



# Detection of Potential Microbial Contaminants and Their Toxins in Fermented Dairy Products: a Comprehensive Review

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## Abstract

Fermented dairy products are dominant constituents of daily diets around the world due to their desired organoleptic properties, long shelf life, and high nutritional value. Probiotics are often incorporated into these products for their health and technological benefits. However, the safety and possible contamination of fermented dairy products during the manufacturing process could have significant deleterious health and economic impacts. Pathogenic microorganisms and toxins from different sources in fermented dairy products contribute to outbreaks and toxicity cases. Although the health and nutritional benefits of fermented dairy products have been extensively investigated, safety hazards due to contamination are relatively less explored. As a preventive measure, it is crucial to accurately identify and determine the associated microbiota or their toxins. It is noteworthy to highlight the importance of detecting not only the pathogenic microbiota but also their toxic metabolites so that putative outbreaks can thereby be prevented or detected even before they cause harmful effects to human health. In this context, this review focuses on describing techniques designed to detect potential contaminants; also, the advantages and disadvantages of these techniques were summarized. Moreover, this review compiles the most recent and efficient analytical methods for detecting microbial hazards and toxins in different fermented dairy products of different origins. Causative agents behind contamination incidences are also discussed briefly to aid in future prevention measures, as well as detection approaches and technologies employed. Such approach enables the elucidation of the best strategies to control contamination in fermented dairy product manufacturing processes.

**Keywords** Fermented dairy · Toxins · PCR · Chromatography · ELISA · Biosensor

## Introduction

Dairy products are among the first fermented foods that humans consumed. Many different types of fermented dairy products (FDP) under different names exist including cheese, yogurt, acidophilus milk (e.g., koumiss), thermophilic sour

milk (e.g., Ayrán), mesophilic sour milk (e.g., cultured buttermilk), acid and alcoholic milk (e.g., kefir) (Macori and Cotter 2018). These products are valued worldwide owing to their long shelf life, organoleptic properties, safety, and improved nutritional and functional properties (Marco et al 2017). Fermented dairy products contain a wide range of nutrients that are necessary for human health as well as protection from malnourishment, e.g., proteins, vitamins, conjugated linoleic acid, bioactive peptides, and minerals (Khan et al. 2019; Saxelin et al. 2003). Some of these nutrients result from the action of the microorganisms in the fermented dairy products (Fernández et al. 2015; Pal et al. 2016).

Fermented dairy products (FDP) can nevertheless be associated with several health hazards. These hazards are linked to distinct contaminant introduction during the fermentation process, leading to sporadic cases or outbreaks. The main categories of these hazards include chemical,

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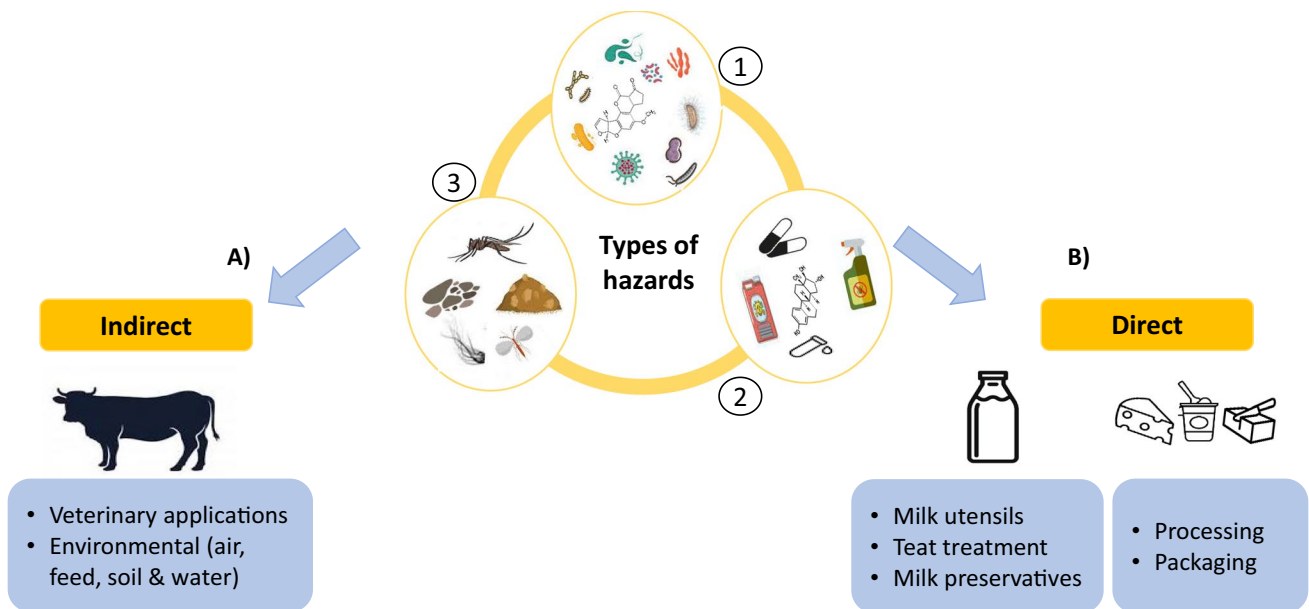
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physical, and biological contaminants (Fig. 1). Chemical hazards include veterinary drug residues (e.g., antibiotics) (Layada et al. 2016), food additives (e.g., nitrates) (Zamrik 2013), pesticides (e.g., organochlorines) (Rusu et al. 2016), biocidal products (e.g., *N*-(3-aminopropyl)-*N*-dodecylpropane-1,3-diamine) (Slimani et al. 2018), heavy metals (e.g., lead and cadmium) (Meshref et al. 2014), chemicals from packaging materials (e.g., di-(2-ethylhexyl) adipate [DEHA]) (S. S. Aly 2016), and volatile organic compounds (e.g., xylenes, 2-ethylhexanol, and triacetin) (Panseri et al. 2014) were found in FDP. Physical hazards are relatively less common, and include whole insects or insect parts, metal fragments, glass pieces, hair or fur, and stones that have been found in these products (Owusu-Kwarteng et al. 2020).

Biological hazards include pathogenic microorganisms and their associated toxic products, e.g., mycotoxins and biogenic amines (Fernández et al. 2015; Maia et al. 2019). These hazards are included during various stages of the production process. For example, during milk collection, the inside of the udder is aseptic area before milk secretion starts (Quigley et al. 2013; Taponen et al. 2019); nevertheless, it is difficult to monitor and maintain the optimum hygiene practices of milk production, especially in underdeveloped regions (Torkar & Teger 2008; Vahedi et al. 2013). Moreover, it was previously reported that the incidence of contamination by certain pathogens such as

*Escherichia coli*, *Staphylococcus aureus*, and *Coxiella burnetii* is higher in dairy products made from raw milk than those made from pasteurized or heat-treated milk. (Verraes et al. 2015) Although pasteurization is a common practice, it is not always enough to totally eliminate spores (Farang et al. 2021; Lindström et al. 2010) as pasteurization ensures inactivation of vegetative pathogenic microorganisms, which increases the safety of products made thereof. Likewise, the traditional processing of the dairy such as fermentation tends to produce a higher risk of contamination, with several underlying factors (Fig. 1) that may lead to bacterial growth and toxin production such as intrinsic factors, e.g., semi-neutral pH, high amount of nutrients, water, or contaminated starter cultures (Kamana et al. 2014). In contrast, extrinsic factors also contribute to contamination including contaminated tools, surfaces, and packaging material sources (Gonfa et al. 2001). Contamination through these sources is usually facilitated by operation deficiencies, e.g., inappropriate temperature and thermal processing, unsafe formulation, insufficient fermentation, and post-processing contamination such as during transportation or storage, in addition to inadequate quality control during manufacture (Lindström et al. 2010).

