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Microbiological quality and antibiotic resistance profile of enteropathogenic *Escherichia coli* isolated from farm milk, curdled milk and fresh milk sold of Ouagadougou and Pabre, Burkina Faso

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ABSTRACT

Background: Milk is a highly perishable foodstuff, which can be contaminated by pathogenic microorganisms. Among these pathogens, Enteropathogenic *Escherichia coli* (EPEC) are known to be responsible for diarrhea in children aged 0-5 years. Aim of this study is to assess microbial load of milk, characterize EPEC strains in curdled and farms milk from markets of Ouagadougou and Pabre cities then assess their antibiotics resistance profile. **Methods:** Standard methods were used for pH and microbiological quality assessment. EPEC were identified by serotyping, and their antibiotic resistance profile was assessed according to CASFM. A total of 102 milk samples were collected. **Results:** Milk pH values ranged from 3.35 to 6.82, with averages of 6.61 for fresh milk, 3.77 for curdled market milk and 6.54 for farms milk. Values of total aerobic mesophilic flora ranged from $1.70 \pm 1.10 \times 10^7$ to $3.56 \pm 1.37 \times 10^8$ CFU/mL, with an average of $3.76 \pm 1.63 \times 10^8$ CFU/mL. Total coliforms (TC), values ranged from $1.94 \pm 0.84 \times 10^5$ CFU/mL to $2.00 \pm 1.00 \times 10^8$ CFU/mL, with an overall average of $3.40 \pm 1.72 \times 10^7$ CFU/mL. Thermotolerant coliform (ThC) averages for fresh milk sampled at markets ranged from $1.47 \pm 0.52 \times 10^3$ CFU/mL to $2.50 \pm 1.01 \times 10^5$ CFU/mL. A total of 125 *E. coli* strains were isolated, of which 9 (7.2%) were enteropathogenic. Study's showed total resistance to penicillin, cefazolin, streptomycin, fusidic acid and tetracycline. However, they were sensitive to cotrimoxazole (100%), chloramphenicol (100%), nitrofurantoin (88.89%), gentamycin (66%) and fosfomycin (55.55%), respectively. **Conclusion:** Study was revealed that curdled and fresh milks sold in Ouagadougou contained pathogenic, some of which were resistant to commonly used antibiotics.

Introduction

Milk is an essential part of the diet of pastoral or agropastoral populations. As a complete food, milk plays an important role in the growth and

maintenance of the body [1]. In Burkina Faso, livestock farming is the second-largest source of exports after cotton, accounting for 26% of exports by value [2], while 70 million liters are imported

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into Burkina Faso every year, notably in the form of powdered milk, worth around 24 to 28 billion FCFA [3]. However, Burkina Faso has a potential production of 250 million liters of milk annually, of which only 5% are valorized [4]. Dairies and traditional producers are the key players in the local milk marketing chain. They provide the link between production and consumption, adapting to the strengths and constraints of both [5]. Demand for milk is growing rapidly, and there is a greater diversity of dairy products in people's diets, including fresh milk, fermented milk, pasteurized milk, yogurt, traditional cheese, cream, butter, gappal and dèguè [6]. What's more, these products are often contaminated with pathogenic germs. Among these germs, the most frequent are *Mycobacteria*, *Brucella*, *Listeria monocytogenes*, *Staphylococcus aureus* and enterobacteria (*Salmonella* and *E. coli*) [7]. Certain strains of *E. coli* are responsible for acute diarrhea and gastritis in humans. Thus, Enteropathogenic *Escherichia coli* (EPEC) are responsible for infantile gastroenteritis in developing countries [8]. In Burkina Faso, numerous studies have been carried out on the physicochemical and microbiological characteristics and consumption of milk and dairy products [9-13]. The work of several authors has shown that milk and dairy products are highly contaminated with total flora and coliforms. Pathogenic germs such as *Escherichia coli*, *Salmonella ssp*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterobacter cloacae* have been identified in these products [14-16]. Nevertheless, few data exist on Enteropathogenic *Escherichia coli* isolated from milks in Ouagadougou and other localities in Burkina Faso. Hence the interest of this study, which was to assess the microbiological quality of fresh and curdled milks sold in the city of Ouagadougou and Pabre. The knowledge of the microbiological quality of the various dairy products will enable essential measures to be taken to ensure the sanitary safety along the production chain to protect consumers' health.

Material and methods

Sampling

The samples included in this study came from the markets of Ouagadougou and Pabre (**Figure 1**). A total of 102 samples were collected in sterile freezer bags, placed in a cooler containing ice boxes and transported to the laboratory. Specifically, 48 samples of farm fresh milk directly

from breeders, 27 samples of curdled milk and 27 samples of market fresh milk were collected. Each type of product (fresh milk and curdled milk) was sampled in duplicate, i.e. 250 mL for microbiological analysis and 250 mL for pH determination. Market samples of fresh and curdled milk were collected from the same producer and sent directly to the laboratory for analysis. Samples were stored in a refrigerator at 4°C and analyzed on the same day.

pH Determination

pH of the samples was measured using an electronic pH meter (WATERPROOF-PC5) by dipping into a 10 mL volume of milk taken from a beaker after calibration at pH 7.02 and 4 [17]. Before any measurement, the pH meter electrode is cleaned, rinsed with distilled water and dried with blotting paper. The pH was then measured by immersing the tip of the pH meter electrode in the milk. The pH value was read after stabilization.

Microbiological analysis

Enumerations of total mesophilic aerobic flora, total and thermotolerant coliforms were carried out on samples of fresh and curdled milk taken from various farms and markets in Ouagadougou and Pabre.

Suspension preparation

Initial suspension was made by adding 10 mL of the sample to 90 mL of physiological water (NaCl 9 ‰). After homogenization, cascade dilutions were carried out up to the millionth (10^{-1} to 10^{-6}). 100 µL of each dilution were inoculated on the surface of each specific medium for the enumeration of microorganisms.

