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Safety and quality assessment of hot-drinks vending machines in Southern Italy

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ABSTRACT

Vending machines (VMs) are common and convenient sources of various food and beverage items. Due to limited available information, concerns have been raised about the hygiene and safety of products dispensed by VMs. This study aims to assess the microbiological contamination of VMs in the Campania region (Italy), by combining microbiological and molecular biology analyses. The results revealed notable microbial contamination in VMs. Total Viable Count exceeded acceptable limits in VMs components, particularly in the inner walls of beverage dispensing nozzles and in the hot-drink product. Coffee and ginseng powders exhibited comparatively lower Total Viable Count values, while chocolate displayed none. Regarding pathogens, *Listeria monocytogenes* and *Salmonella* spp. were not detected in any of the samples subjected to analysis. *Bacillus cereus* (*B. cereus*) and *Staphylococcus aureus* (*S. aureus*) were detected in various VMs components. *B. cereus* contamination was prevalent, exceeding 80% in some surface swabs (water intake pipe, mixer bowl, and dispensing nozzle), and coincided with a high occurrence in the hot-drink final product. *S. aureus* was frequently found in milk powder, dispensing nozzles, and cappuccino samples. This research offers valuable insights into VM hygiene and safety, emphasizing the importance of multifaceted approaches to address microbiological contamination and promote public health. Regulatory compliance with cleaning procedures and proactive measures, such as robust self-monitoring plans and adherence to Good Manufacturing Practices, are crucial in mitigating contamination hazards and safeguarding product quality.

1. Introduction

Food away from home is a widely practiced eating habit, linked to changes in people's lifestyle over the last decades (Godbharle et al., 2022). Variations in consumers' food behavior are associated with the rapid increase in the female workforce; as a result, the busy schedules of both partners in a family have reduced the time available for cooking. Food away from home is generally defined as "full meals and single ready-to-eat items" provided by restaurants, grocery stores, and other markets (Ayala et al., 2008). In this scenario, vending machines (VMs) represent a popular and convenient way for people to access a wide variety of ready-to-eat foods and ready-to-drink beverages. VMs offer a broad range of products at affordable prices, supporting the concepts of timesaving, affordability, and "round-the-clock availability" with service accessible 24 h a day, 7 days a week, whether you're at work, traveling, or in school. During the Covid-19 pandemic, when traditional cafes were closed, and shops had limited supplies (Ratnasri & Sharmilan, 2021), VMs emerged as a safe alternative to crowded environments, such as bars and restaurants, offering hygienically prepared and packaged food and beverages while reducing excessive handling.

Recent data highlight the widespread presence of VMs in various sectors. Private companies, such as factories and offices, account for the majority of VM installations (34% and 17%, respectively). Public administrations, encompassing schools, universities, hospitals, and transit stations, contribute 20% to the distribution (Grech & Allman-Farinelli, 2015; Stoyanov, 2021). In Europe, there are approximately 3.8 million VMs, of which 2.36 million dispense hot-drinks (Boyano Larriba et al., 2019). Italy, in particular, is the international leader in terms of

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installations (820,000), and the world's largest VMs producer and exporter (Statista, 2019). In the Italian market, coffee and other hot beverages represent the largest share of VMs sales (64.5%), followed by water and cold beverages (20%) (Confida, 2022; Statista, 2019).

Hot-drinks VMs typically consist of the following components, from top to bottom: (1) a coffee beans container, where the beans are ground just before brewing to ensure freshness and flavor. (2) powder container compartments. (3) a mixer bowl that blends the powder with water to create the beverage. (4) a dispensing mechanism (dispense nozzle) responsible for delivering the prepared beverage into a cup. (5) a water pipe. There are two main types of hot-drinks VMs based on water supply: automatic and semi-automatic. In automatic machines, the water is directly sourced from the water supply network, ensuring a constant supply of fresh water for each drink preparation. On the other hand, semi-automatic VMs are equipped with a tank containing standing water periodically changed by the operator (Hunter, 1992; Robertson, 1987).

Several factors influence the safety of products dispensed by VMs. One key factor is the quality of raw materials and water used in beverage preparation. Substandard ingredients or contaminated water can pose a risk to consumer health. Operators must ensure the safety and quality of both the powders and the water in the tank (Dragoni & Bonomi, 2004). Regular cleaning and disinfection are another critical aspect. Maintaining the cleanliness of all the internal components is necessary to prevent microbial contamination and ensure product safety (Panisello & Quantick, 2001). Companies responsible for managing hot-drink VMs typically handle machine maintenance. The frequency of cleaning varies depending on factors such as machine type, location, and usage frequency (Dragoni & Bonomi, 2004).