Outbreaks originating from fermented dairy products have been reported in several countries (Cutter 1988; Mungai et al. 2015), and a large number of people still get



**Fig. 1** Types of contaminants of fermented dairy products and their sources: (1) biological, e.g., pathogens and their toxins; (2) chemical, e.g., veterinary drug residues, food additives, pesticide residues, heavy metals, and chemical from packaging materials; (3) physical, e.g., insects and their parts, metal fragments, glass pieces, and hair or

fur. (A) Direct sources during processing and packaging of FD products or by milk utensils, teat treatment and milk preservatives. (B) Indirect sources: by veterinary applications and environment such as soil

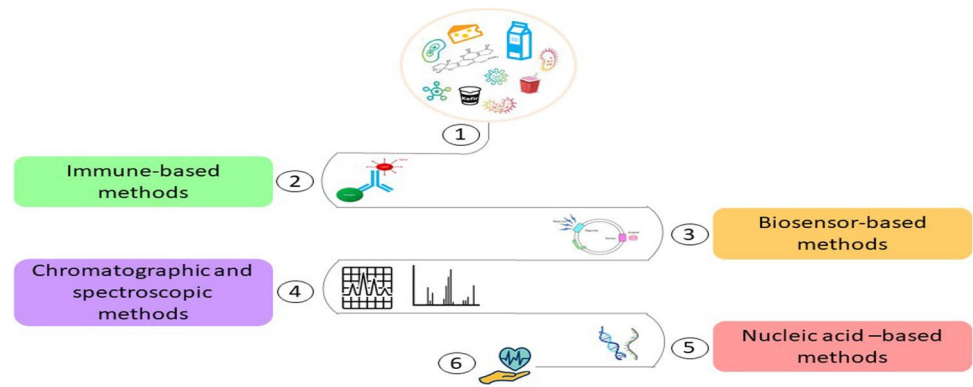
infected every day (Pal et al. 2016). For example, there were 97 cheese-related outbreaks in the USA, between 1998 and 2012, leading to more than 2000 diseases, 221 hospitalizations, and 10 deaths. The most important pathogens in these outbreaks were bacteria (i.e., *Salmonella enterica* [18.5%], *Campylobacter* spp. [13.6%], *Listeria monocytogenes* [12.3%], *Staphylococcus aureus* [7.4%], Shiga toxin-producing *Escherichia coli* [4.9%], *Brucella* spp. [3.7%], *Shigella* spp. [2.5%], *Clostridium perfringens* [2.5%], and *Bacillus cereus* [1.2%]) in addition to viruses (e.g., norovirus that caused one-third of these outbreaks) (Cutter 1988). Pregnant women, neonates, elderly people, and immunodeficient patients, for example, were among the highly affected population by such pathogens after ingestion of some fermented dairy products (Gerner-Smidt 2007). All these facts make us desperately need high quality management and control measures for microbial FDP contaminants including quick and precise detection methods. Besides these pathogens, certain toxins present potential health hazards as mycotoxins and certain biogenic amines. Mycotoxins are biosynthesized as secondary metabolites produced mainly by certain fungal species (*Penicillium*, *Aspergillus*, *Fusarium*, *Claviceps*, and *Alternaria*) (Grout et al. 2020). The most potentially dangerous for humans are the aflatoxins (AFs), ochratoxins (OTs), fusariotoxins (FTs), trichothecenes (TCs), citrinin (CT), and zearalenone (ZEA). AFs are classified as potential human carcinogenic (group I) by the International Agency of Research in Cancer (IARC) (Grenier and Oswald 2011). Other mycotoxins as sterigmatocystin (STC) seemed to be frequently present in cheese (Lund et al. 1995). STC has a certain structure similar to aflatoxins as AFB1 but with lower toxicity (Balogh et al. 2019). Mycotoxins can be found in dairy products via indirect contamination, which results when dairy cows ingest feed containing mycotoxins that pass into the milk or via direct contamination, which occurs because of the intentional or accidental growth of molds introduced either from the environment (airborne), or deliberately inoculated using certain ripening cultures as during cheesemaking (Hymery et al. 2014). Biogenic amines (BAs) are present in lactic acid bacteria-fermented dairy products. BAs are bioactive metabolites produced by certain microorganism either pathogenic or non-pathogenic ones via metabolizing certain amino acids (Benkerroum 2016). Certain microbiota possesses amino acid decarboxylating activity, e.g., lactobacilli produce tyramine, histamine, and putrescine, whereas enterococci form tyramine and enterobacteria which are considered cadaverine and putrescine producers (Schirone et al. 2012). Their levels generally were in the order of milligrams per kilogram; however, they may reach 2 g/kg (Linares et al. 2011). It was reported that the maximum limits of biogenic amines in foods for human consumption as histamine,

tyramine, phenylethylamine, and total BAs is around 50–100, 100–800, 30, and 200–1000 mg/kg, respectively (Ec 2003; Kandasamy et al. 2021).

One of the most serious pathogenic contaminants is *Clostridium botulinum* which is an anaerobic gram-positive bacterium producing neurotoxic proteins (botulinum toxins) which are considered as one of the most lethal substances known (Nigam and Nigam 2010). Botulinum neurotoxins block nerve functions and can lead to respiratory and muscular paralysis. Many botulism outbreaks, noticed in different countries in the last decades, were associated with the consumption of contaminated cheeses (Aureli et al. 2000; Lindström et al. 2010; Pourshafie et al. 1998; Rosen et al. 2020; Townes et al. 1996).

Several recent papers discussed the methods of microbial detection in dairy products to assess milk quality (Ziyaina et al. 2020). Others dealt with the microbial and chemical contaminants regarding the safety profile rather than focusing on the various analytical methods of detection and quantification which present an essential step before judging the safety of the fermented foods (Akinyemi et al. 2021; Owusu-Kwarteng et al. 2020; Sivamaruthi et al. 2019). Others just mentioned the type of the methodology without any details about sample pretreatment, sensitivity, and selectivity (Sivamaruthi et al. 2019). One of the aims of the present review is to provide a tool for the analyst to compare between different methods of analysis and choose the optimum methods of detection and identification that are both faster and highly sensitive which in turn is essential for the successful implementation of any hazard analysis critical control point. The present work covers almost all the well-known microbial toxins as (aflatoxins, sterigmatocystin, ochratoxins, botulinum toxins). This review compiles a broad scope of the recent and efficient various analytical methods used for the identification of the most probable pathogenic microorganisms and their toxins in different types of fermented dairy products. These approaches have not been used for detecting the contaminants in just specific areas but worldwide which supports its efficiency and quality. Although the conventional culture-based methods are still routinely used in most microbiology laboratories for their many advantages, their limitation is also discussed briefly. These limitations make us the need to develop different techniques with high sensitivity and specificity to overcome such limitations and that will produce a successful implementation of any critical control point in hazard analysis. Consequently, each developed, recent, and efficient approach is discussed in detail highlighting its application, sensitivity limits, advantages, and drawbacks. Here, we review various analytical approaches employed for the detection of pathogenic microorganisms and toxins in FDP, including immunologic, chromatographic, and biosensor- and molecular-based ones (Fig. 2). Each analytical

**Fig. 2** Detection of microbial contaminants and toxins in (1) fermented dairy products. These methods including (2) immunologic, (3) biosensor, (4) chromatographic, and (5) molecular methods. These methods are used for detection different pathogenic microorganisms and toxins in different types of fermented dairy products



methodology is discussed in detail highlighting its applications, advantages, and/or any limitations (Table 1) with emphasis on possible future development. Reasons behind contamination are also discussed briefly to aid in future prevention and control measures.

## Detection Methods

Conventional culture-based methods are routinely used in most microbiology laboratories and are the simplest way to detect, identify, and quantify viable pathogens (colony counting) (Haddad & Yamani 2017).

Protocols based on these methods are validated as reference methods according to the European and International Standard Organization (EN ISO) standards, to detect and enumerate foodborne pathogens. While these methods can be inexpensive and give both qualitative and quantitative information on the number and nature of the microorganisms tested, they are greatly restricted by assay time (e.g., *L. monocytogenes* can require up to 7 days to give results) with an initial enrichment step needed in order to detect pathogens (Artault et al. 2001; de Boer and Beumer 1999). It should be mentioned that the drawback associated with the enrichment step is the impossibility to quantify the initial contaminating amounts (Postollec et al. 2011). Moreover, there are many limitations to these methods as low sensitivity (Lee et al. 2014), time-to-result, false-positive counts, and matrix-dependent efficiency (Sohier et al. 2014). Therefore, different techniques with high sensitivity and specificity have been developed to overcome the limitations of conventional methods.