Enumeration of total aerobic mesophilic flora

Total mesophilic aerobic flora was counted as recommended by international standard ISO 4833-1[18]. Plate Count Agar (Liofilchem, Italy) was used for seeding and plates were incubated in an oven at 30°C for 72 h ± 3 h. After the incubation period, colonies were counted. Plates containing between 04 and 300 colonies were used to calculate the number N of microorganisms. The calculation was made using plates from two successive dilutions, using the formula below:

$$N = \frac{\sum C}{(n_1 + 0,1 n_2) d \times v}$$

N = Number of microorganisms per milliliter of product, expressed as a number between

0.1 and 9.9 multiplied by 10^x (where x is the appropriate power of 10).

ΣC : Sum of colonies counted on the plates retained from the two successive dilutions.

n_1 : Number of plates retained in the first dilution.

n_2 : Number of cans retained at second dilution.

d : First dilution retained.

Detection and enumeration of total and thermotolerant coliforms

Coliforms were enumerated according to ISO 4832 [19]. Violet Red Bile Glucose Agar (Liofilchem, Italy) was used for seeding and plates were incubated at 37°C for total coliforms and 44°C for thermotolerant coliforms, in an oven for 24 h $\pm$ 2 h. Purplish colonies with a minimum diameter of 0.5 mm and sometimes surrounded by a reddish zone were counted after the incubation period. The presumed *E. coli* colonies were selected, purified and stored at 4°C in brain heart broth (BioMérieux, France) containing 20% glycerol for future use. To calculate the number N of microorganisms per milliliter of milk sample, the same formula as for total aerobic mesophilic flora was used. However, plates containing between 10 and 150 characteristic colonies at the level of two successive dilutions were retained.

Detection for *Escherichia coli*

Isolation

After inoculation of samples, incubation and enumeration of microorganisms on VRBL medium (Liofilchem, Italy), plates with well-isolated colonies obtained at 37°C and 44°C were selected. Three (3) colonies characteristic of *E. coli* were isolated from each of the selected plates. *E. coli* colonies are small and appear reddish-purple with a red halo.

Biochemical characterization and conservation

Suspect *E. coli* colonies were plated on Mueller Hinton (MH) agar II (Liofilchem, Italy) for 24 h at 37°C. After 24 h, colonies were picked and tested for biochemical characteristics on the minimal gallery (citrate, H₂S, mannitol-mobility, lactose, indole, urea, glucose). *Escherichia coli* that tested positive (urease negative, citrate negative, indole positive, lactose positive,) were stored in cryotubes containing Brain Heart Broth (BioMérieux, France) with 20% glycerol for serotyping.

Identification of EPEC and antibiotic resistance

Serological identification

Serological identification of EPECs from isolated *E. coli* strains was performed by slide agglutination with *E. coli*-specific (Nonavalent, trivalent IV serum) containing somatic O anti-antigens to determine the group. A drop of antiserum was placed on a clear glass slide and mixed with a bacterial culture taken from MH II. The mixture was emulsified with a pipette and then shaken for 5-10 seconds. A control strain was used to compare results (*E. coli* ATCC 25922).

Antibiotic resistance of EPEC

EPEC antibiotic susceptibility testing was carried out on MH agar according to Committee of the French Society of Microbiology (CASFM) [20].

Preparation of bacterial inoculum and inoculation

Bacterial inoculum was prepared from a pure young colony on MH-II agar. A pure colony of the test strain was picked and crushed in physiological water corresponding to the 0.5 McFarland density, i.e. around 1 to 2x10⁸ CFU/mL. Plates were inoculated by flooding, and excess inoculum was removed by aspiration with a sterile syringe and discarded in a vase containing bleach. The inoculated dishes were closed and left to stand in the open air next to the burner for 5 minutes before the antibiotic discs were deposited.

Choice of antibiotics

Total of 14 antibiotics belonging to 8 families were tested on the strains. These molecules were chosen on the one hand according to the recommendations of CASFM [20] and on the other hand according to the antibiotics commonly used in the health system in Burkina Faso.

Antibiotic susceptibility testing

In vitro antibiotic susceptibility testing was done by disc diffusion method on MH agar II. Plates were immediately incubated in an oven for 24 hours at 37°C. Growth inhibition diameter was measured in millimeters and the data was interpreted according to CASFM [20]. The susceptibility of each bacterium to the antibiotics tested was determined by reference to a reading table giving the correlation between inhibition diameter and minimum inhibitory concentration (MIC). Table 1 shows the antibiotics tested as well as the interpretation criteria.

Statistical analysis

Data was entered using Excel 2010 software. Microbiological analyses was performed in duplicate. Quantitative data was processed using

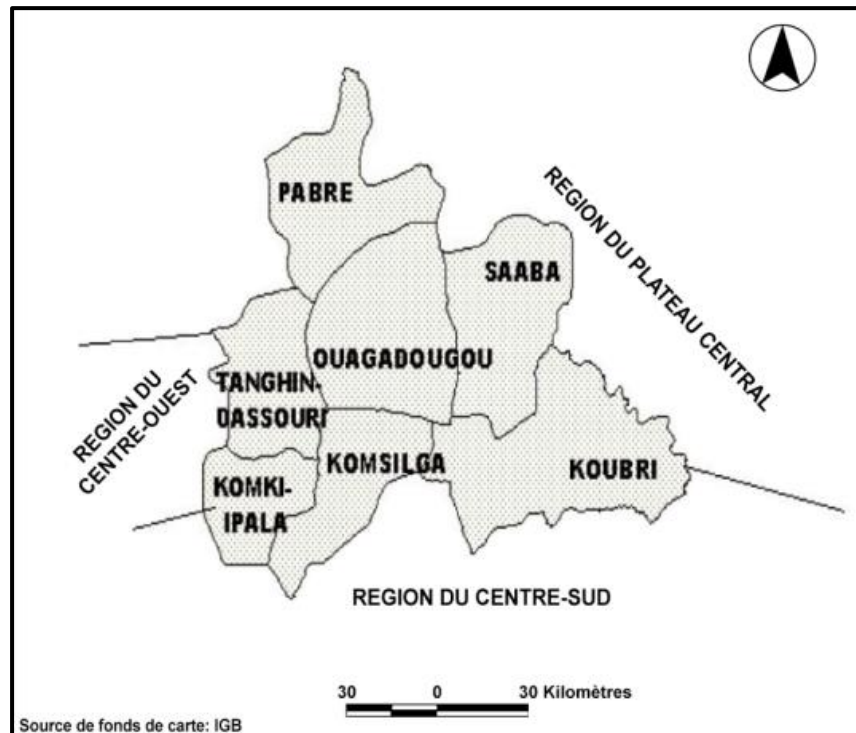
XLSTAT 2016 software to determine averages, standard errors of averages and analysis of variance using Fisher's LSD test at the $p=5\%$ probability threshold.

