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Original Article

Synthesis and Evaluation of the Immunomagnetic Beads for Separation of the *Salmonella typhimurium* from Milk

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Abstract

Salmonella causes zoonotic diseases in humans and many animal species. The bacteria could be spread through fecal-oral transmission and consumption of raw contaminated animal products. Despite the activities which are carried out for the prevention of salmonellosis, it causes economic losses. This study aimed to prepare immunomagnetic beads to separate the *Salmonella* bacteria from experimentally polluted milk samples. The antibodies were purified from the rabbit's hyperimmune sera and coupled to the Fe nanoparticles using diethylenetriaminepentaacetic acid (DTPA) as a linker. The synthesized particles were analyzed using electron microscopy. The limit of bacterial detection by using the immunomagnetic beads coupled with bacterial culture were tested in experimentally contaminated cow milk with *Salmonella*. The separated bacteria were identified by using bacterial culture and biochemical tests. Using immunomagnetic beads (IMB), the *Salmonella* bacteria were removed from milk samples, concentrated in sterilized PBS, and cultured in nutrient agar media. The conventional culture method detected the bacteria in samples polluted with at least 3×10^4 CFU/mL bacteria; however, isolated bacteria were separated from milk samples using IMB and defined on bacterial culture media. The 3 CFU/mL of *S. Typhimurium* were detected in experimentally polluted milk samples using the current immunomagnetic-culture method. The results suggested using the IMB-bacterial culture instead of the conventional culture method.

Keywords: *Salmonella*, milk, Immunomagnetic Separation

1. Introduction

Salmonella species are gram-negative, facultatively anaerobic, flagellated, nonsporulating, straight-rod bacteria with O, H, and Vi antigens. The complex lipopolysaccharide (LPS) functions as an endotoxin. Furthermore, they can carry fimbria. Their fimbria enhances the adhesion of the bacteria to the intestine (1).

Salmonella can cause zoonotic diseases in humans and many animal species. Species of *Salmonella* bacteria are well-known agents of food-borne illnesses such as typhoid, paratyphoid fevers, pneumonia, and bacteremia. Annually, high rates of enteric disease are reported worldwide. The bacteria spread through fecal-oral transmission and consumption of raw contaminated animal products (2). Human infection by *Salmonella* occurs by consuming poultry eggs, undercooked meat, fresh fruits, vegetables, milk, dairy products, and nuts (3). *Salmonella* is mentioned as the second (30%) etiologic agent of food poisoning outbreaks in the United States (4); similar outbreaks rates were reported in other countries, especially by consumption of the polluted milk (5-7). The most common salmonellosis signs include diarrhea, fever, abdominal cramps, and vomiting after ingesting the contaminated products (8). More concerns have been

created due to antibiotic-resistant strains and significant economic defects (9). Early and fast identification of the carriers and the contaminated foods has priority in preventing salmonellosis. Although various activities have been carried out for the care, prevention, and treatment of salmonellosis, it causes significant economic losses in industrial and developing countries (10).

Based on various *Salmonella* strains present in the environment, it is impossible to predict natural dose-response, although an infectious dose of non-typhoid *Salmonella* ranges from100 to10^9 bacteria (11, 12). So, developing a sensitive diagnostic method is critical to detecting variants of *Salmonella*. In existing culture methods, nonselective pre-enrichment followed by selective enrichment is used to isolate the aimed bacteria. Verification of *Salmonella* colonies was performed biochemically and serologically. It takes almost four days to find and remove the negative samples and the other seven days to confirm the *salmonella* colonies (13). Fast diagnosis of pathogens with high specificity and sensitivity might be highly preferred for detecting *Salmonella*. The gold standard method used for *Salmonella* detection, the conventional culture method, has less sensitivity (14). Several methods have been developed to overcome these problems to detect *Salmonella* to increase the sensitivity and decrease the detection time. These methods include immunoassays and nucleic acid-based techniques. Invariably, the immunoassays are rapid and cost-benefit tests compared to genomic evaluation tests. Also, these methods did not require expensive equipment and skilled technicians. Among these strategies, immunomagnetic separation (IMS) could be useful for the rapid and reliable identification of *Salmonella*.