### Immunologic-Based Methods

Immunoassays are bioanalytical methods and based merely on antigen–antibody interactions; the strength of this binding determines the specificity and sensitivity of immunological-based methods which involve the use of mono- and polyclonal antibodies (Zhao et al. 2014). Antigen detection

has been one of the most successful methods in detecting pathogens and their toxins (Foxman 2010).

Enzyme-linked immunosorbent assay (ELISA) has been used to detect different botulinum neurotoxin (BoNT) serotypes in a wide range of milk product matrices, e.g., whole milk, yogurt with minimal fat, and fluid baby milk. In comparison to mouse bioassay (an *in vivo* method to determine safety level), ELISA is faster and easy to perform and less costly to maintain, but it can detect only antigenic components of inactive botulinum neurotoxins. On the other hand, using mouse bioassays, all the functional steps in the intoxication pathway can be measured (Singh et al. 2015). BoNT-producing clostridia and BoNT were assessed using multiplex real-time PCR after DNA enrichment and extraction steps (Anniballi et al. 2013; Benevenia et al. 2022).

Thus, ELISA does not fully replace mouse bioassays, rather it can be used as a preliminary screening method. ELISA has been also used to detect staphylococcal enterotoxin B in yogurt. Pasteurization of the contaminated yogurt rendered this toxin undetectable using ELISA suggesting that this method poorly detects thermally treated staphylococcal enterotoxin B (Principato et al. 2009).

Similar to mini sandwich ELISA, an antibody array has been applied for the simultaneous detection of two pathogenic bacteria (*E. coli* O157:H7 and *Salmonella* spp.) in dairy products using a chemiluminescent detection system (Karoonthaisiri et al. 2009). This system has a similar sensitivity to conventional ELISA for bacterial detection, though with a much shorter assay runtime. The antibody array is inexpensive, as it utilizes a very small amount (micrograms) of the capture and horseradish peroxidase (HRP) antibodies leading to the reduction of the assay cost. Additionally, this array has more advantages including its ability to test multiple samples in parallel. Furthermore, lesser amounts of reagents are required, and the long storage feasibility of the antibody enables its wide adoption for large-scale screening processes in large industrial plants. Other benefits include high sensitivity and absence of cross-reactivity. However, a fluorescent scanner that can read a

**Table 1** Detection methods of pathogens and toxins in dairy products highlighting their applications

Technique	Properties	Applications	Reference
Enzyme-linked immunosorbent assay (ELISA)	<b>Advantages:</b> sensitive, inexpensive, rapid, simple, and reliable <b>Disadvantages:</b> labor-intensive	Identification of - pathogens, e.g., <i>E. coli</i> & <i>S. aureus</i> - toxins, e.g., AFM <sub>1</sub> & botulinum toxin serotypes A, B, E & F	(Anfossi et al. 2008; Singh et al. 2015)
Antibody array using chemiluminescent detecting system and poly-L-lysine glass	<b>Advantages:</b> Relative short run time, test multiple scarce samples in parallel, small amount of reagent is required, long storage of the antibody, sensitive, low cost, no cross-reactivity, absence of non-specific background <b>Disadvantages:</b> expensive fluorescent scanner <b>Advantages:</b> ultrahigh sensitivity	- Detection of <i>E. coli</i> & <i>Salmonella</i> spp.	(Karoonthaisiri et al. 2009)
Quantum bead-based fluorescence-linked immunosorbent assay (FLISA)	<b>Advantages:</b> time-saving, user-friendly, low cost, multiplex detection, suitable for detection of low molecular weight target or if it is present as a single specific antigen	- Detection of aflatoxinM <sub>1</sub>	(Zhou et al. 2019)
Lateral flow immunoassay integrated with competitive and sandwich model	<b>Advantages:</b> low cost, sensitive, performing variety of tests, single-cell detection of the activity of injured and starved bacteria, rapid detection of specific bacterial cell <b>Disadvantages:</b> low signal to noise ratio, provide a low noise to the background imaging system, need for afterwards imaging processing	Detection of - <i>E. coli</i> O157:H7 - AFM <sub>1</sub>	(Wang et al. 2018)
Portable 3D printed smartphone-based fluorescence imager using classical sandwich ELISA	<b>Advantages:</b> Good performance with low detection limit, high specificity, acceptable reproducibility, and excellent biocompatible microenvironment for the immobilized antibody	- Detection of <i>E. coli</i> O157:H7	(M. M. A. Zeinoh et al. 2018)
Biosensor (immunosensor)/electrochemical detection	<b>Advantages:</b> fast analysis (within 3 min), good sensitivity (0.6 log CFU/g or /mL) and accuracy (more than 90%) <b>Disadvantages:</b> need an enrichment step (24 h)	- Detection of <i>E. coli</i>	(X. Zhang et al. 2017)
Biosensor/potentiometric detection	<b>Advantages:</b> portable, ability to observe antibody-antigen interaction, simple, inexpensive, fast, good sensitivity (LOD = 0.02 µg/L), minimized ink usage	- Detection of <i>L. monocytogenes</i>	(Hadjilouka et al. 2020)
Biosensor/chronoamperometric detection	<b>Advantages:</b> short analysis time, no matrix interference	- Detection of AFM <sub>1</sub>	(Abera et al. 2019)
Biosensor/impedimetric detection		- Detection of AFM <sub>1</sub> and M2	(Kanungo et al. 2014)

Table 1 (continued)

Technique	Properties	Applications	Reference
Chromatography (HPLC-FLD)	<b>Advantages:</b> high sensitivity, good accuracy, and reliability <b>Disadvantages:</b> tedious sample preparation, requiring highly professional technicians and a sophisticated instrument	- Detection of AFM <sub>I</sub>	(Mao et al. 2015; Shuib et al. 2017)
ELISA-immunoaffinity column (IAC)	<b>Advantages:</b> specific, no interference peaks, short analysis time <b>Disadvantages:</b> limited toxin adsorption, interferences of an antibody to other matrix components, expensive, time-consuming	- Detection of AFM <sub>I</sub>	(Chavarría et al. 2015)
Chromatography (HPLC-FLD) using dispersive liquid-liquid microextraction (DLLME)	<b>Advantages:</b> high sensitivity, accuracy, no mass spectrometry signal interference, simple, quick, effective, reduced solvent consumption, and high enrichment factors <b>Disadvantages:</b> high expense, complexity, and inefficiency, as well as a high susceptibility to sample pH and low aflatoxins recoveries	- Determination of aflatoxins AFs	(Hamed et al. 2019)
Chromatography (UHPLC-MS/MS)	<b>Advantages:</b> rapid, accurate, automated analysis, high throughput, no analytes loss, reusable cartridge, low consumption of organic solvent, minimal handling of samples <b>Disadvantages:</b> one-step sample extraction, quick, no derivatization step, sensitive, specific, structural determination of contaminant	- Analysis of AFM <sub>I</sub>	(Campone et al. 2016)
Chromatography (LC-MS/MS)	<b>Disadvantages:</b> very high cost and inappropriate for onset testing	- Determination of ochratoxin A and AFM <sub>I</sub>	(Sulyok et al. 2010)
Chromatography (LC-MS/MS) with ESI in multiple reaction monitoring mode	<b>Advantages:</b> selectivity, simultaneous identification & quantification of mycotoxins with good sensitivity LOD (0.006–0.3 µg/kg), and quantification limits LOQ (0.02–1.0 µg/kg), accuracy, and precision	- Detection zearalenones, aflatoxins, ochratoxins, trichothecenes, and fumonisins	(Xie et al. 2015)
Chromatography (Bidimensional LC-MS/MS)	<b>Advantages:</b> powerful analytical platform <b>Disadvantages:</b> constructing databases for protein recognition, grouping redundant proteins, taxonomic and functional annotation are big challenges, requiring dedicated algorithms and software	- Determination of the effect of clostridia spores and lysozyme on microbiota dynamics	(Heyer et al. 2017; Soggiu et al. 2016)
Chromatography (LC-MS/MS)	<b>Advantages:</b> good sensitivity with large linear range from 0.5 to 25 mg/kg and from 10 to 200 mg/kg. The LOD and LOQ were 0.03 and 0.1 mg/kg, respectively <b>Disadvantages:</b> time-consuming for sample preparation and LC run cycle	Determination of sterigmatocystin in cheese	(Veršilovskis et al. 2009)

