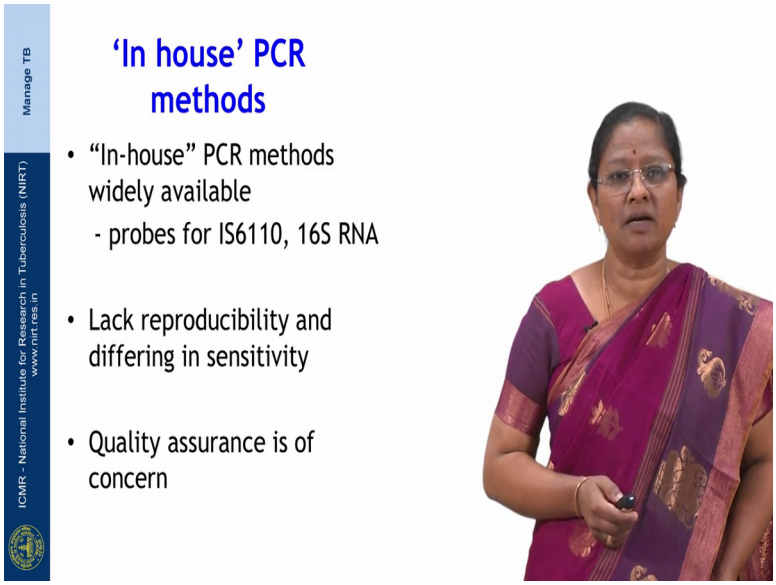

So, there are also are there in house PCR method, that is being done in different laboratories across the countries, which uses the probes the mycobacteria specific probes like the IS6110 and the 16S RNA, but these methodologies or in house PCR methods like reproducibility and different the sensitivity between the different laborites.

The results can be utilized only if the quality is the proper qc and qa are performed on these technologies. So, the quality assurance is of concern and the results from these test should be taken with caution.

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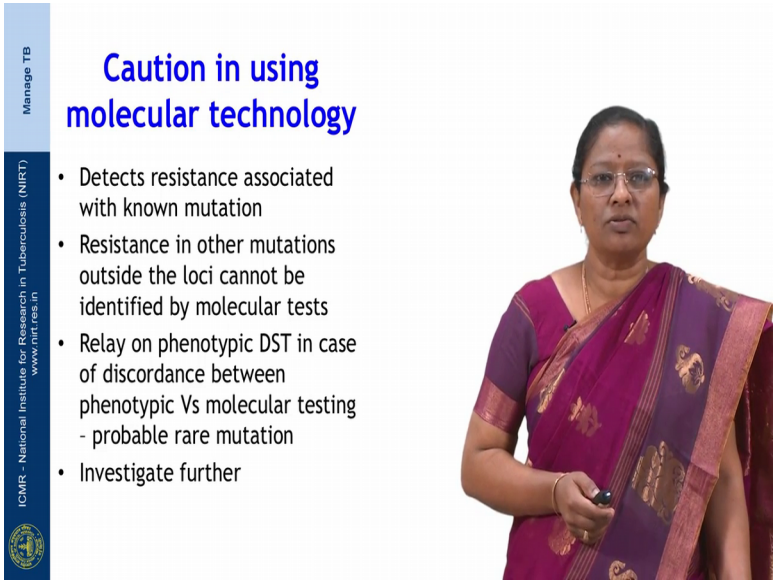

So, coming to the cautions in using the molecule technology, we have to be cautious to know that the resistance reduction for these devices is usually based on the known mutations. The resistances in are mutations other than this loci which are present in the molecule test will not be detected by this methods.

Therefore it is it is better to rely on phenotypic DST, in case of discordance between a phenotypic versus a molecular testing, base possibly it could be due to a rare mutation which is not present on the device.

