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# REVIEW ON MOLECULAR AND BIOCHEMICAL METHODS FOR THE IDENTIFICATION OF STREPTOCOCCUS

Rida Naz, Rabia Amin, M. Anjum Zia

## Abstract

In this review the identification methods of discuss. For the identification of streptococcus firstly microscopic appearance of streptococci observed that are coci shape and form chains and pairs. Further identification of bacteria require biochemical test like catalase, pyr, bacitarcin sensitivity. Steptex is also use commercially for the identification of lancified group it give excellent recognize streptococci of groups A, B, C, and G. API strep 20 is also use to identify bacteria that contain 20 wells. For the fast identification of bacteria molecular methods use. Polymerase chain reaction use with specific primers that target the specific genes of streptococcus. In case of *pyogenes* spy1258, dnase, speB,and the sof genes are key gene target. The amplification of universal sequences that is perseveres in every bacterium. With the one set of universal primer the amplification of the 16sRNA conserved gene present in all bacteria. The amplicon of 996bp produce with all further the RFLP is use to further classify the bacteria.

**Key words:** Streptococcus, 16sRNA, API strep

## Introduction

*Streptococci* belong to genus of Gram-positive bacteria that are largely spread across the animal and human flora normally. They produce many diseases in human and have ability to cause infection that spread very quickly produces due to occurrence of bacteria in usually uncontaminated place. Such readily spreading infection have a significant public health value as a example Annually 800000 deaths in world occur due to pneumococcal disease in children below the age 5 year (Brien *et al.,* 2009).

is cocus shaped gram positive bacteria that occur in chains and pairs. The species of are catalase negative and require enrich media for growth such as blood agar media this ability differentiate the *streptococci* from *staphlylococus*.

Conventionally, Microbiologist sub classify the bacteria by investigating their characteristic pattern that emerge on blood agar media which term as Alpha hemolysis incomplete or partial lysis of RBsc e.g *Streptocous pneumonia*. Beta hemolysis complete lysis of blood cells Streptocous pygenes and gamma no heamolysis(Lancefield, 1993)

*pyogenes* is linked with a large series of diseases that ranges from mild skin infection to serious situation that is able to cause a death like presence of bacteria in blood (bacteraemia) and toxic shock syndrome, flesh eating disease and if these conditions are untreated may direct to rheumatic fever, glomerulonephritis and Pediatric Autoimmune Neuropsychiatric Disorders these are immune mediated post streptococcus infectious (*Cunningham*, 2000).

Asymptomatic carriers of Group A may be present in body with no disease production it is present mainly inside respiratory tract and the skin of persons, but they become active when the immune system of individual become weak and may move to clinical conditions when the defense system of the host become weak or not working well. Person to person transmission occur due to direct contact and respiratory droplets.

The 11-34% is the without illness carriage rate of GAS (Lloyd *et al.,* 2006; Dhaka *et al.,* 2010) and these take part a chief function in the conduction of illness. Quick, precise and timely identification is essential for the association of patients and the carriers to reduce the incidence of invasive streptococus infections (Slinger *et al.,* 2011).

Conventionally The GAS is recognized as a Gram positive cocci show chains shape presenting the beta haemolysis of five percent blood Agar. The biochemical test bacitracin sensitivity and the Pyrollidonyl Aryl Sulfatase test and the lancefied grouping that is base on the

specific cell wall antigen all of these use to differentiate GAS from other beta hemolytic streptococci (Brahmadathan *et al.,* 2006).

Detection of GAS through grouping is also not very authentic because some strains of *Streptococus anginosus* cause the antigenic cross reactions among the other groups ,as like that the strains of *anginosus* and of the *dysgalactiae subsp. Equisimilis* cause antigenic cross reactions (Facklam , 2000).

Now with advancement of technology the fast and reliable method develops for bacteria identification through PCR technology (Kumar, 2015). Polymerase chain reaction specifically target specific genes or transcriptional regulators. For adaption and survival of bacteria in different environment the specific DNA binding proteins the transcriptional regulators perform very important role in gene expression. S*py*1258 is recognized as transcription regulator (TetR/AcrR family this is use as indicator for detection of s pyogenes (Liu *etal.,* 2005).

