Detection of ESBL Genes in *Salmonella enteritidis* Isolated from Clinical Samples

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**Abstract:** *Salmonella enteric* serovar *enteritidis* is currently the most common serovar causing salmonellosis in human. The incidence of gastrointestinal infections caused by *S. enteritidis* has increased during the last decade. The aim of this study was to extract ESBL genes from *S. enteriditis* separated from clinical samples using multiplex PCR. In this study, 29 human fecal samples of Collections microbial Azad University's research were collected. After enrichment and isolation and DNA extraction SipB/C, CmlA/tetR, TEM, PSE-1genes by multiplex PCR were evaluated. A total of 29 clinical samples studied, 6 (68.20%) of the strains were positive for genes SipB / C, CmlA / tetR, TEM specimens were observed. Detection of *S. enteritidis* strains by molecular methods are very accurate and can be done quickly. Studying these genes in various other sources as well as the antibiotics profile is recommended.

**Keywords:** *Salmonella enteritidis*, ESBL gene, Multiplex PCR
1. Introduction

Genus Salmonella is in the family of Enterobacteriaceae, which are more than 2,500 serotypes and are widely spread in the environment and natural inhabitant of the intestines of human and animals and act as intestinal pathogens which can cause a range of diseases in human, animals, wild mammals, reptiles, birds and even insects. Salmon, the American microbiologist, first separated Salmonella cholerae suis from pig excrement at 1885, so this genus was named Salmonella. Salmonella are motile bacilli that ferment glucose and mannose with production of gas but don’t ferment lactose and sucrose. Most of Salmonella produce hydrogen sulphide (H$_2$S) and in most cases are pathogenic to human and animals in the oral form (Mastroeni and Grant, 2013). Salmonella is food-transmitted microorganism and is one of the most important health problems in the world (Ranjbar et al., 2014). This bacterium is one of the most important pathogens in human and animals which can lead to diseases such as enteric fever, gastroenteritis, bacteremia and septicemia in human and is transferred often through direct contact or by consuming food and water contaminated with human or animal feces, as well as by carriers (without symptoms) (Mastroeni and Grant, 2013).

Salmonella enteritidis whose full name is Salmonella enterica subspecies enterica serovar enteritidis, is a rod-shaped, Gram-negative bacteria and without spore which moves by flagella and this is responsible for 99% of diseases in warm-blooded animals (Bakker et al., 2014). This bacterium is the most common Salmonella serotype in Salmonella bacteria because of the large foci among serotypes, therefore using phenotyping methods such as biotyping, phage-typing, etc. is difficult to classify this bacterium. Salmonella enteritidis causes salmonellosis in birds which is transmitted to human through the food chain and is considered one of the most important zoonotic diseases in human and animals. Prevalence of Salmonella enteritidis infections has increased, especially in the recent decades (Harvey et al., 2012). This serotype was the most common serotype of Salmonella in many developed and developing countries including in Iran in the past few years (Budiati et al., 2013). Presence of the infection due to this bacteria in the poultry and studying its dispersion to human food chain using rapid diagnostic methods on zoological products and also studying the genotypic and morphological diversion among its different strains is also possible (Landers et al., 2012).

According to the World Health Organization (WHO), more than 1.4 million annual incidence of diarrhea caused by Salmonella in America suggests that this rate is much higher in less developed countries. Children, pregnant women, elderly people and people with weak immune systems have high risk of developing these infections (Sauteur et al., 2013). From the more than 2500 different serotypes of Salmonella, few of them lead to acute gastroenteritis (Non Typhi) in human in which the symptoms usually begin 8 to 36 hours after consumption of contaminated food and in most cases associated with low-grade fever, nausea and vomiting, diarrhea and abdominal cramps. Although intestinal prevalence
is declined in developed countries but sporadic cases continued to be observed. Statistics provided by the Center of Disease Control (CDC) indicate that 1.4 million people in America are annually infected with Salmonella from which 16,000 cases require hospitalization and 600 die (Tennant and Levine, 2015). The first report of the salmonella food poisoning was by Gartner in 1888 in Germany. In multiple studies, the incidence of Salmonella has been reported 2.74% in USA to 8.3% in Iran. This bacterial infectious cause approximately 25 million infections and approximately 200,000 deaths in the world. Due to the high mortality rate, this is regarded as a serious threat. From the various serotypes of Salmonella, serotypes Typhimurium (Group B) and S. enteritidis is the most common types reported by the CDC (Center of Disease Control) which cause food poisoning infections and have a large outbreak in Asia (Korea, Japan and Thailand, etc.). Salmonella has antigens that are similar to other Enterobacteriaceae. Namination of antigenic and serological typing (serotyping) of Salmonella is done by Kaffmann-White Classification. Salmonella has two major types of antigens (Joshi and Amarnath, 2007). Somatic O antigens in the outer membrane of the cell wall and lipopolysaccharide (LPS) are heat resistant. O antigen has the base of Kaffmann-White Classification (A_Z). These antigens are identified with numbers that may exist in different serogroups and thus can cause cross agglutination reaction in between different serogroups.