Table 1. Characteristics of the antibiotics tested.

Antibiotics	Concentrations (μg)	Resistant (mm)	Sensitive (mm)
Chloramphenicol (C)	30	$\phi < 21$	$\phi \geq 21$
Erythromycin (E)	15	$\phi < 20$	$\phi \geq 20$
Ampicillin (AMP)	10	$\phi < 15$	$\phi \geq 15$
Fusidic acid (FC)	10	$\phi < 26$	$\phi \geq 26$
Fosfomycin (FO)	200	$\phi < 26$	$\phi \geq 26$
Cefazolin (CZ)	30	$\phi < 21$	$\phi \geq 21$
Cefoxitin (CX)	30	$\phi < 23$	$\phi \geq 23$
Gentamicin (GEN)	10	$\phi < 19$	$\phi \geq 19$
Tetracyclin (TE)	30	$\phi < 28$	$\phi \geq 28$
Co-trimoxazole	1.25-23.75	$\phi < 23$	$\phi \geq 23$
Penicillin-GP (PN)	10	$\phi < 25$	$\phi \geq 25$
Kanamycin (KAN)	30	$\phi < 8$	$\phi \geq 16$
Streptomycin (STR)	300	$\phi < 14$	$\phi \geq 20$
Nitrofurantoin (NT)	100	$\phi < 17$	$\phi \geq 17$

ϕ : diameter, μg : microgram [20]

Figure 1. Localities of collected samples (Source: <https://www.igb.bf/>).



Results and discussion

pH of milk analyzed

pH of fresh and curdled market milks

Table 2 shows the pH average values of fresh milk and curd samples from the markets. The average pH of fresh milk collected from markets in

Ouagadougou ranged from 6.37 ± 0.2 to 6.82 ± 0.22 , with an average of 6.61 ± 0.19 . According to FAO [21], the normal pH of fresh milk is close to neutral, between 6.6 and 6.8. Only milk from the Silmig-yiri (6.66 ± 0.21), Toécin-yaar (6.66 ± 0.21) and Tampouy-yaar (6.77 ± 0.2) markets had values similar to those recommended. The average pH

values of milk from the Kamboinsin (6.54 ± 0.24), Pabre (6.52 ± 0.23), Sambin (6.82 ± 0.21) and Katryaar (6.82 ± 0.22) markets were close to those recommended by FAO [21]. **Kas et al.** [22] and **Labiou et al.** [23] reported pH values of 6.59 ± 0.30 and 6.50 respectively, similar to the values obtained. However, **Tankoano et al.** [11] reported lower values than those obtained in this study ($pH = 6.29 \pm 0.06$). Milk pH values above 6.8 could be explained by wetting to increase the income of vendors whose milk pH values analyzed were below 6.6, reflecting the start of fermentation [24]. pH indicates the freshness of the milk (pH between 6.6 and 6.8) [25]. Analysis of variance showed no significant difference between the average pH values of samples from different markets ($p = 0.065$).

For the curdled milks analyzed, mean pH values ranged from 3.35 ± 0.25 to 4.05 ± 0.23 , with an average of 3.77 ± 0.20 (**Table 2**). These values obtained are lower than those reported by **Tankoano et al.** [11], which were 4.17 ± 0.58 to 4.50 ± 0.55 respectively. The average value obtained in the Silmig-yiri market is close to that reported by **Diatia** [26], which was 3.89. The highest value obtained in the Tampouy market is lower than that reported by **Katinan et al.** [27] (5.02 ± 0.16). This difference is justified by several factors such as climate, dairy species, equipment and storage time.

All curd samples have a pH below the maximum value of 4.5 [28]. These results indicate that our samples have acidic pH values. This difference could be due to very thorough fermentation. Curdling begins with reconstitution of milk powder in hot water (between 40°C and 50°C), and this temperature drops to 28°C or 30°C at the end of fermentation, 10 to 13 hours later. The milk is thus acidified very rapidly, thanks to the activity of thermophilic *Streptococcus*. According to **Sabina et al.** [29], the fermentation stage can last up to 72 hours. This could lead to strong acidification of the curdled milk. Moreover, according to **Katinan et al.** [27], artisanal curd production follows an empirical approach, so that certain physical parameters such as fermentation time and temperature vary from one production to another. These parameters could explain the variability of pH values obtained. Analysis of variance showed no significant difference between the mean pH values of curd samples from different markets ($p = 0.171$). Fermentation of fresh milk into curd is carried out by lactic acid bacteria. It can last up to 3 days, and this influences pH.

pH of farm fresh milk

Table 3 shows the mean pH values for farm-fresh milk samples. Mean pH values ranged from 6.48 ± 0.19 to 6.58 ± 0.19 , with an average of 6.54 ± 0.18 . The pH values are close to the normal pH value for fresh milk, which is between 6.6 and 6.8 [21]. The mean value (6.48 ± 0.19) obtained is higher than that reported by **Tankoano et al.** [11] (6.29 ± 0.06), but lower than that of **Labioui et al.** [23], which was 6.55. According to **Amiot** [25], pH variations could be linked to climate, stage of lactation, feed availability and cow health. Analysis of variance showed no significant difference between the mean pH values of samples from different farms ($p = 0.85$).

Microbiological parameters of milk analyzed

Microbiological parameters of fresh market milk

The average loads of the various germs determined in fresh market milks are summarized in **table (4)**.