The S. pneumoniae was first recognized in the late 1800s, and early on was recognized as the most common reason of pneumonia (Musher, 2010).)

In this review discuss all about the identification methods of streptococcus microscopic appearance study, and biochemical test followed by beta hemolysis, catalase test and the PYR and bacitracin test all discuss. And the fast method for various antigen group detection through the streptex and next the identification through the API 20 strep. Molecular method use for bacteria detection explained  Later the Accurate molecular method PCR that target specific genes and 16sRNA gene of bacteria for identification are discussed in detailed.

**Morphology of Streptococcus**

The gram positive bacteria S *pyogenes* and *S pneumoniae* are, no motile, and not form spores; they generally need enrich complex culture media for growth. *S pyogenes* is typically round and egg shaped coccus with a diameter 0.6-1.0 μm .They produce pairs and chains of different length when they divide in one plane. Microscopic appearance of S *pneumonia* is lancet-shaped with a diameter of 0.5-1.25 μm diplococcus, but occasionally hard to differentiate morphologically from other streptococci (Patterson, 1996).

To recognize the colonies S. *pyogenes* in experimental samples, the screening of blood agar media plates for the occurrence of Beta-hemolysis. After 24 hours of incubation at 37C the typical form of S. pyogenes show clear margins with smooth and moist area. The colonies show a white grayish color with a diameter greater than 0.5mm. Heamolysis zone shown on blood agar media this zone is three to four times as big as the diameter of colony. The microscopic appearance of *pyogene* is round shape usually present in pairs and chains colonies (Spellerberg, 2016).

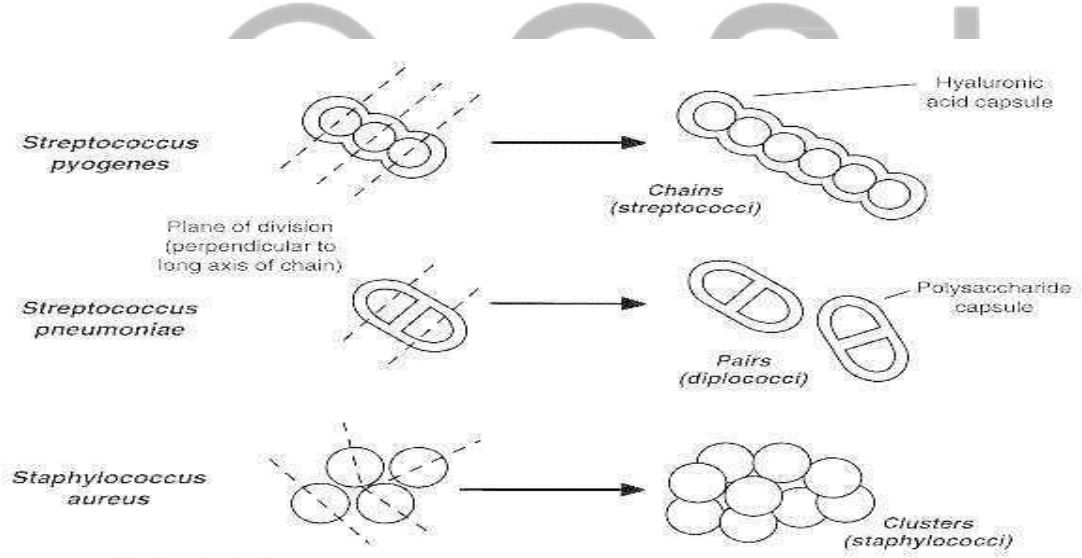


**Fig: 1** Streptococci morphology in comparison with *Staphylococci* (Patterson., 1996)

## Conventional methods for

### *Hemolysis*

On the blood agaer media streptococci show a type of hemolytic response that is used to categorize the streptococci. Beta –hemolysis is connected with complete breakdown of red blood cells around the colony e.g *S.pyogenes* colonies show this type of hemolysis. Alpha-hemolysis is linked with the green colour incomplete or partial hemolysis of Red blood cells around the colonies, e.g strains of *S.pneumonia* show this type of hemolysis. Gamma-hemolytic no hemolysis around the colonies (Parks *et al.,* 2015)

