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LITERATURE REVIEW

Foodborne bacteria in dairy products: Detection by molecular techniques

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Abstract

N. Cancino-Padilla, M. A. Fellenberg, W. Franco, R.A. Ibañez, and E. Vargas-Bello-Pérez. 2017. Foodborne bacteria in dairy products: Detection by molecular techniques. Cien. Inv. Agr. 44(3):215-229. Because of their unique composition and properties, milk and dairy products represent excellent growth media for many pathogenic microorganisms. *Staphylococcus aureus, Salmonella spp., Listeria monocytogenes* and *Escherichia coli* O157:H7 are the most frequent potential pathogens associated with milk or dairy products in industrialized countries and are therefore the main microbiological hazards linked to raw milk and raw cheese. This review summarizes the scientific information about outbreaks related to foodborne pathogens in dairy products and highlights the increasing application of molecular approaches to detect and identify the bacteria responsible for these outbreaks. Molecular techniques have facilitated the rapid detection and identification of foodborne pathogens, which has been crucial for current surveillance and outbreak control.

Keywords: Foodborne diseases, food safety, microbiology, omics techniques.

Introduction

Farm animals represent a major reservoir of pathogens that can be transferred to milk (Arqués *et al*., 2015). *Staphylococcus aureus, Salmonella spp., Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Campylobacter* are the most frequent potential pathogens associated with milk or dairy products in industrialized countries (Jakobsen *et al*., 2011) and are the main microbiological hazards linked to raw milk (Kousta *et al*., 2010; Yang *et al*., 2012; Claeys *et al*., 2013) and raw cheese (Verraes *et al*., 2015). In addition, as with any food matrix, dairy foods present their own inherent source of inhibitors, such as fat or lipid content, calcium concentration, and the presence of indigenous enzymes, e.g., potential inhibitors, that may have a detrimental influence on the overall integrity of an accurate and sensitive assay (Ozer and Akdemir-Evrendilek, 2014).

In natural conditions, the microbial composition of milk is influenced by different parameters, such

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as the microorganisms present in the teat canal, on the surface of teat skin, or in the surrounding air, as well as the animal's feed, the quality of the water supply, and equipment hygiene (Quigley *et al*., 2013). For this reason, the detection of pathogens in dairy products can be a challenge and is needed to ensure safety. One of the most important tasks of food safety is the elimination, or at least the reduction, of foodborne pathogens (Giacometti *et al*., 2013). Therefore, it is necessary to find a way to detect pathogens in their early stages of growth in different food products, which could reduce the number of foodborne outbreaks (Pinu, 2016).

Foodborne disease outbreaks related to dairy products

Because of their unique composition and properties, milk and dairy products represent excellent growth media for many spoilage and pathogenic microorganisms (Nada *et al*., 2012; Claeys *et al*., 2013). Table 1 shows a summary of outbreaks associated with the consumption of different dairy products in the world. Additionally, food derived from animals (beef and dairy products, eggs and fish) has been identified as the main vehicle for the transmission of foodborne pathogens to humans (Ahmed and Shimamoto, 2014; Arqués *et al*., 2015). Additionally, foodborne bacteria can contaminate food products at any point along the production chain: during slaughtering, milking, storage or packaging (Tomat *et al*., 2016).

Several outbreaks have been associated with the consumption of dairy products, particularly cheese and other ready to-eat foods (Melo *et al*., 2015). Cheeses are ready-to-eat food products because they do not undergo any further treatment to ensure their safety before consumption. In addition, contamination of cheeses may occur at several stages in the production chain. Therefore, all the information about bacterial characteristics and susceptibility is necessary to prevent contamination of dairy products with pathogens (Kousta *et al.,* 2010).

