Scientific Research Publishing

Advances in Microbiology, 2026, 16(7), 288-309
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Hygiene and Toxi-Infectious Risks Associated with *Tchakpalo*, a Traditional Beninese Beer

d'Avila Dognon[1], Agossou Damien Pacôme Noumavo[1,2*], Sènan Vodouhe[3], Lucile Adjovi[1], Balbine Gbaguidi[3], Ricardos Aguegue[2], Kouakou Romaric Kouakou[4], Philippe Sessou[5], Farid Baba-Moussa[1]

[1]Laboratory of Microbiology, Food Technology and Phytopathology, Faculty of Science and Technology, University of Abomey-Calavi, Abomey-Calavi, Benin
[2]Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Science and Technology, University of Abomey-Calavi, Abomey-Calavi, Benin
[3]Laboratory of Applied Genetics, Biotechnology and Botany (GEBBA), National Higher School of Biosciences and Applied Biotechnologies, National University of Sciences, Technologies, Engineering and Mathematics, Benin
[4]National Center for Agronomic Research, Research Station of Ferkessédougou, Ferkessédougou, Ivory Coast
[5]Communicable Disease Research Unit, Applied Research Laboratory, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Abomey-Calavi, Benin
Email: *pacome.noumavo@gmail.com

How to cite this paper: Dognon, A., Noumavo, A.D.P., Vodouhe, S., Adjovi, L., Gbaguidi, B., Aguegue, R., Kouakou, K.R., Sessou, P. and Baba-Moussa, F. (2026) Hygiene and Toxi-Infectious Risks Associated with *Tchakpalo*, a Traditional Beninese Beer. *Advances in Microbiology*, **16**, 288-309.
https://doi.org/10.4236/aim.2026.167016

Received: June 17, 2026
Accepted: July 18, 2026
Published: July 21, 2026

Abstract

Tchakpalo is a traditional Beninese beer produced through the spontaneous fermentation of cereals, primarily maize and sorghum. Despite its socio-economic, cultural, and nutritional importance, the conditions under which it is produced and marketed raise concerns regarding its food safety. The present study aimed to assess the microbiological quality of *Tchakpalo* sold in Grand Nokoué (Benin) and to evaluate the level of Good Hygiene Practices (GHP) adoption by producers. Fifty samples of *Tchakpalo* were therefore collected in five municipalities of Grand Nokoué (Cotonou, Porto-Novo, Abomey-Calavi, Sèmè-Podji, and Ouidah). Microbiological analyses focused on Total Mesophilic Aerobic Flora (TMAF), Enterobacteria, *E. coli*, *S. aureus*, and molds. Additionally, the adoption level of GHP was assessed through direct observation of sixty-one *Tchakpalo* producers. The results revealed widespread microbiological contamination of *Tchakpalo*. The prevalence of TMAF and Enterobacteria was 100%, while those of molds, *E. coli*, and *S. aureus* were 80%, 62%, and 52%, respectively. The average microbial loads were 6.57×10^6 CFU/mL for TMAF, 5.21×10^3 CFU/mL for Enterobacteria, 3.44×10^3 CFU/mL for *E. coli*, 0.75×10^3 CFU/mL for *S. aureus*, and 2.51×10^2 CFU/mL for molds. Interpretation of these results considering regulatory guidelines showed that a significant proportion of the samples exhibited poor to unsatisfactory microbiological quality. The assessment of GHPs revealed serious deficiencies at all levels of the production and marketing chain, notably the lack of adequate handwashing, failure to wear protective equipment, the use of untreated water, improper storage of raw materials and the finished product, as well as the lack of training and health monitoring for producers. These results demonstrate that the *Tchakpalo* sold in the Grand Nokoué area presents a concerning level of sanitary quality, strongly associated with the low level of adoption of GHP. Training initiatives, health supervision, and process standardization appear essential to reduce the risks of foodborne illnesses linked to the consumption of this emblematic traditional Beninese beverage.

Keywords

Tchakpalo, Traditional Fermented Beverage, Food Hygiene, Microbiological Quality, Food Safety, Benin

1. Introduction

Traditional beverages are drinks developed by the populations of a specific geographic region using local raw materials, whether cereal-based or not [1]. These beverages have an important place in global food systems due to their availability, unique sensory attributes, nutritional and socio-cultural value, affordability, and ease of production. They are consumed almost daily in Africa by both children and adults and can be fermented or not. Traditional fermented beverages generally result from spontaneous fermentation processes of carbohydrate-rich substrates (particularly cereals), driven by complex microbial communities composed mainly of lactic acid bacteria and yeasts [2]. Although these empirical fermentation practices significantly contribute to improving the organoleptic and nutritional characteristics of these beverages, they can also be a source of microbiological contamination when production conditions are not controlled [3]. Consequently, consuming these indigenous beverages could pose a risk of foodborne illness to consumers. It has been reported that more than two (2) million people worldwide, primarily children and the elderly, die each year from illnesses caused by the consumption of contaminated food and beverages [4].

In Sub-Saharan Africa, the production of traditional fermented beverages is often carried out in environments characterized by weak health oversight, limited adherence to good hygiene and manufacturing practices, and a lack of standardized processes. This is the case with *Tchakpalo*, a traditional fermented beverage made from corn and/or sorghum, produced in Benin and highly appreciated by the local population. As previously demonstrated by Dognon *et al.* [5], the production of *Tchakpalo* in the Grand Nokoué region relies on empirical and heterogeneous processes, involving in particular the use of rudimentary equipment, insufficiently controlled raw materials, and techniques likely to introduce or maintain undesirable microbial flora. Furthermore, *Tchakpalo* producers do not belong to any structured professional organization. This absence of institutional oversight and technical training results in production and marketing practices that fall far short of food industry standards. Consequently, questions arise regarding the health implications associated with these production and distribution conditions. Therefore, assessing the microbiological quality of *Tchakpalo* is an essential and indispensable step in evaluating the risks associated with its consumption.

