Microbiological Quality of Tomatoes (*Solanum lycopersicum* L.) Sold in the Markets of Korhogo (Côte d'Ivoire)

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Authors' contributions

This work was carried out in collaboration among all authors. Author YKM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TS and YTD managed the analyses of the study. Authors KO and RKN managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The present work is part of a sanitary quality control of market garden products in Korhogo. The objective of this study was to know the microbiological quality of tomatoes (*Solanum lycopersicum* L.) sold on the markets of the city of Korhogo.
Place and Duration of Study: The analyses were carried out at the microbiology laboratory of Peleforo Gon Coulibaly University during the months of October, November and December 2020.
Methodology: Germs such as molds, yeasts, mesophilic aerobic germs, total coliforms, *Staphylococcus aureus*, *Escherichia coli* and *Clostridium* were tested and enumerated on 40 tomato samples from the markets of Haoussabougou, Koko, Sinistré and the big market according to conventional microbiology methods.
Results: The average loads of molds and yeasts range from 1.2×10^4 to 6×10^5 CFU/g. The average

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loads of mesophilic aerobic germs vary from $2.3x10^4$ to $2.3x10^5$ CFU/g. The highest loads recorded for *Staphylococcus aureus* and total coliforms were $3.1x10^4$ CFU/g and $1.5x10^4$ CFU/g respectively. *Escherichia coli* ($4x10^1$ CFU/g) was isolated only from tomatoes collected in Haoussabougou. As for the *Clostridium* genus, it was not detected on all the tomatoes analyzed.
Conclusion: In general, the microbial loads of the analyzed samples are higher than the accepted norm. Thus, the tomatoes sold on the markets of the city of Korhogo have an unsatisfactory microbiological quality. It is advisable to clean, disinfect and rinse these tomatoes carefully with drinking water before consuming them raw.

1. INTRODUCTION

Fruits and vegetables are essential components of the human diet that provide essential nutrients to the body, such as vitamins, minerals, and fiber. There is considerable evidence of health and nutritional benefits associated with their regular consumption [1]. Insufficient consumption of fruits and vegetables has been shown to contribute to the increase in chronic non-communicable diseases such as diabetes, cancer, obesity and cardiovascular disease. Despite the benefits associated with eating fruits and vegetables, numerous studies have shown that these foodstuffs consumed in a fresh state, provide an ideal substrate, favorable for microbial contamination [2]. From a safety perspective, these are known to be foods at risk for transmission of pathogenic microorganisms [3]. Over the past two decades, cases of mass food poisoning associated with vegetable consumption, have increased in developed countries, involving pathogens including *Salmonella* and *Escherichia coli* [4]. Reported data show 3816 cases of bloody diarrhea, 845 cases of Hemolytic Uremic Syndrome (HUS) and 54 deaths [5]. Diseases associated with the consumption of contaminated fruits and vegetables are common in many parts of the developing world, but are underestimated due to the lack of reliable survey and surveillance data. Vegetables are eaten raw in the form of salads, ready to use, as an appetizer or as an accompaniment to main courses, in restaurants or during festive ceremonies. Among these salads commonly called condiments, tomatoes (Solanum lycopersicum) are one of them. Raw tomatoes are washed, cut, seasoned or not and are consumed at any time of the day and by all levels of society. Tomato (*Solanum lycopersicum* L.) is a perishable vegetable widely grown and consumed around the world [6]. It is known to be a very profitable crop for smallholder farmers in most developing countries [7]. Due to its nutritional value, taste and accessibility, there has been an increase in demand by consumers [8]. Native to northwestern South America, it is widely cultivated in the world and consumed for its fruit which is very rich in minerals, vitamins, essential amino acids, sugar and dietary fiber. It also contains many vitamins (B, C, ...), iron and phosphorus [9]. Given its nutritional and industrial importance, tomatoes have become one of the most important vegetables in the world; annual global tomato production has increased from 129 to 177 million tons, an increase of 37% between 2010 and 2016 [10]. Despite the health benefits, contamination of fresh tomatoes is of particular concern because these products are likely to be consumed raw, without disinfection, thus posing a potential health risk [11]. Previous studies on the microbiological quality of lettuce and tomatoes from fields and markets in Abidjan reported the presence of pathogens at loads exceeding WHO recommended limits [12,13]. The present work is part of a control of the sanitary quality of market garden products in Korhogo. Thus, the general objective of this study is to know the sanitary quality of tomatoes sold on the markets of the city of Korhogo. Specifically, it is to look for and count certain microorganisms such as aerobic mesophilic germs, total coliforms, yeasts, molds, *Staphylococcus aureus*, *Escherichia coli* and *Clostridium*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The plant material that was the subject of this study was tomato (*Solanum lycopersicum* L.).

