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Bacteriological Examination Of Palm Wine Consumed In Bamenda

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Abstract

Background: Palm wine is a natural alcoholic beverage consumed in most parts of the world especially in the tropics. It is used in many societies as a drink during social events. This beverage is usually gotten from palm trees in general and can be contaminated during the taping, transportation and selling processes. There are many microorganisms present in this wonderful beverage some of which increase the alcoholic content like Saccharomyces cerevisiae and some increase the acidity like Acetic acid bacteria and Lactic acid bacteria.

Aim: This study was aimed at bacteriologically examining palm wine consumed in the city of Bamenda and to determine its quality.

Method:30 palm wine samples were collected from 10 drinking spots from Bamenda I, II and III using stratified and random sampling techniques. Aliquots from these samples were cultured on Cysteine Lactose Electrolyte Deficient Agar (CLED agar) and Salmonella-Shigella Agar (SS agar). Morphological characteristics were examined, Gram stain and biochemical test were then performed on colonies from CLED agar and SS agar.

Results:100% of samples had growth of Salmonella spp with an average bacteria load of 1.9x105 bacteria/mL. 80% of samples showed growth of E. coli with an average bacteria load of 5.8x105 bacteria/mL. Also, 60% of samples had growth of klebsiella spp with an average bacteria load of 5.5x104 bacteria/mL. Vendors that used well water for washing storage containers and cups had higher bacteria loads compared to vendors that used city water with a p-value of 0.024 which is statistically significant.

Conclusions: Due to the quantity of bacteria isolated from palm wine consumed in the city of Bamenda, its quality is poor and can thus serve as a source of infection.

1. Introduction

Palm wine is a natural alcoholic beverage consumed in most parts of the world, especially in the tropics [1]. This beverage contains microbes that have different functions which are beneficial especially to the consumer as some help in the conversion of sucrose from palm sap to alcohol [2]. Palm wine is produced from palm tree through the process of tapping [3]. A study carried out by Ukhun *et al* [4] in Nigeria suggests that the conditions surrounding the tapping and transportation process of palm wine to the drinking spots are very unhygienic thus giving room for contamination with pathogenic and non-pathogenic bacteria. The most common pathogens which most often colonize the intestinal tract cells serve as target cells for infection [5]. Intestinal tract infections often have a common presentation which is diarrhea-like symptoms according to a study carried out by Feng *et al* [6]. Most organisms that present with diarrhoealike symptoms are of the group of bacteria known as Enterobacteriaceae. Members of this group are species such as *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella* and *Shigella* [4]. The Enterobacteriaceae are a large family of Gram negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens such as *Salmonella, Escherichia coli, Shigella* and *Klebsiella* [7]. *Salmonella spp* are facultative intracellular pathogens. Many infections are due to ingestion of contaminated food and water. It is named after Daniel Elmer salmon, a veterinary pathologist of the United States department of agriculture [8]. Salmonella serovars can be divided into two main groups; typhoidal and non-typhoidal Salmonellosis. Most people infected with *Salmonella spp* develop diarrhoea, fever, and abdominal cramps between 12 and 72 hours after infection. Children are at the highest risk for *Salmonella spp* infection. Children under the age of 5 have higher rates of Salmonella infection than any other age group. Young children, older adults, and people with weakened immune systems are the most likely to have severe infections [3]. It can be diagnosed using serological and culture methods in the clinical laboratory [9].

Escherichia coli also known as *E. coli* is a Gram-negative, facultative anaerobe, rod-shaped bacterium. It was discovered by a German bacteriologist called Theodore Escherich. It is commonly found in the lower intestine of warm-blooded organisms (endotherms) [10]. It can also be gotten from contaminated food and though not limited to water. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for intestinal perforations due to food contamination. [11]. Optimum growth of *E. coli* occurs at 37°C, but some laboratory strains can multiply at temperatures up to 49°C [12]. Klebsiella is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule [10]. It is named after the German microbiologist Edwin Klebs in the late nineteenth century. *Klebsiella spp* are found everywhere in nature. The majority of human infections are caused by *K. pneumoniae*, followed by *K. oxytoca*. Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer [13]. Given the above pathogenic strains, it is therefore vital to investigate if they are present in palm wine and thus determine the bacteria load [10]. This will help the community and government in developing policies as to the consumption of this wonderful beverage.

