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A comparative study of indicator bacteria present in ice and soda from Las Vegas food establishments

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A COMPARATIVE STUDY OF INDICATOR BACTERIA PRESENT IN ICE AND SODA FROM LAS VEGAS FOOD ESTABLISHMENTS

By

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ABSTRACT

A Comparative Study of Indicator Bacteria Present in Ice and Soda from Las Vegas Food Establishments

by

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Microbial analysis has long been used as an indicator of water quality. Since the passing of the Safe Drinking Water Act in 1974, microbial standards have been strictly set by the Environmental Protection Agency (EPA) to ensure that the public health is protected from bacterial pathogens. The bacteriological quality of water generally deteriorates as it travels from water treatment facilities through the main distribution system and into private plumbing and distribution systems. For example, Heterotrophic Plate Count (HPC) values typically increase once the water has entered plumbing devices such as beverage vending machines. Upon reaching a private facility, the opportunity for bacterial growth and human contamination is present.

In this study used the EPA water quality standards were used as a reference to analyze ice and soda samples collected from local food establishments for the presence of heterotrophic and coliform bacteria. The samples were evaluated with respect to the U.S. drinking water standards as indicators of the quality of the ice and soda. The study provided important information regarding the quality of the ice and soda dispensed in Las Vegas food establishments. Of the samples analyzed in this study, 33.3% of ice samples and 55.6% of soda samples exceeded the EPA limits set for heterotrophic bacteria

concentration for drinking water. Of the ice samples collected, 72.2% were positive for presumptive coliform bacteria presence, and 88.9% of the soda samples were positive for presumptive coliform presence. No statistical significance was observed between the concentration of heterotrophic bacteria in ice samples (median = 202 CFU/ml) and soda samples (median = 775 CFU/ml). However, the presumptive coliform bacteria data did show that the soda samples (median = 139 CFU/ml) had a significantly higher concentration when compared to the ice samples (median = 3 CFU/ml). The type of food establishment from which the samples were collected did not have a significant influence on the bacteriological quality of the ice and soda. The findings of this study provide important evidence that could have public health implications and may influence future studies related to bacterial contamination of beverages sold in the Las Vegas Valley.

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INTRODUCTION

Purpose of the Study

The purpose of the study was to measure the bacteriological quality of soda and ice that are distributed by Las Vegas casino restaurants, convenience stores, and fast food establishments, and to compare the findings to the existing Environmental Protection Agency (EPA) drinking water quality standards. Ice and soda machines in Las Vegas are regulated by the Southern Nevada Health District under Regulation 96, and are currently visually inspected by local health inspectors along with any ice scoops and the areas surrounding the machines. Typical violations related to ice and soda machines have been reported by the Southern Nevada Health District as, "ice scoop stored on top of dirty ice machine" and "pink slime growth inside soda gun nozzle" (Hynes, 2009). This identifies an obvious health risk, but does not determine the degree in which the "dirty ice machine, slime or growth" may negatively impact human health because the contents of the slime and dirt are not identified. Restaurants also must abide by Clark County regulations (Appendix A), and must be in adherence with the Nevada Administrative Codes (Appendix B). However, the microbial quality of the soda and ice produced by soda fountains and ice machines has not been examined in Las Vegas, Nevada. The findings of this study provide insight into the quality of the soda and ice that many residents and tourists of Las Vegas consume regularly.

Background and Significance

Water Quality

The United States is considered to have a high quality drinking water system that

has a relatively low incidence of exceeding bacteriological quality standards. Since the implementation of standards set by the EPA such as the Safe Drinking Water Act (SDWA) of 1974, there has been a considerable decline in waterborne illness. Water regulations are critical in protecting the public from poor quality drinking water, but indirectly, they are also essential in keeping ingestible liquids other than water safe for consuming. Water is the primary ingredient in the production of ice and soda; therefore, if it has bacterial contamination, then the ice and soda will also be contaminated. Alternatively, contamination in the machinery, parts, and tubing that are involved in the production of ice and soda could also lead to a contaminated final product.

The Southern Nevada Water Authority (SNWA) collects over 35,000 water samples throughout the Las Vegas valley and performs approximately 500,000 water analyses annually in their water quality laboratory (SNWA, 2011). Continuous monitoring of water treatment facilities and distribution points of tap water has shown that the drinking water drawn into private facilities, such as restaurants and convenience stores, meets the bacteriological quality standards of the EPA. Therefore, tap water used to produce ice and soda would not be the likely source of poor microbiological quality of ice and soda.

Bacteriological Indicators of Quality

An accumulation of microorganisms on surfaces, in combination with organic and inorganic substances, can form a matrix known as a biofilm. Biofilms can be responsible for multiple water quality issues, including reduction of dissolved oxygen, odor and flavor changes, colored water from increased corrosion, loss of disinfectant residuals, and

increases in overall bacterial presence (LeChevallier, 2003). However, because access to small and private distribution systems is difficult, biofilm research has been limited (Wende & Characklis, 1990) and is not generally used as an indicator of water quality. Due to the lack of access to likely biofilm locations within distribution systems, such as machinery parts and tubing, other bacterial indicators are used to measure water quality.

Heterotrophic bacteria require organic compounds for growth and can include coliform and non-coliform bacteria. These bacteria are considered normal environmental organisms and are not usually associated with pathogenic bacteria; however, these organisms are consistently used as an indicator of water quality. Heterotrophic plate counts (HPC) have been a useful tool in monitoring the effectiveness of water treatment and to measure re-growth (Reasoner, 1990; World Health Organization [WHO], 2002). The EPA has concluded that heterotrophic plate counts do not correlate with an increased likelihood of fecal contamination (EPA, 2006), but HPC that exceed the EPA limit can potentially interfere with the detection of coliform bacteria (Reasoner, 2004). Therefore, HPC is important in the evaluation of water quality and was used in this study as a general indicator of bacteriological quality of ice and soda.

Coliform bacteria, such as *Escherichia coli,* originate from the intestinal tracts of animals and humans. Due to the origins of some coliform bacteria and the potential harm they can cause to human health, their presence in water has long been used as an indicator of fecal contamination. In an effort to prevent waterborne illnesses, the EPA has created regulations that require drinking water to have no more than 5% of monthly samples test positive for total coliforms, with no tolerance for fecal coliforms, and no more than 500 heterotrophic bacterial colonies per milliliter of water (EPA, 2009). These regulations

are intended to ensure that the water used in private and retail establishments is below the microbiological limits, and safe to consume. Detection of these microorganisms, both pathogenic and non-pathogenic, from samples derived from ice and soda machines is an effective tool in determining the overall bacteriological quality of ice and soda and identifying differences in quality among types of food establishments in the Las Vegas Valley.

Sources of Contamination

Without regular sanitation practices, biofilms may build up in piping and other parts of distribution systems, such as self service ice and soda machine tubing and water holding tanks. There are multiple factors associated with bacterial growth on biofilms including factors like temperature and concentrations of residual disinfectants (Hunter, Colford, LeChevallier, Binder & Berger, 2001), but when conditions are appropriate, the development of biofilms can provide an environment in which heterotrophic bacteria, including coliforms, can survive and even thrive (EPA, 2006). In addition, formed biofilms create an environment for microbes to be protected from disinfectants, reduce environmental stress, and allow for growth and recovery of injured microorganisms (EPA, 2002).

Manufacturers of self-dispense soda machines and ice machines provide instructions in their product manuals for scheduled maintenance to prevent corrosion, contamination, and prolong machine life. For example, Lancer®, a manufacturer of commercial soda fountain dispensers recommends a daily, weekly, monthly, bi-annually and yearly maintenance schedule with specific steps to follow upon the sale of their

machines (Lancer, 2004). These recommended maintenance procedures range from basic cleaning of outer machine parts to more aggressive strategies that require disassembly of the machine and applications of industrial disinfectant chemicals. The growth and viability of any introduced organisms in soda and ice machines can be prevented with thorough sanitization and maintenance procedures, but the regularity of this practice is generally anecdotal in nature and relatively unknown.

Microbial contamination of ice and soda can also occur as a result of insufficient hand washing and food handler hygiene. The existence of coliform bacteria in water, particularly fecal coliforms, has often been used as a reliable indicator of contamination by food handlers, and in some cases, consumer contamination. In addition, previous research has reported that customers using self-serve soda machines sometimes touch the spout from which the ice and the soda are dispensed (White, Godard, Belling, Kasza & Beach, 2010). This contact can allow for bacterial transfer from the customer to the spout, which comes into direct contact with the liquids that are dispensed.

