

Assessment of Heterotrophic Bacterial Count (HPC) Associated with Commercial Freezers in Yenagoa Metropolis

Douye V. Zige*, Faith I. Omeje, Christian K. Anumudu

Department of Microbiology, Faculty of Science, Federal University, Otuoke, P.M.B. 126, Yenagoa, Bayelsa State, Nigeria

*Corresponding author: zighedouye@gmail.com

Received August 15, 2019; Revised September 16, 2019; Accepted September 25, 2019

Abstract Microorganism are known to be present anywhere they can proliferate, its presence dictates its pathogenicity or otherwise. The study was embarked to assess the enteric bacterial quality and potential risk of water at the bottom of selected commercial freezers in Yenagoa metropolis. Serial dilution was adopted for the assessment of Heterotrophic bacterial count (HPC). From the analysis, bacterial count ranged between $1.083 \pm 0.104 \times 10^7$ cfu/mL and $2.0 \pm 0.358 \times 10^7$ cfu/mL. the highest was in sample 4 (S4) and least in sample 10 (S10), the study thus found the presence of heterotrophic bacteria in all samples. This research reveals that freezers S5, S3, S4 and S8 were seriously contaminated, having mean viable bacterial load of 2.47×10^7 cfu/mL, 2.18×10^7 cfu/mL, 2.00×10^7 cfu/mL and 2.00×10^7 cfu/mL, respectively; while freezer S1, S2, S6, S7, S9 and S10 had variably viable bacteria count, the occurrence of heterotrophic bacteria count (HPC) between sampled freezers was statistically very significant ($P < 0.05, 0.01$). Bacteria cells using morphological and biochemical characterization identified in the study include *Escherichia coli* (29.4%), which was the most frequently occurring organism, followed by *Citrobacter spp* (14.7%), *Klebsella pneumonia* (14.7%), *Shigella spp* (11.8%), *Pseudomonas aeruginosa* (11.8%), *Yersinia spp* (8.8%) and *Campylobacter jejuni* (8.8%). *E. coli* were the most frequently isolated bacteria. *E. coli* and other Enteric bacteria isolated from freezers are an indication that food items and water stored in these freezers are not safe from public health stand point. Susceptibility of isolates to antibiotics reveals 8(23.5%) were resistant and 26 (76.5%) were susceptible out of 34 cells identified. High resistance was seen in *Klebsiella spp* which had 2 (60%) and 100% susceptibility was seen among *Citrobacter spp* and *Yersinia spp*, on the other hand other isolate had varying drug resistant patterns. The importance of temperature control and regular efficient cleaning regimes need to be communicated to the public so that, effectual management and cleaning of freezers makes frozen food reliable and less likely to act as significant sources of human food and water borne diseases.

Keywords: Heterotrophic Bacteria Count (HPC), contamination, enteric pathogens, commercial freezers

Cite This Article: Douye V. Zige, Faith I. Omeje, and Christian K. Anumudu, "Assessment of Heterotrophic Bacterial Count (HPC) Associated with Commercial Freezers in Yenagoa Metropolis." *American Journal of Microbiological Research*, vol. 7, no. 3 (2019): 98-101. doi: 10.12691/ajmr-7-3-5.

1. Introduction

The heterotrophic group of bacteria encompass a broad range of bacteria that uses organic carbon sources to grow. Colony counts of heterotrophic bacteria, referred to as HPC, provide an indication of the general load of aerobic and facultative anaerobic bacteria of a water sample. This indicator is also known as standard plate count (SPC), aerobic plate count (APC) and total plate count (TPC) [1]. Heterotrophic plate count (HPC), reflects the load of general aerobic bacteria in the water system, it can be used for detection of all bacteria, but cannot be used in fecal contamination test [2]. Thus, it is no longer used as a health-related indicator [3]. Heterotrophic bacteria are natural inhabitant of the human body and animal. They include; *Proteus*, *Enterobacter*, *Aeromonas*, *Citrobacter*,

Pseudomonas, *Klebsiella*, *Flavobacterium*, *Moraxella*, *Alcaligenese* and *Acinetobacter*, *Bacillus* and *Micrococcus* [4]. The heterotrophic plate counts (HPC), expressed as colony-forming units (CFU), became one of the standard techniques for microbial water quality testing [5]. Health significance and occurrence of injured bacteria in drinking water [6] as well as potentially pathogenic features of heterotrophic plate count bacteria from treated and untreated drinking water has been reported [7]. Heterotrophic bacteria are not indicators of pathogenic conditions but some of them like *Pseudomonas* is opportunists and can cause some infections in skin and lung and others such as *Aeromonas* cause gastroenteritis [8]. With high concentrations of heterotrophic bacteria in the water, it is hard to determine the fecal coliform and pathogenic contaminations [9]. In addition, heterotrophic bacteria count over 1,000 CFU/ml in water samples can cause low sensitivity in tube tests and the membrane filter.

