Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

Prof. Aritri Bir

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Lecture 37 : Molecular Diagnostics in Antimicrobial Resistance Testing

Namaskar. Welcome back to the NPTEL lecture series of Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. So, today we are going to start another new week that is molecular diagnostics in infectious disease where in the previous class we have discussed the syndromic panels and in this class we are going to discuss about the antimicrobial resistance detection technique. So, here are the basic concepts over which we are going to concentrate the classical methods which are already available for detection of antimicrobial resistance, then their automated and semi automated versions and finally, molecular based techniques which can help in detection of antimicrobial resistance. So, the classical methods which are available you can see here these are dilution method, antimicrobial gradient method, disc diffusion method, chromogenic agar media based method and some colorimetric tests. Now, these classical methods remember their results come after at least 18 to 24 hours sometimes 48 hours and that also needs prior bacterial isolation and identification.

So, for that period the treatment are mostly based on empirical approach. Also this time turnaround time the report giving time is prolonged when there is some anaerobes or some slow growing fastidious bacteria. Now, if we see these methods we can have a brief description or brief principles of how these methods are working. So, the dilution method is basically twofold dilution of antibiotics in a liquid growth medium where the standard bacterial suspension is inoculated and following one overnight incubation at 35 degree centigrade the tubes.

Now, the tubes why I am saying tubes because dilution techniques can be tube based also. So, this tubes are examined for visible bacterial growth which appeared as turbidity. Now, the lowest concentration of antibiotic that prevented the growth is represented as minimal inhibitory concentration based on which the resistance can be detected. So, here you can see the modification of the tube are basically done in micro dilution method here you can see the wells are used. So, over the wells twofold dilution means first dilution, second dilution, fourth dilution, eighth dilution like that.

Then antimicrobial gradient method where one gradient based dissipation of antibiotic is used. So, basically this is one establishment of antimicrobial concentration gradient in an agar medium to detect the susceptibility. So, here what is used is gradient strip. So, here you can see this is the gradient strip which has been placed over agar plate and these gradient strip are basically in these agar the below the gradient strip the antibiotic is dissipated or put in such a way that there is one constantly rising gradient. And the strip indicates the strips are labeled and it indicates the antibiotic concentration.

Once again after overnight incubation the reading is taken how the reading is taken the minimal inhibitory concentration is determined by see here you can see the oval shaped growth area is basically demarcated. So, the MIC is determined by the intersection of the lower part of the ellipse here in the test strip. So, this is how antimicrobial gradient method can help in detection of antimicrobial resistance. Then there is this diffusion method one very standardized and well accepted method where bacterial inoculum is plated over one agar plate the agar plate which contains commercially available fixed concentration paper antibiotic discs. So, these are the discs.

Now after the inoculation is given definitely the bacteria grow and so, you can see the zone of growth inhibition around each of the antibiotic disc are measure here. So, this is one qualitative or semi qualitative method where the susceptibility is basically checked by whether that is susceptible whether the antibiotic is intermediately working or whether the strain is basically resistance based on the appearance or non appearance of the inhibitory zone or the bacterial growth. Chromogenic agar is basically one commercially available commercially prepared rather agar which contains chromogen and the property of the bacteria which is exploited here are some specific enzymes which is being produced by the bacteria and those enzymes can metabolize the substrate to release the chromogen from the agar. So, when the chromogen is released it is visually detected by direct observation by some color change over the media. So, this selective and differential media helps in screening of some patients where we want to detect whether there is some bacterial colonization present or not whether some resistant bacteria is present or not.

Similarly cholera metric test in cholera metric test again the bacterial enzyme system is exploited which the enzymes hydrolyze the component in which is incorporated in the bacterial agar sorry in the dilution sorry in the detection solution and in this detection solution there are some indicators some pH indicators which acts as which are dyes basically. So, based on the pH change the color changes and it detects or it indicates the presence of resistant or susceptible pathogens. So, these are the classical method majority of the labs follow and it takes a long time 48 hours is basically a long time to start to wait to start the property treatment or antibiotics. So, basically empirical therapy is the method of choice, but for that the disadvantage is emergence of antimicrobial

resistance. So, to avoid the empirical use of antibiotics what is required detection or identification of specific antibiotics to which the bacteria or the pathogen will be susceptible and that can be start as soon as possible.