Review of Related Literature

Water Cooler Dispensers

Many water cooler dispensers are structurally and functionally similar to the soda dispensers used in food establishments. Both water coolers and soda dispensers deliver an ingestible liquid product that is derived by using tap water through a distribution system of tubing, filtration, and in some cases, carbonation. Therefore, studies conducted on bacteriological quality of water dispensed from water coolers provide information that is relevant to this study, which examined the quality of soda and ice dispensed from soda

fountain machines.

Previous research conducted on water cooler dispensers in Italy clearly showed evidence that the tap water feeding into water cooler dispensers was of higher quality compared to the water dispensed out of the cooler (Liguori, Cavallotti, Amiranda, Anastasi & Angelillo, 2010). This study used the Italian and European drinking water standards as a reference. These regulations for drinking water for human consumption include a Total Viable Count limit of <100 Colony Forming Units (CFU) /ml at 22ºC and < 20 CFU/ml at 37ºC. Liguori et al. (2010) examined the output of non-carbonated water and carbonated water, as well as the tap water that fed the water coolers. Forty-three different water cooler units were examined and analyzed for total bacterial counts. The researchers determined that 71% of the non-carbonated water samples exceeded the standards at 22ºC, and 81% of the samples exceeded the standards at 37ºC. Of the carbonated samples, 86 % and 88% exceeded the standards at 22ºC and at 37ºC, respectively, whereas only 17% of the tap water samples exceeded the standards at both 22ºC and 37ºC. This Italian study demonstrated that these water dispensing systems, which are similar to ice/soda dispensing machines, produced an output with remarkably lower bacteriological quality when compared to the tap water input (Liguori et al., 2010).

Soda and Ice Dispensers

Recent research has also been conducted specifically on the quality of soda fountain beverage samples collected from fast food establishments in the United States. In 2010, 90 beverage samples were collected from 30 fast food establishments in the state of Virginia, and analyzed for microbial content. The study reported that over 70% of the

soda fountain beverages sampled had bacterial growth, with 20% exceeding the HPC limits for drinking water, 48% contained coliform bacteria, and 6.7% had *Escherichia coli* presence (White et al., 2010). This research revealed significant contamination levels in Virginia samples and demonstrated the need to analyze samples in other locations, such as Las Vegas.

Research Questions and Hypotheses

Research Question 1

Will the concentrations of bacteria present in ice and soda samples obtained from three different types of Las Vegas food establishments exceed the EPA water standards for heterotrophic and coliform bacteria?

H_o: The bacteria present in ice/soda samples obtained from food establishments in Las Vegas will not exceed the EPA standards.

Research Question 2

If bacteria are present, which matrix (i.e., soda or ice) will have more coliform and heterotrophic bacteria present?

H_o: There will be no difference in the concentration of heterotrophic and coliform bacteria isolated in soda samples compared to the ice samples.

Research Question 3

Is there a difference in the bacterial concentrations found in ice and soda samples based on the type of food establishment the samples were collected from (i.e., fast food

restaurants, convenience stores, and casino restaurants)?

H_o: There will be no difference in the bacterial counts found in ice/soda between the three different food establishments.

CHAPTER 2

METHODOLOGY

Sample Collection

Thirty-six samples including 18 ice samples and 18 soda samples were collected throughout the Las Vegas Valley in 3 different zip codes between 6:00 a.m. and 12:00 p.m Pacific Standard Time (PST). In each zip code, two casinos, two fast food establishments and two convenience store locations within a radius of 5 miles of each other were targeted for sample collection resulting in 6 ice samples and 6 soda samples per zip code. A specific establishment was not sampled more than once throughout the study (e.g., Wendy's was only sampled one time). All of the samples were collected in the months of September and October of 2011. A minimum volume of 250 ml per sample consisting of one cup of ice and one cup of diet cola without ice (either Diet Pepsi® Soda or Diet Coca Cola® Soda), was collected from three different types of food establishments.

The zip codes that were used for this study were selected to represent both a wide geographic area, as well as a diverse cross section of different socioeconomic characteristics in an effort to represent the Las Vegas Valley as a whole (Appendix C). The median household income, as reported by the 2000 Census in the three zip codes selected, ranged from $23,166 to $60,129. Zip codes that did not include more than one casino in the area were excluded from the sampling selection.

Samples were collected using the procedures and methods approved by the U.S. EPA (Standard Methods for the Examination of Water and Wastewater [SMEWW] 9020, 2005). Ice and diet soda samples were collected in cups provided by the restaurant. An

additional empty cup was obtained at the time of sample collection by the researcher to perform sterility cup controls during microbiological analysis. All of the samples collected from the target locations were stored in an enclosed cooler on ice for transport to the Emerging Diseases Laboratory at the University of Nevada, Las Vegas (UNLV). Before processing and after all of the ice had completely melted, a sodium thiosulfate 10 mg tablet (Brim Technologies, Inc., Randolph, New Jersey) was added to each ice sample (1 tablet per 200 ml) to neutralize the residual chlorine. The total collection and transport time did not exceed 6 hours, and all of the samples were processed in the laboratory within 24 hours of collection.

Every sample collected was labeled upon collection. Sample labeling included the date and time of collection as well as an 8 digit code. The code represented the type of food establishment, a letter to represent sample number, the zip code, and the type of sample. For example, "FFa89145I" indicated that the sample was collected from a fast food establishment (FF), it was the first of two samples from one zip code (a), in the zip code 89145 and contained ice (I).

The food establishments selected for this study fulfilled the following inclusion and exclusion criteria.

FAST FOOD ESTABLISHMENTS

Inclusion Criteria:

- Must be in accordance with the North American Industry Classification System (NAICS) Code 722211- Fast Food Restaurants (U.S. Census Bureau, 2007).
    - Code 722211-Primarily engaged in providing food services (except snack and nonalcoholic beverage bars) where patrons generally order or select

items and pay before eating. Food and drink may be consumed on premises, taken out, or delivered to the customers' location. Some establishments may provide these food services in combination with selling alcoholic beverages.

- Establishment may or may not also offer gasoline from fuel pumps.
- Must offer carbonated fountain soda with ice.

Exclusion Criteria:

- The establishment must not be physically connected to or within another business (i.e., gas station, superstore, and mall).

CONVENIENCE STORES

Inclusion Criteria:

- Must be in accordance with either the North American Industry Classification System (NAICS) Code 445120-Convenience Stores or 447110- Gasoline Stations with Convenience stores (U.S. Census Bureau, 2007).
    - Code 455120- This industry comprises establishments known as convenience stores or food marts (except those with fuel pumps) primarily engaged in retailing a limited line of goods that generally includes milk, bread, soda, and snacks.
    - Code 447110-Engaged in retailing automotive fuels (e.g., diesel fuel, gasohol, gasoline) in combination with convenience store or food mart items. These establishments can either be in a convenience store (i.e., food mart) setting or a gasoline station setting. These establishments may also

provide automotive repair services.

- Must offer carbonated fountain soda with ice.

Exclusion Criteria:

- Not considered a grocery store as defined by North American Industry Classification System (NAICS) Code 4451, 44511, and 44512 (U.S. Census Bureau, 2007).
- Does not have premises inside with tables for food or drink consumption.

CASINO RESTAURANT

Inclusion Criteria:

- Must be in accordance with the North American Industry Classification System (NAICS) Code 722110- Full-Service Restaurants (U.S. Census Bureau, 2007).
    - Code 722110-Primarily engaged in providing food services to patrons who order and are served while seated (i.e., waiter/waitress services) and pay after eating. These establishments may provide this type of food services to patrons in combination with selling alcoholic beverages, providing carry out services, or presenting live nontheatrical entertainment.
- Must have > 100 gambling machines within the same building.
- Must have table games within the same building.
- Must be located inside a main casino building.
- Must offer carbonated fountain soda with ice.