Flagella antigen H, unlike the O antigen has proteinal material that is heat sensitive and will be in two phases; Phase 1 (specific) and Phase 2 (nonspecific).

Vi antigen (derived from the word Virulence) is surface antigen polysaccharide capsule in Salmonella typhi and some strains of Salmonella serotypes C and Dublin (Dublin) (Thomas et al., 2013). These antigen cover somatic and surface antigens. To identify these strains of bacteria we must prepare thick suspension in saline and take in the bain marie 100 °C for 15 minutes to terminate the capsule antigen Vi. The supernatant was discarded and the remaining sediment in the bottom of the tube (containing antigens O and H detected) was used to repeat the serological tests and determine the serotype. ESBLs was determined in Germany for the first time and then quickly were spread among considerable variations bacteria (Gordon et al., 2002). Today, the different ESBLs are growing in world, so they are found all over the world in many different species of bacteria (like Enterobacteriaceae). Nowadays, ESBLs are known as a problem of patients admitted in hospitals around the world. The prevalence of ESBLs are different in clinical strains of countries (Bradford et al., 1998). The ESBL genes linked with multiple resistance to other antibiotics, thus the different genes of incidence of ESBLs due to increased antibiotic resistance, particularly for multi-drug, have created many problems for the treatment of infections (Landers et al., 2012). ESBL-producing bacteria are known as clinical threats and are doctors’ main concern in treating the infections caused by these organisms. Strains with producing this enzymes are responsible for nosocomial infections with prolonged consequences. The best way to identify broad spectrum B-lactamase is an initial screening.
for reduced sensitivity of recommended antibiotics NCCLSand then do confirmatory tests to verify the synergism effect between a marker Combined Disk cephalosporins and beta-lactamase as the inhibitor. The proposed method for screening purposes is National Committee for Clinical Laboratory Standards (NCCLS) and Disk Agar Diffusion test (DAD) and for the approval purposes Combined Disk test (Stürenburg and Mack, 2003) is used. ESBL-producing organisms are clinically important because they show a pattern of widespread drug resistance and they increase morbidity and mortality rates, especially in ICU patients in hospitals. Therefore, it is necessary to apply the optimal treatment strategies and appropriate infection control measures to reduce the prevalence of these organisms (Frey et al., 1988). The aim of this study is determine molecular analysis SipB / C, CmlA / tetR, PSE-1, TEM S. Enteritidis isolates in human resources with multiplex PCR technique.

2. Materials and Methods

2.1. Collection of Bacterial Strains

For this study, 29 samples of human stool samples of patients with acute diarrhea admitted in hospitals in Tehran were collected. The samples contained Salmonella enteritidis. Identification of strains was confirmed by biochemical tests and by agglutination on slide and tube agglutination in Salmonella serotype O and H respectively, were divided. Identification of strains was confirmed by biochemical tests and Salmonella serotype were divided O and H respectively with agglutination on slide and agglutination in tube. All isolates were maintained at 37 ° C in the natural environment and were examined and analyzed for determination of antibiotic resistance.

2.2. Cultivation, Isolation and Identification of Bacteria

Fecal samples were transferred to medium selenite F and they were transferred to the microbiology laboratory. After incubation, they were cultured in the broth SS Agar and were placed for 24 hour at 37 ° C. Salmonella suspect colonies (colonies with black center) isolated and for biochemical testing were planted in the Simon citrate, SIM, MRVP and urea and after 24 hour incubation in 37 ° C, the results were studied. Isolation separated by biochemical characteristics of lactose negative, movable, citrate positive, methyl red positive and urea hydrolysis negative were determined as a Salmonella genus isolates.