Magnetic-based separation of pathogens may have practical applications in laboratories. The IMS method can isolate and concentrate the pathogens and eliminate the inhibitor elements (15). The immunomagnetic beads could enhance the sensitivity of the analytical and diagnostics methods; however, in addition to targeting bacteria, the IMBs may be trapped other microorganisms (16). Also, utilizing the serovar-specific antibodies for preparing the IMB could cause false-negative results because of the pathogenicity of all of the *Salmonella* bacteria in humans; utilization of the aptamer instead of the antibodies was suggested previously (16). The immunomagnetic beads could separate the target bacteria from various samples such as fecal, milk, and water samples. The antigens could be isolated and washed appropriately using a robust magnet to eliminate interfering elements. This study aimed to prepare and evaluate the immunomagnetic beads to separate *Salmonella* from experimentally polluted milk samples. In addition, the limit of detection of bacterial culture and IMB-bacterial culture were calculated and compared in the current study.

2. Materials and Methods

2.1. Preparation of the Bacteria

The *Salmonella typhimurium* (ATCC14028) was cultured in blood agar media and incubated at 37 °C for at least 24 hours. A bacterial suspension was prepared in sterile phosphate buffer saline (PBS), and its OD600 was adjusted to 0.12 nm. The seven 10-fold serial dilutions of this suspension were prepared using sterile PBS. The bacterial counting of prepared suspensions was done using the standard colony counting method (17). Inactivation of the *S. Typhimurium* was achieved by adding 150µL formaldehyde 37% into into 10 mL bacterial suspensions. The prepared suspension was incubated at room temperature for 24 h; then washed 3 times by 10 minutes of centrifugation at 10.000 rpm.

2.2. Preparation of the Samples

The samples were prepared using the induced contaminated milk samples following steps. The seven 1:10 serial dilutions (10^{-1}-10^{-7}) of the *Salmonella typhimurium* and a negative control sample were prepared in sterilized phosphate-buffered saline (PBS). All prepared *Salmonella* concentrations were centrifuged, and the precipitated bacteria were suspended in two cattle milk samples; the milk samples were previously confirmed as *salmonella* negative.

2.3. Preparation of the Hyperimmune Sera

The concentration of inactivated *Salmonella* was adjusted to 4 McFarland standard, and a volume of 0.5 mL was mixed with an equivalent amount of the complete Freund's adjuvant. The prepared antigens were injected subcutaneously and intramuscularly into two rabbits. The booster antigens were prepared by mixing 0.5 mL of the inactivated bacteria at a concentration adjusted to 2 McFarland standard with an equal amount of the incomplete Freund's adjuvant; three sets of boosters were injected as before at 2-week intervals. The micro-agglutination test was used to assess the anti-*Salmonella* antibody titer for each immunized rabbit. The hyper-immune sera were harvested from the immunized rabbits and stored at -20°C.

2.4. Isolation and Purification of the Antibodies

The IgG antibodies were purified by ion-exchange chromatography on the DEAE-C column (Sigma, Number: D3764) according to He, Ren (18) guidelines. Purifying the specific antibodies for *Salmonella* has been done by the following steps of affinity chromatography: Briefly, 5 mg sonicated *Salmonella* antigens were coupled to sepharose 4B (Sigma–Aldrich, Product Number: 4B200) by cyanogens bromide linker. The extracted total IgG was incubated with an activated column; the specific antibodies were released from the column using glycine buffer pH 2.5 and immediately neutralized using tris buffer pH 9.5. The Bradford protein assay and micro-agglutination test checked the quantity and titer of the purified antibody. The purified antibodies were evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were prepared as 11% separating gel and 4% stacking gel, and electrophoresis was performed in running buffer (25 mM Tris base pH 6.8, 192 mM glycine, 1% SDS) at 100V for 90 minutes. Protein ladder molecular weight (Sinaclone, Iran) was run parallel to calculate the proteins' molecular weights. The polyacrylamide gels were stained for 30 minutes with Coomassie blue staining solution, followed by de-staining with 7% acetic acid solution overnight.