In the EU, according to Regulation "EC 178/2002", the "food business operator" is responsible for the safety of dispensed beverages. It's worth noting that the vending sector lacks specific hygiene regulations and generally adheres to the guidelines outlined in Regulation "EC 852/2004" of the European Parliament and of the Council on the hygiene of foodstuffs, particularly regarding the Hazard Analysis Critical Control Points (HACCP) system, and the Regulation "EC 2073/2005" on microbiological criteria for foodstuffs. In this context, competent authorities are tasked with conducting official controls, employing an approach based on risk analysis, as established in Regulation "EU 2017/625".

Despite the widespread consumption of drinks and food from VMs, there is a notable lack of studies focusing on evaluating their hygienic and sanitary aspects. Hence, the objective of this investigation was to assess the hygienic and sanitary conditions of hot-drink VMs located in the Campania Region (Italy), with a particular focus on those situated in densely populated areas and evaluate the potential hazards for consumers.

2. Materials and methods

2.1. Sampling

The present research was carried out between October 2021 to February 2023 in the Campania region (Southern Italy). A total of 221 samples were collected from 30 hot-drink VMs (7 automatic and 23 semi-automatic VMs) in different locations (universities, secondary schools, military schools, military barracks, local health authorities, and companies). The sampling targeted machines with high product dispensing rates (over 1500 products/day). Selection criteria were guided by interviews with food business operators, with a focus on densely populated areas of the Campania Region, in alignment with the 2022 data collected from the "Centro di Riferimento Regionale per la Sicurezza della Ristorazione Pubblica e Collettiva e delle Produzioni Agroalimentari Tradizionali" (C.Ri.P.A.T.). The categories of collected samples are listed in Table 1.

Superficial swab samples were taken using cotton-tipped sterile swabs considering the VM's internal structure (Fig. S1, Supplementary material).

Table 1
Sample number and category.

Sample category	Sample	Total number of samples	% Total samples
Superficial swabs	Water intake pipe, inner walls	23	10.4
	Mixer bowl, inner walls	30	13.6
	Dispensing nozzle, inner walls	30	13.6
Powders	Milk	30	13.6
	Coffee	30	13.6
	Cocoa	30	13.6
	Ginseng	18	8.1
Hot-drink	Cappuccino	30	13.6

The inside walls of the water intake pipe were sampled only from semi-automatic dispensers. The sampling was performed in sterile conditions using disposable material. All samples were immediately refrigerated and shipped in insulated boxes to the Food Inspection laboratory (Department of Veterinary Medicine and Animal Production, University of Naples Federico II) for microbiological and molecular analysis.

2.2. Microbiological analysis

Microbiological analysis were conducted to assess the hygienic quality of the raw materials (coffee beans and powders), the final product (dispensed drink), and the hygienic conditions of the VMs internal structure (superficial swabs). Total Viable Count (TVC), Enterobacteriaceae, *Escherichia coli* (*E. coli*), Enterococci, yeasts and molds were investigated. Briefly, 10 g (or mL) of each sample was weighed and placed in a sterile stomacher bag. The samples were then homogenized using a stomacher machine (BagMixer 400P, Interscience, Saint-Nom-la-Bretèche, France) by adding 90 mL (1:10 w/v or v/v) of peptone water (HiMedia Laboratories, Mumbai, India) for 2 min at 260 rpm. Ten-fold serial dilutions of the homogenized samples were transferred to the corresponding culture media, as specified in Table S1 (supplementary material), and used for microbial enumeration.

Each sample was analyzed in duplicate, and the results of the viable counts were expressed as the mean log colony-forming units per milliliter (CFU/mL) for the beverage, per gram (CFU/g) for powders, and square centimeter (CFU/cm^2) for superficial swabs. The standard error ($\pm$SE) was calculated to indicate the variability within the measurements.