The property of hemolysis is not very reliable for the absolute classification of streptococci, but it is broadly used in fast screening for detection of *S. pyogenes* and *S. pneumoniae.*

## Beta-hemolysis

*Capacity of S. pyogenes* is use to differentiate from other is that they grow on blood agar media that contain sheep blood and this bacteria show the complete breakdown of RBCs while in compare to the other that give incomplete or no breakdown of RBCs Liu etal., 2005). After the finding of β-hemolytic colonies show a characteristic *S. pyogenes* appearance, catalase testing verify that the presence of *Streptococci.*

## Catalase test for differentiation of genus

The microorganism that live in oxygen enrich environment produce catalase enzyme that cancel the dangerous effect of hydrogen peroxide the oxygen metabolites. The catalase enzyme is usually absent in anaerobes, water and oxygen produce by the breakdown of hydrogen peroxide by the action of catalase. The Catalase test is mainly use to differentiate the genus from genus staphylococcus. The genus member is catalase negative whereas the members of genus *staphylococcus* give positive result three percent solution of $H_2O_2$ is mixed with inoculums of bacteria and the solution is visualize for formation of oxygen bubbles .If bubbles produce the catalase positive no bubbles means negative result (Tankeshwar,2011). This test is not particular for streptococcus detection but the strains are classify into the 2 two classes due to hydrogen peroxide production or non producer (Seki *et al.*, 2004).

## Surface Antigen Determination

Lancefield antigen present on the surface of *streptococci.* The differentiates into various species by finding this surface antigen by using antibodies. This method was first developing by Rebecca lancefied (Lancefield, 1933). This antigen grouping sera is available commercially and uses commonly to identify beta hemolytic streptococci. The commercial kits that are available also provided with a substrate for the rapid antigen detection through the agglutination of particular antibodies.

There is good association between the occurrence of antigen and with a particular species but this connection is not hundred percent in the case of A, C, and G antigen group. With the few exceptional cases usually *the* Streptococcus *pyogenes* having the group A antigen. The Occurrence of antigen A group is not restricted to S. pyogene it is also present in *anginosus* group (Facklam, 2002), and also in *dysgalactiae* subsp. *Equisimilis* (Brandt *et al.,* 1999). So for the correct identification of group A require additional tests as for the detection of *S. pyogene* achieved through the pyrrolidonyl-β-naphthylamide (PYR) test and bacitracin sensitivity test.

## Pyrrolidonyl Arylamidase (PYR) Test

For the differentiation of *S.pyogene* from other beta heamolytic *streptococci* like *S. dysgalactiae* subsp. *equismilis* that carry the same morphology the fast calorimetric method the PYR test is commonly used. Through this test the occurrence and activity of pyrrolidonyl aminopeptidase enzyme in streptococcus pyogenes GAS is check. The substrate L-pyrrolidonyl-β-naphthylamide (PYR) hydrolyzes into β-naphthylamide by this enzyme, the addition of cinnamaldehyde reagent generates a red color. In a few minutes this test also performs on strips that is made up of paper and contain adry chromogenic substrate for the enzyme aminopeptidase(Kaufhold *et al.,* 1989).

Manually the PYR agar is used to investigate the presence and activity of aminopeptidase enzyme from the *streptococcal* colony. PYR media were streaked with the inoculating loop containing the streptococcus culture. The plates were incubated for 16 to 20 h at 35°C in a normal atmosphere. From this test enzymatic hydrolysis of L-pyroglutamic acid-f-naphthylamide(PYR) observed . After the hydrolysis of aminopeptidase substrate the free 3- free 3-naphthylamine produce.After that the adding of PYR reagent that is a N,N-dimethylaminocinnamaldehyde on the bacterial colonies. within the two minutes the formation

of red colour indicate the positive result ,and the production of yellow and the light orange colour was consider as negative (Facklam *et al.*,1982).