2.1.2 Culture media

The culture media used for the isolation and enumeration of the various microorganisms are described in Table 1.

2.2 Methods

2.2.1 Sampling

The tomato samples were collected during the months of October, November and December 2020 in four (4) markets of the city of Korhogo which are the big market (BM), Koko market (KM), Haoussabougou market (HM) and Sinistré market (SM). Ten vendors were randomly selected from each of the four markets selected for sampling. A total of 40 samples were analyzed, i.e. 10 samples taken in each market, one sample per vendor. Each sample consisted of 4 tomatoes. The samples were collected in stomacher bags and transported to the laboratory in a cooler for analysis.

2.2.2 Microbiological analyses

2.2.2.1 Preparation of stock suspension and decimal dilutions

Each tomato sample was mixed in sterile bags (stomacher bag) before preparation of stock suspension. The stock suspension was prepared following the method used by [19]. Thus, 10 g of tomatoes were taken and added to 90 mL of previously sterilized buffered peptone water used as diluent. The resulting homogenized mixture constitutes the 10^{-1} dilution stock suspension. One (1) mL of the stock suspension was taken aseptically from a Bunsen burner flame and mixed with 9 mL of diluent in a test tube. This mixture was homogenized and 10^{-2} suspension was obtained. One (1) mL of the 10^{-2} suspension was then homogenized in 9 mL of diluent in a test tube to obtain the 10^{-3} dilution. By the same technique, subsequent dilutions were also made up to the 10^{-5} dilution.

2.2.2.2 Research and enumeration of yeasts and molds

Inoculation for the analysis of yeasts and molds can be done both in the mass and on the surface. However, the technique used in the present work was the surface seeding technique. All manipulations were done in the sterile area of the Bunsen burner flame. Approximately 15 mL of supercooled Dichloran Rose Bengal Chloramphenicol (DRBC) medium were poured into Petri dishes after buckling the neck of the bottle containing the medium. Petri dishes are left on the bench without being half-opened, allowing the media to set in bulk. 0.1 mL of each dilution was taken under aseptic conditions and spread with a sterilized Pasteur rake in each Petri dish containing the agar medium. Petri dishes were then incubated at 30°C for 24 hours for yeasts and 72 hours for molds in the oven. The yeast pasty colonies appear pink due to the assimilation of rose bengal.

2.2.2.3 Research and enumeration of mesophilic aerobic germs

The medium used for the enumeration of mesophilic aerobic germs was Plate Count Agar (PCA). The inoculation was done by incorporating 1mL of each decimal dilution in a Petri dish. Then 15 mL of the previously melted medium maintained in supercooling at 45 °C was poured into the dish containing the inoculum. The mixture was homogenized by manual shaking and then left to cool on the bench at room temperature. After solidification of the medium, a second layer of 4 to 5 mL of the same medium was added to avoid invasion of the plates by certain germs. The seeded Petri dishes were then incubated at 37°C for 24 hours. After this

Table 1. Culture media used for isolation and enumeration of microorganisms

Microorganisms	Culture media	Temperature and incubation time	Method reference
Mesophilic Aerobic Germs	Plate Count Agar	37 °C / 24 h	NF EN ISO 4833-1 [14]
Yeasts	Dichloran Rose Bengal Chloramphenicol	30 °C / 24 h	NF EN ISO 21527-1 [15]
Molds	Dichloran Rose Bengal Chloramphenicol	30 °C / 72 h	NF EN ISO 21527-1 [15]
Total coliforms	RAPID'*E. coli* 2	37 °C / 24 h	NF ISO 16140 [16]
Escherichia coli	RAPID'*E. coli* 2	37 °C / 24 h	NF ISO 16140 [16]
Clostridium	Tryptone Sulfite Neomycin	37 °C / 24 h	NF ISO 6461-2 [17]
Staphylococcus aureus	Baird Parker	37 °C / 24 h	NF EN ISO 6888-1 [18]

incubation period, all colonies present in plates containing between 15 and 150 colonies were counted.

2.2.2.4 Research and enumeration of Staphylococcus aureus

Baird Parker agar was the medium used for the investigation and enumeration of *Staphylococcus aureus*. Inoculation was done by spreading 0.1 mL of the stock suspension or decimal dilutions on the surface of the agar previously poured and cooled in Petri dishes. Incubation was done at 37 °C for 24 hours. Presumptive *Staphylococcus aureus* colonies were black or gray, surrounded by a clear halo. Presumptive *Staphylococcus aureus* colonies present in dishes containing 15 to 150 colonies were counted. Suspected coagulase-positive *Staphylococcus* were confirmed by the coagulase test.