Exclusion Criteria:

- None

Microbiological Analysis

Heterotrophic Bacteria

Culture methods were used to analyze the heterotrophic concentrations of each sample. R2A agar (Difco Laboratories, Sparks, MD) was used for the enumeration of heterotrophic bacteria using membrane filtration and spread plate methods (SMEWW 9215, 2005) with replicates. One hundred microliters (100µl) of each sample, melted ice or soda, were inoculated in duplicate onto R2A agar plates using the spread plate method. One milliliter (1 ml) of each sample was filtered through a mixed cellulose esters, 0.45µm diameter pore size filter (Millipore, Billerica, Massachusetts) using the membrane filtration method and the membrane was placed directly onto the R2A medium. Plates were incubated at 35 ± 2ºC for 5-7 days before counting the heterotrophic bacteria colonies.

All liquid samples (ice and soda) collected were stored for 24-32 hours in the refrigerator at 4 ± 2ºC. The plates were visually inspected within 24-32 hours after inoculation to determine if the preliminary growth showed high concentrations of bacteria on the plate. If high concentrations were observed, a 10^{-2} dilution was inoculated onto R2A agar using the spread plate method and incubated for 5-7 days as indicated above.

Coliform Bacteria

Culture methods were used to enumerate presumptive coliform colonies in samples with Eosin Methylene Blue Agar (Modified) Levine (EMB; Oxoid Ltd., Thermo Fisher Scientific, Waltham, MA). One milliliter (1 ml) of each sample was filtered in

duplicate through a 0.45µm diameter pore size, mixed cellulose esters filter (Millipore, Billerica, Massachusetts) using the membrane filtration method (SMEWW 9222, 2005), followed by a separate filtration of one hundred milliliters (100ml) of each sample in duplicate. Membranes were placed on EMB agar plates and the plates were incubated at 35 ± 2ºC for 24-48 hours. EMB agar is a differentiating medium for Enterobacteriaceae; therefore, any indications of *Escherichia coli* (colonies with green metallic sheen) were also documented. Colony forming units (CFU) on EMB agar were counted and documented as presumptive coliform growth.

Similar to the R2A plates, EMB plates were stored in the refrigerator at 4 ± 2ºC. The plates were visually inspected within 24-32 hours after inoculation to determine if the volumes inoculated had preliminary countable or non-countable colonies. If high concentrations were observed within 24 - 32 hours, a 10^{-2} dilution was inoculated onto EMB agar using the spread plate method and incubated for 24-48 hours before counting bacterial colonies. If low concentrations were observed, a ten milliliter (10 ml) sample was filtered in duplicate using membrane filtration, and incubated for 24-48 hours as indicated above.

Colony Counts

The acceptable limits for counting colonies are 30-300 CFU for spread plates and 20-200 CFU for filter membranes (SMEWW 9215, 2005). All colonies were counted using the established plate limits where possible but the lower limit used in this study was 20 CFU. Any plates with less than the corresponding minimum were recorded as < Lower Detection Limit (LDL) and any plates with more than 300 CFU were recorded as

Too Numerous To Count (TNTC).

Escherichia coli Confirmation

Identification of *E. coli* was performed by isolation of colonies enumerated on EMB agar. All colonies that exhibited a green metallic sheen were documented as likely to have *E. coli* presence, as indicated by the product manufacturer. Colonies from the stored EMB plates were later isolated on fresh EMB plates where possible, and archived in the Emerging Diseases Laboratory at 4°C.

Controls

Growth and sterility controls were performed for each batch of media used in this study according to the media manufacturers' instructions. *Escherichia coli* ATCC 25922 (American Type Culture Collection; ATCC, Manassas, VA) was used as a positive growth control and inoculated onto EMB and R2A plates, incubated at 37ºC and inspected after 24 hours prior to using the media. Sterility controls consisted of incubating un-inoculated agar plates for at least 24 hours. Cup sterility controls were performed by swirling at least twenty milliliters (20 ml) of sterile ultra pure water in each cup and spread plating 100 µl of the sample onto EMB and R2A plates, followed by incubations as described above.

Statistical Analysis

The software programs used in this study for statistical analysis and interpretations were Microsoft Office Excel 2007 and IBM© SPSS 19 statistical student

package. The data collected in this study were used for comparative analysis, and statistical significance to test the hypotheses was determined using a p-value of 0.05. Using the limits as stated above, all the raw counts that were < LDL were changed to 0 to perform statistical analyses, and all samples that were TNTC were changed to 300 CFU/ml to perform statistical analyses and obtain a visual representation of the data.

Basic descriptive summaries to provide frequencies and percentages were used to examine the bacteriological quality of the samples with respect to the EPA coliform and and heterotrophic bacteria standards for drinking water. Shapiro-Wilk's Test of normality was applied to the data that described the CFU/ml of heterotrophic and presumptive coliforms for both ice and soda samples. Non-parametric Mann-Whitney tests were used to determine if there were statistically significant differences between the two types of samples analyzed (i.e., ice and soda). Additionally, contingency tables using Fisher's exact test were used to describe the difference in frequencies of ice and soda samples that exceeded EPA water quality standards.

Lastly, non-parametric independent samples tests were used to determine if there was a statistically significant difference of the medians in bacterial concentrations between the type of food establishments in which the samples were collected. Further analysis using the Kruskal-Wallis one-way analysis of variance was used to rank the bacterial concentrations between the types of food establishments and determine if there was a difference in the medians of bacterial concentrations from which the samples originated.

CHAPTER 3

RESULTS

Sample Location Characteristics

Six different locations were sampled in each of three zip codes in the Las Vegas valley. The zip codes selected did not share any boundaries and represented the central, west and east geographic areas of the valley (Appendix C). Socio-economic status and income level of the zip code locations were intentionally not controlled in this study in order to collect a representative sample of the entire Las Vegas valley. The locations from which the samples were collected also have a wide variability in the number of residential addresses within the zip code and population (Table 1).

Table 1: Characteristics of Las Vegas Valley Locations Sampled

Zip Code	89101	89121	89145
Geographic Location	Downtown/ Central	Boulder Hwy/East	Summerlin/ Northwest
Median Household Income (2000)	$23,166	$40,542	$60,129
Residential addresses	14,324	23,778	9,237
Total Population	52,617	61,669	19,337

* Data were obtained from the 2000 Census.

Bacteriological Quality of Ice and Soda

Heterotrophic Bacteria

The frequencies in which ice an soda samples exceeded the EPA drinking water standards for heterotrophic plate counts per ml were calculated for the total samples, N = 36. Of the 36 samples collected, 17 exceeded the EPA heterotrophic bacteria standard of > 500 CFU/ml. This accounted for 47.2 % of the total samples. These seventeen samples corresponded to 6 of 18 (33.3%) ice samples exceeding the EPA water quality standards, and 10 of 18 (55.6%) soda samples exceeding the standards (Figure 1).

