

# Microbiological Quality of “Rabilé”, a Yeast Used for Fermentation of Dolo, a Local Beer in Dédougou, Burkina Faso

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## Abstract

*Sorghum* beer or dolo is part of the eating habits of part of the population of Dédougou because of its low price compared with industrial beers. Its production is an ancestral tradition that uses traditional equipment and gives dolo organoleptic properties that are not found in industrial beers. The production process involves several stages, including fermentation, which itself comprises natural lactic fermentation followed by alcoholic fermentation using traditional yeasts, which are not controlled in any way. The general aim of this study is to assess the microbiological quality of these fermentative yeasts in the town of Dédougou, in order to contribute to the health safety of the population and the promotion of these local beers. Twenty samples of fermenting yeast were analyzed according to ISO standards, to isolate enterobacteria, total and faecal coliforms according to standard procedures for isolating these micro-organisms. The isolated strains were identified using the API20E gallery. Microbiological analyses revealed the presence of 51.17% enterobacteria, 45.38% total coliforms and 3.45% thermotolerant coliforms. We counted 40% *Escherichia coli*, 20% *Enterobacter cloacae*, 20% *Klebsiella pneumoniae* and 20% *Klebsiella* spp. All the strains detected are capable of surviving in hostile conditions and could harm the quality of the dolo, consumer health and cause real collective food poisoning in the town of Dédougou. This enabled us to assess the microbial quality of these yeasts and to propose more suitable measures for producing and preserving dolo under hygienic conditions to protect consumer health.

## Keywords

Dolo, Yeast, Rabilé, Microbiological Quality, Bacteria

## 1. Introduction

Although the use of microorganisms for useful purposes is not new, the biotechnological “revolution” underway is due to the enormous progress made in the life sciences. Humans have been using fermentation for thousands of years to obtain foods with improved nutritional value [1]. In Africa, certain cereals such as *Sorghum*, maize and millet are often transformed into beverages, the production of which includes an essential alcoholic fermentation stage [2]. This is the case with the use of yeast in the transformation of these foodstuffs into beverages such as dolo. Dolo is a traditional, ancestral beer fermented from *Sorghum* or maize and brewed by women. It’s a popular and much-loved food, with a sour taste and characteristic aroma. The socio-economic importance of dolo production is remarkable, as it is widely used in traditional ceremonies and is an important source of income for local beer maker (dolotières). The relative success of dolo with consumers is attributed to its relatively low price, the therapeutic virtues attributed to it and the improvement in the diet of millions of people to which it contributes [3]. It is generally produced using traditional technology [4]. The ferment of local beer, the dolo, called Rabilé in Burkina Faso [5] is used as condiment for some communities in Burkina Faso as protein source. It is generally harvested from the bottom of a previous fermenting beer resulting from 12 to 13 h overnight fermentation. Mainly studies have been done on dolo production process. Brewing of dolo is a long and complex process, which include several steps that in a general way, these processing steps are almost the same in the principal points of preparation with brewing of industrial beer such as malting, massing, fermentation, filtration and conditioning. The main difference based on ethnical group essentially concerned the frequency of filtration and cooking during mixing and fermentation [6]. Malting is used to germinate the *Sorghum* grains. The objective was to activate synthesis of hydrolytic enzymes ( $\alpha$ -amylases,  $\beta$ -amylases, proteases, etc). Massing is the stage at which starch and the proteins are converted by endogenous hydrolases into fermentable sugars and peptides, respectively. During the massing step, local brewers usually incorporate in the mixture some mucilages obtained from the soaking bulbs of *Abelmoschus esculentus*, *Curculigo pilosa*, *Gladiolus klattianus*, or leaves of *Adansonia digitata*, *Boscia senegalensis*, *Grewia bicolor*, etc. [6] [7] [8] [9]; soaking permits the release of enzymes contained in vegetables bulbs or leaves. *Sorghum* beer is often brewed using extracts of bitter vegetables to impart a bitter taste and flavour as substitutes for hops used for lager beer production [10]. After saccharification, the wort is boiled for approximately 12 h. This step allows desactivation of the enzymes and precipitation of insoluble matters. Prior to the alcoholic fermentation, the wort is incubated with a portion portion of previous brew or dried yeast harvested [11]. Fermentation is the conversion of fermentable sugars into alcohol and carbon dioxide [12]. The wort in fermentation is in agitation due to the release of carbon dioxide and foam. The empirical process involves two types of fermentation: alcoholic fermentation and natural lactic

fermentation. After fermentation, the resulting dolo beer is narrowly filtered so that some yeast may still remain in the final product. This process has a negative consequence on the preservation of dolo because a slow fermentation continues after dolo conditioning and during its sale [13]. Dolo is conditioned in barrels and is conveyed to the places of sale. It may be preserved in cans hermetically closed for approximately three days at room temperature and more if it is stored at low temperature. Quality control for these drinks is limited to tasting. Women brewers assess the quality of these drinks by tasting them before putting them on the market. However, the various stages involved in the production of this beer have not evolved, from open-air drying in the presence of all the circulating micro-organisms, to the lack of good hygiene practices and the use of ferments from previous fermentations without any real knowledge of the exact nature of these ferments. Many of these micro-organisms are the cause of food-borne illnesses (infection, toxi-infection, intoxication) which can have very harmful consequences for consumer health. Despite the socio-economic importance of dolo, once it has been produced it can only be kept for a maximum of two to three days at room temperature. Dolo quickly loses its organoleptic characteristics due to the proliferation of micro-organisms. These organoleptic and microflora changes are attributed to the presence of fermentative flora that produce new substances, but also to the presence of pathogenic germs that cause spoilage [14]. Given the importance of dolo in our society, its promoters are faced with enormous difficulties in the value chain due to quality, which has a negative impact on consumer health. With the aim of improving the organoleptic, nutritional and microbiological qualities of dolo, this study looked at the microflora contaminating the yeasts used to ferment dolo in the town of Dédougou, in order to help improve the microbiological quality of these “dolos”, ensure the health of the population and promote these local beers.