2.3. Molecular analysis

2.3.1. Bacterial strains and culture media

Bacillus cereus ATCC 14579 (*B. cereus*) and *Salmonella enterica* ATCC 13076 (*Salmonella* spp.) were sourced from the Istituto Zooprofilattico Sperimentale del Mezzogiorno. *Listeria monocytogenes* H2048 (*L. monocytogenes*) was provided by the Istituto Superiore di Sanità, while *Staphylococcus aureus* (*S. aureus*) was isolated from food samples. The identification of *S. aureus* isolates was confirmed by using MALDI-TOF mass spectrometry. All these strains were used as reference for preparing the quantification standards for qPCR. The strains were cultured on Trypticase Soy Agar (TSA, Oxoid, Wesel, Germany) for 24 h at 32 °C. Subsequently, a single colony from each strain was inoculated into 5 mL of Brain Heart Infusion broth (BHI, Oxoid, United Kingdom) and incubated further at 37 °C for 24 h (*L. monocytogenes*, *Salmonella* spp., *S. aureus*) and at 30 °C for 48 h (*B. cereus*) achieving an expected bacterial concentration of $\sim 10^9$ CFU/mL for each strain. The cultures were then subjected to ten-fold serial dilution to determine the lower detection limit of the Real-time PCR assay. For each dilution, 100 μL was plated onto Plate Count Agar (PCA, HiMedia Laboratories, Mumbai,

India) and incubated overnight at the specific temperature for each pathogen. The number of CFUs at each dilution step was determined using the plate count method.

For samples analysis, 10 g (or mL) of samples were blended with 90 mL of BHI broth and then incubated overnight at the specific temperature recommended for each pathogen, as previously mentioned.

2.4. DNA extraction and quantification

For the DNA isolation from both bacterial cultures and samples, 1 mL of overnight BHI cultures was centrifuged at 8000 rpm for 10 min. After discarding the supernatants, the cell pellets were used for total genomic DNA extraction with a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol for Gram-positive and negative bacteria, respectively. The extracted DNA was suspended in a final volume of 50 μl of sterile distilled water. DNA quality was verified on 1% agarose gel and DNA concentration was measured using a MaestroNano Pro device (MaestroGen Inc., Taiwán, China). Samples with an A260/A280 ratio ranging from 1.8 to 2.0 were considered. DNA extracts were stored at −20 °C until Real-time PCR analysis.

2.4.1. Validation of primer specificity and PCR optimization

The primer pairs used in this study are presented in Table S2 (supplementary material). The optimal concentration of the selected primer pairs was determined by testing the positive isolates with different concentrations of each primer (900 nM, 600 nM, 300 nM, and 100 nM). The concentration giving the lowest quantification cycle (Cq) value without the formation of a high level of primer dimer was selected. The specificity of primers and absence of unspecific products or primer dimers was confirmed by 2% agarose gel electrophoresis stained with ethidium bromide (data not shown). Primers were optimized to have comparable annealing temperature (Ta) values of 58 °C, thereby allowing all primer sets to be used with the same master mix and analyzed using the same PCR protocol.

2.4.2. Qualitative SYBR green qPCR

Real-time PCR was performed on the reference standards and experimental samples in the StepOnePlus™ Real-time PCR System (Applied Biosystem) using SYBR Green technology. The reactions were performed in a final volume of 20 μl, containing 10 μl of 2X Optimum qPCR Master Mix with SYBR Green (GeneSpin, Milano, Italy), 1 μl of each forward and reverse primer (300 nM), 5 μl of the appropriate DNA template and molecular biology water (BioFroxx, Guangzhou, China). All the qPCR assays were carried out using the following program: a single cycle of DNA polymerase activation for 5 min at 95 °C followed by 40 amplification cycles of 10 s at 95 °C (denaturing step) and 30 s at 58 °C (annealing & extension step). Subsequently, melting temperature analysis of the amplification products was performed by gradually increasing the temperature from 65 to 95 °C in 20 s (±0.5 °C/5s) to assess the specificity of the amplification products. DNA isolated from pure cultures of the reference strains was used as a positive control. For each set of primers, a no-template control was included. The threshold limit setting was performed in automatic mode using software version 2.3. Samples were considered positive when Cq values ≤ 30 were obtained along with the same melting temperature of the positive controls.

To determine PCR amplification efficiencies, slopes of standard curves were calculated through linear regression analysis. The limit of detection (LOD) was calculated as the average minimum number of CFU per PCR reaction required to yield a positive Cq value over the threshold for all fluorescent signals.

2.4.3. Conventional PCR, agarose gel electrophoresis and sequencing

An additional step of PCR end-point was conducted to carry out sequencing of positive samples. This involved amplifying a broader DNA fragment to confirm the presence of the target microorganisms. PCR amplifications were carried out in a 2720 Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). Table S3 reports the primers and conditions used in this study. To determine the presence of amplicons, PCR products were analyzed by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and visualized via ultraviolet transillumination with Universal Hood II Gel Doc System (Bio-Rad, USA). The 100 bp DNA ladder (Solis BioDyne, Tartu, Estonia) was used as a molecular size marker.