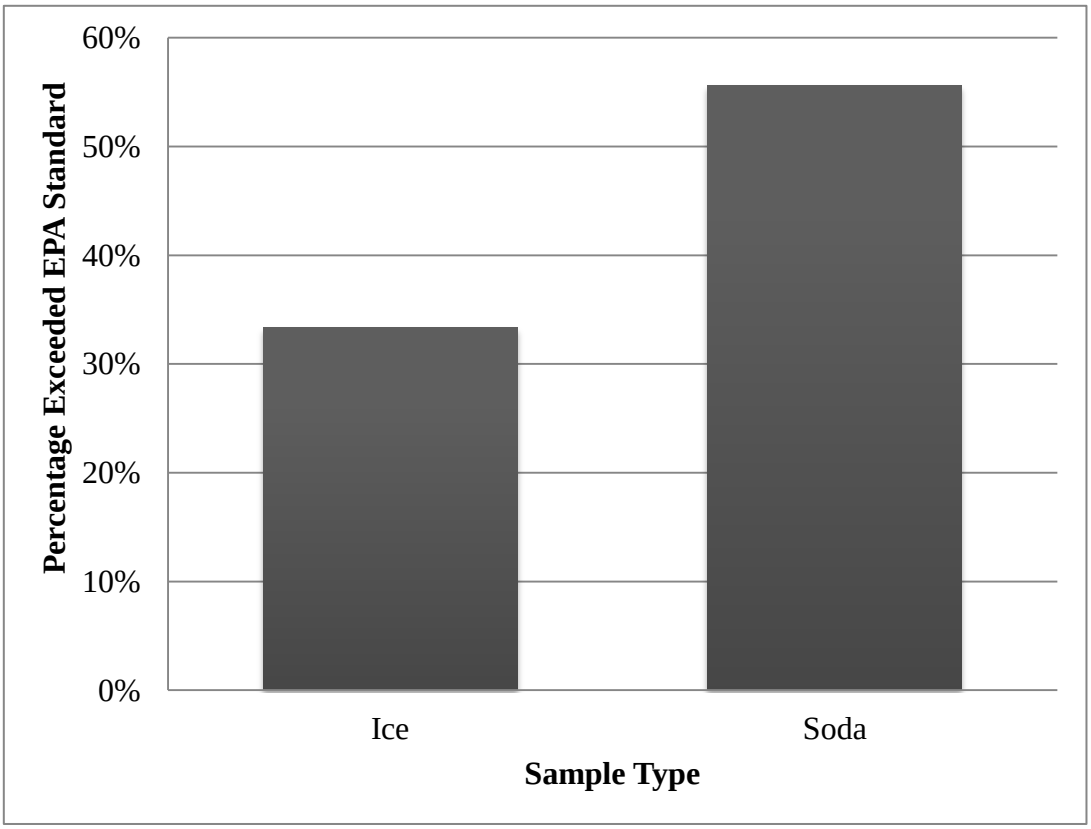


Figure 1: Percentage of Samples with Heterotrophic Plate Count > 500 CFU/ml

Coliform Bacteria

Presumptive coliform bacteria found in the samples were recorded if > 1 CFU/ml was calculated from an average of duplicate plate counts. Of the total samples, 29 of 36 were positive for presumptive coliform presence. These 29 samples corresponded to 13 of 18 (72.2%) of the ice samples exceeding the EPA water quality standard for coliform presence, and 16 of 18 (88.8%) samples of soda exceeding the coliform bacteria standards (Figure 2). The EPA drinking water standards stipulate that < 5% of monthly samples can test positive for total coliforms. The percentage of total samples positive for coliform bacteria in this study was 81%.

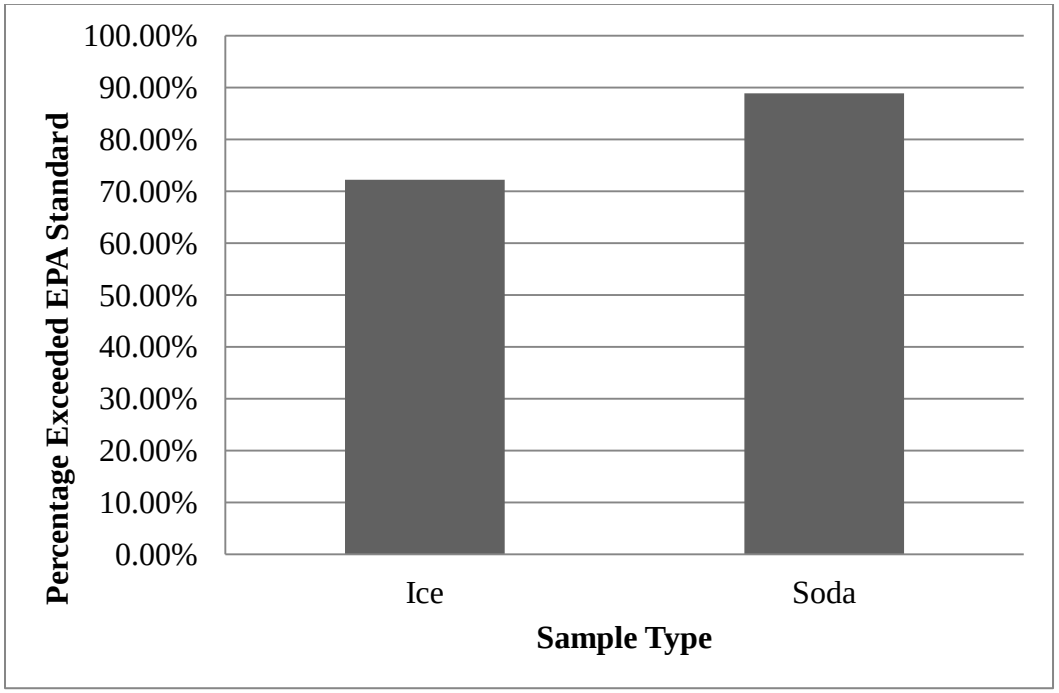


Figure 2: Percentage of Samples with Presumptive Coliform Presence

Identification of Escherichia coli

Presumptive coliform colonies were subcultured onto EMB agar to confirm *E. coli* presence. The selective EMB media used in this study provided a clear description of the visual manifestation of *E. coli* presence. The manufacturer's product description stated that *Escherichia coli* would grow as "isolated colonies, 2-3mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centers by transmitted light." (Oxoid, Ltd.)

Presumptive coliform colonies on 32 samples were streaked for isolation onto fresh EMB plates. Upon visual inspection after incubation for 48 hours at 37ºC, 8 of 32 samples had *E. coli* colonies differentiated by color as described by the manufacturer. Six of these were soda samples collected from all three types of food establishments (e.g., fast food, convenience stores, and casino restaurants). The other two samples containing *E. coli* were ice samples collected from one fast food restaurant and one casino restaurant (Table 2).

Table 2: *Escherichia coli* Presence in Isolated Samples

	Frequency		Totals
	Ice	Soda	
***E. coli* Confirmed**	2	6	8
No Presence of *E.coli*	15	9	24
Total	17	15	32

Summary of Exceedance of EPA Standards

Data showed that 33.3% of the ice samples exceeded the heterotrophic bacteria standard and 72.2% exceeded the coliform standard. In soda samples, 55.6% of the samples collected exceed the heterotrophic limits, and 88.9% of samples exceeded the coliform standard for water set by the EPA (Tables 3 and 4).

Comparative Analysis of Sample Types

Contingency tables were developed to determine if there was a statistically significant difference between the number of samples that exceeded heterotrophic and coliform bacteria EPA standards between the ice and soda samples (Tables 3 and 4).

Table 3: Heterotrophic Bacteriological Quality of Samples, N = 36

	Frequency, (%)		Totals
	Ice	Soda	
Exceeded EPA Standard	6 (33.3%)	10 (55.6%)	16 (44.4%)
Did Not Exceed EPA Standard	12 (66.6%)	8 (44.4%)	20 (55.6%)
Total	18 (100%)	18 (100%)	36 (100%)

* Exceeded EPA Standard if > 500 CFU/ml from a mean of duplicate plate counts

The statistical analysis showed no significant differences between the ice and soda when comparing the number of samples that exceeded or did not exceed the

heterotrophic bacterial standard (Fisher's exact test $p = .505$), or with samples that exceeded or did not exceed the coliform standard (Fisher's exact test $p = .402$).

Table 4: Presumptive Coliform Presence in Total Samples, N = 36

	Frequency, (%)		Totals
	Ice	Soda	
Presence	13 (72.2%)	16 (88.9%)	29 (80.6%)
No Presence	5 (27.8%)	2 (11.1%)	7 (19.4%)
Total	18 (100%)	18 (100%)	36 (100%)

* Presumptive Coliform presence if > 1 CFU/ml from a mean of duplicate plates

Shapiro-Wilk's Test of normality showed that the data for this study were not normally distributed for heterotrophic plate counts ($W = .323$, $p = .000$) or for presumptive coliform bacteria ($W = .736$, $p = .000$). Attempts to transform the data for normal distribution failed with standard deviation and logarithmic transformation methods. Therefore, the quantifiable concentrations (CFU/ml) of presumptive coliforms and heterotrophic bacterial counts were statistically analyzed using the non-parametric Mann-Whitney Test. This analysis showed that there was no significant difference between the number of heterotrophic microorganisms recovered from the ice (Mean rank = 15.58) compared to the soda samples (Mean rank = 21.42) at 37ºC ($U = 109.5$, $Z = -1.662$, $p = .097$). However, there was a statistically significant difference in the concentration of presumptive coliforms in ice samples (Mean rank = 12.86) when

compared to the concentration of presumptive coliforms in soda samples (Mean rank = 24.14) (U= 60.5, Z = -3.228, p = .001) (Figure 3).

