Typing of Streptococcus Pyogenes Isolates from Patients with Acute Pharyngitis by Random Amplified Polymorphic DNA Fingerprinting Analysis

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Abstract

Background: Streptococcus pyogenes is (group a β-hemolytic Streptococcus GAS) is an important gram-positive bacteria causes a wide spectrum of clinical diseases ranging from mild pharyngitis to life-threatening invasive infections. Random amplified polymorphic DNA (RAPD) fingerprinting is a PCR based technique which has been successfully used to appear genetic variation between closely related strains within the same species, it is more reliable technique for the typing of GAS isolates in epidemiological investigation. The current study aims to investigation genetic diversity of Streptococcus pyogenes isolates from acute pharyngitis patients by RAPD-PCR fingerprinting method.

Subject and methods: One hundred and twenty-five pharyngeal swabs were collected during the periods 21/10/2021 to 25/1/2022 from patients with acute pharyngitis. The standard bacteriological methods used for isolation and identification of S. pyogenes. Twenty clinical isolates of S.pyogenes, were genotyped by the RAPD-PCR fingerprinting method achieved by three arbitrary primers OPA13, OPA14 and P14

Results: out of 125 pharyngeal swabs, only 40 swabs give positive culture for S.pyogenes with a percentage of 32%. Amplification of genomic DNAs from the GAS isolates with OPA13 primer showed best results than other primers (OPA14 and P14) resulted 15 polymorphic DNA segment, phylogenetic analysis showed a high degree of genetic diversity, classified GAS isolates into 4 main clusters

Conclusion: The DNA fingerprinting by using RAP-PCR analysis is an effective method for evaluation the genetic diversity of GAS isolates

Keywords: Streptococcus pyogenes, Pharyngitis, RAPD-PCR, DNA fingerprinting

1. Introduction

Group a streptococcus (GAS) is synonymous with Streptococcus pyogenes, the only species within this group of β-hemolytic streptococci, so called group a β-hemolytic streptococcus (GABHS) is a Gram positive one of the leading pathogenic bacteria that infects children and adolescents, and is associated with a wide spectrum of infections and disease states [1].

That can cause both non-invasive and invasive disease (GAS), as well as nonsuppurative sequelae. This includes pharyngitis, scarlet fever, impetigo, cellulitis, type II necrotizing fasciitis, streptococcal toxic shock syndrome, acute rheumatic fever (ARF) and post-streptococcal acute glomerulonephritis (PSAGN) [2].Streptococcus pyogenes the most common bacterial cause of pharyngitis.

The classic presentation of GAS pharyngitis includes sudden onset of fever and sore throat with inflammation of the tonsils noted on exam in the absence of viral respiratory features. Tender anterior cervical lymphadenopathy is often present. Palatal petechial, strawberry tongue, red swollen uvula, or scarlatiniform rash may also be present. GAS pharyngitis can occur at all ages and it is most common in school-aged children with a peak at 7-8 years of age. Pharyngitis caused by GAS is rare in children less 3 years and become much less common in late adolescence [3, 4].

Epidemiological investigations of GAS infections and outbreaks usually used several methods for typing GAS emm typing and Multi-Locus Sequence Typing (MLST) both methods rely on sequencing of one emm gene (emm typing >200 serotypes or seven genes (MLST typing). In general, these are the two most widely used methods of geneotyping of S.pyogenes .Both methods are relatively fast and uncomplicated, but require specialized equipment and can be too expensive for routine use. In addition, they both have too low resolution to distinguish between closely related strains [5].

Pulsed field gel electrophoresis (PFGE) has been for many years the method of choice used to investigate differences between strains at the genome level. But PFGE analysis requires specialized equipment, skilled personnel, and is difficult to analyze and compare [6] Additional methods for typing GAS isolates named the phage profiling method [7].

Recently Random amplification polymorphism DNA (RAPD) have been widely used as non-sequencing based tools for genotyping [8]. RAPD-PCR is a simple, rapid, easy, and inexpensive method that can be performed in a moderate laboratory. In this method, a single short primer (8-12 nucleotides) is used in each reaction which its melting temperature (Tm) is low Primers can attach randomly to several DNA sequences in the genome The number and the positions of binding primer sites are unique for each bacterial strain (Amplified segments of DNA in RAPD PCR technique are random [9]. Differences between the generated RAPD patterns from the different DNAs indicate polymorphism between strains [10]. In

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Iraq, information regarding genotyping of GAS isolates is largely lacking

The current study aims to determine the genetic diversity and relationships of GAS isolates associated with pharyngitis cases by using RAPD-PCR analysis.

