Molecular approaches for detection and identification of foodborne pathogens

S. Boughattas [1] (PhD), R. Salehi [2]* (PhD)

1. Department of Environmental and Biological Chemistry, College of Agriculture, Life and Environmental Sciences, Chungbuk National University, Cheongju, Chungbuk, South Korea (Republic of)
2. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Foodborne pathogens comprise microorganisms such as viruses, bacteria and parasites that can be transmitted by food and affect public health worldwide. The most common viruses transmitted via food are *hepatitis A virus* and *Norwalk-like caliciviruses*. Also, the most common bacteria involved in foodborne illnesses are *Campylobacter jejuni, Clostridium perfringens, Salmonella* spp, *Escherichia coli O157:H7*; and the most important parasites that can cause these conditions are *Giardia duodenalis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Trichinella spiralis*, *Taenia saginata and/or solium*, *Entamoeba histolytica, Anisakis* spp. and *Diphyllobothrium* spp. Because of their eventual small number in the sample, their detection and identification is not always easy. On the other hand, conventional methods like cultures are almost labor intensive, time consuming and costly. Recently, molecular techniques have been developed for rapid, sensitive and specific identification. The most common molecular methods are polymerase chain reaction (PCR)-based techniques. In this article, the sensitive and specific molecular tests for routine detection and identification of foodborne pathogens are reviewed.

Introduction

Food safety is one of the most important areas in public health worldwide. Foodborne disease, foodborne infection and/or foodborne poisoning result from infection with viruses, bacteria or parasites (Tauxe, 2002).

Until recently, cultural techniques were the gold standard for detection and identification of microorganisms contaminating food stuffs (Jasson et al., 2010), but conventional methods are labor intensive, time consuming, and costly (Singh et al., 2013). Therefore, it is necessary to apply novel tools to address microbial food safety beyond merely determining the presence of particular contaminating pathogens in food matrixes. Advances in biotechnology have resulted in the development of rapid methods that minimize manipulation, provide results in less time, and reduce cost, such as DNA based assay: polymerase chain reaction (PCR), DNA hybridization, and DNA microarray. In addition, these methods are not only capable of detecting microbial agents in food samples but also provide an opportunity to trace the origin of contamination through precise genotyping of the contaminating pathogen, which is the basis of molecular epidemiology.

PCR is a powerful technique that revolutionized molecular biology by offering applications in the diagnosis of microbial infections and genetic diseases, as well as in detection of pathogens in food (Calvo et al., 2001). Assays based on PCR are now accepted as methods for rapidly confirming the presence or absence of specific pathogens in foods, and PCR based kits are commercially available for detection of specific foodborne pathogens. There are many different protocols based on PCR such as Nested PCR, Hot start PCR, Real time PCR etc. used for foodborne pathogens' detection and they will be discussed bellow.

DNA hybridization or gene probe assays consist of detection of DNA or RNA targets using complementary labeled nucleic acid probes. One format is colony hybridization, involving transfer of colonies from the surface of an agar medium onto a solid support, cell lysis, denaturation of the DNA, and linkage to the membrane support, followed by hybridization with a labeled probe (Boileau et al., 1984).

*Corresponding author
Email: r_salehi@med.mui.ac.ir

DNA microarray also named DNA chip or biochip is a collection of microscopic DNA spots attached to a solid surface. This method allows running many genetic tests in parallel (Rasooly and Herold, 2008).

Another molecular technique used for detection of foodborne pathogens is Nucleic Acid Sequence Based Amplification (NASBA). NASBA is a method in which RNA is used to be amplified for identification. It has been employed for detection of *Campylobacter* Spp., *L. monocytogenes, Salmonella* Spp., *Cryptosporidium* Spp., and foodborne viruses using 16S rRNA and mRNA as target molecules (Cook, 2003).

Randomly Amplified Polymorphic DNA (RAPD) is another PCR based technique (Wolska and Kot, 2013). This method has been used for many foodborne pathogens.

Amplified Fragment length polymorphism (AFLP) is mainly used for molecular typing, detecting restriction fragments of the genome using PCR (Hamza et al., 2013).

There are many more increasing number of molecular analysis methods that are available for characterizing microorganisms in their environments.