It is within this context that the present study was conducted, with the aim of: (i) assessing the microbiological compliance of *Tchakpalo* samples sold in Grand Nokoué (Benin), and (ii) evaluating the adoption level of good hygiene practices byproducers during the production and sale of *Tchakpalo*.

2. Materials and Methods

2.1. Study Area

This study was carried out in the five (5) largest urban municipalities in southern Benin that make up the Grand Nokoué area, consisting of: the economic capital, Cotonou; the administrative capital, Porto-Novo; and three residential municipalities: Abomey-Calavi, Sèmè-Podji, and Ouidah [6]. The choice of these municipalities is justified, among other reasons, by the limited documentation on the *Tchakpalo* production sector in these areas. The few existing studies are concentrated in Central Benin, the region of origin for this traditional beer. However, it is now consumed nationwide, particularly in major cities where it is experiencing considerable growth [7].

2.2. Sampling

Fifty (50) samples of *Tchakpalo* were collected at random from vendors of the beverage (one sample per vendor), at a rate of ten (10) samples per municipality. The samples, collected in sterile zip-top bags under the usual conditions of sale and consumption of the beverage, were transported to the laboratory in a pre-disinfected cooler and kept at 4˚C using dry ice (**Figure 1**).

(a) (b)

Figure 1. Sample collection of *Tchakpalo*. (a): Sample collected; (b): Packaging for transport.

2.3. Microbiological Analysis

Since *Tchakpalo* is a liquid product, it was directly used as the stock solution. Decimal dilutions were prepared under aseptic conditions according to the method described by Speck [8]. One milliliter of the stock solution was added to 9 mL of saline to prepare the 10^{-1} dilution. One milliliter of the 10^{-1} dilution was then withdrawn and transferred to a second tube containing 9 mL of physiological saline to obtain the 10^{-2} dilution. The procedure was repeated until all necessary decimal dilutions were obtained. Details regarding the microorganisms identified in *Tchakpalo* are presented in **Table 1**. Although the isolation of the various microbial genera, notably *E. coli* and *S. aureus*, was performed on highly reliable, specific isolation media, several routine confirmatory tests were conducted. These included Gram staining, catalase, indole, and staphylococcal coagulase tests.

Table 1. Microorganisms and culture conditions.

Microorganism	Reference	Culture medium	Culture Conditions	Colony counted
Total Mesophilic Aerobic Flora	ISO4833-2 [9]	Plate Count Agar	30˚C 72 h	All microbial colonies.
Enterobacteria	ISO21528-1 [10]	Eosin-Methylene Blue Agar	37˚C 24 h	Dark purple colonies, bluish colonies, brownish colonies, amber colonies.
Escherichia coli	ISO16649-2 [11]	Tryptone-Bile-Glucuronate Agar	44˚C 24 h	β-glucuronidase-positive colonies (blue to blue-green).
Staphylococcus aureus	ISO6888-1 [12]	Baird-Parker Agar (potassium telluride + egg)	37˚C 24 h	Black colonies with a pale halo.
Molds	ISO21527-2 [13]	Sabouraud Agar with Chloramphenicol	25˚C 5 days	All mold colonies.

ISO: International Organization for Standardization.

2.4. Criteria for Interpreting Microbiological Results

The interpretation of the microbiological analysis results was carried out in accordance with the recommendations of Food Standards Australia New Zealand (FSANZ) and the Quebec Ministry of Agriculture, Fisheries and Food (MAPAQ). A three-class system (Satisfactory, Marginal, and Unsatisfactory) was used to interpret these results (**Table 2**).

2.5. Assessment of the Adoption Level of Good Hygiene Practices by Producers in *Tchakpalo*

The assessment of the adoption level of good hygiene practices by female producers in *Tchakpalo* was conducted using direct observation during the production and marketing monitoring phase, involving sixty-one (61) producers (09 in Ouidah, 16 in Abomey-Calavi, 07 in Cotonou, 08 in Sèmè-Podji, and 21 in Porto-Novo). They were selected based on their availability and, above all, their willingness to allow us to monitor production with them (signed consent form). The information collected focused on personal hygiene, raw materials and the final product, production and sales equipment, the production and sales environment, and finally, training and health monitoring. In total, twenty-nine (29) hygiene criteria were included in this assessment.

Table 2. Criteria for interpreting microbiological results.

Microorganism	Microbiological criteria			References
	Satisfactory	Marginal	Unsatisfactory	
Total Mesophilic Aerobic Flora	$<10^5$	$[10^5; 10^6]$	$>10^6$	[14]
Enterobacteria	$<10^2$	$[10^2; 10^4]$	$>10^4$	[15]
Escherichia coli	<3	$[3; 10^2[$	$\geq 10^2$	[15]
Staphylococcus aureus	$<10^2$	$[10^2; 10^3[$	$\geq 10^3$	[15]
Molds	$<10^2$	$[10^2; 10^3]$	$>10^3$	[14]

TAMF: Total Mesophilic Aerobic Flora; ***E.*:** Escherichia; ***S.*:** Staphylococcus. **CFU:** Colony-Forming Unit; **FSANZ:** Food Standards Australia New Zealand; **MAPAQ**: Quebec Ministry of Agriculture, Fisheries, and Food.