For standard laboratory recognition procedures, the *S.pyogene* with PYR positive that illustrate the characteristic morphology of *S. pyogenes* now able to possibly recognize as *S. pyogenes* Spellerberg *etal.*, 2016). PYR test is considered to be specific for identification of *S.pyogenes* but some other β-hemolytic streptococcal species *iniae* and *porcinus* that is mainly animal associated species and very rarely occur in human medical sample are also PYR positve (AbrAhAM & SiSTlA, 2016; Spellerberg *et al.*, 2016).

To evade the fake positive results occur by other Species of bacterial that is PYR positive that is present in mixed culture so to avoid this situation the test must be perform on pure cultures.

## Bacitracin Sensitivity Test

Bacitracin test is use to differentiate the *S. pyogene* from the extra beta-hemolytic *streptococci* and non A group due to increase sensitivity to bacitracin (Maxted *etal.*, 1953). For obtaining better specificity in *streptocous pyogene* identification bacitracin test also with the Lancefield antigen A recognization are use because the other member of B hemolytic streptococci that have Antigen A group show resistance toward bacitracin. This analysis is also use to differentiate *S. pyogenes* from other PYR-positive β-hemolytic *streptococci* species, such as S. iniae and S. porcinus.

To perform a bacitracin sensitivity test, firstly the strain that is tested is sub-cultured on (SBA) blood agar plate, since on the primary plate placing the bacitracin disc that can give changeable results. The strain that is being tested is streaked with a pure culture of individual colonies on blood agar media plate and a disk having 0.04 U of bacitracin is settle on the blood agar plate. The incubation of plate overnight at 35°C in five percent carbon dioxide any region of inhibition around the disc point out the sensitivity of the bacterial strain (Kumar *et al.*, 2003; Amrouche *et a*l., 2004; James & McFarland, 1971).

## Streptex for antigen detection

The detection of beta hemolytic streptococci by a traditional lancefied method is very time wasting and technically challenging (Fuller, 1938; Lancefield, 1938). A new better and fast method of slide agglutination of trypsinised by using streptococcal suspension is developed in 1956 (Rosendal, 1956).The more modification of this method is done by the integration of the staphylococcal protein A coagglutination method. The result of this method is the immediate floc formation of the streptococcal suspension with trypsinization(Christensen *et al.,* 1973; Maxted *et al.,* 1976).

These processes depend on the availability of protein A and the homologous sera. For the small scale making of the antisera for the coagglutination individuallly not possible in a very busy clinical practice. Now this method is modified recently by Wellcome Laboratories through replacement of polystyrene latex particle for staphylococcal protein A and next the pronase enzyme is applied for the antigen removal (Ederer *et al.,* 1972).

This method is advertising commercially as streptex and use for the reorganization of A, B, C, D and F lancefied groups. Steptex is also use commercially for the identification of lancified group it give excellent recognize streptococci of groups A, B, C, and G. but its weakness is the limited accessibility of reagents and the deficiency of a group D antiserum (Waitkins etal.,1979)

## API strep evolution

The streptococcaceae family member has a significant impact in spreading a range of diseases in man and animal. The group A *pyogenes* produces infections and the group B causes pneumonia and the edometritis (inflammation of uterus lining) in adults. Meningitis is also produce in newborns due to group B streptococcus (Robinson *et al.,* 1979; Barton *et al.,* 1973; Bayer *et al.,* 1976 ) and also due to Strep dysgalactiae (Quinn *et al.*,1978).

Group G streptococci also produce endocarditis ( Buoza et al., 1979).A wide Varity of infection occur due to the viridans streptococci like the septicaemia and deep-seated abscesses is also cause by this streptococci (Parker et al., 1976).

Generally the microbiologist depend on the sereological method for the classification the streptococci these method is base on the lancefied grouping of surface antigen(Lancefield, 1993). Various commercial kits are present to perform this test (Waitkins *et al.,* 1979; Slifkin *et al.,* 1980).

But the limitation of this method is this is only give beneficial results for beta-haemolytic streptococci and gives variable results, for D group members. The additional chemical test required for the identification of alpha- and the non-haemolytic streptococci. An effort to join a variety of reactions into one solid and fast system. (API) created the API-STREP method that utilizes the reactive enzymes of the API ZYM (Waitkins *et al.*, 1976: Waitkins *et al.*, 1980).

