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Molecular Study of spy1258 gene in Streptococcus Pyogenes Isolated from Pharyngitis Patients in Fallujah City

Nada Zaidan Khalaf[1], Laith Moslih Najeeb[2], Asraa Adnan Abdul-Jalil[3]

[1]Post graduate Student, University of Anbar, College of Sciences, Department of Biology, Iraq,
[2]Assist Prof. Dr. University of Anbar, College of Sciences, Department of Biology, Iraq,
[3]Assist Prof. Dr. University of Anbar, College of Pharmacy, Iraq

Abstract

Background : *Streptococcus pyogenes* (GAS) strict human pathogen that give rise to a wide range of diseases. GAS is the most current bacterial cause of pharyngitis and firstly effect school-age children 5-15years of age. SPY1258 gene is a specialized gene group in GAS and its considered specific for GAS and can be used for its detection as a markers.

Method : A total of 300 throat swab were collected from patients suffering from pharyngitis referred to Fallujah general teaching Hospital, Fallujah taeching Hospital for women and chiladren and Amerya general Hospital , Iraq during a peroid between 2018-2019.

Theses samples were submit for bacteriology and molecvular tests.

Results : Out of all samples screened 43 isolates (14.3)were identified as ß – hemolytic streptococci and only 13 isolates were detected as streptococcus pyogenes by bacteriology and biochemical test and also by PCR GAS isolates exposed in order to detect the particular gene (SPY1258). Antibiotic sensetivity test showed a high incidence of bacterial resistance to macrolids.

Conclusion : Bacitracin sensetive is non- specific test for the identification of *Streptococcus pyogenes*, and should be used further test, for best result should confirmed by SPY1258 gene. The SPY1258 gene can be used for accurate molecular diagnosis of *Streptococcus pyogenes* .

Key words: *Streptococcus pyogenes, SPY1258, pharyngitis*

Introduction

Streptococcus pyogenes , a group A streptococci (GAS), is strict human pathogen that give rise to a wide range of diseases , from moderate to acute invasive diseases . *Streptococcus pyogenes* is countable for over than 500000 death each year [(1)].

β - hemolytic *streptococci* produce a toxin that forms a clear zone of hemolysis on blood agar, confirmation its strength to devastate red blood cells. This hemolysis is referred to toxins created by *Streptococcus pyogenes* called “Streptolysins” which can destroy the red blood cells and also the white blood cells which is responsible for destroying pathogen [(2)]

The skin and mucus membranes of the human host are the only reservoirs for *Streptococcus pyogenes* in nature . The major reservoirs of *Streptococcus pyogenes* are the school aged children (5-15) years [(3)] .

Pharyngitis (sore throat) is one of the most widespread situation encountered by the family practitioner [(4)].

Streptococcus pyogenes is the most current bacterial cause of pharyngitis and firstly affect school-age children 5 – 15 years of age [(5)] .

The universal development of antibiotic resistance between GAS strains , and the economic crisis emerge out of it in healthcare industries has presupposed the need for an alternative agent with novel texture features[(6)]

SPY1258 gene is a specialized gene group in *Streptococcus pyogenes* which was encodes transcriptional regulators . As transcriptional regulators represent fundamental molecular combination in the adaptation and existence of *Streptococcus pyogenes* [7] .

Materials and Method

Three hundred swabs were collected from children with pharyngitis ranging in age from 4 to 14 years , who refeered to fallujah general teaching Hospital , fallujah teaching Hospital for women and children , The health center of the primary health care sector in fallujah , health centers in refugee camp and special medical clinics in the fallujah city , During the peroid from November 2018 to May 2019 .

Throat swab was taken from the pharynx of each patient , and immediately placed in a trypton soya broth with 5% blood and transferred to TSA, the plates were incubated at 37℃ for 18 to 24 hours under 5- 10% Co2 , in the candel Jar [8].

Identification of streptococcus pyogenes :

The identification of the colonies were confirmed based on morphological and growth features , including the phenotypically discrete colonies , beta- hemolysis on blood Agar plate. Biochemical test and Bacitracin discs susceptibility were used in order to confirmed streptococcus pyogenes. GAS diagnosis was confirmed by using vitek 2 compact.

Antimicrobial susceptibility testing :

the standard disc diffusion method on Muller – Hinton agar supplemented with5%blood was done in order to confirmed GAS isolates and Antimicrobial susceptibility . Incubated for at lest 24hours at 37℃ in air inrichment with 5-10% carbondioxide . The aproper disc with known concentration were used such as Penicillin G , Ceftriaxon , Vancomycin , Azithromycin , Erythromycin , clindamycin , Levofloxacin ,chloramphenicol , Ampicillin suleactum.