So, for that some methods are invented which are based on this micro dilution susceptibility testing, but the devices are mostly semi automated or automated. Now, these semi automated or automated machines how they help they give some customized test reports those customized test reports of patient helps in giving the reports in versatile ways. Number 1 it is attached to a data management system where a computer software is used. So, basically this is one digitalized platform. Now, these data can be used in two way number 1 is rapid individual patient report.

So, the patients are getting reports in a very rapid way another is it can store data. So, basically this is one archive of epidemiological data for some specialized purpose like the hospital antibiogram can be checked, the infection prevention reports can be checked, what is the trend of the organism which is running currently in the locality or in the hospital that can be identified some summary can be prepared and also the cumulative susceptibility data can be drawn. So, the archive of these automated and semi automated devices are helpful in many way. So, basically these automated and semi automated system they are based on some softwares and this is actually integrated with the laboratory information system which can be accessed from different platform. So, what is the advantage definitely it minimizes the uncertainty and bias.

So, man to man variation and report interpretation can be the variations can be avoided. Similarly, the sample handling time the test time all this can be reduced. So, the help in saving time, but there are limitations the major limitation is it cannot detect as a whole these techniques cannot detect the underlying mechanism of the antimicrobial resistance. These are some examples of automated and semi automated devices. Wi-tech 2 system is one very commonly used platform.

This is basically broth micro dilution detection based and antibiotic sensitivity testing system which contains multiple wells over which 17 to 20 antimicrobial agents are plated. So, the resistance to those antimicrobial agents can be checked simultaneously. Now, if the bacterial isolate is not previously identified. So, in that case another card can be inserted which is helping in detection of the bacterial strain and after that the antimicrobial susceptibility can be checked over another card. So, Wi-tech 2 can identify the bacterial strain when it is not identified and after that 17 to 20 antimicrobial agents over which the susceptibility can be checked in a simultaneous pattern.

Phoenix system is another such platform where bacterial identification as well as antimicrobial susceptibility both can be checked. So, the principle is based on some

oxidation reduction indicator that is Resazurin dye or Alamar blue. So, based on their oxidation and reduction there is the changes that can be detected and the basic indicator is basically the growth of bacteria. And this bacterial growth are checked over various concentration of antimicrobial agents. Then micro scan walk away plus system it also helps in detection of antimicrobial susceptibility testing.

It has 3 panel configuration which gives combo panels can give break point combo panels and also can identify the MICs. So, these are some automated and semi automated devices which are basically based on the micro dilution susceptibility testing, but as they are automated and semi automated they are basically saving time. So, now coming to the molecular based techniques for resistance detection. Now, the previous techniques or the classical methods and the automated and semi automated methods as well they can check the antimicrobial agents to which the pathogens are susceptible or resistance, but the mechanism of resistance cannot be detected. For that what is required is to identify the resistance gene and those can be detected by molecular based techniques.

So, what are the different molecular based techniques currently available? Some methods are amplification based methods the amplification of target gene is required after that hybridization based technique where hybridization nucleic acid probes are utilized for targeted gene identification and also sequencing based approaches where genome sequencing is done to detect the resistant conferring mutations or resistance gene through the whole genome of the bacteria. So, these are the different principles based on which the molecular techniques are going to detect the resistance gene. Now coming to the amplification based method the polymerase chain reaction is one very important one you all know the principles by this method basically the target gene is identified and amplified. Then how they are detected by electrophoresis by southern blotting where the probes are used restriction fragment length polymorphism by which the mutation can be analyzed single strand conformational polymorphism by which again the mutation can be analyzed also DNA fingerprinting. Then different molecular beacons can be utilized you all have read already.

So, apart from that what can be done to identify the specific gene is those can be sequenced by different DNA sequencing techniques currently LAMP technique is available that is loop mediated isothermal amplification. So, these are the techniques where the specific genes are identified. So, first is the amplification of the gene after that identification of the gene. Now the limitation is once again the resistance mechanism how the underlying the targeted or the identified genes are mutating or they are conferring the mutation that is hard to crack by PCR based techniques. Again the genes can be identified by some array platform where DNA microarray can be utilized to identifies numerous target genes simultaneously and finally, identification of unknown

genes can be done by whole genome sequencing.