2.5. Preparation of the Fe Nanoparticles

An in-house synthesis procedure was followed to prepare Fe3O4 nanoparticles. The matrix solution was prepared by mixing the Ethylene glycol (37.5 mL) and n-octylamine (25 mL); the prepared solution was heated to 150 °C into a three-neck round-bottom flask using an oil bath. Anhydrous FeCl3 (2.4 g) was dissolved in another solution which contained ethylene glycol (10 mL) and Milli-Q water (3.0 mL). The iron (III) solution was slowly added to the flask and further stirred up and heated at 180 °C for 24 h. The iron nanoparticles were absorbed using a robust magnet and washed three times with acetone (19).

2.6. Synthesis of the Immunomagnetic Beads

The Fe nanoparticles 1 mL (130 mg/mL) were mixed with Tris-HCl 0.1 M, pH 9.6, which contained DTPA 200 mg, and incubated overnight at room temperature. After 3 sets of washing by PBS, the purified anti-*Salmonella* antibody 1 mg was added to the activated Fe nanoparticles. Samples were shaken occasionally to prevent bead sedimentation for 2 hours at room temperature and incubated overnight at 4°C. Un-reacted groups of the Fe nanoparticles were blocked by adding 4 mg/mL of skimmed milk. The mixture was incubated for 2 hours at room temperature and washed 3 times with sterilized PBS. The prepared immunomagnetic beads were dispersed in a filtered storage buffer, including sterilized PBS containing BSA 1%. The produced conjugates were aliquoted and stored at 4°C. The antibody coupling to the Fe nanoparticles was confirmed using the Bradford protein assay (15).

2.7. TEM Analysis of the Immunomagnetic Beads

Fe nanoparticles, DTPA-activated Fe nanoparticles, and immunomagnetic beads were spread on aluminum folie and dried in an incubator 60◦C for 72 hours. Transmission Electron Microscopy (TEM) (LEO, E906) was used to determine the morphology and size of the synthesized particles. The particle size is reported as the average observed size, which is the

average particle size of approximately 100 individual particles from multiple TEM images.

2.8. Separation of the *Salmonella* from Milk Samples

Two sets of seven 1:10 serial dilutions (two replicates) of the *Salmonella* bacteria were prepared, as described, in 1 mL of milk samples. One group was cultured in nutrient agar media as control samples, and 250 µL of IMB was added to the second set of samples. The prepared samples were shacked at 37°C for 45 minutes. The bead-bacterium complexes were collected using a robust magnet. The sample supernatant was carefully aspirated off with a sterile Pasteur pipette. Beads were washed three times with 1 mL PBS containing 0.05% Tween 20 (PBS-T) and were finally resuspended in 100 µL PBS. The sediment of the IMB was cultured in a nutrient agar medium. The plates were incubated for 48 hours at a 37◦C incubator; the growing colonies of *Salmonella* were confirmed by using biochemical tests, including production of H2S, dulcitol and glucose fermentation, negative urease, and indole reaction; the positive colonies were counted manually (Figure 1).

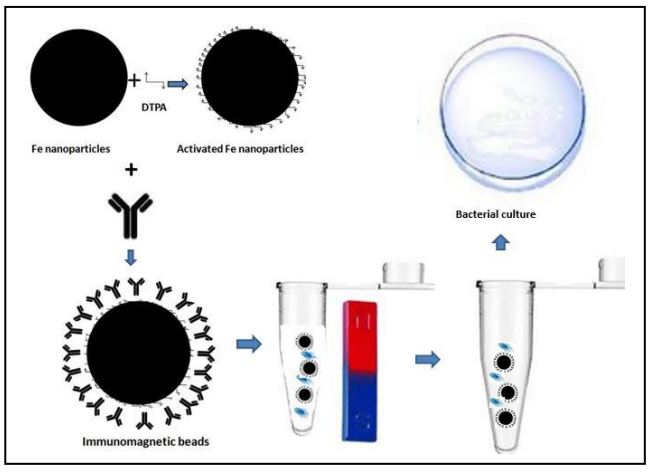

Figure 1. The schematic process of *Salmonella* separation and detection using immunomagnetic beads. The magnetic Fe nanoparticles were activated using DTPA; the purified anti-salmonella antibodies were coupled to the activated Fe nanoparticles. The preparedimmunomagnetic beads were placed in polluted samples and removed using a magnet after 45 minutes of incubation on a shaker. The removed immunomagnetic beads were transferred to bacterial culture media and incubated at 37 °C for 48 hours. The growing colonies were identified based on biochemical tests.