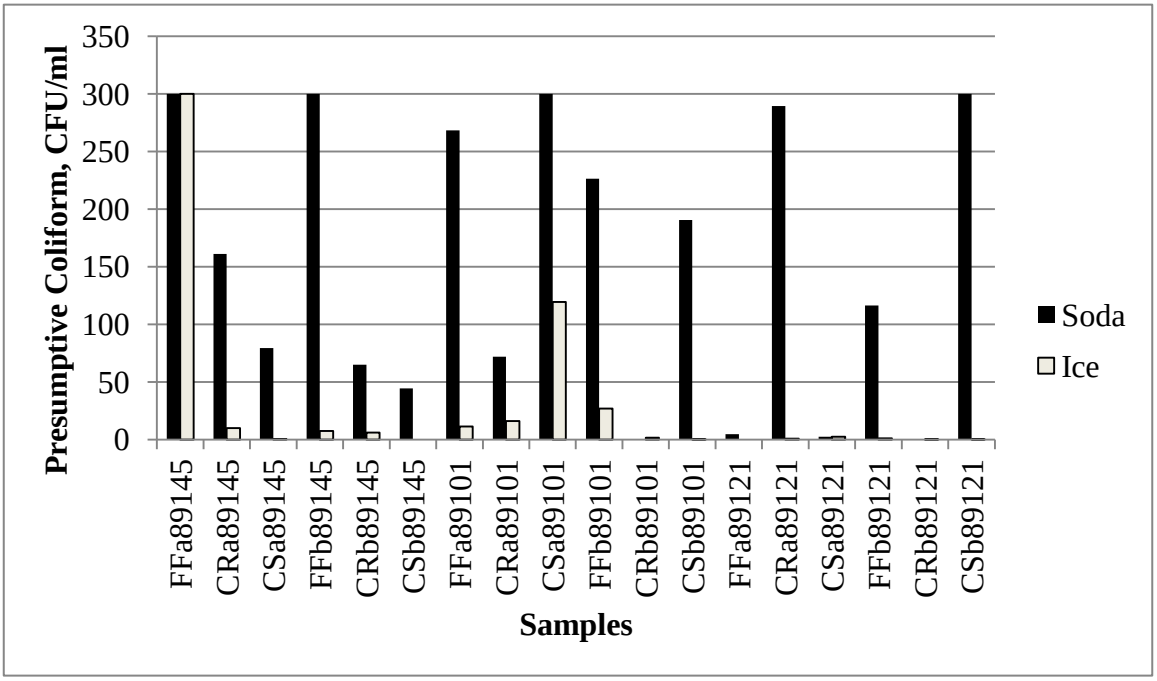


Figure 3: Presumptive Coliform (CFU/ml) from Ice and Soda Samples
* Note: All colonies counted as < LDL were changed to 0; all colonies counted as TNTC were changed to 300 for this bar graph.

Comparative Analysis Between Food Establishments

A total of 3 different types of food establishments were used as collection locations for the soda and ice samples. Frequency data were used to initially compare the concentration and percentage of bacteria between groups (Figures 4 and 5). Non-parametric statistical analysis was used to determine whether there was a statistical significance of the bacteriological quality of samples based on the type of food

establishment from which samples were collected. Among the 36 samples collected 12 samples were from casino restaurants, 12 samples were from convenience stores, and 12 were collected from fast food establishments.

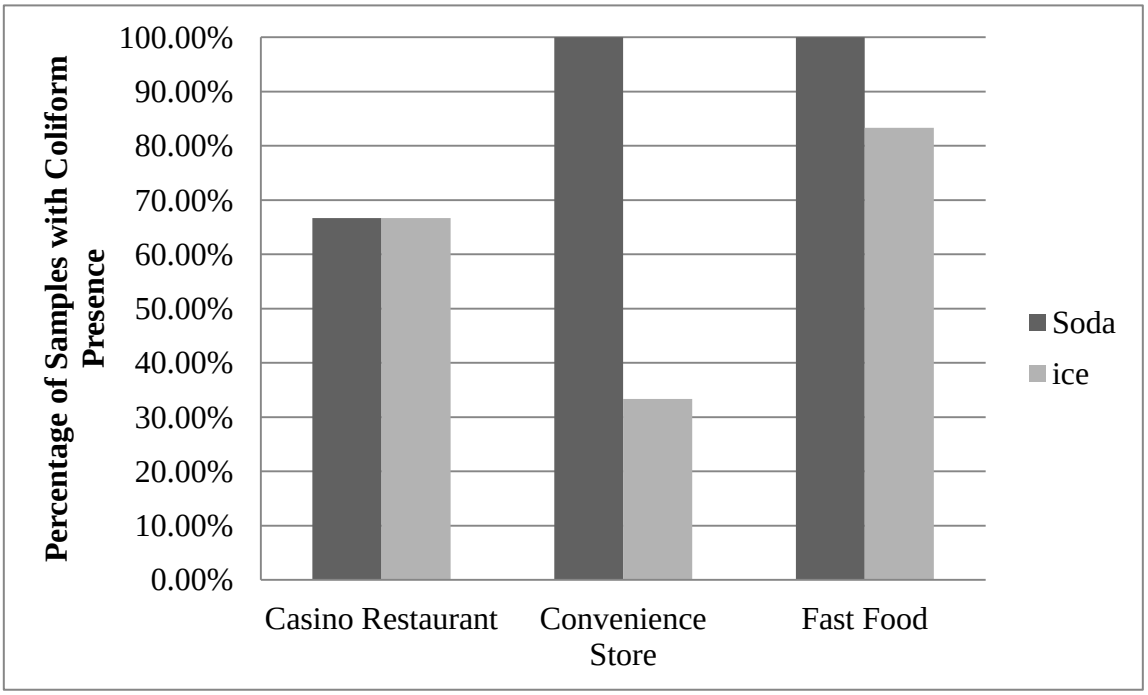


Figure 4: Percentage of Presumptive Coliform Bacteria Presence by Food Establishment

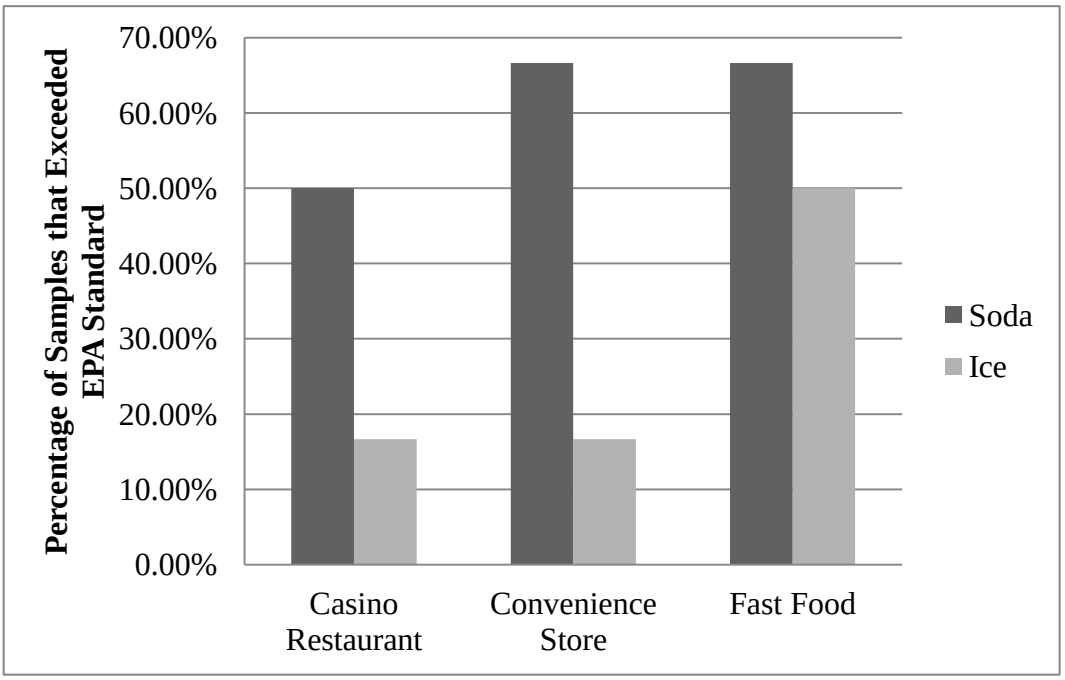


Figure 5: Percentage of Heterotrophic Bacteria > 500CFU/ml by Food Establishment

Non-parametric independent samples median tests demonstrated that there was no statistically significant difference between the concentration of presumptive coliform bacteria collected from different types of food establishment (Ice $p = .513$, Soda $p = .135$) and there was no significant difference in the concentrations of heterotrophic bacteria between the different types of food establishments (Ice $p = .513$, Soda $p = .513$) (Table 5).

