Isolation and identification of *Salmonella typhimurium* bacteria with detection of type-1 fimbriae coding gene by polymerase chain reaction (PCR) technique

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Abstract

Two hundred faeces sample were collected from cattle with different age and sex in Al- Diwaniyah Province. The study was conducted in the period between November 2016 and November 2017. *Salmonella typhimurium* bacteria identified by routine methods such as culturing on selective media, biochemical test and agglutination test using monovalent and multivalent antisera. PCR was can detection type-1 fimbriae gene coding for *fimC* of *Salmonella typhimurium*. Results showed that *Salmonella* isolates were 14.5% in the bovine fecal samples. Also, the serotyping of isolates by using monovalent and polyvalent antisera revealed that all *Salmonella* isolates in cows were *S. typhimurium.* The PCR technique was used for detection of type-1 fimbriae coding gene by specific primer for *fimC* gene. All *S. typhimurium* isolates in cows appeared to be contained this gene show one distinct band MW.289 bp when electrophoresed on agarose gel. The results of this score indicated that the PCR technique potentate a loud specify in the disclosing of *S. typhimurium* especially the serotype that encoded to *fimC* gene type-1 fimbriae isolated from cows in comparison to other routine diagnostic tests.

Keyword: *Salmonella typhimurium*, Type-1 fimbriae, Cows, Feases, PCR
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عزل وتشخيص جرثومة *Salmonella typhimurium* والكشف عن الجين المشفر لتخليق النوع ألـهدبي الأول باستعمال تقنية تفاعلات سلسلة البلمرة (PCR)

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الخلاصة

تم جمع ٢٠٠ عينة براز في الفترة من شهر تشرين الثاني عام ٢٠١٦ ولغاية شهر تشرين الثاني ٢٠١٧ من ألأبقار بأعمار وأجناس مختلفة في مزارع ومجازر محافظة الديوانية . وقد تم عزل وتشخيص جرثومة السالمونيلا باستخدام طرق الزرع الجرثومي للعينات على أوساط زرعيه انتقائية بالإضافة إلى استعمال الاختبارات الكيميوحيوية واختبار التلازن باستعمال المصول المضادة متعددة واحادية التكافؤ وكذلك استخدمت تقنية تفاعل سلسلة البلمرة (PCR) للكشف عن وجود الجين الهدب النوع الأول (Type -1- fimbriae) المشفر للأهداب الخاصة بجرثومة *S.typhimurium* . أظهرت النتائج أن نسبة عزل جراثيم السالمونيلا *Salmonella* spp في عينات براز الحيوان كانت 14.5% وأظهرت النتائج التنميط المصلي لعزلات جرثومة السالمونيلا والتي استخدم فيها المصل المضاد متعدد وأحادي التكافؤ أن جميع عزلات السالمونيلا في الأبقار من النمط المصلي *S.typhimurium*. وعند استخدام تقنية الـPCR للكشف عن الجين المشفر لتخليق Type-1-(fimbriae) أظهرت جميع عزلات النمط المصلي *S.typhimurium* في الأبقار احتوائها على هذا الجين وذلك لوجود حزمة واحدة ناتجة من عملية التضخيم للحامض النووي الـ DNA والتي حجمها 289 زوج قاعدي عند ترحيلها على هلام الاكاروز. كشفت نتائج هذه

الدراسة ان تقنية تفاعل سلسلة البلمرة لـ PCR أظهرت سرعة التشخيص في الكشف عن النمط المصلي لجرثومة السالمونيلا لاسيما المشفرة للجين المسؤول عن تشفير النوع ألهدبي الأول المعزولة من الأبقار مقارنة بالفحوصات الأخرى الزرعية والكيموحيوية والمصلية.