Reports from developed countries indicated that milk and dairy products are implicated in 1-6% of the total bacterial foodborne outbreaks (Ahmed and Shimamoto, 2014), with 39.1% attributed to milk, 53.1% to cheese and 7.8% to other milk products (Claeys *et al*., 2013). In 2013, 2.14% of foodborne outbreaks were attributed to the consumption of cheese and dairy products (11 and 7 outbreaks, respectively) in Europe (Dalzini *et al*., 2016).

The most common source of reported outbreaks in the USA has historically been raw (unpasteurized) milk (Taylor *et al*., 2013). In their study, Bianchi *et al.* (2013) concluded that unpasteurized milk can be a vehicle for a variety of microorganisms (*Listeria spp*., *Salmonella*, and *Campylobacter*) and that outbreaks related to cheeses made with unpasteurized milk are also common (Gould *et al*., 2014). The development of a disease after consumption of contaminated dairy products made from raw milk depends on several factors, such as the pathogenicity of the bacteria strain, the number of ingested microorganisms, the physiological state of the microorganism, and the health condition of the consumer at the moment of ingestion (Verraes *et al*., 2015).

Bacteria involved in dairy product contamination

Staphylococcus aureus, Salmonella spp.*, Listeria monocytogenes* and *Escherichia coli* O157:H7 are the most frequent potential pathogens associated with milk or dairy products in industrialized countries (Jakobsen *et al*., 2011) and are therefore the main microbiological hazards linked to raw milk (Kousta *et al*., 2010; Yang *et al*., 2012; Claeys *et al*., 2013) and raw cheese (Verraes *et al*., 2015). Raw milk provides a potential growth medium for the development of bacteria that can be controlled or destroyed through the pasteurization process (Arqués *et al*., 2015). However, the number of people consuming unpasteurized products continues to increase all over the world due to a growing demand for natural and unprocessed foods (*Fusco and Quero*, 2014).

Table 1. Summary of outbreaks associated with the consumption of different dairy products in the world

Year	Country	Product involved	Pathogen	Cases	Reference
North America					
1998	Canada	Cheese	*Salmonella enterica*	80	Ahmed et al., 2000
1998-2011	United States	Cheese (Unpasteurized milk)	*Salmonella* *Listeria monocytogenes* *Escherichia coli*	13 4 4	Gould et al., 2014
2002	Canada	Soft Ripened Cheese	*Listeria monocytogenes*	130	McIntyre et al., 2015
2005	United States	Raw milk	*Escherichia coli* O157:H7	18	Denny et al., 2008
2007	United States	Pasteurized Milk	*Listeria monocytogenes*	5	MMWR, 2008
2008	Canada	Cheese (Pasteurized milk)	*Listeria monocytogenes*	38	Gaulin et al., 2012
2001-2010	United States	Raw milk	*Campylobacter spp.* STEC *Salmonella spp.*	407 31 39	Robinson et al., 2014
2010	United States	Gouda cheese (Unpasteurized milk)	*Escherichia coli* O157:H7	19	McCollum et al., 2012
2010-2012	United States	Raw milk	*Campylobacter spp.* STEC	40 8	Mungai et al., 2015
2012	United States	Ricotta salata cheese	*Listeria monocytogenes*	6	Heiman et al., 2016
2013	Canada	Gouda cheese (Unpasteurized milk)	*Escherichia coli O157*	29	Gill and Oudit, 2015
2014	United States	Ice cream	*Listeria monocytogenes*	2	Rietberg et al., 2016
2015	United States	Ice cream	*Listeria monocytogenes*	4	Pouillot et al., 2016
Europe					
1996-1997	United Kingdom	Formula-dried milk	*Salmonella enterica*	17	Threlfall et al., 1998
1997	France	Morbier cheese (Unpasteurized milk)	*Salmonella enterica*	113	De Valk et al., 2000
1998-1999	Finland	Butter	*Listeria monocytogenes*	25	Lyytikäinen et al., 2000
1997-2001	France	Cheese (Unpasteurized milk)	*Staphylococcus aureus*	70	Kérouanton et al., 2007
2004-2005	France	Powdered Infant Formula	*Salmonella enterica*	136	Brouard et al., 2007
2005-2007	Netherlands	Raw milk	*Campylobacter jejuni*	38	Heuvelink et al., 2009
2006	Netherlands	Hard cheese	*Salmonella Typhimurium*	38	Van Duynhoven et al., 2009
2006-2007	France	Cheese (Unpasteurized milk)	*Salmonella enterica*	23	Dominguez et al., 2009
2006-2007	Germany	Cheese (Pasteurized milk)	*Listeria monocytogenes*	34	Koch et al., 2010
2007	Austria	Milk, cacao milk or vanilla milk	*Staphylococcus aureus*	166	Schmid et al., 2009
2008	Spain	Infant Formula	*Salmonella kedougou*	21	Rodríguez-Urrego et al., 2010
2009	Austria	Quargel cheese	*Listeria monocytogenes*	25	Fretz et al., 2010
2009	Germany	Quargel cheese	*Listeria monocytogenes*	8	Fretz et al., 2010
2009	Czech Republic	Quargel cheese	*Listeria monocytogenes*	1	Fretz et al., 2010
2009	France	Soft cheese (Unpasteurized milk)	*Staphylococcus aureus*	23	Ostyn et al., 2010
2012	Portugal	Cheese (Pasteurized milk)	*Listeria monocytogenes*	30	Magalhães et al., 2015
2012	Spain	Latin-style fresh cheese (Pasteurized milk)	*Listeria monocytogenes*	2	De Castro et al., 2012

