

# Assessment of food safety and foodborne zoonotic bacterial and fungal Pathogens in pig meat sold in slaughterhouses of Kinshasa, Democratic Republic of the Congo

## Abstract

Foods produced from animals are major reservoirs for many of the foodborne pathogens such as *Campylobacter* species, non-Typhi serovars of *Salmonella enterica*, Shiga toxin-producing strains of *Escherichia coli* and *Listeria monocytogenes*. The zoonotic potential of foodborne pathogens, as well as their ability to produce toxins that cause disease or even death, are enough to highlight the gravity of the situation. In this research, it is evidenced that animals act as vehicles in transmission of foodborne pathogens such as *Candida albicans*, *Citrobacter freundii*, *Escherichia coli*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Salmonella spp*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Yersinia enterocolitica*. their impact, and their current status. This study contributes to improving the health of the meat consumed by the Kinshasa population and popularization of national and international opinion about the dangers posed by the consumption of these meats if there is infection. Matete market with 42.8% of samples contaminated with *Staphylococcus aureus*, three out of six samples may be declared unfit for human consumption and samples from other sites may also be declared unfit for consumption because the bacterial count revealed were above the threshold recommended by the FAO. These were 33.3 % (case 2/6) of samples contaminated with *Staphylococcus aureus*, Central Market 33.3% *Staphylococcus aureus* and *Salmonella spp*, Gambela 33.3% (*Staphylococcus aureus* and *Salmonella spp*), Liberté market 20% contaminated with *Salmonella spp* samples. Since no effective interventions have eliminated these pathogenic bacteria from animals and food, we indicated that they will continue to cause outbreaks and deaths around the world.

**Keywords:** Pork, *Enterobacteriaceae*, Mushrooms, slaughterhouse, FAO

## INTRODUCTION

Of infectious diseases in humans, 60% of them are caused by pathogens shared with wild or domestic animals (Taylor *et al.*, 2001). Such pathogens and diseases include leptospirosis, cysticercosis and echinococcosis, toxoplasmosis, anthrax, brucellosis, rabies, Q fever, Chagas disease, type A influenzas, Rift Valley fever, severe acute respiratory syndrome (SARS), Ebola hemorrhagic fever, and the original emergence of HIV (IRLI, 2012; Grace *et al.*, 2011; Molyneux *et al.*, 2011; WHO, 2006; Karesh *et al.* 2005; Daszak *et al.*, 2000). The classification of zoonotic diseases takes into account the route of transmission (vector-borne or foodborne), the type of pathogen (macroparasites, microparasites, protozoa, viruses, bacteria, worms, ticks or fleas) or the degree of human-to-human transmissibility. (Lloyd *et al.*, 2009).

The zoonotic potential of foodborne pathogens and their ability to produce toxins causing disease or even death is enough to recognize the seriousness of the situation. The seriousness of the situation is recognizable by the zoonotic potential of foodborne pathogens and their ability to produce toxins thereby causing disease or even death. The magnitude of this problem is illustrated by the significant proportion of the 1.5 billion annual diarrheal episodes in children under 3 years of age that are caused by enteropathogenic microorganisms, resulting in more than 3 million deaths per year (EFSA-ECDC, 2016).

As a result, foodborne illnesses represent less than 1% to 10% of the actual incidence (Scallan *et al.*, 2011). The reality on the importance of farm animals as vectors of pathogenic bacteria is real; for example, beef is thought to be the vector of 7% of the 1.7 million cases of foodborne illness recorded between 1996 and 2000 in England and Wales (Anderson *et al.*, 2009).

Growing global human population and urbanization, gross national income (GNI) per capita, globalization and changes in consumption patterns (more protein in the diet) have increased the consumption of dairy products. animal origin (Dhama *et al.*, 2013). Forecasts suggest that consumption of these products will reach 376 million tonnes by 2030 (Dhama *et al.*, 2013).