Table 5: Hypothesis Test Summary for Comparison of Food Establishment Type Using Independent-Samples Median Test

Null Hypothesis	P value
The medians of ice coliform CFU/ml are the same across types of food establishments.	.513
The medians of soda coliform CFU/ml are the same across types of food establishments.	.135
The medians of ice heterotrophic CFU/ml are the same across types of food establishments.	.513
The medians of soda heterotrophic CFU/ml are the same across types of food establishments.	.513

* Asymptomatic significances are displayed ($p = 0.05$).

To further analyze the data between categories of food establishments, Kruskal-Wallis one-way analysis of variance by ranks was applied. The analysis showed no significant difference between the number of presumptive coliform bacteria in ice between food establishments ($p = .354$) or in presumptive coliform concentration in soda between food establishments ($p = .199$). Similarly, there was no statistical difference in heterotrophic concentrations in ice ($p = .586$) and soda ($p = .203$) between the types of food establishments. This additional analysis provided supplementary evidence that there is no significant difference between the type of food establishment from which the samples were collected in terms of bacteriological quality.

CHAPTER 4

DISCUSSION

Summary of Findings

Bacterial Quality of Samples

The samples collected for this study were equally distributed between ice and soda as well as the type of food establishment in which the samples were collected. Culture analysis results clearly illustrated that there is a quality issue regarding the output of ice and soda from local food establishments. The high concentration of both heterotrophic and presumptive coliform bacteria discovered by this study raises questions regarding the food handler practices of the employees working at these establishments, and the frequency of recommended maintenance and sanitization of the machines that produce ice and carbonated soda for public consumption.

Possibilities of outside contamination include customer or employee cross contamination of the outer parts of the machine that are in contact with the ice or soda dispensed. However, the introduction of bacteria into ice and soda by food handlers is likely a lesser concern because the majority of the samples collected were taken from self service fountain soda machines. Twenty six of 36 samples were collected by the researcher or the staff from self service soda fountain machines in which the employee had little or no contact with the cup, soda, or ice. This greatly reduces the chance of contamination by the worker and suggests that bacterial introduction to ice and soda was derived from another source. Although the presence of fecal coliform bacteria such as *E. coli* in a restaurant setting typically indicates contamination by food handlers, the majority of the members of the coliform family have been shown to originate, not just

from an intestinal source, but also from nonenteric environments that include biofilms within drinking water distribution systems (Toranzos, McFeters, Borrego, & Savill, 2007).

A more likely explanation for the elevated bacterial levels present in the samples is a lack of machine maintenance and sanitization. Environmental bacteria that reside in water could be amplified by biofilm buildup in the tubing and machinery parts in contact with the water, ice and soda syrups. Without proper and frequent sanitary measures, the biofilms created would likely provide an environment that is conducive for bacterial replication and growth for both pre-existing bacteria as well as any bacteria that may be introduced into the system by an outside source.

The concentration of bacterial colonies present in the samples was shown to be highly variable between the sites of collection. This variability may be explained by the maintenance performed by individual establishments or the age of the machine itself. Normal wear of soda fountain machines would likely influence bacterial survival and reproduction. Over time, machinery parts will deteriorate and possibly create divots and scratches within the distribution system increasing the total available surface area that could provide microhabitats for microorganisms and biofilm formation (Wende and Characklis, 1990). Further evaluation of the machines in which the samples were collected would be needed to determine which, if any, of these factors influenced the concentrations of bacteria present.

The first research question asked: Will the concentrations of bacteria present in ice and soda samples obtained from three different types of Las Vegas food establishments exceed the EPA water standards for heterotrophic and coliform bacteria?

Of the 36 samples collected, 17 exceeded the EPA heterotrophic bacteria standard of > 500 CFU/ml, and 29 of 36 were positive for presumptive coliform presence, exceeding the EPA water quality standard for coliform presence. In addition, 8 of the samples produced *E. coli* isolates; therefore, the null hypothesis for this research question has been rejected.

Differences in Sample Type

Ice and soda have different chemical characteristics that can affect the growth and survival of bacteria contained within them. Initial observations comparing the ice and soda sample contamination suggested that there was a difference between the two types of samples; however, the only statistical difference between the types of samples was in the amount of presumptive coliform bacteria present. Although statistical differences could not be shown for heterotrophic bacteria, the percentage and frequency of the soda samples that exceeded both the heterotrophic and coliform standards were consistently higher when compared to the number of ice samples that exceeded the standards. Certain chemical and physical properties of ice and soda could enhance or reduce the rate and opportunity for bacterial growth. This study suggests that the soda has poorer quality, in terms of bacterial concentrations, when compared to the quality of the ice samples.

The volume of one ice sample collected from a casino restaurant was slightly under 250 ml after it melted. Duplicate plates for the one hundred (100 ml) analysis (membrane filtration method) could not be inoculated and the final count was derived from one plate rather than the average of two plate counts. The CFU/ml calculated was not an outlier within the data set; therefore, it was included in the complete data set.

The second research question asked: If bacteria are present, which matrix (i.e., soda or ice) will have more coliform and heterotrophic bacteria present? The frequency in which ice and soda exceeded the standards was not statistically different between the type of samples, but the analysis of quantifiable counts of colony forming units per ml revealed a difference. Soda and ice samples did not differ in heterotrophic plate counts, but were statistically different in presumptive coliform colony counts per ml. For this reason the null hypothesis for this question was rejected.

Evaluation of Food Establishments

Three different types of food establishments were chosen in this study to represent common places in which people eat and drink at in Las Vegas. These establishments included popular fast food establishments, convenience stores and casino restaurants. Eighteen locations were sampled with a breakdown of 6 fast food establishments, 6 convenience stores, and 6 casino restaurants. Chain restaurants (e.g., McDonald's, Wendy's, and 7-Eleven) were not sampled more than once in this study in order to eliminate a bias toward or against any one particular chain. The way in which the samples were dispensed varied across food establishment types. All of the samples collected from the casino restaurants were dispensed by personnel from the restaurant, whereas 8 of 12 samples from the fast food restaurants were dispensed by the researcher, and all 12 samples collected from convenience stores were dispensed by the researcher.

Comparison analysis of the three different types of food establishments did not substantiate a difference in the bacterial concentrations between them. This evidence indicates that the soda fountain machines or ice machines are similar across all categories

of establishments. These results also imply that the way in which the sample was collected, personnel-dispensed or researcher-dispensed, did not affect the concentration of microorganisms isolated.

The third research question asked: Is there a difference in the bacterial concentrations found in ice and soda samples based on the type of food establishment the samples were collected from (i.e., fast food restaurants, convenience stores, and casino restaurants)? The non-parametric analysis applied to answer this question showed that there is no statistically significant difference in bacterial concentrations based on the type of food establishment that the sample was collected from. The null hypothesis for this research question could not be rejected, and therefore, was retained.

Overview

Contamination of *E. coli*, a known pathogenic bacterium, was observed in some of the samples, which indicates fecal contamination. This kind of contamination could lead to gastrointestinal illness in the people who are ingesting soda and/or ice. The health implications associated with *E. coli* presence in food have been well documented. While multiple strains of *E. coli* have been discovered and determined to have differences in virulence and pathogenicity, any discovery of this species of bacteria in ice and soda beverages signify potential health threats to consumers. Between 1998 and 2009, there were 308 reported illnesses, 154 hospitalizations and 6 deaths reported in the state of Nevada from consumption of food contaminated with *E. coli* serotype O157:H7 (Centers for Disease Control and Prevention [CDC], 2009). Although these data only reflect the impact of one serotype of *E. coli*, the importance of monitoring microbial quality,

specifically *E. coli*, of ingestible items is evident.

In this study, the percent of total samples (ice and diet soda) that contained *E. coli* was 22.2% (8 of 36). This was similar to the results found by White et al. (2010), in which 20% (20 of 90) of the ice, soda, and diet soda samples were found to have *E. coli.* This study showed that Las Vegas samples had a higher bacterial concentration overall when compared to the Virginia study. Our study showed that 47.2% of the samples exceeded the EPA standard of > 500 CFU/ml, while the White et al. (2010) study, found that 20% of their total samples exceeded >500 CFU/ml. Similarly, this study resulted in higher concentrations of presumptive coliform presence, with 80.6% of the samples containing presumptive coliforms compared to the 48% found by White et al. (2010).