Organisms such as *Pseudomonas* and *Flavobacterium* can prevent the growth of coliform and prevent the observed gas production in lactose fermentation and with dispersed growth on the membrane filter, and interfere with coliform on M-Endo medium, and therefore coliform detection could be hindered. Increase in heterotrophic bacteria could be a sign of trouble in treatment, repair, installation or influence of microbial growth in the distribution system and presence of biofilm [10], and one reasons which may increase the risk of gastroenteritis [11].

Yenagoa metropolis harbours many kinds of businesses including operators of restaurants, superstores, cold rooms which store wide variety of foods such as meat, fish, shellfish, vegetables, alcoholic and non-alcoholic beverages and water in several containers etc. These food items are majorly stored in deep freezers as a means of storage but due to infrequent light supply in this part of the world, food items are thawed with a resultant draining of water down the bottom of freezers. This water accumulates and becomes a breeding ground for heterotrophic bacteria which become active once taken out of the freezers [12,13,14]. This study was aimed at determining the Heterotrophic bacteriological quality of water that collects at the bottom of freezers in selected stores in Yenagoa metropolis, to assess and establish the possible public health risk posed by these restaurants.

2. Materials and Methods

2.1. Collection of Water Sample

Sample was collected from the bottom of commercial freezers in Yenagoa, Bayelsa state. A total of 10 samples were collected in triplicate. The samples were randomly collected from cool rooms, restaurant and from stores within the city capital. The sample was collected in sterile glass bottle using disposable sterile hand gloves. These were labeled sample 1 to sample 10 respectively and taken to the laboratory for bacteriological analysis [15].

2.2. Bacteriological Analysis

2.2.1. Total Heterotrophic Counts

The method of [16] was adopted with slight modification as samples collected were moderately turbid and, hence ten-fold serial dilutions (10^{-3} - 10^{-7}) were prepared for all samples using sterile distilled water [17]. 1mL aliquots of samples diluted to 10^{-3} - 10^{-7} were plated on already prepared nutrient agar in Petri dishes in triplicates and the total heterotrophic count THC was determined by pour plate technique [18] using nutrient agar (Oxoid, England). These cultures were incubated at 37°C for 48hours after which bacterial colonies were counted.

2.2.2. Identification of Bacterial Isolates

Identification of bacteria isolated was based on cultural, Morphological and cellular (Gram's reaction and motility) characteristics along with biochemical characterization such as Indole, catalase, coagulase, oxidase methyl red, Voges proskauer and Citrate utilization (IMViC) reactions as well as carbohydrate utilization using Klinger iron agar

(KIA) test were employed to establish the identity of each isolate. The cultural media used initially for the presumptive isolate were selective media *Salmonella Shigella* Agar, Macconkey agar TCBS, Simon Citrate Agar, Xylose Lysine Deficiency (XLD), The method of [16,19,20] was adopted in the cultural and biochemical identification of isolates.

2.3. Statistical Analysis

Differences in contamination level of the sampled freezers with respect to bacterial counts were determined using the statistical method, analysis of variance (ANOVA) with SPSS version 20.

3. Result/Discussion

Table 1 presents the Mean heterotrophic bacterial counts in water samples from freezers, the bacterial count ranged between $1.083 \pm 0.104 \times 10^7$ cfu/mL and $2.0 \pm 0.358 \times 10^7$ cfu/mL. the highest was in sample 4 (S4) and least in sample 10 (S10), the study thus found the presence of heterotrophic bacteria in all samples. The bacteriological quality was better than that reported by [21], who had reported that 16% of the bottled water samples from Canada had bacterial counts higher than 1000cfu/mL. The quality was also superior to those samples analyzed by [22,23] who reported that more than 50% of the water samples had bacterial counts higher than 1000cfu/mL. The results were comparable to those reported by [24] in the bottled drinking water samples sold in retail outlets of Nigeria. This research reveals that freezers S5, S3, S4 and s8 were seriously contaminated, having mean viable bacterial load of 2.47×10^7 , 2.18×10^7 cfu/mL, 2.00×10^7 and 2.00×10^7 respectively; while freezer S1, S2, S6, S7, S9 and S10 has variable viable bacteria count, these counts may be as a result of poor sanitary practices.