And also the aesulin and hippurate breakdown hydrolysis reaction and the glycogen and raffinose manitol fermentation process along also with. This system is easy and efficient but only alter is the availability of less data base API assess by (Waitkins *et al.*, 1981). Afterward API-20 STREP formed.

## Method

This process allows the direct inoculation from a pour plate.After the incubation the reagents are apply the ZYMA and the ZYMB are add to PYRA to LAP cupules. In the HIP cupule ninhydrin is added to the HIP cupule, and the one drop of 40 percent potassium hydroxide is added into the VP cupule and on 1 drop of six percent alpha naphtol that is in the ethyl alcohol also added into VP capule. These tests need 10 min to build up and the result is compare with the manufacturer guide table sheet. The ESC Aesculin hydrolysis, and the Arginine dihydrolase (ADH) reactions and the carbohydrate reactions result are interpret after the four hours and 24 hours (Tillotson ,1982).



## Advantages and limitation

Rarely although the API-20 STREP have troubles in distinguish the groups C and the G groups, it prove outstanding in recognize the viridans and the members of the group D and also identified accurately the A and B lancified group. API-20 STREP method prove rapid, reliable and simple to carry out and available at suitable cost (Tillotson , 1982).

For the recognization of the beta-hemolytic *streptococci* belong to group A the API 20S system is establish to be technically precise. 96 strains of b heamolytic *Streptococci* collected randomly and out of which 48 group A beta- heamolytic *Streptococci* 100 percent identification through the API 20S is done(Keville & Doern, 1982).

# Molecular Methods

## PCR followed by specific genes

For the identification of minute quantity of any pathogen even the dead one the Polymerase chain reaction (PCR) is the essential technique that is use. In medical samples a variety of strategies to PCR amplification of bacterial DNA are available. The primary method that is depend upon the use of species-specific primers, but this process lack the ability to identify bacterial infection absolutely(Sauer *etal.,* 2005).

For the recognization of *S.pyogenes* spy1258, dnase, speB, and the sof genes are the key gene target (Kumar *et al.,* 2011; Dunne *et al.,* 2013; Slinger *et al.,* 2011). The sepB code for the potent virulence factors. The serum opacity factor is coded by *sof* gene and just 40 to 50 percent expressed by GAS. *Dnase*B is not extremely particular because other than the *S. pyogene* it also shows in lower quantity by beta-heamolytic . In all GAS *Spe*B gene is present this gene is chromosomally encoded structural gene (Louie *et al.,* 1998).

For adaption and species specific *maintenance Spy*1258 gene is require which is putative transcriptional regulator gene and is particularly present in *S. pyogenes* (Kumar etal., 2011).

It was also noted that in the other genome of bacteria that is accessible at gene bank the same sequence of spy1258 is completely missing. In a variety of clinical samples for the fast detection of *S.pyogenes* this specific gene was use in various studies. The utilization of PCR primers that is produced from that gene creates an amplification of DNA fragment of 407bp from the *S.pyogenes*. The other type of and bacteria not produce this type of fragment so this is confirmed that the spy1258 gene is a special layout that is present only in *S.pyogenes* (Liu *et al.,* 2005)

Another study that is conducted by Al Saudi, by using the spy1258F forward and spy1258R reverse primer in PCR. Notice that from every *S.pyogene* particular DNA piece of the possible size (450bp) was produce (Al Saudi., 2015).This result was agreed with all earlier result (Liu *et al.,* 2005; Dunne *et al.,* 2013 ; Schabauer *et al.,* 2014) .So from all of the studies this is confirmed that the spy1258 is specifically present in *S. pyogene* only.

In all the *pyogenes* the Dnase B gene appears to be special to that organism. In the PCR amplicification particular primers of that dnase B gene are used and a specific fragment of 140bp amplicon produce this verify that this gene is present in S.pyogenes only (Saudi *et al.,* 2015) and the result agree with previous study (Slinger *et al.,* 2011).

The Streptococcus pyrogenic exotoxin B gene (speB) is a chromosomally programmed structural gene of *S.pyogene*. This gene is pyrogenic and heart poisonous virulence factor of *S. pyogene.* The PCR base finding of *pyogence* by means of particular primers of speB in human the complete time reported for overall analysis is 80 minute for disease detection.

