Genotyping of Streptococcuspneumoniae Isolated from Upper Respiratory Tract Infections by RAPD analysis

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Streptococcus pneumoniae, commonly called the pneumococcus,is responsible for high rates of morbidity and mortality worldwide. Genotyping of S. pneumoniaeby using RAPD technique have largely replaced the phenotyping methods. this study was suggested and designed to study the genotyping of S. pneumoniaeby using RAPD analysis method. In this study, randomly amplified polymorphic DNA (RAPD) analysis was performed by using arbitrarily primer (S1254) and PCR technique.Strains were considered different from one another if their patterns differed by one prominent band in three repeated experiments.

In the current study, 31 patterns (different types of S. pneumoniae) were yielded out of 32 isolates composed 4-10 fragments ranging from (64-1922) pb in size of which one permenant band (134 pb) was obtained from most isolates.The dendrogram generated when these (32) different isolates of S. pneumoniae were compared is and this dendrogram appeared two main clusters, whereas the first includes 26 isolates with 3 subcluster, and the second include the remaining 6 isolates with two subcluster. The first contain 4 isolates and the second included 2 isolates . The higher homology 66% were appeared between isolates S_{13} and S_{15}, followed by 60% between isolates S_1 and S_{32}. These of RAPD-PCR method it possible to identify non-serotyped strains, and shows the necessity of this molecular typing technique for typing S. pneumoniaestrain from patients with LRTI.

Intruduction:

S.pneumoniae is the major cause of community- acquired pneumonia in adults and serious respiratory infections in children in the United States. An estimated three to five million deaths occur annually in children under 5 years of age due to acute respiratory infections, for which Streptococcus pneumoniais the most important pathogen (Obaro, 2011).Differentiation of S. pneumoniafrom other viridans group streptococci, has conventionally been based on phenotypic characteristics, most commonly by demonstrating optochin OPT susceptibility and/or solubility in bile (Facklam,2002).

Molecular genetic analyses based on the 16S rRNA gene have provided a potent means of characterization at the species level (Stackebrandt and Goebel, 1994). Unfortunately, S. pneumoniae shares over 99% 16S rRNA gene identity with S. mitis and S. oralis (Suzuki etal., 2005), complicating molecular identification among them.Genetic transformation , exchanges of virulence factors among streptococcal species and the appearance of new closely related streptococcal species, such asS.pseudopneumoniae, confirm that problems in taxonomy and pneumococcal identification will continue in the future(Arbique et al., 2004; Fink, 2005).

Molecularmethods for typing are useful in studying a short-term (the spread of an isolate of a hospital or local community) as well as epidemiological follow-up . For S.pneumoniaeepidemiology, accurate typing methods, such as pulsed-field gel electrophores (PFGE) and restriction fragment length polymorfism (RFLP) (Schlegel et al., 2003) and multilocus sequence typing (MLST) (Spratt 1999), have been developed.The PFGE method is based on the evaluation of total chromosomal DNA, and the obtained PFGE type is compared with similarity and diversity patterns. It is commonly used and considered as a gold standard (Shaaly et al., 2005). Several modifications of the RFLP analysis have been used in the epidemiological follow-up of S.pneumoniae(Doit et al.,2002 ;Schlegel et al., 2003).

Molecular studies on S. pneumoniae have received a little attention in Iraq. In light of the medical importance and to demonstrate the molecular relation between isolates, this study was suggested and designed to study the Genotyping of S. pneumoniae by using RAPD analysis method.

Materials and Methods

Bacterial strains.

In this study 74 pneumococcal isolates recovered from 600 sputum of patients with clinical symptoms of LRTI (pneumonia, COPD and TB)During theperiod of from November, 2012 - February, 2013 obtained from consultants clinic for respiratory and chest in Al-Sadder Medical City .All isolates were diagnosis by microscopic ,colonial morphology,and by used Conventional biochemical test . STREPO-SYSTEM 9R and VITEK-2 copact using GPcards were used for confirmed and accurte final diagnosis test ,

DNA extraction.

For genetic analysis, we selected 32 of S.pneumoniae isolates **.** S.pneumoneae isolates were cultured on tryptic soy agar suplimented with 5% sheep blood and inoculated individually into TSB and incubated at 37˚C/24h. Genomic DNA Extraction Kit (Geneaid) was used for DNA extraction**.**

Detection of DNA by Agarose Gel Electrophoresis

Gel electrophoresis was used for detection of DNA by UV transilluminator according to Sambrooket al. (2001).

Genotyping of Isolates by RAPD Method

An arbitrary primer was used for RAPD-PCR assay of S.pneumoniae DNA sample (100 ng) using a modified protocol of Duarte etal., (2005) in a 25-µl PCR reaction volume. And RAPD-PCR products were resolved by electrophoresis on ethidium bromide (0.5 µg/ml) pre-stained 1.0% agarose gel using a 1 Kb DNA ladder (Fermentas) for size extrapolation.The bands were detected by used UVI B and software (version 12.14). Isolates were considered different from one another if their patterns deferred by one prominent band in three repeated experiments.