2. MATERIALS AND METHODS

2.1 Study design.

This study was a descriptive community based cross sectional study in the city of Bamenda which involved popular spots where palm wine was sold

2.2 Study setting

This study was carried out in the city of Bamenda and laboratory base analysis was done at Ringland Medical Diagnostic laboratory. Ringland medical is a hospital located at Foncha street, opposite

St John catholic church Bamenda being the capital. The region is one of the two English speaking regions of

in the city of Bamenda North west region of Cameroon with a population of 1.8 million and a population density of 104.3 people /km^2. In addition, this region is also known for its academic activities including both English and French-speaking Cameroonians and currently has four universities (1 state university and 3 private universities). The main public hospital for the region is the Bamenda Regional Hospital and other private

and faith-based hospitals such as the St Blaise clinic, the Mbingo Baptist Hospital and the St. Elizabeth Catholic General Hospital, Shisong which increase access to healthcare in the region. Ringland medical is located in the city's capital Bamenda and it remains a centre for health in the region and provides health services to the more than 10,000 inhabitants. The main public hospital for the region is the Bamenda Regional Hospital and other private and faith-based hospitals such as the St Blaise clinic, the Mbingo Baptist Hospital and the St. Elizabeth Catholic General Hospital, Shisong which increase access to healthcare in the region. Ringland medical is located in the city's capital Bamenda and it remains a centre for health in the region and provides health services to the more than 10,000 inhabitants of the Bamenda town and the entire population of the North West Region. The administration of the hospital is led by a director who in turn has several subordinates.

2.3 Study period

This study was conducted for one month. It started from 1st May and ended on the 3th of June 2017.

2.4 Target population

The target population of this study was vendors of palm wine in the city of Bamenda.

2.5 Sampling technique and Sample size

A stratified and random sampling technique was used to collect samples from the various selling spots by first stratifying Bamenda into three Municipalities which are Bamenda I, Bamenda II and Bamenda III. In each municipality, a random sampling technique was used by selecting popular drinking spots at random and then skipping 5 drinking spots before collecting again. From each selling spot, three kinds of samples (fresh sample, overnight sample and refrigerated fresh samples) were collected. In total, 30 samples were collected and analyzed.

2.5 Data collection

2.5.1 Sample collection

Palm wine samples were bought from popular drinking spots around the city of Bamenda which were randomly selected to avoid being biased. These samples were collected using sterile urine containers from the various spots and brought immediately to the laboratory for analysis.

Figure 1: Sample collection

2.6 Laboratory procedure

2.5.1 Preparation of culture media

A culture medium is a combination of chemicals which can either be in a solid, liquid or semi-solid state and functions in supporting the growth of microorganisms. Different types of media are used to grow different types of organisms. Two types of culture media were used in this study. These are Cysteine Lactose Electrolyte Deficient (CLED) agar and Salmonella-Shigella (SS) agar. They were prepared according to the manufacturer's instructions as follows

2.5.1.1 Cysteine Lactose Electrolyte Deficient (CLED) Agar

CLED agar is a selective and differential medium commonly used in the microbiology department of the laboratory. It is selective and differential for Gram negative organisms. It is composed of lactose, pancreatic digest of gelatin and casein, beef extract, cysteine, pH indicator bromothymol blue and agar. CLED Agar was manufactured by Liofilchem s.r.l. Bacteriology products company based in Italy. It was prepared following the manufacturer's instructions as follows [49];

- 36.2g of powdered CLED agar media was to be dissolved in 1000ml of distilled water but just 900ml was needed for the study. The required amount of powdered CLED agar was thus prepared using simple proportion as follows;

If 36.2g are needed to dissolve in 1000mls of distilled water, Then, Xg will be dissolved in 900ml. Calculating for Xg, a cross multiplication was made and Xg found to be 32.58g.

- The Bunsen burner flame was lid and the rest of the procedure done close to the flame to prevent contamination.
- This amount was measured using an electric scale balance by placing the powdered agar on a sterile A4 paper after zeroing it.
- The powdered agar was then transferred to a previously sterilized clean bottle and 900ml of distilled water (measured using a syringe) was then added to the bottle and corked tightly.
- The agar was then swelled to allow the formation of a homogenate and then heated using a gas cooker till it completely dissolved.