The interpretation of the results for the sensetivity testing were done depending on clinical and laboratory standard institute (CLSI).

Effect of Trans-cinnamic acid and 4-amino-2-hydroxybenzoic acid as antibacterial agent :

In our study we tested the effectiveness of some ligand (Trans- cinnamic acid and 4- Amino- 2-hydroxybenzoic acid) against the viable cells of different strains of *Streptococcus pyogenes* . The method of Agar diffusion by wells was followed to prove the inhibitory activity of ligands . In this study , four different concentrations (25mg/ml , 50 mg/ml , 75mg/ml , 100mg/ml) of each ligand were applied on five sensitive and resistance strain of *Streptococcus pyogenes* and the inhibitory activity of these ligands determined by measurement of the zone of inhibition .

DNA Extraction :

GAS isolates were inoculated on Brain heart infusion broth and incubated at 37℃ for 24 hours. After then genomic DNA extracted from a fresh brain heart infusion broth by using the instructions above .

SPY1258 gene polymerase chain reaction :

SPY1258gene were synthesized from Alpha DNA (Alpha DNA co , caued) Which were designed according to [7] Table(1) .

Table (1) : Sequence of primer sets used for PCR amplification of SPY1258 gene.

Gene	Primer name	Primer Sequence	Product length
SPY1258	SPY1258 (F)	5’ AAAGACCGCCTTAACCACCT3’	407bp
	SPY1258 (R)	5’ TGCCAAGGTAAACTTCTAAAGCA 3’	
Company		Alpha DNA / Monteral	

The PCR reaction kit (Gotaq gene master mix) was chosen from the promega and the PCR program which are used in amplification of the DNA target were included in a total volume of 25 µL in 0.5 mL eppendorf tube containing 5 µL templet DNA, 12.5 µL PCR master mix, 2.5 µL of each primer, 2.5 µL PCR water. The reaction for *eaeA* was included in a total volume of 25 µL in 0.5 mL eppendorf tube containing 2 µL templet DNA, 12.5 µL PCR master mix, 2 µL of each primer, 6.5 µL PCR water.

The PCR amplified products were detected and analyzed in 1.3gm% agarose gel electrophoresis with presence of (0.5mg/ml) ethidium bromide , and the detection of the specific band 407bp in UV transluminater.

Results

The results of our study showed that from 300 throat samples from children with pharyngitis , 43 (14.3%) samples were ß – hemolytic streptococci , 73 (24.4%) isolates of Alpha – hemolytic streptococci , 87 (29%) isolates of Staphylococcus spp. , 23(7.6%) isolates of candida and 74 (24.7%) throat swab were no growth , table (2) .

Table (2) :Number and percentage of isolated bacterial isolates .

Type of Microorganism	Number of isolate	%
ß – hemolytic streptococci	43	14.3%
α-hemolytic streptococci	73	24.4%
Staphylococcus.spp	87	29%
Candida.spp	23	7.6%
Negative throat swab	74	24.7%
Total isolates	300	100%

Out of 43 ß- hemolytic streptococci isolates , GAS were detected in 13(4.3%) . The isolates of *streptococcus pyogenes* were identified according to the phenotypic identification criteria . The streptococcus pyogenes isolates belonged to 10(3.3%) male and 3(1%) female patients .

Out of 43 ß – hemolytic streptococci there 30 isolates belonging to bacteria other than *Streptococcus pyogenes* were diagnosed , such as *streptococcus Salivarius* , *Streptococcus alactolyticus , Streptococcus agalactiae* and *Granulicatella adiacens* .

The results from susceptibility test of *streptococcus pyogenes* are presented in figure (1) , showing that chloramphenicol revealed high activity with 92% of the strain susceptible . In the present study observed the good activity of ß – lactams antibiotic against the streptococcus pyogenes with 84% and 70% of the isolates were susceptible to the Ampicillin sulbactam and Penicillin G respectively .

As for Ceftrixone, it showed greater activity than Penicillin were 76% of strain were sensetive. In this study of clindamycin and Vancomycin were also good active against *streptococcus pyogenes* with susceptibility rate 76% of isolates.

Levofloxacin also revealed good activity 69% of isolates were susceptibile.

In this present study *streptococcus pyogenes* revealed high resistance to macrolid antibiotic, the percentage of resistance for Erythromycin and Azithromycin of *streptococcus pyogenes* were 69% and 38% respectively .

The result depicted in figure (1) showed the percentage of bacterial resistant to antibiotic .