Mean total mesophilic aerobic flora (TMAF) loads ranged from $1.70 \pm 1.10 \times 10^7$ to $3.56 \pm 1.37 \times 10^8$ CFU/mL, with a mean of $3.76 \pm 1.63 \times 10^8$ CFU/mL. All samples exceeded the **AFNOR** [30] (2×10^5 CFU/mL). This high level of contamination is probably linked to a lack of hygiene on the part of saleswomen and at the level of sales equipment [31]. In addition, the temperature and lack of respect for the cold chain favor bacterial proliferation [31, 32]. High TMAF loads in fresh milk have been reported by **Bonfoh et al.** [32] (1.3×10^8 CFU/mL), **Barro et al.** [33] (4.9×10^9 CFU/mL), **Koussou et al.** [34] (4.6×10^7 CFU/mL), **Labioui et al.** [23] (2.6 to 12×10^6 CFU/mL). Statistical analysis showed a significant difference for total aerobic mesophilic flora ($p = 0.001$).

For total coliforms (TC), mean values ranged from $1.94 \pm 0.84 \times 10^5$ CFU/mL to $2.00 \pm 1.00 \times 10^8$ CFU/mL, with an overall average of $3.40 \pm 1.72 \times 10^7$ CFU/mL. Samples from all 9 markets were highly contaminated, with mean values well above the **AFNOR** [30] below 10^2 CFU/mL. Previous work has also revealed high levels of contamination in fresh milk samples. **Tankoano et al.** [1] and **Ounine et al.** [35] found total coliform loads of $2.7 \pm 1.8 \times 10^6$ CFU/mL and 1.07×10^7 CFU/mL respectively. As for **Bachtarzi et al.** [36], the total coliforms counted were of the order of 5.3×10^5 CFU/mL. The difference was not significant ($p = 0.3$). Thermotolerant coliform (TCh)

averages for fresh milk sampled at the markets ranged from $1.47 \pm 0.52 \times 10^3$ CFU/mL to $2.50 \pm 1.01 \times 10^5$ CFU/mL. The overall mean obtained was $7.14 \pm 6.95 \times 10^4$. None of the samples complied with the **AFNOR** [30] (<10 CFU/mL).

Milk was highly contaminated, with loads ranging from 1.1×10^3 CFU/mL to 1.3×10^5 CFU/mL, while the average fecal coliform loads in the fresh milk samples of **Tankoano et al.** [11] and **Belarbi.** [37] were $1.6 \pm 1.2 \times 10^6$ CFU/mL and 1.5×10^3 CFU/mL, respectively.

In sum, samples from all 9 markets show high levels of contamination for TC and ThC. These levels of contamination are closely dependent on general hygiene conditions and the health status of animal [38]. According to **Kouamé-Sina et al.** [24], the absence of a cold chain and high temperature of fresh milk (31.9°C) encourage the rapid proliferation of germs in milk. In addition, the addition of water, which is not always potable, is a significant source of contamination. The difference is not significant, with p-values of 0.2 respectively.

Microbiological parameters of market curds

The average loads of the various germs determined in market curdled milks are summarized in **table (5)**. Mean TMAF loads ranged from $9.94 \pm 5.31 \times 10^6$ CFU/mL to $2.70 \pm 0.82 \times 10^{10}$ CFU/mL, with an overall mean of $4.48 \pm 1.50 \times 10^9$ CFU/mL. All samples from the 9 markets showed very high loads compared with the normal recommended load of 10^3 CFU/mL [30]. This high contamination is thought to be linked, on the one hand, to a lack of hygiene in the production and sale of curdled milk and, on the other, to the sales environment, which generally takes place in the street and often in uncovered containers [16]. So, **Tankoano et al.** [11], and **Compaoré et al.** [12] had reported high contamination of curdled milk with mean TMAF values of $2.7 \pm 2.1 \times 10^{10}$ CFU/mL; 5.5×10^8 CFU/mL, respectively. These differences indicate a diversity in the observance of hygiene measures, resulting in variations in contamination levels. Milk curd production requires 125 to 250 g of commercial yoghurt (ferment) for 5 to 10 L of reconstituted milk. This large quantity of ferment could explain the high levels of aerobic mesophilic germs [27]. Statistical analysis showed a significant difference for total aerobic mesophilic flora ($p=0.007$).

The total coliform loads ranged from $1.45 \pm 1.03 \times 10^4$ CFU/mL to $1.27 \pm 0.65 \times 10^7$

CFU/mL, while. Thermotolerant coliforms from $4.41 \pm 1.10 \times 10^3$ CFU/mL to $3.05 \pm 0.21 \times 10^1$ CFU/mL. The overall means obtained for CT and CTh were $2.61 \pm 1.13 \times 10^6$, $7.05 \pm 2.33 \times 10^2$, respectively. No sample met the **AFNOR** (<10 UFC/mL) [30]. **Tankoano et al.** [11]; **Katinan et al.** [27] and **Compaoré et al.** [12] reported averages of $5.6 \pm 4.3 \times 10^4$ CFU/mL, $2.80 \pm 4.86 \times 10^4$ CFU/mL and $2.2 \pm 2.7 \times 10^5$ CFU/mL, respectively. These averages are closer to those obtained for samples from Kamboinsin, Silmig-yiri and Tanghin markets. Coliforms are part of commensal flora of digestive tract of humans and animals. Their presence in milk indicates a lack of hygiene on the part of those involved in production and processing. The inadequate sanitary quality of fermented milks has been highlighted [12, 31]. Statistical analysis showed a significant difference for fecal coliforms ($p=0.028$). The high level of coliforms and other pathogenic microorganisms in curdled milk is thought to be linked to a lack of good personal, environmental and sanitary hygiene practices, on the one hand, and to the water and utensils used during curd production, on the other hand [39]. Statistical analysis showed a significant difference for thermotolerant coliforms ($p=0.001$). The results of the microbiological analyses showed that 33.33% (i.e. 3 samples out of 9) of fresh milk samples had higher TMAF values than the curdled milk samples. This could be explained by the fact that the curdled milks had very low pH values (≤ 4.6), which would limit the growth of microorganisms in curdled milk. Indeed, acid production by lactic acid bacteria inhibits the growth of pathogens by lowering the pH of the medium [40]. The presence of coliforms and other microorganisms in milk implies possible bacterial contamination of both the utensils and the water used in the manufacturing process [41]. However, some curd samples have a higher TMAF than fresh milk. According to **Maïwore et al.** [42], the high level of microorganisms in curdled milk is due, on the one hand, to a lack of good personal, environmental and sanitary hygiene practices and, on the other, to the water and utensils used during curdling.