- The dissolved agar was then transferred to an Autoclave and was autoclaved for 15 minutes at a temperature of 121^{o}
- After autoclaving, the medium was allowed to cool down to a temperature of between 40^{o}C and 45^{o}C and 25ml was then poured into sterile brand new petri-dishes very close to the Bunsen burner flame.
- The petri-dishes were covered and allowed to stand for at least 30 minutes to solidify.
- After solidification, they were placed in an incubator overnight for 24 hours as a test for sterility. The plates that passed the sterility testing by showing no growth were transferred to the refrigerator for storage and preservation while those that showed growth were discarded and new ones prepared.

Figure 2: Preparation of CLED Agar Medium

2.5.1.2 Salmonella-Shigella (SS) Agar

SS agar is a selective and differential medium commonly used in the microbiology department of the laboratory. It is selective and differential for Gram negative organisms. It is composed of lactose, pancreatic digest of gelatin and casein, beef extract, cysteine, pH indicator bromothymol blue and agar. SS Agar was manufactured by Liofilchim products company based in Italy. It was prepared following the manufacturer's instructions as follows [50]

- 52g of powdered SS agar media was to be dissolved in 1000ml of distilled water but just 900ml was needed for the study. The required amount of powdered SS agar was thus prepared using simple proportion as follows;

If 52g are needed to dissolve in 1000mls of distilled water.

Then, Xg will be dissolved in 900ml.

Calculating for Xg, a cross multiplication was made and Xg found to be 32.58g.

- The Bunsen burner flame was lid and the rest of the procedure done close to the flame to prevent contamination.
- The above calculated amount was measured using an electric scale balance by placing the powdered agar on a sterile A4 paper after zeroing it.
- The powdered agar was then transferred to a previously sterilized clean bottle and 900ml of distilled water (measured using a

syringe) was then added to the bottle and corked tightly.

- The agar was then swelled to allow the formation of a homogenate and then heated using a gas cooker till it completely dissolved.
- After forming the homogenate, the medium was allowed to cool down to a temperature of between $40^{o}C$ and $45^{o}C$ and 25ml was then poured into sterile brand new petri-dishes very close to the Bunsen burner flame.
- The petri-dishes were covered and allowed to stand for at least 30 minutes to solidify.
- After solidification, they were placed in an incubator overnight for 24 hours as a test for sterility. The plates that passed the sterility testing by showing no growth were transferred to the refrigerator for storage and preservation while those that showed growth were discarded and new ones prepared

Figure 3: Preparation of SS Agar Medium

2.5.2 Gram stain technique

The procedure was done through the following steps [49]

- Sterile glass slides were labeled. A drop of sterile normal saline was placed on each sterile glass slide and smears were made on the glass slides.
- The bacteria smear on the slide was heat fixed by passing it over the Bunsen burner flame.
- Each slide was covered with the crystal violet stain for one minute.
- The slide was rapidly and carefully washed using clean water.

- Grams iodine was added to each slide for 30 seconds. This step fixes the crystal violet stain on to the bacteria cell wall.
- Each slide was tilted to remove excess iodine.
- Each slide was rapidly decolourized with acetone for 20 seconds and then washed with clean water.
- The slides were then counter stained with Carbol fuschin for 20 seconds and washed with clean water thereafter.
- The slides were dried and a drop of immersion oil added to each. They were then examined microscopically using the oil immersion objective (X100 objective)

2.5.3 Indole test procedure

Indole test was used to differentiate *E. coli* colonies from *Klebsiella spp* colonies. It is used to determine the ability of an organism to split amino acid Tryptophan to form the compound indole. The production of indole was detected by the addition of kovac's reagent. This reagent contains 4 (p)-dimethylamino benzaldehyde which reacts with indole to produce a red complex chemical compound. The procedure was carried out as follows [49];

- The two isolated colonies were picked up using a sterile wire loop and inoculated unto the surface of two different tryptophan broths.

- They were then labeled immediately after inoculating unto the surface of the tryptophan broth.

- The two inocula were then incubated in an incubator for 24 hours.

- 0.5 ml of kovac's reagent was then added to the two test tubes and colour change observed

- The appearance of a reddish colour was confirmation that the colonies were that of *E. coli* while the non-appearance of a reddish colour was indicative of *Klebsiella spp* colonies as they don't split tryptophan to indole.

2.5.4 Dilution of samples

Samples were diluted in test tubes with a dilution factor of 1000. A micropipette was used to transfer samples from one test tube to the next as seen in figure 4 below

Figure 4: Dilution of samples.

2.5.5 Bacteria enumeration

Samples were diluted and the dilution factor which was 1000 taken note of. The viable plate count enumeration technique was used to count the colony forming units.