2.2.2.5 Research and enumeration of total coliforms and Escherichia coli

RAPID'*E. coli* 2 agar was used for the enumeration of total coliforms. Inoculation was done by incorporating 1 mL of the stock suspension and decimal dilutions into sterile Petri dishes. A volume of 12 to 15 mL of supercooled medium at 45°C was poured into the Petri dishes containing the inoculum and the mixture was homogenized by gentle shaking. After solidification, a second layer of 4 mL of the same medium was poured in. The Petri dishes were incubated for 24 hours at 37 °C in the oven. After the incubation period, pink-purple (E. coli) and blue (other coliforms) colonies were counted. However, plates containing 15 to 150 colonies were considered.

2.2.2.6 Research and enumeration of clostridium

The medium used was tryptone sulfite neomycin agar (TSN). For inoculation, 1 mL of the initial suspension and / or decimal dilutions is taken and then inoculated into the mass of TSN agar previously melted. The reading was taken after 24 hours of anaerobic incubation. The black colonies visible on the Petri dishes were counted.

- Expression of Results :

Germ count calculations were performed according to [20] which determines the number N of colonies according to the following formula :

$$N = \frac{\sum C}{(n1 + 0{,}1 \times n2)d.v}$$

N : Number of microorganisms in CFU/g of product
ΣC : Sum of colonies counted on the Petri dishes retained at two successive dilutions
v : Volume of inoculum collected (0.1 mL or 1mL)
n1 : Number of Petri dishes retained at the first dilution
n2 : Number of Petri dishes retained at the second dilution
d : Dilution at which the first counts are obtained

2.2.3 Frequency of isolation of microorganisms tested

The frequency of isolation is the percentage of occurrence of a species, genus or group of microorganisms in relation to all samples analyzed. It was determined by the following formula :

$$FI = \frac{Ns}{Nt}$$

F_I : Frequency of isolation
Ns : Number of samples presenting the same species or genus or group
Nt : Total number of samples (40 samples)

2.2.4 Microbiological criteria for fruits and vegetables

To define the microbiological quality of the analyzed tomatoes the microbiological reference criteria for fruits and vegetables [21] were used (Table 2).

Table 2. Microbiological reference criteria for fruits and vegetables [21]

Microorganisms	M
Mesophilic aerobic germs	10^7 CFU/g
Total coliforms	10^3 CFU/g
Escherichia coli	10^3 CFU/g
Yeasts	5×10^6 CFU/g
Molds	10^4 CFU/g
Staphylococcus aureus	10^3 CFU/g
Clostridium	10^4 CFU/g

M: limit of acceptability beyond which the results are no longer considered satisfactory

2.2.5 Statistical analysis

The results obtained were subjected to analysis of variance (ANOVA) with Statistica software version 7.1. In case of significant difference between the studied parameters, the classification of the averages is done according to the Newmann-Keuls test. The significance level is $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Average mold loads on the tomatoes analyzed

Fig. 1 shows the average mold loads of the different samples which vary from $1.2 \times 10^4 \pm 6.10^3$ to $6.10^5 \pm 5.10^5$ CFU/g product. The highest contamination ($6.10^5 \pm 5.10^5$ CFU/g of product) was observed in samples collected at the Haoussabougou market. However, samples from the large market have the lowest mold load ($1.2 \times 10^4 \pm 6 \times 10^3$ CFU/g). Statistical analyses revealed that there was no significant difference ($P > 0.05$) between the mold loads of tomato samples from the Haoussabougou and Koko markets on the one hand, and the big market and Sinistré market on the other.

3.1.2 Average yeast loads on the tomatoes analyzed

The average yeast loads obtained on the tomato samples from the different markets shown in Fig. 2 range from $2.6 \times 10^4 \pm 2.7 \times 10^3$ to $1.3 \times 10^5 \pm 9.5 \times 10^4$ CFU/g. Tomato samples from the Sinistré market were the most loaded with yeast, with an average of $1.3 \times 10^5 \pm 9.5 \times 10^4$ CFU/g. In contrast, tomato samples collected from Koko market have the lowest yeast load with a value of $2.6 \times 10^4 \pm 2.7 \times 10^3$ CFU/g. The yeast load of tomatoes from Sinistré market is significantly ($P < 0.05$) higher than that of the other markets which show no significant difference ($P > 0.05$).