Continuation of Table 1...

Year	Country	Product involved	Pathogen	Cases	Reference
2013	Germany	Ice cream	*Staphylococcus aureus*	13	Fetsch et al., 2014
2014	Switzerland	Soft cheese (Unpasteurized milk)	*Staphylococcus aureus*	14	Johler et al., 2015
2014	Finland	Raw milk	*Yersinia pseudotuberculosis*	43	Pärn et al., 2015
Asia					
2000	Japan	Reconstituted milk	*Staphylococcus aureus*	13420	Asao et al., 2003
South America					
2006	Argentina	Cream	*Staphylococcus aureus*	53	López et al., 2008
2007	Paraguay	Ultrapasteurized milk	*Staphylococcus aureus*	400	Weiler et al., 2011
2008	Chile	Brie and Camembert cheese	*Listeria monocytogenes*	165	Montero et al., 2015

†STEC: Shiga toxin–producing *Escherichia coli*

Outbreaks due to cheese made from unpasteurized milk are often caused by *Salmonella* (34%), *Campylobacter* (26%), *Brucella* (13%), and Shiga toxin–producing *Escherichia coli* (11%) (Gould *et al*., 2014). *Listeria* is killed by pasteurization, and outbreaks of this bacterial strain have rarely been associated with pasteurized dairy products, including cheese (Koch *et al.,* 2010). These foodborne bacteria are of concern for the dairy industry because they have been identified in different dairy products and have been implicated in outbreaks (Kousta *et al*., 2010; Yang *et al*., 2012; Claeys *et al*., 2013; Ricci *et al*., 2013; Robinson *et al.,* 2014; Tayel *et al*., 2015; Pouillot *et al*., 2016).

Listeria monocytogenes has been involved in numerous outbreaks occurring after consumption of contaminated milk and dairy products throughout the world (Wang *et al.,* 2015; Dalzini *et al*., 2016). In 2015, dairy products were identified as the main sources of listeriosis (Quero *et al.,* 2014; Jackson *et al.,* 2016). Raw milk can be contaminated with *Listeria monocytogenes* from unclean equipment during milking, during storage in bulk tanks or during transport to the cheese processing plant, where hygienic control measures may not be adequate (Melo *et al.,* 2015). Because of its high case-fatality rate, listeriosis is, after salmonellosis, the second most frequent cause of foodborne infection-related deaths in Europe (Arqués *et al*., 2015; Dalzini *et al*., 2016). However, outbreaks from *Listeria monocytogenes* are not common compared with those caused by pathogens such as *Salmonella* (Todd and Notermans, 2011).