Microbiological parameters of farm-fresh milk

The average loads of the various germs determined in the farms' fresh milk are summarized in **table (6)**. Analysis of farm-fresh milk showed that total aerobic mesophilic flora (TMAF) ranged from $1.87 \pm 1.78 \times 10^6$ CFU/mL to $5.55 \pm 2.26 \times 10^7$ CFU/mL, with an overall mean of $2.05 \pm 1.13 \times 10^7$

CFU/mL. All samples had averages above the AFNOR (2×10^5 UFC/mL) [30]. However, samples from 5 farms out of 16 had values close to the same standard. Awareness-raising and training in good hygiene practices on the part of some dairy farmers may be at the root of this difference in sample load. Indeed, milk from a perfectly healthy animal treated aseptically is normally devoid of microorganisms [43]. On leaving the udder, the number of germs is very low 5×10^3 CFU/mL. The increase in the number of germs depends on the hygienic conditions under which handling is carried out, i.e. the state of the animal's property, specifically the udders, the surrounding environment (stable, milking parlor), the teat, the milk collection equipment (milking bucket, milking machine) and finally the milk storage and transport equipment (cans, vats, tanks) [43, 44]. Results indicating high microbial loads in farm-fresh milk have been published by **Bonfoh et al.** [32] (1.3×10^8 CFU/mL), **Koussou et al.** [34] (4.6×10^7 CFU/mL) and **Aggad et al.** [38] (38.4×10^6 CFU/mL).

For total coliforms (TC) and thermotolerant coliforms (ThC), mean values ranged from $7.41 \pm 4.72 \times 10^4$ CFU/mL to $4.83 \pm 2.12 \times 10^6$ CFU/mL and from $1.82 \pm 1.29 \times 10^3$ CFU/mL to $1.57 \pm 1.86 \times 10^6$ CFU/mL. The overall averages obtained were $2.02 \pm 1.01 \times 10^6$ for TC and $3.56 \pm 2.60 \times 10^5$ for ThC. None of the samples complied with AFNOR [30], below 10^2 CFU/mL and 10 CFU/mL for CT and CTh, respectively. Studies carried out in Côte d'Ivoire by **Kas et al.** [22]; in Morocco by **Labioui et al.** [23] and in Côte d'Ivoire **Katinan et al.** [27] had reported loads of coliform averages to 3.85×10^3 , 2×10^4 CFU/mL and $2.80 \pm 4.86 \times 10^4$, respectively. These values are below the maximum average of $4.83 \pm 2.12 \times 10^6$ CFU/mL. However, **Ounine et al.** [35] reported high total coliform loads of 1.07×10^7 CFU/mL. Studies by **Tankoano et al.** [11] and **Kheira et al.** [45] reported average thermotolerant coliform loads of 1.5×10^3 CFU/mL and $1.6 \pm 1.2 \times 10^6$ CFU/mL respectively. The samples from the various farms showed high levels of contamination for TC and ThC. These levels of contamination are closely dependent on the general hygiene conditions of milking, milkers, transport equipment and the health status of the animal [38]. According to **Kouamé-Sina et al.** [24], milk quality deteriorates rapidly from milking to sale. This rapid deterioration in the microbiological quality of milk is partly linked to

the hygienic conditions of milking, in particular the cleanliness of udders and collection utensils (farmers' cans), and the time taken to deliver the milk to the dairy. Statistical analysis showed no significant differences for total mesophilic aerobic flora, total coliforms and thermotolerant coliforms.

***Escherichia coli* and EPEC prevalence**

Prevalence of *E. coli* in isolated strains

Table 7 shows the prevalence of *Escherichia coli* in the strains isolated. Of the 357 strains isolated from the various samples, the proportion of presumptive *E. coli* was 28% (125/357), including 37% (62/168) in farm milk, 43% (45/105) in fresh market milk and 28.6% (18/84) in curdled milk. These abnormally high rates of presumptive *E. coli* are thought to be linked to the non-conformity of street foods and the environment in which dairy products are sold and milked, as reported by several authors [30, 46]. In Côte d'Ivoire, studies by **Dadié et al.** [47] on unpasteurized milk reported a proportion of 10.6% of presumed *E. coli*. According to our results, unpasteurized milk presents health risks for consumers.

Prevalence of EPEC in milk

Table 8 shows the prevalence of EPEC in the milks studied. The figure 2 show an example of positive and negative reaction found during the experiment. The prevalence of EPEC in milk samples was 9 (7.5%), with 3 (5.3%) in farm fresh milk, 5 (14.2%) in market fresh milk and 1 (3.5%) in market curd. These results show that farm fresh milk, market fresh milk and market curd are contaminated with EPEC and represent a risk factor for the development of EPEC infection among consuming children. **Dadié et al.** [47] reported a prevalence rate of 2.7% in unpasteurized milk. According to several authors, EPEC is the leading cause of diarrhea in infants and children under five years [48,49]. In view of these results, hygiene measures such as washing hands and containers during milking should be practiced.

Sensitivity of isolated strains to antibiotics

To assess the antibiotic resistance of EPEC strains isolated from farm fresh milk, market fresh milk and curdled milk sold in the city of Ouagadougou and Pabre a total of 14 antibiotics belonging to 8 families were tested on 9 EPEC strains. The results are shown in **table (9)**.

Table 2. pH of samples analyzed.