3.1.3 Average loads of mesophilic aerobic germs on the analyzed tomatoes

Tomatoes sold on the Korhogo market are highly contaminated with mesophilic aerobic germs. This contamination varies from one market to another (Fig. 3). The highest load of mesophilic aerobic germs was observed in tomatoes sold at the large market ($2.3 \times 10^5 \pm 3.6 \times 10^4$ CFU/g) while the lowest load of mesophilic aerobic germs was observed in tomatoes sold at the Haoussabougou market ($2.3 \times 10^4 \pm 2.3 \times 10^4$ CFU/g). Statistical analysis of the results
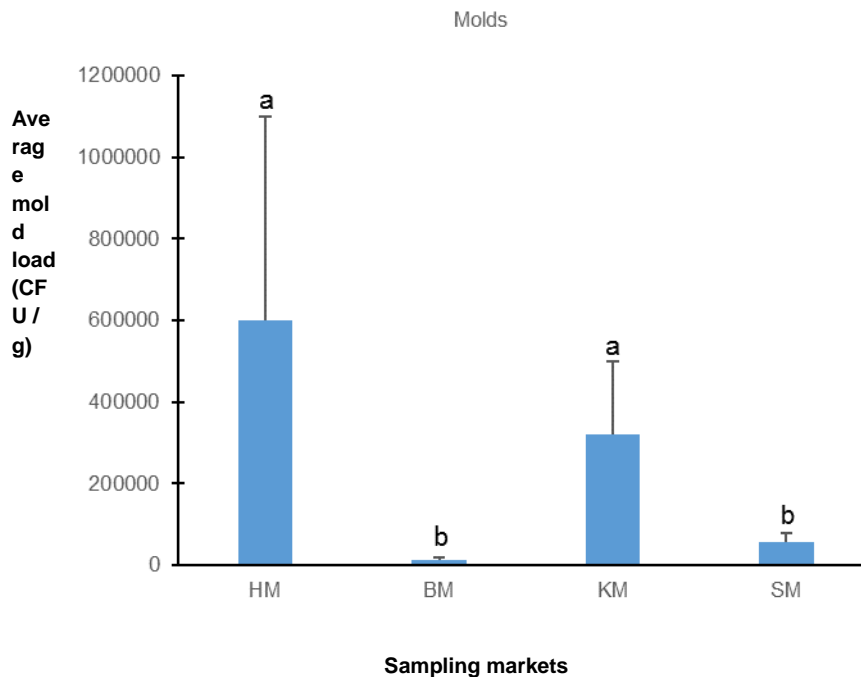

Fig. 1. Average mold loads observed on the different tomato samples
Values with the same letter are not statistically different (P > 0.05).
HM: Haoussabougou market; BM: Big Market; KM: Koko market; SM: Sinistré market
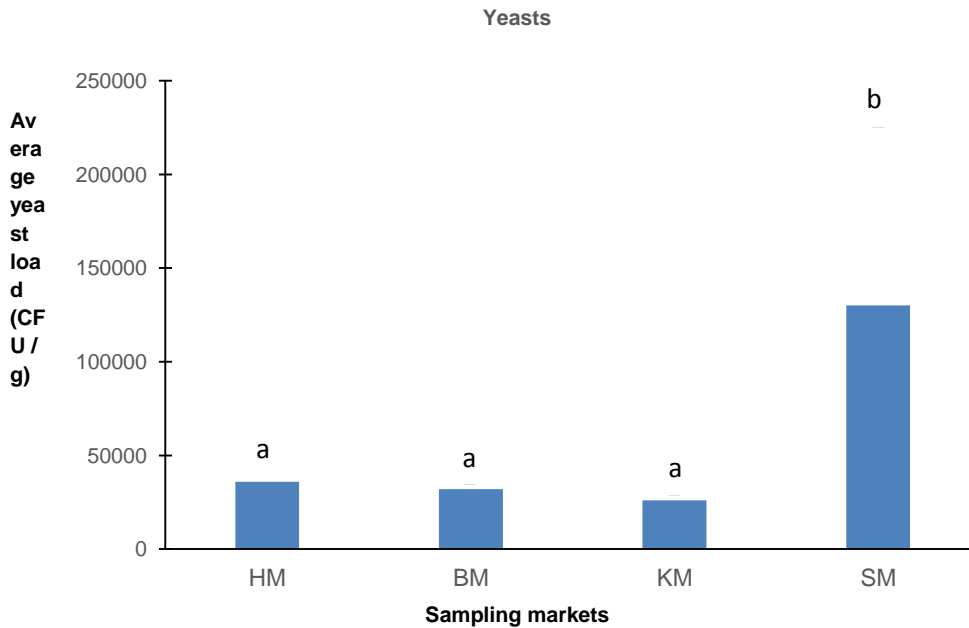
Yeasts

Fig. 2. Average yeast loads observed on the different tomato samples
Values with the same letter are not statistically different (P > 0.05).
HM: Haoussabougou market; BM: Big Market; KM: Koko market; SM: Sinistré market

revealed a significant difference (P < 0.05) between the samples analyzed. Thus, the mesophilic aerobic germs loads of the samples taken at the big market and at Koko are significantly higher than those obtained from the Haoussabougou samples.