Table 1 (continued)

Technique	Properties	Applications	Reference
Quantitative polymerase chain reaction (qPCR)	<p><b>Advantages:</b> detects DNA (in viable or non-viable cells), easily automated for screening of food samples in a short time, high-throughput analysis capabilities, specificity and high sensitivity</p> <p><b>Disadvantages:</b> cannot differentiate between live and dead cells</p>	<ul style="list-style-type: none"> <li>- Detection of STEC and <i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i></li> </ul>	(Botsaris et al. 2010) Farrokh et al. 2013)
Quantitative reverse transcription PCR (RT-qPCR)	<p><b>Advantages:</b> simple, sensitive, and reproducible technique for quantification of relative transcript levels, with the highest sensitivity for detecting low abundance mRNA</p> <p><b>Disadvantages:</b> difficulty in detection of enteric viruses, a good normalization is needed, expensive due to probes</p>	<ul style="list-style-type: none"> <li>- Detection of norovirus</li> <li>- Quantification of staphylococcal enterotoxin</li> </ul>	(Duquenne et al. 2010)
Multiplex polymerase chain reaction (mPCR)	<p><b>Advantages:</b> an alternative to MVLTST for the rapid screening of epidemic clone strains, used to perform preliminary screening prior to gene expression studies in vitro or in situ</p> <p><b>Disadvantages:</b> a PCR simplex for each virulence-associated gene is used because mPCR could not be developed although testing of various protocols and amplification conditions have been reported</p>	<ul style="list-style-type: none"> <li>- Detection of virulence-associated genes and epidemic clone-markers in <i>L. monocytogenes</i> isolates</li> </ul>	(Lomonaco et al. 2012)
PCR-restriction fragment length polymorphism (PCR-RFLP)	<p><b>Advantages:</b> No prior sequence information is required nor oligonucleotide synthesis</p> <p><b>Disadvantages:</b> requires a large number of DNA samples</p>	Typing of <i>egc</i> loci	(Viçosa et al. 2013)
Immunomagnetic separation-polymerase chain reaction (IMS-PCR)	<p><b>Advantages:</b> high sensitivity, rapid</p> <p><b>Disadvantages:</b> low detection limit, false-positive results due to usage of non-specific primers</p>	Direct detection of <i>Brucella abortus</i> and <i>B. melitensis</i>	(Öngör et al. 2006)
PCR based on conversion NTP-binding genes	<p><b>Advantages:</b> fast, reliable, easily incorporated, phage monitoring in all stages of fermented dairy industry, specifying contaminated milk in processes that do not require a vulnerable starter organism or phage deactivating conditions</p>	Detection and identification of <i>Lactobacillus casei</i> and <i>L. paracasei</i> phages	(Binetti et al. 2008)

Table 1 (continued)

Technique	Properties	Applications	Reference
Next-generation sequencing	<p><b>Advantages:</b> high specificity more than RT-PCR, more effective for strains identification than traditional sequencing techniques, fast, powerful tool for insight into community biodiversity &amp; function, identification of new genes &amp; metabolic pathways encoded in the community</p> <p><b>Disadvantages:</b> complex in procedure and analyses, expensive</p>	Assessment of bacterial and fungal diversity and pathogenic strains from shotgun metagenomic data	(Aaron M. Walsh et al. 2017; Xu et al. 2015)

plate format is usually expensive and limit its use in simple laboratory setups (Karoonthaisiri et al. 2009).

Mycotoxins have been extensively analyzed using ELISA. Aflatoxin M1 (AFM1) is the only mycotoxin for which maximum levels have been set (0.5 & 0.05 ppb in the milk used for cheesemaking in China/USA and EU, respectively) (Hymery et al. 2014). ELISA-based methods have shown to be efficient, fast, and inexpensive screening methods for AFM1. AFM1 has been identified using ELISA-based methods in milk and buttermilk in Kenya revealing that all examined samples encompassed mycotoxins surpassing the recommended detection limit (5 ng/kg) (Kuboka et al. 2019). Two-thirds of the tested samples showed AFM1 level exceeding 50 ng/kg. Many factors contributed to this contamination including the general public's poor knowledge of aflatoxins' nature, which affects their management, and poor agriculture practices. For example, instead of green forage, some farmers relied on feed concentrates and stored feed. Additionally, the prevailing high humidity was conducive for fungal growth and mycotoxin production in fermented foods (Kuboka et al. 2019). In Jordan, the analysis of fermented milk (buttermilk) samples was also performed using ELISA (Omar 2012). All samples were shown to be contaminated with AFM1 at a range of 47.97–2027.11 ng/kg. It is important to point out that farm pastures in Jordan are not commonly available, and nourishing milkers with feedstuffs is popular, particularly in the dairy industry, which contributed to the reported results (Omar 2012). Moreover, AFM1 is primarily dissolvable in milk's watery phase or adsorbed to the casein particles leading to high levels in buttermilk (Gürbay et al. 2006; Var and Kabak 2009). Finally, AFM1 was detected in several dairy products using ELISA in many countries. In Turkey for example, AFM1 quantity has been reported surpassing the permissible limits (50 ng/kg) in raw milk, UHT milk, and yogurt samples (Temamogullari and Kanici 2014) and ranged from 51 to 850 ng/kg in different cheeses (Atasever et al. 2010).

Recent advances in ELISA-based methods were incorporated to improve the technique sensitivity. The sensitivity of conventional HRP-based ELISA catalytic tetra-methylbenzidine is often insufficient for the identification of AFM1 as it ranges between 0.1 and 10 ng/mL (Swierczewska et al. 2012). Novel signal transducers have been applied to improve ELISA's sensitivity, but these procedures are not compatible with the standard ELISA platform (Song et al. 2018). Recently, quantum bead-based fluorescence-linked immunosorbent assay was used for AFM1 determination in yogurt and pasteurized and powdered milk. Quantum dot beads (QB) with a diameter of 150 nm were used as the carrier of competing antigen. QB utilization instead of HRP protein has led to increased fluorescent signal and decreased binding affinity of the competing antigen to antibodies and less time consuming than conventional HRP-based ELISA. Detection limits as low as 0.6 pg/mL in yogurt, 0.5 pg/mL in



pasteurized, and 0.72 pg/mL in milk powder were observed (Zhou et al. 2019).

In comparison to conventional ELISA that requires professional operation, lateral flow immunoassay is an approach that is relatively easy to use, time-saving, and inexpensive (Wang et al. 2018). Gold nanoparticle-based lateral flow immunoassay (GNP-LFI) combined with competitive and sandwich systems could detect pathogens, e.g., *E. coli* and mycotoxins (i.e., AFM1) in milk. Nevertheless, the detection limits for *E. coli* O157:H7 and AFM1 were at  $1.58 \times 10^4$  colony-forming units (CFU)/mL and  $50 \text{ pg} \cdot \text{mL}^{-1}$ , respectively (Wang et al. 2018).

A portable 3D-printed smartphone-based fluorescence imager was also used recently to detect *E. coli* in yogurt using classical sandwich ELISA and the specific recognition of antibody to *E. coli* O157:H7 (M. M. A. Zeinhom et al. 2018). With a detection limit of 1 CFU/mL, this device was able to detect and quantify *E. coli* O157:H7 from 1 to  $10^6$  CFU/mL. The imager embraces a long-pass thin-film interference filter, a compact laser diode-based photo source, and excellent insert lenses to minimize noise and losses. Compared to other fluorescent-labeled techniques, this device exhibited great potential owing to its multipurpose and high-quality camera. Additionally, it presents a proper platform for performing a variety of tests including nucleic acids and small molecules due to its feasibility. Moreover, the whole process could be finished rapidly within 2 h. Despite all of these advantages, fluorescent detection on this device is still a challenge owing to the low signal-to-noise ratio and the need for afterwards image processing as in the case of other smartphone-based devices (M. M. A. Zeinhom et al. 2018).