Trends in human behavior regarding their drinking preferences (e.g., soda with or without ice) should be considered when analyzing the implications of this study. Bacterial concentrations of combined samples were not tested in this study. Although it is common for people to consume their fountain soda with ice in it, the ice and soda samples were analyzed for bacteriological quality separately in this study with each type of sample resulting in poor bacteriological quality. Consequently, the combination of ice and soda may yield a higher overall concentration of bacteria than either ice or soda by itself. The findings of this study suggest that food establishments that serve ice and soda in Las Vegas should consider a monitoring system in which a maintenance schedule is adhered to, in an effort to prevent possible foodborne illness for their patrons.

Limitations and Considerations of this Study

The primary limitation of this study was the relatively small sample size. A larger

sample size would provide a more complete representation of the bacteriological quality of soda and ice in local food establishments. The design of this study was developed to represent the Las Vegas Valley; therefore, it did not control for factors such as socioeconomic strata and geographic locations. As a result, these factors must be taken into account when interpreting the data and may have had some influence in the variability of the data collected. There are undoubtedly differences in the chemical properties of soda and ice that inhibit or promote bacterial growth; however, this research study did not address these differences.

Presumptive coliform bacteria were not confirmed (as coliforms), with the exception of *E. coli*; therefore, further microbiological analysis is needed to confirm coliform presence. The manufacturer of the EMB media suggests further analysis to ensure coliform presence such as the IMViC (Indole, Methyl Red, Voges-Proskauer, and Citrate tests) or sub-culturing with the use of the RapID system to seek out a specific organism (L. Weldon, Molecular Technical Service Representative-Oxoid products, personal communication, August 15, 2011). Further, the EMB media used to enumerate presumptive coliform bacteria did not differentiate all of the colonies that were visible. Some colonies could be identified by the color according to the manufacturer, but most of the colonies enumerated remained unidentifiable because they were present in high concentrations and could not be isolated. Although the agar was used for differentiation of *E. coli*, it also can be used for the identification of non-coliform bacteria, including coagulase positive staphylococci and *Candida albicans*. Therefore, the numbers reported in this study are estimates and may be assumed to be higher than the actual CFU/ml of presumptive coliform bacteria.

Alternatively, the use of culture methods in this study may have resulted in an underestimation of the number of CFU/ml enumerated. Culture methods provide valuable information on the microbial quality of the samples collected; however, unlike Polymerase Chain Reaction (PCR) methods, this method is limited to the enumeration of healthy, non injured viable microorganisms. Injured organisms are likely not included in the counts of the enumerated colonies by culture. In addition, the filtration method used in this study could cause additional stress and injury to the microorganisms within the samples, which could result in an underestimation of the bacterial contamination. This is an important factor because although injured pathogenic microorganisms exhibit a temporary decrease or loss in virulence, they can recover from injury in which virulence can be completely restored (Singh & McFeters, 1990).

Conclusions and Recommendations for Future Research

This study determined the quality of ice and soda distributed by local food establishments using the EPA drinking water standards as a reference. The findings suggest that high concentrations of bacteria in ice and soda are prevalent in Las Vegas, and raises questions about the microbiological quality of these products. Given the consistent testing of drinking water carried out by local water authorities, the water feeding the soda fountain distribution systems can be assumed to meet the drinking water standards. Consequently, the quality problems reported by this study are not likely to be derived from the water entering the system, but rather the soda fountain or ice machine itself.

The study only scraped the surface of a potential contamination problem and quality issue in ice and soda. Further research should be conducted to determine the source of this contamination with the aim of implementing preventative measures. It also may be necessary to determine the pathogenic burden of these results. Specific types of bacteria could be identified in the future to determine whether the high concentrations of bacteria could impact the health of people consuming these food items. Given the high bacterial concentrations that resulted from this study, future studies may want to incorporate more dilutions, more replicates, and use of selective media or selective methods for the identification and quantification of pathogens to determine the health risk associated with drinking iced sodas from local restaurants.

The results of this study provide evidence that the quality of the ice and soda from local establishments often do not meet federal drinking water standards and may be a health risk for those who consume them. Studies such as this may influence the future of ice machine and soda fountain regulations, and could establish the need for more stringent policies toward maintaining low microbial contamination in these products.

96.05.0400. Tubes, Lines, and Cold Plates for Beverages or Drainage; Sinks and Drain Boards.

1. Drop-in cold plates, carbonator tanks, bottle holders, beverage tubing, service bins, and similar devices (except bin level controls) are not acceptable in potable ice pans or bins. When installed in potable ice bins, cold plates shall be constructed integrally with the bin.

96.06.0500. Drains.

1. Dishwashing machines, warewashing machines, warewashing sinks, ice bins, ice machines, walk-in refrigerators, steam kettles, potato peelers, utility sinks, food preparation sinks, and similar types of enclosed equipment in which food, portable equipment, or utensils are placed, shall not be directly connected to the sewage system. Each waste pipe from such equipment shall discharge into an open, accessible, floor sink, floor drain, or other suitable fixture that is properly trapped and vented. Indirect connections of drain lines from other equipment used in the preparation of food or washing of equipment and utensils may be required by the Health Authority when, in its opinion, the installation is such that backflow of sewage is likely to occur.

96.06.0200. Ice.

1. Ice shall be made from water meeting the requirements of Section 96.06.0100.1, in an icemaking machine that is located, installed, operated, and maintained so as to prevent contamination of the ice; or shall be obtained from a source approved by the Health Authority.
2. Ice shall be handled, transported, and stored in such a manner as to be protected against contamination. If block ice is used, the outer surfaces must be thoroughly rinsed so as to remove any soil before it is used for any purpose.
3. If ice crushers are used, they shall be maintained in a clean and sanitary condition and shall be protected from contamination at all times.
4. If ice is used, approved containers and utensils shall be provided for storing, serving, and transporting it in a sanitary manner. Ice buckets, other containers, and scoops, unless they are of the single-service type, shall be of a smooth, impervious material, and designed to facilitate cleaning. Only sanitary, food-grade containers shall be used for storage of any ice used in the food establishment.
5. All multi-purpose containers within the food establishment, used for ice, must be labeled in 4 inch letters: "ICE ONLY."

APPENDIX B
NEVADA ADMINITRATIVE CODES

NAC 446.275 Requirements for installation and maintenance of equipment installed before and after certain dates. (NRS 439.150**,** 439.200**,** 446.940**)**

1. Equipment that was installed in a food establishment before October 14, 1988, and does not meet all of the requirements of NAC 446.230 to 446.275, inclusive, may be acceptable in that establishment if it is in good repair, capable of being maintained in a sanitary condition and the surfaces which may come into contact with food are not toxic.

2. All new and replacement equipment installed after August 12, 1992, and before May 23, 1996, must:

(a) Comply with all applicable standards of the NSF International in effect as of January 31, 1988; or

(b) In the absence of any applicable standard, be approved by the health authority.

3. All new and replacement equipment installed after May 23, 1996, must:

(a) Comply with all applicable standards of the NSF International in effect as of May 23, 1996; or

(b) In the absence of any applicable standard, be approved by the health authority.

4. A copy of the standards of the NSF International may be purchased from the NSF International, P.O. Box 130140, Ann Arbor, Michigan 48113-0140, at the following prices:

No. 1. Soda Fountain and Luncheonette Equipment.....	$35
No. 12. Automatic Ice Making Equipment.....	30
No. 18. Manual Food and Beverage Dispensing Equipment.....	30

NAC 446.255 Tubes for beverages. (NRS 439.150**,** 439.200**,** 446.940**)** Tubes which convey beverages or ingredients for beverages to the head of a dispenser may not touch stored ice that is intended for use as food.