2.3. DNA Extraction

DNA extraction was performed with Molecular Biological System Transfer kit and done according to the provided instructions. After the above steps, to check the quality of extracted DNA, the obtained solution containing DNA was transferred on agarose gel electrophoresis.
2.4. PCR

According to the studies and comparing different sequences used as target for detection of Salmonella species, the following primers were used to identify multi-drug resistance in Salmonella strains (Carlson et al., 1999). The sequencing primers used in this study are presented in table 1. Primers according to the manufacturer's instructions, prepared in concentration of 100 ppm / μl in distilled water and in concentration of 10 pm/μl were used 20 μl in PCR reaction.

<table>
<thead>
<tr>
<th>primer</th>
<th>Primer sequences</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SipB/C</td>
<td>5-ACAGCAAAATGCGGATGCTT-3</td>
<td>Carlson et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5-GCGCGCTCAGTGTAGGACTC-3</td>
<td></td>
</tr>
<tr>
<td>CmlA/tetR</td>
<td>5-CGCTCCTTCGATCCCGT-3</td>
<td>Carlson et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5-GCTGCGTTCATCTACAACAGAT-3</td>
<td></td>
</tr>
<tr>
<td>PSE-1</td>
<td>5-CTTGGTTCCGCGCTATCTG-3</td>
<td>Carlson et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5-TACTCCGAGCACCACACCCGAGG-3</td>
<td></td>
</tr>
<tr>
<td>TEM</td>
<td>5-GCACGAGTGTTACATCGA-3</td>
<td>Carlson et al., 1999</td>
</tr>
<tr>
<td></td>
<td>5-GGTCTCCTCGATCAGGCAG-3</td>
<td></td>
</tr>
</tbody>
</table>

Multiple PCR reactions was used, for each primer pair SipB / C, CmlA / tetR, PSE-1, TEM, respectively, individually with values in the volume of 20 ml. It should be noted that all PCR reactions were performed with negative control sample that has all the necessary components for PCR except the template DNA.

2.5. PCR Optimization

Optimization is the determination of the appropriate reactants concentration and appropriate temperature bonding primers (Ta) to obtain appropriate PCR products. For this purpose, for each of parameters such as Ta and number of cycles, several tests were carried out; Then PCR products and primers and the reaction conditionswere surveyed and temperature and cycleswere chosen which in thesesteststhe best temperature was 50° and cycles were 40. PCR products were kept for short time in the refrigerator at 4 ° C and for a long time at - 20 ° C.

2.6. Multiplex PCR Products Electrophoresis Using Agarose Gel and Sign Images

Agarose gel electrophoresis is a standard way to check and separate DNA fragments generated by PCR. The advantages of this method is its simplicity, rapidity and the ability to dismantle DNA. Positions and places in the gel are detected by staining with low concentrations of erythrogel and observed directly affected by UV radiation.
3. Results and Discussion

Genomic DNA was extracted from standard strains and then were spread on agarose gel (Figure 1).

![Figure 1: Genomic movement patterns on agarose gel. (Rows 1-2 are Ladder 50 bp and S. Enteritidis standard strains, respectively.)](image)

Results multiplex PCR reactions for the detection of *Salmonella enteritidis* strains in human subjects

According to the results of a series of 29 clinical samples, only 6 (20.68%) of the strains were positive and 23 samples (79.32%) of the strains were negative.

**Confirming the gene SipB / C with M-PCR**

From the set of 29 clinical samples, 3 samples for gene SipB / C with a piece weight 232 bp was positive that be included 10.3% of the isolates.

**Confirming the gene CmlA/tetR with M-PCR**

From the set of 29 clinical samples, 2 samples for gene CmlA / tetR with a piece weight 260 bp was positive that be included 6.90% of all isolates.

**Confirming the gene TEM with M-PCR**

From the set of 29 clinical samples, 1 sample for TEM gene with a piece weight 291 bp was positive that be included 3.44% of the isolates.

**Confirming the gene PSE-1 with M-PCR**

In none of the clinical samples gene PSE-1 with a piece weight 132 bp was found.
Table 2: Results of Multiplex-PCR strains of *Salmonella enteritidis* (total samples: 29)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SipB / C (232 bp)</td>
<td>3 (10.3%)</td>
</tr>
<tr>
<td>CmlA/tetR (260 bp)</td>
<td>2 (6.90%)</td>
</tr>
<tr>
<td>TEM (291 bp)</td>
<td>1 (3.44%)</td>
</tr>
<tr>
<td>PSE-1 (132 bp)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2: Identifies *Salmonella enteritidis* by Multiplex PCR using 4 pair primers. SipB / C, CmlA / tetRA, PSE-1, TME on the left side of the field marker bp50, columns positive control, negative control column and the column 1-11 clinical isolates of *Salmonella enteritidis* formed a band of primer CmlA / tetR.