2. Material and Methods

Study population and Pharyngeal swab collection

Acute pharyngitis patients were the study population.125 pharyngeal swabs were collected during the periods 21/10/2021 to 25/1/2022 from patients enrolled to Educational Hilla Hospital and Al-imam Al-Sadq General Teaching Hospital.

The patients included to this study whose aged 1-60 years and with symptoms of acute pharyngitis and diagnosed by the specialist physician. Whereas, the cases excluded from this study were pregnant women and tonsillectomy cases and those who took antibiotics within 2 weeks of sample collection.

Ethical approval

The necessary ethical approval from ethical committee of the hospitals, private clinic and patients and their followers must obtained. Moreover, all subjects involved in this work are oral informed and the agreement required for doing the experiments are obtained from each one preceding the collection of specimens. This study was approved by the committee of publication ethics at college of science for women, University of Babylon, Iraq.

Bacterial isolation and identification

The pharyngeal swabs were transported using Brain heart infusion broth transport media with cold box containing ice pack to Babylon University, Microbiology Research Laboratory. The specimens were inoculated on 5% Columbia blood agar plates and incubated at 37 °C in candle jar with 5% CO2 atmosphere for 24 h [1]. Catalase test was done from 24 h growth of β-hemolytic colonies to differentiate catalase negative, this catalase negative Streptococcus species was subjected to bacitracin test, the Bacitracin susceptible Streptococcus isolates subjected to rapid identification using Vitek 2 compact system to confirm identifying Streptococcus pyogenes.

RAPD- PCR amplification

Genomic of DNA was prepared from overnight grown streptococcal cultures, using promega DNA extraction kit and according to the manufacturer instructions. The concentration of extracted DNA was measured and confirmed by NanoDrop Spectrophotometer (Thermo Fisher).

Amplification was carried out using three random primer sets for RAPD-table1. The DNA amplification reaction was carried out in a 25µl volume containing 5µl DNA, 12.5µl GoTaq® Green Master Mix (Promega), 2.5µl of primer (10 pMol), and 5 µl of nuclease free water; A single primer was used in each reaction. Amplifications were performed in a DNA thermal cycler (Perkin Elmer Cetus, USA) with conditions, each consisting of initial denaturation 94c for 4 min followed by 40 cycles .denaturation at 94°C for 1 min, annealing using temperature gradient at 35±5°C for 1 min and extension at 72°C for 2 min a final extension 72c for 7 min. Amplified products were resolved in 1.5% percent agarose gel, stained with ethidium bromide and visualized in a UV trans illuminator.

The phylogenic investigation of GAS isolates conducted by the amplified polymorphic DNA segments which analyzed by Paleontological Statistics version 4.0

Oligonucleotide primers used in RAPD-PCR Protocol of S. Pyogenes

Name of primer	Sequence 5´ 3´	Size (bp)	GC content (%)	Tm a (°C)	Range No. of distinct RAPD product	reference
OPA13	CAGCACCCAC	10	70	34	8	Moghaddam et al.2019
OPA14	GACCGCTTGT	10	60	32	8	
P14	GATCAAGTCC	10	50	30	1	

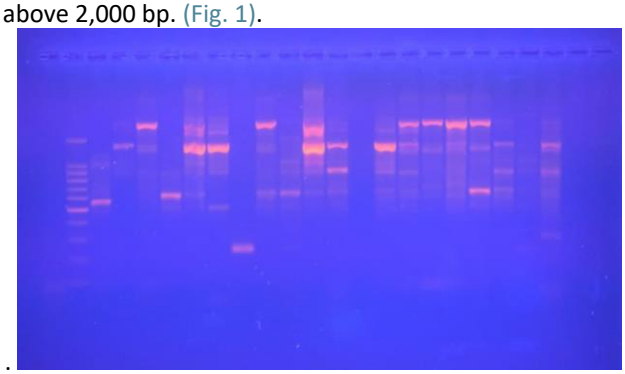
3. Results

Occurrence of Streptococcus pyogenes

The results of the current study showed that out of 125 pharyngeal swabs, only 40 swabs give positive culture for S.pyogenes with a percentage of 32%. The isolates were negative for catalase enzyme test and susceptible for bacitracin with beta-hemolytic zone around pin colony as well as microscopic examination revealed that a Gram positive cocci with long chain arrangement. Rapid identification test was achieved by using Vitek 2 compact system for confirming the diagnosis of S.pyogenes isolates, it had a confidence rate at 99% for S. pyogenes. Twenty GAS isolates were selected for investigation the genetic diversity among them.