2.6. Data Processing and Statistical Analysis

Data processing and statistical analyses were performed using R software (RStudio 2024.04.1 + 748; RStudio Team, Boston, MA, USA, 2020). Descriptive statistics (mean, standard error, and proportions) were used to summarize the data. Microbial counts (CFU/mL) were log10-transformed prior to statistical analyses to improve normality and homogeneity of variances. Differences in microbial loads among municipalities were assessed using one-way analysis of variance (ANOVA). When significant effects were detected, means were separated using the Student-Newman-Keuls (SNK) post-hoc test. Differences in the prevalence of microorganisms between municipalities were evaluated using the chi-square test. Statistical significance was set at $p < 0.05$. The significance levels were interpreted as follows: $p < 0.05$ (significant), $p < 0.01$ (highly significant), and $p < 0.001$ (very highly significant).

3. Results

3.1. Prevalence of Potentially Pathogenic Microorganisms in *Tchakpalo*

Table 3 presents the prevalence of certain microorganisms with potential toxin-producing capabilities in *Tchakpalo* samples collected from various municipalities in Grand Nokoué. There is a total prevalence (100%) of Total Mesophilic Aerobic Flora (TMAF) and Enterobacteria, followed in descending order by molds, *E. coli*, and finally *S. aureus*.

The prevalence of *E. coli* varies significantly from one municipality to another ($p < 0.001$), with proportions ranging from 0% (Sèmè-Podji) to 100% (Cotonou), with an overall prevalence of 62%. This variability indicates a heterogeneous distribution of fecal contamination across municipalities. In contrast, the overall prevalence of *S. aureus* (52%) and molds (80%) does not vary significantly between municipalities ($p > 0.05$). This lack of significance suggests relatively homogeneous contamination by these microorganisms across the Grand Nokoué region. These results highlight widespread contamination of *Tchakpalo* sold in the municipalities of Grand Nokoué. They reveal systemic deficiencies in hygiene and control of production processes, as shown in Figure 2 and Figure 3. It can be noticed that the processors work on the floor in direct contact with soil, not in an appropriate production room with soil revetment like tile.

Table 3. Prevalence (%) of potentially pathogenic microorganisms in *Tchakpalo.*

Municipalities	TAMF	Enterobacteria	*E. coli*	*S. aureus*	Molds
Ouidah (n = 10)	100	100	80	50	80
Abomey-Calavi (n = 10)	100	100	40	60	90
Cotonou (n = 10)	100	100	100	60	90
Sèmè-Podji (n = 10)	100	100	0	40	70
Porto-Novo (n = 10)	100	100	90	50	70
P-value	nd	nd	***	°	°
Grand Nokoué (n = 50)	100	100	62	52	80

TAMF: Total Aerobic Mesophilic Flora; ***E.***: Escherichia; ***S.***: Staphylococcus. The chi-square test was used to assess whether the difference in prevalence was statistically significant between municipalities, with a significance threshold set at $p < 0.05$. ˚ = $p > 0.05$ (not significant); = $p < 0.05$ (significant); = $p < 0.01$ (highly significant); = $p < 0.001$ (very highly significant); **nd**: not determined.

Figure 2. *Tchakpalo* production environment at a producer's site (Municipality of Abomey-Calavi, Benin).

Figure 3. The sales practice of *Tchakpalo* ((Municipality of Ouidah, Benin)).

3.2. Abundance of Microorganisms in the *Tchakpalo*

The load of TAMF, Enterobacteria, *E. coli*, *S. aureus*, and molds in the *Tchakpalo* are presented in Table 4. This table reveals high levels of contamination for all microorganisms studied. TAMF has an overall average of 6.57×10^6 CFU/mL, with highly significant differences between municipalities ($p < 0.001$). The highest concentrations were observed in Porto-Novo (20.44×10^6 CFU/mL), while the lowest were recorded in Sèmè-Podji (0.10×10^6 CFU/mL). Enterobacteria showed an average concentration of 5.21×10^3 CFU/mL, also characterized by highly significant differences ($p < 0.001$), with a maximum in Abomey-Calavi (15.03×10^3 CFU/mL) and a minimum in Sèmè-Podji (0.32×10^3 CFU/mL). Microbial loads of *E. coli* follow a similar trend, with an overall average of 3.44×10^3 CFU/mL and highly significant variability among municipalities ($p < 0.001$). The absence of this bacterium in Sèmè-Podji (0 CFU/mL) contrasts with high values in Abomey-Calavi (9.70×10^3 CFU/mL). Regarding *S. aureus*, the average concentration is 0.75 $\times 10^3$ CFU/mL, with highly significant differences ($p < 0.01$).

The lowest values were observed in Sèmè-Podji. Finally, mold had an average concentration of 2.51×10^2 CFU/mL, with significant differences ($p < 0.05$). *Tchakpalo* from Porto-Novo had higher mold counts. In contrast, samples from Sèmè-Podji recorded low microbial loads. Overall, the results show that *Tchakpalo* sold in the municipalities of Grand Nokoué has high microbial loads of potentially pathogenic microorganisms. There is also significant inter-municipal variability for all microbiological parameters evaluated.

Table 4. Abundance of *Tchakpalo* in mesophilic and toxin-producing flora.