This growing demand for animal products leads to intensive animal production and product processing, with an increased circulation of food on a global scale. This condition could lead to

faulty processing practices and an increased risk of contamination with foodborne pathogens at any point in the farm-to-fork chain. Contagion from animals and animal products is a serious concern as it is difficult to control. Various factors could be involved in contamination, including the environment (associated wildlife, water from different sources, disposal of animal manure, etc.) and human handling of animals (slaughtering and processing practices, and procedures storage, etc.) (Sofos, 2008).

In the Democratic Republic of Congo (DRC), suspected outbreaks of foodborne illnesses were reported in various literatures. In most cases, the etiologies of foodborne zoonoses remain undetermined

Some characteristics of animal production and food consumption habits in DRC that may promote zoonotic disease transmission include (1) high density of both human and animal populations living in close proximity;(2) a predominance of smallholder production systems with mixed species and little/no biosecurity; (3) the presence of abattoirs and wet markets operating with rudimentary hygiene, limited cold chain for distribution and low levels of meat inspection; (3) widespread consumption of raw/undercooked blood, meat, fish, organ tissues, raw leafy vegetables and wild animal products and (4) use of untreated wastewater and sewage for agriculture.

Pig production and slaughtering systems, however, have not evolved at the same pace as pig population growth. Pigs are typically raised extensively or semi-extensively, although more and more professional pig farmers are converting to a complete intensive system. Due to the absence of professional pig slaughterhouses, pigs are generally slaughtered on slaughter slabs or in the streets. According to the Animal Slaughterhouse and Meat Inspection Act, all slaughtered animals and meat should be examined by a meat inspector. However, the Government of DRC has so far been unable to implement these regulations.

Pigs are vectors of several zoonotic agents, which can by direct contact or by indirect routes of transmission be transmitted to humans. Some of these agents are transmitted via food through faecal contamination of meat during the slaughter process. Besides bacteria and parasites, pigs harbor various viruses in their gastrointestinal tract, which do not necessarily cause disease in animals (CDC, 2015; Kreuzer *et al.*, 2012). Some of these viruses are closely related to human

viruses and are therefore suspected of having zoonotic potential. For some of them, zoonotic transmission has already been proven.

Evidence of food-borne diseases in low- and middle-income countries is still limited, but recent studies suggest that the most significant also comes from biological hazards (Grace, 2015), with an estimated 20 % of all human illnesses and death associated with endemic zoonoses (Grace *et al.*, 2011).

Given the growing demand for pork and the disproportionately slow progress of production systems, the importance of pork as a vector of zoonotic agents is likely to increase. Consumption of raw or undercooked pork can be a source of various zoonoses, including parasitic zoonoses such as trichinellosis, taeniasis and toxoplasmosis (Dorny *et al.*, 2009). Assessing and monitoring the potential threat emerging from this growing market will therefore become increasingly important for the protection of public health. For this purpose, we were carrying out a cross-sectional study in slaughterhouses in the Province of Kinshasa, in which, moreover, the results should help to estimate the risk of zoonotic bacterial and fungal transmissions through the pig food chain and to highly exposed people such as slaughterers, veterinarians and consumers.

Microbial pathogens can cause disease by the consumption of animal products contaminated with microorganisms or their toxins. In this research, it is evidenced that animals act as vehicles in transmission of foodborne pathogens such as *Candida albicans*, *Citrobacter freundii*, *Escherichia coli*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Salmonella spp.*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Yersinia enterocolitica*,

## **2. Material and method**

### **2.1 Study area**

Slaughter points were selected according to the different districts of the city of Kinshasa.

#### **(a) Slaughterhouse of the Liberté market**

Located within the Marché de la Liberté on Lumumba Boulevard, in the Commune of Masina, Bitabe District, Tshangu District, in the Eastern part of the city of Kinshasa.

#### **(b) Slaughterhouse of the Matete market**

Located in the market of the same name, Quartier Mutoto, next to the Maison Communale and the Tribunal de Grande Instance of Matete, District of Mont-Amba, in the South-eastern part of the city of Kinshasa.

**(c) Slaughterhouse at the Bumbu market**

Located in the market of the revolution at the crossroads of the Liberation and Revolution avenues, Lubudi district, Bumbu Commune, Funa District, in the western part of Kinshasa. Here, there are nearly 1,000 pig slaughters per year.

