Original Article

Indian Journal of Communicable Diseases
Volume 2 Number 2, July - December 2016
DOI: http://dx.doi.org/10.21088/ijcd.2395.6631.2216.1

Author Affiliation:
*MD Student, Department of Clinical Sciences, Saint James School of Medicine, 1480 Renaissance Drive, Suite 300, Park Ridge, IL 60068, USA.
**Departments of Microbiology and Epidemiology, Saint James School of Medicine, Albert Lake Drive, The Valley, Anguilla – 2640 (British West Indies)

Reprint Request:
Harish C. Gugnani,
Departments of Microbiology and Epidemiology, Saint James School of Medicine, Albert Lake Drive, The Valley, Anguilla – 2640 (British West Indies)
E-mail:
harish.gugnani@gmail.com

Total Bacterial Count and Presence of Escherichia coli in Raw Ground Beef Samples Obtained From Three Major Supermarkets in Bonaire, Dutch Caribbean

John Melling*, Christopher Smith*, Harish C. Gugnani**

(Received on 19.08.2016, **Accepted on** 02.09.2016)

Abstract

Background: There is no information on the microbiological quality of retailed ground beef in Bonaire, Dutch Caribbean. The objective of this study was to determine the total bacterial counts and detect the presence of *Escherichia coli* in samples of ground beef .sold in three major supermarkets in Bonaire.

Methods: A total of 36 samples of raw ground beef comprising 12 from each of the three supermarkets collected over a period of 4 weeks were examined. Dilutions of each sample were inoculated on three plates of nutrient agar by standard procedure. The plates were incubated at 37^0C/room temperature for 48 hours to determine the total bacterial count as colony forming units per gram (CFU/g). Additionally, 5 samples were used from each of the supermarket to detect the presence of *E. coli*.

Results: It was found that the mean bacterial count of CFU/g of ground beef samples from supermarket 1 was significantly higher (224,800) than that from the supermarkets 2 (5280) and 3 (4800). The mean *E. coli* count in the samples from supermarkets 1 and 2 was 52 and 9 CFU/g respectively.

Conclusion: This study is the first attempt to assess the overall microbiological quality of raw ground beef retailed in Bonaire by demonstrating significant differences in the means of total bacterial CFU/g, and counts of *E. coli* in samples from different supermarkets. This information can guide the producers of ground beef and the management of the supermarkets to recognize the good microbial quality of ground beef and to prevent bacterial contamination during handling.

Keywords: Raw Ground Beef; Bonaire; Total Bacterial Count, *Escherichia Coli*.

Introduction

Raw meats sold in supermarkets often contain *Salmonella*, *Escherichia coli, Staphylococus aureus*, and other bacteria [1]. These bacteria cause thousands of cases of illness, some of which result in hospitalizations and mortality each year, and their several strains have developed resistance to common antibiotics [2,3]. Cross contamination can also occur when safe food handling procedures are not followed. Therefore, the quality of the retailed raw meat is important for the health of the local population. Total bacterial counts (also called Aerobic Plate Counts or APC) and presence of *E. coli* in meats are used to help determine general hygiene, quality and safety of meat products [4-6]. These counts help determine the shelf life of meat. Lower initial bacterial counts are associated with increased shelf life, while higher initial bacterial counts lead to more rapid development of slime and other effects of meat spoilage [7]. Increased shelf life allows consumers more freedom in timing of meat purchase and meat preparation. While proper cooking destroys most of these bacteria, many people consume raw beef (as in steak tartar) or undercook or improperly cook the meat before eating. Thus it was considered worthwhile investigating the bacteriological quality of raw ground beef sold in supermarkets in Bonaire from the public health point of view.

Methods

Sample Collection

Separate samples of raw ground beef were collected (by purchase) from the regular chilled displays in the three largest supermarkets located on the island of Bonaire on each Monday, Wednesday

and Friday over a period of 4 weeks from November 11 to December 6, 2013. This comprised a total of 36 samples. Additional samples were obtained prior to November 11 for use in refining the total bacterial count method and preparing the Gram stained smears of the ground beef on microscopic slides. All samples were placed in an iced cooler and transported to laboratory for prompt processing.