Specific fragment of 423bp of speB gene is produce. That can to be utilizing as a genetic marker for the early detection of rheumatic heart diseases because this amplicon of size 432bp don't show similarity with other organism (Kaushal *et al.,* 2012).

The invasive and non invasive diseases and the chronic rheumatic fever is produce by a human pathogen *pyogenes*. Rheumatic heart disease is a follow-up of rheumatic fever consequences from an untreated strep throat basis harm of the heart valve. The PCR base finding of *pyogence* by means of particular primers of virulent Sof gene in human the complete time reported for overall analysis is 1 hour for disease detection time reported so far for the confirmation of the disease. Specific Amplicon of 228 base pair of sof gene is produce. This can be use as a genetic pointer for *S. pyogenes* detection because it does not show homology with other organisms (Kumar *et al.,* 2011). All the main gene target of streptococcus pyogenes discusses in table: 2

**Table: 2 Key gene targets of Streptococcus pyogenes**

| **Genes** | **Primer sequence**<br>**5'----------→ 3'** | **Product size** | **Refrences** |
|---|---|---|---|
| spy1258 | spy1258 forward<br>5_-AAAGACCGCCTTAACCACCT-3_<br>spy1258 reverse<br>5_-TGGCAAGGTAAACTTCTAAAGCA-3_). | 407bp | Liu *et al.*, 2005) |
| Dnase **B** | Forword primer<br>5 TGA TTC CAA GAG CTG TCG TG 3<br>Reverse primer<br>R 5 TGG TGT AGC CAT TAG CTG TGT T 3 | 104bp | (Slinger *et al.*, 2011)<br>(Saudi *e tal.*, 2015) |
| SepB | Forward primer<br>(5'-GTA GCAACACATCCTGTAGCTGCA-3')<br>reverse primer<br>(5'- AGGTGCAC GAAGCG CAG AAG ATAT -3') | 423bp | (Kaushal *et al.*, 2012). |
| Sof | forward primer<br>(5'-TAGCCCCGACAGTT TTAGGA-3')<br>revers primer<br>(5'-AGGCTGGAGTAGTGCCTGAA-3') | 228bp | (Kumar etal., 2011) |

**PCR followed by 16S rRNA and RFLP**

The second method that is base on the amplification of universal sequences that is perseveres in every bacterium (McCabe *et al.*, 1995). For bacteria identification in laboratory Culture is mainly a very careful and time consuming process and need 8 hours of incubation and further extra time is require to perform biochemical tests and immunological analysis to find the bacteria. The 16SrRNA genes common in almost every bacterial pathogens present in body fluids has been sequenced (Bottger, 1989; Lane *et al.*, 1985; Wilson *et al.*, 1990).

With the one set of universal primer the amplification of that gene that is depends on16sRNA conserved gene present in all bacteria (Oreisen *et al.,* 1994; Radstrom *et al.,* 1994). Nowadays for the detection and identification of bacteria digestion of PCR product through restriction enzyme is performed in 4.5 hours. The restriction fragment length polymorphism layout of universal PCR product universal for different bacterial species are different and same pattern with same species, the 16SrRNA gene the preserved fragment generate a 996bp can be differentiate through this method.

To find and identify the bacteria in medical sample the Amplification and sequence examination of the 16SrRNA gene can be helpful (Jenkins *et al.,* 2012). With discovery of polymerase chain reaction and DNA sequencing method the comparison study of gene sequence of bacteria species disclose that a 16SrRNA gene is highly conserved inside the species and between the species of same genus so for the specie level identification of bacteria this is use as a new Gold standard method (Olsen *et al.,* 1993; McCabe *et al.,* 1995). For the epidemiologic and the taxonomic differentiation of bacteria the rRNA gene and RFLP examination, or ribotyping, has been used (Bruneau *et al.,* 1994). Many studies exposed that the ribotyping is very stable and reproducible method. And this is appropriate typing scheme for finding the molecular epidemiology of genetically different bacteria (Maslow *et al.,* 1993).