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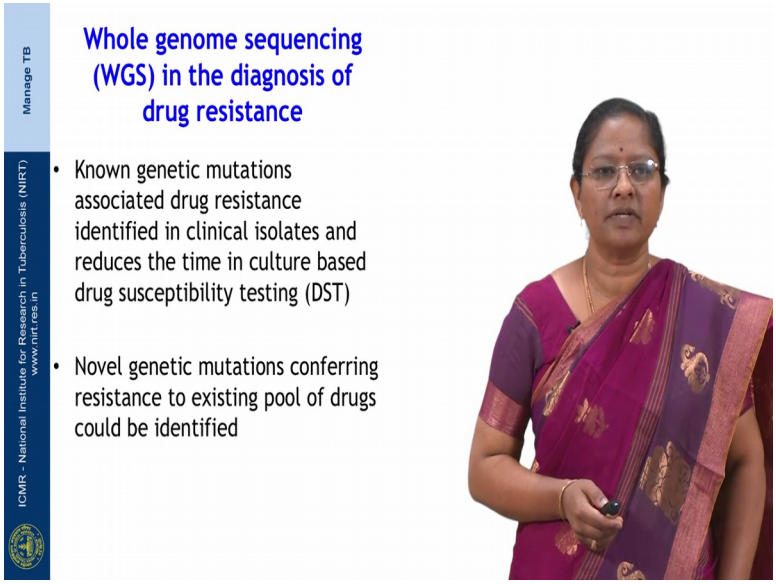

Therefore, it is required to investigate further before the patient is put on treatment. Moving on to whole genome sequencing for the diagnosis of drug resistance, currently we are not using whole genome sequencing for patient management in India however it has been put it implementation in some of the developed countries.

What actually happens here is, the complete genome of the micro bacteria is sequenced and the non genetic mutations associated with drug resistance for the clinical isolates are identified in one stretch and this reduces the time, when compared to culture based of susceptibility testing.

And as I mentioned earlier, any novel genetic mutations conferring resistance to the pool of drugs could also be identified in the whole genome sequencing, which is not possible by the other technology molecular technologies.

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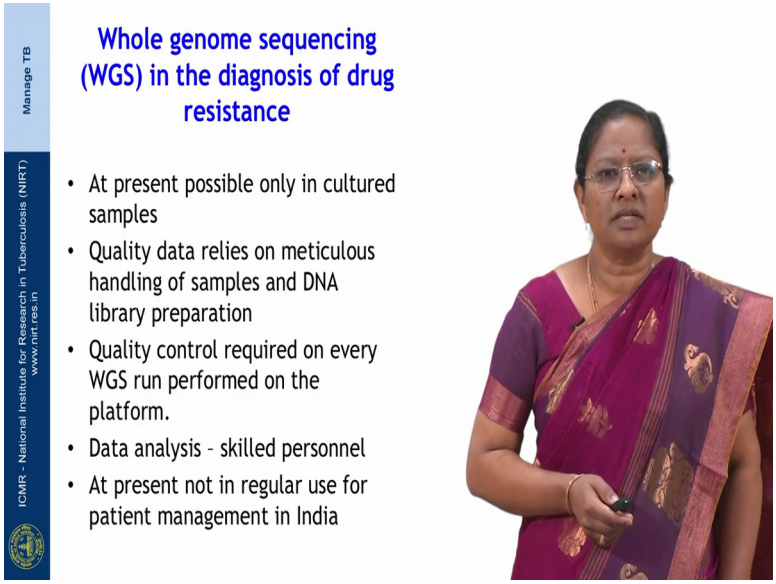

The whole genome sequencing is currently possible only in cultured samples, and the quality data from these depends on mediculous handling of the sample as well as the DNA library preparation.

Stringent quality control is required on every WGS run that is performed in this platform. And since there is enormous amount of data that will be available on the whole genome sequencing, data analysis required skilled personnels and we do have open softwares which can give the drug resistance prediction results.

At present this is not in regular use for patient management in India. The whole genome sequencing can also be used for drug surveillance studies and also for doing outbreak investigation studies.

