Repeated detection of microbes in beverages dispensed from soda fountain machines and the effect of flushing on microbial density

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1. Introduction

Eliminating sources of microbial contamination is fundamental to ensuring the quality and safety of foods and beverages in the food industry. Numerous recent studies have focused on the sources of microbial contamination during food and feed production, and the safety risks posed by biofilm formation on food processing equipment and food surfaces (for a review, see Van Houdt and Michiels, 2010). Biofilms, aggregated communities of microbes that colonize solid surfaces, can form on a multitude of materials (Faille and Carpentier, 2009), can contain pathogenic bacteria (e.g. Listeria and Salmonella species.), are resistant to disinfectants, and, thus, pose a risk to public health by the constant release of planktonic cells and bacterial endotoxins (Bridier et al., 2011; Keskinen et al., 2008; Krolasik et al., 2010; Marchand et al., 2012; Poimenidou et al., 2009; Van der Veen and Abee, 2011; Walker and Marsh, 2007).

Although many studies have focused on preventing or eliminating biofilms from machinery used in food processing facilities, little direct attention has been given to specialized equipment used to handle foods and beverages in restaurants, including soda fountain machines (SFM). Water and soda syrup are dispensed from storage facilities to machines used in food processing facilities, little direct attention has been given to specialized equipment used to handle foods and beverages in restaurants, including soda fountain machines (SFM). Water and soda syrup are dispensed from storage facilities to

2. Methods

Few studies have examined patterns of microbial contamination in soda fountain beverages. In this study, patterns of microbial contamination in beverages dispensed from soda fountain machines (SFM) sampled in June 2009 and then again 13 months later were compared. Over 70% of beverages contained microbes in both years, suggesting that contamination of beverages dispensed from SFMs can continue for long periods of time. In addition, the impact of disinfecting the dispensing nozzles and plastic tubing of SFMs, as well as the impact of machine use on microbial contamination was assessed. Managers from 26 establishments (fast-food and convenience stores) were interviewed about their SFM disinfecting practices and no correlation was found between the self-reported disinfecting practices and levels of microbial contamination in beverages dispensed from SFMs. Furthermore, in a direct study of two SFMs with an established disinfecting regimen, CFU/mL in beverages increased significantly immediately after disinfecting of plastic tubing yet returned to pre-disinfecting concentrations within 11 days. These results suggest that disinfecting may disturb microbial communities, resulting in increased planktonic microbes, but not the ultimate removal of planktonic microbes in the beverage lines had been reduced by flushing. As there are currently no regulations regarding the disinfecting of SFM tubing or periodic inspections of beverages dispensed from SFMs, it would be valuable for consumers to encourage increased surveillance of SFMs, and to dispense some beverage before filling their cups.

3. Results

The tubing bore size and surface of this tubing provide conditions favorable for the formation of biofilms (Chaberny et al., 2006; IMI Cornelius Inc., 2009; Liaqat and Sabri, 2008; Yu et al., 2010; Zanetti et al., 2009). Numerous studies of dental unit water lines (DUWLS) tubing, which are similar to those used in SFMs, have shown formation of persistent biofilms in DUWLS which can contain opportunistic pathogenic bacteria, such as Legionella and Pseudomonas species (for a review see Coleman et al., 2009). While DUWLS have been relatively well studied, only two studies that we are aware of have examined patterns of microbial contamination in beverages dispensed from SFMs. White et al. (2010) sampled beverages from 30 SFMs in fast food restaurants and convenience stores in the Roanoke Valley, VA and found...
detectable levels of microbes in more than 70% of the beverages. In many
cases, the levels of microbial contamination exceeded that allowed for
municipal drinking water as set by the U.S. Environmental Protection
Agency US EPA (2012) — 20% of beverages had a heterotrophic plate
count (HPC) > 500 colony forming units (CFU)/mL and 48% contained
coliform bacteria. In a similar study, Hertin (2011) found that all sampled
beverages dispensed from 18 SFMs in Las Vegas Valley, NV, had detect-
able levels of microbes and a very large percentage exceeded EPA stand-
dards for drinking water (55% had a HPC > 500 CFU/mL and 86%
contained coliform bacteria).