2.6 Ethical consideration

Administrative authorization was obtained from the Regional Delegation of Public Health North West region of Cameroon and from Ringland medical hospital.

2.7 Results and Interpretations

The growth of bacteria indicates contamination so the total number of bacteria present in the sample was calculated using the formula below [49]:

NO of bacteria/mL = (No of colonies X dilution factor)/volume of sample

2.8 Data management and analysis

Data collected was keyed into Microsoft excel sheet in a Universal Serial Bus flash drive. The data was later imported into IBM statistical software package for social sciences (SPSS) version 22.0. The Chi-square test was used to compare bacteria load based on the type of palm wine. All analysis was done at 95% confidence level with Pearson value less than 0.05 considered being statistically significant.

RESULTS

3.1 Results

A total of 30 samples were collected from 10 drinking spots and analyzed. Three samples were collected from each drinking spot. The three samples were Fresh Palm wine, Overnight Palm wine and Refrigerated Palm wine. Other parameters like storage condition of the palm wine was assessed to check if the palm wine was covered or left open, the type of water used in washing storage containers and cups was also

assessed from this study, 2 sources of water were used for washing storage containers and cups. Majority of the drinking spots (60%) used well water while the remaining 40% used city water. The location of the various drinking spots was taken into consideration with 3 drinking spots from Bamenda I, 4 drinking spots from Bamenda II and 3 other drinking spots from Bamenda III. This information is summarized on Table 4.1 below. *Salmonella spp, klebsiella spp* and *E. coli* were isolated from the various samples as seen in figure 3.1 below

Figure 5: Colonies of E. *coli* (light yellowish with distinct and single colonies), colonies of *Klebsiella spp* (thick yellowish with mucoid colonies) seen on CLED agar (A) andcolonies of *Salmonella spp* (transparent colonies

Table 1: Summary of type of water used, storage conditions and location of the various drinking spots in Bamenda

DRINKING SPOTS	BACTERIA LOAD (bacteria/mL)			STORAGE CONDITIONS	TYPE OF WATER USED	LOCATION OF SELLING SPOT
	Salmonella spp	*Escherichia coli* specie	*Klebsiella spp*			
1	$1.4xi10^{5}$	$9.0x10^{4}$	$6.0x10^{4}$	Closed containers	Well water	Bamenda I
2	$3.4x10^{3}$	$1.5x10^{3}$	$1.8x10^{3}$	Open containers	Well water	Bamenda III
3	$6.0x10^{4}$	$4.0x10^{4}$	0	Open containers	City water	Bamenda II
4	$4.0xx10^{4}$	0	0	Closed containers	City water	Bamenda I
5	$2.2x10^{3}$	$1.4x10^{3}$	$1.0x10^{3}$	Open containers	Well water	Bamenda III
6	$1.6x10^{5}$	$9.0x10^{4}$	0	Closed containers	Well water	Bamenda I
7	$5.0x10^{5}$	$2.0x10^{4}$	$3.0x10^{4}$	Closed containers	City water	Bamenda II
8	$3.2x10^{5}$	$1.8x10^{5}$	$1.4x10^{5}$	Open containers	Well water	Bamenda II
9	$2.0x10^{4}$	0	$4.0x10^{4}$	Open containers	City water	Bamenda III
10	$1.3x10^{5}$	$6.0x10^{5}$	0	Closed containers	Well water	Bamenda II

3.1.1 Determination of bacteria load in fresh, overnight and refrigerated palm wine sold in some popular drinking spots in Bamenda