Sample Processing and Total Bacterial Count Determination

From each sample of ground beef one gram was aseptically weighed on an electronic balance cleaned with an alcohol swab, and placed into a sterile test tube containing 10 mL of sterile distilled water. It was agitated for 2 minutes and 0.01 (equivalent to 0.01g) quantities of the suspension were delivered to each of the three prepared petri dishes of sterile nutrient agar (Oxoid), using a sterile inoculating loop. The inoculum was spread uniformly using a sterilized glass spreader. In a prior test, this quantity of meat was found to yield acceptable numbers of colony counts per dish (30-300). Inoculated petri dishes were incubated for 48 hours initially in both at Bonaire ambient room temperature (22^0C-30 0C) and at 37^o C in the Bonlab located a little faraway. Both incubation methods yielded similar results. Therefore, for ease of work, the petri dishes were then incubated for 48 hours in ambient temperature. All bacterial colonies (including those of pinpoint size) appearing on the agar medium were manually counted and recorded for each sample. Total bacterial counts were expressed as CFU/g calculated by multiplying the number of colonies on the plate by the dilution factor (1,000).

Determination of E. Coli Counts in the Samples

Five of the 36 samples were randomly selected for determination of counts of *E.* coli in the same manner as for *S. aurues*, using sterile petir dishes (plates) of MacConkey agar in place of Nutrient agar.

Statistical Analysis

The means of Aerobic Plate Count (APC) were first compared using the one-way ANOVA test. Assumptions of normality and equal variance were tested using the Shapiro-Wilk and Levene tests respectively. If the ANOVA demonstrated significance, more detailed results were found by comparing the means using unpaired t-tests. As two hypotheses were being tested on each data set, the Bonferroni-adjusted significance level of p=0.025 was used to correct for Type-I error. All analysis was made using actual data numbers; however, for simplicity, CFU results are reported by rounding to the nearest ten.

The ratios of "*E. coli* present" to "*E. coli* not present" were compared using Fisher's Exact Test as only five samples were used. Mean *E. coli* colony counts of each of the five samples were analyzed for normal distribution using Shapiro-Wilk test, and compared using unpaired t-test. A Bonferroni-adjusted p-value of .025 was employed.

Gram Staining

Loopfulls of round, white and yellow colonies appearing in the plates were suspended in sterile distilled water. Smears prepared by spreading a loopfull of the suspensions on microscopic slides were stained by Gram stain procedure using crystal violet as the primary dye and safranin as a counter stain following a standard procedure.

Results

Total Bacterial Counts

Total bacterial count of individual samples ranged from 1,200 to 282,000 CFU/g. Three of these from supermarket 3 fell outside the acceptable range of aerobic plate counts (below 30-300 CFU/g). The

Table 1: Total aerobic bacterial plate count in colony forming units (CFU/g) in 12 samples of ground beef from three supermarkets in Bonaire

Date of sample	Supermarket 1	Supermarket 2	Supermarket 3
11-Nov	193000	7200	10200
13-Nov	207000	5500	9200
15-Nov	233000	4200	9000
18-Nov	251000	3200	3900
20-Nov	276000	4100	2800*
22-Nov	205000	3900	4100
25-Nov	263000	3700	5600
27-Nov	220000	3900	1200*
29-Nov	259000	6400	4000
2-Dec	282000	5100	6200
4-Dec	38000	4800	5300
6-Dec	261000	5700	1900*

*CFU count per plate fell outside the generally accepted range of 30-300

counts in individual samples are shown in Table 1. Microscopic examination of Gram stained smears of different colored colonies showed the presence of both Gram positive and Gram negative bacteria in rods and sometimes in cocci. Since the study was aimed at determining the total bacterial counts in the samples, no record was kept of the relative umber of Gram positive and Gram negative bacteria. The mean APC CFU/g with 95% confidence interval are given in Table 2.