**(d) Slaughterhouse of the Gambela market**

Located within the Gambela Market at the intersection of Gambela and Sport Avenues in the Commune of Kasa-Vubu, Funa District, in the heart of the city centre. The market serves part of the Commune of Kalamu, Limete, Ngiri-Ngiri, Bumbu, Kinshasa, Bandalungwa and Kintambo.

**(e) Slaughterhouse of the Marché Central**

Located in the central market of the city of Kinshasa at pavilion 6, Commune de la Gombe, District of Lukunga, in the northern part of the city of Kinshasa, this slaughterhouse provides meat to the inhabitants of the surrounding neighbourhoods and those coming from various backgrounds across the country.

## **2.2. MATERIALS**

### **2.2.1 Biological material**

The study material consists of 30 pork samples taken from five slaughterhouses in a few markets in Kinshasa. At each site, 6 samples are taken at peak times (1:00 pm to 3:00 pm) depending on the availability of meat.

### **Sampling equipment**

They were used to collect, store and transport the samples under aseptic conditions (without the influence of the external environment) from the sampling sites to the laboratory for further analyses.

**Table 1:** Different types of cultural media by purpose

Enrichment	Isolation	Identification	Enumeration
Selenite broth Alkaline peptone water	Mac Conkey Mannitol Salt Agar (M.S.A) Sabouraud Chloramphenicol (S.C) <i>Salmonella-Shigella</i> agar (S.S.A) Hektoene Methylene Blue Eosin Agar (E.M.B)	PCA on surface	Candida ID Trycase sulfite Neomycin agar (TSN) Sulfure-Indole Mobilité (SIM) Sodium Citrate (Media Simmons) Urée-indole-milieu Kligler

## 2.3 METHODS

### 2.3.1 Sample collection

After identification of the different killings, the meat samples were taken (200 gr per sample) and placed in sterile glass jars and then in the isothermal tank charged with a cold accumulator.

Thirty samples of pork meat are taken and then divided by killings into six (6) pieces and sent to the laboratory for analysis.

### 2.3.2 Macroscopic and microscopic analyses

#### (a) Macroscopic analyses

It was based on the observation of the conditions under which the meat was spread in the killings and the physical appearance of the meat.

#### (b) Microscopic analyses

We used two sub-methods: quantitative and qualitative.

##### - Quantitative analysis

This sub-method uses two techniques, dilution and enumeration. Dilution was carried out after inoculation of the sample in selenite broth and peptone water; using a dropper, a 0.001 ml sample of each sample was placed in 1 ml of physiological water which represents a dilution of 10<sup>-3</sup>.

Enumeration is performed from the diluted solution after incubation at 37°C for 24 hours. One drop or 0.001 ml of each sample is taken from the platinum loop and stained in a zig-zag pattern

on the surface of the medium for enumeration. These boxes were incubated at 37° C for 24, 48, or 72 hours depending on the strain to be isolated. This was how the counting of colonies that was grown were carried out.

The total number of colonies for a sample was calculated by multiplying the number of colonies after counting by the inverse of the dilution or inoculum as shown in the formula below (Larpent, and Larpent-Gourgaud, 1990):

$$F = N \times f \times d$$

Where F is the formula to calculate the total number of colonies for a sample, N is the number of colonies; f is the factor and d is the dilution to use.

Under aseptic conditions, we incorporate one drop of each dilution into the Plate Count Agar medium and incubate at 30 to 37°c for 72 hours to count total mesophilic germs.