3.1.4 Average *Staphylococcus aureus* load on the tomatoes analyzed

Fig. 4 shows the loads of *Staphylococcus aureus*. In general, tomatoes sold in the markets of Korhogo contain high loads of *S. aureus*. These loads range from $8.1 \times 10^3 \pm 6.10^3$ to $3.1 \times 10^4 \pm 1.5 \times 10^3$ CFU/g. Tomatoes sold at the Sinistré market had the lowest *S. aureus* load of $8.1 \times 10^3 \pm 6.10^3$ CFU/g, while the big market had the highest *S. aureus* load ($3.1 \times 10^4 \pm 1.5 \times 10^3$ CFU/g). Statistical analysis showed that there was no significant difference (P > 0.05) between the average S. aureus loads on tomatoes from the Haoussabougou, Sinistré and Koko markets.

3.1.5 Average total coliform loads on the tomatoes analyzed

The average total coliform loads of the different tomato samples ranged from $2.2 \times 10^3 \pm 9.5 \times 10^2$ to $1.5 \times 10^4 \pm 5.5 \times 10^3$ CFU/g (Fig. 5). The highest total coliform load was observed in samples from the large market ($1.5 \times 10^4 \pm 5.5 \times 10^3$ CFU/g), while the Haoussabougou market recorded the

lowest load ($2.2 \times 10^3 \pm 9.5 \times 10^2$ CFU/g). Statistically, there is no significant difference (P>0.05) between the mean total coliform loads of tomatoes collected at the big market and at Koko and Sinistré markets.

3.1.6 Average *Escherichia coli* loads on the tomatoes analyzed

The results shown in Fig. 6 indicate that tomatoes sold in the big market, Koko and Sinistré markets were not contaminated with *Escherichia coli*. Tomatoes sold in the Haoussabougou market recorded $4.6 \times 10^1 \pm 4 \times 10^1$ CFU/g as *Escherichia coli* load.

3.1.7 Average *Clostridium* loads on tomatoes analyzed

No *Clostridium* was observed on tomatoes sold in the big market, Haoussabougou, Koko and Sinistré markets.

3.1.8 Frequency of isolation of germs tested

The presence of yeasts and molds was observed on all the tomato samples analyzed (100%). Total coliforms, mesophilic aerobic germs and *Staphylococcus aureus* were isolated at the same frequency (91.66). *E. coli* was isolated in 8.33% of the samples analyzed. No presence (0%) of *Clostridium* was observed on the tomato samples analyzed (Fig. 7).
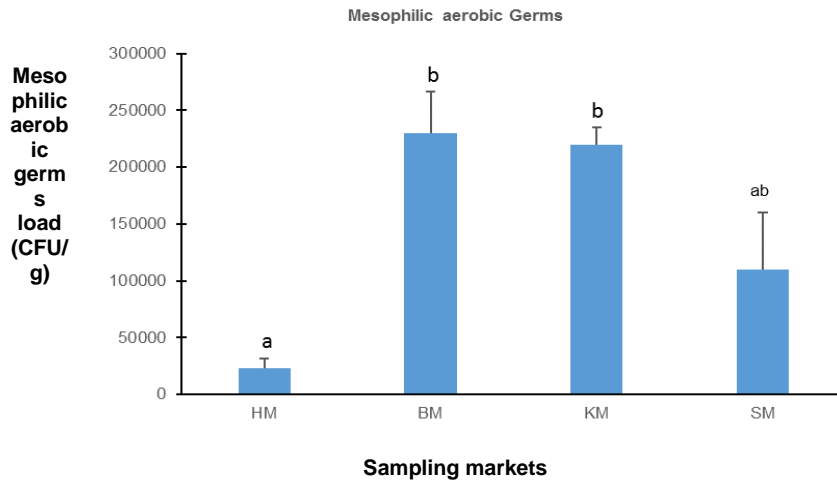
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Mesophilic aerobic Germs