RAPD fingerprinting analysis

Amplification of genomic DNAs from the GAS isolates with OPA13 primer showed best results than other primers (OPA14 and P14), based on the number, intensity and size range of RAPD bands. In RAPD pattern consisting of 15 distinct DNA fragments, generally ranging from 250 to above 2,000 bp. (Fig. 1).



(Fig. 1). 1.5 % Gel electrophoresis of RAPD –PCR products using OPA13 primer. M: is DNA marker 100-1500bp. Lane 1-20: GAS samples resulted the DNA polymorphic segments at length size 250-above 2000bp visualized by Ethidium bromide under Uv transeluminator

In phylogenetic analysis, this study showed a high degree of genetic diversity in GAS isolates they were classified into 4 main clusters

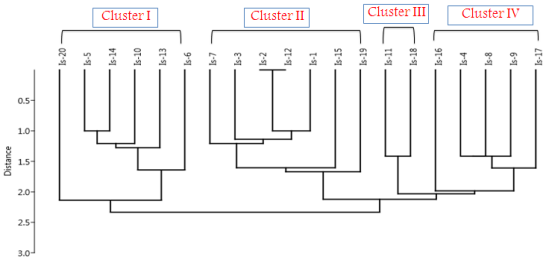


Figure (1): RAPD-PCR dendrogram phylogenetic tree analysis of Streptococcus pyogenes isolates by using (Paleontological Statistics version 4.0). The Cluster analysis using (algorithm Ward's method) were showed 4 clusters within 15 polymorphic variants between 20 S. pyogenes isolates.

Table: RAPD-PCR Cluster analysis and polymorphic variants for S. pyogenes isolates

No. of Cluster	No. of Isolate	No. of polymorphic variants
I	Is-5, Is-6, Is-10, Is-13, Is-14, Is-20	5
II	Is-1, Is-2, Is-3, Is-7, Is-12, Is-15, Is-19	6
III	Is-11, Is-18	1
IV	Is-4, Is-8, Is-9, Is-16, Is-17	3
Total: 4	20	15

4. Discussion

The most common bacterial cause of pharyngitis is infection by Group A β-hemolytic streptococcus (GABHS), commonly known as strep throat. 5–15% of adults and 15–35% of children in the United States with pharyngitis have a GABHS infection [11].

In a local studies [12, 13] concerned with isolating S.pyogenes from pharyngitis patients of the percentage of it were 30% and 52.2% respectively

The reasons for the discrepancy in the rates of infection with GAS are not relatively understood and may be due to the different conditions of the study in terms of demographic of patients registered in each study.

The incidence and prevalence of both invasive and non-invasive GAS infections in developing countries are largely unknown. Systematically collected data are essential for a functioning disease-control program and, thus, the measurement of incidence and temporal trends are an essential first step toward reducing the burden of GAS disease in developing countries [14].

The molecular typing of GAS isolates by using RAPD pattern, it classified the isolates into four main cluster which were determined by converting RAPD data into algorithm Ward's method and analyzed by Paleontological Statistics version 4.0 to produce a phylogenetic tree

The pharyngeal GAS isolates in the present study revealed a high degree of genetic variations which can be generated by mutations.

A previous study noted that the RAPD and PFGE techniques could be efficient tools in epidemiological studies of GAS [8, 15].

Introduced that RAPD-PCR-High resolution melting curve (HRM) analysis as a potential alternative method to differentiate non-dysenteriae Shigella species from clinical samples. They found RAPD-PCR-HRM assay more sensitive and specific than ERIC-PCR-HRM as the potential of alternative method for differentiation of non-dysenteriae Shigella [8].

5. Conclusion

The DNA fingerprinting by using RAP-PCR analysis is an effective method for evaluation the genetic diversity of GAS isolates. The large variation in the genetic material may result from mutations due to for Excessive use of antibiotics during course of infection

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