## Biosensor Methods

Biosensors are defined by the International Union of Pure and Applied Chemistry as integrated receptor-transducer devices that capture a biological signal and convert it into a detectable electrical signal (McNaught and Wilkinson 1997). Biosensors are analytical devices that comprise a biological material (biorecognition element) and a physicochemical (e.g., electrochemical, magnetic) transducer of various types. The bioreceptors (e.g., antibodies, enzymes, nucleic acids) recognize the target analyte, whereas the physical transducer translates the response into an electrical signal (Dunne et al. 2005; Parker and Tothill 2009). Biosensor that is based on using antibody (as a bioreceptor) for specific molecular recognition of antigens (immunologic reaction) is called immunosensor (Lim and Ahmed 2019). Biosensors are simple, low-cost, portable, facile to run, and sensitive and allow rapid “real-time” monitoring and multiple analyses that are essential for successful implementation of any hazard analysis critical control point (HACCP) (Ali et al. 2020; Suherman et al. 2021).

A portable cell-based biosensor device was developed for *L. monocytogenes* detection in milk and cheese. It depends on measuring the cell membrane potential changes according to the principle of the bioelectric recognition assay. This technique offers a fast analysis (results within 3 min) concomitant with good sensitivity ( $0.6 \text{ log CFU/g}$  or /mL) and accuracy (more than 90%) (Hadjilouka et al. 2020). An immunosensor with high sensitivity has been developed for *E. coli* electrochemical assay by utilizing poly (diallyldimethylammonium chloride)-functionalized graphene oxide (GO-PDDA) and gold nanoparticles (AuNPs) via a simple sonication-induced assembly. Additionally, the (dAb-Au-THi) nanoprobe were constructed as biorecognition elements by exploiting the amplification effect of AuNPs to load detection antibody (dAb) and enormous thionine (THi). This approach was utilized on fresh milk, as well as expired and unexpired yogurt (X. Zhang et al., 2017). The assay showed high sensitivity (detection limit of 35 CFU/mL), selectivity, and reproducibility for *E. coli* and hence represents a potential platform to determine contaminated dairy products with pathogenic bacteria (X. Zhang et al. 2017).

A flexible biosensor has been used to analyze AFM1 in dairy samples by a chronoamperometric technique using dispense-printed electrodes, which was operated using single-walled carbon nanotubes (SWCNTs) functionalized on dispense-printed electrodes and coated with monoclonal antibody (mouse IgG). The sensor exhibited a relative rapid response (25 min) with a low limit of detection ( $\text{LOD} = 0.02 \text{ } \mu\text{g/L}$ ) compared to unfunctionalized sensor ( $\text{LOD} = 0.049 \text{ } \mu\text{g/L}$ ) or simple sensor ( $\text{LOD} = 0.039 \text{ } \mu\text{g/L}$ ) using antibody-modified screen-printed carbon working electrode with carbon counter and silver-silver chloride pseudo-reference electrode (Parker and Tothill 2009; Abera et al. 2019). Screen printing is a common technique for biosensor fabrication (Yamanaka et al. 2016) that requires a mask to structure biosensors, high amount of materials, and a precise thickness modulation. In this regard, the dispense-printing enables the use of any printing material and production of capricious shapes, presenting a layout for custom-made and application-tailored biosensors (Abera et al. 2019). An impedimetric immunosensor without a label has been developed for the analysis of AFM1 and AFM2 in a flow-based setup in milk products. This sensor could analyze aflatoxin M1/M2 in a complex matrix, e.g., drinking yogurt. It could also detect these toxins at low levels as  $1 \text{ ng/L}$  under the flow configuration (Kanungo et al. 2014).

## Chromatographic Methods

### Sample Pre-treatment Methods

To remove interferences and achieve satisfactory recoveries, it is crucial to implement a successful protocol for the cleanup and extraction of the targeted analyte which

are generally time consuming. The cleanup methods rely mainly on liquid/liquid extraction, solid-phase extraction (SPE), or both. For example, aflatoxins are rather soluble in polar organic solvents (chloroform, methanol, acetonitrile, etc.) than in non-polar organic ones such as hexane (Garcia et al. 1994) though liquid–liquid extraction has been used to extract aflatoxins from different food matrices (Hamed et al. 2017). SPE has been used as a replacement for conventional liquid–liquid extraction as it is more sample- and solvent-efficient, selective, and flexible due to the availability of a wide range of stationary phases for different analytes (K. Zhang and Banerjee 2020). Immunoaffinity-based extraction methods are very close to SPE methods where the sorbent is containing immobilized antibodies that present a specificity or a high affinity towards a target analyte or a group of structural analogs (Scott and Trucksess 1997). Immunoaffinity-based extraction was applied for the purification of AFM1 from different fermented dairy products (Iha et al. 2011; Chavarría et al. 2015).

A wide range of chromatographic methods have been utilized for detecting microbial products in FDP. Thin-layer chromatography (TLC) is a standard method of Association of Official Analytical Chemists (AOAC) for AF analysis since 1990 (Vaz et al. 2020). Although TLC was employed to analyze AFM1 in different cheese samples (Filazi et al. 2010), it suffers from low accuracy with no adequate LOQ (Mulunda et al. 2013; Vaz et al. 2020). High-performance liquid chromatography coupled with UV detector was used for patulin detection, whereas HPLC coupled with fluorescence detector (HPLC-FLD) is used for the quantitative detection of AFM1 and OTA (Oztürk Yilmaz and Altinci A 2018; Pattono et al. 2013). Moreover, liquid chromatography coupled with mass spectrometry (LC–MS) was recommended as the best choice to properly carry out mycotoxin quantification in food by different agencies as US Food and Drug Administration (FDA), Brazilian Health Surveillance Agency (ANVISA), and the Chinese Pharmacopoeia Commission (CP) (Medina et al. 2021). In addition, LC/MS is considered the most sensitive technique for mycotoxin analysis in cheese (Hymery et al. 2014; Kokkonen et al. 2005). However, HPLC methods generally require a sophisticated instrument, highly professional technicians, and tedious sample preparation (Mao et al. 2015; Shuib et al. 2017). The HPLC-FLD approach based on the core–shell column (core–shell silica consists of a solid core coated with a layer of porous silica (Hayes et al. 2014) has been developed to detect AFM1 in raw milk samples, with a detection limit (LOD) of 0.005  $\mu\text{g}/\text{kg}$  and a quantification limit (LOQ) of 0.015  $\mu\text{g}/\text{kg}$ . This core–shell column increased the analytical performance in comparison with a conventional fully porous column with no expensive hardware updates. It was observed under the same instrumental conditions that the peak height of column with 2.6- $\mu\text{m}$  core–shell particles

(4.6 mm  $\times$  100 mm) is 159% and 368% of traditional totally porous 3- $\mu\text{m}$  particles (4.6 mm  $\times$  150 mm) and 5- $\mu\text{m}$  particles (4.6 mm  $\times$  250 mm) columns. Furthermore, without solvent replacement, this approach simplified sample preparation, allowing for the direct analysis of raw milk samples (Mao et al. 2015). HPLC-FLD method was also used with post-column photochemical derivatization to detect AFM1 in milk and FDP. AFM1 could be detected using this method at levels below 0.004  $\mu\text{g L}^{-1}$ . Unlike chemical derivatization, the method of photochemical derivatization does not involve organic and chlorinated solvents making it more environmentally friendly.

A reversed-phase HPLC-FLD method was developed and validated for the determination of AFM1 levels in various types of cheese, yogurt, and milk beverages in Brazil using immunoaffinity chromatography (IAC) for the prior purification and isolation of AFM1 (Iha et al. 2011). The recovery was relatively low and ranged from 71 to 76% for cheese and yogurt, respectively. However, it displayed satisfactory sensitivity with an LOD and LOQ of 3 ng/kg and 10 ng/kg, respectively.

AFM1 was also detected in pasteurized milk and fresh soft cheese using IAC as a purification step prior to HPLC-FLD analysis for samples collected from different cheese brands in Costa Rica (Chavarría et al. 2015). Although IAC cleanup-based approach offers advantages in providing column specificity to analytes, toxin adsorption appeared rather limited due to antibody interferences with other matrix components. Enzymatic hydrolysis-based extraction was thus applied for fat and protein removal from cheese to improve AFM1 recovery in cheese and other dairy foods. AFM1 levels in analyzed milk and cheese surpassed acceptable limits (FDA limit: 500 ng/L and EC limit: 50 ng/L) (Chavarría et al. 2015). Aflatoxin G1 was also detected in white cheese in Saudi Arabia using the same platform, with processing, ripening, and storage condition suggested to be the source of contamination (M. M. Aly et al. 2010). The same approach was also tested in other countries including Portugal, Turkey, and South Korea (Kim et al. 2000; Martins and Martins 2004; Sahin et al. 2016).