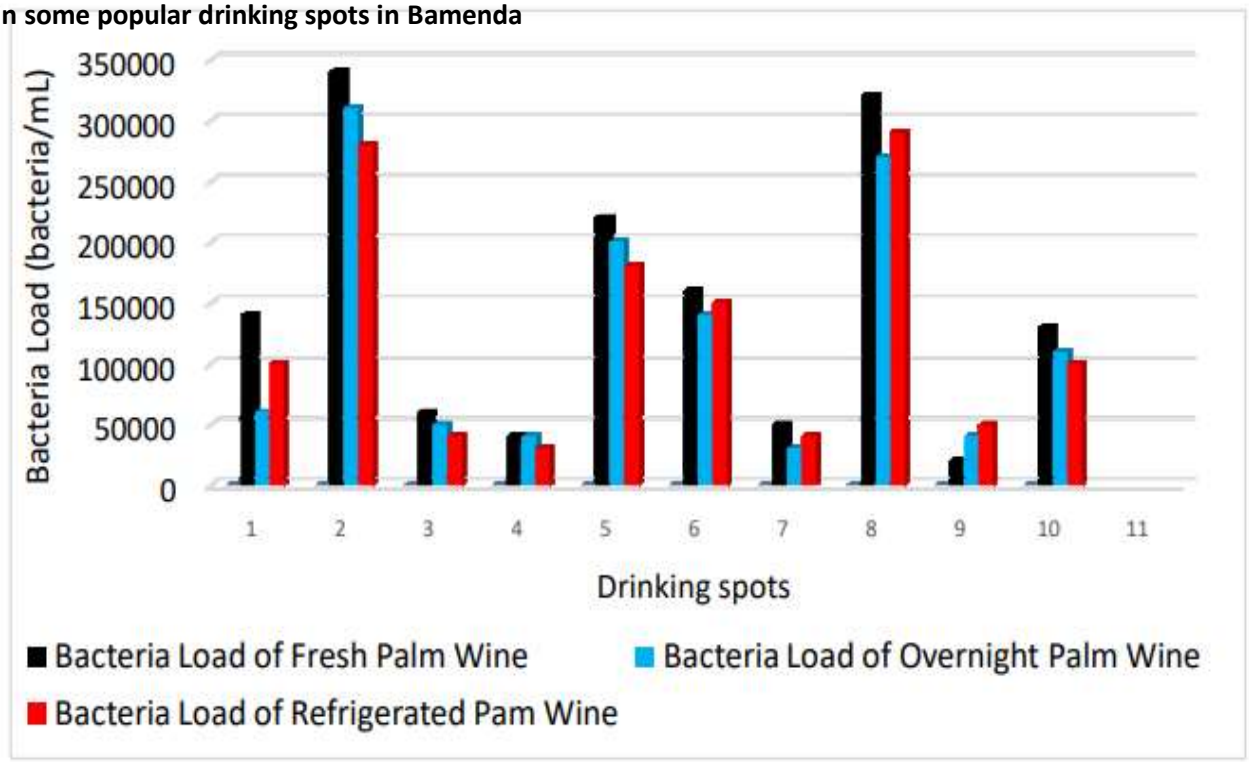
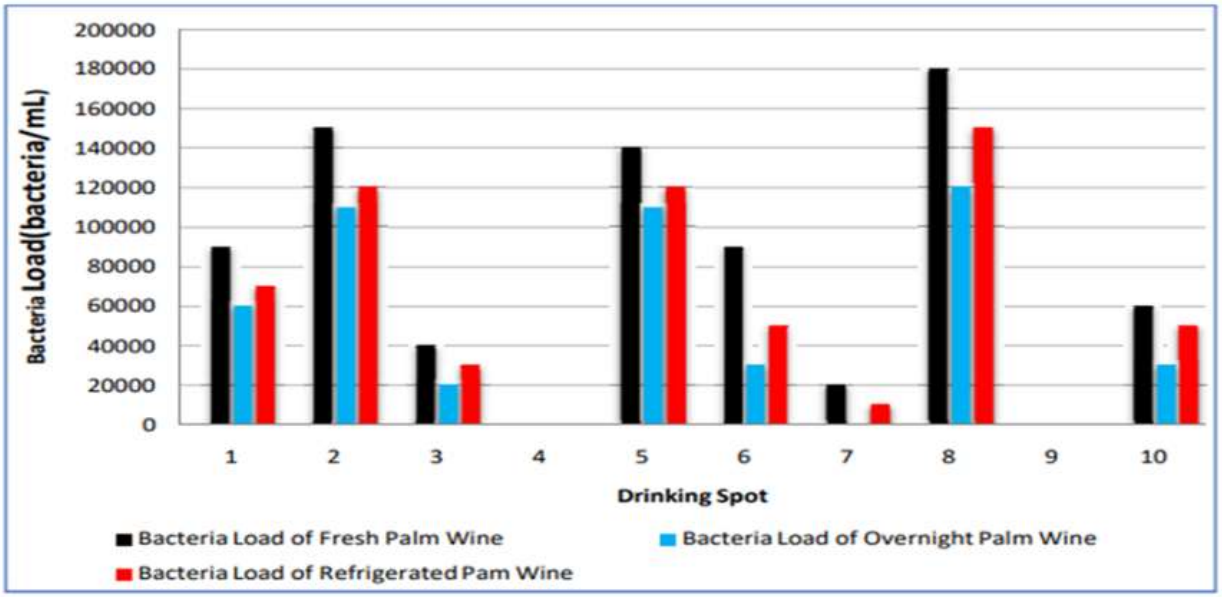