Viruses

Viruses are very small microorganisms ranging from 15 to 400 nm comprised of protein and nucleic acid. The most common viruses transmitted via food are *hepatitis A virus* and *Norwalk-like caliciviruses*. The other viruses transmitted are genus of *Enterovirus*, *Hepatovirus, Hepatitis E virus, Astrovirus, Rotavirus, Adenoviridae, Arenavirus, Flavivirus, Hantavirus, Aphthovirus* and *Aichi.*

However, foods are rarely tested for viral contamination because culture of the foodborne viruses is very difficult. Moreover, their direct detecting suffers from some problems such as standardization, inhibition of enzymes used in RT-PCR, false positive results and etc. (Atmar and Estes, 2001; Koopmans and Duizer, 2004; Lees, 2000). Infection with gastro-enteric viruses is routinely diagnosed by examination of stool samples using electron microscopy, passive particle agglutination tests, or enzyme inked immune-sorbent assays (ELISA); but their sensitivity is not high enough to detect low number of viruses. Meanwhile, molecular techniques such as polymerase chain reaction (PCR) and reverse transcription polymerase chain reaction (RT-PCR) could increase the chance of viruses' detection (Bosch et al., 2004; Buesa et al., 2002; Di Pinto et al., 2004; Koopmans et al., 2001). For example, sapoviruses and noroviruses have been detected using RT-PCR (Hutson et al., 2004) and human adenoviruses have been screened by real-time-RT nested PCR assay (Myrmel et al., 2004). Also, Arenavirus has been detected in patients using a nested RT-PCR assay (Park et al., 1997). RT-PCR and real-time PCR methods were reported to detect hepatitis A (Hutson et al., 2004; Lee et al., 2012; Stals et al., 2013).

Molecular biology techniques have been used for detection of the most common foodborne viruses like *Norovirus* and hepatitis A in shellfish, but none are usually available for other foods virus. The variability of the *Norovirus* genome hinders the development of a detection kit for routine use. An appropriate method for extraction and molecular detection of human enteric viruses from food was reported by Leggitt and Jaykus (2000).

Bacteria

Bacteria are the most common organisms causing foodborne diseases. The most frequent pathogens involved in this illness are *Campylobacter jejuni, Clostridium perfringens, Salmonella* spp and *Escherichia coli O157:H7*. Other common genera of bacteria can bear foodborne diseases: *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Shigella* spp., *Staphylococcus aureus, Staphylococcal enteritis, Streptococcus, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Yersinia enterocolitica* and *Yersinia pseudotuberculosis.* Less common bacterial agents are also reported such as *Brucella* spp., *Corynebacterium ulcerans, Coxiella burnetii* and *Plesiomonas shigelloides.*

The subtractive hybridization methods have been studied on *L. monocytogenes* and *E. coli* (Pradel et al., 2002; Sorsa et al., 2004; Wu and Muriana, 1995; Wu and Muriana, 1999). In most molecular studies, the target gene in bacteria, 16S rDNA was assessed by a variety of gel-based methods including PCR, Length heterogeneity PCR (LH-PCR), Restriction Fragment Length Polymorphisms (RFLP), Denaturing Gradient Gel Electrophoresis (DGGE), and Temperature Gradient Gel electrophoresis (TGGE).

Among the molecular techniques, ribotyping using a combination of the restriction enzymes *SphI* and *PstI* has been used for *Salmonella.* Also, typing of *Campylobacter* has been done with flagellar (*fla*) gene as a target. Gene Trak kits are available for detection of *Salmonella, Listeria, Campylobacter,* and *E. coli O157:H7* (Fung, 2002).

Different typing and sub-typing methods appear to group strains or isolates at different levels. This would be valuable for *E. coli O157:H7* because the potential virulence of the organism depends on serotype. Regardless, typing of foodborne pathogens would be useful for epidemiologic investigations.

Typing and sub-typing may be achieved using PCR for specific gene or gene variants. Each serotype of *E. coli* presents different manifestations that depend on different sets of virulence genes. Various molecular methods were developed for the identification of specific genes and gene variants (Fukushima et al, 2003; Nishiki et al., 2012). It has been demonstrated that *stx* genes are appropriate target for sub-typing of *E. coli O157:H7* as well as *sopE* gene for *Salmonella* sub-typing (Ehrbar et al., 2002; Mirold et al., 2001).