## **2. Materials and Methods**

### **2.1. Study Framework**

This is a cross-sectional study of local yeast samples called “rabilié” used in the fermentation of dolo in the town of Dédougou, obtained after sampling in some of the town’s place of sale of dolo called cabarets. The samples were analyzed in the Food Microbiology Department of the National Public Health Laboratory (LNSP).

### **2.2. Biological Material**

The biological material consisted of 20 samples of dolo yeast taken from 20 dolo sellers “dolotières” in the town of Dédougou during the period from December 2020 to March 2021. During sampling, we homogenized the batches of fermenting yeast to be collected in order to ensure as uniform a distribution as possible, then collected 300 g per sample in sterile bags using a spoon cleaned with alcohol. To identify each sample, we coded the packaging. This code has two com-

ponents: The first is a number referring to the producer's sector and the second is a letter from the French alphabet referring to the producer. As the samples were taken, they were placed in a sterile plastic bag and then stored at room temperature in a cooler that had been disinfected with alcohol. First of all, we made sure that we were wearing protective equipment (gloves, muffler, gown and cap) and then proceeded to disinfect the equipment with alcohol. Using a clean, sterile spoon, we removed around 300 g of yeast, placed it in a sterile sampling bag, sealed it and then labelled the bag with a marker. Once the sampling was complete, the samples were transported to the laboratory as quickly as possible (in less than 24 hours) for microbiological analysis.

### 2.3. Microbiological Analysis

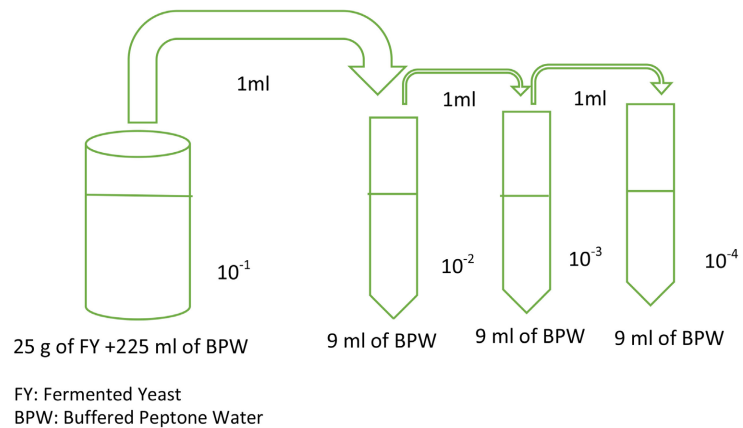
The microbiological analysis was carried out at the Laboratoire National de Santé Publique (LNSP), in the Direction de Contrôle des Aliments et de la Nutrition Appliquée (DCANA), in the Service de la Microbiologie Alimentaire (SMA). The aim of this analysis was to determine and identify the microflora contaminating the yeast used to ferment dolo in the town of Dédougou, in accordance with ISO 21528-1:2004 [15], ISO 21528-2:2004 [16] and NF ISO 4832:2006 [17]. These international standards provide general guidelines for the enumeration of Enterobacteriaceae and coliforms in products intended for human consumption or animal feed. The parameters sought are as follows: the enumeration of coliforms and thermotolerant coliforms and the detection and enumeration of Enterobacteriaceae.

### 2.4. Preparation of the Stock Solution and the Various Dilutions

The preparation of the stock solution suspensions and sample dilutions was carried out in accordance with international standard ISO 6887-1: 2017 [18], which describes the general rules for the preparation of the stock suspension and decimal dilutions. Thus, 25 g of fermentation yeast were introduced into 225 ml of buffered peptone water (BPW). From this solution, a series of cascade dilutions was carried out by pipetting 1 ml of the previous dilution into 9 ml of BPW for the next dilution. Using a test carried out prior to the study, we determined that the optimum dilutions were between  $10^{-1}$  (SM) and  $10^{-4}$ . **Figure 1** shows how decimal dilution was done.

### 2.5. Plating

Inoculation was carried out in depth using a sterile micropipette, 1 ml of each dilution was transferred to each corresponding petri dish. Approximately 15 ml of medium was poured, homogenized and left to dry for approximately 15 minutes at room temperature. A second layer of medium was poured onto the first solidified layer. The various operations (preparation of dilutions and inoculation) were carried out in a fume hood with the benzene burner switched on.



**Figure 1.** Decimal dilution.

## 2.6. Incubation

The petri dishes were incubated in an oven preset at 37°C (VRBG for total coliforms and VRBG for enterobacteria) and 44°C (VRBL for heat-tolerant coliforms) for 24 to 48 hours before enumeration.

## 2.7. Counting

After the specified incubation period, all the characteristic pinkish-purple colonies grown on the agar of each plate are counted to give the number of Enterobacteriaceae found per milliliter or per gram of sample when the test is carried out according to the specified method.

## 2.8. Expression of Results

General case (counting characteristic and/or non-characteristic colonies): dishes containing at least 10 colonies [ $10 \leq \text{colonies} \leq 150$  characteristic and/or non-characteristic]: calculate the number  $N$  of micro-organisms per milliliter or per gram of product as a weighted average, using the following formula:

$$N = \frac{\sum C}{1.1 \times d}$$

$N$ : Number of thermotolerant coliforms per milliliter or per gram of product;

$\sum C$ : Sum of colonies counted on the 2 plates retained from 2 successive dilutions, at least one of which contains at least 10 colonies;

$d$ : dilution corresponding to the first dilution retained ( $d = 1$  if the liquid product seeded directly is retained).