Fig. 3. Average mesophilic aerobic germs loads observed on the different tomato samples
Values with the same letter are not statistically different (P > 0.05).
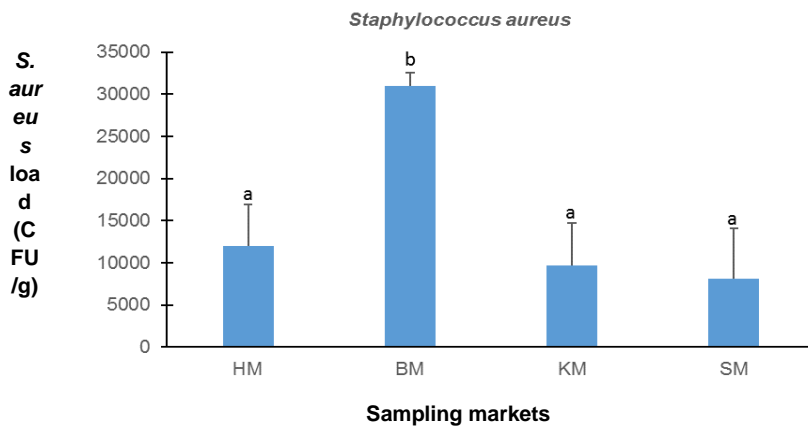*HM: Haoussabougou market; BM: Big Market; KM: Koko market; SM: Sinistré market*

Staphylococcus aureus

Fig. 4. Average *Staphylococcus aureus* loads observed on the different tomato samples
Values with the same letter are not statistically different (P > 0.05).
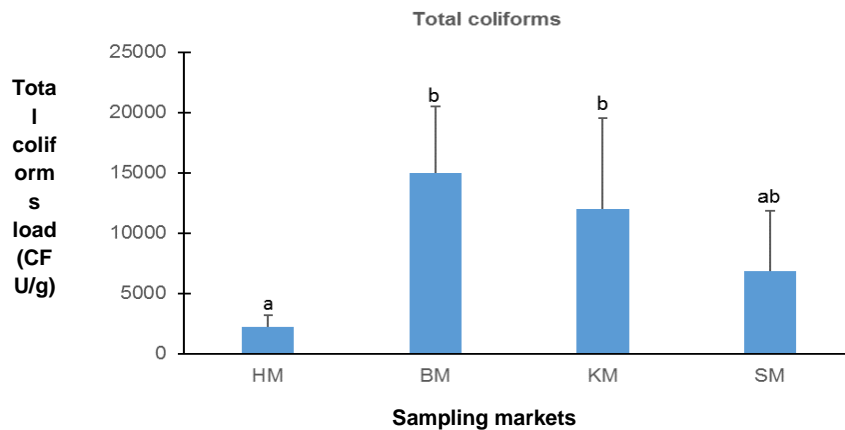*HM: Haoussabougou market; BM: Big Market; KM: Koko market; SM: Sinistré market*

Total coliforms

Fig. 5. Total coliform loads detected on the different tomato samples
Values with the same letter are not statistically different (P > 0.05).
HM: Haoussabougou market; BM: Big Market; KM: Koko market; SM: Sinistré market
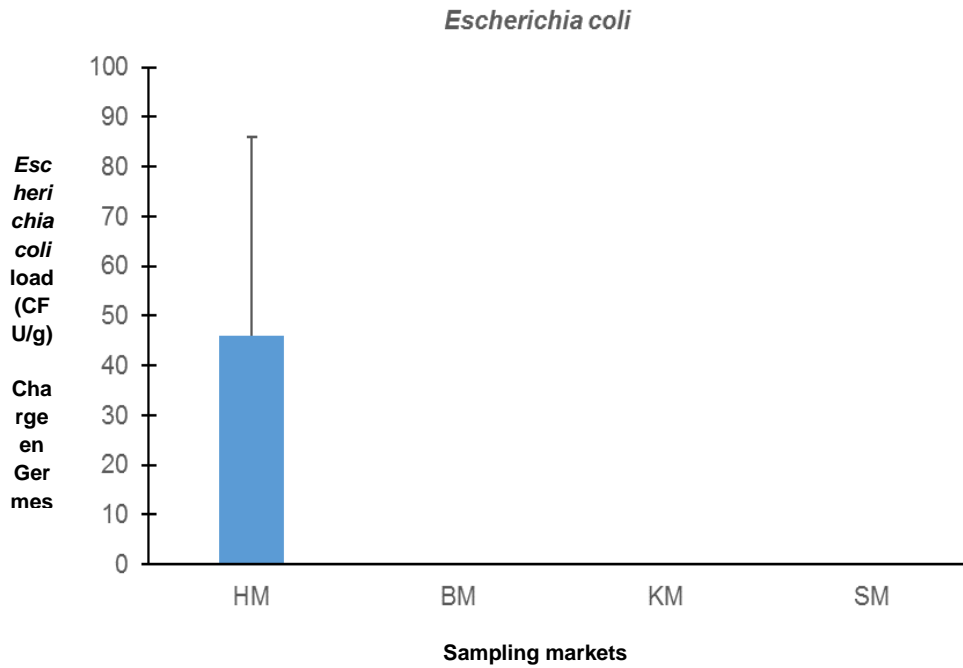
Escherichia coli