Repetitive DNA sequences are universally present in the bact-

erial genome that is used for *Enterobacter* and *Salmonella* (Anderson et al., 2010; Fendri et al., 2013; Healy et al., 2008; Hiett and Seal, 2009; Kilic et al., 2010; Ye et al., 2011). As these elements are widespread in gram-negative bacteria, they can be used for major sub-typing way. This method was used for: the characterization of *V. cholera* strains involved in an epidemic episode in Ukraine (Clark et al., 1998), for sub-typing of *V. parahemolyticus* strains causing an outbreak associated with oysters on Canada's west coast (Marshall et al., 1999) and for typing *Salmonella* isolates (Mezal et al., 2013; Van Lith and Aarts, 1994).

Variable number of Tandem Repeat (VNTR) is another sub-typing method used for foodborne pathogens, such as *Salmonella typhimurium* (Lunestad et al., 2013; Prendergast et al., 2011).

Pulsed-field gel electrophoresis (PFGE) is another molecular tool for typing of the foodborne pathogens such as *E. coli O157:H7* (Barrett et al., 1994) that uses different protocols depending on restriction enzymes, pulse conditions, run times, and size standards.

Another molecular tool for molecular characterization of food-borne bacteria is PCR-RFLP (Marty et al., 2012). In this method, after amplification step, restriction digestion analysis is done for sub-typing gene variants. This method has been used for diagnosis of *C. jejuni* (Steinhauserova et al., 2002) and *C. coli* (Petersen and Newell, 2001).

Multi-locus sequence typing (MLST) is a recent molecular tool for bacterial pathogens typing. Within this method, bacterial isolates are classified directly by approximately seven housekeeping genes (Maiden et al., 2013). This method has been used for *C. jejuni* (Nielsen et al., 2010), *Salmonella enteric* (Achtman et al., 2013) and *E. coli* (Adiri et al., 2003). However, this method was not successful for *E. coli O157:H7* due to the clonality of this organism (Noller et al., 2003).

Parasites

A parasite is a smaller organism than its host, from which it derives nourishment and protection. Several parasites are involved in foodborne illness. These organisms live and reproduce themselves within the tissues and organs of infected human and animal hosts, and are often excreted in feces. The most reported ones are *Giardia duodenalis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Trichinella spiralis*, *Taenia saginata/solium*, *Entamoeba histolytica, Anisakis* spp, and *Diphyllobothrium* spp.

An important point of view is that the detection methods for parasites in food and water are derived from clinical settings. This is a problem because the parasites load in food is different from the load in clinical samples. On the other hand, culture of all parasites is impossible. Therefore, concentration methods for parasites detection in food samples are necessary. For water

samples, filtration is used to concentrate large volumes of water for detection of *Cryptosporidium* and *Giardia*. Also, meat mincing and digestion are used for meat-borne parasites such as *Toxoplasma gondii, Trichinella spiralis* and *Anisakis simplex.*

The techniques used for parasites detection comprise RFLP analysis for differentiation of *Cyclospora* spp and *Eimeria* spp (Orlandi and Lampel, 2000), PCR using SNP-oligonuleotides, Real-Time PCR and multiplex-nested-PCR for identification of *Cyclospora* spp (Lee et al., 2010; Orlandi et al., 2003).

The mouse and cat bioassays for detection of *T. gondii* tissue cysts needs six to eight weeks and therefore is not suitable for slaughterhouse testing or monitoring of commercial meat products (Gamble and Murrell, 1998; Warnekulasuriya et al., 1998). Another monitoring method is tissue culturing but it is not sensitive as mouse assay (Lindsay et al., 1991).

The most frequently parasite associated with the consumption of contaminated water is *Giardia duodenalis* for which five outbreaks have been reported in food contamination especially vegetables that are eaten raw by infected or infested food handlers. Concentration techniques such as floatation and microscopy diagnosis have been described for *Giardia* cysts as the same as *Cryptosporidium* oocysts and less for *T. gondii* oocyst (Borchardt et al., 2009; Dumètre and Dardé, 2003; Isaac-Renton et al., 1998; Kourenti et al., 2003; Kourenti and Karanis, 2006; Sotiriadou and Karanis, 2008).