Round the calculated result to 2 significant figures.

Record as the result  $N$  the number of microorganisms per ml (liquid products) or per g (other products), expressed as a number between 1.0 and 9.9 multiplied by  $10^x$  (where  $x$  is the appropriate power of 10) or a whole number to 2 significant figures.

## 2.9. Estimation of Small Numbers

- Case of a dish (test sample or stock suspension) containing fewer than 10 colonies

If the dish contains fewer than 10 colonies, but at least 4 ( $4 \leq \text{colonies} \leq 10$ ), calculate the result as specified for the general case and express it as the estimated number NE of microorganisms per ml (liquid products) or per g (other products).

If the total is between 3 and 1 ( $1 \leq \text{colonies} \leq 3$ ), the precision of the results is so low that the result should be expressed as follows: Thermo-tolerant coliform is present but with:

NE' = less than 4 microorganisms/ ml (liquid products) or,

NE' = less than 40 microorganisms/ per g (other products)

In the case of a can (test sample or stock suspension) containing no characteristic colony, report the result as follows:

“NE” = Less than 1 thermotolerant coliform/ml (Liquid products),

“NE” = Less than 10 thermotolerant coliform/g (Other products).

Where d is the dilution ratio of the parent suspension.

## 2.10. Strain Identification Procedures

### 2.10.1. Sub Culturing

Under sterile conditions, an isolated colony representative of the strain is taken using a Pasteur pipette or a platter and spread on a new plate of culture medium.

**Table 1** shows how the colony number has been chosen for sampling.

### 2.10.2. Detection of *Escherichia coli*

Methylene Blue Eosin (MBE) agar was used. Five colonies isolated on VRBL and VRBG agar were picked and each was then transferred to EMB agar. After incubation for 24 hours at 37°C, colonies with a metallic green sheen were isolated.

### 2.10.3. Detection of *Salmonella* and *Shigella*

To test for *Salmonella* and *Shigella*, Hektoen agar and Xylose Lysine Deoxycholate (XLD) agar were used in accordance with ISO 6579-1:2017 [19]. Research and characterization were carried out in the following stages:

- Non-selective pre-enrichment: the previously prepared stock solution (25 g of the sample in 225 mL of EPT) was incubated at 37°C for 18 h  $\pm$  2 h.

**Table 1.** Choice of colony number for sub culturing.

Number of characteristic colonies counted	Number of characteristic colonies to be sampled
$\leq 5$	Collect all characteristic colonies
6 - 30	5
31 - 40	6
41 - 55	7
56 - 70	8
71 - 90	9
91 - 110	10
111 - 135	11
136 - 150	12

Selective enrichment: a double enrichment was carried out using the pre-enrichment. For the first enrichment, 0.1 mL of the pre-enrichment was introduced into 10 mL of Rappaport Vassiliadis (RV) broth and incubated at 42°C for 24 hours. For the second enrichment, 1mL of the pre-enrichment was introduced into 10 mL of Muller Kauffmann Tetrathionate (KTT) broth supplemented with brilliant green and incubated at 37°C for 24 hours.

- Selective isolation: 0.1mL of each RV and KTT broth was streaked onto Hektoen agar and XLD agar and incubated at 37°C for 24 hours. Characteristic black colonies were confirmed by biochemical tests using the Api20E gallery.

### 2.11. Confirmation Test

The confirmation test using the Api20E gallery enabled us to identify the different species of enterobacteria. It is a gallery of 20 ready-to-use micro-tubes enabling 23 biochemical tests to be carried out in order to identify GRAM-bacilli belonging to the Enterobacteriaceae family.

## 3. Results

### 3.1. Isolation of Microorganisms from Yeast Samples (Rabilé)

The microbiological analysis of the twenty samples rabilé, the local yeast taken from various vendors gave the results shown in **Table 2** below:

**Table 2.** Representation of the microbial load of rabilé.

Sample code	Enterobacteria	Total coliforms	Thermotolerants coliforms
6MY	$<1.0 \times 10$	$<1.0 \times 10$	$<1.0 \times 10$
1R	$1.0 \times 10$	$<1.0 \times 10$	$<1.0 \times 10$
5SN	$<1.0 \times 10$	$<1.0 \times 10$	$<1.0 \times 10$
5P	$2 \times 10^3$	$1 \times 10^3$	$1 \times 10^2$
5N	$1 \times 10^2$	$8 \times 10^1$	$4 \times 10^1$
4Y	$<1.0 \times 10$	$<1.0 \times 10$	$<1.0 \times 10$
1B	$2 \times 10^2$	$8 \times 10^1$	$1 \times 10^1$
5GM	$3 \times 10^2$	$2 \times 10^2$	$1 \times 10^2$
6P	$6 \times 10^1$	$<1.0 \times 10$	$<1.0 \times 10$
5LA	$1 \times 10^2$	$1 \times 10^2$	$<1.0 \times 10$
2SN	$<1.0 \times 10$	$<1.0 \times 10$	$<1.0 \times 10$
3CF	$3 \times 10^1$	$<1.0 \times 10$	$<1.0 \times 10$
5SG	$5 \times 10^2$	$1 \times 10^2$	$2 \times 10^1$
3BB	$7 \times 10^1$	$<1.0 \times 10$	$<1.0 \times 10$
6N	$4 \times 10^1$	$3 \times 10^1$	$<1.0 \times 10$
4F	$2 \times 10^3$	$1 \times 10^3$	$4 \times 10^1$
6D	$1 \times 10^3$	$7 \times 10^2$	$17 \times 10^1$
1A	$2 \times 10^3$	$2 \times 10^3$	$5 \times 10^2$
3F	$3 \times 10^2$	$2 \times 10^2$	$8 \times 10^2$
5T	$2 \times 10^4$	$2 \times 10^4$	$7 \times 10^1$