Both of these studies offered a snapshot of levels of microbial con-
tamination of soda fountain beverages in different regions of the
United States. If biofilms form in SFMs, then it is likely that microbial
contamination of soda fountain beverages will persist over time.
Therefore, in order to determine if microbes were still detectable in
soda fountain beverages, we reaccessed beverages dispensed from a
subset of establishments sampled by White et al. (2010). In addition,
we also examined the impact of disinfecting (both self-reported and
observed) and the impact of machine use on levels of microbial con-
tamination of soda fountain beverages.

2. Materials and methods

2.1. Part 1: Reexamination of contamination levels of beverages dispensed
from SFMs

Beverages delivered from SFMs that had been tested for microbial
contamination at 10 Roanoke, VA establishments by White et al.
(2010) in June 2009 were reexamined in July 2010. Where possible,
three different beverages (a sugar soda, a diet soda, and water)
were collected between 0830 and 1030 Eastern Standard Time
(EST) from each soda fountain. However, two soda fountains did
not dispense water, thus 10 sugar sodas, 10 diet sodas, and 8 waters
were collected and microbial contamination was assessed.

In the White et al. (2010) study, beverages were collected by dis-
{}##pensing approximately 7 mL of each beverage directly into a 15 mL
sterile disposable test tube. In 2010, beverages were dispensed into pur-
chased 0.35–0.65 L self-serve cups (similar to Hertin, 2011) and then
decanted into 15 mL sterile test tubes outside of each establishment. All
tubes were capped and immediately stored in a chilled cooler. In
order to determine whether the beverage cups presented an additional
source of contamination, we conducted a pilot study in which self-serve
cups from 46 establishments were collected and swabbed to assess the
level of cup microbial contamination. Each cup was swabbed with a
sterile cotton tip for 10 s which was then placed in 5 mL of sterile saline.
The saline (500 μL) was then plated on Tryptic Soy Agar (TSA, obtained
from Fischer Scientific, Pittsburgh, PA), incubated, and CFUs were
counted 48 h later. No microbes were detected in seventy percent of
the cups sampled and only four percent (N = 2) had more than 3
CFU/mL. Thus, cups were not the source of microbial contamination
found in beverages.

Following the White et al. (2010) protocol, upon return to the lab-
{}##oratory, 500 μL of beverage samples were plated using the spread
plate technique onto three Eosin Methylene Blue Agar (EMB, obtained
from Carolina Biological Supply, Burlington, NC), and three TSA
plates. All plates were incubated at 37 °C for 48 h. For each agar
type, control plates with no inoculum were also incubated.

In order to determine heterotrophic plate counts (HPC), CFUs on TSA
plates were counted and triplicate plate counts were averaged for each
beverage sample. Plates with more than 300 colonies were designated
as too-many-to-count (TMTc), and for conservative statistical analysis,
these plates were assigned a value of 300 CFU. If the average plate count
was ≤ 1 CFU/mL, the beverage was characterized as having no detect-
able microbes (Sutton, 2011). Microbial colonies from EMB plates
were visually characterized as either coliform bacteria or non-coliform
bacteria (any colony that was purple or green in color indicated lactose
fermentation and categorized as a coliform).

The HPC (in CFU/mL) and the number of coliform CFUs/mL were
then compared between the 28 beverages collected in 2009 and 2010
using Wilcoxon Signed Rank tests in the PASW Statistics 18 (SPSS,
2008). Non-parametric analyses were employed as colony counts were
not normally distributed.

2.2. Part 2: Comparison of self-reported disinfecting patterns and
 contamination levels

In July 2010, employees (primarily management personnel) from
47 fast food establishments in Virginia and North Carolina were
contacted by telephone and asked to participate in a survey examin-
ing the disinfecting practices of their soda fountain dispensers.
Three beverages (a sugar soda, a diet soda and a water where possible)
were collected from each of the establishments contacted had been sampled
earlier for microbial contamination using the same methodology
described in Part 1; however, none of the managers were informed
that beverages from their establishments had been collected and an-
alyzed. Thirty seven of these establishments were part of a broader
regional study (Goddard et al., unpublished data) and 10 were from
the study described in Part 1. Fifty-five percent of those contacted
were willing to answer questions (18 of 37 in the broader regional
study and 8 of 10 in the study described above). Participating man-
agers were asked to describe their staff’s disinfecting practices relat-
ging to the dispensing nozzles and the plastic tubing that carries
syrup and water to the SFM (e.g. frequency of disinfecting, personnel
involved).