Markets	Number of samples		pH	
	FMM	LCM	FMM	CMM
Kamboinsin-yaar	3	3	6.54 ± 0.24 ^{ab}	3.67±0.23 ^{abc}
Silmig-yiri	3	3	6.66 ±0.21 ^{ab}	3.35±0.25 ^{bc}
Katr-yaar	3	3	6.82 ± 0.22 ^a	3.92±0.24 ^{ab}
Sambin-yaar	3	3	6.82 ± 0.2 ^a	3.89±0.23 ^{ab}
Marché de Pabré	3	3	6.52 ± 0.23 ^{ab}	3.88±0.23 ^{ab}
Toécin-yaar	3	3	6.66 ± 0.21 ^{ab}	3.72±0.23 ^{abc}
Toukin-yaar	3	3	6.37 ± 0.00 ^b	3.70±0.00 ^{abc}
Tanghin-yaar	3	3	6.37 ± 0.2 ^b	3.8±0.23 ^{abc}
Tampouy-yaar	3	3	6.77 ± 0.2 ^a	4.05±0.23 ^a
Average	-	-	6.61±0.19	3.77±0.20

FMM= Fresh market milk; CMM= Curdled market milk; Values followed by identical letters are statistically non-different ($p > 0.05$).

Table 3. pH of farm fresh milk samples analyzed.

Farm code	Number of samples	pH
ELFF1	3	6.52±0.19 ^a
ELFF2	3	6.52±0.19 ^a
ELFF3	3	6.54±0.21 ^a
ELFF4	3	6.54±0.2 ^a
ELFF5	3	6.5±0.19 ^a
ELFF6	3	6.53±0.19 ^a
ELFF7	3	6.58±0.19 ^a
ELFF8	3	6.56±0.22 ^a
ELFF9	3	6.57±0.19 ^a
ELFF10	3	6.55±0.19 ^a
ELFF11	3	6.52±0.22 ^a
ELFF12	3	6.53±0.19 ^a
ELFF13	3	6.53±0.19 ^a
ELFF14	3	6.57±0.00 ^a
ELFF15	3	6.56±0.21 ^a
ELFF16	3	6.48±0.19 ^a
Average	-	6.54±0.18

Values followed by identical letters are statistically non-different ($p > 0.05$).

Table 4. Average microbiological parameters of fresh market milk.

Markets	TMAF (CFU/mL)	TC (CFU/mL)	ThC (CFU/mL)
Kamboinsin-yaar (n=3)	1.61±0.68×10 ^{8b}	6.81±3.94×10 ^{7ab}	2.5±1.01×10 ^{5a}
Silmig-yiri (n=3)	2.69±1.08×10 ^{7b}	8.58±2.79×10 ^{5b}	1.39±0.84×10 ^{5b}
Katr-yaar (n=3)	3.08±1.64×10 ^{8a}	9.46±4.41×10 ^{6b}	1.40±0.78×10 ^{5ab}
Sambin-yaar (n=3)	2.70±1.53×10 ^{8a}	6.18±3.47×10 ^{6b}	6.02±1.98×10 ^{4ab}
Pabre market (n=3)	1.61±0.67×10 ^{8b}	1.95±0.63×10 ^{7ab}	1.47±0.52×10 ^{3b}
Toécin (n=3)	1.89±0.77×10 ^{9b}	2.00±1.00×10 ^{8b}	1.03±0.15×10 ^{4b}
Toukin-yaar (n=3)	1.70±1.10×10 ^{7b}	6.51±3.77×10 ^{5a}	6.07±3.78×10 ^{3b}
Tanghin-yaar (n=3)	3.56±1.37×10 ^{8b}	1.94±0.84×10 ^{5b}	6.67±4.70×10 ^{3b}
Tampouy-yaar (n=3)	1.87±0.83×10 ^{8b}	1.45±0.87×10 ^{6b}	1.01±3.31×10 ^{5b}
Average (n=27)	3.76±1.63×10 ⁸	3.40±1.72×10 ⁷	7.14±6.95×10 ⁴
AFNOR [30]	2.10 ⁵ CFU/mL	<10 ² CFU /mL	<10 CFU /mL

Values followed by different letters are statistically different ($p < 0.05$). TMAF=Total mesophilic aerobic flora, TC=Total coliforms, ThC=Thermotolerant coliforms.

Table 5. Average microbiological parameters of market curds.

Markets	Number of sample	TMAF (CFU/mL)	TC (CFU/mL)	ThC (CFU/mL)
Kamboinsin-yaar	3	9.94±5.31×10 ^{6b}	8.47±4.05×10 ^{4b}	2.27±1.28×10 ^{2cd}
Silmig-yiri	3	1.70±0.68×10 ^{7b}	1.45±1.03×10 ^{4b}	9.09±6.43×10 ^{1d}
Katr-yaar	3	1.24±0.17×10 ^{10a}	1.93±0.24×10 ^{5b}	2.73±1.29×10 ^{2cd}
Sambin-yaar	3	2.70±0.82×10 ^{10a}	5.18±2.90×10 ^{6b}	3.05±0.21×10 ^{1d}
Marché de Pabré	3	3.76±2.19×10 ^{8b}	4.29±0.38×10 ^{6b}	3.94±1.93×10 ^{2bc}
Toecin-yaar	3	3.06±1.60×10 ^{9b}	1.59±0.60×10 ^{5a}	5.90±2.25×10 ^{2a}
Toukin-yaar	3	3.44±1.76×10 ^{9b}	1.27±0.65×10 ^{7b}	4.41±1.10×10 ^{3b}
Tanghin-yaar	3	1.10±0.47×10 ^{7b}	6.36±1.93×10 ^{4b}	1.82±1.29×10 ^{2cd}
Tampouy-yaar	3	3.00±0.49×10 ^{8b}	7.77±1.96×10 ^{5b}	8.61±3.30×10 ^{1d}
Average	-	4.48±1.50×10 ⁹	2.61±1.13×10 ⁶	7.05±2.33×10 ²
AFNOR [30]	-	10 ³ CFU /mL	<10 CFU /mL	<10 CFU /mL

Values followed by different letters are statistically different ($P < 0.05$). TMAF=Total mesophilic aerobic flora; TC=Total coliforms; ThC=Thermotolerant coliforms

Table 6. Microbiological parameters of farm fresh milk.