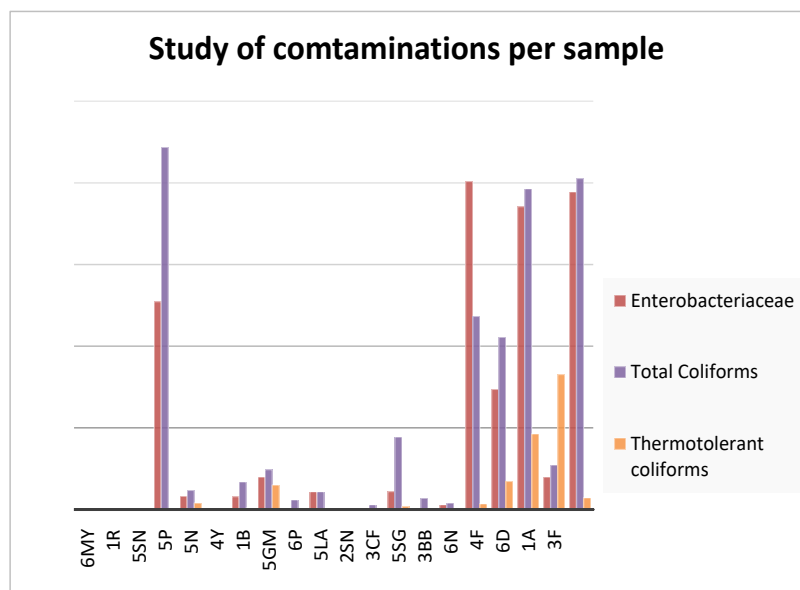
Three types of microorganisms were present in all the samples tested. The results shown in the diagrams in **Figure 2** indicate a very wide variability in the contamination of yeasts used in alcoholic fermentation.

A large proportion of the yeasts used for dolo fermentation in the town of Dédougou are highly contaminated with total coliforms, enterobacteria and fecal coliforms or thermotolerant coliforms. **Figure 3** gives the summary of the different types of microorganisms (faecal and total coliforms and enterobacteria).

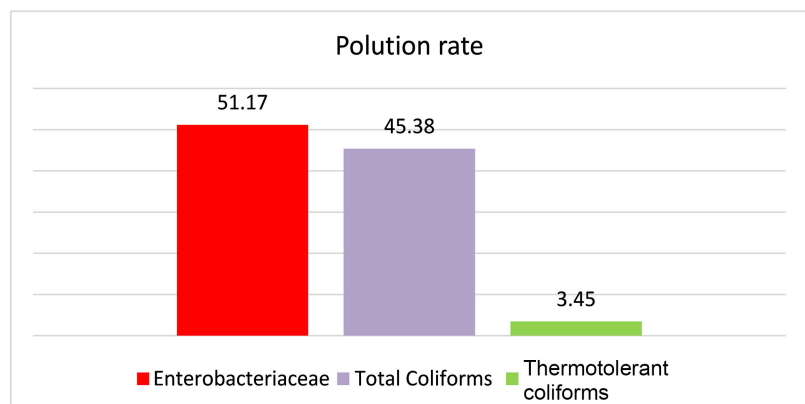
The results show that a large proportion of these fermentation yeasts were heavily contaminated with enterobacteria, *i.e.* 51.17% and 45.38% total coliforms, but also thermotolerant (faecal) coliforms at 3.45%.

### 3.2. Identification of Isolated Strains

Isolated microorganism strains were identified using the API20E Gallery. **Table 3** show the results of identifications of different strains isolated.



**Figure 2.** Overall assessment of the presence of Enterobacteriaceae.



**Figure 3.** Overall contamination rate of yeast samples studied.



**Table 3.** Identification of strains isolated from all samples.

Sample code	Isolated strains	Number of colonies/ml
6MY	-	
1R	-	
5SN	-	
5P	<i>Klebsiella pneumoniae</i>	$1 \times 10^2$
5N	<i>Escherichia coli</i>	$4 \times 10^1$
4Y	-	
1B	-	
5GM	<i>Enterobacter cloacae</i>	$2 \times 10^2$
6P	-	
5LA	-	
2SN	-	
3CF	-	
5SG	<i>Klebsiella spp.</i>	$3 \times 10^2$
	<i>Escherichia coli</i>	$4 \times 10^2$
3BB	-	
6N	-	
4F	-	
6D	-	
1A	-	
3F	<i>Escherichia coli</i>	$2 \times 10^2$
5T	-	

Potentially contaminating strains were not found in all the samples tested.

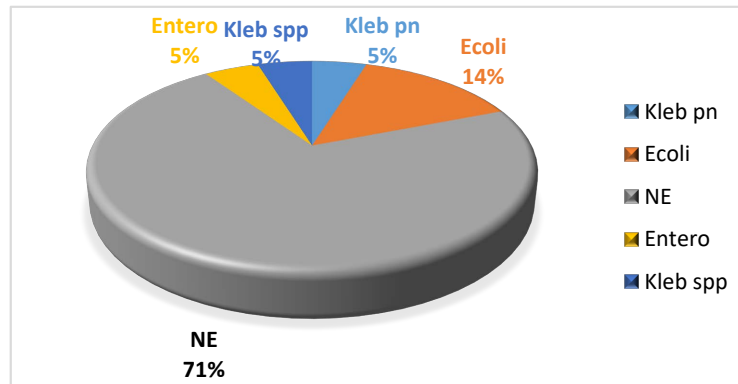
**Figure 4** shows the presence rate of strains in the various samples.