Staphylococcus aureus is a ubiquitous pathogen; thus, the sources of this bacteria for dairy products contamination are diverse (Rosengren *et al*., 2010). This bacterium is commonly found in a wide variety of mammals and birds and can be transferred to food mainly by dairy animals that have mastitis and by human carriers during food processing (Hennekinne *et al*., 2012). Contamination of *Staphylococcus aureus* can have a broad occurrence in raw dairy products, with frequencies between 5 and 100% in cheeses (Verraes *et al*., 2015). The number of *Staphylococcus aureus* in raw milk or other dairy products needs to be less than 10^4 CFU g^{-1}, according to the US FDA regulations (Yu *et al.,* 2016). During the manufacture of cheese, natural staphylococcal contamination is a minor component of the total microbial population, and the initial *Staphylococcus aureus* contamination is usually below 10^3 CFU ml^{-1} of raw milk (Duquenne *et al.,* 2010). Foods of animal origin with high protein contents such as milk and dairy products, meat, meat products, salads and bakery products favor the growth of bacteria, and this type of food has been frequently

incriminated in *Staphylococcus aureus* outbreaks (Fetsch *et al.,* 2014). This bacterium can grow in an extensive range of temperatures, pH values, sodium chloride concentrations and water activity and it can also produce staphylococcal enterotoxins, which are responsible for staphylococcal food poisoning (Schelin *et al.,* 2011).

Staphylococcus aureus is present in raw materials and food and can be inactivated with heat treatment, but enterotoxins are heat resistant and may persist in food even after heat treatment. Staphylococcal enterotoxins are active even after boiling for 30 min and may remain stable at 121 °C for 28 min (Necidova *et al.,* 2016). Minimum pasteurization treatments are based on European legislation, which set the heat treatment at 72 °C for 15 s or 63 °C for 30 min. High-temperature pasteurization is defined as heat treatment of milk at not less than 85 °C. In addition, in the Chilean food regulations (Reglamento Sanitario de los Alimentos), dairy products are treated at ultra-high-temperatures (UHTs) 130 °C and 145 ºC for 2 or 4 s to ensure inactivation of bacteria and toxins.

Salmonella spp. is the most frequent cause of food-borne outbreaks, and human salmonellosis is the second most frequently reported zoonosis in the European Union (Wuyts *et al*., 2013). Milk is a food that has a high chance of contamination by *Salmonella* spp. (Riyaz-Ul-Hassan *et al.,* 2013), mainly before leaving the farm, usually because of fecal contamination during the milking process (Ahmed and Shimamoto, 2014). Additionally, *Salmonella* spp. can be transmitted to humans via the consumption of contaminated dairy products (Vignaud *et al.,* 2017), especially unpasteurized or insufficiently pasteurized milk and cheeses, which cause outbreaks of salmonellosis in humans (De Valk *et al.,* 2000; Ahmed and Shimamoto, 2014). Finally, the main reservoirs of Shiga toxin-producing *Escherichia coli* are ruminants, contaminating milk through subclinical mastitis or feces, and the bacteria can persist in milking equipment (Arqués *et al*., 2015).

Foodborne bacteria detection

The detailed characterization of isolates is critical for the investigation of common outbreak sources in order to identify the source, implement control measures and/or take steps to remove the implicated food from the market place (Bopp *et al*., 2016).

Conventional methods

Traditional methods for the detection of bacterial pathogens in foods have been widely used because they are sensitive and inexpensive and can give both qualitative and quantitative information on the number and the nature of the microorganisms present in the food sample (Zhao *et al*., 2016). The conventional methods for detection of these pathogens involve identification and confirmation based on culturing on selective media along with biochemical tests and immunological assays (Quigley *et al.,* 2013). These methods are standard methods; however, they are extremely laborious, time consuming (requiring several days), and often inconclusive (Singh *et al*., 2011; Chen *et al*., 2015; Bopp *et al.,* 2016; Yu *et al*., 2016). For these reasons, there is an increasing demand for more rapid methods of foodborne pathogen detection (Zhao *et al*., 2014), in order to complement or replace the traditional microbial culture procedures with more advanced, sensitive, and rapid microbial detection methods.