Targete gene	DNA sequence (5'-3')	Reference
RAPD1254	CCG CAG CCA A	Duarte et al. (2005)

Table (1): Program used to amplify the RAPD 1254

Stage	Temperature (time)	
Initial denaturation	94C° for 5min	2cycle
Denaturation	94C° for 1min	40cycle
Annealing	37C° for 1min	
Extension	72C° for 2min	
Final extension	72C° for 10min	

Results and Discussion:

In this study, randomly amplified polymorphic DNA (RAPD) analysis was performed by using arbitrarily primer (S1254) that described by Duarte et al. (2005) and PCR technique. The PCR-RAPD products were resolved by 2% agarose gel electrophoresis. Strains were considered different from one another if their patterns differed by one prominent band in three repeated experiments.

In the current study, 31 patterns (different types of S. pneumoniae) were yielded out of 32 isolates composed 4-10 fragments ranging from (64-1922) pb in size of which one permenant band (134 pb) was obtained from most isolates (figure 1).

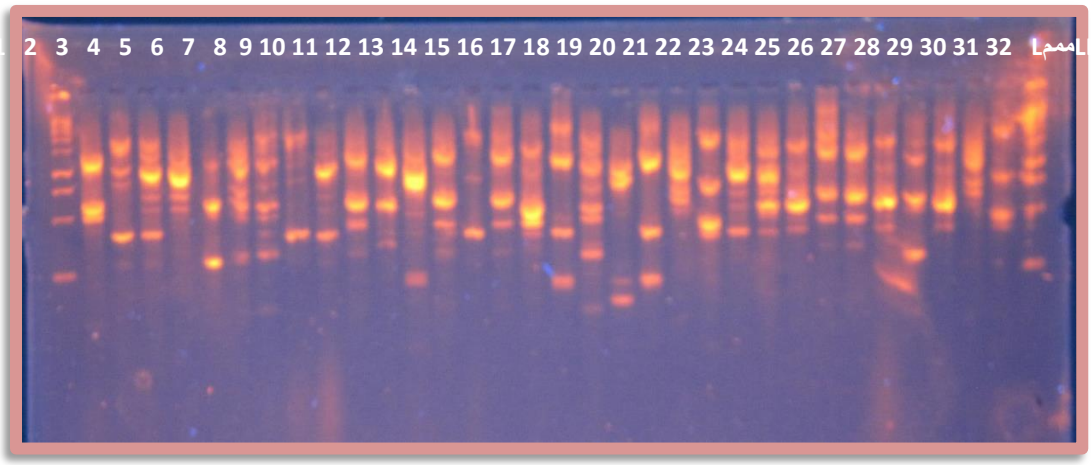

Figure (1) RAPD amplified products of the S. pneumoniaeusing random primers (1254)

These of RAPD-PCR method it possible to identify non-serotyped strains, and shows the necessity of this molecular typing technique for typing S. pneumoniaestrain from patients with LRTI.

The dendrogram generated when these (32) different isolates of S. pneumoniaewere compared is shown in figure (2). This dendrogram appeared two main clusters, whereas the first includes 26 isolates with 3 subcluster, and the second include the remaining 6 isolates with two subcluster. The first contain 4 isolates and the second included 2 isolates (figure 2). The higher homology 66% were appeared between isolates S_{13} and S_{15}, followed by 60% between isolates S_1 and S_{32}.

Kilian et al., (2008) investigated the evolutionary history of the pneumococcus and its close commensal relatives using a polyphasic phylogenetic strategy and analysed of this unique cluster of closely related species with very distinct pathogenic potentials. Surprisingly,Arbiqueet al., (2004) demonstrated that S. pneumoniae is but one of several hundred distinct phylogenetic lineages of a cluster of otherwise

commensal streptococci known as S. mitis or S. pseudopneumoniae in which S. pneumoniae is no more genetically divergent from other members of the cluster than individual lineages of S. mitis are from each other. The name S. pseudopneumoniae was recently assigned to one of these lineages.

Because the number of strains has been increasing year by year, the possibility of an alternative method for typing pneumococci was investigated. Therefore, a number of genotyping techniques such ribotyping (Shundi et al., 2000), pulse field gel electrophoresis (Gonzalez–Rey et al., 2003), random amplified polymorphic DNA (RAPD) analysis (Fica et al., 2003) and multilocus sequence typing (Enright et al., 2001) are being applied worldwide for typing of isolates. In order to simplify the typing work and reduce cost and time, the Fenollet al., (1997) used RAPD test routinely in the laboratory, based on the SGT distribution of the pneumococci isolated in Spain.

RAPD have been also used for subtyping of GAS isolates mainly in western countries (Gonzalez–Rey et al., 2003 ;Fica et al., 2003). The RAPD assays were performed at constant DNA concentrations and the experiments were repeated several times by taking proper precautions. In the present study, RAPD results were reproducible when samples were run in large gels simultaneously. Thus, all these facts suggest the usefulness of this technique in disease outbreak detection.Nandi et al., (2008) demonstrated the benefit of RAPD fingerprinting in comparison to other molecular methods in identifying and characterizing S. pneumoniae isolates obtained from pharyngitis and RF/RHD cases.