The amplicons were subsequently purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Sequencing of the amplified products was conducted by Bio-Fab Research (Rome, Italy). All gene sequences were then subjected to BLAST analysis against the GenBank database.

2.5. Statistical analysis

For the statistical analysis, the Kruskal-Wallis test was performed using GraphPad Prism 8.4.3 software to assess the statistical significance of differences among microbiological parameters across the collected samples. The Kruskal-Wallis test was chosen due to the ordinal nature of microbiological counts and the non-normal distribution of the data. Statistical significance was considered for p values ≤ 0.05.

3. Results

3.1. Microbiological analysis

Among the swabbed surfaces (Fig. 1), the dispensing nozzle displayed the highest mean TVC, with an average of about 3.92 ± 0.35 log CFU/cm^2. On the other hand, the mixer bowl had the lowest TVC, with an average of around 2.27 ± 0.23 log CFU/cm^2. Similarly, enterococci counts were highest in the dispensing nozzle, with an average of approximately 2.60 ± 0.30 log CFU/cm^2. Conversely, the water intake pipe had the lowest mean enterococci count, averaging around 1.13 ± 0,1 log CFU/cm^2. Yeast and mold counts followed a similar trend, with the dispenser nozzle having the highest mean counts and the mixer bowl showing the lowest counts among the surfaces. Considering the overall contamination levels, the sample that appears to be the most contaminated is the dispensing nozzle, with the highest mean TVC and Enterococci counts, followed by cappuccino.

Considering the powders used in the formulation of various VM products (Fig. 2), the data reveals distinct patterns. The milk powder showed the highest mean TVC, with an average of approximately 3.01 ± 0.23 log CFU/g. In contrast, coffee powder exhibited the lowest TVC, with an average of around 1.03 ± 0.03 log CFU/g. Similarly, the highest mean count of enterococci was found in milk powder, averaging at about 1.12 ± 0.09 log CFU/g, while coffee powder had no detectable enterococci. Yeasts and molds also exhibited varying levels among the powders. The highest mean yeast and mold counts were observed in milk powder, with averages of approximately 1.14 ± 0.11 log CFU/g and 1.15 ± 0.08 log CFU/g, respectively. Enterobacteriaceae were detected solely in milk powder sample, with an average count of approximately

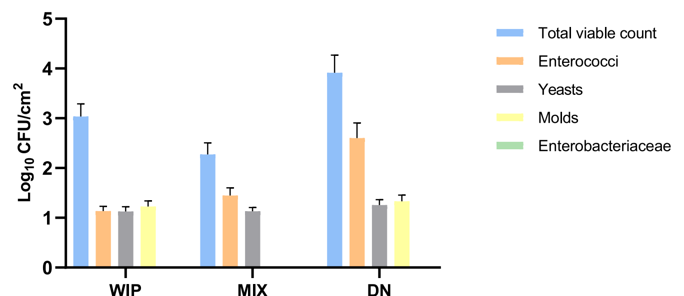

Fig. 1. Average contamination values of superficial swabs
WIP: water intake pipe; MIX: mixer bowls; DN: dispensing nozzle.

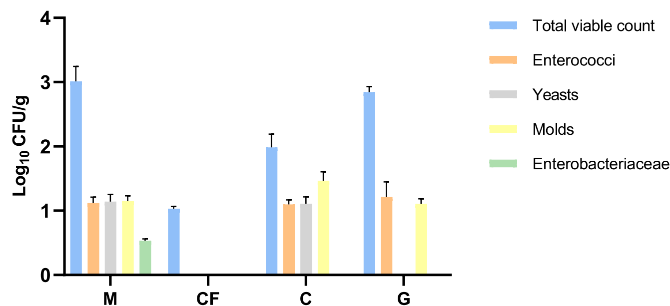

Fig. 2. Average contamination values of powders.
M: milk; CF: coffee; C: chocolate; G: ginseng.

0.53 ± 0.03 log CFU/g.

As reported in Fig. 3, the TVC for cappuccino was approximately 3.98 ± 0.30 log CFU/mL. Enterococci were also detected, with an average count of around 2.11 ± 0.30 log CFU/mL whereas yeast and mold counts were approximately 1.00 ± 0.03 log CFU/mL each.