Molecular genetic techniques

Due to their limitations, conventional methods are now giving way to molecular diagnostic methods based on DNA analysis, such as polymerase chain reaction (PCR), multiplex PCR and real-time quantitative PCR (qPCR), which have been used for rapid and reliable detection of foodborne pathogens (Chiang *et al.,* 2012). In addition, typifying methods are also largely used for accurate genetic characterization in outbreak investigations. Table 2 shows the molecular genetic

Table 2. Selection of molecular genetic techniques used for foodborne detection

Bacteria	Approach	Target gene or enzyme used	Detection	Dairy product	Reference
Listeria monocytogenes	qPCR	hly	4 log CFU mL^{-1}	Raw milk, pasteurized milk, kulfi, ice cream, paneer, and infant foods	Singh *et al*., 2011
	qPCR	hlyA	3.63 log	Raw milk	Quero *et al*., 2013
	qPCR	hlyA	CFU g^{-1} 4.9×10^3 CFU g^{-1}	Milk	Paul *et al*., 2015
	Multiplex PCR	htpG	1 CFU mL^{-1}	Quargel chesse	Chiang *et al*., 2012
	PFGE	AscI and ApaI	$N \times 10^4$ CFU ml^{-1}	Cheese (pasteurized milk)	Schoder *et al*., 2014
	PFGE	AscI		Cheese (pasteurized milk)	Koch *et al*., 2010
	PFGE	AscI and ApaI			Gaulin *et al*., 2012
Salmonella	qPCR	invA	3 log CFU mL^{-1}	Raw milk, pasteurized milk, kulfi, ice cream, paneer, and infant foods	Singh *et al*., 2011
	Multiplex PCR	Random DNA fragment	$N \times 10^4$ CFU mL^{-1}	Milk	Chiang *et al*., 2012
	qPCR	Stn	25 to 500 cells	Milk	Riyaz-Ul-Hassan *et al*., 2013
	MLVA	-	-	Cheese (raw milk)	Vignaud *et al*., 2017
	PCR	*Salmonella Enteritidis* and *Typhimurium*	3,03% of samples 1% of samples	Milk Cheese (raw milk)	Ahmed and Shimamoto, 2014
	PCR	hilA	5 bacteria ml^{-1} 10^3 bacteria ml^{-1}	Milk Ice-cream	Marathe *et al*., 2012
Staphylococcus aureus	qPCR	egc	10^2 to 10^3 CFU mL^{-1}	Raw milk	Fusco and Quero, 2014
	Multiplex PCR	hsp	$N \times 10^4$ CFU ml^{-1}	Milk	Chiang *et al*., 2012
E. coli O157	qPCR	Rfb	1 CFU mL^{-1}	Raw milk	Paul *et al*., 2013
E. coli O157: H7	Multiplex PCR	Random DNA fragment	$N \times 10^4$ CFU mL^{-1}	Milk	Chiang *et al*., 2012
E. coli O157: H7	qPCR	stx1, stx2 and stx2f	4×10^6 to 40 CFU mL^{-1}	Milk	Derzelle *et al*., 2011
E. coli O157:H7	PCR	stx1, stx2 and rfb	2,5% of samples 1,1 % of samples	Raw milk Cheese	Ahmed and Shimamoto, 2014
E. coli O157	WGS	-	-	Raw milk	Butcher *et al*., 2016

qPCR: Quantitative polymerase chain reaction
PFGE: Pulsed field gel electrophoresis
MLVA: Multiple-locus variable-number tandem repeat analysis
PCR: Polymerase chain reaction
WGS: Whole genome sequencing

techniques applied to dairy products to detect and identify foodborne bacteria.

