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ORIGINAL ARTICLE

Rapid Molecular Approach for Simultaneous Detection of *Salmonella* spp., *Shigella* spp., and *Vibrio cholera*

Q7 Reza Ranjbar [a,*], Ali Naghoni [a], Davoud Afshar [b], Farhad Nikkhahi [c], Mohsen Mohammadi [d]

[a]*Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
[b]*Department of Microbiology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.*
[c]*Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
[d]*Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran.*

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Abstract
Objectives: Gastrointestinal tract infection is still one of the serious public health problems in many geographic areas and is endemic in most countries including Iran. Early detection of the gastrointestinal tract pathogens can be extremely important. The aim of the current study was to apply a shortened time-multiplex polymerase chain reaction (PCR) for rapid and simultaneous detection of *Salmonella* spp., *Shigella* spp., and *Vibrio cholera*.
Methods: The standard and clinical strains of *Salmonella* spp., *Shigella* spp., and *V. cholerae* were used in the assay. Multiplex PCR was performed and optimized based on amplification of *invA*, putative integrase, and *ompW* genes for detecting *Salmonella* spp., *Shigella* spp., and *V. cholerae*, respectively. The specificity of the assay was evaluated by testing 12 different bacterial species.
Results: Only *Salmonella* spp., *Shigella* spp., and *V. cholerae* strains had positive results when subjected to the assay using multiplex PCR. The assay showed a high sensitivity, and no amplification products were observed in multiplex PCR with any of the other microorganisms.
Conclusion: Our study indicated that the *invA*, putative integrase, and *ompW*-based multiplex PCR assay appears to be an efficient method for rapid and simultaneous detection of *Salmonella* spp., *Shigella* spp., and *V. cholerae*.

1. Introduction

Worldwide, gastrointestinal tract infections are the second most important cause of death; about 25 million enteric infections occur each year. These infections cause significant morbidity and death in children younger than 5 years in particular and in elderly people. It has been estimated that 4–6 million children die each year because

*Corresponding author.
E-mail: ranjbarre@gmail.com (R. Ranjbar).

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of diarrheal diseases, particularly in the developing countries [1]. Numerous outbreaks of diarrheal illness caused by various microorganisms have been reported. Microorganisms such as *Shigella*, *Salmonella*, *Vibrio*, *Escherichia coli*, *Campylobacter jejuni*, *Giardia lamblia*, *Cryptosporidium*, and *Rotaviruses* have been reported to be the most important causes of diarrheal outbreaks. *Salmonella* spp., *Shigella* spp., and *V. cholerae* are the most important bacterial causes of diarrhea in Iran [2–5].

The diseases caused by all of these microorganisms could be serious, resulting in death. *V. cholerae* causes cholera, a disease with endemic or pandemic potential characterized by watery diarrhea and vomiting, leading to severe and rapidly progressing dehydration and shock [6]. The symptoms are caused by cholera toxin, which is produced by pathogenic strains of *V. cholerae*. Many efforts have been made to introduce a more effective vaccine, but many researches have shown that the vaccination has no role for cholera; however, new oral vaccines are displaying egregious promise [7].

Shigellosis and salmonellosis are caused by *Shigella* spp. and *Salmonella* spp., respectively. These organisms are likely to be the common cause of diarrhea worldwide. *Shigella* spp. are the causative agents of inflammatory diarrhea and dysentery, thus presenting a serious challenge to public health authorities worldwide [5]. Although shigellosis has no known animal reservoirs, we are still lacking an effective vaccine owing to poor immune responses to oral vaccines and existence of multiple serotypes [8].

Unlike *Shigella*, *Salmonella* spp. (except *Salmonella enterica* subspecies Typhi) are found in many animals. Thus, salmonellosis is well recognized as zoonosis disease [9]. The prevalence of *Salmonella* infection varies depending on the waste disposal, water supply, food preparation practices, and climate. Gastroenteritis is the most common disease among children caused by *Salmonella* [5].

The traditional methods for detection of bacterial infections are still primarily based on culture and serological methods that may take several days to be completed. There has been a general move toward molecular methods for microbial detection, which are based less on phenotypic features and more on stable genotypic characteristics. In recent years, polymerase chain reaction (PCR) and similar nucleotide-based methods have become potentially powerful alternative approaches in microbiological diagnostics because of their higher user-friendliness, rapidity, reproducibility, accuracy, and affordability. These methods have also gained momentum in terms of use for rapid, specific, and sensitive detection of foodborne pathogens [10–15].

Multiplex polymerase chain reaction is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction [16]. This technique is a powerful molecular method in microbiological diagnostics that allows the simultaneous amplification of more than one target sequence in a single PCR reaction, saving considerable time and effort, and decreasing the number of reactions to be performed in order to assess the possible presence of foodborne pathogens [16–18].

In this study, we describe a multiplex PCR assay for the rapid and simultaneous detection of *Salmonella* spp., *Shigella* spp., and *Vibrio cholera*.

2. Materials and methods

2.1. Bacterial strains

The bacterial strains were obtained from the Pasteur Institute of Iran and used in this study (Table 1). Clinical Q1 isolates of the three most important foodborne bacterial pathogens including *Salmonella* and *Shigella* were obtained from patients admitted to Children's Medical Center and Baqiyatallah Hospitals in Tehran, Iran, during 2012–2014. Subsequently, identification of the

Table 1. Bacterial strains included in this study, and performance of the multiplex PCR assay for detecting *Salmonella*, *Shigella*, and *Vibrio cholera*.

Strains	Reference	Multiplex PCR results
Salmonella serovar Albany	ATCC 51960	+
Salmonella serovar Enteritidis	ATCC 4931	+
Salmonella serovar Hadar	ATCC 51956	+
Salmonella serovar Reading	ATCC 6967	+
Salmonella serovar Typhi	ATCC 19430	+
Salmonella serovar Typhimurium	ATCC 14028	+
Citrobacter freundii	ATCC 8090	–
Escherichia coli	ATCC 25922	–
Shigella flexneri	PTCC 1234	+
Shigella soneii	ATCC 9290	+
Staphylococcus aureus	PTCC 1189	–
Vibrio cholerae	PTCC 1611	+

ATCC = American Type Culture Collection (USA); bp = base pair; PCR = polymerase chain reaction; PTCC = Persian Type Culture Collection (Iran).

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