3.2. Analytical sensitivity and qualitative SYBR green qPCR

Real-time PCR assay conditions were preliminary optimized using SYBR Green I reactions. Among tested combinations of primer concentration (900 nM, 600 nM, 300 nM, and 100 nM) at different reaction volumes (10 μL and 20 μL), the final concentration of 300 nM of each primer was found to produce the optimal amplification signal for both primer combinations (data not shown).

The Real-time PCR amplification efficiencies for all pure cultures of pathogens were in line with the MIQE guidelines for qPCR experiments. The correlation coefficient values, standard curve slopes, Y-intercept values, and LOD results are reported in Table 2.

From the results of the Real-time PCR analysis, it was determined that the only two pathogens detected among the samples were *B. cereus* and *S. aureus*. *L. monocytogenes* and *Salmonella* spp. were not detected in any of the samples subjected to analysis.

As reported in Table 3, the swab samples taken from the water intake pipe, dispensing nozzle, and mixer bowl showed varying levels of contamination with *B. cereus*. The water intake pipe exhibited the highest contamination level, with *B. cereus* present in 91.3% of the samples. The dispensing nozzle and the mixer bowl also displayed a notable contamination rate, with *B. cereus* detected in 83.3% and 80% of the samples, respectively. In contrast, the powder samples showed distinct contamination patterns. Notably, milk exhibited the highest contamination level among the powders, with 73.3% of the samples testing positive for *B. cereus*. Coffee and ginseng had lower occurrences

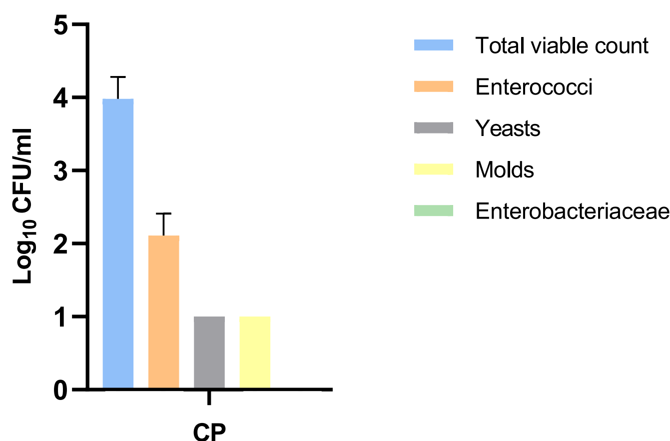

Fig. 3. Average contamination values of hot-drink.
CP: cappuccino.

Table 2
Sensitivity assay results obtained by pure culture genomic DNA of each pathogen microorganism.

Pathogen microorganism	Efficiency	Coefficient	Slope	LOD (CFU/mL)
Bacillus cereus	98.370	0.998	−3.362	10^1
Listeria monocytogenes	101.375	1	−3289	10^1
Staphylococcus aureus	101.866	0.997	−3278	10^1
Salmonella spp.	98.370	0.998	−3,3616	10^1

Table 3
Percentage of positive samples for *B. cereus* and *S. aureus*.

% of positive samples (n/N)		
Samples	*Bacillus cereus*	*Staphylococcus aureus*
Water intake pipe	91.3 (21/23)	21.7 (5/23)
Mixer bowl	80 (24/30)	13.3 (4/30)
Dispensing nozzle	83.3 (25/30)	26.6 (8/30)
Milk	73.3 (22/30)	26.6 (8/30)
Coffee	40 (12/30)	n.d.[a]
Cocoa	n.d.[a]	n.d.[a]
Ginseng	33.3 (6/18)	n.d.[a]
Cappuccino	70 (21/30)	16.6 (5/30)

[a] n.d., not detected.

of *B. cereus*, with 40% and 33.3% of samples testing positive, respectively. Chocolate powder exhibited no detectable presence of *B. cereus* in any of the samples. Cappuccino demonstrated moderate levels of *B. cereus* contamination, with 70% of samples testing positive.

In terms of the presence of *S. aureus*, the highest occurrence was observed in milk samples and the dispensing nozzle, both with a prevalence of 26.6%, followed by the water intake pipe (21.7%). In contrast, *S. aureus* was not detected in coffee, chocolate, and ginseng samples. Cappuccino samples also showed a moderate occurrence of *S. aureus* (16.6%).

3.3. Conventional PCR and sequencing

The presence of *B. cereus* and *S. aureus* evaluated by Qualitative SYBR Green qPCR was confirmed for all positive samples by sequencing. The amplicons were subjected to sequencing, and the obtained sequences were compared against the GenBank database. The results unequivocally confirmed the identification of *B. cereus* and *S. aureus*, with similarity scores ranging between 99% and 100%.