In addition use of PCR based RAPD method for typing of GAS was found to be highly discriminatory. As reported earlier, selection of primers, optimization of PCR condition and combination of different primers play an important role in discriminating the isolates by RAPD (Seppälä et al., 1994). Nandi et al., (2008) used five arbitrarily selected primers were tested. The majority of arbitrary primers used, produced distinctly reproducible patterns in all the isolates studied. These result confirm the result of the present study in which the primer used (S1254) in this study gave a high difference between isolates. Although, Iwalokun et al., (2012) demonstrated the limitations of RAPD in epidemiological characterization of S. pneumoniaeisolates in this environment. Therefore, for better phylogenetic grouping of S. pneumoniaeand improved understanding of serotype switching, typing techniques such as multilocus sequence typing (MLST) are required and this method was used for better understanding of clonal diversity, dissemination and pathogenicity of S. pneumoniaeat regional, national and country levels.

Genotypic similarity, as was found in the majority of the paired samples, indicates clonal relatedness and implicates that pneumococci present at different sites in the upper respiratory region of a patient originate from a single source (Tonnaeret al., 2005). In conclusion the primer used in this study is the best for genotyping of S. pneumoniaedue to it gives high varieties.

Despite advances in our understanding of the epidemiology and pathogenesis of pneumococcal infections, the precise genetic factors that predispose a given pneumococcal clone for disease versus carriage remain unknown (Pettigrew et al., 2006).

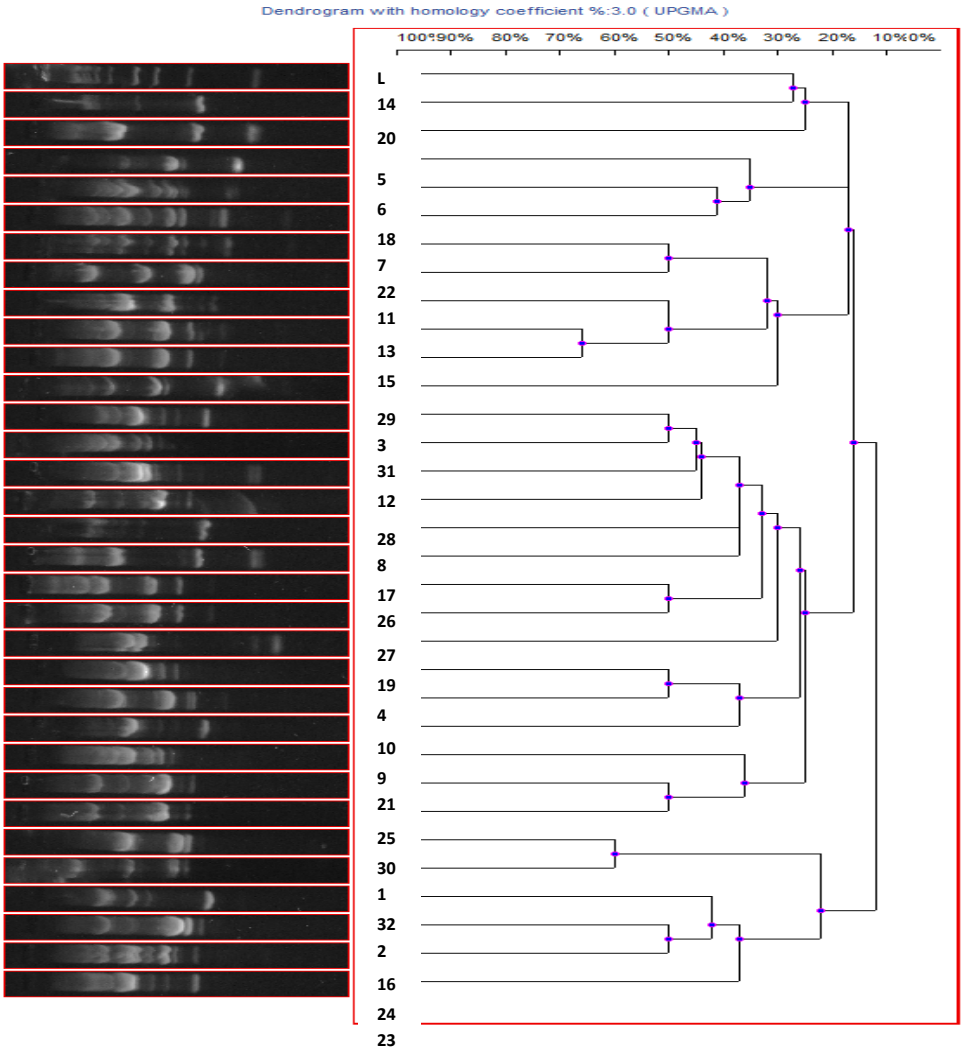

Figure (2): Dendrogram showing genetic relationship of 32 S. pneumoniae isolates produced by RAPD with primer 1254.

Conclusion:RAPD analysis is the faster and simplest method used for typing of S. pneumonia by using primer S1234.

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