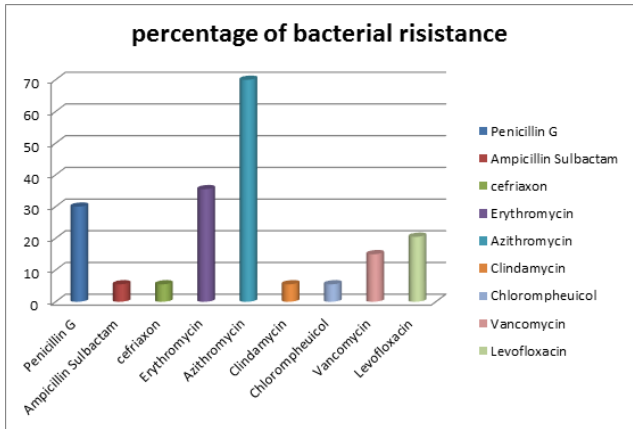

Figure (1) : percentage of bacterial resistant to Antibiotic .

The results revealed high effect of Trans – cinnamic acid and 4-amino-2-hydroxybenzoic acid. Trans-cinnamic acid showed good activity at concentration 75mg/ml and the concentration 100mg/ml . figure (2) .

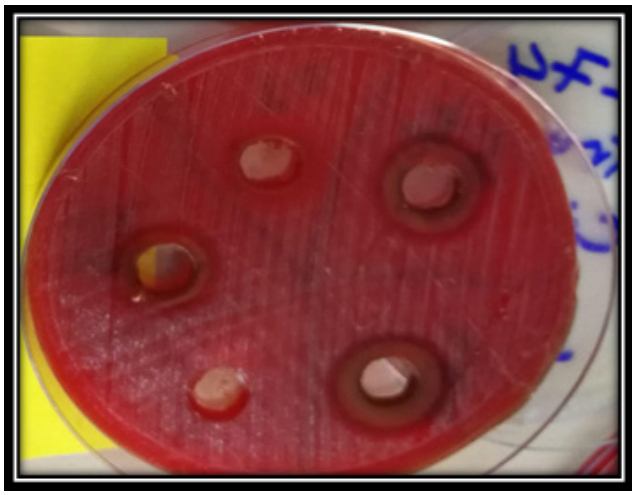

Figure (2) : The results described as high sensitive to the new ligands at concentration 75mg/ml and 100mg/ml .

While 4-amino-2-hydroxybenzoic acid revealed very good activity against *Streptococcus pyogenes* at all concentration figure (3) .

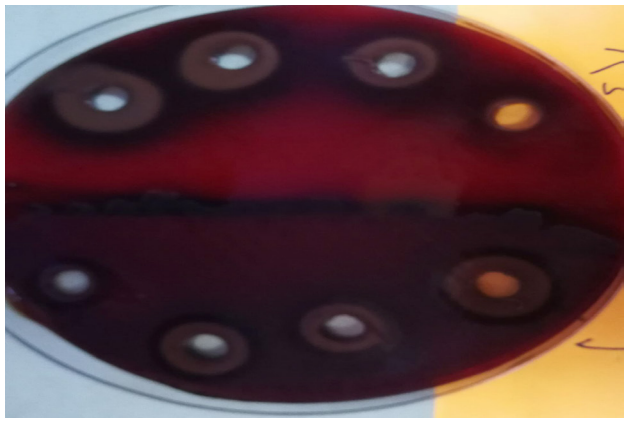

Figure (3) : Excellent activity of 4- amino -2-hydroxybenzoic acid .

The results in the current study were shown that the occurrence of *streptococcus pyogenes* 13from the total 300 isolates. In the present study, the diagnosis of *streptococcus pyogenes* isolates was confirmed by conventional PCR .

The results depicted in figure (4) reveals the presence of the diagnostic bands of the SPY1258 gene, which has a molecular weight of 407base pairs in all 13 isolate 100% , that gave positive result to vitek examination .