3.4. Statistical analysis

The Kruskal-Wallis test revealed a statistically significant difference among the groups (Kruskal-Wallis statistic = 22.60, $p < 0.001$).

4. Discussion

Our study represents one of the first comprehensive microbiological analyses of hot-drink VMs, encompassing raw materials, final product safety, and internal hygienic conditions. Notably, such an in-depth investigation is scarce in the existing literature, as demonstrated by the limited number of studies addressing these critical aspects (Hall, Saltmarsh, et al., 2007, Hall et al., 2012, 2012; Hunter, 1992; Longo et al., 2013; Nelms and Barnes-Josiah, 1997; Robertson, 1987; Vallone & Bonomi, 2013).

It is important to recognize that our sampling period, from October 2021 to February 2023, coincided with the ongoing Covid-19 pandemic. Interestingly, global trends and reports by food business operators suggested an overall increase in VMs usage during this period (Chenarides et al., 2021; Heins, 2023), likely influenced by the circumstances of the pandemic. To contextualize our study further, it is crucial to note that in

Italy, while restaurants reopened in June 2021, the restoration of regular dining activities, including the removal of the green pass requirement, only occurred in March 2022 (Gili et al., 2023). During this period, food business operators reported a roughly 20% increase in services from VMs, aligning with international trends. This information offers valuable insights into the impact of pandemic-related circumstances on hot-drink VMs usage and, consequently, the potential implications for hygiene and safety practices within this framework.

The microbiological analysis conducted in this study sheds light on critical aspects of VMs hygiene and safety. Scientific literature has defined TVC threshold values, which serve as critical indicators of the potential hazard of foodborne illnesses associated with surfaces in direct contact with beverages (Bean & Griffin, 1990; Garayoa et al., 2016). Typically, acceptable limits fall within the range of 50–10^2 CFU/cm^2. Bacterial counts exceeding 10^3–10^4 CFU/cm^2 are considered high, and levels surpassing 10^4 CFU/cm^2 are deemed unacceptable. Notably, in this research the highest TVC related to superficial swabs was observed in the inner walls of the beverage dispensing nozzle (10^4 CFU/cm^2) and the water intake pipe (10^3 CFU/cm^2). Thus, our results showed high contamination levels, aligning with previous studies that have consistently identified the nozzles as the most heavily contaminated components within hot-drink VMs (Hunter, 1992; Robertson, 1987; Vallone & Bonomi, 2013). The reason for this outcome may be related to the dispensing nozzle position, prone to external environmental contamination, representing an optimal surface for microbial colonization and proliferation. Additionally, the high presence of Enterococci in dispensing nozzles can be attributed to the frequent usage of these machines by a large number of people. Human interactions with the machines, such as product dispensing, can introduce enterococci into the machine's components (Verónica et al., 2015). These bacteria are well-documented for their ability to form biofilms, which act as protective shields, making them resistant to routine cleaning efforts (Ch'ng et al., 2019).

Regarding powders, the microbiological results are particularly noteworthy. In detail, milk powder exhibited the highest level of contamination (10^3 CFU/g), possibly due to its composition favorable for bacterial growth (Sekhon et al., 2021) and/or storage conditions. This finding aligns with the results presented by Vallone & Bonomi (2013), but is higher than what was reported by Longo et al. (2013), who observed a contamination level of 10^2 CFU/g in a similar context. Some colonies isolated from milk powder on Plate Count Agar (PCA) medium, with a typical fimbriate morphology, were identified by MALDI-TOF as *Bacillus licheniformis*. This is a *Bacillus* species that is notably present in powdered milk. Although not pathogenic, it negatively impacts milk and dairy products' quality, being responsible for the alteration of the organoleptic properties (Ramirez-Olea et al., 2022). This underscores the need for strict quality control measures to prevent the degradation of product characteristics. Conversely, coffee powder displayed the lowest contamination levels which is consistent with previous studies (Longo et al., 2013), highlighting the antimicrobial properties of coffee, attributed to its acidity and high-temperature brewing process (Rawangkan et al., 2022; Singh Arora et al., 2009). In the case of chocolate powder samples, the minimal contamination observed aligns with findings reported by Longo et al. (2013) and Vallone & Bonomi (2013), suggesting that suggesting that chocolate-based products may have certain natural properties that discourage microbial growth (Dumbrava et al., 2020). In fact, cocoa, a key component of chocolate, contains polyphenols with potential antimicrobial activity (Nsor-Atindana et al., 2012; Todorovic et al., 2017). The presence of Enterococci in milk, chocolate, and ginseng powders can instead be attributed to the adaptability of these bacteria to different substrates and growth conditions. The presence of Enterococci in milk has historically been associated with fecal contamination. However, recent research (Braiek & Smaoui, 2019) has identified Enterococci in both raw and pasteurized milk (ALzubaidy et al., 2019; Dapkevicius et al., 2021), which suggest a potential hazard of post-production contamination.