Introduction

Salmonella is a widespread disease in the world. It infects both humans and animals (zoonosis disease) and causes serious injuries that can lead to death of animals (1). Salmonellosis is common in the calf from 1to 10 week of age. Also, the disease can occur in adult cows and consider one of the serious economic diseases that affect the beef cattle (2). Infection often occurs by eating contaminated food; although cows are infected with several serotypes, the most important species are *S. typhimurium* and *S. Dublin* (3). The severity of the disease and the lack of effective treatment as well as the spread of the disease was motivated to focus research on improving the efficiency of the diagnostic method for this bacteria and study its epidemiology and pathogenesis, in recent years, a large number of molecular diagnostic techniques have been used to facilitate the investigation of pathogenic bacteria and to promote epidemiological studies by using molecular markers to identify the nature of the relationship between the strains obtained from different sources and to determine the source of infection. Several studies have indicated the use of a PCR technique to investigate the virulence factors of *Salmonella*, including *S. typhimurium* and *S. Enteritidis*, by detecting the encoded gene for the formation of type-1 fimbriae *fimC* gene (4). According by, this study was aimed to detect of *fim C* gene in *Salmonella typhimurium* using PCR technique. The purpose of this study was to contrast the sensitivity and specificity of PCR with conventional isolation and properties methods presently used in diagnostic laboratories.

Materials and methods

Samples collection

Total of 200 feces samples were collected from cows and calves aged from one day to four years from different parts of Al-Diwaniyah city. The collection began from November 2016 until November 2017. The feces were collected directly from the rectum and transferred to the laboratory by cool box.

Identification of *Salmonella* spp isolates

After culturing on Selenite broth, a loopful of broth was lined on surface of SS, XLD and BG agar plate and then incubated at 37 C° for 24 hrs. The biochemical characters of non - lactose fermenting bacteria was delineates by utilizing TSI agar and Urease test and the other biochemical tests (5).

Serological identification

The specific antisera were used in agglutination test on the glass slide for bacterial suspension (polyvalent -O- group, polyvalent -H- phase I and *Salmonella* antisera).

PCR method

DNA extraction and purification

The DNA of all dissociating were demodulator and purified utilizing genome DNA purification kit, Presto TM mini g DNA Bacteria Kit (Geneaid, USA) the extracted DNA was checked by nanodrop spectrophotometer and store in -20°C until used.

Primers

Specific primers used for the disclosure specific sequence of *fim*C gene coding for biosynthesis of fimbriae C of *Salmonella typhimurium* (6), which is accoutered by Bio Corp company (Canada) (Table 1).

Table 1: The primer of the *fim C* gene for *S. typhimurium*

Primer		Sequence	Amplification	Reference
fim c gene	F	AGCGAGCCCA AAAGTGAAA	289bp	6
	R	ATCTTGAGATG GTTGCCGAC		

PCR reaction was used to detect *fimc* gene for *S. typhimurium* in a bacterial strain. PCR was performed by use 5 µl of the template DNA, 12 PCR water Bioneer (South Korea). Amplification was carried out in a thermocycler (Eppendorf mastercycler®) (Bioneer-south Korea). Agarose gel electrophoresis 1.5% of PCR prolific was carried out using mM Tris-Borate- EDTA (TBE) buffer at 70V for 2 hour, and the DNA bands were stained with ethidium bromide (Sinaclon, Iran) 100 bp DNA ladder was used to confirm the specific size.

The PCR assay was done at 95°C for 5 minutes and then for 30 cycles of 94°C for 30 second, 58°C for 40 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes, with a final hold at 4°C in a thermal cycler (Thermo cycler, Eppendorf, Germany).

The target DNA amplification (*fim*C gene)

The isolates were amplificated on the gel by using the electrophoresis apparatus, the band produced by binding between DNA and primers, the monadic bands show under the UV light by using stain called ethidium bromide.

Statistical analysis

Chi-square test was used to analysis of the results at $P<0.05$. The sensitivity and the specificity and the accuracy were calculated by applying several specific formula used for this purpose (7).

Result

Culture and biochemical tests

The colonies on *Salmonella-Shigella* agar were circular, pale yellow convex with a black center of 2-3 mm (Figure 1), while on Brilliant green agar it smooth, bright, round, translucent, pink, with a reddish-pink center colour agar. Colonies appear small circular, smooth, convex, red, with a black center on XLD agar (Figure 2).

