BACTERIAL ENUMURATION AND DETECTION IN *Rhynchophorus phoenicis* (AFRICAN PALM WEEVIL LARVA*)* SOLD WITHIN YENAGOA METROPOLIS

ABSTRACT

Microbial contamination of *Rhynchophorus phoenicis* (larva of the African palm weevil) was examined from street vendors. *R. phoenicis* larvae sold on the street were purchased from six vendors in the following location including Akenfa, Ekeki, Kpansia, Opolo, Swali and Etegwe in Yenagoa Metropolis, Bayelsa State, Nigeria. The pour plate method was used to assess the total viable count (TVC), Total colony count (TCC) and Total streptococcal count (TSC). Samples were homogenized before the plate counting method was applied at respective concentration ranging from 0.1ml-10^{-7}ml, however 10^{-}7ml concentration was used for viable colony counts and aseptically inoculated onto MacConkey agar, Nutrient agar and blood agar for identification of potential pathogens. Results revealed TVC ranging from 48.5 ± 5.4^{a} to $117.5\pm8.5^{b,}$ TCC ranging from 44.0 ± 5.1^{ac} to 70.0 ± 8.6^{ab} and TSC ranging from 44.8 ± 4.8^{a} to 60.0 ± 6.7^{a}Similarly bacteria identified in all samples based on morphological and biochemical characteristics were *Staphylococcus aureus, Staphylococcus cholermidis, Streptococcus* sp, *Escherichia coli, Salmonella* sp, *Enterobacter* spp, *Pseudomonas* spp, *Proteus* spp and *Klebsiellae* spp. Street-sold *R. phoenicis*, can be a source of food poisoning if not handled properly during, handling, processing and hawking.

Keywords: *Rhynchophorus phoenicis, Vendor,* Bayelsa State and Coliform

INTRODUCTION

The street food plays an important role in the nutritional requirements of urban dwellers in many cities of developing countries. Millions of people on a daily basis patronize different variety of street food including the larva *R. phoenicis* that are relatively cheap and accessible [11]. However, food-borne diseases associated with street food are a major public health problem [13]. Patronizer of such food are more interested in the fact that these foods are cheap and easily accessible,neglecting the safety, quality, and hygiene [8] Diarrhea diseases from street food occur due to improper use of good hygienic practices (GHPs) [5]. Vendors are often not educated, unlicensed, untrained in food handling and hygiene practices [9], many of them work under unsanitary conditions with no knowledge of the health implication associated with food-borne disease [2]. Unsanitary practices are common among vendors of larva of *R. phoenicis,* especially among children who hawk this delicacy. Children who hawk this delicacyusually leave the covers open and, in most cases, flies are seen around, hence the possibility food borne pathogens [14]. In Bayelsa State of Nigeria the roasted street vended *R. phoenicis* popularly known asBayelsa "suya" are in most cases exposed sources of contamination like dust and sand and this is due to the fact that the food is not properly covered and handled [4]. The improper application of holding temperatures, time, cleanliness, and sanitation in street food selling operations are additional risk factors linked to *R. phoenicis* contamination. According to information now available, *R. phoenicis* sold on the street has large concentrations of bacteria, including other diseases causing organisms [3].

Without proper protection, the street-sold African Palm Weevil is sold and carried from one location to another, exposing it to dust and other harmful environmental factors and, as a result, allowing harmful organisms to enter the *R. phoenicis* and cause food poisoning [1]. Estimating the number of microorganisms in food is routinely done to evaluate its microbiological quality or to confirm its presumed "safety"[6]. To determine the health risks of the food, this technique necessitates the collection of food samples, the performance of microbiological tests or analyses, and the evaluation of the results. In light of this, the

purpose of this study is to ascertain the level of bacteria present in the *R. phoenicis* consumed by the people of Bayelsa State.

MATERIALS AND METHODS

Study Area

This study was conducted in Yenagoa Metropolis, the capital of Bayelsa State, while the facilities of the Federal Medical Center were used for laboratory analysis.

Collection of Samples

The twelve (12) samples used for this study were *R. phoenicis* Samples were purchased by four different street vendors around Yenagoa Metropolis. Samples were placed in sterile ziplock polythene bags, appropriately labeled, and transported to the Federal Medical Center (FMC), Yenagoa microbiology laboratory for analysis within two hours of collection.

Preparation of sample

Portions of the *R. phoenicis* sample sold on the street were homogenized in a mortar and pestle using a sterile peptone broth diluent. An amount of 10 g of homogenate from each Rhynchophorus phoenicis sold on the street was aseptically removed with a sterile spoon and carefully transferred into a sterile test tube containing 90 ml of peptone medium, then the Homogeneous samples of samples were diluted to 10-5cfu/g and 10^{-6}cfu/g [15].

Enumeration of total viable count (TVC)

0.1 ml of each 10-fold dilution was transferred in duplicate onto a sterile petri dish using a micropipette for each dilution for the determination of total bacterial count. The diluted samples were inoculated on the petri dish and the already prepared nutrient agar was poured on the petri dish containing the inoculated sample using pour plate technique under aseptic condition usually close to a flame. The plates were kept in an incubator at 37°C for 24 hrs.

After incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in particular dilution was multiplied by the dilution to obtain the total viable count. The total viable count was counted using the Quebec colony counter (Reichert, USA) and expressed as colony forming unit per ml of sample homogenate (cfu/ml) [15].

Enumeration of total coliform count (TCC)

The sampling procedure, dilution and pour plate methods are similar to those for total live bacteria sampling. Only when coliform counts are counted, MacConkey agar is used. The calculation of the TCC is similar to the calculation of the total viable count [15].

Enumeration of total streptococci count (TSC)

The sampling, dilution, and inoculation procedures were similar to those performed for the total viable count. Only when streptococcus is counted, blood agar is used. The calculation of the TSC is similar to the calculation of the possible totals[15].

Identification of isolated bacteria

The method of [16] was used with minor modifications to isolate foodborne bacteria. Ten (5 g) of each food sample was homogenized using a sterile mortar and pestle, the resulting homogenate was aseptically added to 9 ml of prepared food broth. Direct plating of overnight broth culture was done aseptically on Eosin Methylene Blue, *Salmonella-Shigella*Agar,

chocolate agar, nutrient agar and Macconkey agar and incubated at 37°C for 24-48hrs. After incubation, the streaked plates were examined for colonies with different cultural characteristics and cultured in the respective medium. Pure colonies are obtained by subculture on nutrient agar. All putative isolates were further identified using standard biochemical methods [7]. The differential properties of these isolated strains were read as described in [10].

Statistical analysis

Results were analyzed using Microsoft Excel, 2016. One-way ANOVA was used to test for variation in microbial counts from different sites at a probability of 0.05%. The source of variations was monitored using the Bonferroni post-test.

Result and discussion

Microbial Enumeration

Samples of street vendor *R. phoenicis* from six locations in Yenagoa Metropolis yielded significant bacterial growth. The presence of these organisms in *R. phoenicis* plants may be due to *R. phoenicis* containing sufficient amounts of all the nutrients necessary for bacterial growth. When establishing colonies on plates containing *R. phoenicis* sold on the street, the number of colonies was counted with an electronic counter. The mean count and standard error of each duplicate plate were obtained using Microsoft Excel 2007 and expressed as mean log colony-forming units ± standard error per gram (mean log CFU ± SEM/g). The total colony count of *R. phoenicis* samples collected from different vendors are presented in Table 1.

Table 1: Total colony count of street vended *R. phoenicis* sold by vendors at Yenagoa metropolis

Sample site	TVC ($X10^7$cfu/g)	TCC ($X10^7$cfu/g)	TSC ($X10^7$cfu/g)
Akenfa	63.8 ± 6.3^{A}	52.5 ± 6.4^{A}	54.0 ± 5.4^{A}
Ekeki	68.5 ± 9.8^{A}	70.0 ± 8.6^{AB}	53.5 ± 8.8^{A}
Swali	68.3 ± 3.3^{A}	44.0 ± 5.1^{AC}	44.8 ± 4.8^{A}
Kpansia	73.3 ± 5.7^{A}	47.0 ± 2.5^{AB}	60.0 ± 6.7^{A}
Opolo	48.5 ± 5.4^{A}	52.5 ± 1.5^{AB}	48.5 ± 5.0^{A}
Etegwe	117.5 ± 8.5^{B}	60.5 ± 1.2^{AB}	59.5 ± 5.0^{A}

All values are mean ± standard error. Different superscripts in the same column are significantly different ($P\geq0.05$). TVC: total viable count, TCC: total coliform count, TSC: total streptococcal count.

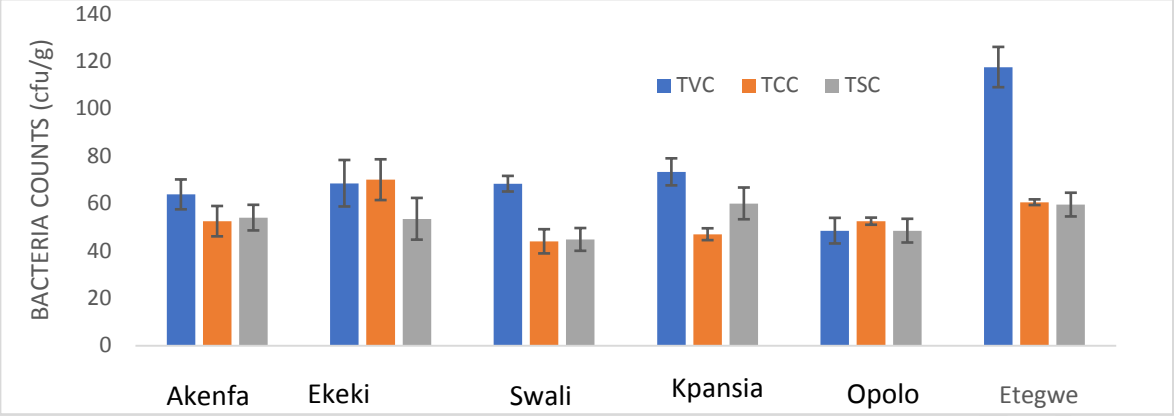

Figure 1. A graph showing the mean count of bacteria genera from different sampling sites