Microorganisms		Ouidah (n = 10)	Abomey-Calavi (n = 10)	Cotonou (n = 10)	Sèmè-Podji (n = 10)	Porto-Novo (n = 10)	*p-value*	Grand Nokoué (n = 50)	Microbiological criteria			References
									S	M	U	
TAMF ($\times 10^6$ UFC/mL)	$\bar{x}$	0.35^{b}	10.21^{b}	1.84^{b}	0.10^{b}	20.44^{a}	***	6.57	$< 10^5$	[10^5; 10^6]	$>10^6$	[14]
	$\sigma_{\bar{x}}$	0.80	1.13	0.21	0.07	10.52		8.80				
Enterobacteria ($\times 10^3$ CFU/mL)	$\bar{x}$	2.23^{b}	15.03^{a}	2.366^{b}	0.32^{b}	6.13^{b}	***	5.21	$< 10^2$	[10^2; 10^4]	$>10^4$	[15]
	$\sigma_{\bar{x}}$	0.61	5.40	1.45	0.19	3.45		5.87				
E. coli ($\times 10^3$ UFC/mL)	$\bar{x}$	1.60^{b}	9.70^{a}	1.80^{b}	0.00^{b}	4.13^{b}	***	3.44	< 3	[3; 10^2[	$\geq 10^2$	[15]
	$\sigma_{\bar{x}}$	2.08	1.27	1.75	0.00	2.54		3.79				
S. aureus ($\times 10^3$ UFC/mL)	$\bar{x}$	1.03^{a}	0.57^{a}	1.29^{a}	0.28^{b}	1.10^{b}	**	0.75	$< 10^2$	[10^2; 10^3[	$\geq 10^3$	[15]
	$\sigma_{\bar{x}}$	0.29	0.04	0.53	0.25	0.20		0.54				
Molds ($\times 10^2$ CFU/mL)	$\bar{x}$	2.27^{a}	1.76^{a}	3.66^{a}	0.83^{b}	4.05^{a}	*	2.51	$< 10^2$	[10^2; 10^3]	$>10^3$	[14]
	$\sigma_{\bar{x}}$	1.17	1.67	1.00	0.27	1.43		1.33				

TAMF: Total Aerobic Mesophilic Flora; ***E.***: Escherichia; ***S.***: Staphylococcus. **CFU**: Colony-Forming Units; $\bar{x}$: Mean; $\sigma_{\bar{x}}$: Standard Error. On the same row, means marked with different letters are significantly different at the 5% level according to the Student-Newman-Keuls test. ˚ = p > 0.05 (not significant); = p < 0.05 (significant); = p < 0.01 (highly significant); = p < 0.001 (very highly significant); **FSANZ**: Food Standards Australia New Zealand; **MAPAQ**: Quebec Ministry of Agriculture, Fisheries, and Food.

3.3. Global Microbiological Quality of the *Tchakpalo* Samples Analyzed

The evaluation of the microbiological quality of the *Tchakpalo* samples (Figure 4), based on the interpretation criteria recommended by FSANZ (Food Standards Australia New Zealand) and MAPAQ (Quebec Ministry of Agriculture, Fisheries, and Food), reveals a generally concerning situation. In fact, the majority of the samples analyzed fall into the mediocre to unsatisfactory categories, indicating insufficient sanitary quality of the product sold throughout the municipalities of Grand Nokoué. TAMF levels far exceed compliance thresholds, classifying the majority of samples as unsatisfactory (52%). Only 22% of samples had a relatively acceptable TAMF microbial load. No sample from *Tchakpalo* had a satisfactory Enterobacteria load. The majority (78%) were of mediocre quality. For *E. coli*, 82% of the samples analyzed exceeded acceptable limits, indicating significant fecal and environmental contamination.

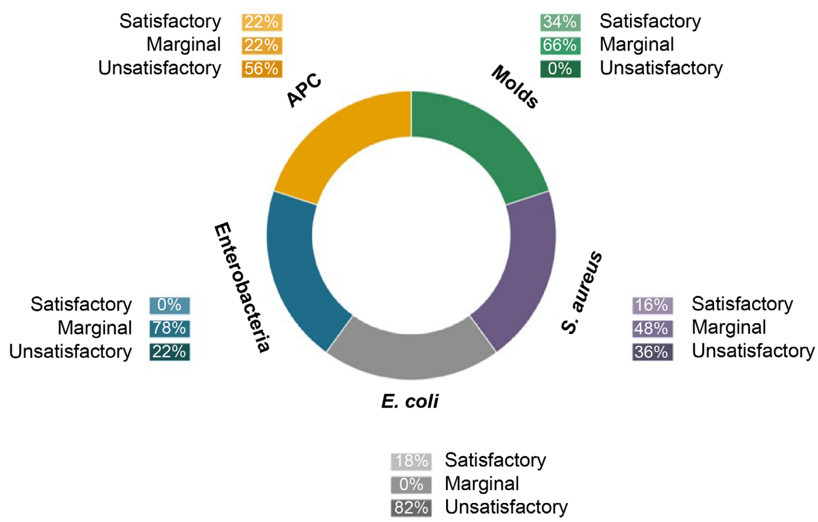

Figure 4. Microbiological compliance of the *Tchakpalo* samples analyzed.

3.4. Adoption Level of Good Hygiene Practices by *Tchakpalo* Producers

Table 5 presents the assessment results regarding the adoption level of Good Hygiene Practices (GHP) by *Tchakpalo* producers included in this study. Direct observations reveal a generally very low level of GHP adoption, regardless of the hygiene component considered. Even basic hygiene requirements are not met throughout the production and sales chain.

In terms of personal hygiene, no producer washes her hands properly. There is also a complete lack of use of personal protective equipment. Furthermore, direct handling of the product with bare hands is systematic (100%) with no usage of gloves, as is the concurrent use of banknotes, coins, and phones during production and sales (100%) were observed. Furthermore, only 29.51% of producers demonstrate satisfactory personal hygiene, while 73.77% engage in risky behaviors during

Table 5. Hygiene practices profiles of *Tchakpalo* producers (n = 61).

