

Automatic Vending-Machines Contamination: A Pilot Study

Rosa Cardaci¹, Sandra Burgassi¹, Davide Golinelli², Nicola Nante^{1,2}, Mario A. Battaglia³, Daiana Bezzini³ & Gabriele Messina^{1,2}

¹ Department of Molecular and Developmental Medicine, Laboratory of Environmental Hygiene, University of Siena, Siena, Italy

² Post-Graduate School of Public Health, University of Siena, Siena, Italy

³ Department of Life Sciences, University of Siena, Siena, Italy

Correspondence: Gabriele Messina, Post-Graduate School of Public Health, University of Siena, Via Aldo Moro 2, 53100, Siena, Italy. Tel: 39-05-7723-4084. E-mail: gabriele.messina@unisi.it

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Abstract

Hot-drinks vending machines are disseminated worldwide and millions of drinks are served every day. Because of a small number of studies on hot-drinks related illnesses, the aim of this pilot study was to identify the presence and load of bacterial species, potentially harmful for consumers, within hot-drinks vending machines external critical surfaces. This preliminary cross sectional study was carried out in April 2015 at the University of Siena, Italy. Samples were taken from the critical surfaces of 4 hot-drinks vending machines (VM); the analyzed VM's critical surfaces were: Dispense Areas, Nozzles and Glass-Holders. The samples were sown on selective culture media: Plate Count Agar (PCA) at 22 °C and 36 °C, Slanetz and Bartely Agar (SBA) and Mannitol Salt Agar (MSA). Total Viable Count (TVC) at 36 °C and 22 °C was assessed for mesophilic and psychrophilic contamination. Results were expressed in terms of average CFU/cm². Descriptive and statistical analyses were performed in order to assess which surface was the most contaminated. The nozzles resulted to be the most contaminated critical surface, showing average values over the limits in all the culture media (PCA 36 °C, PCA 22 °C, MSA and SBA). The statistical analysis showed that the nozzles were significantly more contaminated ($p < 0.05$) than Dispense Areas and Glass-holders both in PCA 36 °C and in PCA 22 °C. Given the high number of CFU/cm², VM may constitute a potential threat for consumer's health, reason for which further studies are recommendable.

Keywords: automatic vending machines, critical surfaces, contamination, hot-drinks, food-borne illness risk

1. Introduction

1.1 Background

A Vending Machine (VM) is an operational machine located at either a client site or in a public location designed specifically for the sale and dispensing of food, drinks, and other goods. The automatic vending machines (VMs) were imported from the United States and appeared for the first time in Italy in the early 50's (European-Vending-Association, 2014). At the beginning they were only used for the distribution of bottled drinks; after a few decades, VMs were also used to dispense espresso coffees. The increasingly sophisticated and organized image and the range of products offered, together with the different eating habits of the population have made their use increase. VMs can be frequently found in crowded workplaces such as hospitals, schools and universities. The 6 biggest markets in Europe are Italy, France, United Kingdom, Germany, Spain and the Netherlands, which make up around 80% of the total European market, generating a turnover of €11.3 billion annually (European-Vending-Association, 2014). Coffees are produced from coffee beans grounded and pressed on a filter where the hot water pass through the coffee powder. The other drinks are instead produced from soluble powder; each of these is carefully collected and separated into specific containers, mixed with water in a mixer once the beverage is selected. Coffee and drinks are then collected in a plastic cup and served to the user. The powders are obtained from a freeze-drying process, a preservation system used to maintain the product in good condition as long as possible before its use. The microbiological quality of these drinks can be influenced by the microbial load of powders, in which even after the heat drying process mold spores, bacteria, and microbial spores can survive. The microbiological quality of the water used (which must be water for human

consumption), and its temperature (which should not be lower than +80 °C), results to be very important (Dragoni & Bonomi, 2008). Moreover, for the good quality of the beverages and to minimize the risk of contamination, hygiene, cleaning procedures, times elapsing between one cleaning and the other, the idle time of the distributor (i.e. weekend, holidays) and the complexity of the machines, also assume a great importance (Cantoni, 1984; Vallone & Bonomi, 2013). The vending machines companies, for the correct HACCP application (required by European Regulation 852/2004 and Regulation 853/2004), provide a cleaning and sanitizing procedures plan to be done periodically. Under the HACCP system, food business operators ensure that all stages of production, processing and distribution of food under their control satisfy the hygiene requirements laid down in the European Regulation. For this reason, all the vending machines need accurate cleaning and sanitizing procedures, as they put in direct contact the food product with the mechanic components of the machine, but the frequency of cleaning depend on the type of machine, the frequency of use, and the location. The contact between foods/beverages and the external surfaces of the machine can result in the transmission of bacterial species or spores which can represent a risk for consumer's health. The purpose of the cleaning and sanitization plan is to eliminate or reduce the contamination risk to acceptable levels (Dragoni & Bonomi, 2008). Thus, VMs external surfaces might be at risk of microbial contamination due to poor hygienic conditions or inappropriate cleaning procedures.