2.9. Statistical Analysis

The results were analyzed using descriptive statistics; the diagrams were drawn with Excel software version 13. The statistical significance was evaluated using the one-way analysis of variance (ANOVA). A *P* value of less than 0.05 was considered significant in statistical analyses.

3. Results

3.1. Bacterial Counting

Counting of the bacterial culture of OD=0.12 nm revealed the presence of approximately 3×10^8 CFU/mL in the initial concentration of the bacterial solution; this number was equal to McFarland 1.

3.2. Antibody Purification

The rabbits produced an appropriate antibody titer after three sets of immunization. The micro-agglutination, SDS-PAGE, and Bradford analysis showed suitable antibody purification using ion-exchange chromatography and affinity purification (Figure 2). The hyperimmune sera have an anti-*Salmonella* titer equal to 1/1024; the purified antibody using diethylaminoethyl cellulose (DEAE-C) (600 µg/mL) and affinity-purified antibodies (150 µg/mL) have 1/512 and 1 /512 titer, respectively.

3.3. Synthesis of the Immunomagnetic Beads

The TEM image of the prepared Fe3O4 nanoparticles is shown in figure 3; the particles' diameters were 176 nm. The immunomagnetic particles have a diameter equal to 260 nm (Figure 4). These prepared nanoparticles showed a suitable distribution in different matrixes, including water, phosphate-buffered saline, and milk samples.

3.4. Detection of the Bacteria in Milk Samples

Seven 1:10 serial dilution of the bacteria concentration was used to prepare the control and test samples. The growing colonies were observed in control samples which contained at least 3×10^4 CFU/mL of *Salmonella* bacteria; this level was documented as the detection limit of bacterial culture. Incubation of the milk samples with 250 µL of the prepared immunomagnetic beads resulted in the collection of all of the *Salmonella* bacteria; in

other words, immunomagnetic beads removed all of the *Salmonella* bacteria from samples supernatant. Also, the bacterial colony was successfully observed in all concentrations of the polluted samples (Table 1). These results showed a detection limit of immunomagnetic beads separation-bacterial culture method was 3 viable cells/mL of *Salmonella* in experimentally polluted milk samples.

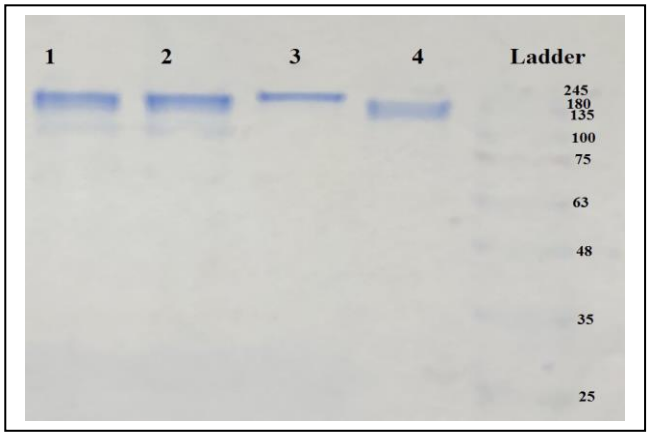

Figure 2. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) of the purified antibody using affinity and ion exchange (I.E.) chromatography. 1, 2) IgG purified by I.E., 3, 4) purified antibody using affinity chromatography Ladder) marker protein including 245, 180, 135, 100, 75, 63, 48, 35, 25, 20, 17 and 11 kDa, respectively