Table 2: Mean aerobic bacterial plate (CFU/g) with confidence intervals from 3 supermarkets in Bonaire

Supermarket	Mean CFU/g	95% Confidence Interval
1	224,000*	182,370-265,630
2	4,800	4,040-5,570
3	5,280	3,430-7,140

*Denotes that this mean is significantly higher than other means (p=1.488E-16)

Testing the One-Way ANOVA Assumptions

The Shapiro-Wilk Test

This test was employed to test the samples for normality. The bacterial count of 38,000 CFU/g from one of the samples from supermarket 1 (December 4) was found to be an outlier (defined as below Quartile 1-1.5xInterquartile Range). When the mean CFU/g for supermarket 1 was calculated without the outlier, the outlier was more than 6 standard deviations from the mean. The cause of this observation could not be known. It could represent an error in method or a pertinent observation, even if unlikely. For this reason, the data point was not dropped from the sample set completely. The analysis was run with and without this outlier. With the outlier included, supermarket 1 data yielded a Shapiro-Wilk statistic of W = 0.745 and a related p-value of 0 .0024 (n=12), indicating the population is not normally distributed. At the 5% significance level for normality, W = 0.859 is the critical value. With the outlier taken out of the data set, the 5% significance level the critical value is W = 0.850, supermarket 1 data yielded a Shapiro-Wilk statistic of W = 0.927 and a related p-value of 0.3823 (n=11), indicating normal distribution.

Supermarket 2 yielded a Shapiro-Wilk statistic of W = 0.941 and a related p-value of 0.5152 (n=12). At the 5% significance level, W = 0.859 is the critical value. Therefore, the null hypothesis was accepted that the population is normally distributed. Store 3 yielded a Shapiro-Wilk statistic of W = 0.937 and a related p-value of 0.4546 (n=12). At the 5% significance level, W = 0.859 is the critical value. Therefore, the null hypothesis was accepted that the population is normally distributed. As both supermarket 2 and supermarket 3 are normally distributed, we will accept the normal distribution of the data from supermarket 1 as well that was found excluding the outlier, keeping this exclusion in mind as we draw our conclusions.

Results from the Shapiro-Wilk test are represented in Table 3.

Table 3: Results from Shapiro-Wilk Test for Normal Distribution concerning APC CFU/g of raw ground beef samples from three supermarkets in Bonaire

Supermarket	W-Statistic	P-value	Critical value for W at 5% significance level	Result of Hypothesis
1*	0.927	0.3823	0.85	Accept, Normal Distribution
2	0.941	0.5152	0.859	Accept, Normal Distribution
3	0.937	0.4546	0.859	Accept, Normal Distribution

1* number of samples (n)=11. *Calculated without outlier. Store 2 and Store 3, n=12.

The final assumption for using one-way ANOVA analysis is that the variances are equal. The Levene Test for Equality of Variances yielded a W-statistic of 5.0029 (p=0.0129), indicating that the variances are different. However, the one-way ANOVA test is robust in this case as the sample sizes are equal; the effect on Type I error is minimal [11].With assumptions of independence, normal distribution and equal variances addressed, one-way ANOVA test was employed.

One-Way ANOVA

A one-way ANOVA test was run testing the

hypothesis that the mean APC CFU/g of all three samples were equal. The ANOVA yielded an F ratio of 133.717 and associated p-value of 1.488E-16. Therefore, the null hypothesis was rejected; at least one mean APC CFU/g was significantly different from the others.

Unpaired t-Tests

Mean APC CFU/g from supermarket 1 and supermarket 2 were compared. Using the unpaired t-test, the t-value was found to be11.588 (p=1.654E-07). This is well within the predetermined significance of p=0.025, indicating a significant difference between supermarket 1 and supermarket 2 means, with supermarket 1 having a much larger mean APC CFU/g for raw ground beef. An unpaired t-test comparing means from supermarket 1 and supermarket 3 yielded a t-statistic of 11.578 (p=1.653E-07),again indicating a significant difference between Store 1 and Store 3 means, with supermarket 1 having a much larger mean. The third unpaired t-test comparing means of supermarket 2 and supermarket 3 resulted in a t-statistic of -0.526 (p=0.609), indicating no significant difference between the means of supermarket 2 and supermarket 3. The results of the unpaired t-tests analyzing the means of the APC CFU/g are shown in Table 4.