Out of a total of 20 samples analyzed, 15 samples (71%) were free of the microorganisms of interest; *E coli* was detected in 02 samples (14%), and strains of *Klebsiella pneumoniae*, *Klebsiella spp.* and *Enterobacter cloacae* were detected in just one sample each (5% contamination).

## 4. Discussion

### 4.1. Possible Pollution Sources and Transmission Routes of Dolo Production

The different processes used for dolo production affect probably its compound and nutritional quality and needed biochemical control. The ferment obtained at the end of the dolo-making process is dried, usually stored in the open air and reused for the next fermentation. This is a real source of contamination for this ferment through the air, animals, insects, humans, physical equipment and water used to sell dolo, etc. The greatest complexity lies in the hygiene conditions that



**Figure 4.** Strain presence rates detected.

are not mastered by the women brewers. They can sell drinks containing micro-organisms that can be the cause of certain illnesses that can harm people's health. In view of the physical, chemical and biological parameters, the manufacturing process for this traditional drink needs to be improved, in compliance with hygiene rules and quality control assessment in the various production chains, in order to enhance the value of its sale on a national and even international scale [20]. Studies on Burkina Faso [21] [22], Centrafic Republic [20] [23] and Ivory Coast [23] report that the local yeast rabilé content yeast strains such *Rhodotorula muciliginosa*, *Candida pseudorhangii*, *Candida heliconiae*, *Shizosaccharomyces pombe* and *Sporobolomyces odoratus* and others contaminant strains that could cause food intoxication [20] [22]. The diversity of yeasts strains contained in Rabilé can be explained by the fact African traditional alcoholic beverage production results from spontaneous fermentation and as a result, both desirable and non-desirable strains are present in the product [14]. Cookings are generally short due to lack of fuel. This could result in the selection of potentially pathogenic heat-resistant strains.

The presence of Enterobacteria in a foodstuff such as dolo is a real public health problem, given the number of consumers in the town of Dédougou, but it also makes preservation difficult for producers. Because of this instability, producers are forced to adapt their production to the volume of daily consumer demand, at the risk of incurring huge losses. Production is therefore carried out on a daily basis, making the women's work extremely arduous. These organoleptic changes are attributed to the presence of fermentative flora that produce new substances, but also to the presence of pathogenic germs that cause spoilage [24]. The overall aim of this study was to assess the microbiological quality of these fermentative yeasts.

Microbiological analysis of twenty samples of dolo fermenting yeast taken from various vendors revealed that contamination of dolo fermenting yeast ranged from  $<1.0 \times 10$  to  $2 \times 10^4$  cfu/mL for enterobacteria;  $1.0 \times 10$  to  $2 \times 10^4$  cfu/mL for total coliforms; and 3.45% for thermotolerant coliforms ( $<1.0 \times 10$  to  $8 \times 10^2$ ). The samples were taken from 20 different locations. After analysis, it

appears that some do not have contaminants (6M; 1R, 5SN, 4Y, 6P, 2SN, 3CF and 3BB); Unlike everyone else. The question actually is why this difference? The manufacturing process appears in the first line. However, as our study was carried out in the same town of Dédougou, with practically the same cultural practices, the different stages of manufacturing are essentially the same. This difference could be explained by the techniques of recovery, drying and conservation of these ferments called rablié. The contaminants detected in the starter culture could be due to the conditions of treatment of this yeast after fermentation, since the different stages of fermentation are practically similar. This results are similar to those reported by Mogmenga and collaborators [21] in seven localities of Burkina Faso.

Firstly, it should be noted that total Enterobacteriaceae count is used as a marker of faecal contamination and good manufacturing practice. It is therefore an indicator of the quality of food processing (ISO 21528-1-2017 [25], ISO 21528-2-2017 [26]). A high number of this marker would be indicative of a poor manufacturing process or possible subsequent contamination of the final product, implying a hygiene and health risk for the consumer. The rate of presence of these bacteria was 51.17%, well above that of Ernest LANGO and collaborators [20], and Maoura and collaborators [27], where the rate is respectively 5.26 and 36.84% (in a local drink called Bili-Bili).

Secondly, coliforms are indicators of food and water contamination, so enumerating these bacteria in dolo yeast is an indispensable tool for determining its quality [28]. Overall, our study revealed a coliform contamination rate of 45.38% with values ranging from  $<1.0 \times 10$  to  $2 \times 10^4$  cfu/mL. These results are slightly better than those of James *et al.* (52%) with  $2.4$  to  $1.9 \times 10^4$  cfu/ml, but unsatisfactory compared to those of Kayodé and collaborators (13.64%) with  $1 \times 10^1$  to  $4.2 \times 10^2$  cfu/mL [4].

Finally, fecal coliforms or thermotolerant coliforms are a subgroup of total coliforms capable of fermenting lactose at a temperature of  $45.5^\circ\text{C}$ . They are common bacteria found in the digestive tract of humans and animals. The species most frequently associated with this group of bacteria is *Escherichia coli* and, to a lesser extent, certain species of the genera *Citobacter*, *Enterobacter* and *Klebsiella* [29]. The detection of thermotolerant coliforms in a product should raise serious suspicions of contamination of fecal origin. They are good markers of food handling hygiene [30]. The highest rate of presence of these bacterial groups we detected was 3.45%. This rate is better than that found by Coulibaly and collaborators [31], who found 12.63%. However, it remains very high compared with those found respectively by Sawadogo-Lingani and collaborators [6] (2.54%) and Djè and collaborators (1.85%) [3].

## 4.2. Identification of Bacterial Species

After isolating the various contamination strains, we were able to identify four bacterial species.

