Short Communication

Outer Membrane Protein C (*ompC*) Gene as the Target for Diagnosis of *Salmonella* Species Isolated from Human and Animal Sources

Alaa Abdel-Kadhim Jawad [1] and Alaa H. Al-Charrakh [2*]

1. College of Veterinary Medicine, Al-Qadisiya University, Al Diwaniyah, Iraq
2. Department of Microbiology, College of Medicine, Babylon University, Hilla, Babylon Governorate, Iraq

Abstract

Background: The use of selective and differential plating media is a simple method for the isolation of *Salmonella* spp. Recently, there has been a general move toward molecular methods of *Salmonella* detection and typing.

Methods: A total of 1200 different specimens collected from human and animal sources were involved in his study. 600 stool specimens from patients suffering from diarrhea and 600 specimens from gall bladder (bile) of cattle from Al-Diwaniya slaughter house, Iraq were used. *Salmonella* spp. were isolated and identified using bacterial culturing on selective media and colonies were tested by API 20Eand then serotyping through polyvalent antisera and conformation by Polymerase Chain Reaction (PCR). PCR was used to detect *ompC* gene encoding biosynthesis of outer membrane protein C of *Salmonella* genus.

Results: The results revealed that the rate of *Salmonella* isolates was 0.5% (3/600) from human and 1% (6/600) from animals. The PCR technique revealed that 9 isolates of *Salmonella* spp. harbored *ompC* gene. The results of this study revealed that the PCR technique had a high specificity in detection of *Salmonella* spp., in comparison to culture and biochemical test, Mini API 20 E and serological tests. The present study found no significant differences between human and animal isolates.

Conclusion: Detection of *ompC* gene is a good method for detection of *Salmonella* species isolated from clinical specimens. It has a high specificity in comparison with other tests, with its advantages of greater speed and effectiveness than conventional detection methods.

Avicenna J Med Biotech 2016; 8(1): 42-45

Keywords: Detection, Gene, Polymerase chain reaction, *Salmonella*

*** Corresponding author:**
Alaa H. Al-Charrakh, Ph.D.,
Department of Microbiology
College of Medicine, Babylon
University, Hilla, Babylon
Governorate, Iraq
Tel: +96 47813216822
E-mail:
ahani67@gmail.com;
aalcharrakh@yahoo.com
Received: 1 May 2015
Accepted: 13 Sept 2015

Introduction

Enteric pathogens such as *Salmonella enterica* (*S. enterica*) cause significant morbidity and mortality. *S. enterica* serovars are a diverse group of pathogens that have evolved to survive in a wide range of environments and across multiple hosts [1]. Infection begins with the ingestion of contaminated food or water so that salmonellae reach the intestinal epithelium and trigger gastrointestinal disease. In some patients, the infection spreads upon invasion of the intestinal epithelium, internalization within phagocytes, and subsequent dissemination [2]. It has been estimated that there are more than 3 million deaths associated with Gram-negative enteric pathogens worldwide due to diarrhea and enteric fever each year [3]. The use of selective and differential plating media is a simple method for the isolation of *Salmonella* spp. A wide variety of selective and differential media has been developed for this purpose, including xylose lysine desoxycholate agar (XLD), Hektoen Enteric (HE) agar, and bismuth sulfite (BS) agar [4]. There has been a general move toward molecular methods of *Salmonella* detection and typing and PCR has become a potentially powerful alternative in microbiological diagnostics. While it is easy to amplify DNA derived from pure cultures, problems arise if the sample investigated is as complex as clinical specimen or food, since the PCR is easily inhibited by numerous substances, including humic acids, fats, and proteins. The *ompC* gene contains sequences unique to *Salmonella* isolates and demonstrates that this gene is a suitable PCR target for detection of *Salmonella* strains [5].

The aim of this study was to evaluate the rapid detection test to identify salmonellosis using the PCR technique for the detection of *ompC* gene of *Salmonella* genus, and to compare the PCR with traditional iso-

Copyright © 2016, Avicenna Journal of Medical Biotechnology. All rights reserved.

lation and characterization methods currently used in diagnostic laboratories.