	Hygiene criteria	Observations	
		No (%)	**Yes (%)**
Personal hygiene	Proper hand washing (before, during, and after production)	00	100
	Use of protective equipment (hairnet, apron, gloves, etc.).	00	100
	General personal hygiene (clothing, nails, bracelets, watches, necklaces, etc.)	129,51	70,49
	Risky behaviors (coughing, blowing one's nose, eating, drinking, smoking, etc.)	26,23	73,77
	Handling with bare hands	100	00
	Use of cash, coins, and cell phones during production/sales	100	00
Raw materials and finished ducts	Use of tap water	70,49	29,51
	Water treatment (heating, chemicals, etc.)	00	100
	Proper handling of grains (sorting, washing, etc.)	70,49	29,51
	Raw materials and finished products are stored together.	44,26	55,74
	Storage on the floor	100	00
	Storage at room temperature for several hours	100	00
Production and sales equipment	Use of production and sales equipment for household purposes	93,44	06,56
	Washing equipment before and after each use	100	00
	Use of potable water and detergent for washing equipment	49,18	50,82
	Proper washing of equipment used to serve customers (gourds, bowls, cups, bottles, etc.) after each customer/use.	09,80	91,20
	Protection of clean equipment from dust, insects, etc.	20	80
	Equipment or containers made of stainless steel	00	100
Production and sales environment	Protection against pests (insects, rodents, etc.).	00	100
	Protection against dust, smoke, exhaust fumes, etc.	00	100
	Proximity to latrines, dumps, or sewage	08,20	91,80
	Regular waste removal (garbage, wastewater, etc.) from production and sales sites	29,50	70,50
	Presence of animals (dogs, cats, poultry, etc.) at production and sales sites	08,20	91,80
Health training and monitoring	Training (formal or informal) in food hygiene	00	100
	Annual medical examination	00	100
	Sanitary inspection	00	100
	Voluntary exclusion in cases of illness	32,79	67,21
	Presence of visible lesions on the body (hand, arm, etc.).	131,15	68,85
	Good general health at the time of production/sale	91,20	09,80

the production and sale of *Tchakpalo*. These practices indicate significant exposure of the product to human-derived contamination. Regarding raw materials and finished products, 70.49% of producers use tap water. However, none of them treat the water before use. Raw materials and finished products are stored under inadequate conditions (stored directly on the floor, 100%; prolonged exposure to room temperature, 100%). Furthermore, 44.26% of producers store raw materials and finished products in the same environment, thereby increasing the risk of cross-contamination.

An assessment of the management of production and sales equipment shows that no stainless steel or similar materials are used. Much of the production and sales equipment is also used for domestic purposes (93.44%). Systematic washing of equipment is reported (100%), but it is often performed without ensuring the use of potable water or appropriate detergents (50.82%). Even more concerning, only 9.80% of producers properly wash the utensils used for service after each customer, indicating a high potential for cross-contamination. Environmental conditions are also a cause for concern. No effective protective measures against insects, rodents, dust, smoke, and exhaust fumes were observed. At several production and sales sites, the presence of waste (70.50%) and domestic animals (8.20%) was noted.

Finally, the results regarding training and health monitoring indicate that no female producer has received training in food hygiene, undergoes an annual medical examination, or is subject to regular health inspections. Although 91.20% of the producers appeared to be in good health at the time of the assisted production sessions, only 32.79% reported voluntarily suspending their activities or entrusting them to someone else when they are sick. Overall, these results reveal very low compliance with good hygiene practices in the *Tchakpalo* sector in the Grand Nokoué area, with major shortcomings at all levels of the production and marketing chain.

4. Discussion

The results obtained in this study highlight widespread microbiological contamination in the *Tchakpalo* samples analyzed in the study area. These results confirm that *Tchakpalo* sold in the Grand Nokoué exhibits significant microbiological contamination, consistent with several previous studies on traditional fermented beverages. In Benin, a recent study by Dognon *et al.* [5] highlighted the variability of *Tchakpalo* production processes and their empirical nature. This situation constitutes a major microbiological risk factor. Similarly, Konfo *et al.* [7] reported that cereal-based fermented beverages in Benin frequently exhibit high levels of hygiene indicator microorganisms due to poorly controlled processing conditions. [16] attributed this high contamination to inadequate hygiene conditions during the production, storage, and sale of fermented beverages. The consistent presence of Enterobacteria and the high prevalence of *E. coli* observed in this study corroborate the findings of [4]. These authors demonstrated that traditional fermented beverages in sub-Saharan Africa are often exposed to fecal contamination. Indeed, Enterobacteria serve as an indicator of fecal or environmental contamination, often linked to the use of contaminated water or inadequate hygiene practices [17] [18]. The detection of *E. coli* in food is one of the most widely used hygiene criteria in the food industry [19]. *E. coli* is a key indicator of fecal contamination in food and beverages [20]. It has also been detected in traditional fermented beverages in Ghana, notably in *Emuduro*, a traditional beer characteristic of the Hausa people [21]. These risks are common in artisanal systems for the production of fermented beverages, particularly in West Africa, where health oversight remains limited [22].

In fact, in the municipalities of Grand Nokoué, *Tchakpalo* is produced in homes under unsanitary conditions that expose the production process (raw materials, equipment, water, intermediate products, finished products, etc.) to various types of contamination, particularly fecal and environmental contamination. Furthermore, the sales environment and the way *Tchakpalo* is sold pose a major risk of contamination. The sales environment (primarily along roadsides) exposes the beverage to contamination from air, dust, exhaust fumes, etc. Food placed close to the ground and exposed to dust has been linked to foodborne illnesses such as cholera and diarrheal diseases [23]. Several foodborne outbreaks have been linked to poor practices and poor hygiene among food handlers [24]. These observations are also consistent with recent studies by Paez-Lerma *et al.* [25] and [20] on traditional fermented beverages.