Overall, our study revealed the presence of 51.17% ( $<1.0 \times 10$  to  $2 \times 10^4$  cfu/mL) enterobacteria, as well as 45.38% ( $<1.0 \times 10$  to  $2 \times 10^4$  cfu/mL) total coliforms and 3.45% thermotolerant coliforms ( $<1.0 \times 10$  to  $8 \times 10^2$ ). These results are unsatisfactory compared with those found by Ernest and collaborators [20] (5.26% to 36.84% enterobacteria), Sawadogo-Lingani and collaborators [6] (37.73% enterobacteria), Dahouenon and collaborators [32] ( $3.9 \times 10^1$  to  $4.6 \times 10^1$ /mL). Our results are slightly better than those of Bayoi and collaborators [33] who counted  $1.1 \times 10^3$  to  $2.4 \times 10^4$  total coliforms and  $1.02 \times 10^2$  to  $6.1 \times 10^2$  thermotolerant coliforms. These results are slightly less satisfactory than those of numerous researchers who have studied dolo and pito (Burkina Faso, Ghana) [6]; tchapalo (Ivory Coast) [3]; ikigage (Rwanda), chibuku (Zimbabwe) [34], who indicate that the analyses carried out on the various traditional *Sorghum* beers reveal an approximate consistency.

In the course of our study, we were able to make a general assessment of the presence of microflora contaminating the yeast used to ferment dolo in the town of Dédougou (enterobacteria). In general, the non-conformity of certain fermenting yeasts results from production, drying and storage procedures which are carried out in an artisanal manner and do not follow a protocol capable of guaranteeing their microbiological quality as well as compliance with hygiene rules and Good Manufacturing Practices. What's more, these producers do not receive any prior training from the relevant authorities to ensure the health safety of these local beverages (according to some producers).

Specifically, the presence of Enterobacteriaceae in fermentation yeast is very high. Nearly 50% of samples exceeded the threshold of  $1.0 \times 10$  CFU/g of faecal coliforms required by the standard; compared with beers from other countries, where the fermenting yeast contains other dominant flora (lactic acid bacteria) and a low presence of enterobacteria. In the light of these results, it seems clear that the major problem with the presence of these microorganisms is generally attributed to contamination of faecal origin, and can be explained to some extent by open-air drying exposed to dust and other volatile animals that are possible reservoirs of microorganisms, but also by production and preservation techniques. After selecting our colonies for the confirmation test, we did not detect the presence of *salmonella* in the fermentation yeast, thus reducing the rate of pathogenic microorganisms. The study as a whole provides information on the microflora contaminating dolo fermenting yeast.

### **4.3. How to Improve the Fermentation Process to Reduce the Presence of Harmful Microorganisms and Improve the Quality and Health Safety of Dolo**

The presence of pathogenic germs (enterobacteria, total and fecal coliforms) revealed that the process of ferment production need to be ameliorated for suitable and safety exploitation. It is necessary to improve the manufacturing process of this traditional beverage by respecting the rules of hygiene and an evaluation of quality control in the various production chains in order to enhance its sale on a

national and even international scale. It would also be necessary to increase and respect cooking times in order to completely eliminate potentially pathogenic heat-resistant strains. Rabilé is used as a condiment, and also a ferment, then, we suggest the use of molecular biology techniques for the characterization of traditional starter Rabilé. Such an approach would lead to an improvement of Rabilé quality and also avoid food intoxication that Rabilé consumption can induce. Also we suggest the packaging of rablé in sterile conditions and proceed to their popularization among women brewers for the next fermentations, but also among the population for their consumption, because it constitutes a balanced source of proteins [35].

## 5. Conclusion

The aim of our work was to assess the microbiological quality of the yeast “rabilé” used to ferment local beer called dolo in Dédougou, mainly in terms of enterobacteria. Our study revealed that the microbiological quality of yeast used to ferment dolo (rabilé) in Dédougou is unsatisfactory. Microbiological analyses revealed the presence of 51.17% enterobacteria, 45.38% total coliforms and 3.45% thermotolerant coliforms. We also counted 40% *Escherichia coli*, 20% *Enterobacter cloacae*, 20% *Klebsiella pneumoniae* and 20% *Klebsiella* spp. No *Salmonella* strains were detected. In view of these results, we can conclude that the contamination of these enterobacteria is the result of an inadequate process or sanitation. Producers therefore need to improve their yeast production, drying, preservation and treatment techniques before use. In the future, it would be interesting to set up a quality control and monitoring committee, provide training for female producers, draw up national quality control standards, carry out further microbiological studies on dolo yeast throughout the country, and also carry out antibiograms on isolated strains to determine their antibiotic resistance profile. According to most of studies of local beer fermentation process, there are not a significant difference between the different process steps. Unless there are cooking defects in certain situations, the heat used for the other stages before fermentation is such that there is little chance of having contaminants. In this case, the critical step lies more at the level of fermentation which requires good ferments and hygienic fermentation conditions in order to guarantee the hygienic quality of the dolo. Then, the use of specific starter culture is better to reduce the growth of harmful microorganism. Given the emergence and evolution of this sector, we expect these results to improve in the years to come. With new operators needing to master manufacturing procedures in their ongoing quest for professionalism to meet the challenges of market access, in a particularly competitive environment.