Materials and Methods

Samples collection

A total of 1200 different specimens collected from human and animal sources were involved in this study, of which 600 stool specimens from patients suffering from diarrhea admitted to Al-Diwaniya Teaching Hospital for Maternity and Children (Al-Diwaniya province, Iraq) and 600 specimens from gall bladder (bile) of cattle from Al-Diwaniya slaughter house were collected. This study was conducted during the period that extended from May 2013 to April 2014.

From each specimen, 25 *gm* of human stool with a sterile wood spatula or animal bile were directly injected into an Erlenmeyer flask and 225 *ml* of buffered peptone water were added to obtain 1 part sample+9 part buffer, then mixed and incubated at 37°*C* overnight (16-20 *hr*). After that, 1 *ml* of the pre enrichment broth was transferred to 10 *ml* Tetrathionate broth and incubated at 37°*C* overnight (18-24 *hr*). Moreover, 0.1 *ml* (100 *ul*) of the pre-enrichment broth was also transferred to 10 *ml* Rappaport-Vassiliadis soy peptone (RVS) broth and incubated at 41°*C* overnight.

Isolation and identification of Salmonella isolates

For each specimen, a loopful (10 μl) from the incubated Tetrathionate broth (I) and RVS broth (II) was spread on XLD and on BGA agar plates and incubated at 37°*C* overnight (18-24 *hr*). For biochemical confirmation and serotyping, two suspected colonies (grown on XLD and BGA agar) were plated onto non-selective medium (nutrient agar) [6]. Colonies that showed biochemical characteristics similar to that of *Salmonella* spp. were tested by Mini API 20E then serotyping. The identified isolates were then confirmed by PCR with *ompC* primers for detection of *Salmonella* genus [7].

DNA extraction and purification

One colony of each strain (cultured on solid medium) was inoculated into 5 *ml* of BHI (Broth Heart Infusion) and grown overnight at 37°*C*. From these cultures, DNA was purified from bacterial cells using Genomic DNA Mini kit (Geneaid, UK). Chromosomal DNAs obtained were used as templates for all PCR experiments. The PCR reactions were carried out in a thermal cycler (Clever, U.K). Before PCR assay, and in order to quantify the DNA concentration ($ng\mu l^{-1}$), the quantification of DNA samples was carried out by means of a spectrophotometric reading using 1 μl aliquots of Genomic DNA with a NanoDropTM spectrometer (NanoDrop Technologies), adopting the manufacturer's recommendations. The concentration of DNA was estimated from absorbance at 260 *nm*. DNA profiles were performed using bacterial DNA and loading buffer according to the manufacturer's instructions (Bioneer, Korea).

Detection of ompC gene by PCR

The *ompC* gene was detected in the present study. This gene is encoding biosynthesis of outer membrane protein C of *Salmonella* genus. The primer sequence of this gene (provided by Bioneer Co., S. Korea) was (*ompC F*: ATC GCT GAC TTA TGC AAT CG, *ompC R*: CGG GTT GCG TTA TAG GTC TG) 204 *bp*. Temperature for *ompC F* was 49.7°*C*, and for *ompC R* 53.8°*C*. PCR was performed at the following conditions: 95°*C* 2 *min* 1×, 95°*C* 60 *s*, 57°*C* 60 *s*, 72°*C*, 120 *s* 30×, and 72°*C* 5 *min* 1× [8]. Each primer was prepared by dissolving it in 1000 μl of deionized distilled water to obtain stocks concentration of 12 *picomole/µl*. Green master mix, 2× (Promega, USA) includes: 1: Taq DNA polymerase, 2: dNTPs which include 400 μM of each dATP, dGTP, dCTP, dTTP, 3: 3 *mM* of $MgCl_2$ (magnesium chloride), and 4: Yellow and blue dyes as the loading dye. After PCR, the profiles of amplification products were detected by gel electrophoresis. Five μl of total reaction mixture was loaded on a 1.5% agarose gel and electrophoresed at 100 *V* at 70 *mA* for 60 *min*. The amplified DNA fragments were visualized by UV illumination after agarose gel electrophoresis and ethidium bromide staining by standard procedures.