[Bd. of Health, Food Establishments Reg. Art. 5 § 5.2 subsecs. 5.2.5-5.2.7, eff. 9-17-82]—(NAC A 9-16-92; 5-23-96)

NAC 446.280 Location of equipment. (NRS 439.150**,** 439.200**,** 446.940**)**

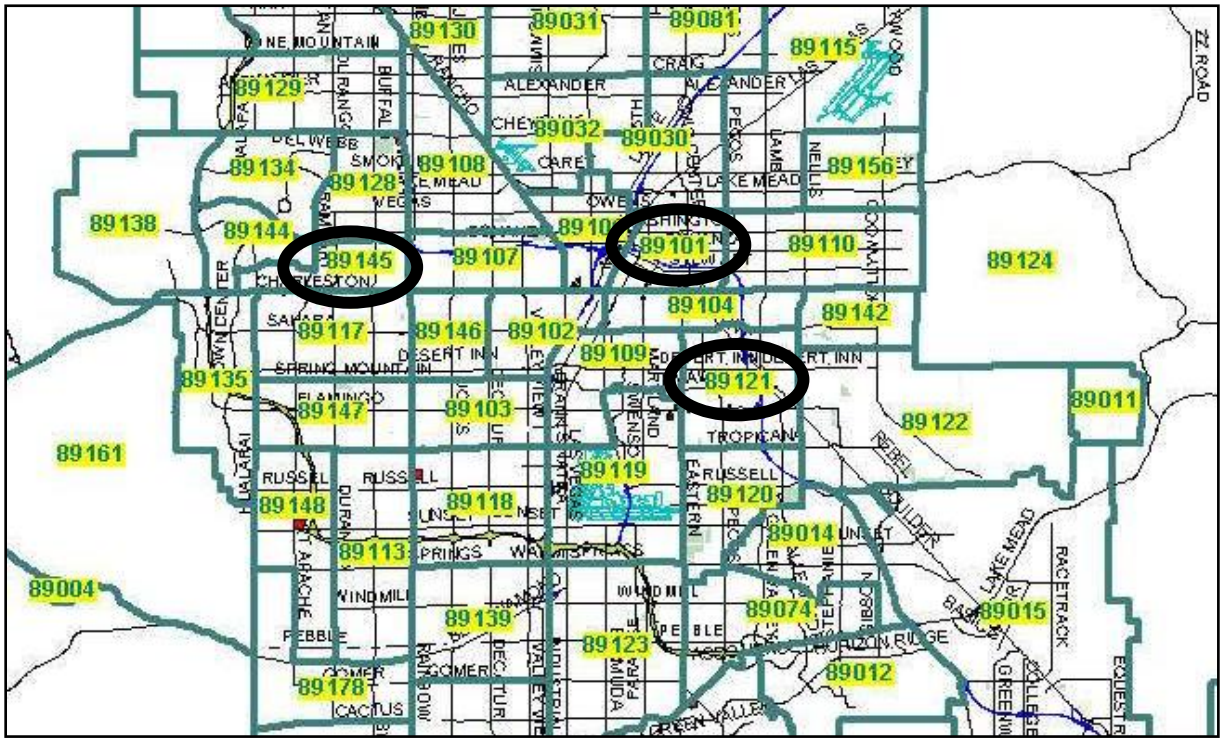
1. Equipment, including ice makers and equipment for storing ice, may not be located under exposed or unprotected sewer lines or waterlines, open stairwells or other sources of contamination.

2. The requirement of subsection 1 does not apply to automatic sprinklers required by law.

[Bd. of Health, Food Establishments Reg. Art. 5 § 5.3 subsec. 5.3.1, eff. 9-17-82]

APPENDIX C

LAS VEGAS ZIP CODE MAP



APPENDIX D

LIST OF ACRONYMS

CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
EMB	Eosin Methylene Blue Agar (Modified) Levine
EPA	Environmental Protection Agency
HPC	Heterotrophic Plate Counts
LDL	Lower Detection Limit
NAICS	North American Industry Classification System
PCR	Polymerase Chain Reaction
PST	Pacific Standard Time
SDWA	Safe Drinking Water Act
SMEWW	Standard Methods for the Examination of Water and Waste Water
SNWA	Southern Nevada Water Authority
TNTC	Too Numerous to Count
UNLV	University of Nevada, Las Vegas
WHO	World Health Organization

REFERENCES

Centers for Disease Control and Prevention (2009). Foodborne Outbreak Online Database (FOOD) Retrieved from http://wwwn.cdc.gov/foodborneoutbreaks/

Eaton, A., Clesceri, L., Rice, E., Greenberg, A., & Franson, M. A. (2005). 9215 Heterotrophic Plate Count. *Standard Methods for the Examination of Water and Wastewater* (21 ed., pp. 9.34-9.36). Washington, D.C.: American Public Health Association, American Water Works Association, & Water Environment Federation.

Eaton, A., Clesceri, L., Rice, E., Greenberg, A., & Franson, M. A. (2005). 9222 Standard Total Coliform Membrane Filtration Procedure. *Standard Methods for the Examination of Water and Wastewater* (21 ed., pp. 9.60-9.64). Washington, D.C.: American Public Health Association, American Water Works Association, & Water Environment Federation.

Eaton, A., Clesceri, L., Rice, E., Greenberg, A., & Franson, M. A. (2005). 9020 Quality Assurance/Quality Control. *Standard Methods for the Examination of Water and Wastewater* (21 ed., pp. 9.2-9.14). Washington, D.C.: American Public Health Association, American Water Works Association, & Water Environment Federation.

Environmental Protection Agency. (2002). *Health Risks from Microbial Growth and Biofilms in Drinking Water Distribution Systems*. Washington D.C.: Government Printing Office.

Environmental Protection Agency. (2006). *Distribution System Indicators of Drinking Water*. Washington D.C.: Government Printing Office.

Environmental Protection Agency. (2009). *2009 Edition of the Drinking Water Standards and Health Advisories*. Washington D.C.: Government Printing Office.

Hunter, P., Colford, J., LeChevallier, M., Binder, S., & Berger, P. (2001). Waterborne Diseases. *Emerging Infectious Diseases, 7*(3), 544-545.

Hynes, M. (2009, March 11). Restaurant Report. *Las Vegas Review-Journal.* Accessed from http://www.lvrj.com/taste/41077952.html.

Lancer® (2004, March 03). Installation and Service Manual for the Delta III Dispenser. Lancer Series 9000. Accessed from http://www.lancercorp.com// images/search_tech_bulletins/28043703.pdf

LeChevallier, M. (2003). Conditions Favouring Coliform and HPC Bacterial Growth and on Water Contact Surfaces. *Heterotrophic Plate Counts and Drinking-Water Safety: The Significance of HPCs for Water Quality and the Human Health* (pp. 177-197). Cornwall, UK: Publish on behalf of WHO by IWA Publishing.

Liguori, G., Cavallotti, I., Arnese, A., Amiranda, C., Anastasi, D., and Angelillo, I. (2010). Microbiological quality of drinking water from dispensers in Italy. BMC Microbiology, 10, 19

McEachern, J., (2011). Las Vegas zip code map. Retrieved August 10, 2011, from http://www.act4u.com/zip/ZipCodes.html

NAICS Main Page. (n.d.). Census Bureau Home Page. Retrieved August 10, 2011, from http://www.census.gov/eos/www/naics/

Nevada Administrative Code (Last Revised 2008). Preparation and Service of Ice. Retrieved from http://www.leg.state.nv.us/nac/NAC-446.html#NAC446Sec175

Singh, A., & McFeters, G. (1990). Injury of Enteropathogenic Bacteria in Drinking Water. In Mcfeters, G. (Ed.), *Drinking Water Microbiology* (pp. 368-379). New York City: Springer-Verlag New York Inc..

Southern Nevada Health District. (2010) Governing the Sanitations of Food Establishments. Approved by the Board of Health March 25, 2010. Government Printing Office.

Southern Nevada Water Authority (2011). Water Quality Report. Retrieved from http://www.snwa.com/wq/report.html

Toranzos, G., McFeters, G., Borrego, J., & Savill, M. (2007). Detection of Microorganisms in Environmental Freshwaters and Drinking Waters. *Manual of Environmental Microbiology* (pp. 249-264). Washington: ASM Press.

Wende, E & Characklis, W. (1990). Biofilms in Potable Water Distribution Systems. In Mcfeters, G. (Ed.), *Drinking Water Microbiology* (pp. 249-268). New York City: Springer-Verlag New York Inc.

White, A., Godard, R., Belling, C., Kasza, V., & Beach, R. (2010). Beverages Obtained from Soda Fountain Machines in the U.S. Contain Microorganisms, including coliform bacteria. *International Journal of Food Microbiology, 137*, 61-66.

World Health Organization, Protection of the Human Environment, Water, Sanitation and Health. (2002). *Heterotrophic Plate Count Measurement in Drinking Water Safety Management*. Geneva: Government Reporting Office.

VITA

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