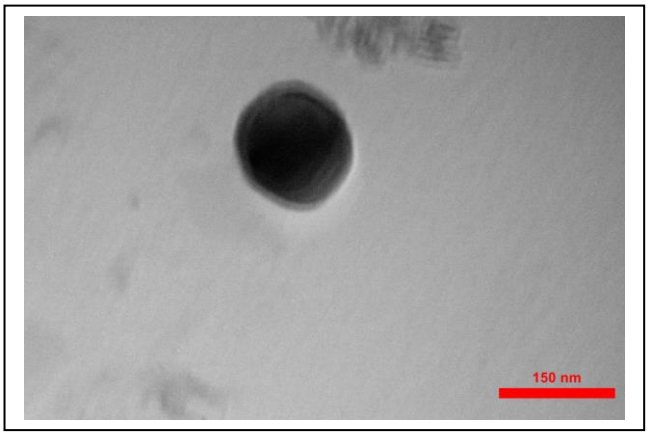

Figure 3. Transmission Electron Microscopy (TEM) images of the prepared Fe nanoparticles using solvothermal method beads

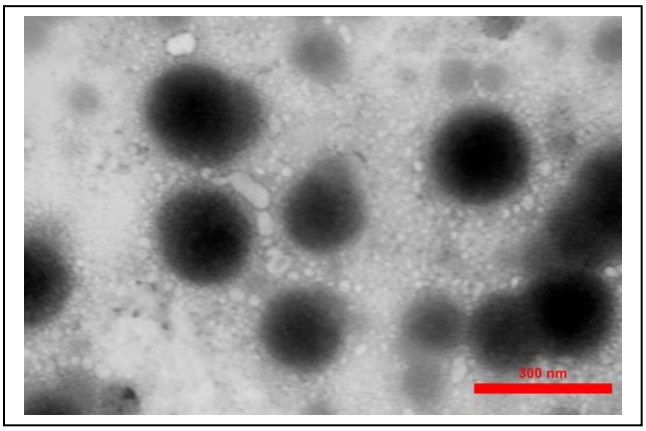

Figure 4. Transmission Electron Microscopy (TEM) images of the prepared immunomagnetic beads using DTPA as a linkerc

Table 1. Detection of *Salmonella typhimurium* in milk samples using immunomagnetic beads and bacterial culture. The immunomagnetic beads were mixed with the contaminated milk samples; the separated bacteria were cultured and counted manually. Bacterial concentrations greater than 3×10^5 have too many growing colonies in both control and IMB plates. The uppercase and lowercase letters show a significant difference

Bacterial concentration	3×10^5	3×10^4	3×10^3	3×10^2	3×10^1	3
Control	341 ± 57^{A}	101 ± 34^{A}	0^{A}	0^{A}	0^{A}	0^{A}
IMB	1148 ± 120^{a}	370 ± 37^{a}	134 ± 18^{a}	70 ± 15^{a}	59 ± 8^{a}	28 ± 11^{a}
Supernatant of IMB	0	0	0	0	0	0

4. Discussion

This research evaluated the immunomagnetic bead (IMB) separation method for separating *Salmonella* in milk samples. Experimentally polluted samples were prepared by making 1:10 serial dilution of *S. Typhimurium* in cow milk. Incubation of IMB samples for 45 minutes has relevant results. Using IMB, all diluted *Salmonella* bacteria were concentrated in 100 µL of sterilized PBS and cultured in nutrient agar media. At least 3×10^4 CFU/mL bacteria were detected using the conventional culture method in polluted samples; however, using IMB, all concentrations of the bacteria were separated from milk samples and defined on a culture medium. This tested method enhanced the sensitivity compared to the usual method of culture. The used bead size in the current study was 260 nm; previously, Chen and Park evaluated the effects of the bead size on the selective and non-specific binding of the prepared IMB to separate the *Salmonella*. They suggested that the smaller beads (almost 100 nanometers) could bind to the bacteria non-specifically. However, in 1000 nm beads, the available particles were still low, and the diffusion was slower than the 500 nm IMBs. They found the highest recovery in the 500 nm beads in comparison to 100 and 1000 nm (20). The used beads in the current study have an average size of 176 nm; these medium particles let remove all of the targets in milk samples. The particles' aggregation was resolved by adding the triton compound to the beads in the preparation process.