Statistical analysis

Statistical analysis was carried out using SPSS version 17. Variables were presented as frequencies and percentages. Pearson's chi square (χ^2) test was applied and p-value was considered significant at $p<0.01$ [9].

Results

The isolation rates of *Salmonella* spp. were 0.5% (3/600) from human and 1% (6/600) from animals using the conventional culture methods on enrichment and selective media (Table 1).

The confirmed diagnosis of *Salmonella*_spp. was performed by Mini API 20 E system and the results were obtained based on interpretation kit chart and results of Mini API 20 E system. The isolates showed positive results for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization and H_2S production. Sugar fermentations showed positive results for glucose, mannose, inositol, sorbitol, rhamnose, melibiose, arabinose, while they were negative for beta-galactosidase (ONPG), urease, tryptophan deaminase, indole production, acetoin production, and gelatin hydrolysis. They were also negative to sucrose and amygdalin.

Table 1. Isolation rates of *Salmonella* spp. from human and animals sources

Isolate source	No.	Positive result	Negative result	χ^2 value
Human	600	3	597	Calculated χ^2=1.008
Animal	600	6	594	Tabulated χ^2=6.6349 df= 1
Total	1200	9	1191	No significant (p<0.01)

Table 2. Different lab techniques used for diagnosis of *Salmonella* spp

Test	Culture and biochemical	Mini API 20E	Serotyping	PCR
	+ve isolates	+ve isolates	+ve isolates	+ve isolates
Study group	9	9	9	9

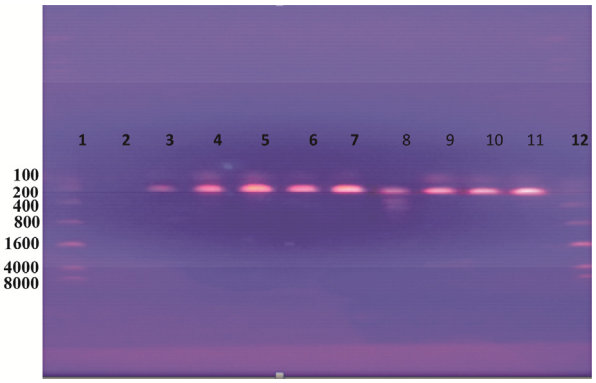

Figure 1. DNA amplification of a 204 *bp* of *Salmonella* spp. Detecting *ompC* gene using PCR. Lane 1: ladder; lane 2: negative; lanes 3, 4, 5, 6, 7, 8, 9, 10, and 11: positive results as *Salmonella* spp.; lane 12: 8000 *bp* marker (Ladder).

Validity of different techniques in diagnosis of salmonella isolates

Nine isolates gave positive results for culturing and biochemical tests, Mini API 20E (at 99.9% likelihood), serotyping by polyvalent antisera (unpublished data) and gave positive results for PCR tests (Table 2).

Amplification of target DNA (ompC gene)

The results of PCR amplification (performed on all DNA samples extracted from the isolates) were confirmed by electrophoresis analysis. Depending on DNA marker (8000 *bp* DNA ladder), the result of this estimation revealed that the amplified DNA was 204 *bp* for *ompC* gene (Figure 1).