## Conflicts of Interest

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

## References

- [1] Gotcheva, V., Pandiella, S.S., Angelov, A., Roshkova, Z. and Webb, C. (2001) Monitoring the Fermentation of the Traditional Bulgarian Beverage Boza. *International Journal of Food Science and Technology*, **36**, 129-134. <https://doi.org/10.1046/j.1365-2621.2001.00429.x>
- [2] Assiedu, J.J. (1991) La transformation des produits agricoles en zone tropicales: Approche technologique. Karthala, Paris.
- [3] Djè, M.K., N'Guessan, K.F., Djeni, T.N.D. and Dadie, T.A. (2008) Biochemical Changes during Alcoholic Fermentation in the Production of "Tchapalo", a Traditional *Sorghum* Beer. *International Journal of Food Engineering*, **4**, Article 2. <https://doi.org/10.2202/1556-3758.1408>
- [4] Kayode, A.P., Polycarpe, A.A., Joseph D.H., Anita R.L. and Robert M.J.N. (2005) Quality of Farmers' Varieties of *Sorghum* and Derived Foods as Perceived by Consumers in Benin. *Ecology of Food and Nutrition*, **44**, 271-294. <https://doi.org/10.1080/03670240500187302>
- [5] Ibrahim, K., Marius, K.S., Aly, S., Iliassou, M., Ousmane, K. and Alfred, S.T. (2016) Isolation and Molecular Identification of Yeast Strains from "Rabile" a Starter of Local Fermented Drink. *African Journal of Biotechnology*, **15**, 823-829. <https://doi.org/10.5897/ajb2016.15265>
- [6] Sawadogo-Lingani, H., Lei, V., Diawara, B., Nielsen, D.S., Møller, P.L., Traoré, A.S., et al. (2007) The Biodiversity of Predominant Lactic Acid Bacteria in Dolo and Pito Wort for the Production of *Sorghum* Beer. *Journal of Applied Microbiology*, **103**, 765-777. <https://doi.org/10.1111/j.1365-2672.2007.03306.x>
- [7] Dicko, M.H., Searle-Van Leeuwen, M.J.F., Beldman, G., Ouedraogo, O.G., Hilhorst, R. and Traoré, A.S. (1999) SHORT CONTRIBUTION Purification and Characterization of  $\beta$ -Amylase from *Curculigo pilosa*. *Applied Microbiology and Biotechnology*, **52**, 802-805. <https://doi.org/10.1007/s002530051595>
- [8] Dicko, M.H., Searle-Van Leeuwen, M.J.F., Hilhorst, R. and Traore, A.S. (2000) Extraction, Partial Purification and Characterization of  $\beta$ -Amylase from the Bulbs of *G. klattianus*. *Bioresource Technology*, **73**, 183-185. [https://doi.org/10.1016/S0960-8524\(99\)00164-9](https://doi.org/10.1016/S0960-8524(99)00164-9)
- [9] Dicko, M.H., Searle-Van Leeuwen, M.J.F., Traore, A.S., Hilhorst, R. and Beldman, G. (2001) Polysaccharide Hydrolases from Leaves of *Boscia senegalensis* Properties of Endo-(1 $\rightarrow$ 3)- $\beta$ -D-Glucanase. *Applied Biochemistry and Biotechnology*, **94**, 225-241. <https://doi.org/10.1385/ABAB:94:3:225>
- [10] Adenuga, W., Olaleye, O.N. and Adepoju, P.A. (2010) Utilization of Bitter Vegetable Leaves (*Gongronema latifolium*, *Vernonia amygdalina*) and *Garcinia kola* Extracts as Substitutes for Hops in *Sorghum* Beer Production. *African Journal of Biotechnology*, **9**, 8819-8823.
- [11] Sefa-Dedeh, S., Sanni, A.I., Tetteh, G. and Sakyi-Dawson, E. (1999) Yeasts in the Traditional Brewing of Pito in Ghana. *World Journal of Microbiology and Biotechnology*, **15**, 593-597. <https://doi.org/10.1023/A:1008955300156>
- [12] Mohamed Abdoul-Latif, F., Bassolé, N.I.H. and Dicko, M.H. (2013) Proximate Composition of Traditional Local *Sorghum* Beer Dolo Manufactured in Ouagadougou. *African Journal of Biotechnology*, **12**, 1517-1522.
- [13] Mariam Egunlety, A., Viera-Dalode, G., Houssou, P. and Dossou, J. (2019) Procédé de Production, caractéristiques microbiologique, physicochimique et sensorielle du chakpalo, bière traditionnelle de maïs en Afrique de l'Ouest. *Annales de l'Université de Parakou-Série Sciences Naturelles et Agronomie*, **9**, 61-78.