The hot-drink product showed a high contamination level (10^4 CFU/mL) compared to powders used in the formulation of hot-drink products. Powders are characterized by low water activity (aw), which inhibits microbial growth (Raposo et al., 2015), but the addition of hot water increase the water activity, providing a favorable environment for microbial multiplication (Cardaci et al., 2017). During the passage through dispensing nozzle the beverage accumulates further contaminating microorganisms. Notably, these findings differ from those reported by Longo et al. (2013) and Vallone & Bonomi (2013), who documented a lower TVC of 10^3 CFU/mL for the dispensed product.

Regarding the evaluation of pathogenic microorganisms, the absence of *L. monocytogenes* and *Salmonella* spp. in all the VMs subjected to control aligns with the findings reported by Longo et al., 2013, suggesting compliance with safety standards. These pathogens are typically subjected to rigorous monitoring and control measures in food products and raw materials, as outlined in Regulation "EC 2073/2005". The lack of detection in our study might be attributed to the robust surveillance and control programs in place for these pathogens at the earlier stages. Conversely, the presence of *B. cereus* and *S. aureus*, not mandated by the regulation for this category of products, highlights their distinct prevalence in our VMs samples. This discrepancy underscores the importance of nuanced regulatory considerations, shedding light on potential variations in monitoring intensity for different pathogens across diverse food contexts. The percentage of *B. cereus* contamination evaluated in this study was very high, overcoming the 80% of positive samples of all surface swabs performed (80% - Mixer bowl, 83.3% - Dispensing nozzle, 91.3% - Water intake pipe). *B. cereus*, a ubiquitous microorganism associated with the environmental dust (Cayemitte et al., 2022), could contaminate the water pipe during water tank replacement. The operator must pay attention to the exposure of the water pipe during handling procedures. Internal conditions within the water pipe may facilitate microbial growth, possibly due to stagnant water, irregular flow, or surfaces that encourage bacterial adhesion. Factors such as nutrient availability within the pipe, water temperature, and inadequate maintenance practices could further promote contamination (Makris et al., 2014). *B. cereus* was detected in 73.3% of milk powders, which could be related to possible contamination from the environment or improper handling and storage (Kumari & Sarkar, 2016). Moreover, a high percentage (70%) of *B. cereus* was also found in the final hot-drink. These findings may be a consequence of the contamination of milk powder and surfaces accumulating in the final product. *B. cereus* presence aligns with previous studies that have reported incidents of bacterial food poisoning linked to hot-drinks dispensed from VMs. Nelms and Barnes-Josiah (1997) documented a case of food poisoning attributed to *B. cereus* contamination in hot chocolate from a VM located in a plant cafeteria. Similarly, Hall et al. (2007b; 2012) identified *B. cereus* as the most significant microbiological hazard potentially associated with hot-drink VMs. A critical concern associated with the presence of *B. cereus* in VMs is its potential to produce spores. The spores can survive in powdered food products for extended periods (Pal et al., 2014). When conditions become favorable, such as when the powdered product is rehydrated with hot water in a VM, the spores can germinate, leading to the growth and proliferation of vegetative cells (Putri, 2017). This rebirth of vegetative cells can result in the production of toxins, which are heat-stable and can cause foodborne illnesses upon ingestion (Drobniewski, 1993). This phenomenon underscores the importance of not only detecting the presence of *B. cereus* but also addressing the potential for spore formation and subsequent toxin production.

S. aureus was detected at a higher rate (26.6%) in the inner wall of the dispensing nozzle. This can be justified by the direct exposure of the dispensing nozzle to the surrounding environment. Particularly noteworthy is the elevated detection rate of *S. aureus* in milk powder (26.6%), surpassing the 3.4% reported by Longo et al. (2013). This could be indicative of unsanitary processing conditions or post-processing contamination (Xing et al., 2016) that conditioned the presence in 16.6% of cappuccino samples. This bacterium can produce heat-stable

toxins, and even a small amount of these toxins in food can lead to foodborne illnesses (Kadariya et al., 2014). Its detection suggests a potential risk of toxin contamination.