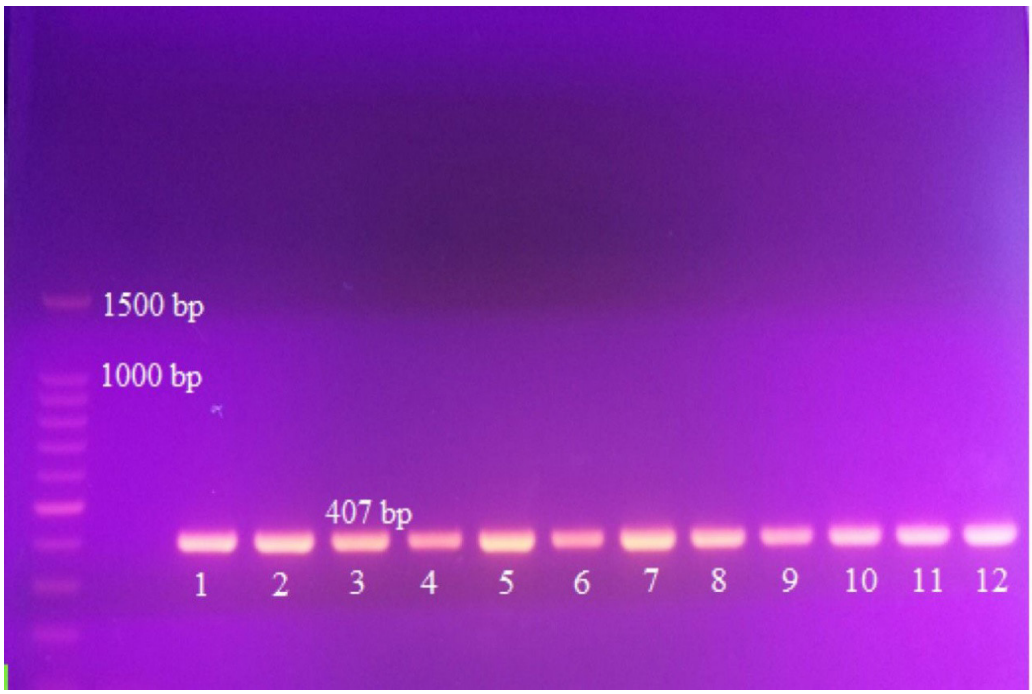

Figure (4) : Gel electrophoresis for PCR product of SPY1258 gene.

Discussion

Our study revealed that *Streptococcus pyogenes* was detected based on bacteriologicl methods (Bacitracin procedure and vitek compact 2) and identified by PCR technique. In our study observed that the SPY1258 gene was generated from whole 13 *Streptococcus pyogenes* strains only,but not from all 43 ß – hemolytic streptococci isolated, this results conferm that SPY1258gene was

definit gene only for *Streptococcus pyogenes* and can be used as amarker for its detection [9]. These results were in a greement with studies conducted by[10] this study revealed that SPY1258 gene was particular for GAS only. But not from another species of the genus *Streptococcus*.

And also a greement with results of [11] showed that the SPY1258 gene was present in all *Streptococcus pyogenes* isolates our study finding were disagreement with [9]who reported that out of 24 *Streptococcus pyogenes* isolate only 21 isolates had this gene.

This study shows that the prevalence of (3.3%)in males was higher than that recorded in females (1%).

These results agreed with that reported by Al-Gebori [12] in Iraq that indicuted male have more prevalence of infection than females . while our study results was in disagreement with [13] who reported the females were more susceptibile and response for infection with males ti ratio (3:1)the prevalence of *streptococcus pyogenes* infection in this study was (4.3%) this is in agreement with finding of [14] who reported a prevalence of 5.3% for *streptococcus pyogenes* pharyngitis . This results were approach to the ration 2.96% obtained by the researcher [15] who reported the prevalence of *streptococcus pyogenes* infection was (2.5%). The results in this study was higher than the results of (1.96%) reported by [16].

The result is lower than a percentage (15.3%) reported by [17].

The lower prevalence of GAS reported in this study, me be due to the fact that major of the pateints examined were found to be on antibiotic as most pharyngitis is viral in origin, streptococcus pyogenes pharyngitis is responsible for around 15% over all cases and 80-90% of cases are caused by viruses.

Our study finding that the percentage of resistance for the Erythromycin and Azithromycin were 69% and 38% respectively . This result agreement with findings of [18] who reported high macrolid resistance (40-70%) in Taiwan. This result is dramatically different from that has been repoeted in Saudia Arabia showed that the resistant was only 6.3% [19].

The main reason for this high rate of resistance that found in the current study, could be attributed to high misuse of Macrolid antibiotic in Iraq.

The present study revealed excellent activity of trans-cinnamic acid and 4-amino-2-hydroxybenzoic acid against *Streptococcus pyogenes* .

This result is in agreement with a work carried out by [20] Who reported that the cinnamic acid is considered and interesting for the evolution of novel antimicrobials.

This finding is in agreement with a research done by [21] who revealed that the paraaminobanzoic acid is one of a series of propargyl-linked antifolates , which is very active against GAS and dihydrofolate reductase enzyme from bacterial.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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