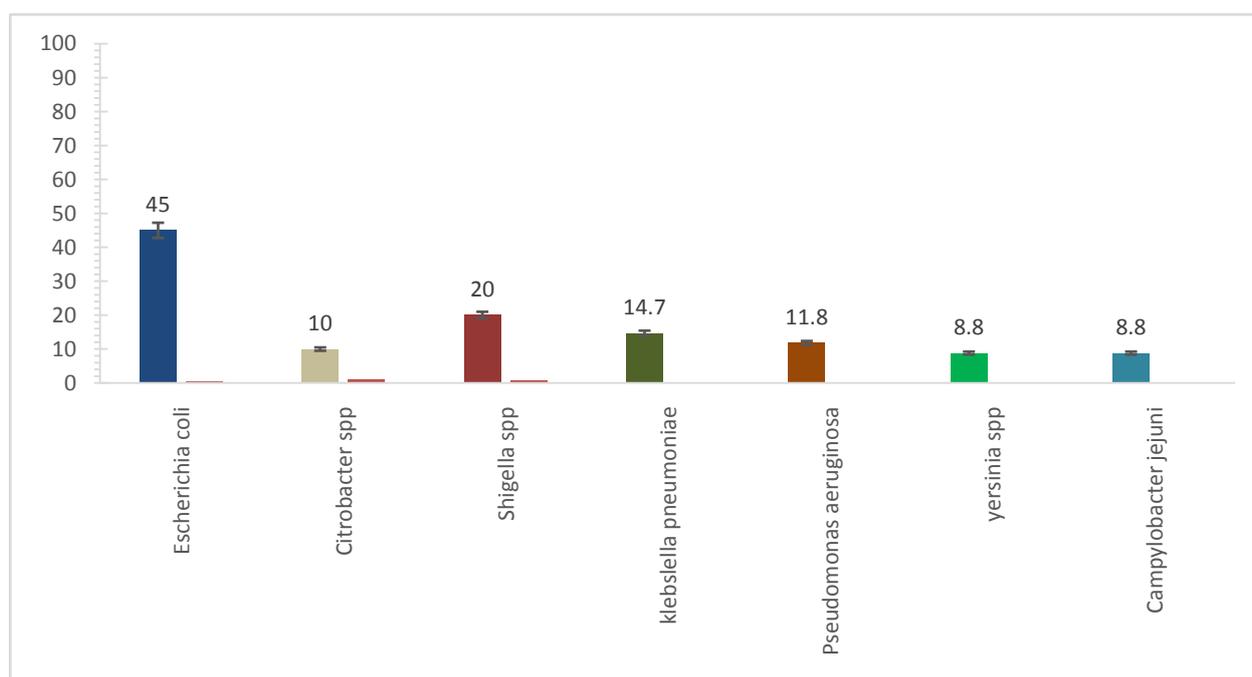
Figure 1 presents the incidence of enteric bacteria pathogens in the sample waters. the result shows that *Escherichia coli* was (29.4%), which was the most frequently occurring organism, followed by *Citrobacter spp* (14.7%) and *Klebsella pneumonia* (14.7%), *Shigella spp* (11.8%) and *Pseudomonas aeruginosa* (11.8%), *Yersinia spp* (8.8%) and *Campylobacter jejuni* (8.8%). *E coli* were the most frequently isolated bacteria *E. coli* and other Enteric bacteria isolated from freezer is an indication food items and water stored in these freezers are not safe from public health stand point [25,26,27]. These may be associated to food handler's hygiene practices, especially hand washing practices after using the rest room [28].

Table 2 presents the percentage of bacterial cells resistance and susceptibility to antibiotics, the result showed that 8(23.5%) were resistant and 26 (76.5%) were susceptible out of 34 cells identified. High resistance was seen in *Klebsiella spp* which had 2 (60%) and 100% susceptibility was seen among *Citrobacter spp* and *Yersinia spp*, on the other had other isolate had varying drug resistant patterns. Results of the antibiotic sensitivity testing revealed that most of the Enteric bacteria isolated from water sample from freezers were mostly susceptible to antibiotics used for the investigation.