Molecular techniques for pathogens are being developed for various aspects of detection, such as sensitivity, rapidity, and selectivity (Zhao *et al*., 2014). Studies have shown that identification systems based on molecular genetic techniques are more discriminating than phenotypic methods and often provide more accurate taxonomic information about a particular strain, which is very important in pathogen surveillance (Henri *et al*., 2016). Additionally, these approaches allow the detection of very low numbers of organisms in the sample and high throughput of many samples for routine analysis (Lee *et al.,* 2015).

One of the advantages of DNA-based pathogen detection assays is the high level of specificity, as they detect specific nucleic acid sequences in the target organism by hybridizing them to a short synthetic oligonucleotide complementary to the specific nucleic acid sequence (Zhao *et al*., 2014). These methods have also become valuable tools for investigating foodborne outbreaks and identifying the responsible etiological agents (Riyaz-Ul-Hassan *et al.,* 2013).

Polymerase chain reaction

PCR is a technique that amplifies a specific DNA sequence, producing thousands to millions of copies. These methods usually detect specific genes in strains of bacteria isolated from contaminated foods (Hennekinne *et al.,* 2012). This technique has been recognized as one of the most promising rapid microbiological methods for the detection and identification of bacteria in a wide range of foods (Auvolat and Besse, 2016). It has been used to detect foodborne bacterial pathogens such as viable *Escherichia coli* O157:H7, *Salmonella* and *L. monocytogenes* in food (Lee *et al.,* 2015; Wang *et al*., 2015). PCR is widely used to detect *Staphylococcus aureus* (Kim *et al*., 2001; Vancraeynest *et al.,* 2007; Yang *et al.,* 2007) because of its high sensitivity and specificity (Chen *et al.,* 2015).

It is important to consider that PCR requires precise and expensive instruments, and macromolecules such as proteins and fat present in milk and ice cream could interfere with the PCR assay. Removal of these macromolecules is essential to prevent PCR inhibition (Marathe *et al*., 2012).

Real-time quantitative PCR (qPCR)

The use of qPCR has provided several advantages over conventional PCR such as quantification, real-time and *in situ* analyses, in addition to automation (Riyaz-Ul-Hassan *et al.,* 2013). In this technique, the PCR products are detected as they accumulate. The amount of generated PCR product is proportional to the increase in a fluorescent signal, which is monitored during the exponential phase (Auvolat and Besse, 2016). This technique permits rapid identification and quantification of bacteria (Singh *et al.,* 2011; Quigley *et al.,* 2013).

Several attempts have been made to develop qPCR assays for the detection of *L. monocytogenes* and *Salmonella* spp. over a wide range of food products including beef, seafood, fresh produce, and dairy products (Singh *et al.,* 2011). The sensitivity of qPCR when applied to a food matrix is generally quite limited when compared with other enumeration methods. Consequently, in most cases, qPCR is not suitable for the accurate enumeration of, for example, low levels of *L. monocytogenes* in food (Auvolat and Besse, 2016).

Multiplex PCR

Multiplex PCR involves the simultaneous detection or amplification of multiple target sequences in a single reaction by using different primers for each target. Multiplex PCR has the potential to produce

considerable savings of time and effort within the laboratory without compromising test utility (Elnifro *et al.,* 2000).

DNA microarray

In DNA arrays, specific DNA sequences are synthesized in a 2-D or 3-D array on a surface to which the DNA is covalently or non-covalently attached. The DNA array is used to probe a solution containing a mixture of labeled nucleic acids, and the binding (by hybridization) of these "targets" to the "probes" on the array allows the measurement of the relative concentrations of the nucleic acid in the sample (Bumgarner, 2013). Biochips may allow the simultaneous detection and identification of multiple microorganisms in a relatively short time, and they are being described as a powerful tool for the detection of foodborne pathogens (Chiang *et al.,* 2012). However, the high cost associated with this approach is the main restriction limiting its application in the dairy and food industries on a routine basis (Singh *et al.,* 2011).