The isolates of *S. pyogene* when use in amplification of 16S rRNA with universal primers PCR product of desire size 996bp and a non specific band of 160 base pair also generate that may be due non- specific amplification from every DNA sample (Saudi *et al.,* 2015).

Using the universal primer for amplification followed by the restriction fragment length polymorphism is easily and sensitive way of finding and classify bacteria (Kumaravelu *et al.,* 2008).

Therefore by using PCR primer that target at the conserved area of 16S rRNA genes, it is possible to detecting DNA of approximately every bacteria and further recognition of the bacterium is complete by the restriction endonucleases digestion method (Lu *et al.,* 2000) or PCR product nucleotide sequencing (Harris & Hartly, 2003).The PCR products of *S.pyogene* treated with the restriction enzymes HeaIII formed 460bp, 330bp, 135bp and 95bp, four bands on gel. The restriction pattern of PCR product of different species of have different pattern while the restriction pattern of PCR product of same species create a same pattern (Saadi *et al.,* 2015).

In other study that is conducted on the cerebrospinal fluid for detection of microbes. Use the method of 16SRNA amplification of specific sequence of DNA followed by the RFLP of the polymerase chain reaction product (Lu J *et al.*, 2000). Treatment of HeaIII restriction enzyme RFLP bands of 460bp, 330bp, 135bp and 95bp produced in case of *S. pyogene* (Kalghatgi *et al.*, 2008).

Table; 2 16Srna gene target and RFLP pattern

| Genes | Primer sequence | Product size | RFLP pattern with HEAIII enzyme | Refrence |
|---|---|---|---|---|
| 16S rna | U1)F 5'CCAGCAGCCGCGGTAATACG-3',<br><br>(U2)R 5'-ATCGGTTACCTGTTACGACTTC3 | 996bp | 460 bp,<br>330 bp,<br>135 bp, and<br>95 bp | Kalghatgi *et al.*, 2008<br>Alsaudi *et al.*, 2015 ) |



## Plasmid profile analysis

One of the old methods for the epidemiological study of bacteria is the investigation of plasmid profile. In this method the plasmid DNA is isolated from bacteria and then run on the agarose gel electrophoresis. This method is easy to interpret the outcome results and simple to carry out. But the plasmid is the extrachromosomal mobile element that is able to suddenly lost and readily attain by the bacteria. The bacterial isolates that are associated epidemiologically is able to simply show the plasmid profiles differently (Trindade et al., 2003). This is also describing that the plasmids are the transposons that carry the resistant determinants. That is able to suddenly

gone and attain by a bacteria and rapidly altering the plasmid DNA composition. Plasmids live into a range of spatial shapes that is straight and nick and supercoiled shapes when this apply to electrophoresis result produce a diverse movement velocities. And the reproducibility of this method is influence by this (Hartstein et al. 1995)

## Random amplified polymorphism deoxyribonucleic acid (RAPD)

This PCR based method that uses that arbitrary primer to amplify the piece of the target DNA at random in the low -stringency polymerase chain reaction situation (Wassenaar and Newell, 2000.). A set finger prints of diverse length that is specific to each strain are produce after the amplification of one or more sequences of DNA by this procedure. (Farber 1996, Trindade et al.2003)

The RAPD technique is first apply in 1990 to observe the DNA sample of Human. After that the various authors explain the utilization of Random amplified polymorphism deoxyribonucleic acid technique for microorganisms (Babalola, 2002). This method applies the random primer with no need of the information regarding the nucleotide sequences of any specie. The amplify product show a polymorphism that is utilize like a genetic marker (Williams et al., 1990)

## Multilocus sequence typing (MLST)

This is a nucleotide base clear and easily carried method that uses the inner fragment sequence of seven housekeeping genes for the typing of bacteria (Maiden *et al.*, 1998; Spratt 1999; Urwin and Maiden 2003).