There are works indicating that PCR is a powerful tool for *T. gondii* detection due to the time required for detection of tissue cysts in meat products (Dumètre and Dardé, 2003; Jones and Dubey, 2010). Some molecular based routine techniques that have been used for *T. gondii* are RT-PCR, TaqMan PCR, PCR-enzyme immunoassay oligoprobe, nested PCR and multiplex PCR, (Boughattas et al., 2014; Buchbinder et al., 2003; Burg et al., 1989; Lin et al., 2000; Putignani et al., 2011; Schwab and McDevitt, 2003; Zintl et al., 2009). The usual target genes used in these studies were *ITS-1* rRNA (Jauregui et al., 2001), *18s* rRNA (Kupferschmidt et al., 2001; MacPherson and Gajadhar, 1993), a 529bp repeat element (Aigner et al., 2010; Homan et al., 2000; Opsteegh et al., 2010), the single copy P30 gene (Buchbinder et al., 2003; Warnekulasuriya et al., 1998) and *SAG1-4, SAG5C, GRA4* (Contini et al., 2005; Meisel et al., 1996).

Conclusion

The emerging foodborne pathogens are likely to cause serious health public problems, especially in developing countries where they lead to considerable morbidity and mortality rates. These organisms comprise viruses, bacteria and parasites. Quick, sensitive, specific and easy techniques for detection of these pathogens are needed; some of the appropriate techniques are molecular based methods such as PCR, DNA hybridization and

DNA microarray. These methods have been set up for detection and identification of foodborne pathogens and they might become the new gold standards for the identification of foodborne pathogens.

Conflicts of interest

None declared.

Acknowledgement

References

Achtman M., Hale J., Murphy R.A., Boyd E.F., Porwollik S. (2013). Population structures in the SARA and SARB reference collections of *Salmonella enterica* according to MLST, MLEE and microarray hybridization. *Infection, Genetics and Evolution*. 16: 314-25.

Adiri R.S., Gophna U., Ron E.Z. (2003). Multilocus sequence Typing (MLST) of *Escherichia coli O78* strains. *FEMS Microbiology Letters*. 222: 199-203

Aigner C.P., Silva A.V., Sandrini F., Osório Pde S., Poiares L., Largura A. (2010). Real-time PCR-based quantification of *Toxoplasma gondii* in tissue samples of serologically positive outdoor chickens. *Memorias do Instituto Oswaldo Cruz*. 105: 935-7.

Anderson P.N., Hume M.E., Byrd J.A., Hernandez C., Stevens S.M., Stringfellow K., Caldwell D.J. (2010). Evaluation of repetitive extragenic palindromic-polymerase chain reaction and denatured gradient gel electrophoresis in identifying *Salmonella* serotypes isolated from processed turkeys. *Poultry Science*. 89: 1293-300.

Atmar R.L., Estes M.K. (2001). Diagnosis of noncultivatable gastroenteritis viruses, the human caliciviruses. *Clinical Microbiology Reviews*. 14: 15-37.

Barrett T.J., Lior H., Green J.H., Khakhria R., Wells J.G., Bell B.P., Greene K.D., Lewis J., Griffin P.M. (1994). Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli O157:H7* by using pulsed-field gel electrophoresis and phage typing. *Journal of Clinical Microbiology*. 32: 3013-3017.

Boileau C.R., d'Hauteville H.M., Sansonetti P.J. (1984). DNA hybridization technique to detect *Shigella* species and enteroinvasive *Escherichia coli*. *Journal of Clinical Microbiology*. 20: 959-961.

Borchardt M.A., Spencer S.K., Bertz P.D., Ware M.W., Dubey J.P., Alan Lindquist H.D. (2009). Concentrating *Toxoplasma gondii* and *Cyclospora cayetanensis* from surface water and drinking water by continuous separation channel centrifugation. *Journal of Applied Microbiology*. 107: 1089-1097.

Bosch A., Pintó R.M., Comas J., Abad F.X. (2004). Detection of infectious rotaviruses by flow cytometry. *Methods in Molecular Biology*. 268: 61-68.

Boughattas S., Ayari K., Sa T., Aoun K., Bouratbine A. (2014). Survey of the parasite *Toxoplasma gondii* in human consumed ovine meat in Tunis City. *PLOS ONE*. 9: e85044.

Buchbinder S., Blatz R., Rodloff A.C. (2003). Comparison of real-time PCR detection methods for B1 and P30 genes of *Toxoplasma gondii*. *Diagnostic Microbiology* and *Infectious Disease*. 45: 269-271.