For the total viable count, the bacterial load was highest in the sample obtained from Tumbia sample site (117.5±8.5) followed by Kpansia (73.3±5.7), Ekeki (68.5±9.8), Swali (68.3±3.3), Akenfa (63.8±6.3) and then Opolo (48.5±5.4). However, the total viable counts at the different sample sites are significantly different at (P≥0.05) significant level. For the total coliform count, the coliform load was highest at Ekeki sampling site (70.0±8.6), followed by Etegwe (60.5±1.2), Akenfa and Opolo with the same mean value but different standard error value (52.5±6.4 and 52.5±1.5), Kpansia (47.0±2.5) and then Swali (44±5.1). However, the coliform counts at the different sampling sites are significantly different at (P≥0.05) significant level.

For the total streptococcal count, the load was highest at Kpansia sampling site (60.0±6.7), followed by Etegwe (59.5±5.0), Akenfa (54.0±5.4), Ekeki (53.5±8.8), Opolo (48.5±5.0) and then Swali (44.8±4.8). However, the total Streptococcal count at the different sample sites is not significantly different at (P≥0.05) significant level.

Isolation of bacteria from street vended *Rhynchophorus phoenicis*

Nine bacteria genera were isolated from the street vended *Rhynchophorus phoenicis* and their occurrence in different vended point is shown in table 2 below.

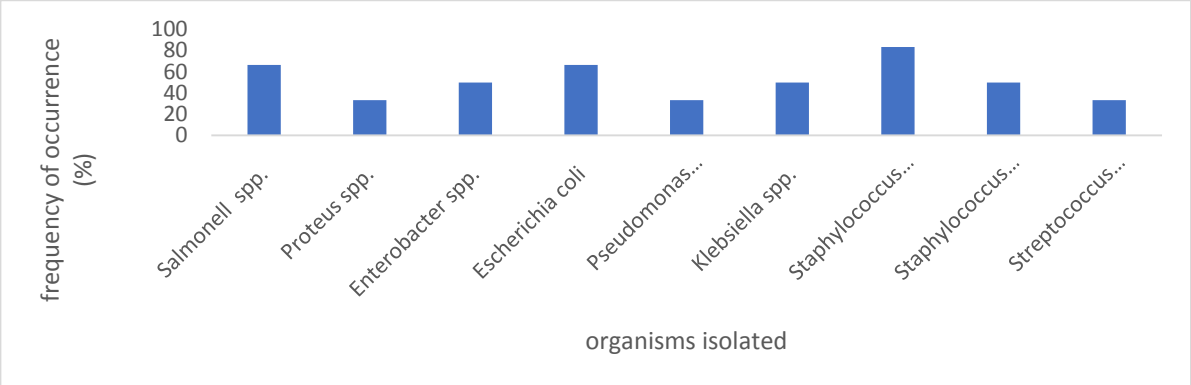

Figure 2: A graph of frequency of occurrence of the isolate in the sampled sites

The total number of findings recorded in this study indicates microbial diversity (difference in shape or species) in these sites. Contamination with these organisms in this study could be due to the condition of the street food tables, the equipment used during food preparation and

processing, and the sanitation practices of the food court. street vendors and handlers, identified variation in bacterial contamination. Although most of the organisms found in this study are normal flora of various parts of the human and animal body, some of them have been associated with a variety of disease problems. *Staphylococcus aureus* has the highest percentage of occurrence and *Proteus and Streptococcus* has the lowest percentage of occurrence, similar studies by [3] also isolated *Staphylococcus aureus* and *Proteus* species from the weevil. *Staphylococcus aureus* cause food intoxication, when allowed to incubate in certain foods and produce heat stable enterotoxins that renders food dangerous even though it appears normal [12].

Conclusion and Recommendation

R. phoenicis sold on the street is a popular street food in the state of Yenagoa Bayelsa. All ages, all walks of life eat pho sold on the street. It was served quickly, was also delicious and reasonably priced. The majority of street centres are located next to waste disposal sites and dusty roads or streets with heavy traffic of people and vehicles. From the results of this survey, it can be concluded that; First, food served in vending operations is not relatively safe to consume, and *R. phoenicis* sold on the street contains foodborne bacteria that can cause infections and food poisoning. Second, the presence of specific microorganisms in these foods and on surfaces, even when detected in low quantities, indicates the need for improved infrastructure, especially regarding the provision of adequate sanitation facilities. Third, the situation of selling *R. phoenicis* on the street in Yenagoa municipality is happening very blindly. Street vendors have little or no knowledge of food hygiene practices and do not pay due attention to personal hygiene and properly cover up the Mangrove trees sold on the street during their self-sale. motion. Finally, the bacteria from the food contained in this street food are resistant to antibiotics and cannot be treated with these antibiotics.

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