Table 4: P-Values Derived from unpaired t-tests analyzing mean APC CFU/g of raw ground beef from three supermarlets in Bonaire n=12 for each supermarket

Null Hypothesis	t-Statistic	P-Value	Result
There is no difference between mean APC CFU/g of Store 1 and Store 2.	11.588	1.654E-07*	Reject Hypothesis; There is a difference.
There is no difference between mean APC CFU/g of Store 1 and Store 3.	11.578	1.653E-07*	Reject Hypothesis; There is a difference.
There is no difference between mean APC CFU/g of Store 2 and Store 3.	-0.526	0.609	Accept Hypothesis; There is no difference.

*Significance Level p =0 .025.

Occurrence of E. Coli in Ground Beef

Five samples from each of the three supermarkets were tested for presence of *E. coli*. E. *coli* was present in all five samples from supermarket 1, in four samples from supermarket 2, and in none of the five samples from supermarket 3. This resulted in a mean colony count of 52. *E. coli* was present in 3 of the 5 samples from supermarket 2, the colony counts being 26, 6, 0, 13 and 0, resulting in a mean colony count of 9. None of the five samples from supermarket 3 yielded *E. coli*.

Table 5: Observed mean *E. coli* colony counts from raw ground beef from three supermarkets in Bonaie

Sample no	Date of collection	SM # 1	SM # 2	SM # 3
1	8-Nov	96	26	0
2	27-Nov	30	6	0
3	29-Nov	69	0	0
4	2-Dec	37	13	0
5	4-Dec	28	0	0
Mean Colony Count		52	9	0

Table 6: Results from Shapiro-Wilk Test for Normal Distribution Concerning *E. coli* colony counts from raw ground beef samples. 5 from each of three supermarkets in Bonaire

From three supermarket	W-Statistic	P-value	Critical value for W at 5% significance level	Result of Hypothesis
#1	0.8496	0.1933	0.762	Accept, Normal Distribution
#2	0.8759	0.2909	0.762	Accept, Normal Distribution
#3	NA	NA	0.762	Included in analysis*

*For the scope of this study, we include mean=0 in analyzing data.

The data sets were tested for a normal distribution using the Shapiro-Wilk test. Store 1 yielded a statistic of W=0.8496 (p=0.1933), indicating normal distribution. Store 2 yielded a Shapiro-Wilk statistic

of W = 0.8759 (p=0.2909), also indicating normal distribution. Store 3 presented an interesting situation. A total of zero counts were observed with no standard deviation or variance. Therefore, a Shapiro-Wilk statistic could not be found, as division by zero is undefined. For the limited scope of this study, we included the data from marker 3 anyway and kept this in mind in concluding our study. No outliers were found in the data sets.

Results from Shapiro-Wilk test are represented in Table 6.

With the assumption of normal distribution met, unpaired t-tests were employed on the data. comparing means of supermarket 1 and supermarket 2, a t-statistic of 3.229 (p=0.032). This is above the Bonferroni-adjusted significance of p=0.025. Therefore, we must accept the hypothesis as it relates to our sample. There is not a significant difference represented between the mean colony counts of supermarkets 1 and 2. Mean *E. coli* colony counts of Store 1 and Store 3 were compared yielding a t-statistic of 3.638 (p=0.022), indicating a significant difference. The test comparing markes 2 and 3 indicated no significant difference between means with a t-statistic of 1.844 (p=0.139).

The results of the unpaired t-tests analyzing the means of *E. coli* colonies are represented in Table 7.

Table 7: P-Values Derived from Unpaired t-tests analyzing mean *E. coli* colony counts (ECC) of raw ground beef

Null Hypothesis	t-Statistic	P-Value	Result
There is no difference between mean ECC of Store 1 and Store 2.	3.229	0.032	Accept Hypothesis; There is no difference.
There is no difference between mean ECC of Store 1 and Store 3.	3.638	0.022	Reject Hypothesis; There is a difference.
There is no difference between mean ECC of Store 2 and Store 3.	1.844	0.139	Accept Hypothesis; There is no difference.

No of samples for each supermarket, Bonferroni-adjusted significance level p=0.025.