Also, the mentioned authors suggested that the beads' particle size increased after the antibodies' coupling. Our research prepared nonmagnetic beads that directly interacted with the target bacteria; however, other research mainly used commercial immunomagnetic beads which interacted with the targets indirectly (21). In other research, samples were often incubated in enrichment media after preparing the experimentally polluted samples (22, 23). Appling enrichment media could enhance the test sensitivity from 10^4-10^5 CFU/mL to 2 CFU/mL (23). The nonselective pre-enrichment of experimentally polluted milk samples for 12 hours reduced the detection limit of *Salmonella* by using the immunomagnetic beads-PCR method to 1-10 CFU/mL (24). Our research evaluated the actual amount of bacteria in experimentally polluted milk samples. The obtained detection limit of 3 CFU/mL of whole milk without pre-enrichment suggested the success of the developed IMB-culture method.

Detection of the *Salmonella* contamination in biological samples has been analyzed with different sensitive methods such as polymerase chain reaction, ELISA, and agglutination test. The obtained detection limits were 10^6 CFU/mL for indirect competitive ELISA (25), 2.5×10^5 CFU/mL for surface plasmon resonance (26), 1.25×10^6CFU/mL by using immune-chromatography strip (27), 1 CFU/mL by using pulsed-field gel electrophoresis (24), and 1 to 300 CFU/mL by using Real-Time PCR (28, 29). Mansfield and Forsythe (23) used immunomagnetic beads to separate the 6 strains of *Salmonella*, which were incubated overnight in enrichment culture media; combining the IMB and ELISA detected at least 10^5-10^6 cells/mL. However, immunomagnetic beads separation followed by qualitative PCR had a detection limit of 18 CFU/mL of *Salmonella* from pork and milk samples. Previously other researchers suggested that IMS culture was more sensitive than direct culture for the isolation of *Salmonella* (30, 31). Taha, Mohamed (24) used immunomagnetic separation followed by culturing in CHROMagar Plus media and Real-Time PCR to detect *Salmonella* in experimentally polluted chicken meat samples. After 4 hours of pre-enrichment, the assays showed a detection limit equal to1.6 CFU/mL. A combination of IMS and Real-Time PCR detected 18 CFU/mL of *Salmonella* (32). Successful detection of *Salmonella* was reported by Brandao, Liebana (33); the researcher obtained a limit of detection equal to 1 CFU/25 mL of milk by using 8 hours pre-enrichment period and a magneto immunosensor method. Also, colorimetric paper-based analytical devices were combined with immunomagnetic separation (IMS) for detecting *Salmonella typhimurium* by Srisa-Art, Boehle (34); the

method had a limit of detection equal to 10^3 CFU/mL of milk without any pre-enrichment. Bai, Cui (35) detected *Salmonella* in experimentally polluted milk using a method based on extraction of mRNA via magnetic capture probes and RT-qPCR; they reported sensitivity of the method was equal to 10^4 CFU/mL and 10 CFU/mL after 12 hours of incubation in enrichment media (35). In recent years, various receptors have been developed for detecting *Salmonella* in milk and other food products using an antibody, aptamer, nucleic acid probe, bacteriophage, and lectin. Among all these, antibodies have various advantages, such as high affinity and specificity (36). The immune library produces a protein structure based on the antibody's binding region, called nanobody. He et al. confirmed the application of the nanobody library in the detection of *Salmonella* (18). The current study focused on detecting the biological samples infected with *Salmonella*, but we should keep care about the medical treatment of the infected humans and animals. According to the various climate region of Iran, natural remedies, mainly medicinal plants, should be in means for successful treatment because of their direct antibacterial effects and benefits in managing the clinical symptom (37).

The 3 CFU/mL of *S. Typhimurium* in experimentally infected milk samples were detected in culture media without any pre-enrichment using the current immunomagnetic-culture method. The current method for producing IMB is more economical than other techniques, such as the EDC-NHS method. In conclusion, the results strongly suggested using the IMB-bacterial culture instead of the conventional culture method. Utilization of the illustrated method of IMB synthesizes for separation of the other *Salmonella* serotypes from clinical samples could be proposed in complementary research.