Buesa J., Collado B., López-Andújar P., Abu-Mallouh R., Rodríguez Díaz J., García Díaz A., Prat J., Guix S., Llovet T., Prats G., Bosch A. (2002). Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. *Journal of Clinical Microbiology*. 40: 2854-2859.

Burg J.L., Grover C.M., Pouletty P., Boothroyd J.C. (1989). Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *Journal of Clinical Microbiology*. 27: 1787-1792.

Calvo J.H., Zaragoza P., Osta R. (2001). Technical note: A quick and more sensitive method to identify pork in processed and unprocessed food by PCR amplification of a new specific DNA fragment. *Journal of Animal Science*. 79: 2108-2112.

Clark C.G., Kravetz A.N., Dendy C., Wang G., Tyler K.D., Johnson W.M. (1998). Investigation of the 1994-5 Ukrainian *Vibrio cholerae* epidemic using molecular methods. *Epidemiology and Infection*. 121: 15-29.

Contini C., Seraceni S., Cultrera R., Incorvaia C., Sebastiani A., Picot S. (2005). Evaluation of a Real-time PCR-based assay using the lightcycler system for detection of *Toxoplasma gondii* bradyzoite genes in blood specimens from patients with toxoplasmic retinochoroiditis. *International Journal of Parasitology*. 35: 275-283.

Cook N. (2003). The use of NASBA for the detection of microbial pathogens in food and environmental samples. *Journal of Microbiological Methods*. 53: 165-174.

Di Pinto A., Conversano M.C., Forte V.T., La Salandra G., Montervino C., Tantillo G.M. (2004). A comparison of RT-PCR-based assays for the detection of HAV from shellfish. *the New Microbiologica*. 27: 119-124.

Dumètre A., Dardé M.L. (2003). How to detect *Toxoplasma gondii* oocysts in environmental samples? *FEMS Microbiology Reviews*. 27: 651-661.

Ehrbar K., Mirold S., Friebel A., Stender S., Hardt W.D. (2002). Characterization of effector proteins translocated via the SPI1 type III secretion system of *Salmonella Typhimurium*. *International Journal of Medical Microbiology*. 291: 479-485.

Fendri I., Ben Hassena A., Grosset N., Barkallah M., Khannous L., Chuat V., Gautier M., Gdoura R. (2013). Genetic Diversity of Food-Isolated *Salmonella* Strains through Pulsed Field Gel Electrophoresis (PFGE) and Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR). *PLoS One*. 8: e81315.

Fukushima M., Kakinuma K., Hayashi H., Nagai H., Ito K., Kawaguchi R. (2003). Detection and identification of *Mycobacterium* species isolates by DNA microarray. *Journal of Clinical Microbiology*. 41: 2605-2615.

Fung D.Y. (2002). Predictions for rapid methods and automation in food microbiology. *The Journal of AOAC International*. 85: 1000-1002.

Gamble H.R., Murrell K.D. (1998). Detection of parasites in food. *Parasitology*. 117:S97- S111.

Hamza S., Naseem S., Bashir E., Rizwani G.H., Hina B. (2013). Trace element geochemistry of Manilkara zapota (L.) P. Royen, fruit from winder, Balochistan, Pakistan in perspective of medical geology. *Pakistan Journal of Pharmaceutical Sciences*. 26: 805-811.

Healy B., Mullane N., Collin V., Mailler S., Iversen C., Chatellier S., Storrs M., Fanning S. (2008). Evaluation of an automated repetitive sequence-based PCR system for subtyping *Enterobacter sakazakii*. *Journal of Food Protection*. 71: 1372-1378.

Hiett K.L., Seal B.S. (2009). Use of repetitive element palindromic PCR (rep-PCR) for the epidemiologic discrimination of food-borne pathogens. *Methods in Molecular Biology*. 551: 49-58.

Homan W.L., Vercammen M., De Braekeleer J., Verschueren H. (2000). Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *International Journal for Parasitology*. 30: 69-75.

Hutson A.M., Atmar R.L., Estes M.K. (2004). Norovirus disease: changing epidemiology and host susceptibility factors. *Trends in Microbiology*. 12: 279-287.

Isaac-Renton J., Bowie W.R., King A., Irwin G.S., Ong C.S., Fung C.P., Shokeir M.O., Dubey J.P. (1998). Detection of *Toxoplasma gondii* oocysts in drinking water. *Applied and Environmental Microbiology*. 64:2278-2280.