1.2 Aim

Previous studies (Hall, Short, Saltmarsh, Fielding, & Peters, 2007; Nelms, Larson, & Barnes-Josiah, 1997) were focused on assessing the contamination of coffee powders and/or beverages, without considering if and how the surfaces in direct contact both with beverages and/or with cups/glasses might be contaminated as well.

The main purpose of this pilot study was to evaluate the hygienic conditions of hot-drinks vending machines located in an highly crowded area; in particular, to identify the presence and the microbial load of potentially harmful bacterial species on external surfaces which can be considered a proxy of contamination for the beverages and, consequently, a risk for the consumers.

2. Method

2.1 Setting, Research Design and Sample Size

This preliminary cross sectional study was carried out in April 2015 at the University of Siena, Italy. A total of 12 samples from 4 hot-drinks VM.

2.2 Sampling Procedures

The samples, using cotton-tipped sterile swabs, were taken all in one day (Monday) at 11 a.m. from 4 machines belonging to the same company. In order to assess the hygienic conditions inside the dispensing area 3 external critical surfaces at the risk of contact with the beverages were identified: i) Dispense Area, ii) Nozzles, iii) Glass-Holders. After sampling these surfaces, the swab samples were transported into the laboratory of the Department of Molecular and Developmental Medicine (University of Siena), and placed into tubes containing 1.5 ml of phosphate buffered saline (PBS) for 30 minutes. Each suspension of PBS was then double seeded in two 90 mm Petri dishes containing the following culture media: Plate Count Agar (Oxoid) at 22 °C (PCA 22 °C), to assess the non-specific Total Viable Count (TVC) due to psychrophilic organisms; Plate Count Agar (Oxoid) at 36 °C (PCA 36 °C), to assess the non-specific Total Viable Count due to mesophilic organisms; Mannitol Salt Agar (Oxoid) at 36 °C (MSA), to detect *Staphylococcus* and *Slanetz And Bartley* Agar (Oxoid) at 36 °C (SBA), to detect *Enterococcus*. The culture media were prepared and sterilized according to the manufacturer's instructions. The plates were incubated for 48 hours and then counted, enumerating the number of colony forming units (CFU) developed.

2.3 Data Analysis

Data was collected using Microsoft Excel. For the microbiological evaluation of the hygienic state of food surfaces and equipment, the literature proposes standard values fixing the numbers of bacteria (mesophilic aerobic germs) beyond which the surfaces are incorrectly cleaned/sanitized. The Italian Guidelines on surface sanitation ("*Microbiological Control of Surfaces*") highlights the limits of microbial population beyond which a surface suffers from an incorrect cleaning procedure and limits beyond which there can be a risk of deterioration in the quality of the food which potentially come in contact with the surface. The acceptable levels do not exceed 50 CFU/cm² (Regione-Emilia-Romagna, 1997). The parameters indicated in Table 1 were taken as a reference and as a standard of comparison for the subsequent description of the results.

Results were expressed in terms of average CFU/cm² from the swabs of the 4 VM for each of the 4 culture media. To obtain the CFU/cm² the area of each surface has been calculated. A descriptive analysis was performed.

Shapiro-Wilk test rejected the hypothesis of normal data distribution and Mann-Whitney test was used in order to verify which of the 3 critical surfaces was the most contaminated. Stata SE, version 12 (Stata Corporation, College Station, TX, USA) was used and statistical significance was set at $P \leq 0.05$.

3. Results

The dispense areas of the vending machines showed values over the limits on Plate Count Agar at 36 °C and at 22 °C (Table 2). Respectively, the average TVC was 63 (SD 20) CFU/cm² in PCA at 36 °C and 54 (SD 28) CFU/cm² in PCA at 22 °C. The clamps holding the plastic cups (Glass-holders) showed average values exceeding the limits in PCA at 36 °C and at 22 °C, in Mannitol Salt Agar (Staphylococcus) and in Slanetz and Bartley Agar (Enterococcus) (Table 2). The average TVC was 457 (SD 647) CFU/cm² in PCA at 36 °C and 408 (SD 674) CFU/cm² in PCA at 22 °C. In Mannitol Salt Agar the average bacterial load was 127 (SD 242) CFU/cm². In Slanetz and Bartley Agar the average bacterial load was 405 (SD 776) CFU/cm².