This a genetic techniques that is depend on the sequencing of the seven genes of about 450 to 500 base pairs fragment of each gene use for the bacteria that contain the high level of variation inside that housekeeping genes. This method also has a high resolution power. This method allows the detection of same microorganism and the very closely linked through the detection of variation in the various loci. Basically they work as a marker along the evolution they stay stable and utilize into the hug time scale for the link of strains or for the various geographical areas (Losada *et al.*, 2013). With the idea of the electrophoresis of multilocus enzyme the multilocus sequence typing (MLST) was find out .MLEE), (Maiden *et al.*, 1998; Spratt 1999; Trindade *et al.*, 2003)

## Pulsed field gel electrophoresis (PFGE)

This methods is use to separate the bigger piece of the DNA molecule in the agarose gel electrophoresis through the application of electric currents that at a time alter the 3 direction not like conventional method in which the current fellow just in one way(Schwartz and Cantor 1984;Arbeit 1999; *Trindade et al.,* 2003).

By the action of restriction enzymes and the endonucleases the whole chromosomes are break down to produce a fragments of DNA of various length in the pulsed field gel electrophoresis theses fragments of different sizes are also called the RFLP and these patterns are very specific for certain stains and species (Shi *et al.,* 2010).

The PFGE is a gold standard method for the various researchers for investigation of occurrence of pathogen and for the further epidemiological study (Alonso *et al.,* 2005).

## Amplified Fragment Length Polymorphism

This technique is base on the amplification of the particular subset of the piece of DNA that produces after the action of restriction enzyme breakdown basically this is a genomic fingerprinting method. This is initially, functional only for the categorization of plant genomes, now amplified fragment length polymorphism is also utilize for the bacterial typing. AFLP with 2 variation have explained, the initially the 2 restriction enzyme and the two primer are use for amplification and secondly use the 1 primer and 1 restriction enzyme.

Firstly the DNA of bacteria is isolated and purify after that the 2 restriction enzyme EcoRI and the Msel is apply for digestion. After that with the RFLP fragments adaptor are attaché that contain the sequence for the primer binding and the every restriction site. Afterwards, the restriction fragments are bound to adaptors that contain each restriction site and a sequence that is homologous to a binding site of the PCR primer. The polymerase primer that is use for the amplification are homologous to the adaptor and contain a 1 or 2 particular bases in the3’ends (Sammarco *et al.,* 2014; Paixão *et al.,* 2013). The primer that is use for the amplification is radio labeled and for the occurrence and lacking of the pieces of the DNA to recognize the polymorphism through the PAGE denaturing investigation (Blears *et al.,* 1998; Wassenaar and Newell, 2000)

## CONCULSION

In this review the identification methods of Streptococcus discuss. For the identification of streptococcus firstly microscopic appearance of streptococci observed they are coci shape that that form chains and pairs..

Bacterial culture studies of blood agar media show the zone of hemolysis around the bacterial colonies. Further identification of bacteria require biochemical test like catalase, pyr, bacitarcin sensitivity use. Gram positive Streptococi are catalse negative, and PYR positive but some other strains of streptococci are also positive so for the further identification we perform bacitracin sensitivity test because Streptococus pyogenes is sensitivity to bacitracin. For bacteria identification in laboratory Culture is mainly a very careful and time consuming process and need 8 hours of incubation and further extra time is require to perform biochemical tests and immunological analysis to find the bacteria. Steptex is also use commercially for the identification of lancified group it give excellent recognize streptococci of groups A, B, C, and G. but its weakness is the limited accessibility of reagents and the deficiency of a group D antiserum. API strep 20 is also use to identify bacteria that contain 20 wells these wells contain chromogenic substrate for different reactions that produce different colour after reaction and the result read from the reading table.API 20strep is excellently identify the group D and group A and B. Next So for fast and correct identification of bacteria less time consuming molecular methods use. Polymerease chain reactions use with specific primers that target the specific genes of s.pyogenes. In case of s.pyogenes spy1258, dnase, speB,and the sof genes are key gene target. Specific size of amplicon produces that compare with marker.

The second method that is base on the amplification of universal sequences that is perseveres in every bacterium. With the one set of universal primer the amplification of that gene that is depends on16sRNA conserved gene present in all bacteria. The amplicon of 996bp produce with all further the RFLP is use to further classify the bacteria. RFLP pattern of same size produce in case of same species while different RFLP produce with different species.

We find that the molecular method by using PCR that target the specific genes and the 16sRNAgenes is found to be very effective fast and accurate method than the conventional methods.

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