Jasson V., Jacxsens L., Luning P., Rajkovic A., Uyttendaele M. (2010). Alternative microbial methods: An overview and selection criteria. *Food Microbiology*. 27: 710-730.

Jauregui L.H., Higgins J., Zarlenga D., Dubey J.P., Lunney J.K. (2001). Development of a real-time PCR assay for detection of *Toxoplasma gondii* in pig and mouse tissues. *Journal of Clinical Microbiology*. 39: 2065-2071.

Jones J.L., Dubey J.P. (2010). Waterborne toxoplasmosis--recent developments. *Experimental Parasitology*. 124: 10-25.

Kilic A., Bedir O., Kocak N., Levent B., Eyigun C.P., Tekbas O.F., Gorenek L., Baylan O., Basustaoglu A.C. (2010). Analysis of an outbreak of

Salmonella enteritidis by repetitive-sequence-based PCR and pulsed-field gel electrophoresis. *Internal Medicine*. 49: 31-36.

Koopmans M., Vinjé J., Duizer E., de Wit M., van Duijnhoven Y. (2001). Molecular epidemiology of human enteric caliciviruses in The Netherlands. *Novartis Foundation Symposium*. 238: 197-214.

Koopmans M., Duizer E. (2004). Food-borne viruses: an emerging problem. *International Journal of Food Microbiology*. 90: 23-41.

Kourenti C., Heckeroth A., Tenter A., Karanis P. (2003). Development and application of different methods for the detection of *Toxoplasma gondii* in water. *Applied and Environmental Microbiology*. 69: 102-106.

Kourenti C., Karanis P. (2006). Evaluation and applicability of a purification method coupled with nested PCR for the detection of Toxoplasma oocysts in water. *Letters in Applied Microbiology*. 43: 475-481.

Kupferschmidt O., Krüger D., Held T.K., Ellerbrok H., Siegert W., Janitschke K. (2001). Quantitative detection of *Toxoplasma gondii* DNA in human body fluids by TaqMan polymerase chain reaction. *Clinical Microbiology and Infection*. 7: 120-124.

Lee K.B., Lee H., Ha S.D., Cheon D.S., Choi C. (2012). Comparative analysis of viral concentration methods for detecting the HAV genome using real-time RT-PCR amplification. *Food and Environmental Virology*. 4: 68-72.

Lee S.H., Joung M., Yoon S., Choi K., Park W.Y., Yu J.R. (2010). Multiplex PCR detection of waterborne intestinal protozoa: microsporidia, *Cyclospora*, and *Cryptosporidium*. *The Korean Journal of Parasitology*. 48: 297-301.

Lees D. (2000). Viruses and bivalve shellfish. *International Journal of Food Microbiology*. 59: 81-116.

Leggitt P.R., Jaykus L.A. (2000). Detection methods for human enteric viruses in representative foods. *Journal of Food Protection*. 63:1738-1744.

Lin M.H., Chen T.C., Kuo T.T., Tseng C.C., Tseng C.P. (2000). Real-time PCR for quantitative detection of *Toxoplasma gondii*. *Journal of Clinical Microbiology*. 38: 4121-4125.

Lindsay D.S., Dubey J.P., Blagburn B.L., Toivio-Kinnucan M. (1991). Examination of tissue cyst formation by *Toxoplasma gondii* in cell cultures using bradyzoites, tachyzoites, and sporozoites. *Journal of Parasitology*. 77: 126-132.

Lunestad B.T., Truong T.T., Lindstedt B.A. (2013). A multiple-locus variable-number tandem repeat analysis (MLVA) of *Listeria monocytogenes* isolated from Norwegian salmon-processing factories and from listeriosis patients. *Epidemiology & Infection*. 141: 2101-2110.

MacPherson J.M., Gajadhar A.A. (1993). Sensitive and specific polymerase chain reaction detection of *Toxoplasma gondii* for veterinary and medical diagnosis. *Canadian Journal* of *Veterinary Research*. 57: 45-48.

Maiden M.C., van Rensburg M.J., Bray J.E., Earle S.G., Ford S.A., Jolley K.A., McCarthy N.D. (2013). MLST revisited: the gene-by-gene approach to bacteria genomics. *Nature Reviews Microbiology*. 11: 728-736.