Farm	Number of samples	TMAF (CFU/mL)	TC (CFU/mL)	ThC (CFU/mL)
ELFF1	3	2.23±1.00×10 ^{7abc}	2.97±1.78×10 ^{6ab}	1.85±1.69×10 ^{5b}
ELFF2	3	3.01±1.72×10 ^{7a}	2.15±0.83×10 ^{6b}	1.03±1.07×10 ^{5b}
ELFF3	3	2.10±1.26×10 ^{7ab}	4.83±2.12×10 ^{6b}	1,56±1,17×10 ^{5b}
ELFF4	3	2.67±1.55×10 ^{7abc}	1.56±0.83×10 ^{6ab}	2.49±1.5×10 ^{5b}
ELFF5	3	1.06±0.61×10 ^{7abc}	2.82±1.63×10 ^{6ab}	4.36±1.13×10 ^{5b}
ELFF6	3	1,32±0,64×10 ^{7bc}	3.09±1.83×10 ^{6ab}	2.50±1.73×10 ^{5b}
ELFF7	3	1.17±0.62×10 ^{7bc}	2.27±0.64×10 ^{6b}	9.46±4.82×10 ^{5b}
ELFF8	3	1.39±0.65×10 ^{7ab}	9.18±1.98×10 ^{5ab}	2.79±0.56×10 ^{5b}
ELFF9	3	5.42±0.54×10 ^{6c}	1.23±0.72×10 ^{6ab}	1.05±0.64×10 ^{5b}
ELFF10	3	5.21±2.79×10 ^{6bc}	7.41±4.72×10 ^{4b}	3.91±1.93×10 ^{4b}
ELFF11	3	8.39±3.96×10 ^{6bc}	2.90±1.16×10 ^{6b}	7.59±4.36×10 ^{5b}
ELFF12	3	5.64±3.04×10 ^{6abc}	3.36±0,57×10 ^{6a}	1.57±1.86×10 ^{6b}
ELFF13	3	1.05±0.60×10 ^{7c}	2.54±1.02×10 ^{5b}	1.85±1.05×10 ^{5b}
ELFF14	3	1.79±0.98×10 ^{7bc}	1.82±1.03×10 ^{5b}	1.82±1.29×10 ^{3b}
ELFF15	3	1.87±1.78×10 ^{6abc}	3.27±1.80×10 ^{5b}	<10 ^a
ELFF16	3	5.55±2.26×10 ^{7c}	1.07±0.23×10 ^{6b}	7,91±4,18×10 ^{4b}
Average	-	2.05±1.13×10 ⁷	2.02±1.01×10 ⁶	3.56±2.60×10 ⁵
AFNOR [30]	-	2.10 ⁵ CFU/mL	<10 ² CFU/mL	<10 CFU/mL

Values followed by different letters are statistically different ($P < 0.05$). TMAF=Total mesophilic aerobic flora; TC=Total coliforms; ThC = Thermotolerant coliforms.

Table 7. Prevalence of *E. coli* in isolated strains.

Samples (n=119)	FFM (n=56)	MFM (n=35)	MCM (n=28)	Total number
Number of strains by site	N=168	N=105	N=84	N=357
Number of presumptive <i>E. coli</i>	62(37%)	45(43%)	18(21.4%)	125(28.6%)

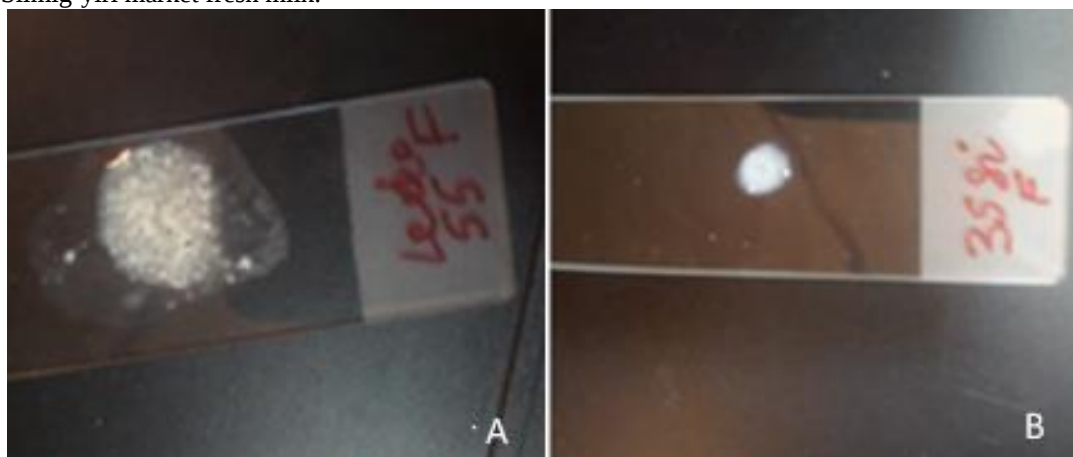
Legend: FFM = Farm fresh milk; MFM= Market fresh milk; MCM = Market curdled milk; N=Number of samples, N=Number of strains

Table 8. Prevalence of EPEC in milks.

Samples	Nonavalent	Trivalent I	Trivalent II	Trivalent III	Trivalent IV
	12 serotypes	O111, O26, O55	O86, O119, O127	O125, O126, O128	O114, O124, O142
F. farms	3(5.3%)	-	1(1.7%)	2(3.6%)	-
F. markets	5(14.2%)	1(2.8%)	1(2.8%)	3(8.6%)	-
Curdled milk	1(3.5%)	-	-	1(3.5%)	-
Total EPEC	9(7.5%)	1(0.8%)	2(1.7%)	6(5%)	-

Table 9. Antibiotic sensitivity testing of isolated strains.

Families	Antibiotics	Sensitive strains		Resistant strains	
		Number	Percentage	Number	Percentage
β -lactamines	Penicillin.G	0/9	00.00	9/9	100
	Cefazolin	0/9	00.00	9/9	100
	Cefoxitin	1/9	11.11	8/9	88.89
	Ampicillin	4/9	44.44	5/9	55.56
Aminosides	Gentamicin	6/9	66.67	3/9	33.33
	Kanamycin	3/9	33.33	6/9	66.67
	Streptomycin	0/9	00.00	9/9	100
Glycopeptides and association	Fusidic acid	0/9	00.00	9/9	100
	Fosfomycin	5/9	55.56	4/9	44.44
Macrolides	Erythromycin	02/9	22.22	7/9	77.78
Phenicoles	Chloramphenicol	9/9	100	0/9	00.00
Tetracycline	Tetracyclin	0/9	00.00	9/9	100
Sulfamides and association	Co-trimoxazole	9/9	100	0/9	00.00
Nitrofurans	Nitrofurantoin	8/9	88.89	1/9	11.11

Figure 2. A: Positive reaction with strain Led55 from farm fresh milk; **B:** Negative reaction with strain Sil58 from Silmig-yiri market fresh milk.