Discussion

In this study, it was found that *Salmonella* spp. infection is one of the causes of diarrhea. This may reflect the fact that *Salmonella* spp. is one of etiologic agents of diarrhea that infect human and animals especially during the summer, and that *Salmonella* is a zoonotic bacterial agent, and *Salmonella typhimurium* was the most common serovar found in animals and in human specimens. In this study, 3 isolates from human (0.5%) and six (1%) from animals were recovered using the conventional culture methods on enrichment and selective media. Results revealed no significant differences between human and animal isolates ($p< 0.01$). Traditional *Salmonella* detection methods are based on cultures using selective media and characterization of suspicious colonies by biochemical and serological tests [7]. Other studies conducted in Iraq, also revealed prevalence of *Salmonella* spp. at different rates of 7.9, 8.47 and 10%, [10-12] respectively. *Salmonella* detection by using conventional media, such as *Salmonella-Shigella* agar (SS), is based on lactose fermentation and H_2S production and the number of false-positive results with these media that may occur necessitates conducting time-consuming and expensive additional testing [13]. According to Mini API 20E results, all the 9 isolates (by culture and biochemical tests) were detected as *Salmonella* spp. at 99.9% likelihood level. Nucera *et al* [14] evaluated API 20E and PCR for the identification of *Salmonella* spp. isolates. They found that API 20E had the highest similarity with PCR tests at the 99.9% likelihood level and the validation of both PCR and API 20E (at the 99.9% likelihood level) showed that they are accurate diagnostic tests. Results also revealed that the isolates (No=9) were confidently identified as *Salmonella* spp. by detection of *ompC* gene. The specific PCR product (*ompC* gene) is a 204 *bp* fragment which was visualized by gel electrophoresis. All *Salmonella* isolates (detected by phenotypical tests) gave positive results with the PCR. The *ompC* gene contains sequences unique to *Salmonella* isolates and demonstrates that this gene is a suitable PCR target for detection of *Salmonella* strains.

Conclusion

PCR technique has a high specificity in comparison with other tests, with its advantages of greater speed and effectiveness than conventional detection methods. Detection of *ompC* gene is a good method for detection of *Salmonella* species isolated from clinical specimens.

References

1. Foley SL, Johnson TJ, Ricke SC, Nayak R, Danzeisen J. Salmonella pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev 2013;77 (4):582-607.
2. Fàbrega A, Vila J. Salmonella enterica serovar typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev 2013;26(2):308-341.
3. Donnenberg MS. Pathogenic strategies of enteric bacteria. Nature 2000;406(6797):768-774.
4. Park SH, Ryu S, Kang DH. Development of an improved selective and differential medium for isolation of Salmonella spp. J Clin Microbiol 2012;50(10):3222-3226.
5. Pickup RW, Rhodes G, Hermon-Taylor J. Monitoring bacterial pathogens in the environment: advantages of a multilayered approach. Curr Opin Biotechnol 2003;14 (3):319-325.
6. Mikoleit ML. Isolation of Salmonella and Shigella from Faecal Specimens. Atlanta, USA: WHO Global Foodborne Infections Network; 2010 Nov. 15 p.
7. Alvarez J, Sota M, Vivanco AB, Perales I, Cisterna R, Rementeria A, et al. Development of a multiplex PCR technique for detection and epidemiological typing of salmonella in human clinical samples. J Clin Microbiol 2004;42(4):1734-1738.
8. Kwang J, Littledike ET, Keen JE. Use of the polymerase chain reaction for Salmonella detection. Lett Appl Microbiol 1996;22(1):46-51.

9. Belle GV, Fisher LD, Heagerty PJ, Lumley T. Biostatistics a Methodology for the Health Sciences. 2nd ed. USA: A John Wiley & Sons, Inc., Publication; 2004. 888 p.

10. Jawad AA. Detection of fim A, rfbj (B) and fimC Genes of Salmonella Isolates using singleplex and multiplex polymerase chain reaction [master's thesis]. [Qadisiya, Iraq]: College of Medicine Al-Qadisiya University; 2010.

11. Al-Janabi JK. Microbiological study of some causative agents of diarrhea in children under five year of age in Al-Diwaniya city [dissertation]. [Qadisiya, Iraq]: College of Education Al-Qadisiya University; 2006.

12. Al–Karawiy HAM. Isolation and identification of Salmonella typhimurium and detection of gene encoded type-1-fimbria by using polymerase chain reaction. [master's thesis]. [Basra, Iraq]: College of Veterinary Medicine, University of Basrah; 2008.

13. van Dijk S, Bruins MJ, Ruijs GJ. Evaluation and implementation of a chromogenic agar medium for salmonella detection in stool in routine laboratory diagnostics. J Clin Microbiol 2009;47(2):456-458.

14. Nucera DM, Maddox CW, Hoien-Dalen P, Weigel RM. Comparison of API 20E and invA PCR for identification of Salmonella enterica isolates from swine production units. J Clin Microbiol 2006;44(9):3388-3390.