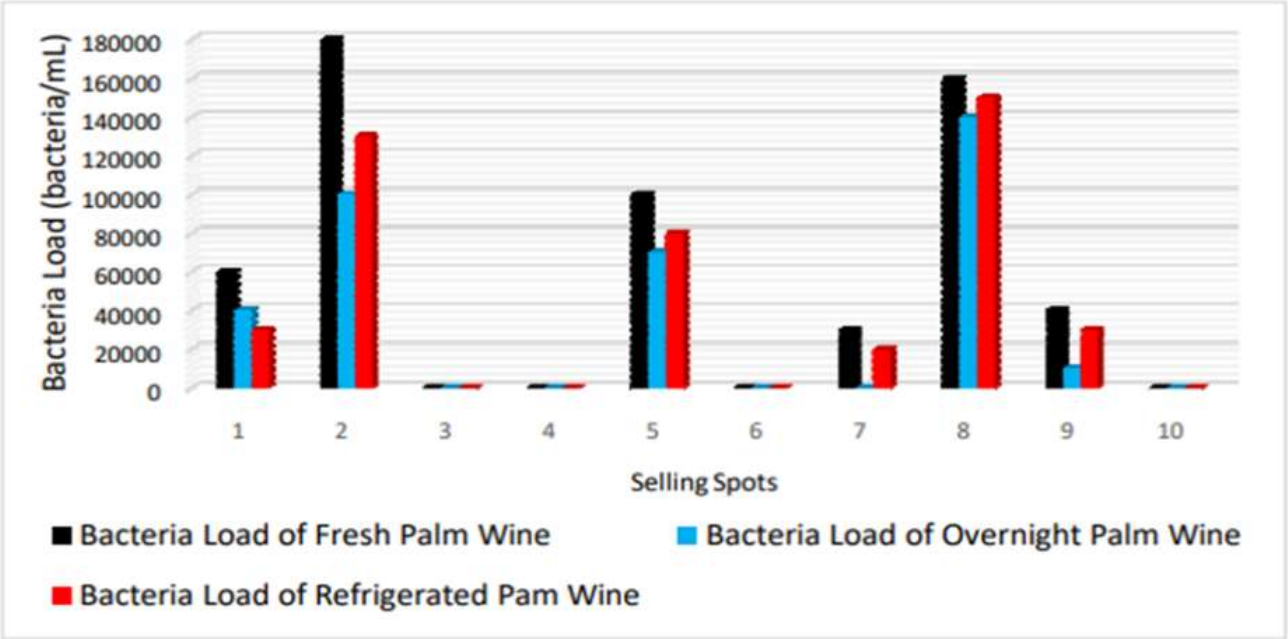
Figure 6: Bacteria Load of *Salmonella spp* with respect to the various drinking spots From the Histogram above, *Salmonella spp* was isolated from all the samples collected from the various drinking spots. Out of these 10 drinking spots, spot 2 had the highest bacteria load of $3.4x10^5$bacteria/mL and $3.1x10^5$ bacteria/mL in Fresh palm wine and Overnight Palm wine respectively. The highest bacteria load in refrigerated palm wine was observed in spot 8 with a bacteria load of $2.9x10^5$bacteria/mL. The lowest bacteria load of *Salmonella spp* in Fresh palm wine was observed in spot 9 ($2.0x10^4$bacteria/mL) while the lowest bacteria load in overnight palm wine was observed in spot 7 ($3.0x10^4$bacteria/mL). For refrigerated palm wine, the lowest bacteria load of *Salmonella spp* was observed in spot 4 ($3.0x10^4$bacteria/mL) out of the 10 drinking spots analyzed.