The nozzles were the most contaminated critical surface, showing average values over the limits in all the culture media (Plate Count Agar at 36 °C and at 22 °C, Mannitol Salt Agar and Slanetz and Bartley Agar). The average TVC was 2063* (SD 1042) CFU/cm² in PCA at 36 °C, and 2063* (SD 1042) CFU/cm² in PCA at 22 °C. In Mannitol Salt Agar the average bacterial load was 569 (SD 686) CFU/cm². In Slanetz and Bartley Agar the average bacterial load was 635 (SD 881) CFU/cm². These results, concerning dispense areas, glass-holders and nozzles, showed values exceeding the limits fixed by the surfaces sanitizing guidelines.

The statistical analysis showed that the nozzles were significantly more contaminated ($p < 0.05$) than the surfaces of dispense areas and glass-holders both in PCA at 36 °C and in PCA at 22 °C. On the other hand, this difference was not statistically significant in MSA and SBA (Table 2).

Table 1. Limits of microbial load

| Bacterial Load (CFU/cm ²) | |
|---------------------------------------|--------------------------|
| <50 | Acceptable |
| 50-10 ² | Limit of acceptability |
| 10 ² -10 ³ | High bacterial load |
| 10 ³ -10 ⁴ | Very high bacterial load |
| > 10 ⁴ | Not acceptable |

Note. Microbial population beyond which a surface suffers from an incorrect cleaning procedure.

Table 2. CFUs of dispense area, glass-holders and nozzles

| | DISPENSE AREAS | | | | GLASS-HOLDERS | | | | NOZZLES | | | |
|--------------|-----------------------------|-----|-----|--------|-----------------------------|-----|------|--------|-----------------------------|------|------|--------|
| | CFU/cm ² (SD) | Min | Max | Median | CFU/cm ² (SD) | Min | Max | Median | CFU/cm ² (SD) | Min | Max | Median |
| PCA36 | 63 (20) | 31 | 78 | 76 | 457 (647) | 35 | 1500 | 170 | 2.063* (1.042) | 1500 | 3750 | 1500 |
| PCA22 | 54 (28) | 12 | 78 | 64 | 408 (674) | 26 | 1500 | 51 | 2.063* (1.042) | 1500 | 3750 | 1500 |
| MSA | 9 (8) | 1 | 22 | 7 | 127 (242) | 0 | 615 | 0 | 569 (686) | 0 | 1617 | 263 |
| SBM | 34 (36) | 76 | 2 | 30 | 405 (776) | 0 | 1995 | 0 | 635 (881) | 0 | 2040 | 20 |

Note. *Nozzles were significantly more contaminated ($p < 0.05$) than dispense areas and glass-holders in PCA at 36 °C and in PCA at 22 °C. The results of PCA 36 °C and 22 °C of the nozzles have been both estimated and have to be interpreted as "uncountable" number of CFU. SD: Standard Deviation.