Marshall S., Clark C.G., Wang G., Mulvey M., Kelly M.T., Johnson W.M. (1999). Comparison of molecular methods for typing *Vibrio parahaemolyticus*. *Journal of Clinical Microbiology*. 37: 2473-2478.

Marty E., Buchs J., Eugster-Meier E., Lacroix C., Meile L. (2012). Identification of staphylococci and dominant lactic acid bacteria in spontaneously fermented Swiss meat products using PCR-RFLP. *Food Microbiology*. 29: 157-166.

Meisel R., Stachelhaus S., Mévélec M.N., Reichmann G., Dubremetz J.F., Fischer H.G. (1996). Identification of two alleles in the GRA4 locus of *Toxoplasma gondii* determining a differential epitope which allows discrimination of type I versus type II and III strains. *Molecular and Biochemical Parasitology*. 81: 259-263.

Mezal E.H., Stefanova R., Khan A.A. (2013). Isolation and molecular characterization of *Salmonella enterica serovar javiana* from food, environmental and clinical samples. *International Journal of Food Microbiology*. 164: 113-118.

Mirold S., Ehrbar K., Weissmüller A., Prager R., Tschäpe H., Rüssmann H., Hardt W.D. (2001). *Salmonella* host cell invasion emerged by acquisition of a mosaic of separate genetic elements, including *Salmonella* pathogenicity island 1 (SPI1), SPI5, and sopE2. *Journal of Bacteriology*. 183: 2348-2358.

Myrmel M., Berg E.M., Rimstad E., Grinde B. (2004). Detection of enteric viruses in shellfish from the Norwegian coast. *Applied and environm-ental Microbiology*. 70: 2678-2684.

Nielsen L.N., Sheppard S.K., McCarthy N.D., Maiden M.C., Ingmer H., Krogfelt K.A. (2010). MLST clustering of *Campylobacter jejuni* isolates from patients with gastroenteritis, reactive arthritis and Guillain-Barré syndrome. *Journal of Applied Microbiology*. 108: 591-599.

Nishiki I., Minami T., Chen S.C., Itami T., Yoshida T. (2012). Expression of the serum opacity factor gene and the variation in its upstream region in *Streptococcus dysgalactiae* isolates from fish. *The Journal of General and Applied Microbiology*. 58: 457-463.

Noller A.C., McEllistrem M.C., Stine O.C., Morris J.G.J.R., Boxrud D.J., Dixon B., Harrison L.H. (2003). Multilocus sequence typing reveals a lack of diversity among *Escherichia coli O157:H7* isolates that are distinct by pulsed-field gel electrophoresis. *Journal of Clinical Microbiology*. 41: 675-679.

Opsteegh M., Langelaar M., Sprong H., den Hartog L., De Craeye S., Bokken G., Ajzenberg D., Kijlstra A., van der Giessen J. (2010). Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR. *International Journal of Food Microbiology*. 139: 193-201.

Orlandi P.A., Lampel K.A. (2000). Extraction-free, filter-based template preparation for rapid and sensitive PCR detection of pathogenic parasitic protozoa. *Journal of Clinical Microbiology*. 38: 2271-2277.

Orlandi P.A., Carter L., Brinker A.M., da Silva A.J., Chu D.M., Lampel K.A., Monday S.R. (2003). Targeting single-nucleotide polymorphisms in the 18S rRNA gene to differentiate *Cyclospora* species from Eimeria species by multiplex PCR. *Applied and Environmental Microbiology*. 69: 4806-4813.

Park J.Y., Peters C.J., Rollin P.E., Ksiazek T.G., Gray B., Waites K.B., Stephensen C.B. (1997). Development of a reverse transcription-polymerase chain reaction assay for diagnosis of lymphocytic choriomeningitis virus infection and its use in a prospective surveillance study. *Journal of Medical Virology*. 51: 107-114.

Petersen L., Newell D.G. (2001). The ability of Fla-typing schemes to discriminate between strains of *Campylobacter Jejuni*. *Journal of Applied Microbiology*. 91: 217-224.

Pradel N., Leroy-Setrin S., Joly B., Livrelli V. (2002). Genomic subtraction to identify and characterize sequences of Shiga toxin-producing *Escherichia coli O91:H21*. *Applied and Environmental Microbiology*. 68: 2316-2325.