Molecular subtyping methods

Molecular subtyping has been an instrumental tool for the surveillance and outbreak investigation of foodborne illness for several years (Deng *et al.,* 2016) and has proven critical for identifying clusters that warrant further investigation (Jackson *et al.,* 2016). These approaches are essential epidemiological tools for detecting the outbreak of foodborne diseases, and must provide strong discriminatory power and high epidemiological concordance (Liu *et al.,* 2016). The molecular typing methods that are commonly used to detect bacterial disease outbreaks include pulsed field gel electrophoresis (PFGE), multiple-locus variable-number tandem repeat analysis (MLVA), and clustered regularly interspaced short palindromic repeat and multiple-virulence-locus sequence typing (CRISPR-MVLST) (Liu *et al.,* 2016). Recently, these have been powered by whole-genome-sequencing (WGS) technologies.

Pulsed field gel electrophoresis

PFGE has been described as the gold standard for subtyping the genus and species to provide further discrimination among bacterial pathogens (Wuyts *et al.,* 2013; Taylor *et al.,* 2015; Adkins *et al.,* 2016) and is used by the National Molecular Subtyping Network for Foodborne Disease Surveillance in United States (PulseNet) (Bopp *et al.,* 2016). Initially, PFGE was used to type *Escherichia coli O157:H7* and then was developed to enable typing of various important bacteria, such as *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Salmonella* (Liu *et al.,* 2016).

This is the most commonly used molecular subtyping method for surveillance and outbreak detection because of its high discriminatory power and reproducibility (Deng *et al*., 2016). PFGE was used for the molecular subtyping of *Listeria monocytogenes* isolates in a commercial cheese made from pasteurized milk that caused an outbreak in Germany from October 2006 through February 2007 (Koch *et al.,* 2010) and in Quebec, Canada, in 2008 (Gaulin *et al.,* 2012). Additionally, in 2010, this technique was useful in determining the genotypic diversity of *Listeria monocytogenes* in the acid curd cheese that caused a multinational outbreak between 2009 and 2010 (Schoder *et al.,* 2014).

However, PFGE is time-consuming and laborious to perform (Liu *et al*., 2016), which makes it less suitable for typing many isolates, and it requires rigorous standardization of the protocols (Bertrand *et al.* 2015). PFGE also lacks the discriminatory capacity and phylogenetic basis of more advanced methods (Jackson *et al.,* 2016).

Multiple-locus variable-number tandem repeat analysis

MLVA is a molecular subtyping method based on amplification and fragment size analysis of the number of repeats in the variable-number tandem repeats region of the bacterial genome (Bertrand *et al.* 2015). It is rapid and highly reproducible, and the results are easily interpreted and standardized among laboratories. Bacteria that have been typed by MLVA include *Salmonella* (Wuyts *et al.,* 2013), *Escherichia coli* and *Vibrio parahaemolyticus* (Liu *et al.,* 2016).

This method has proven very useful in investigating foodborne outbreaks because it utilizes the naturally occurring variation in the number of tandem repeat DNA sequences, and it has facilitated analysis since it requires no specific technical expertise (Vignaud *et al.,* 2017). MLVA was applied to a raw milk cheese outbreak in France in 2012 to subtype *Salmonella enterica* subspecies *enterica* serovar Dublin, which is one of the most frequently isolated Salmonella strains in humans in that country (Vignaud *et al.,* 2017).

Whole genome sequencing

PFGE and MLVA often do not provide sufficient resolution to differentiate between outbreaks (Taylor *et al*., 2015). Recently, whole genome sequencing has offered that discriminatory power with the potential to enhance epidemiological investigations and elucidate transmission pathways (Phillips *et al*., 2016). The use of next-generation sequencing technology for WGS allows for the sequencing of large numbers of isolates, and novel bioinformatics tools can be used for comparative genomics and analysis of the phylogeny of the isolates (Revez *et al.,* 2014). WGS has been a very useful and powerful tool for establishing potential links between clinical, food and environmental isolates of pathogens, which could allow the identification of the source of contamination and remove contaminated foods from markets (Deng *et al.,* 2016).