HPLC-FLD has been reported for the identification of aflatoxins B1/B2/G1/G2/M1 in yogurt using dispersive liquid–liquid micro extraction. Limits of quantifications for AFs were found below 50 ng kg<sup>-1</sup> (Hamed et al. 2017) providing high sensitivity, accuracy, and no mass spectrometry signal interference allowing for the determination of these mycotoxins at trace levels. However, this method has several drawbacks including high cost, complexity, inadequacy, and high sensitivity to the sample pH, thus low recovery for some AFs (Hamed et al. 2019, 2017).

The analysis of AFM1 in dairy products (i.e., whole and skimmed cow's milk, yogurt, goat's milk, and powdered baby milk) has been achieved via online solid phase extraction

(SPE) combined with ultra-high-performance liquid chromatography coupled with tandem MS (SPE-UHPLC-MS/MS). This quick and automatic analytical method allows for the accurate determination of AFM1 in less than 20 min by salt-induced liquid–liquid extraction after removal of protein. Compared to offline SPE, online SPE offers high throughput that does not involve loss of analytes, allows reusing cartridges, and involves use of small amounts of organic solvent and minimal sample handling. Moreover, method quantitation limits were  $< 4.0 \text{ ng kg}^{-1}$ , that is *ca.* 25-fold lower than AFM1 acceptable limits, and with no significant matrix interference effects. As such, this method can be applied for the regular screening and quality assurance of fermented dairy products (Campono et al. 2016).

Core–shell poly (dopamine) magnetic nanoparticles were used as dispersive solid phase extraction (dSPE) sorbent to extract 6 mycotoxins including  $\alpha$ -zearalanol,  $\beta$ -zearalanol,  $\alpha$ -zearalenol,  $\beta$ -zearalenol, and zearalenone from complex matrices such as dairy and yogurt before their LC–MS analysis providing good linearity. Dopamine has the ability of self-polymerization in aqueous phase under weak alkaline conditions resulting in the formation of a surface-adhesive film onto a diversity of materials. LODs were between 0.21 and  $4.77 \text{ }\mu\text{g/L}$  for milk samples versus 0.29 and  $4.54 \text{ }\mu\text{g/kg}$  for yogurt samples. Magnetic nanoparticles are most widely used in dSPE owing to their ability to be easily separated by an external magnet from the matrix. This method provides fast, simple, cheap, and environmentally friendly approach for the extraction of mycotoxins (González-Sálamo et al. 2017).

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) provides high selectivity, which allows for multi-analytes to be determined without sample cleanup. Most of the existing LC approaches that cover many fungal metabolites are currently used for qualitative screening of fungal metabolites rather than for quantitative analysis (Nielsen & Smedsgaard 2003), which is partly a consequence of the availability deficiency of acceptable criteria. The use of MS provides quantitative detection of mycotoxins in crude extracts, as long as the extraction competences and matrix impacts are appropriately defined for all the analyte/matrix combinations under investigation. Consequently, LC–MS/MS-based multi-mycotoxin approach was used for the semi-quantitative detection of mycotoxins such as fumonisin B1 and B2 and ochratoxin A (OTA) in moldy crème fraiche (French soured cream) samples collected from private households in Austria, several of which were above the regulatory limits. The contamination levels ranged from 300 to  $16,000 \text{ }\mu\text{g/kg}$  (Sulyok et al. 2010).

LC–MS/MS was also reported for the detection of OTA in hard cheese in Northern Italy using a one-step sample extraction method. The extraction was performed with

acetonitrile/water found optimal for OTA solubility and low extraction of lipophilic interferences. Furthermore, avoiding the use of IAC reduced the time and cost as well as random errors and prevented systemic error sources (Biancardi et al. 2013). In addition to OTA, other mycotoxins, i.e., roquefortine C and mycophenolic acid, were also detected in blue cheese after fat removal by methanol and single liquid extraction. The LOQ was at  $5 \text{ }\mu\text{g kg}^{-1}$ . However, while this method is suited for multi-toxin detection simultaneously from complex matrix with high selectivity and sensitivity, it failed to detect cyclopiasonic acid. In China, mycotoxins including zearalenone, AF, OT, trichothecene, and fumonisin were detected in dairy, powdered milk, yogurt, and cheese sample using low-temperature cleanup followed by SPE and LC–MS/MS in a multiple reaction monitoring (MRM) mode. This proposed approach has shown satisfactory validation parameters in different matrices comprising detection, sensitivity, matrix effects, linearity, LOD ( $0.006\text{--}0.3 \text{ }\mu\text{g/kg}$ ), LOQ ( $0.02\text{--}1.0 \text{ }\mu\text{g/kg}$ ), accuracy, and precision. In addition, it has been utilized in regular examination for simultaneous identification and quantification of mycotoxins from dairy samples (Xie et al. 2015). OTA and citrinin produced by *P. verrucosum* were detected in crème fraiche using LC–ESI–MS/MS-based method (Sulyok et al. 2010). Direct injection and tandem mass spectrometry (DI–MS/MS) combined with (LC–MS/MS) was used for profiling AFs upon ingestion in dairy cows fed no sequestering agents using receiver-operator characteristic (ROC) curves. The results revealed that arginine, methylhistidine, alanine, and citrulline were potential biomarkers of AF intake in milk cows fed no sequestering agents, with high selectivity and susceptibility (area under the ROC curve = 0.986) (Ogunade et al. 2019).

A chromatographic method was developed for the determination of sterigmatocystin in cheese based on immunoaffinity column (IAC) for an efficient sample cleanup prior to analysis via LC–MS/MS. This leads to high recoveries from cheese samples reaching 104% with a good precision (RSD) of 2.9% alongside a good limit of quantification of  $0.6 \text{ }\mu\text{g/kg}$  (Marley et al. 2015) which is about 3 order of magnitude lower than that reported with classical SPE followed by LC–MS/MS analysis (Veršilovskis et al. 2009).

A metaproteomic approach based on bidimensional LC–MS/MS permitted the identification of various protein levels of microorganism subjected to different stresses which was used to determine the effect of *Clostridium* spores and lysozyme on microbiota functional dynamics in Italian hard cheese (Grana Padano cheese), with aging process identified as the main source of contamination (Soggiu et al. 2016). Although this approach presents a potential platform for complex matrixes, it requires complex software and hardware for protein functional annotation and data visualization (Heyer et al. 2017).

Many analytical methods were used to assess biogenic amines such as ion-exchange chromatography with colorimetric detection, GC–MS, HPLC–MS/MS, and HPLC–DAD (Rabie et al. 2011; Kandasamy et al. 2021; Loizzo et al. 2013) and capillary electrophoresis coupled with contactless conductivity detection (Adımcılar et al. 2018). Hazardous levels (> 100 mg/kg) of biogenic amines including histamine and tyramine were reported in Mesh and blue cheese in Egypt using ion-exchange chromatography-colorimetric detector, based on protein separation according to their surface charge. These amines are derived from microbially mediated decarboxylation of free amino acids, e.g., *Escherichia* and *Klebsiella* (Rabie et al. 2011).

### Molecular Biological Methods

Molecular methods based on nucleic acids and genomic markers correspond to specific nucleic acid sequences, and they are nonculture-dependent methods. They have the advantage of high specificity and discriminative ability

between closely related microorganisms at the genus level. Most molecular biological approaches are based on identifying specific sequence targets in microorganisms (Feron et al. 2020).

Conventional polymerase chain reaction (PCR), “real-time” PCR, and next-generation DNA sequencing are the most widely utilized molecular-based techniques that could be applied in a qualitative or quantitative setting to identify and/or quantify one or multiple targets simultaneously (Fig. 3; Table 1). However, these methods require good DNA isolation techniques and expensive equipment and may detect viable and non-viable pathogens (Rico-Munoz et al. 2019).