E. coli is a commensal bacterium found in the intestinal tract of mammals. Certain pathogenic strains of *E. coli* have been linked to several diseases in animals and humans [26]. The presence of *E. coli* in ready-to-eat food is also an indicator of the potential presence of enteric pathogens in that food [27]. Thus, the presence of *E. coli* in food makes it unsafe for human consumption. *E. coli* should not be detected in ready-to-eat food, even if a tolerance level is permitted. The U.S. Centers for Disease Control and Prevention (CDC) has estimated that *E. coli* O157:H7 infections cause 73,000 illnesses, 2,200 hospitalizations, and 60 deaths per year in the United States [28]. In addition to foodborne illnesses, *E. coli* strains can cause serious diseases such as diarrhea, dysentery, peritonitis, mastitis, sepsis, pneumonia, meningitis, hemorrhagic colitis, hemolytic uremic syndrome, and death [29]. In developed countries, *E. coli* is the most common pathogen associated with childhood diarrhea and is an emerging antimicrobial-resistant enteropathogen [30]. Based on their specific virulence properties, serotypes, and various epidemiological and clinical characteristics, seven distinct groups of diarrheagenic *E. coli* are recognized, namely: enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), diffuse-adhering *E. coli* (DAEC), and Shiga toxin-producing *E. coli* (STEC) [19].

With regard to *S. aureus*, its prevalence and abundance are closely linked to the rudimentary and manual nature of *Tchakpalo* production processes. Indeed, *S. aureus* is part of the normal flora of the skin and nasal passages in 20 to 50% of healthy individuals [31]. People working in the food sector therefore constitute a significant source of contamination. *S. aureus* is thus a good indicator of skin and mucosal contamination. This ecological specificity explains its constant presence in artisanal food products [18]. In Ethiopia, Erkihun *et al.* [32] showed that a significant proportion of food handlers (20%) were carriers of *S. aureus*. In Ivory Coast, Attien *et al.* [33] reported the frequent presence of *S. aureus* in street-sold beverages, including those sold hot, when hygiene conditions are inadequate. These results support the hypothesis that *S. aureus* contamination of *Tchakpalo* is primarily of human origin. Practices such as inadequate handwashing, direct handling of the product with bare hands, etc., may explain the high levels of *S. aureus* in *Tchakpalo* from Grand Nokoué. Furthermore, it should be noted that the presence of *S. aureus* in food poses a particular risk when the strains are enterotoxigenic. Indeed, staphylococcal foodborne illness (SFI) is primarily caused by a specific group of toxins known as enterotoxins [34]. Staphylococcal enterotoxins (SETs) are heat-stable and can cause food poisoning even after the product has been heat-treated. To date, more than 30 different SETs have been described, and SEA, SEB, SEC, SED, and SEE are the most commonly implicated in foodborne toxi-infections [31]. These heat-stable toxins can resist proteolytic enzymes (pepsin and trypsin). This property allows them to retain their biological activity even after cooking or passage through the gastrointestinal tract. According to the World Health Organization, STIs are among the leading causes of foodborne outbreaks worldwide. ASF is characterized by a sudden onset of symptoms (1 to 6 hours after ingestion), including vomiting, abdominal pain, and stomach cramps [35]. FDI can lead to hospitalizations, particularly among the YEPI (Young, Elderly, Pregnant women, and Immunocompromised Individuals) [36]. Thus, the prevalence and abundance of *S. aureus* observed in samples from *Tchakpalo* in the Grand Nokoué region suggest a potential public health risk, particularly if production and marketing conditions are not improved.

The fungal flora of interest in this study is molds. Although certain molds may be involved in the development of aromas in fermented food products, the majority of them are part of the spoilage flora, particularly in beverages. In *Tchakpalo* produced in the municipalities of Grand Nokoué, toxin-producing molds (Fusarium spp., Aspergillus spp., and Penicillium spp.) were present, with an average microbial load of 2.51×10^2 CFU/mL. The presence of these molds may be due to poor hygiene [37]. They may also originate from the grains used in *Tchakpalo* production. Indeed, [38] demonstrated that the grains used in traditional fermentations are frequently contaminated by potentially mycotoxin-producing fungal species, particularly those belonging to the genera Aspergillus and Penicillium. The high prevalence and large number of molds in *Tchakpalo* pose a serious public health problem, as these fungal genera are highly toxinogenic. Their pathogenicity is closely linked to the production of a group of toxins known as mycotoxins. Mycotoxins are secondary metabolites produced by various species of mold. Mycotoxin production is primarily associated with the genera Aspergillus, Penicillium, Fusarium, Claviceps, and Alternaria, and occurs under specific conditions [39]. There are more than 500 mycotoxins that are toxic and harmful to human, animal, and plant health. The most common and best-studied mycotoxins are those that pose the greatest risks to human health. These include aflatoxins, ochratoxins, fumonisins, zearalenone, trichothecenes, patulin, and citrinin [40]. Prolonged dietary exposure to these has been linked to cancers and diseases of the kidneys, liver, and immune system. Unfortunately, there is no single solution in the fight against mycotoxins. It is therefore important to take all necessary measures to reduce and control mycotoxin levels in food products. Molds can produce pectinases, which are degrading enzymes with a high potential to alter the taste, odor, and appearance of beverages. In light of the foregoing, it can be unequivocally concluded that the consumption of *Tchakpalo* in the municipalities of Grand Nokoué exposes consumers to serious risks of foodborne illness. However, these findings contrast with certain studies conducted on other African fermented beverages. For example, Chelule *et al.* [41] showed that in Mahewu (a South African fermented beverage), enterobacteria gradually disappear during fermentation due to the acidification of the medium. Similarly, Holzapfel [42] demonstrated that lactic fermentation, by lowering the pH and producing bacteriocins, constitutes an effective barrier against pathogens. The persistence of high microbial loads in *Tchakpalo* therefore suggests: insufficient acidification of the medium, post-fermentation recontamination, or limited dominance of lactic acid bacteria. From a public health perspective, the results confirm that traditional fermented beverages have a dual nature. As Tamang *et al.* [43] point out, they can have beneficial effects (nutritional value, improved digestibility, supply of functional microorganisms, etc.), but they can also serve as vectors for pathogens when hygiene conditions are insufficient.