Then coming to mass spectrometry yes proteomics technique can also be exploited to identify bacterial resistance. Now if you remember in case of mass spectrometry what is important the indicator is mass to charge ratio of what of the ionizing protein. So, who is the ionizing protein here? Here we are talking about ribosomal proteins abundant ribosomal proteins which are directly isolated from bacterial isolate or cell pellets. So, the basic mass spectrometric principle is actually exploiting rapid ionization of such proteins from the bacteria using energy absorbent matrix laser pulse and detects the PMS or the peptide mass fingerprint of that specific bacteria. So, basically particular microbe can be identified at the level of genus even or species or strain by matching its PMF pattern that is the actually rather that is the fingerprint or identification mark for that specific bacteria which is obtained from the open database of PMF.

So, here the ribosomal proteins of different bacteria is or different pathogen is basically exploited to check MS. Now mass spectrometry definitely is one very good technique, but that is costly and also it needs expertise. So, there are some semi quantitative technique of MALDIAT of MS that is MALDI, Bio-typer Antibiotic Susceptibility Test Rapid Assay. So, that is that is MBT-ASTRA which basically measure the relative growth rates of bacterial isolates which is exposed to antibiotic and that is compared to control samples which are not treated not exposed to antibiotics for a short duration the exposure is for short duration. Now the area under the curve which basically calculate the bacterial growth is compared.

So, the if the bacterial strain is resistant there will be lesser curve lesser area under curve if the bacterial strain is susceptible there will be sorry. If the then the software tool basically helps to calculate the area under curve the area under curve which denotes the bacterial growth. Now those bacteria which are resistant to the antibiotic they will grow. So, the area under curve will be high whereas, the susceptible bacteria the area under curve will be lesser than the resistant one. Now MBT-RESIST assay is here where the bacterial are grown in parallel to two distinct culture medium one containing carbon 12 and the other containing carbon 13.

Now the mass spectra of the bacteria grown on this isotope level medium are basically compared. So, in presence of antibiotic one culture media content 12C another culture media content 13C. Now if the MZ ratio is checked what is seen the resistance strain can thrive in the presence of antibiotic and that incorporate 13C into the polypeptide whereas, the susceptible strain which are grown on 12C they are not grown. So, basically there is a shift in the peak to the higher side in case of the resistant bacteria because they incorporate the heavier one heavier carbon the 13C. So, that that is based on the MBT-RESIST assay is based on the peak shifting of this MZ ratio in the mass

spectrum.

So, these are the different modification of mass spectrometry which can be used for rapid detection of antibiotic resistance. So, these are the antimicrobial susceptibility testing method where phenotypical method are basically based on the classical techniques, dilution techniques, dis diffusion techniques, gradient test, chromogenic assays and automated devices. Then comes the molecular based technique the commonest one is PCR amplification followed by the sequencing or sequencing via NGS or identification of the genes via different probe based or hybridization method also the proteins RNA proteins can be identified by mass spectrometry based assay. Now, this is the resistome or resistance profile where the clinical specimen is used for either genetic analysis or genomic analysis. So, that in the genetic analysis the specific gene target is amplified then it is goes for the detection or presence or abundance of such gene via single gene detection or multiple gene detection.

Similarly, microarray is used to identify multiple target genes simultaneously. Now, coming to the genomic analysis here the whole microbiome or whole genome is basically sequenced for identification of different resistance genes which can be stored in a database and further utilized to check the resistance or resistance profile. So, these are the current methods which are available for identification of the bacterial strain or antibiotic resistance. So, majority are basically identification for my bacterial strain or microscopic technique followed by strains and after that the MIC values are determined the genes the strains can be identified by mild it of nucleic acid amplification test different immuno diagnostics and probe based detection technique. Now, for rapid tests it takes around 30 to 40 hours, but in a polymicro sample it takes 0.

5 to 8 hours. So, these are the different available techniques based on which the antimicrobial resistance can be checked. So, we have learned that molecular antibiotic resistance mechanism detection or molecular anti microbial resistance can be directly detected via some identification of some resistance gene. The methods available are amplification based method hybridization method as well as sequence based method also mass spectrometry can be utilized to identify different pathogens based on their PMF pattern and these techniques take shorter time, but they are expensive and the extensive trainings are also needed for conduction and interpretation of the results. These are the summary. Thank you and see you in the next class. Thank you.