In order to determine if reported disinfecting practices were associ-
ated with patterns of contamination, the average HPC for all sampled
beverages from each establishment was calculated and then compared
between establishments reporting daily disinfecting of nozzles (N = 17),
discharging every other day (N = 3), and disinfecting weekly
(N = 6) using a Kruskal–Wallis one-way analysis of variance (K-W
ANOVA). In addition, the HPCs were compared between establishments
that reported that the plastic tubing was disinfected by employees (self,
N = 6), disinfected by an outside company (N = 13), was not
disinfected (N = 2), and those uncertain of the disinfecting patterns
(N = 5) using a K-W ANOVA.

2.3. Part 3: Analysis of beverages from soda fountain machines with
 established disinfecting regimens

To better understand the impacts of disinfecting and beverage use
on microbial colonization of SFMs, in 2011 we carried out a more de-
tailed study of beverages dispensed from two self-serve SFMs located
in the dining facility on the Hollins University campus. In this facility,
nozzles were removed and disinfected on a daily basis and every
3 months the plastic tubing in the machine was disinfected by per-
sonnel from the distributor (PepsiCo Inc.).

Five days prior to disinfecting, we examined the contamination levels
of 16 beverages (11 sugar sodas, three diet sodas, one caffeine free
soda, and one water) by collecting 10 mL of each beverage in a 15 mL
sterile plastic collection tube at 0700 EST. Samples were plated in triplicate
on TSA as described above in Part 1. An average HPC was determined for
each beverage. To disinfect the lines, the tubing was removed from the
{}##ryp bag (located in a storage room > 30 m from machine) and a
{}##cfectant and then water was flushed through each line and through the
{}##hine. After the tubing was reattached to the syrup bags and the
{}##ine readied for use a second beverage sample was immediately
{}##lected and processed as described above. A third sample of each
{}##verage was collected at 0700 EST 11 days later which allowed for
{}##ays of patron use post-disinfecting (the first 4 days post-
{}##fecting occurred during spring vacation when the dining facility
was closed). The average HPC for each of the three beverage samples

ences in HPC in beverages sampled from establishments that reported (most commonly a 10% bleach solution). However, there were no differ-

3.2. Comparison of self-reported disinfecting patterns and contamination levels

All establishments reported that nozzles were disinfected by removing them from the machine and soaking them in a disinfecting solution (most commonly a 10% bleach solution). However, there were no differences in HPC in beverages sampled from establishments that reported disinfecting nozzles daily, every other day, or weekly (Table 1, K-W ANOVA, N = 26, df = 2, KW = 2.37, P = 0.306).

While all establishment managers were confident on the frequency of disinfecting nozzles, some were not certain of the frequency of the disinfecting of the plastic tubing. Five of the managers (19%) were uncertain about whether or not the plastic tubing was ever disinfected. Two of the managers (8%) indicated that the plastic tubing in their SFMs was never disinfected, while half of the managers (N = 13) indicated that their tubing was disinfected by an outside company (typically the Coca-Cola Co. or PepsiCo Inc. distributor of the machines). Finally, six of the managers (23%) indicated their employees disinfected the tubing. There was no difference, however, in the level of contamination (HPC) of beverages sampled from establishments with different tube disinfecting regimens (Table 1, KW-ANOVA, N = 26, df = 3, KW = 1.93, P = 0.588).

3.3. Analysis of soda fountain machines with established disinfecting regimens

Disinfecting of the plastic tubing in the two SFMs on the Hollins University campus did not reduce microbial contamination in the 16 beverages (Fig. 2). In fact, immediately afterwards there was a significantly higher HPC in the beverages when compared to the samples taken before disinfecting and 11 days after disinfecting (Friedman Test, N = 16, $\chi^2 = 52.01, df = 2, P < 0.0005$). Moreover, 14 of the 16 beverages (88%) collected immediately after disinfecting had HPC > 500 CFU/mL, while none of the samples collected before or 11 days after disinfecting exceeded this EPA drinking water regulation (US EPA, 2012). Post hoc tests revealed there was no difference in microbial density in beverages sampled 5 days before or 11 days after disinfecting.