All EPEC strains were resistant to penicillin GP (100%), cefazolin (100%) and cefoxitin (88.89%). Indeed, similar studies by **Nikiéma et al.** [50] and **Renata et al.** [51] revealed resistance of Enteropathogenic *Escherichia coli* to cefazolin (100%) and penicillin G (83%). Other authors found EPEC resistance to cefoxitin (70.4%) [52]. The resistance of EPEC strains to penicillin and cephalosporins (Cefazolin; cefoxitin) could be

explained by the synthesis of penicillinase and cephalosporinase, which are enzymes responsible for resistance. These hypotheses are confirmed by studies carried out by **InVS** [53], which have shown that beta-lactam resistance is mainly due to extended-spectrum beta-lactamases (ESBL), an enzyme that hydrolyzes all penicillins or cephalosporins with the exception of cephamycins and carbapenems. The high rates of resistance

observed with these antibiotics could be explained by their overuse in the treatment of human and animal diseases.

All strains showed total resistance to streptomycin, but were sensitive to gentamicin and kanamycin, with rates of 66% and 33.33% respectively. **Adrian et al.** [54] reported resistance of EPEC to penicillin G (83%). Indeed, EPECs possess enzymes capable of modifying resistance to antibiotics such as kanamycin (46%), gentamicin (48%) [55]. The resistance of strains to kanamycin and streptomycin could be explained by the presence of enzymes such as streptomycin phosphotransferase, streptomycin adenylyltransferase and kanamycin phosphotransferase (APH (3')) types I and II. The presence of antibiotic-resistant *E. coli* in products is of particular concern, as it is the most widespread Gram-negative pathogen in humans and a frequent cause of bacteremia in both the community and hospitals [56].

Strains showed sensitivity to fosfomycin (55.55%). Authors had reported sensitivity of clinical Enteropathogenic *Escherichia coli* strains to fosfomycin (65.63%) [52]. The decline in sensitivity to fosfomycin is thought to be due to the overuse of antibiotics in agriculture and livestock farming.

Three antibiotics belonging to three families (macrolides, tetracyclines, phenicols) were tested on our strains. The strains showed complete sensitivity to chloramphenicol. However, high levels of resistance were observed to tetracycline (100%) and erythromycin (77.78%). Chloramphenicol is a broad-spectrum antibiotic that inhibits protein synthesis. It is also less widely used in livestock farming. These two factors could explain the effectiveness of this antibiotic on the EPEC strains isolated. The excessive use of tetracyclines and macrolides in livestock farming and agriculture seems to explain the high levels of resistance observed. Studies carried out in Burkina Faso by **Konaté et al.** [57] on clinical strains of EPEC showed that the latter were resistant to tetracycline (92.3%) and erythromycin (100%), but sensitive to chloramphenicol (84.6%).

As for sulfonamides and combinations, the strains isolated showed 100% sensitivity. Previous studies in Burkina Faso by **Nadembega et al.** [58] and **Ouedraogo et al.** [59] reported high sensitivity rates to cotrimoxazole of 86.05% and 87.5% respectively. Other authors such as **Konaté et al.**

[57] reported a high sensitivity rate to cotrimoxazole (84.6%).

The strains were 88.89% sensitive to nitrofurantoin. Used in *Staphylococcus aureus* infections, nitrofurantoin has been identified as an antibiotic for the relief of uncomplicated urinary tract infections. Studies by **Park et al.** [60] reported that 98.1% of urinary *E. coli* were sensitive to nitrofurantoin.

Conclusion

This study aimed to investigate total and thermotolerant coliforms, characterize EPEC in farm milk, curdled milk and milk from markets in Ouagadougou and Pabre and assess antibiotic resistance. The study showed that all milk samples analyzed were highly contaminated with total aerobic mesophilic flora, especially market curdled milk. High coliform values were observed in all milk samples, showing that these milks present a health risk for consumers, and particularly for children. The study also demonstrated the presence of EPEC strains in market fresh milk, farm fresh milk and market curdled milk. A total of 125 *E. coli* strains were isolated, 9 of which (7.2%) were enteropathogenic. The conditions under which curdled and fresh milk is sold in certain outlets are precarious, and the environment inappropriate and unsuitable. Some milkers are unaware of good milking practices. Milk sold in markets and supplied by farms is heavily contaminated with coliforms. This indicates a lack of knowledge of good hygiene, milking and transport practices on the part of suppliers and vendors. Antibigrams show the emergence of resistance to Penicillin G, Cefazolin, Cefoxitin, Kanamycin, Streptomycin, Fusidic acid, Erythromycin and Tetracycline. In view of these results, measures need to be taken regarding the rational and controlled use of antibiotics in livestock farming, in order to reduce the emergence of new resistant strains. It should also be pointed out that EPEC strains isolated during this study remain sensitive to certain aminoglycosides (gentamycin), glycopeptides and combinations (fosfomycin), phenicols (chloramphenicol), nitrofurans and certain sulfonamides and combinations (cotrimoxazole). To improve the quality of artisanal dairy products, the sales environment should be away from dumps and sewage drains. Milking, storage and sales utensils must be clean and not exposed to dust, air or sunlight.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to submit this manuscript for publication.

Availability of data and materials

All data and materials were presented in this manuscript.

Authors' contributions

BW, HC, and SNS collected the samples. BW, HC, SZ, SB, BK, AT, SNS and AS analyzed and interpreted the data. BW, HC, SZ, SB, BK, AT and SNS wrote the manuscript. BW, HC, AT and AS reviewed the article critically. All authors have read and approved the final version of the manuscript.

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Competing interests

The authors declare that there are any potential sources of conflict of interest.

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