Figure 7: Bacteria load of Escherichia coli with respect to the various Drinking Spots



From the Histogram above, *Escherichia coli* was isolated from samples collected from 8 drinking spots out of a total of 10 drinking spots. Out of these 8 drinking spots, spot 8 had the highest bacteria load of $1.8x10^5$bacteria/mL and $1.5x10^5$ bacteria/mL in Fresh palm wine and refrigerated Palm wine respectively. The highest bacteria load in overnight palm wine was still observed in spot 8 with a bacteria load of $1.5x10^5$ bacteria/mL. The lowest bacteria load of *Escherichia coli* in Fresh palm wine was observed in spot 7 ($2.0x10^4$ bacteria/mL) while the lowest bacteria load in overnight palm wine was observed in spot 3 ($2.0x10^4$ bacteria/mL). For

refrigerated palm wine, the lowest bacteria load ($1.0x10^4$bacteria/mL) out of the 10 drinking spots analyzed. *Escherichia coli* was not isolated from spot



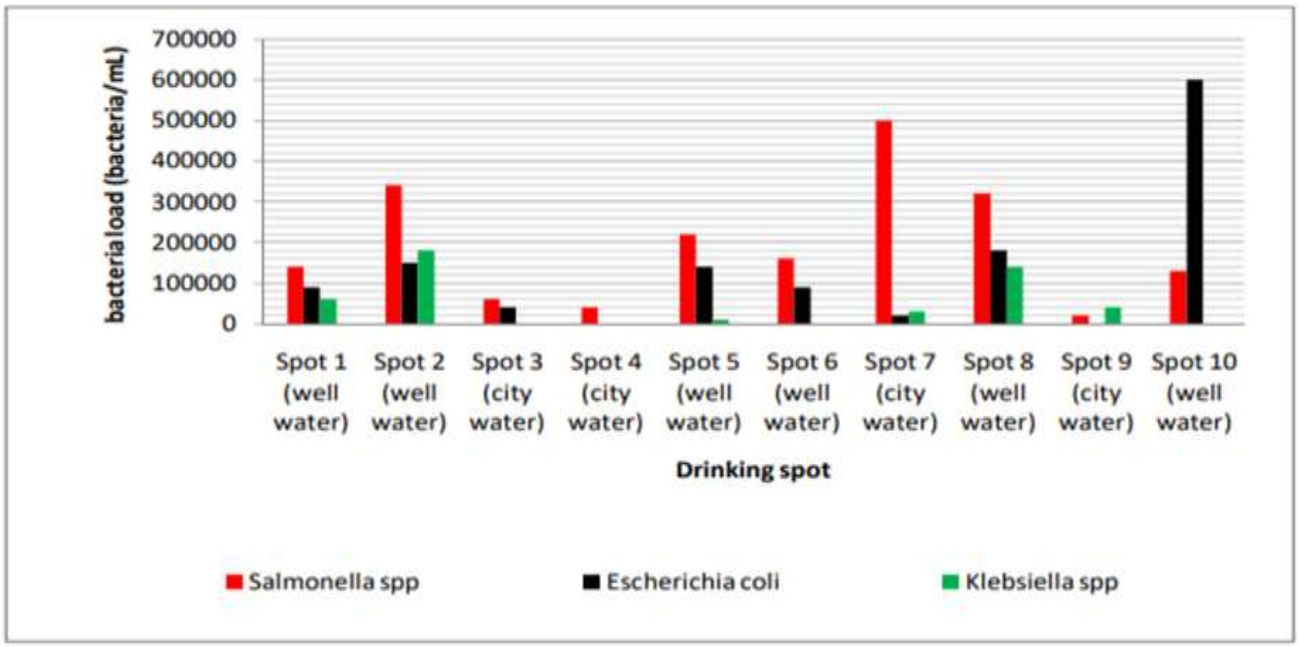
of *Escherichia coli* was observed in spot 7 4 and 9

Figure 8: Bacteria load of *Klebsiella spp* with respect to the various Drinking spots.

From the Histogram above, *Klebsiella spp* was isolated from samples collected from 7 out of a total of 10 drinking spots. Out of these 7 drinking spots, spot 2 had the highest bacteria load of $1.8x10^5$ bacteria/mL. Spot 8 then had a bacteria load of $1.6x10^5$bacteria/mL in overnight palm wine and $1.5x10^5$ bacteria/mL in refrigerated Palm wine. The highest bacteria load in overnight palm wine was still observed in spot 8 with a bacteria load of $1.5x10^5$ bacteria/mL. The lowest bacteria load of *Klebsiella spp* in Fresh palm wine was observed in spot 7 ($3.0x10^4$ bacteria/mL) while the lowest bacteria load in overnight palm wine was observed in spot 9 ($1.0x10^4$ bacteria/mL). For refrigerated palm wine, the lowest bacteria load of *Klebsiella spp*was observed in spot 7 ($2.0x10^4$bacteria/mL) out of the 10 drinking spots analyzed. *Klebsiella spp* was not isolated from spot 3, 4, 6 and 10