Authors' Contribution

Conceived and designed the experiments: M. Kh. and D. Gh.

Performed the experiments: M. Kh., D. Gh. and Sh. S. M.

Analyzed the data: M. Kh. and D. Gh.

Research space and equipment: M. Kh. and D. Gh.

Contributed reagents/materials/analysis tools: M. Kh. and D. Gh.

Ethics

All ethical standard were approved by the ethics committee of the Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Andino A, Hanning I. Salmonella enterica: survival, colonization, and virulence differences among serovars. Sci World J. 2015;2015:520179.
2. Gillespie IA, O'Brien SJ, Adak GK, Ward LR, Smith HR. Foodborne general outbreaks of Salmonella Enteritidis phage type 4 infection, England and Wales, 1992-2002: where are the risks? Epidemiol Infect. 2005;133(5):795-801.
3. Ehuwa O, Jaiswal AK, Jaiswal S. Salmonella, Food Safety and Food Handling Practices. Foods. 2021;10(5).
4. Dewey-Mattia D, Manikonda K, Hall AJ, Wise ME, Crowe SJ. Surveillance for Foodborne Disease Outbreaks - United States, 2009-2015. MMWR Surveill Summ. 2018;67(10):1-11.
5. Marth EH. Salmonellae and Salmonellosis Associated with Milk and Milk Products. A Review1. J Dairy Sci. 1969;52(3):283-315.
6. Sharp JCM, Paterson GM, Forbes GI. Milk-borne salmonellosis in Scotland. J Infect. 1980;2(4):333-40.
7. Small RG, Sharp JC. A milk-borne outbreak due to Salmonella dublin. J Hyg (Lond). 1979;82(1):95-100.
8. Crum-Cianflone NF. Salmonellosis and the gastrointestinal tract: more than just peanut butter. Curr