Figure 3: Identifies *Salmonella enteritidis* by Multiplex PCR using 4 pair primers. CmlA / tetR, PSE-1, TEM from the left to the column marker bp50, columns positive control, negative control column and column *Salmonella enteritidis* 12-22 clinical isolates formed two bands of primer SipB / C and CmlA / tetR.
Figure 4: Identifies *Salmonella enteritidis* by Multiplex PCR using 4 pair primers.

SipB / C, CmlA / tetRA, PSE-1, TME on the left side of the field marker bp50, columns positive control, negative control column and the column 23-29 clinical isolates of *Salmonella enteritidis* formed a band of primer TEM.

*Salmonella* is a group of Gram-negative bacilli from family Enterobacteriaceae which are common features and there have over than 2500 serotypes and they widely distributed in the environment. *Salmonella* as intestinal pathogens caused a wide range of diseases including enteric fever, gastroenteritis, bacteremia, septicemia and etc. *Salmonella* usually are transferred through direct contact or by consuming food and water contaminated with human or animal feces and by carriers without symptoms (Mersch-Sundermann *et al*., 2004).

*Salmonella* contamination manifested in two common infectious diseases forms, typhoid fever and gastroenteritis with septicemia or without sepsis. More than 2500 different serotypes of *Salmonella*, a limited number of them leading to acute gastroenteritis (non-Typhi) in humans and symptoms of diseases usually begin 8-36 hours after consumption of contaminated food and in most cases associated with mild fever, nausea, vomiting, diarrhea and abdominal cramps. In fact, based on the type of disease, the strains of *Salmonella* are usually classified in two types; typhoid and non-typhoid (Tennant and Levine, 2015). Although the prevalence of intestinal form declined in developed countries but sporadic cases be observed. Statistics provided by the Center of Disease Control (CDC) indicate that 1.4 million people in America with *Salmonella* infections annually that 16,000 of them
require to hospitalization and 600 people die. In other words, infections with Salmonella typhi and other Salmonellas still has great deal of importance in developing and developed countries.

One of the most important serotypes of Salmonella is S. enteritidis that has important role in causing infections in humans. The prevalence of these bacteria (S. enteritidis) increases during the last decades that this is known the most common type of Salmonella serving the human infections in many developing countries such as Iran. World Health Organization (WHO) statistics show there are a dramatic increase in the number of cases of Salmonella enteritidis infection in different regions of Europe, North America and South American countries. The results of study that was conducted in Spain, showed that Salmonella enteritidis are the most common cause of salmonellosis after Salmonella typhimurium. In many developing countries such as Brazil, the prevalence of food borne and nosocomial infections due to Salmonella enterica serotype Enteritidis still being discussed as an important health problem (Koutsolioutsou et al., 2001).

This is more than 50 years that used antibiotics to treat quickly and effectively infections. During this time, many changes has been created in type and sensitivity of antibiotics and bacterial resistance (Neves and Martins, 1967). One of the major problems in the treatment of infectious diseases is pathogenic bacteria resistance to antibiotics. Drug resistance is a complex concept involved in which several factors including the type of organism causing the infection, the germs in the body, the distribution of the antibiotic in the body, the drug concentration at the site of infection, the immune status of patients and influence each other. Resistance to antibiotics is 2 form; natural and acquired (Martins et al., 2010). In intrinsic resistance, the normal or wild cell is able to inhibit the antibiotic and posseses chromosomal pressure, whereas acquired resistance is caused by exposure of sensitive and normal population to different agents and resistance-sensitive strains. The most common ways of acquiring resistance is divided into 2 categories; genetic chromosomal resistance and plasmid resistance (Vieira-Pinto et al., 2011).

The acquired resistance is a result of mutation in chromosomal gene or in connection with plasmids, transposons and integrons. Bacteria can easily access the resistance genes located on the motile genetic agents such as plasmid, transposons and integrons, which can be transferred from one bacteria to another. In all efforts for reducing the antibiotic resistance, 2 factors must be kept in mind: First, excessive use of antibiotics and second, easiness of the expansion of the resistance gene. Although there has been many alerts about this subject, still it hasn’t been taken seriously in the medical society and the way of its expansion and the effects of drugs on this matter has been left unknown (Lira et al., 2008, Wild et al., 2011).