Prendergast D.M., O'Grady D, Fanning S., Cormican M., Delappe N., Egan J, Mannion C., Fanning J., Gutierrez M. (2011). Application of multiple locus variable number of tandem repeat analysis (MLVA), phage typing and antimicrobial susceptibility testing to subtype *Salmonella enterica* serovar *Typhimurium* isolated from pig farms, pork slaughterhouses and meat producing plants in Ireland. *Food Microbiology*. 28: 1087-1094.

Putignani L., Mancinelli L., Del Chierico F., Menichella D., Adlerstein D., Angelici M.C., Marangi M., Berrilli F., Caffara M., di Regalbono D.A., Giangaspero A. (2011). Investigation of *Toxoplasma gondii* presence in farmed shellfish by nested-PCR and real-time PCR fluorescent amplicon generation assay (FLAG). *Expermental Parasitology*. 127: 409-417.

Rasooly A., Herold K.E. (2008). Food Microbial Pathogen Detection and Analysis Using DNA Microarray Technologies. *Foodborne Pathogens and Disease*. 5: 531–550.

Schwab K.J., McDevitt J.J. (2003). Development of a PCR-enzyme immunoassay oligoprobe detection method for *Toxoplasma gondii* oocysts, incorporating PCR controls. *Applied Environmental Microbiology*. 69: 5819-5825.

Singh A., Poshtiban S., Evoy S. (2013). Recent advances in bacteriophage based biosensors for food-borne pathogen detection. Sensors (Basel). 13: 1763-1786.

Sorsa L.J., Dufke S., Schubert S. (2004). Identification of novel virulence-associated loci in uropathogenic *Escherichia coli* by suppression subtractive hybridization. *FEMS Microbiology Letters*. 230: 203-208.

Sotiriadou I., Karanis P. (2008). Evaluation of loop-mediated isothermal amplification for detection of *Toxoplasma gondii* in water samples and comparative findings by polymerase chain reaction and immunofluorescence test (IFT). *Diagnostic Microbiology* and *Infectious Disease*. 62: 357-365.

Stals A., Van Coillie E., Uyttendaele M. (2013). Viral genes everywhere: public health implications of PCR-based testing of foods. *Current*

Opinion in Virology. 3: 69-73.

Steinhauserova I., Ceskova J., Nebola M. (2002). PCR/restriction fragment length polymorphism (RFLP) typing of human and poultry *Campylobacter jejuni* strains. *Letters in Applied Microbiology*. 34: 354-358.

Tauxe R.V. (2002). Emerging food-borne pathogens. *International Journal of Food Microbiology*. 78: 31-41.

Van Lith L.A., Aarts H.J. (1994). Polymerase chain reaction identification of *Salmonella* serotypes. *Letters in Applied Microbiology*. 19: 273-276.

Warnekulasuriya M.R., Johnson J.D., Holliman R.E. (1998). Detection of *Toxoplasma gondii* in cured meats. *International Journal of Food Microbiology*. 45: 211-*2*15.

Wolska K., Kot B. (2013). Twitching motility activity, biofilm formation, and genetic typing for clinical isolates of *Pseudomonas aeruginosa* by random amplified DNA PCR. *Acta Microbiologica et Immunologica*

Hungarica. 60: 313-328.

Wu F.M., Muriana P.M. (1995). Genomic subtraction in combination with PCR for enrichment of *Listeria monocytogenes*-specific sequences. *International Journal of Food Microbiology*. 27:161-174.

Wu F.M., Muriana P.M. (1999). Cloning, sequencing, and characterization of genomic subtracted sequences from *Listeria monocytogenes*. *Applied and Environmental Microbiology*. 65: 5427-5430.

Ye Y., Wu Q., Zhang J., Lu J., Lin L. (2011). Isolation of *Salmonella* from meat samples and characterization by enterobacterial repetitive intergenic consensus-polymerase chain reaction and antibiotics test. *Foodborne Pathogens and Disease*. 8: 935-937.

Zintl A., Halova D., Mulcahy G., O'Donovan J., Markey B., DeWaal T. (2009). In vitro culture combined with quantitative TaqMan PCR for the assessment of *Toxoplasma gondii* tissue cyst viability. *Veterinary Parasitology*. 164: 167-172.