4. Discussion

The resulted contamination of the VMs critical surfaces can represent a serious public health issue. VMs are widespread worldwide and they represent an important resource for consumers. The VMs contribution to the society is important, and their presence everywhere is a clear evidence. On the other hand, the magnitude of the VMs diffusion, together with the possibility and the risk that their products may be contaminated, can represent an important public health challenge. Risk assessment within the vending industry may encounter many problems. Many guidelines and articles identify limits values of CFU/cm² (Table 1) beyond which a surface in direct contact with food and/or drinks may become at risk of causing food-related illnesses (Bean & Griffin, 1990; Garayoa, Yanez, Diez-Leturia, Bes-Rastrullo, & Vitas, 2016). Robertson (1987) sampled the nozzles, drip trays, and dispense areas of both hot and cold machines. He found that the Total Viable Count of the nozzles was 100-fold higher than the other 2 areas (Robertson, 1987); his results are similar to the results of this pilot study. These results are also similar to those of another study (Vallone & Bonomi, 2013), in which the nozzles showed a microbial contamination higher than other components/surfaces of the machines. The same Authors suggested that this part of the dispenser can be affected by external environmental contamination and by beverages residues, which may constitute a substrate for microbial proliferation. This kind of process can lead to a transfer of the microbial population to the human host and, consequently, to food-related illnesses. The contamination of the surfaces sampled in our pilot study exceeded the limit values fixed by the guidelines. This may represent a risk for consumers' health. There has been one published case of bacterial food poisoning from a hot-drinks vending machine. Nelms (Nelms et al., 1997) reported on a case of food poisoning (*Bacillus Cereus*) in a Minneapolis manufacturing plant, where employees reported vomiting and nausea after drinking hot chocolate from a vending machine based in the plant cafeteria (Hall et al., 2007). In Italy the provision of food and beverages through Automatic Dispensers is governed by D.Lgs. 193/2007 which transposed the European regulations 852/2004 "Food hygiene", and n. 853/2004, 854/2004 and 882/2004 named "specific rules of hygiene for food of animal origin". The D.Lgs. 193/2007 requires the food business operators to develop, implement and maintain permanent cleaning procedures based on the principles of HACCP (Hazard Analysis Critical Control Points). This system identifies the risks for human health and the safety and preventive measures for their elimination and/or their control (Dragoni & Bonomi, 2008). Kassa et al. (2001) also stated the importance of direct visual inspection, as another measure for preventing potential risk of food-borne illnesses (Hall et al., 2007; Kassa, Harrington, Bisesi, & Khuder, 2001). As mentioned above, previous studies focused on hygiene and contamination of the internal mechanical parts of the vending machines, on the microbiological quality of the powders used to prepare the beverages, and/or on the beverages direct contamination (Hall et al., 2007; Nelms et al., 1997; Vallone & Bonomi, 2013). The main purpose of our study was rather to highlight whether a microbial population on the critical surfaces may contaminate the beverages; therefore, we wanted to assess whether there can be a potential risk for consumer's health, considering that cross-contamination is involved in many food-borne outbreaks (Rossvoll et al., 2015). The nozzles contamination, in particular, widely exceeded the limits; moreover, the nozzles were significantly more contaminated than the other VM external surfaces. As mentioned in the introduction, another element to be considered in order to make a VMs microbiological risk assesment is the beverages temperature. High temperatures can lead to thermal-shock, able to eliminate the thermolabile bacterial flora. The Italian guidelines report that the dispended coffee should be at temperatures above +80 °C. On the contrary, in many studies (Longo & Giuffrida, 2013; Vallone & Bonomi, 2013) the temperature of the delivered beverages rarely exceeded the critical limit of 65-70 °C. This factor may increase the risk of contamination. The culture media used in this study have only permitted the identification of the Total Viable Count (PCA at 22 °C and at 36 °C), not specific for human pathogens. In addition, unlike other previous studies no direct analyses of the products (coffee or other drinks) have been performed. For these reasons no comments can be made on the total number of pathogens present within the hot-drinks.

Another limit can be the low number of VM that we examined, and the fact that not all the model of VMs are assembled the same. These limits do not allow us to state that the problem of poor hygiene and maintenance procedures is widespread worldwide. On the other hand, a previous survey (Robertson, 1987) found that only 20% of operators questioned used the prescribed cleaning protocol from the machine manufacturer.

Although this was a preliminary study, the results showed a significant contamination of the analyzed surfaces. These results, comparable with those of previous studies (Robertson, 1987; Vallone & Bonomi, 2013), demonstrate that the VMs critical surfaces contamination can be high and that nozzles were the most contaminated surface. Moreover, the nozzles are the external part of the machine more directly in contact with the beverages; this fact may amplify the risk of cross-contamination. One reason for the presence of this high bacterial flora on the nozzles surface can be the attributable to its high level of humidity and to the user's direct

contact with these surfaces when collecting the beverages.

In conclusion, this study demonstrates that the safety of vending machines is not absolute. VMs may, in fact, represent a potential threat to human health. For these reasons, VMs maintenance procedures and their cleaning and sanitization plans (application of appropriate cleaning procedures to be carried out by trained and sensitized personnel) play a fundamental role and need to be carried out properly.

As stated by other authors, it should be noted that all the concerns raised above could be addressed through the application of a correct good manufacturing practice (GMP) (Longo & Giuffrida, 2013). It is recommended that manufacturers and operators who deal with the maintenance of the VMs are aware of the risks; moreover, the producers should entrust to more modern cleaning and engineering/hardware technologies in the manufacture of the VMs, in order to avoid as much as possible the direct contact between the components of the machine, the final product and the consumers' hands.

Competing Interests Statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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