Interactions between fast growth, high density of cells, genetic process of mutation, natural selection and also bacteria’s high potential in genetic transfer cause compromise and diversity in an abnormal measure and therefore it seems that the bacteria’s compromise happens very quickly in a
environment containing antibiotics (resistance). It has to be noted that the ESBL-producing bacteria are serious problems in treating bacterial infections. In the past few years we have noticed the increase in this kind of resistance in bacterial strains including Salmonella. Resistance to antibiotics is also an important threat for people’s health all around the world and therefore it has to be followed more severely (Broz et al., 2012). Mechanisms of bacteria in antibiotic resistance are different. One of these mechanisms is producing betalactamase in the bacteria (Gonzalez-Escobedo and Gunn, 2015). Betalactamase is a bacterial enzyme which can inactivate antibiotics such as Penicillins and Cephalosporins by hydrolysing the betalactam ring. These enzymes have the ability to break down broad-spectrum 3rd generation Cephalosporines such as Ceftazidime, Cefotaxime, Ceftriaxone and Monobactams (Aztreonam). But these enzymes don’t have any effects on Cephamycins (Cefoxitin and Cefotetan) and Carbapenems (Imipenem and Meropenem), and their activity is inhibited by Clavulonic acid, Sulbactam and Tazobactam (Chen et al., 2014).

Ampicillin, Chloramphenicol and Sulfamethoxazole have been used for treating diseases caused by Salmonella in the past, but due to appearance of resistant strains all over the world, Fluroquinolones and Cephalosporines are used as alternatives. Therefore 3rd generation Cephalosporines became common in treating severe Salmonella infections. This fact resulted in strains resistant to some drugs and also ESBL-producing Salmonellas. Broad-spectrum Betalactamases is now known as a problem in hospitalized patients all over the world. Prevalence of Broad-spectrum Betalactamase is different in clinical species in various countries (Kröger et al., 2012). On the other hand, ESBL genes are connected to other antibiotics by developing multiple resistances, so that indication and prevalence of different ESBL genes has caused many problems in treating the relevant infections by increasing the antibiotic resisttance, especially in the form of multiple drugs. Bacteria producing this kind of enzymes such as Betalactam-producing bacteria in Salmonella enteritidis and other members of Entrobbacterciaeae are known as clinical threats and have caused great concern among doctors in treating the infections related to these organisms. At the beginning the resistance was limited to hospital infection which could cause long infections with undesirable consequences (Mathur et al., 2012). But nowadays this kind of resistance is reported from various parts of the world including our country as well, which has occurred due to doctors’ excessive prscription and wrong usage of antibiotics by patients (Hajjar et al., 2012).

According to the increasing prevalence of Salmonella enterica serotype enteritidis between food consumers, especially high risk patients (hospitalized elderly and children) and also increased resistance to 3rd generation Cephalosporines and development of betalactam-producing strains in Salmonella enteritidis and other members of Enterobacteriaceae, different genes in these enzymes should be studied in every region in order to control this type of resistance in Enterobacteriaceae strains, quick and appropriate treatment of infections which are suspected to be due to broad-spectrum
betalactam-producing organisms and also getting more information on the amount of prevalence. Since *Salmonella enteritidis* possesses high hemogenity among Salmonella serotypes, it is typed using phenotypic methods such as biotyping, phage typing, etc. (Okoro *et al*., 2012). Since these methods are time-consuming, not economical and unreliable, they are not to be used in broad epidemiological studies. Therefore, quicker and more specific methods are needed, such as molecular methods and using the polymerase chain reaction method (PCR) which has high senstivity and specificity for detecting infective agents. Multiplex PCR is a very specific, effective and appropriate method which can judge on samples suspected to be Salmonella using multiple primers simultaneously in less than 12 hours, so in this study, ESBL genes in *Salmonella enteritidis* isolated from clinical samples were studied using multiplex PCR.

The pattern of multiplex PCR was compared by observation and calculation of their location in gel based on their molecular weight and comparing them to the molecular marker of each sample and the pattern of the gene. Direct detection of ESBL genes using multiplex PCR is an appropriate criterion for identifying the susceptibility of strains in producing ESBL genes. Different studied have been done in various contries on molecular identifaction of ESBL in *Salmonella enteritidis* using multiplex PCR.