Fig. 6. *Escherichia coli* loads detected on the different tomato samples
HM: Haoussabougou market; BM: Big Market; KM: Koko market; SM: Sinistré market
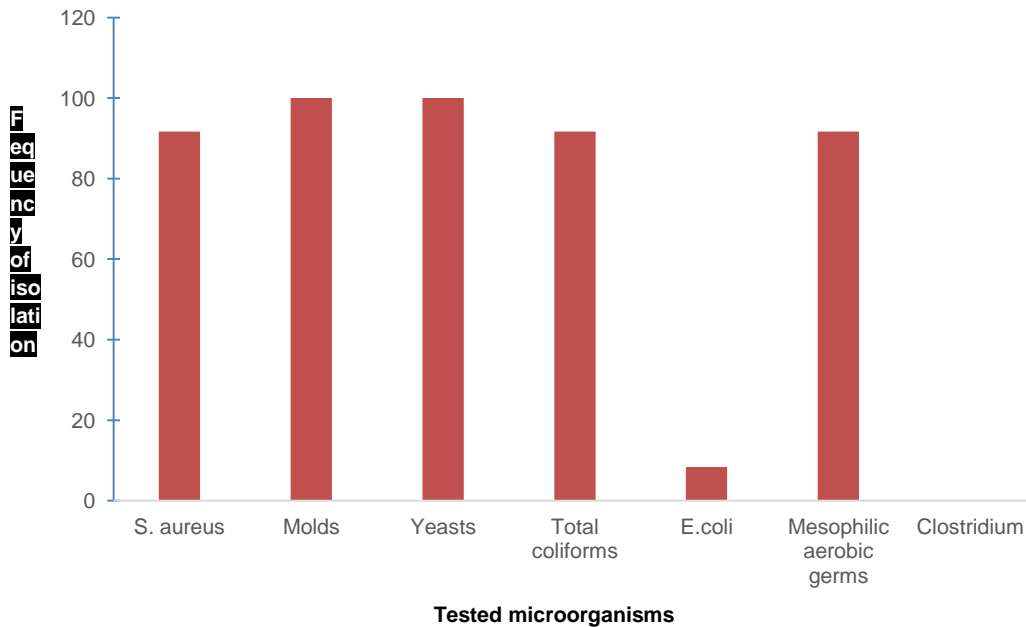

Fig. 7. Frequency of isolation of microorganisms tested

3.2 Discussion

The objective of this study is to evaluate the microbiological quality of tomatoes sold in the markets of the city of Korhogo. To do this, 40 samples of tomatoes were taken and analyzed. The loads of microorganisms obtained (molds, yeasts, mesophilic aerobic germs, total coliforms, *Staphylococcus aureus*, *Escherichia coli* and

Clostridium) were compared to the recommendations for fruits and vegetables. Regarding molds, the loads obtained on tomato samples from different markets vary from 1.2×10^4 to 6×10^5 CFU/g. These loads are above the microbiological criterion ($< 10^4$ CFU/g) [21]. These results are in agreement with those of [22]. Indeed, in a similar study these authors detected molds in vegetable salads (tomato,