Table 1. Results of Total Heterotrophic Bacteria counts for Different locations

Locations	Mean Cont. (X 10 ⁷ cfu/ml)	Standard Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
S1	1.417ab	0.597	0.065	2.89	1.00	2.10
S2	1.673abc	0.327	0.857	2.490	1.30	1.92
S3	2.183cd	0.407	1.172	3.195	1.90	2.65
S4	2.000bcd	0.173	1.570	2.430	1.80	2.10
S5	2.470d	0.352	1.596	3.344	2.10	2.80
S6	1.760abcd	0.622	0.215	3.305	1.08	2.30
S7	1.800abcd	0.265	1.143	2.457	1.50	2.00
S8	2.007bcd	0.358	1.118	2.896	1.80	2.42
S9	1.540abc	0.394	0.563	2.517	1.12	1.90
S10	1.083a	0.104	0.825	1.342	1.00	1.20

S1 – S10 are sampling codes for different sampling locations. Data expressed as Mean± Standard Deviation, differences in alphabetical subscript indicates significant difference.

**Figure 1.** Percentage incidence of Enteric bacteria isolated**Table 2. Percentage of Total Heterotrophic Bacteria Resistance to Antibiotics**

Bacteria cells	No of cells	Resistance	susceptible
<i>E coli</i>	10	3 (30%)	7 (70%)
<i>Citrobacter spp</i>	5	0 (0%)	5 (100)
<i>Shigella spp</i>	4	1 (25%)	3 (75%)
<i>Klebsiella spp</i>	5	2 (60%)	3 (40%)
<i>Pseudomonas auroginosa</i>	4	1 (25%)	3 (75%)
<i>Yirsinia spp</i>	3	0 (0%)	3 (100%)
<i>Campylobacter spp</i>	3	1 (33.3%)	2 (66.7 %)
Total	34	8(23.5%)	26 (76.5%)

4. Conclusion

These results from the study revealed that most commercial freezers used in Yenagoa metropolis are of public health concern as a wide range of waterborne and food borne infections may spread through these contaminated commercial freezers. This study has shown that food and water borne pathogens can survive in water at the bottom of commercial freezers and therefore pose a risk of cross-contamination among food and water handlers. Thus, a number of potential food and water related

pathogens including *Escherichia coli*, *Campylobacter* spp, *Salmonella* spp, *Shigella* spp etc were encountered in this study. These results suggest that the heterotrophic bacteria in high concentrations in water do not have any effect on determining the fecal coliform and pathogens however the level of contamination is likely to be influenced by hygiene practice of freezer cleaning and maintenance. The study also finds out that there was significant difference among the different water samples, the spread, growth and survival of food and waterborne pathogens can be controlled with correct food storage and preparation

practices, regular cleaning and disinfection of food contact sites. As we rely more and more on refrigeration as a means of food preservation, it is crucial that the public be made aware that the freezers can in fact represent a significant niche for the persistence and dissemination of enteric pathogens. The importance of temperature control and regular efficient cleaning regimes need to be communicated to the public so that, effective management and cleaning of freezers makes them consistently reliable elements of the chilled food chain, and less likely to act as significant sources of human food borne diseases.