Real-time “quantitative” polymerase chain reaction (qPCR)–based method was validated to detect *L. monocytogenes* in soft cheese samples that were naturally contaminated showing higher performance than the international organization for standardization (ISO) 11,290–1 standard method (Gianfranceschi et al. 2014). This trial included twelve laboratories from six European countries, and the limit of detection reached 10 CFU per 25 g of sample

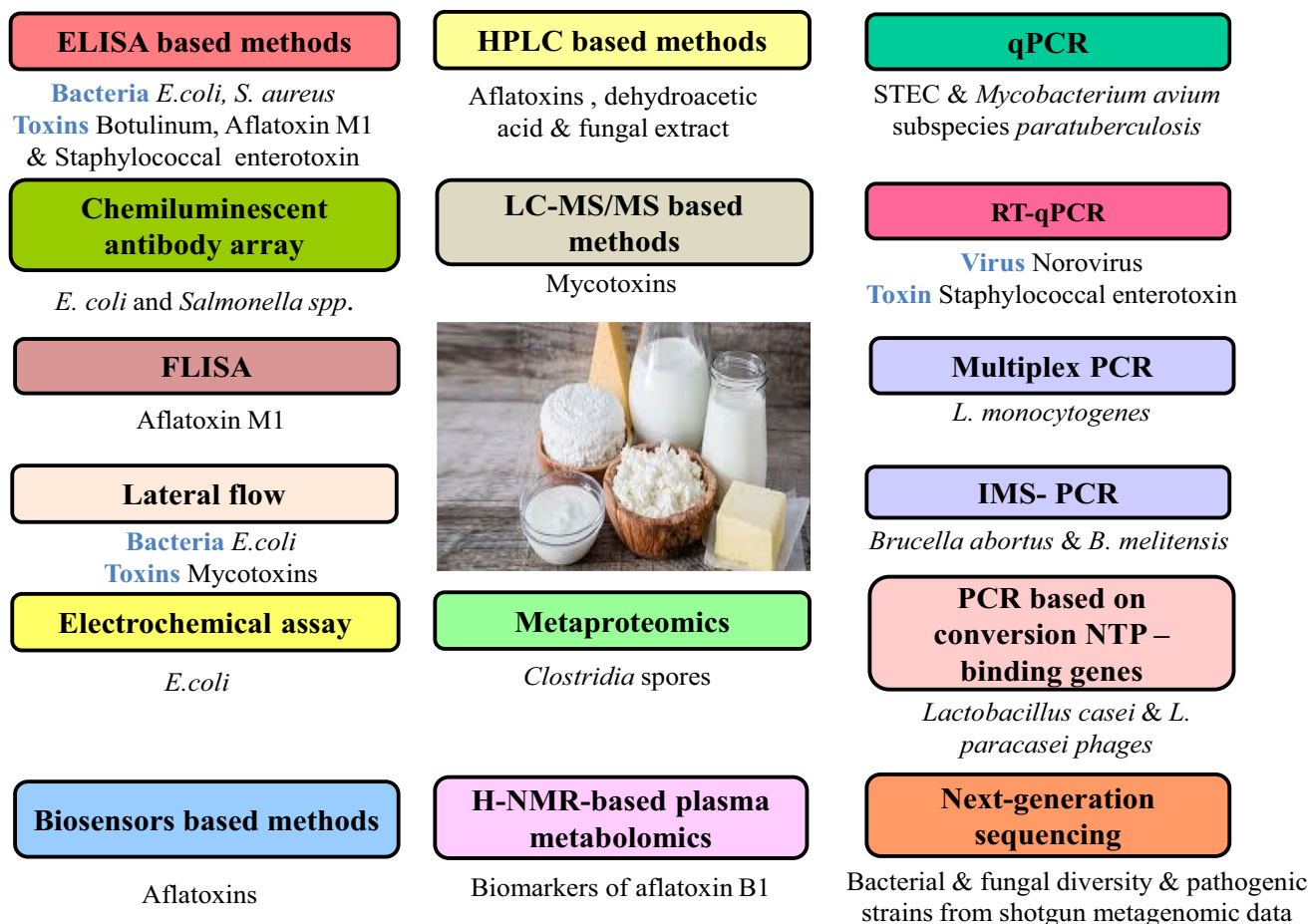


Fig. 3 Different approaches for the detection of different pathogenic microorganisms and toxins in fermented dairy products

indicating outstanding sample-to-laboratory concordance values (N75%). Additionally, relative sensitivity, specificity, and accuracy had excellent values (97.6%, 96.7%, and 82.7%, respectively). This methodology has been based on an ISO compatible enrichment coupled with a bacterial DNA extraction and a consolidated “real-time” PCR assay. Nevertheless, the complexity of the DNA extraction procedure in this method could contribute to false-negative results (Gianfranceschi et al. 2014). Rapid detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in processed bulk tank milk in Cyprus was also reported using qPCR and combined phage IS900 PCR. In contrast, viable *M. paratuberculosis* was detected in cheese samples from different milk types by only using qPCR. It is important to highlight that the qPCR method detects DNA from viable as well as dead cells, whereas the phage-based method only detects DNA from viable cells. Loss of viability associated with these methods is not as strong as in the case of conventional culturing methods (Botsaris et al. 2010).

The quantitative reverse transcription PCR (RT-qPCR) method was applied to quantify the expression of staphylococcal enterotoxin A- and D-encoding genes (i.e., *sea* and *sed*) in semi-hard and soft cheeses. The multiple house-keeping reference genes were used for expression of data normalization. Furthermore, this normalization strategy helped in determining gene expression levels for even low abundance species during all stages of cheese manufacture (Duquenne et al. 2010). RT-qPCR application in pasteurized feta cheese samples in Egypt revealed low levels of *seb* gene (encode *S. aureus* enterotoxin), with such contamination mostly attributed to faulty processing and poor handling and hygienic practices (Zeinhom et al. 2015). PCR-restriction fragment length polymorphism (PCR-RFLP) was applied to explore the genetic variability and the distribution of *egc* loci in different staphylococcus aureus strains. The existence of individual enterotoxin gene clusters (*egc*) of *S. aureus* in raw milk and cheese was determined using multiplex (*seg*, *sei*, *sem*, and *seo*) or simplex (*sen* and *seu*) PCR. In spite of a little iteration of *egc*-carrying isolates identified by PCR-RFLP, a large number of isolates were found to be carriers of the entire collection of individual *egc*-related genes suggesting the existence of these staphylococcal enterotoxins' genes outside *egc* loci or the presence of variants of *egc* locus. It was also suggested that geographical and sample origin influenced the incidence and prevalence of the various *egc* classes. Although no prior sequence information is required nor oligonucleotide synthesis for RFLP, it requires a large number of DNA samples (Viçosa et al. 2013).

In a study for the identification of *L. monocytogenes*, serotyping, using pulsed field gel electrophoresis, virulence-related genes (*actA*, *inlJ*, *plcA*, *prfA*, *hlyA*, and *iap*) were

detected in almost all isolates (94%) from PDO Gorgonzola cheese using PCR. In addition, epidemic clone V (one of serotypes 1/2a) of *L. monocytogenes* was also detected in many samples using multiplex PCR assay. Multi-virulence-locus sequence typing methods (MVLST) represent a sensitive approach for the detection of virulence genes but are considered expensive and time consuming. Thus, multiplex PCR screening may be more useful or rapid in assessing the potential for epidemics and public health threats caused by *L. monocytogenes* in PDO Gorgonzola cheese and other milk products. It should be noted that one isolate was misidentified as *L. monocytogenes* probably owing to atypical phenotype and resulted negative by PCR, later identified as *L. innocua* by 16S rRNA gene sequencing analysis (Lomonaco et al. 2012). In Egypt, molecular characterization with positive amplification of *nuc*, *coa*, and *mecA* genes was used to detect methicillin-resistant *Staphylococcus aureus* (MRSA) in raw milk, Damietta cheese, and Kareish cheese. The mean *S. aureus* counts in raw milk, Damietta cheese, Kareish cheese, and yogurt ranged at  $3 \log^{10}$  CFU/g. In addition, all isolates were reported to contain genes encoding hemolysin (*hla*) and staphylococcal enterotoxins (*sea*, *seb*, and *sec*) using PCR technique. The reason for contamination was attributed to a lack of hygienic procedure in the manufacturing, retail, or storage processes (Al-Ashmawy et al. 2016).