Gastroenterol Rep. 2008;10(4):424-31.
9. Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, et al. The emergence in Taiwan of fluoroquinolone resistance in Salmonella enterica serotype choleraesuis. N Engl J Med. 2002;346(6):413-9.
10. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004;82(5):346-53.
11. Blaser MJ, Newman LS. A review of human salmonellosis: I. Infective dose. Rev Infect Dis. 1982;4(6):1096-106.
12. Oscar T. Dose-response model for 13 strains of salmonella. Risk Anal. 2004;24(1):41-9.
13. Anon. Microbiology–General Guidance on Methods for the Detection of Salmonella. 1993.
14. Alocilja EC, Radke SM. Market analysis of biosensors for food safety. Biosens Bioelectron. 2003;18(5):841-6.
15. Khosravi M, Nouri M, Mohammadi A, Mosavari N, Constable PD. Preparation of immunomagnetic beads coupled with a rhodamine hydrazine immunosensor for the detection of Mycobacterium avium subspecies paratuberculosis in bovine feces, milk, and colostrum. J Dairy Sci. 2021;104(6):6944-60.
16. Bell RL, Jarvis KG, Ottesen AR, McFarland MA, Brown EW. Recent and emerging innovations in Salmonella detection: a food and environmental perspective. Microb Biotechnol. 2016;9(3):279-92.
17. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Enterobacteriaceae. Clinical Vet Microbiol. 1. 2nd ed2013. p. 239-74.
18. He Y, Ren Y, Guo B, Yang Y, Ji Y, Zhang D, et al. Development of a specific nanobody and its application in rapid and selective determination of Salmonella enteritidis in milk. Food Chem. 2020;310:125942.
19. Dupont D, Luyten J, Bloemen M, Verbiest T, Binnemans K. Acid-Stable Magnetic Core–Shell Nanoparticles for the Separation of Rare Earths. Ind Eng Chem Res. 2014;53(39):15222-9.
20. Chen J, Park B. Effect of immunomagnetic bead size on recovery of foodborne pathogenic bacteria. Int J Food Microbiol. 2018;267:1-8.
21. Ahmed S, Rubahn H-G, nter, Erdmann H. Development of an Immunomagnetic Separation Method for Viable Salmonella Typhimurium Detected by Flow Cytometry. Online J Biol Sci. 2016;16(4).
22. Kumar S, Balakrishna K, Batra HV. Enrichment-ELISA for Detection of Salmonella typhi From Food and Water Samples. Biomed Environ Sci. 2008;21(2):137-43.
23. Mansfield LP, Forsythe SJ. The detection of Salmonella using a combined immunomagnetic separation and ELISA end-detection procedure. Lett Appl Microbiol. 2000;31(4):279-83.
24. Taha EG, Mohamed A, Srivastava K, Reddy P. Rapid detection of Salmonella in chicken meat using immunomagnetic separation, CHROMagar, ELISA and real-time polymerase chain reaction (RT-PCR). Int J Poult Sci. 2010;9(831):e835.
25. Bang J, Shukla S, Kim YH, Kim M. Development of indirect competitive ELISA for the detection of Salmonella typhimurium. Rom Biotechnol Lett. 2012;17:7194-204.
26. Barlen B, Mazumdar SD, Lezrich O, Kämpfer P, Keusgen M. Detection of Salmonella by Surface Plasmon Resonance. Biosensors. 2007;7(8):1427-46.
27. Wang W, Liu L, Song S, Tang L, Kuang H, Xu C. A highly sensitive ELISA and immunochromatographic strip for the detection of Salmonella typhimurium in milk samples. Sensors (Basel). 2015;15(3):5281-92.
28. Ding T, Suo Y, Zhang Z, Liu D, Ye X, Chen S, et al. A Multiplex RT-PCR Assay for S. aureus, L. monocytogenes, and Salmonella spp. Detection in Raw Milk with Pre-enrichment. Front Microbiol. 2017;8:989.
29. Rastegar H, Ashtiani HA, Andalibi M, Nehshabouri SH, Akbari M, Rassam H, et al., editors. Detection, isolation and assessment of Salmonella entiritidis in milk by conventional culture methods and real-time PCR in Iran. 2013.
30. Lynch MJ, Leon-Velarde CG, McEwen S, Odumeru JA. Evaluation of an automated immunomagnetic separation method for the rapid detection of Salmonella species in poultry environmental samples. J Microbiol Methods. 2004;58(2):285-8.
31. Yang X, Li H, Wu Q, Zhang J, Chen L. Comparison of Direct Culture, Immunomagnetic Separation/culture, and Multiplex PCR Methods for Detection of Salmonella in Food. Food Sci Technol Res. 2015;21(5):671-5.
32. Wang J, Li Y, Chen J, Hua D, Li Y, Deng H, et al. Rapid detection of food-borne Salmonella contamination using IMBs-qPCR method based on pagC gene. Braz J Microbiol. 2018;49(2):320-8.

33. Brandao D, Liebana S, Campoy S, Alegret S, Isabel Pividori M. Immunomagnetic separation of Salmonella with tailored magnetic micro and nanocarriers. A comparative study. Talanta. 2015;143:198-204.
34. Srisa-Art M, Boehle KE, Geiss BJ, Henry CS. Highly Sensitive Detection of Salmonella typhimurium Using a Colorimetric Paper-Based Analytical Device Coupled with Immunomagnetic Separation. Anal Chem. 2018;90(1):1035-43.
35. Bai Y, Cui Y, Suo Y, Shi C, Wang D, Shi X. A Rapid Method for Detection of Salmonella in Milk Based on Extraction of mRNA Using Magnetic Capture Probes and RT-qPCR. Front Microbiol. 2019;10:770.
36. Wang M, Zhang Y, Tian F, Liu X, Du S, Ren G. Overview of Rapid Detection Methods for Salmonella in Foods: Progress and Challenges. Foods. 2021;10(10).
37. Jalali H, Mozaffari Nejad AS, Ebadi AG, Laey G. Ethnobotany and Folk Pharmaceutical Properties of Major Trees or Shrubs in Northeast of Iran. Asian J Chem. 2009;21:5632-8.