3.4. Analysis of contamination level in beverages sampled after machine use

Dispensing –0.95 L of a beverage significantly decreased the number of microbes in soda fountain beverages (Fig. 3). The average HPC for beverages decreased by 55% (Wilcoxon Signed Rank Test, N = 30, $Z = –4.14, P = 0.001$), while the average coliform count decreased by 75% (Wilcoxon Signed Rank Test, N = 30, $Z = –4.05, P = 0.001$).

4. Discussion

Over 70% of the beverages dispensed from SFMs (20 of 28) examined by White et al. (2010) and re-sampled 13 months later in this study showed microbial contamination. These findings suggest that beverages dispensed from SFMs are easily re-contaminated or can be contaminated over long periods of time, similar to findings from studies of DUWLs (Coleman et al., 2009; Merritt et al., 2001).

Disinfecting regimens may influence patterns of microbial contamination; however, beverages dispensed from SFMs with a higher frequency of self-reported nozzle disinfecting did not have fewer

![Fig. 1. Average (±SE) HPC and coliform counts for 28 beverages (10 soda, 10 diet, 8 waters) collected from 10 SFMs sampled in 2009 (White et al., 2010) and 2010.](image-url)
Given that there are over 195,000 fast food establishments in the U.S. and countless other establishments (e.g., convenience stores), it is likely that immunocompromised individuals, which number over 8.5 million in the U.S. (Kemper et al., 2002), regularly come in contact with contaminated SFM beverages (Fitzgerald, 2007). While we did not isolate and identify any of the microbes in this study, Hertin (2011) and White et al. (2010) both isolated E. coli from soda fountain beverages. While most strains of E. coli are non-pathogenic, its presence indicates fecal contamination which could be linked to other more pathogenic microbes. Additionally, White et al. (2010) isolated several species of Gram-negative bacteria (Chryseobacterium meningosepticum, Klebsiella pneumoniae, and Stenotrophomonas maltophilia) which are known to be opportunistic pathogens and of particular risk to the immunocompromised (Brooke, 2012; Gungor et al., 2003; Hung et al., 2008; Struve and Krogfelt, 2004). While disinfecting SFMs does not appear to eliminate microbial contamination, perhaps ensuring regular disinfecting of plastic lines may reduce the potential exposure to microbes by ensuring that contamination remains at acceptable levels (e.g., HPC < 500 CFU/mL). Beverages dispensed from the SFMs in the Hollins University cafeteria have been regularly sampled for 2 years and have never exceeded an HPC > 500 CFU/mL except immediately following disinfecting. These machines are disinfected every 3 months.

Disinfecting regimens have been studied extensively with DUWL systems where biofilm formation can occur in as little as two weeks with HPC values exceeding 10,000 CFU/mL (Lin et al., 2011). Tuttlebee et al. (2002) found that weekly disinfecting of DUWLs with hydrogen peroxide ($H_2O_2$) resulted in an HPC < 200 CFU/mL (recommendation of the American Dental Association). However, if disinfecting was halted, water quality in the DUWLs deteriorated with contamination levels exceeding recommended amounts within three weeks. A more recent study has shown that the biofilms in these lines are tenacious, persisting even after exposure to 7% $H_2O_2$ for 24 h (Lin et al., 2011). For DUWLs, Lin et al. (2011) found that the only effective way to remove biofilms was to disinfect frequently with a 2% $H_2O_2$ in conjunction with constantly mixing a 0.5% $H_2O_2$ solution in with the municipal water.

Mixing $H_2O_2$ into waterlines of SFMs would likely alter the taste of the beverage, and would therefore be a less desirable option for controlling contamination. While other tubing materials which may be less likely to support the formation of biofilms are being explored (e.g., PTFE, see Sachetti et al., 2007; Kargar et al., 2012), there may be strategies that consumers can use to reduce their exposure to microbes from soda fountain beverages. We found that dispensing ~0.95 L of a soda beverage after a period of presumed low use decreased HPC by 55% and coliform CFUs by 75% in beverage samples. Similar strategies have been recommended for DUWLs (U.S. Department of Health, 2003), where one study reported a 50% reduction in microbial CFU/mL after three minutes of flushing from DUWLs (Rice et al., 2006).

Data from our study and those of others (Hertin, 2011; White et al., 2010) strongly suggest that microbial contamination of SFMs results from biofilm formation in the plastic tubing of SFMs. It would be useful to confirm this with direct analysis of SFM tubing, as well as to understand the rates at which sterile SFM tubing is colonized by microbes and the potential pathogens that can grow under these conditions.

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