The very low level of BPH adoption observed in this study is a key factor contributing to the high levels of microbiological contamination previously identified in *Tchakpalo*. The hygiene deficiencies noted largely account for the high prevalence of enterobacteria, *E. coli*, *S. aureus*, and molds in the *Tchakpalo* samples analyzed. Indeed, environmental conditions are often identified as major factors contributing to the deterioration of the sanitary quality of commercially available traditional fermented beverages, particularly in West Africa. Similar observations were reported by Tankoano *et al.* [44] regarding Dolo, a traditional fermented beverage from Burkina Faso. In that study, more than half of the Dolo production facilities visited exhibited major deficiencies regarding handler hygiene, water quality, and protection of products against environmental contamination. The authors established a significant relationship between these poor practices and the high levels of thermotolerant coliforms observed in Dolo. Irregularities in handwashing procedures and the lack of personal protective equipment constitute major hygiene failures. In fact, handwashing generally amounts to little more than rinsing the hands. Very few female producers wash their hands properly with soap before and during production or after handling a contaminated object. Similar results were obtained by Byakika *et al.* [45] during a study on the influence of food processors' knowledge, attitudes, and practices on the microbiological quality of Obushera, a traditional fermented sorghum-based beverage in Uganda. These authors highlighted low levels of adherence to good hygiene practices among

Obushera vendors, including a lack of systematic handwashing, failure to wear personal protective equipment, and constant exposure of Obushera to the external environment. These authors also reported high levels of contamination by coliforms and staphylococci, confirming the influence of hygiene practices on the product's microbiological quality. In fact, the hands of food handlers are recognized as one of the main sources of food contamination. Poor handling practices facilitate the transfer of pathogenic microorganisms from the skin, mucous membranes, or the environment to ready-to-eat foods. Handling *Tchakpalo* simultaneously with banknotes, coins, cell phones, and other objects likely to carry contaminants during sales also constitutes a significant factor in cross-contamination.

Many studies have shown that frequently touched surfaces, particularly mobile phones and coins, can harbor a wide variety of potentially pathogenic microorganisms and contribute to their spread into food [46]. Research by Islam *et al.* [47] reported bacterial contamination in 99% of coins in circulation in Bangladesh. The authors isolated several potentially pathogenic bacteria, including *E. coli*, *S. aureus*, Salmonella typhimurium, and Klebsiella aerogenes, some of which exhibited multidrug-resistant profiles. Additionally, Appiah *et al.* [48] also demonstrated that banknotes and coins serve as significant reservoirs of pathogenic microorganisms (*S. aureus*, *E. coli*, *Salmonella* spp., and *Klebsiella* spp.). These findings confirm that coins and banknotes constitute a potential vector for cross-contamination capable of compromising the food safety of items handled simultaneously with them. The simultaneous handling of *Tchakpalo* with bare hands, along with coins and banknotes, observed among all female producers, constitutes an additional source of contamination for this beverage. The improper practices observed regarding the use of cell phones during *Tchakpalo* production and sales activities could contribute to cross-contamination of the product. Indeed, Almizwary *et al.* [49] reported that cell phones are significant reservoirs of potentially pathogenic microorganisms such as *S. aureus*, *E. coli*, Pseudomonas spp., and Klebsiella spp. According to these authors, frequent handling of these devices without proper hand hygiene increases the risk of transferring contaminants to ready-to-eat foods and beverages. The authors emphasize that poor hand hygiene after using a phone promotes cross-contamination. Khoothiam *et al.* [50] also demonstrated the presence of *S. aureus* on the mobile phones of food vendors in Thailand. The authors also identified strains carrying enterotoxin genes and exhibiting antibiotic resistance, thereby highlighting the potential role of mobile phones in the transmission of pathogenic microorganisms to food.

Although most producers use tap water, the fact that this water is not treated before being used in production and sales represents another potential source of contamination. In fact, during sales, producers add water directly to the *Tchakpalo*. It should be noted that even when water comes from a distribution network considered safe, its quality can deteriorate during transport, storage, or handling due to inadequate practices that promote microbial recontamination. In Ivory Coast, Seki *et al.* [51] demonstrated that bacterial contamination of water increased significantly after storage in households. These authors identified storage conditions, container cleanliness, and handling practices as key factors contributing to the deterioration of water's microbiological quality. The risk is even greater for *Tchakpalo* producers, who rely on water from wells or boreholes. In fact, groundwater extracted from wells and boreholes can be contaminated by seepage from latrines, septic tanks, domestic wastewater, household waste, or agricultural activities located near the collection points [52]. Olalemi *et al*. [53] reported the presence of enteric bacteria indicative of fecal contamination in several groundwater sources used for human consumption in Nigeria. In Benin, Behanzin *et al*. [54] reported the presence of *E. coli* in 45.83% of the well and borehole water samples analyzed in Sèmè-Podji. More recently, Adanlokonon *et al*. [55] demonstrated that all wells studied in the municipality of Agbangnizoun were contaminated with bacteria indicative of fecal pollution. These results confirm that untreated groundwater can be a significant source of microbiological contamination of food and beverages, particularly when used as a raw material in the production of *Tchakpalo*. These findings therefore underscore the need to strengthen quality control of the water used by producers and to promote appropriate treatment before its incorporation into the production process, particularly during the sale of the product.