High levels of contamination in yogurt, doogh, and kashk fermented dairy products with *E. coli* strains were identified in Iran using PCR and antimicrobial susceptibility testing. Results indicated that O157 and non O157 Shiga toxin-producing *E. coli* (STEC) strains were the most common in samples, and that the most frequently observed characteristics of *E. coli* isolated strains were O157 serogroups; the *stx1*, *stx2*, *eaeA*, and *ehly* putative virulence genes; *tetA*, *tetB*, *blaSHV*, and *citm* antibiotic resistance genes; and tetracycline, penicillin, ampicillin, and streptomycin resistance genes. As observed in other cases, contamination might be due to cross-contamination and poor hygienic practices during processing and packaging of dairy products (Dehkordi et al. 2014).

Immunomagnetic separation–polymerase chain reaction (IMS-PCR) was used for direct identification of *Brucella abortus* and *Brucella melitensis* in soft cheese samples using sheep anti rabbit IgG, providing higher sensitivity than other procedures. The limit of detection (LOD) with (IMS-PCR) was as low as  $3 \times 10^2$  bacteria/mL, while the other PCR assay method without IMS yielded a ten-fold higher LOD ( $3 \times 10^3$  bacteria/mL). This methodology cannot only be used for the detection of brucellosis in complex food samples, e.g., FDP, but rather in large-scale epidemiological studies due to its pace and high sensitivity. However, it is liable to yield false-positive results due to the usage of non-specific primers (Öngör et al. 2006).

Detection of bacteriophages infecting *L. casei* and *L. paracasei* in artificial contaminated milk and other commercial samples (fermented milk and cheese whey) was achieved by targeting a highly conserved region of nucleoside triphosphate (NTP)-binding genes that regulates the replication modules of *L. casei* phages  $\phi$  A2 and  $\phi$  AT3 using PCR. This region was chosen due to similarity between both genomes as well as completely sequenced genomes of  $\phi$  A2 and  $\phi$  AT3. Additionally, the ability of this method to detect the phages in industrial samples aside from its simplicity poses it for employment in dairy industry routines for use in processes that do not require a vulnerable starter organism or phage deactivating conditions, considering the significance of phage monitoring in all stages of the dairy industry. This method has previously been used to successfully identify bacteriophages that infect a variety of lactic acid bacteria (LAB) including *Lactococcus lactis* (Labrie & Moineau 2000), *S. thermophilus* (Labrie & Moineau 2000), and *Lactobacillus lactis* (Zago et al. 2006) and, simultaneously, phages of three bacterial species of industrial relevance such as *Lactococcus lactis*, *S. thermophilus*, and *Lactobacillus lactis* (Binetti et al. 2008).

DNA sequencing has increasingly been utilized for microbial detection in FDP. This approach revolutionized several fields including food microbiology. *Rhizomucor variabilis*, a causative of foodborne illnesses in the USA, was detected in unopened vials of Greek yogurt using DNA sequencing of internal transcribed spacer region-1 (ITS-1). Highly accurate Sanger sequencing is usually used for rapid identification of sporadic cases, monitoring, and outbreak investigations (Sulaiman et al. 2014). Next-generation sequencing was used to detect microbial pathogens in homemade yogurt and to further study microbial diversity of yogurt based on its geographical origin and manufacturing process, and to further provide insight into the selection of starter cultures to be employed in manufacture (Xu et al. 2015). The microbiome in fermented dairy beverage (Nanu) was characterized using a combined 16S rRNA gene analysis and shotgun metagenomic sequencing approach. Metagenomic sequencing techniques are considered to be more effective for strain identification than culturing approaches, aside from its robustness and ease of identification of genes and metabolic pathways that are encoded within the microbial community. However, sequencing procedures are relatively complex and expensive and requires bioinformatics skill and expertise for data mining (Walsh et al. 2017). These tools were recently reviewed by Jagadeesan et al. (2019). In another study, bioinformatics-based mining of novel gene targets was reported for the identification of *Cronobacter turicensis* in contaminated powdered infant formula using PCR. All 4232 gene sequences of *C. turicensis* z3032 were aligned by using the Basic Local Alignment Search Tool (BLAST). Primer set CT11, targeting gene CTU\_19580, was selected for the development of the PCR assay. The detection limits of specific PCR assay were as low

as 760 fg/ $\mu$ L for genomic DNA (ca. 158 copies/ $\mu$ L of DNA) detecting *C. turicensis* with initial cell titer of 8.5 CFU per 10 g powdered infant formula (Chen et al. 2019). Although PCR-based methods have been widely used for the identification of pathogenic bacteria, they provide no information about the bacterial colony location and distribution within food matrices such as soured cream, yogurt, and cheese. Microscopic methods such as confocal laser scanning microscopy (CLSM) and cryo-scanning electron microscopy (cryo-SEM) can thus be used for that purpose (Hickey et al. 2015). A novel filtration-based RT-PCR method was developed for rapid and sensitive quantification detection of viable *Salmonella enterica* and *L. monocytogenes* in yogurt providing excellent detection, quantitative limits, and high accuracy compared to traditional microbiological methods (D'Urso et al. 2009).

## Conclusion and Future Perspectives

While fermented dairy products hold many health benefits, their contamination could cause serious health and economic losses warranting for effective early detection to be implemented in the fermented dairy industries, along the entire process until products reach consumers. Factors leading to such hazards include the susceptibility of the dairy product to cross-contamination during processing, or the contamination at the source. Herein, we reviewed the most recent and effective detection techniques, which target the most virulent microorganisms and or their associated toxins highlighting their advantages and limitations. Numerous detection methods are currently used to reduce the pathogen burden in fermented dairy products. Immunoassay-based methods (e.g., ELISA) are used for the detection of serious toxins (e.g., AFM1). Such methods are usually rapid, inexpensive, and in need of minimum laboratory equipment and authorization, but they are unable to measure all functional steps in intoxication pathways and hinder multi-residue methods and the detection of non-target analytes. Other novel analytical methods based on biosensors could be thus used for the analysis of these toxins. They are highly sensitive, fast, and easy to operate and pave the way to custom-made. Different chromatographic-based approaches have also provided the immense ability for detecting major pathogenic bacteria, mycotoxins, and biogenic amines. Chromatographic methods have numerous advantages such as high sensitivity and accuracy, but they also have some setbacks including cost, complexity, and tedious sample preparation. Molecular-based methods such as different types of PCR-based methods have been reported for the detection of bacteria and viruses. Although these methods pose good advantages including detection of DNA whether it is in viable or non-viable cells, easily automated for screening of food samples in short-time high-throughput

analysis capabilities, they cannot differentiate between live and dead cells. Next-generation sequencing methods are powerful to gain insight into community biodiversity and function, identify novel genes, and determine which metabolic pathways are encoded in the community. However, most of these tools produce short reads and require complicated procedures and analyses in addition to their high cost. Bioinformatics approaches are also robust tools to analyze and dissipate genomic data and of potential to be considered in dairy analysis. They provide high specificity in pathogenic strains identification in a short time. Whole genome sequencing (WGS) can be used for improvement of supplier and raw material management in addition to optimizing efforts on environmental pathogen verification programs. Combining two or more of these detection strategies will improve sensitivity and overcome some of each method's drawbacks and limitations. All the discussed analytical methodologies have advantages and disadvantages, yet it is crucial to be aware of the limitations of each methodology, whereas the preferred methods of detection and identification are both faster and highly sensitive which are essential for successful implementation of any hazard analysis critical control point. Finally, detection methods can help the producer to meet the safety and quality control measurements, while maintaining fermented dairy product flavor characteristics. Understanding physiochemical parameters is the way to control these hazards before finding a suitable way for their prevention. Development in the area of microbial contaminants and toxin discrimination is highly required because the benefits of rapidly detecting and determining spoilage/pathogenic bacteria and toxins earlier in fermented dairy products manufacture would be highly advantageous in terms of both safety and economic needs.

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## Declarations

**Ethics Approval** Informed consent not applicable.

**Conflict of Interest** Alaa S. El-Sayed declares that he has no conflict of interest. Hany Ibrahim declares that he has no conflict of interest. Mohamed A. Farag declares that he has no conflict of interest.

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