Soo Jin Yang and *et al* (2012) have studied antibiotic resistance in Salmonella enterica serovar enteritidis and typhimorium isolated from animals in Korea and realized that enteritidis strains in this study had 5 different patterns of drug resistance and 2 patterns of PCR multiplication. 2 out of 3 of the enteritidis Ampicillin-resistant isolates had the blaFM gene. Thet also noticed that there were differences between the two DT104 isoalted from Korea and America, such as missing the cmlA/tetR and blaPSE-1 (Seo *et al*., 2012).

Hasman *et al* (2013) studied the genetic of broad-spectrum betalactamase resistance gene in Salmonella strains isolated from chicken, beef and patients in Netherlands and realized the high genetic diversity in the samples. They also realized that the blaTEM-52 in Salmonella enterica, Enteritidis, Enteritidis type 14b, Paratyphi B, Virchow type 11 and Typhimuruium 507 ,blaTEM-20 in S. java, ParatyphiBvar and blaTEM-63 in S. isangi is dominant. They concluded that there was genetic diversity in ESBL genes from Salmonella enterica isolated from animals, food products and human (Dolejska *et al*., 2013).

Cheng and *et al* (2006) studied and evaluated the drug resistance genes in Salmonella typhimurium in Taiwan. They showed that this method is more appropriate than other methods such as phage typing and biotyping in epidemiological searches (Chiu *et al*., 2006).

Riano *et al* (2006) defined and identified the characteristics of ESBL produced by Salmonella enterica in the livestock feed in Spain for the first time, which led to the fact that 3 strains of avian S. enterica (2 strains of Virchow and one strain of enteritidis) contain blaCTX-M-9 and blaTEM-1b, whereas ESBL produced by the risen serotype of pigs contain blaSHV-12 and blaTEM-1b. This
research also showed that CTX-M is the dominant ESBL in enterica strains in livestock feed (Riano et al., 2006).

Boisramee-Gastrin et al. (2011) studied the Salmonelllas in admitted children. During this study, 55 families who had adopted 61 kids from Bamako orphanage were studied. 92 species of Salmonella were isolated from stool samples of 30 children. Isolates belonged to Salmonella enterica from different serovars from which enteritidis andbabelsberg were the most common. PCR and DNA sequencing showed that all ESBL-producing isolates contained blaTEM-1, 21 had blaSHV-12 and the remaining 5 had blaCTX-M-15 (Boisramé-Gastrin et al., 2011).

Due to the importance of ESBL genes in Salmonella enteritidis, molecular identification of SipB/C, PSE-1, TEM, CmlA/tetR was studied using the multiplex PCR. Therefore 20 clinical samples were chosen from the microbial collection of Azad university science and research center of Shahriar, the samples were studied using the SipB/C, PSE-1, TEM and CmlA/tetR primers and then its detection and amplitude were studied.

Out of 29 clinical samples, 6 (20.86%) were positive, and 23 samples (79.32%) were negative. 3 samples out of 29 were positive for sipB/C with the weight of 232 base pair which is 10.3% of the isolates. 2 samples were positive for CmlA/tetR with a piece weighing 260 base pair, which is 6.90% of the isolates. 1 out of 29 samples was positive for TEM with a piece weighing 291 base pair, which is 3.44% of the isolates. PSE-1 weighing 132 base pair was not detected in any of the samples.

Results using M-PCR from this study is somehow different from the studies done by other researchers. For example, unlike Korea, not all the following enteritidis samples contained SipB/C, and only 3 samples had this gene. Also, strains which were studied in this research, like the 2 samples in Korea, had TEM, and they had CmlA/tetR unlike samples from Korea and similar to the samples from America.

4. Conclusion

Diagnosis and detection of broad-spectrum betalactamase-producing strains of Salmonella enteritidis has great deal of importance due to the prevalence of food-related diseases and hospital infections. Reaching this goal and preventing the consequences is based on quick diagnosis of this pathogen using rapid and precise methods such as molecular methods like multiplex PCR. With this method, due to its high sensitivity and specificity, pathogenic genes can be detected in the shortest time possible, and with the right treatment on the right time, resistant strains and the gene transfer in human and animal populations can be prevented.

In this research, molecular detection of PSE-1, TEM, CmlA/tetR and SipB/C were studied using multiplex PCR, in which the results from the M-PCR method were different from studies of
other researchers in various parts of the world. This difference might be due to geographic diversities and ecological sources. Most studies were done in American and European countries and in Iran, there aren’t many studies available on molecular identification of Salmonella enteritidis pathotypes using M-PCR, which can explain the differences between the results.

References


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