The observed storage conditions also contribute to an increased risk of microbiological contamination. Storing raw materials and *Tchakpalo* together on the floor at home, as well as at room temperature for long periods, are major factors contributing to the microbial contamination of *Tchakpalo*. Mishra *et al*. [56] recommend separating raw materials and finished products in storage areas to limit the risk of recontamination. Indeed, in artisanal production systems, prolonged exposure of ready-to-eat foods to room temperature can promote the proliferation of certain undesirable microorganisms and accelerate their microbiological spoilage. Sikombe *et al*. [57] demonstrated that storing Mabisi, a traditional fermented beverage from Zambia, at room temperature led to significant changes in the microbial flora and reduced the product's microbiological stability, whereas refrigeration significantly improved its shelf life. The joint storage of raw materials and *Tchakpalo* on the floor at home, combined with prolonged storage at room temperature, also constitutes a potential source of contamination that may further compromise the microbiological quality of this beverage. These observations highlight the importance of improving storage practices to enhance the food safety of *Tchakpalo*.

In environmental terms, *Tchakpalo* is exposed to external contaminants. The lack of adequate protection against dust, insects, rodents, exhaust fumes, etc., combined with the proximity to latrines, garbage dumps, wastewater, or domestic animals, creates conditions conducive to cross-contamination. Indeed, food and beverages sold in open environments are particularly vulnerable to contamination from ambient air, dust, and various environmental vectors [58]. Insects and rodents are also recognized as mechanical vectors for many pathogens. Their presence near food can facilitate the transfer of fecal bacteria from waste, excrement, or contaminated water to products intended for human consumption. The complete lack of food safety training, medical supervision, and health inspections reflects a significant lack of institutional oversight in the *Tchakpalo* sector in Benin. Several studies have shown that food handlers who have not received any food safety training generally exhibit inadequate levels of hygiene knowledge and practices. Indeed, training in food safety is one of the key determinants of improved hygiene practices and reduced risks of food contamination [59].

Furthermore, the lack of regular medical monitoring represents an additional risk factor. Food handlers may be asymptomatic carriers of pathogenic microorganisms that can be transmitted to food during preparation or distribution. In a study conducted among butchers in Almada (Portugal), Oliveira *et al*. [60] assert that health status is an essential component of GHPs designed to prevent foodborne illnesses. Fekadu *et al*. [59] demonstrated that establishments subject to regular health inspections generally exhibit a higher level of compliance with food safety requirements than those not subject to any supervision. In our study, the low levels of hygiene observed among *Tchakpalo* producers. Fekadu *et al*. [59] showed that establishments with regular health inspections generally exhibit better compliance with food safety requirements than those without any supervision. In our study, the low hygiene levels observed among *Tchakpalo* producers are one of the main factors explaining the poor microbiological quality of this beverage in Grand Nokoué.

Thus, the low adoption of Good Hygiene Practices (GHP) observed in this study appears to be a major determinant of the degraded microbiological quality of *Tchakpalo*. This confirms that, in artisanal production systems, improving food safety necessarily involves strengthening the capacities of producers, standardizing processes, and establishing a minimum level of health oversight. It is therefore necessary to implement training, awareness, and health inspection programs to improve the safety of this traditional beer and reduce the risk of foodborne illness among consumers.

5. Conclusion

This study highlights widespread microbiological contamination of *Tchakpalo* sold in the municipalities of Grand Nokoué, characterized by a high prevalence and loads of microorganisms indicative of poor hygiene and health risks. The results also show a very low adoption level of good hygiene practices by producers, with major deficiencies concerning personal hygiene, water quality, storage conditions, equipment maintenance, the production environment, and health oversight of the sector. These deficiencies are likely the main factors explaining the poor microbiological quality observed. Beyond the mere presence of potentially pathogenic microorganisms, these results raise the question of the actual pathogenicity of the strains circulating in the *Tchakpalo*. In this regard, it appears essential to further investigate in order to characterize the virulence factors and antibiotic resistance profiles of the microbial strains, particularly those of *S. aureus* and *E. coli* isolated. Such an approach will allow for a more precise assessment of the risks to consumers and a better understanding of the potential role of this traditional beverage in the spread of pathogenic and multidrug-resistant bacteria. In a context marked by the global emergence of antibiotic resistance, these investigations are fully aligned with the One Health approach, which recognizes the close interactions between human health, animal health, and the environment.

Acknowledgments

The authors thank the *Tchakpalo* producers involved in this study.

Funding

This study was funded by the authors.

Author Contributions

d'Avila DOGNON: Conceptualization, Formal analysis, Data curation, Funding acquisition, Methodology, Writing – original draft.

Agossou Damien Pacôme NOUMAVO: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

Sènan VODOUHE: Conceptualization, Methodology, Funding acquisition, Supervision, Validation, Supervision, Writing – review & editing.

Lucile ADJOVI: Methodology, Formal analysis, Investigation, Writing - original draft, Funding acquisition.

Balbine GBAGUIDI: Methodology, Formal analysis, Writing - original draft.

Ricardos AGUEGUE: Conceptualization, Writing – review & editing.

Kouakou Romaric KOUAKOU: Conceptualization, Writing – review & editing

Philippe SESSOU: Conceptualization, Writing - review & editing.

Farid BABA-MOUSSA: Supervision

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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