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Detection Flagellin Gene of Enteric Isolates of *Salmonella enterica serovar Typhi* Using Conventional PCR Technique

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Abstract

The study was conducted to study the virulence gene (Flagellin gene) of *Salmonella typhi*. Stool samples were collected from patients with are ranging (15 – 60) years of the both sexes who suffering from diarrhea and visited AL-Yrmouk Teaching Hospital in Baghdad Governorate for the period between March to September 2017..The samples were cultured on suitable media then Api20 test was done in order to confirm the diagnosis, the polymerase chain reaction (PCR) was tested to diagnose the Flagellin gene responsible for the virulence of the bacteria. We conclude from the present study that flagellin gene in *S.typhi* is responsible for pathogenicity in this bacteria.

Keywords: Flagellin, gene, *Salmonella*, *Typhi*, Typhoid fever.

الخلاصة

أجريت هذه الدراسة لمعرفة جينات الضراوة لجراثيم سالمونيلا تايفي حيث تم جمع عينات الخروج من المرضى البالغين الذين تتراوح اعمارهم من (١٥ – ٦٠) سنة ومن كلا الجنسينالذين يعانون من الإسهال والمراجعين لمستشفى اليرموك التعليمي في محافظة بغداد للفترة من شهر اذار الى شهر ايلول ٢٠١٧. حيث تم زرع العينات على الأوساطالزرعية المناسبة وبعدها تم عمل فحص Api20 وذلك لتأكيد التشخيص بعد ذلك تم اجراء فحص تفاعل البلمرة المتسلسل PCR لتشخيص جين الفلاجلين المسؤول عن ضراوة البكتيريا .نستنتج من هذه الدراسة وجود جين الضراوة الفلاجلين في جراثيم السالمونيلا تايفي والذي يعتبر مسوؤل عن الامراضية في هذه البكتريا .

Introduction

Typhoid fever consider the main problem of public health and spread in developing countries, the main causes belong to *Salmonella typhi* bacteria[1][2]. The cases of typhoid fever are large amount in the world and very difficult to detect because the clinical Signs are widely spread [3]. The World Health Organization Proven cases of typhoid fever were 17 million [4]. The pathogenicity of *S. typhi* depends on virulence factor which causes the disease in human, The villi or fimbria which present on the cell surface of *S. typhi* which playing an important roles in the process of adhesion and colonization to the human cells [5]. Flagella in *S. typhi* is responsible for motility and play critical role in host cell invasion and escape from immunity by intra cellular site.

Keeping the concept of the introduction the same, different documents have different styles to introduce the written text. For example, the introduction of a Functional Specification consists of information that the whole document is yet to explain. If a User guide is written, the introduction is about the product. In a report, the introduction gives a summary about the report contents.

Materials and Methodologies

Samples collection

167 stool samples were obtained from diarrhea cases from patients who attended to AL-Yarmouk Teaching Hospital in Baghdad Governorate during the study period from March to September 2017.The samples were transferred in cold conditions to the laboratory for diagnostic tests.

Identification of S.typhi

Morphological and cultural characteristics of bacterial isolates

Microscopical characteristics of bacterial cells as well as cultural properties of the colonies grown on specific culture media were determined using standard methods as primary diagnosis of *S. typhi* [8].

Biochemical Tests andAPI 20 E System

The bacterial isolates were identified using different biochemical tests such, oxidase, catalase test, in addition to growth on Kligler Iron agar [9]. Bacterial identification of *S.typhi* was conformed using API 20E strips (Biomerieux, France).

Extraction of the total DNA

The extraction of DNA was done by extraction kit.

Amplification of the total DNA

Specific primers and the amplification conditions were presented in Table 1.

Table 1: Primers used in this study.

Primers	Sequence		Amplicon size
*fliC*18	F	ACT GCT AAA ACC ACT ACT	363 bp
	R	TGG AGA CTT CGG TCG CGT AG	

Table 2:Amplification conditions used in this study.

PCR cycle	repeat	Temperature	Time
Initial denaturation	1	94ºC	5min
Denaturation	30	94ºC	20sec.
Annealing		50-65ºC	30sec
Extension		72ºC	30sec
Final extension	1	72ºC	7min

Results and Discussion

Distribution of S. typhi Positive Culture

A total of 45 (26.9%) *S. typhi* isolates were obtained from 167 patients with clinically suspected typhoid fever. The results were confirmed with positive cultures.

Distribution of Flagellin Gene

Detection of flagellin gene performed by PCR using primers according to [11], as shown in Figure 1.

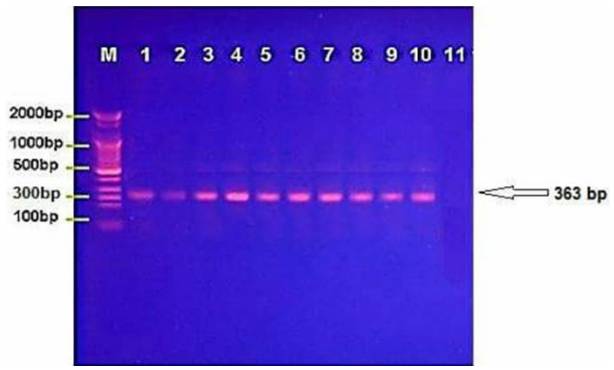

Figure 1: Agarose gel electrophoresis (1%agarosee,7v/cm^2 for 30 min) of flagellin gene of *S.typhi*. (363bp) amplicon size compared with M line (M100 bp DNA ladder).

Typhoid fever caused by *S.typhi*is a worldwide health problem in different countries [12]. This problem is particularly prominent in developing countries because of several correlated factors like unplanned urbanization with growing of peri-urban towns that lack safe water supply, lacking of sanitation services, increased local migration of great sums of workers, in addition to inefficacies of vaccine preparations programs [13].

The results showed isolation and identification of *S.typhi* from diarrhea samples these results in agreement with [14] which recorded the morphological properties of the *S.typhi* exhibited Gram negative, small Virulence of *S. typhi* possessed an important factor the infection of human by typhoid fever, these factors are fimbria or ill present on the cell surface of *S.typhi* which possess effective role in the infection human [18]. The role of motility of *S.typhi* in pathogenicity was Penetration of the intestinal mucosa which consider the important step in the causes of infection because it allows bacteria to pass through the epithelial barrier, the Flagella are consist of a protein called flagellin which also act as an antigen [19].

The results detected the presence of flagellin gene which responsible for the pathogenicity of *S.typhi*,, who reported that flagellin genes might be responsible for an amplified risk for emerging severe disease. The study of [7] found that flagellin gene present in three types which responsible for the gastrointestinal bleeding and make the *S.typhi* more invasive compared to others microorganism.

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