3.1.2 Comparison of Total Bacteria load in Fresh, Overnight and Refrigerated palm wine

Table 2: Comparison of total bacteria Load in Fresh, Overnight and Refrigerated palm wine



3.1.2 Comparison of Total Bacteria load in Fresh, Overnight and Refrigerated palm wine

DRINKING SPOT	TOTAL BACTERIA COUNT (bacteria/mL)	
	Fresh Palm Wine	Overnight Palm Wine
1	$2.9x10^5$	$1.6x10^5$
2	$6.7x10^5$	$5.4x10^5$
3	$1.0x10^5$	$7.0x10^4$
4	$4.0x10^4$	4.010^4
5	$4.6x10^5$	$3.8x10^5$
6	$2.5x10^5$	$1.7x10^5$

Table 3: Significances of the comparison of total bacteria Load in Fresh Overnight and Refrigerated palm wine

Parameters	**P-values (Significance)**
1) Total bacteria in Fresh and	**0.034**
2) Total bacteria in Overnight	**0.035**
3) Total bacteria in fresh and	0.617

From table 4.2 above, all the 10 drinking spots showed bacteria growth in total. Drinking Spot 10 had the highest total bacteria load ($7.3x10^5$ bacterial/mL) for Fresh palm wine while drinking spot 8 had the highest total bacteria load for overnight palm wine ($5.5x10^5$ bacteria/mL) and Refrigerated ($5.9x10^5$ bacteria/mL) Palm wine. After the comparison of the various bacteria load in Fresh and Overnight, the difference was statistically significant with a P-value of 0.034. Comparing total bacteria load in Overnight and Refrigerated palm wine, the P-value was 0.035 which was also statistically significant. The comparison between total bacteria load in Fresh and Refrigerated Palm wine, it was found to be statistically insignificant with a P-value of 0.617. A general reduction in bacteria load was observed from Fresh palm wine to overnight palm wine. Also, there was a reduction in total bacteria load in refrigerated Palm wine compared to total bacteria load in fresh Palm wine

Table 4: Significances of the comparison of total bacteria Load in Fresh palm wine the types of water used in washing storage containers and cups

Parameters	**P-values**
1) Total bacteria load in fresh palm	**0.024**
2) Total bacteria load in fresh palm	**0.043**

From the histogram above, out of the 10 drinking spots, 4 of them used city water for washing of storage containers and cups for drinking palm wine and 6 of them used well water. *Salmonella spp* was isolated from all the 10 drinking spot with the highest occurring in drinking spot 7 ($5.0x10^5$ bacteria/mL) that used city water. The least was isolated from drinking sport 9 ($2.0x10^4$ bacteria/mL) that as well used city water. *Escherichia coli* specie was also isolated from all the drinking spots except drinking spot 4 and 9. The highest bacterial load was isolated from spot 10 ($6.0x10^5$ bacteria/mL) using well water and the least from spot 9 ($2.0x10^4$ bacteria/mL) using city water. *Klebsiella spp* was isolated from spot 1, 2, 5, 7, 8 and 9. The highest bacteria load of *Klebsiella spp* was isolated from drinking spot 2

($1.8x10^5$ bacteria/mL) and the least was isolated from spot 7. Generally, comparing total bacteria load in fresh palm wine with the use of well water and city water for washing storage containers and cups were significant with P-values being 0.024 and 0.043 respectively.

CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

Conclusions

100% of samples had growth of *Salmonella spp* with an average bacteria load of $1.9x10^5$ bacteria/mL. 80% of samples showed growth of *E. coli* with an average bacteria load of $5.8x10^5$ bacteria/mL. Also, 60% of samples had growth of *Klebsiella spp* with an average bacteria load of $5.5x10^4$ bacteria/mL. The comparison of the total bacteria load in fresh palm wine, overnight palm wine and refrigerated palm wine showed a high bacteria load in fresh palm wine ($6.4x10^5$ bacteria/mL) secondly by refrigerated palm wine ($5.9x10^5$ bacteria/mL) which were statistically significant. The least total bacteria load was recorded in overnight palm wine ($5.5x10^5$ bacteria/mL. Well water should not be used in washing storage containers and cups used in serving palm wine. This is because the well waters are not treated and contribute significantly (P = 0.024) to the contamination of palm wine. Due to the quantity of bacteria isolated from palm wine consumed in the city of Bamenda, its quality is poor and can thus serve as a source of infection.

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