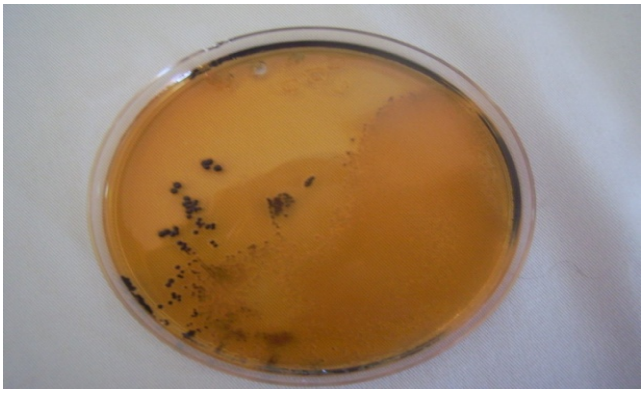

Figure 1: Colonies of *S. typhimurium* on SS agar, colonies appear smooth, convex, pale with a black center.

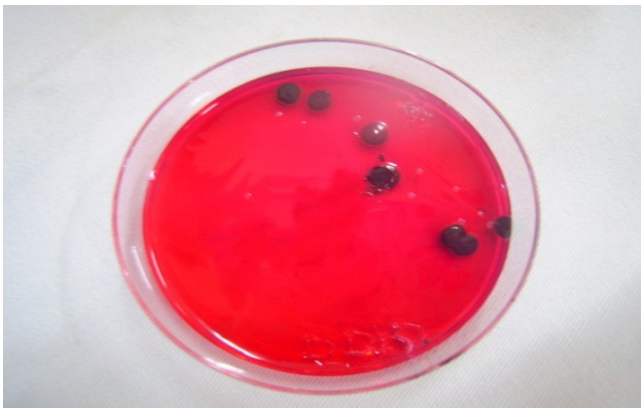

Figure 2: Colonies of *Salmonella typhimurium* growth on XLD agar. Colonies appear smooth, convex and red colour; it has a black center.

The percentage of *Salmonella* spp isolation was 14.5% (29/200) utilities the classical culture methods of the feces on media (enrichment and selective) (Table 2).

A 28 cases represent 100% was positive in culture and biochemical analysis tests. It included 26 cases that represent 89.7% gave positive findings for PCR tests, while 24 fettles that represent 92.3% was positive for serotyping methods and PCR (Table 3).

The control group that used in our study consist of 21 cases. The control group was given negative results in culture methods and PCR examination (Table 4).

Table 2: Number and percentage of *Salmonella* disestablished that isolated by culture methods

Salmonella spp isolation	No.	%
Positive	29	14.5
Negative	171	85.5
Total	200	100

Comparison between PCR (using *fimC* gene) and routine tests

The accuracy PPV and NPV, The Specificity and the sensitivity of the PCR test were 100, 87.5, 68, 89.6, 100% respectively. while it were 89.6, 100, 68, 100, 87.5% in culture and biochemical tests respectively. PCR Results (using *fimC* gene) and serotyping test 100, 92.3, 72, 92.3, 100% respectively.

Amplification of target DNA (*fim*C gene)

The score of PCR amplification which was officiated on the DNA extracted off all the studied isolates were asserted by the electrophoresis analysis. By this parsing, the strands of DNA resulted off the prospering binding between specific primers and isolates extracted DNA. These successful bindings emerged as single bands under the UV light using ethidium bromide as a determined DNA stain. The electrophoresis was also wield to discretion DNA weight adopting on DNA marker (100 bp DNA ladder) and the score of this appreciation disclosed that the descant DNA is 289 bp for *fimC* gene (Figure 3).