E. coli was Found in All the 5 Samples From Supermarket 1, and in 2 Samples Each from Markets 2 and 3.

The proportions of presence of *E.coli* were compared using Fisher's Exact Test. The data was independent, there are no outliers and the variables meet the classifications for use in the contingency table of Fisher's Exact Test. The test comparing proportions of *E. coli* colony present in samples from market 1 and 2 yielded a p-value of 0.4444, which is above the Bonferroni-adjusted significance of p=0.025, indicating no significant difference between proportions from Store 1 and Store 2. The test comparing proportions from markets1 and 3 yielded a p-value of 0.0079, indicating a significant difference between proportions. Comparison of proportions from markets 2 and 3 yielded a p-value of 0.1667, indicating no significant difference between the proportions.

These hypotheses, p-values and results concerning proportions of *E. coli* presence as found using Fisher's Exact Test are represented in Table 8.

Table 8: P-values derived from Fisher's Exact Test analyzing proportions of presence of *E. coli* colonies in samples of raw ground beef

Null Hypothesis	P-Value	Result
There is no difference between the proportions of *E. coli* presence in beef from Store 1 and Store 2.	0.4444	Accept Hypothesis; There is no difference.
There is no difference between the proportions of *E. coli* presence in beef from Store 1 and Store 3.	0.0079	Reject Hypothesis; There is a difference.
There is no difference between the proportions of *E. coli* presence in beef from Store 2 and Store 3.	0.1667	Accept Hypothesis; There is no difference.

No. of samples for each supermarket=5, Bonferroni-adjusted significance level p=0.025. Note: Fisher's Exact Test does not have a "test-statistic" but computes the p-value directly.

Results Summary

It was found that the means of APC CFU/g of the sampled raw ground beef were different. Specifically, the raw ground beef from Store 1 has a mean APC CFU/g that is higher than both Store 2 and Store 3 at a p=0.025 significance level, while the means of APC CFU/g from Store 2 and Store 3 are not statistically different from each other. Additionally, the only difference in proportions of *E. coli* presence and mean colony counts at a significance level of p=0.025 was found between Store 1 and Store 3, with Store 1 having a higher proportion of presence and mean colony

count of *E. coli*. The presence of Gram-negative bacteria was seen in each of the six Gram stained slides.

Discussion

Studies of this nature that assess the quality of ground beef using total bacterial counts and *E. coli*as markers have been done in several parts of the world [3,4,5,6,12,13]. It is interesting to note that our results pertaining to total bacterial countl fall within standards of health as suggested by the Institute of Food Science and Technology (IFST) [14]. The IFST suggests that the maximum level for CFU/g obtained from APC at any point in the shelf life of a raw meat product is 10^7 colonies per gram of meat. This is good news for the consumers and producers of raw ground beef in Bonaire. However, there is still a significant difference between the beef from Store 1 and the other two locations. Additionally, the means of APC CFU/g from Store 2 and Store 3 are similar to numbers reported in nationwide US studies of raw ground beef, while the mean APC CFU/g from Store 1 is considerably larger [5,6]. For example, the mean APC CFU/g from our Store 1 would have fallen within the top 6.3% of 1,719 samples reported in one US study [5]. This suggests that although the Store 1 data is within limits due to health, its overall bacterial levels are greater than normal. As discussed earlier, this can negatively impact the shelf life of the meat [7] and poses possible health concerns for Store 1 consumers who undercook their beef or allow cross contamination.

Future research could be more quantitative and specifically address the relatively poor bacteriological quality of the beef retailed in supermarket 1 (total bacterial count/g and E. oli count/g being higher than in that from the markets 2 and 3) Questions could be answered such as: Addressing the questions:What are the handling procedures of raw ground beef in Store 1 versus the other stores? What is the timeframe of beef processing and sales? These kinds of questions could help Store 1 address the specific differences in procedure that may be leading to increased total bacteria and *E. coli* in their ground beef, thus allowing Store 1 to improve their product.