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onion, lettuce) with loads between 10^4 and 10^5 CFU/g. Statistical analyses revealed that there were significant differences (P < 0.05) between mold loads of tomato samples from different markets. The variation in mold loads could be explained by variation in production and harvesting conditions, poor sanitation, poor storage and unhygienic practices of tomato handlers. Yeast loads ranged from 2.6×10^4 to 1.3×10^5 CFU/g. These values do not reach the acceptability threshold (< 5×10^5 CFU/g). The yeast load of tomatoes from the Sinistré market is significantly (P < 0.05) higher than that of the other markets. This yeast contamination may be due to storage locations, ambient air, market cleanliness as well as the containers used [23]. With regard to mesophilic aerobic germs, the highest load was observed on tomatoes sold at the big market (2.3×10^5 CFU/g) while the lowest load was observed on tomatoes sold at the Haoussabougou market (2.3×10^4 CFU/g). In all four markets, loads were below the threshold value (10^7 CFU/g) set relative to microbiological criteria for fruits and vegetables [21]. These loads are lower than those reported by [24] on a microbiological study of fresh vegetables. The sources of contamination of the samples with mesophilic aerobic germs are diverse: the environment, handling of the product by producers and traders. A high mesophilic aerobic germ load would promote high spoilage of the product and pose a risk for the presence of pathogens [25]. Statistical analysis of the results revealed a significant difference (P < 0.05) between the samples analyzed. Indeed, [26] showed that the variability in mesophilic aerobic germs from one vendor to another could depend on the density of street traffic that influences environmental hygiene thus leading to tomato contamination. Tomatoes sold in Korhogo markets contain high loads of *Staphylococcus aureus*. These loads range from 8.1×10^3 to 3.1×10^4 CFU/g and are above the microbiological criterion (< 10^3 CFU/g) applicable to tomato [21]. This result is consistent with a microbiological study conducted on fresh vegetables in Ethiopia where Staphylococcus aureus loads were around 10^3 CFU/g [27]. Statistical analysis showed that there was no significant difference (P > 0.05) between the average S. aureus loads on tomatoes from the Haoussabougou, Sinistré, and Koko markets. The contamination of tomatoes with *S. aureus* could be due to skin and nose contact, the environment, and packaging and storage conditions [28]. With respect to total coliforms, the highest load was observed in the samples from the big market, i.e. 1.5×10^4 CFU/g,

while the Haoussabougou market recorded the lowest load (2.2×10^3 CFU/g). However, there was no significant difference (P > 0.05) between the average total coliform loads of tomatoes collected at the big market and at the Koko and Sinistré markets. All samples from the sampling sites had average loads above the accepted standard (< 10^3 CFU/g). The high total coliform load promotes product spoilage and is a risk for the presence of pathogens [29]. The presence of total coliforms in samples is indicative of poor hygiene in production, which may result from the producer, contact equipment, and/or the immediate product environment [30]. The high microbial load of products recorded in different markets could be attributed to factors such as inadequate storage facilities, personal hygiene of vendors, lack of adequate waste disposal and sanitation facilities [31]. Coliforms are referred to as the "hygiene test" [32], because they report on general hygiene. Of all the tomato samples analyzed only those from the Haoussabougou market were contaminated with *Escherichia coli* (4.6×10^1 CFU/g). The *E. coli* load recorded is below the accepted standard [21]. *E. coli* is an indicator of fecal contamination that shows the risk of the presence of pathogenic germs. Indeed, this germ informs about the lack of cleanliness of handling, the lack of cleanliness of the premises (production site and market) and the equipment used for production and harvesting [33]. According to [34] in Côte d'Ivoire, [35] in Morocco, and [36] in Belgium, irrigation water for urban vegetable crops was the main source of microbial contamination in production. No *Clostridium* was detected in any of the samples analyzed. This microorganism colonizes soils [33]. The absence of this germ can be explained by the fact that it develops in the absence of oxygen (strict anaerobic). Probably, from the harvest to the sale, the analyzed tomatoes were kept in the open air to avoid rotting. The presence of yeasts and molds was observed on all tomato samples analyzed (100%). Total coliforms, mesophilic aerobic germs and Staphylococcus aureus were isolated at the same frequency (91.66). *E. coli* was isolated in 8.33% of the samples analyzed. With regard to accepted standards, it appears that tomatoes sold on the markets of the city of Korhogo are generally of unsatisfactory microbiological quality. Hygiene measures at the level of producers (watering, production and harvesting equipment, storage conditions) and sellers (packaging, containers, place of sale) must be applied.

4. CONCLUSION

The main objective of this study was to evaluate the microbiological quality of tomatoes sold in the markets of the city of Korhogo in order to preserve consumer health. This study highlighted the presence of molds, yeasts, mesophilic aerobic germs, *Staphylococcus aureus*, total coliforms, and often *Escherichia coli* on tomatoes. Loads vary from sample to sample, and by market. Thus, the study showed that the frequency of isolation of molds and yeasts is higher than that of other germs. This study revealed an excessively high level of non-compliance. The high microbial loads noted in this study reflect poor hygiene at the production level, and poor handling at harvest and post-harvest. These tomatoes constitute a potential risk for the consumer that will become a real risk if mistakes are made during the disinfection process. The microbiological contamination must be taken care of to prevent risks to the health of consumers, adopting effective programs to fight against this contamination, respecting the hygiene from production, transport, market to the plate of the consumer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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