WGS has been recently used to understand outbreak sources and the transmission patterns of bacteria, including *Escherichia coli, Campylobacter, Listeria spp. and Salmonella spp.* (Lambert *et al*., 2015; Clark *et al.,* 2016; Jackson *et al.,* 2016; Wilson *et al*., 2016). Furthermore, WGS has the potential to discriminate between sporadic and outbreak isolates which may be indistinguishable by current methods of subtyping (Phillips *et al.,* 2016). WGS usefulness in food safety is undeniable; however, this approach is expensive and is not currently in place in the majority of public health laboratories (Bopp *et al*., 2016). Additionally, analysis of WGS data can be difficult due to the extensive computational capacity and bioinformatics skills needed for genomic comparisons and to determine a threshold to establish relatedness (Burall *et al.,* 2016).

General remarks

There are different examples of outbreaks associated with the consumption of dairy products, and the identification of causative bacteria is very complex. Novel molecular techniques have been crucial for accuracy in the detection of foodborne bacteria in diverse types of dairy products (including pasteurized milk), and it is probable that without these molecular approaches, the outbreaks' etiological agents would not have been correctly identified.

On the other hand, the dairy industry requires fast, sensitive and cost-effective technology for the detection of foodborne pathogens in order to fulfill food safety requirements and to establish routine quality control testing. For example, due to the increasing demand for raw products and the conditions (i.e., transport temperature and humidity) between the processing and storing of dairy products, the application of advanced analytical

methods for pathogen detection could ensure safety and prevent outbreaks due to consumption of contaminated products, reducing the economic losses related to removal of products, negative corporate images and court costs.

Molecular typifying methods are powerful tools for accurate genetic characterization of foodborne pathogens, and the dairy industry and governments might apply them extensively, implementing standard protocols for foodborne pathogens in developed and developing countries, ensuring the food safety of dairy products regardless of their origin. Molecular techniques can be used in different industries such as food and pharmaceutics; however, they require expensive equipment and reagents, and their setup requires highly technical skills. Additionally, infrastructure will be another important factor to consider, since most of these techniques require controlled environments to avoid contamination and misleading results. In the case of the dairy industry, qPCR and PCR techniques are more feasible to implement since they are quick and cost-effective and do not require much skill to perform, compared to other molecular techniques.

Resumen

N. Cancino-Padilla, M. A. Fellenberg, W. Franco, R.A. Ibañez, y E. Vargas-Bello-Pérez. **2017. Bacterias transmitidas por los alimentos en los productos lácteos: detección por técnicas moleculares. Cien. Inv. Agr. 44(3): 215-229.**Debido a su composición y propiedades únicas, la leche y los productos lácteos representan un gran medio de crecimiento para muchos microorganismos patógenos. *Staphylococcus aureus, Salmonella spp., Listeria monocytogenes y Escherichia coli O157: H7* son los patógenos más frecuentes asociados con la leche o los productos lácteos en los países industrializados. La identificación de estos patógenos a través de métodos tradicionales o dependiente de cultivo requieren mucho tiempo, por lo cual diferentes investigaciones han centrado sus esfuerzos en el desarrollo de métodos de detección rápida para identificar bacterias transmitidas por los alimentos. Conocer el método más apropiado para la identificación de patógenos podría ser útil para la vigilancia y el control de brotes, y también para mejorar las normas de higiene en las prácticas alimentarias con el objetivo de minimizar la contaminación microbiológica.

Palabras clave: Inocuidad de alimentos, microbiología, patógenos trasmitidos por los alimentos, técnicas biotecnológicas.

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