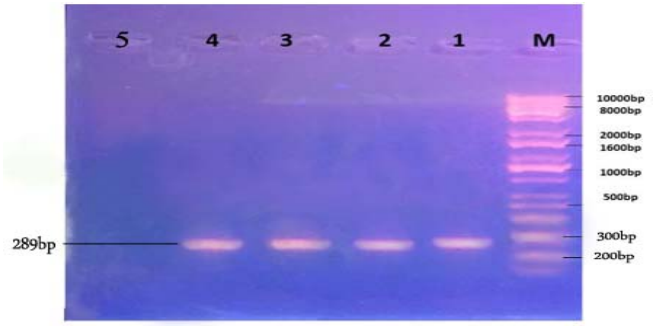

Figure 3: *fimC* gene of *S. Typhimurium* at 289 bp in PCR technique, Lane (1-4) positive, while M: 100 bp represent marker.

Table 3: Comparison between PCR findings and other tests for detecting *Salmonella* spp

Test		PCR method				Total N.	Chi-P≤0.05
		positive		negative			
		No.	%	No.	%	No.	
Culture method	positive 29	26	89.7	3	10.3	50	X^2=15.429
	negative 21	0	0	21	100		Significant
Stereotyping method	positive 26	24	92.3	2	7.7	50	X^2=15.42
	negative 24	0	0	24	100		Significant

Table 4: All laboratory methods that used in detection *Salmonella* spp.

The Test	The culture and biochemical test		The serotyping		PCR	
	+	-	+	-	+	-
Study group	29	0	26	3	24	5
Control	0	21	0	21	0	21

Discussion

The animal considers the first source of *Salmonella* infection, and the presence of rodents helps to transfer the infection of different *Salmonella* spp. The infected patients may become a carrier for these bacteria so they will become a source of infection, as *S. typhimurium* infect both humans and animals and not specialized for a particular host. Therefore, it becomes one of the common pathogens in zoonotic diseases (8).

The high incidence of *Salmonella* infection through food contamination and the severity of the disease, led researchers in this area to focus their efforts on finding a method to diagnose these bacteria with high speed and sensitivity (9,10).

Salmonellosis is one of the major zoonotic foodborne diseases worldwide (11) and it's prevalence in animals poses a continuous threat to man (12). Diagnosis of *Salmonella* spp depends on bacterial isolation from clinical specimens based on selective media and their characterization by biochemical and serological tests. The diagnosis by routine methods is expensive regarding effort and time; it may take between 7-2 days (13). Currently, the modern diagnostic laboratories have been characterized by reducing the time and effort in the diagnosis of *Salmonella* through the use of several modern techniques like PCR technology. PCR is one of the advanced techniques in microbiology as it provides advancement in the solving of problems of diagnosis and detection of the bacterial serotypes (9,14).

The PCR technique based on the amplification of very small piece of DNA sequence, therefore, most microorganisms in the world are well documented and easily diagnosed using this technique, it has become possible to identify different bacteria such as *Salmonella* bacteria with precision, high speed and less effort (15). In this study, all 26 isolates were positive results by the PCR *fimC* gene. Wherever, the product was a 289-bp fragment, it confirms by ethidium bromide stain in gel electrophoresis. This result was similar to the result obtained by (6) by using the same primers for this gene in different spp of enteric bacteria namely, *E.coli*, *Salmonella arizonae*, *Citrobacter spp* and *Enterobacter spp.* These results revealed that the amplification of 289 bp fragments for the mentioned bacteria especially *S. typhimurium* which gave obvious results comparing with other types of the tested bacteria. The *fimC* gene has specific sequences to *S. typhimurium* spp. Also, the gene can use for detection of *S. typhimurium* isolates (16). *Salmonella spp* were detected in faces by using many of the media, like XLD agar BG agar and SS agar. Also, the isolates tested with H_2S production and lactose fermentation. The false-positive number was high (17). There are significant differences at P<0.05 between classical method and PCR results because the percentage of *Salmonella spp* depend on classical methods has low specificity as compared with PCR. Therefore, there are many false- positive (18,19). This study concluded that the use of PCR technology required only four hours after the primary isolation from Selenite broth. Also, It is provided diagnostic information, which took several days in the routine test for detection of *Salmonella*. The result showed a high degree of specialization and sensitivity in the detection of *Salmonella* (20,21) as well as it provided the effort, speed and cost in diagnosing the pathogenic bacteria of like *Salmonella* species.

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