Limitationss

We predict that most objections to this study will be because of the relatively smaller sample sizes and narrow scope, which were part of the limitations of this particular study. While *E. coli* was detected in several of our samples, there arr various different kinds of *E. coli*, many of which are opportunistic [15]. Therefore, we cannot make direct conclusions as to the health risk involved, only that further research needs to be done. The presence of Gram-negative bacteria in the Grram-stained slides, along with the presence of *E. coli* in the raw beef samples, suggest that other gram negative pathogenic bacteria such as *Salmonella* or *Shigella* may be present in the beef. While it was not within the scope of this study to further describe specific bacteria, this is an excellent suggestion for further research. Future studies could focus on other bacteria often present in ground beef such as *Salmonella*, *Shigella*, *Campylobacter jujuni*, *Listeria*, *monocytogenes* or *Staphylococcus aureus* which along with *E. coli* can all cause disease or death [1,2]. Research could select for specific types of *E. coli*, including 0157:H7 as this strain is particularly connected with human illness and is usually meant to be undetectable in raw meat [3,15,16]. Gram-negative bacteria as a general group are more resistant to antibiotics than gram-positive bacteria [17]. Research could also address whether or not all of these bacteria present in the beef are resistant to antibiotics, a growing concern [3].

Another objection to our study could be the handling of the outlier observed in the Store 1 data. Further research, increasing the samples of total bacterial count, could bear on our decision to assume normality of distribution. The same principle applies to the low counts of TBC observed in three of the Store 3 samples; our study results will become more significant (or less) as future research increases the studies of total bacterial counts.

It was only within this study scope, and therefore, a limitation, to include three of the major supermarkets on the island of Bonaire with a relatively short time-frame of sample collection. Samples were taken over one month's time and not throughout a whole year. For this reason, our analysis may not reflect possible seasonal differences [6]. Clearly, there are also other sources from which consumers obtain their meat, beyond the three selected supermarkets. These other stores could undergo these studies as well and over a longer time-frame to form an even more complete picture of the quality of the raw ground beef for sale in Bonaire.

Conclusion

Consumers have many options when it comes to purchasing meat and it will be an advantage for them

to have access to the data from this study which compares levels of bacterial counts in locally available meats. From this study, local consumers can gain a better understanding of which store provides the highest quality and safety relative to total bacterial count and then weigh other factors such as convenience and cost and make consumption choices that best meet the needs of their circumstances.

This study also provides a framework for the supermarkets to assess their own requirements for purchase and handling of meat. Stores with higher quality as related to total bacterial count can use that as a selling point for their consumers, can find pride in providing quality products and can continue to use safe meat handling procedures. Stores with lower quality meat can be made aware of the findings and assess the practices leading to these circumstances. This information may be used to implement improved meat handling guidelines and thereby benefit the supermarket and consumer alike.

Finally, studying the quality of local raw ground beef through *E. coli* and aerobic plate counts can be a platform for encouraging safe handling of ground beef in Bonaire. As people come to understand that raw ground beef does contain bacteria, they may be motivated to follow safe meat handling guidelines. These guidelines include keeping meat cold and clean until cooking, washing surfaces before and after coming into contact with the raw meat, and cooking meat to safer temperatures [7]. This research can serve as a foundation for education and more studies dealing with meat safety and food poisoning prevention.

This study aimed to answer the question, "Is there a difference between the total bacterial counts of raw ground beef sold at three of the major supermarkets in Kralendijk, Bonaire, Dutch Caribbean?" Our null hypothesis was that there was no difference in the total bacterial counts in meats available to consumers. To a lesser degree, this study also assessed whether *E. coli* bacteria were present in the meat sold at the same three supermarkets. The null hypothesis was that no *E. coli* was present and that there was no difference between the three supermarkets.

Consumers and the supermarkets can benefit from these findings in many ways.

This study serves as a good foundation in beginning to assess the overall quality of raw ground beef in Bonaire. Statistical comparisons allow us to see that there are differences in the means of total bacterialCFU/g between different supermarkets. This information allows consumers to recognize where the highest quality of beef can be obtained as it relates to contamination, and to a lesser degree, overall prevalence of *E. coli*. The study can help specific producers see that improvementscan be made in their product. In general, the overall presence of bacteria and specifically*E. coli* should encourage the safe-handling of meat by everyone involved in the process. Areas for further research are suggested as a means of building upon the groundwork laid here.

Acknowledgments

We would like to thank Jefferson at the Bonaire Bonlab for helping with supplies, agar preparation, and sterilization. Also, thank you to Saint James School of Medicine for partially funding this research project. Additionally, John and Chris would like to thank their wives, Marielle and Stephanie. Their continuous love and support throughout the research process contributed to our success. Moreover, they are credited for inspiration in writing this document and for help in the editing process.

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