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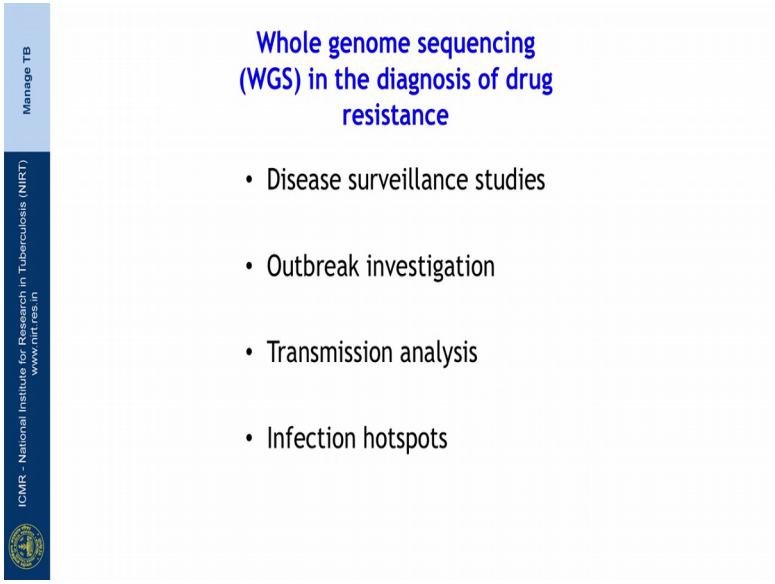

Basically it can track down the strain from where the contacts of established or had the infection and therefore, can also do a transmission analysis and in addition it can also help in tracking and identifying infection hotspots for preventive strategies.

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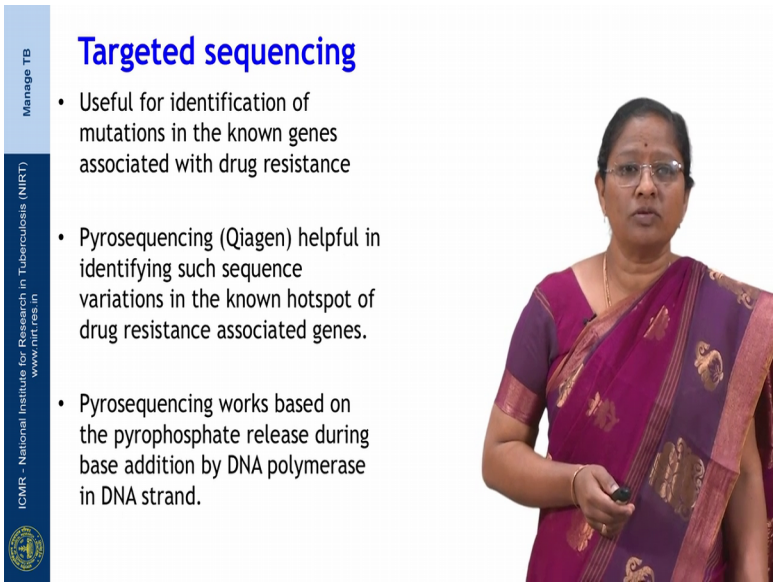

So, other than whole genome sequencing, you also have targeted sequencing. So, the targeted sequencing is useful for identification of mutations to the known genes associated with drug resistance.

The pyrosequencing technologies one such technology, which is helpful for identifying specific genes or specific resistance in the known hotspots of drug resistance associated genes.

The pyrosequencing which works based on the sequencing by synthesis methodology, where the pyrophosphate is released during the base addition by a DNA polymerase in a DNA strand.