References

- [1] Sophie, V Understanding microbial indicators for drinking water assessment: interpretation of test results and public health significance. *National Collaborating Centre for Environmental Health*, pg1-12. 2013.
- [2] Hsu, F.-C., Shieh, Y., Van Duin, J., Beekwilder, M and Sobsey M.D Genotyping male-specific RNA coliphages by hybridization with oligonucleotide probes. *Appl Environ Microb*, 61: 3960-3966. 1995.
- [3] Edberg, S.C and Smith, D.B Absence of association between total heterotrophic and total coliform bacteria from a public water supply. *Appl Environ Microbiol*. 55: 380-4. 1988.
- [4] Bartram, J., Cotruvo, J., Exner, M., Fricker, C and Glasmacher A. Heterotrophic plate counts and drinking-water safety: the significance of HPCs for water quality and human health, Ed, IWA Publishing, pp. 7-16. 2003.
- [5] Le Chevallier, M.W., Seidler, R.J and Evans, T.M Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol*. 40, 922-930. 1980.
- [6] McFeters, G.A., Le Chevallier, M.W., Singh, A and Kippin, J.S. Health significance and occurrence of injured bacteria in drinking water. *Water Sci. Technol*. 18 (10), 227-231. 1986.
- [7] Pavlov, D., de Wet, C.M., Grabow, W.O and Ehlers, M.M Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water. *Int. J. Food Microbiol*. 92 (3), 275-287. 2004.
- [8] Helmer, R and Hespagnol, I. Water pollution control: a guide to the use of water quality management principles. Supply W, Council SC, Organization WH, Press C. ed. E & FN Spon; London, pp. 400-402. 1997.
- [9] Allen, M.J., Edberg, S.C and Reasoner, D.J. Heterotrophic plate count bacteria—what is their significance in drinking water? *Int J Food Microbiol*, 92pp: 265-274. 2004.
- [10] Ainsworth, R., Water, S and WHO. Safe piped water: managing microbial water quality in piped distribution systems/edited by Richard Ainsworth, editor. .pp: 25-130. 2004.
- [11] Prévost, M., Rompré, A., Coallier, J., Servais, P., Laurent, P., Clément, B and Lafrance, P. Suspended bacterial biomass and activity in full-scale drinking water distribution systems: impact of water treatment. *Water Res*, 32pp: 1393-1406. 1998.
- [12] White, B. A and West, E.M. Personal hygiene and kitchen hygiene. *J. Food Serv. Inst.*, 5: 220-36. 1992.
- [13] Davies, R.J. The national sanitation training program: Canadian Restaurant Associations Answers to Safe and Sanitary Food Service. *J. Food Technology*, 39: 367-9. 1995.
- [14] Eja, M. A., Etok, C.A., Asikong, B.E., Mbot, C.I and Arikpo, G.E. Incidence of enteric bacterial pathogens in water found at the bottom of commercial freezers in Calabar, Southeastern Nigeria. *Southeast Asian J. Trop. Med. Public Health*, 37 (2): 394-399. 2006.
- [15] Baker, F.J and Silverton, R.E. Introduction to medical laboratory technology. (sixth edition), Butterworth and co-publishers limited, UK. 2: 2-392. 1985.
- [16] Ntekpe M. E., Ukpanah, U.A., Edem, A.B., Edet, E and Umoeakam, N.M. Sanitary Practices and Occurrence of Coliform Bacteria in Freezers Used in Some Restaurants in Uyo Metropolis. *Int. J. Food Nutr. Saf*. 3(2): 71-80. 2013.
- [17] American Public Health Association. Standard methods for the examination of water and wastewater, 20th eds. APHA, Washington, D. C. 1998
- [18] Atlas, R.M and Bartha, R. Degradation and mineralization of petroleum by two bacteria isolated from coastal waters. *Biotechnological Bioengineering*. 14: 297-308. 1992.
- [19] Cheesbrough, M. District Laboratory Practice in Tropical Countries. (4th edition). Cambridge university press, UK. p143-157. 2006.
- [20] Iquo, B.O., Itodo, S.E., Blessing, F.O and Usang, A.I. Microbial Contamination of House Hold Refrigerators in Calabar Metropolis-Nigeria." *American Journal of Epidemiology and Infectious Disease*. 5(1)1-7. 2017.
- [21] Warburton, D.W., Peterkin, P.L., Weiss, K and Johnston, M. Microbiological quality of bottled water sold in Canada. *Can. J. Microbiol*. 32, 391-393. 1986.
- [22] Richards, J., Stokely, D and Hipgrave, P. Quality of drinking water. *Br. Med. J*. 304, 571. 1992.
- [23] Hunter, P.R., Burge, S.H and Hornby, H. An assessment of the microbiological safety of bottled mineral waters. *Rivista Italiana d' Igiene* 50, 394-400. 1990.
- [24] Ogan, M.T. Microbiological quality of bottled water sold in retail outlets in Nigeria. *J. Appl. Bacteriol*. 73, 175-181. 1992.
- [25] McCoy, J. H. The safety and cleanliness of waters and foods. *J. Applied Bacteriology*, 24: 365-67. 1991.
- [26] World Health Organization. Guidelines for Drinking Water Quality. vol. 2. WHO, Geneva. 1996.
- [27] Brooks, G. F., Carroll, K.C., Butel, J.S., Morse, S.A and Mietzner, T.A. Jawetz, Melnick and Adelberg's Medical Microbiology, 25th eds. p213-5. 2010.
- [28] Conway, P.L. Human colonic bacteria: role in nutrition, physiology, and pathology. In *Microbial ecology of the human large intestine*. Gibson and G. T. Macfarlane, eds. CRC press, Boca Raton, FL, p1-24. 1995.