- <https://doi.org/10.56109/aup-sna.v9i2.55>
- [14] Lyumugabe, F., Kamaliza, G., Bajyana, E. and Thonart, P.H. (2010) Microbiological and Physico-Chemical Characteristic of Rwandese Traditional Beer Ikingage. *African Journal of Biotechnology*, **9**, 4241-4246.
- [15] The International Organization for Standardization (ISO) (2004) ISO 21528-1:2004: Microbiologie: Directives générales pour le dénombrement sans revivification des Enterobacteriaceae; Technique NPP et méthode par comptage des colonies. <https://www.iso.org/fr/standard/34565.html>
- [16] The International Organization for Standardization (ISO) (2004) ISO 21528-2:2004 Microbiologie: Directives générales pour le dénombrement sans revivification des Enterobacteriaceae; Technique NPP et méthode par comptage des colonies. <https://www.iso.org/fr/standard/34566.html>
- [17] ISO (2006) ISO 4832:2006 Microbiologie des aliments, Méthode horizontale pour le dénombrement des coliformes, Méthodes par comptage des colonies. <https://www.iso.org/fr/standard/38282.html>
- [18] ISO (2017) ISO 6887-1:2017 Microbiologie des aliments; Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen micro-biologique; Partie 1: Règles générales pour la préparation de la suspension mère et des dilutions décimales. <https://www.iso.org/fr/standard/63335.html>
- [19] ISO (2017) ISO 6579-1 :2017 Microbiologie des aliments: Méthode horizontale pour la recherche des *Salmonella* spp.; Amendement 1: Annexe D: Recherche des *Salmonella* spp. dans les matières fécales des animaux et dans des échantillons environnementaux au stade de la production primaire <https://www.iso.org/fr/standard/56712.html>
- [20] Lango-Yaya, E., Lebon Bondom, R., Agboko, F.M., Worowounga, X., Yangakola, M. and Rafai, D.C. (2020) Contribution à l'étude des caractéristiques physico-chimiques et microbiologiques des boissons traditionnelles faites à base de sorgho: Cas de Bili-Bili et de son dérivé le Dora-Bonga. *Afrique SCIENCE*, **17**, 35-46. <http://www.afriquescience.net> <https://doi.org/10.19044/esj.2021.v17n21p115>
- [21] Iliassou, M., Kounbèsiounè Marius, S., Ibrahim, K. and Sababénédyo Alfred, T. (2017) Evaluation of Hygienic Quality of Ferment of Local Beer Dolo Used as Condiment in Burkina Faso. *African Journal of Biotechnology*, **16**, 1449-1456.
- [22] Mogmenga, I., Dadiré, Y., Somda, M.K., Keita, I., Ezeogu, L.I., Ugwuanyi, J., et al. (2019) Isolation and Identification of Indigenous Yeasts from "Rabilé", a Starter Culture Used for Production of Traditional Beer "Dolo", a Condiment in Burkina Faso. *Advances in Microbiology*, **9**, 646-655. <https://doi.org/10.4236/aim.2019.97040>
- [23] Aka, S., Djeni, N., N'Guessan, K., Yao, K. and Dje, K. (2008) Variabilité des propriétés physico-chimiques et dénombrement de la flore fermentaire du tchapalo, une bière traditionnelle de sorgho en Côte d'Ivoire. *Afrique SCIENCE*, **4**, 274-286. <http://www.ajol.info/index.php/afsci/article/view/61688>
- [24] Pale, S., Taonda, S.J.B., Bougouma, B. and Mason, S.C. (2010) Sorghum Malt and Traditional Beer (Dolo) Quality Assessment in Burkina Faso. *Ecology of Food and Nutrition*, **49**, 129-141. <https://doi.org/10.1080/03670241003697573>
- [25] ISO (2017) ISO 21528-1:2017 Microbiologie de la chaîne alimentaire-Méthode horizontale pour la détection et dénombrement des Enterobacteriaceae. Partie 1: Détection de Enterobacteriaceae. <https://www.iso.org/fr/standard/55228.html>
- [26] ISO (2017) ISO 21528-2:2017 Microbiologie de la chaîne alimentaire-Méthode hori-



- zontale pour la détection et dénombrement des Enterobacteriaceae. Partie 2: Nombre de colonies technique. <https://www.iso.org/fr/standard/63504.html>
- [27] Maoura, N., Mbaiguinam, M., Vang Nguyen, H., Gaillardin, C. and Pourquie, J. (2005) Identification and Typing of the Yeast Strains Isolated from Bili Bili, a Traditional *Sorghum* Beer of Chad. *African Journal of Biotechnology*, **4**, 646-656. <https://doi.org/10.5897/AJB2005.000-3117>
- [28] OMS (2011) Escherichia coli entérohémorragique (ECEH). <https://www.who.int/news-room/fact-sheets/detail/e-coli>
- [29] Keith, E.G., Allen, M.J. and Rice, E.W. (1999) Comparison of Escherichia Coli, Total Coliform, and Fecal Coliform Populations as Indicators of Wastewater Treatment Efficiency. *Water Environment Research*, **71**, 332-339. <https://doi.org/10.2175/106143098X121752>
- [30] Payment, P. (1999) Poor Efficacy of Residual Chlorine Disinfectant in Drinking Water to Inactivate Waterborne Pathogens in Distribution Systems. *Canadian Journal of Microbiology*, **45**, 709-715. <https://doi.org/10.1139/w99-063>
- [31] Coulibaly, W.H., N'Guessan, K.F., Coulibaly, I., Djè, K.M. and Thonart, P. (2014) Les levures et les bactéries lactiques impliquées dans les bières traditionnelles à base de sorgho produites en Afrique subsaharienne (synthèse bibliographique). *Biotechnologie, Agronomie, Société et Environnement*, **18**, 209-219.
- [32] Dahouenon-Ahoussi, E., Degnon, R.G., Euloge A.S. and Sohounhloue, D.C.K. (2012) Stabilisation de la bière produite à partir de matières amylacées locales (*Sorghum bicolor* et *Musa acuminata*) par adjonction de l'huile essentielle de *Cymbopogon citratus*. *Journal of Applied Biosciences*, **51**, 3596-3607. <https://www.m.elewa.org/>
- [33] Bayoï, J., Djoulde, R. and Etoa, F. (2016) Technologie de fabrication, propriétés physico-chimiques et microbiologiques de la bière « kapsiki blanche » produite dans les monts Mandara au Nord-Cameroun. *Afrique SCIENCE*, **12**, 123-134.
- [34] Togo, C.A., Sara, B., Feresu, S.B. and Mutukumira, A. (2002) Identification of Lactic Acid Bacteria Isolated from Opaque Beer (*Chibuku*) for Potential Use as a Starter Culture. *The Journal of Food Technology in Africa*, **7**, 93-97. <https://doi.org/10.4314/jfta.v7i3.19239>
- [35] Ouédraogo, N., Savadogo, A., Somda, M.K., Zongo, C. and Traore, A.S. (2012) Essai de production de protéines d'organismes unicellulaires par des souches de levures à partir de résidus de tubercules de patate douce et d'igname. *Biotechnologie, Agronomie, Société et Environnement*, **16**, 463-467.