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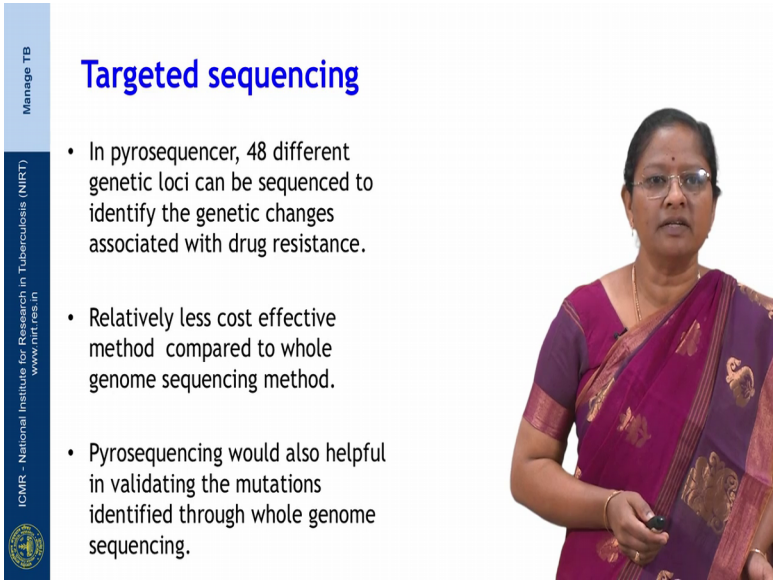

In pyrosequencing, we can do 48 different genetic loci sequencing to identify the genetic changes associated with drug resistance.

It is relatively less cost effective when compared to the whole genome sequencing and you can do an in depth sequencing. The pyrosequencing would also help in validating the mutations where there is a discordance between a phenotypic DST and the whole genome sequencing.

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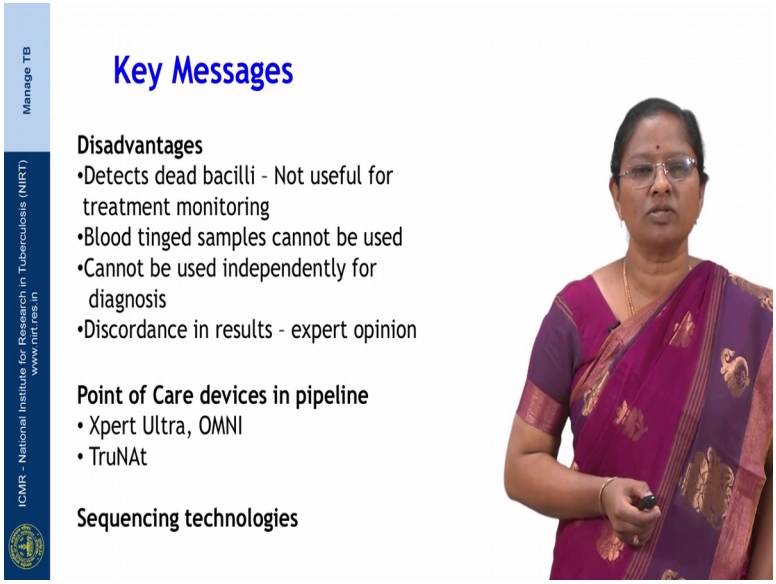

So, the key messages or the take home message from this session or that, the molecular diagnosis which is the recent development has been a trendsetter for diagnosis and for diagnosis of TB and drug resistance prediction. This is more sensitive and it has a quick turnaround time and it has significantly enhance the early detection of disease and drug resistance.

The currently available tools that is used in the programmatic setup for diagnosis of a m tuberculosis includes the expert MTB RIF and the line probe assay for first slide and the second line is under assessment and its soon to be rolled out. So, let us also discuss about the disadvantages which are involved in using the molecular diagnosis, one of it is that it detects dead bacilli therefore, it is strongly recommended not to use for patient monitoring or patient who are on treatment.

And also blood test samples cannot be used in molecular diagnostic devices and it cannot be used independently for diagnosis in case of discordant results, it is better to have an expert opinion and also look into the phenotypic DST results.

We also touched upon the point of care devices, which are in pipeline including the Xpert Ultra the OMNI and the truNAT, which are currently being evaluated. So, I also mentioned about the sequencing technologies, which can be used if there is a discrepancy between the phenotypic results and the currently available molecular diagnosis results.

Thank you.