The findings of this study provide valuable insights into the hygiene and safety aspects associated with VMs, emphasizing critical areas that warrant attention, with reference to the beverage dispensing nozzle. Regular and thorough cleaning, including the use of disinfectants to disrupt biofilms, is essential to maintain the hygiene and safety of VM products and prevent potential health risks (Cardaci et al., 2016), notably related to *B. cereus* and *S. aureus* contamination (Hall, Saltmarsh, et al., 2007).

In Italy, the domestic law governing the quality and safety of VM products is outlined in Italian Legislative Decree 193/2007, which follows recommendations of European Union Regulations (Reg. 852/2004, EC 853/2004, and EU 625/2017 concerning hygiene rules and official controls for food of animal origin). Under Italian Legislative Decree 193/2007, food business operators are mandated to establish, implement, and maintain permanent cleaning procedures grounded in the principles of HACCP. During this study, audits were conducted with VM operators responsible for maintenance and cleaning operations. Operators revealed that the current cleaning frequency occurs daily for 6 out of 30 VMs and twice a week for 21 out of 30 VMs. These cleaning operations typically involve brief scrubbing along internal surfaces during the powder-filling process. Additionally, a more comprehensive sanitization is carried out automatically along the internal pipes using boiling water or food-grade acids after approximately 15,000 beverages are dispensed. This irregular deep cleaning frequency, contingent on usage, poses potential hazards, particularly in critical environments like hospitals or schools where vulnerable groups like "Young, Old, Pregnant, Immunosuppressed" (YOPI) may be affected. The risk intensifies in high-contamination zones, notably dispensing nozzles, emphasizing the need for specific attention. The results of this study show that the current cleaning operation strategies may be inadequate to guarantee consumer safety. To address this issue, a strategic implementation of preventive actions at critical control points becomes crucial for mitigating potential hazards. These measures encompass a systematic monitoring of temperature facilitated by sensors, regular microbial testing, and immediate corrective actions when deviations are detected. Considering the prevalence of specific foodborne pathogens, it is imperative to introduce additional preventive measures. For pathogens like *S. aureus*, a robust emphasis on stringent handwashing practices and continuous staff training is essential to minimize contamination risks. Conversely, for *B. cereus*, it is essential to implement a more rigorous control of the quality of raw materials. Comprehensive quality checks for raw materials can significantly reduce the likelihood of contamination, thereby enhancing the overall safety of VM products.

While our study provides valuable insights into the hygiene and safety of hot-drink VMs, it is confined to the Campania region. To achieve a more comprehensive understanding, future research should broaden its geographical scope by including diverse regions across Italy and Europe. Such an expansion would not only enhance the study's applicability but also contribute to a more nuanced and extensive assessment of the factors influencing VMs hygiene and safety practices on a broader scale. Furthermore, conducting periodic analyses on the same machines would provide additional perspectives into the long-term effectiveness of cleaning procedures.

A systematic approach based on hazard analysis allows for the identification of potential critical control points and the development of appropriate preventive measures. However, in Campania and other Italian regions official monitoring plans have not yet been implemented. Consequently, business operators lack directives for enhancing their cleaning and maintenance procedures. The establishment of guidelines for official controls by competent authorities and monitoring plans by food business operators, constitute effective strategies for mitigating contamination hazards and ensuring the production of safe and high-quality products.

5. Conclusions

This study has contributed to advancing scientific knowledge and addressing the existing gap concerning the hygiene and safety issues associated with hot-drink VMs. Our results significantly underscore the necessity of implementing appropriate self-monitoring strategies, promoting rigorous adherence to the principles of Good Manufacturing Practices, optimizing the timing and methods of sanitization, and fostering the efficacy of official control activities. It is imperative that official controls on VMs are integrated into scheduled documents, such as "monitoring plans", based on a risk assessment approach. These measures are crucial for enhancing consumer protection and ensuring the safety of VMs.

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CRediT authorship contribution statement

Iolanda Venuti: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Marina Ceruso:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology. **Tiziana Muscariello:** Investigation, Formal analysis, Data curation. **Carmela Vallone:** Methodology. **Paolo Sarnelli:** Project administration, Funding acquisition, Conceptualization. **Giovanni Battista Varcasia:** Project administration, Funding acquisition, Conceptualization. **Tiziana Pepe:** Writing – review & editing, Visualization, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2024.110376.

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