**Table 2:** Culture conditions and strains to be enumerated from the media used

Media	Incubation	Strains to be counted
Mac Conkey cristal purple	37° to 44°C ; 24 to 48 hours	Thermo tolerant coliforms
Sabouraud chloramphenicol	37°c ; 5 days	Yeasts and moulds
Mannitol Salt Agar (PSA)	37°C ; 4 to 48 hours	<i>Staphylococcus aureus</i>
Trycase Sulfite Neomycin	46°C in aerobics; 72 hours	<i>Clostridium sulfato reducer</i>
Hektoene and Eosine blue methylene	37°c ; 24 to 48 hours	<i>Salmonella</i> and <i>Shigella</i>
Pyocyanosis	37°C ; 24 to 48 hours	<i>Pseudomonas aegunosa</i>

- **Qualitative analysis**

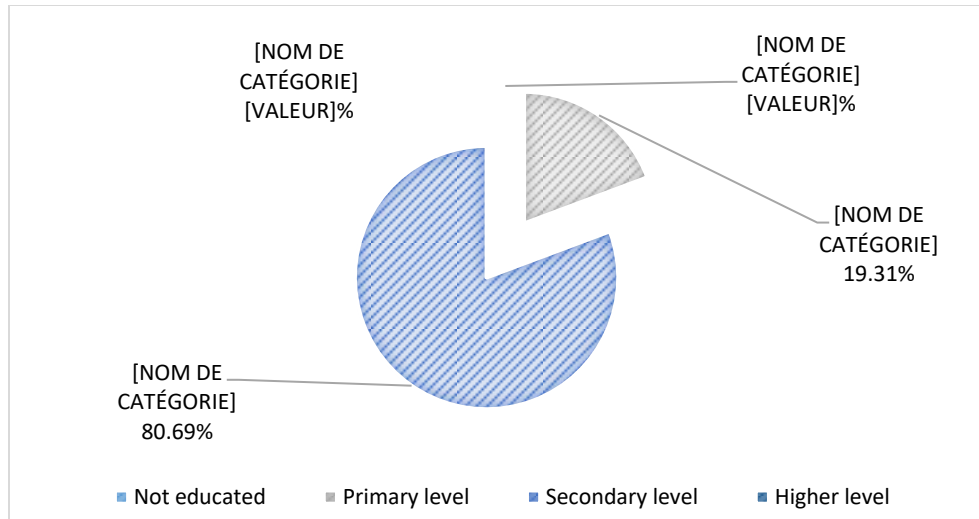
This sub-method used three techniques: Seeding, Isolation and Identification.

**3. Results and discussion**

**3.1 Socio-demographic parameters**

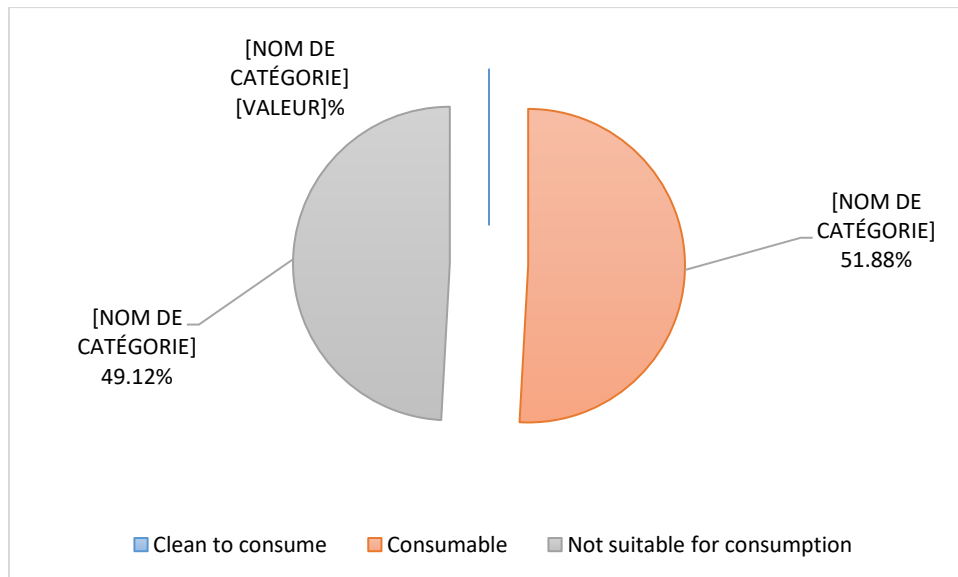
We were interested in the level of education of the vendors, the types of transport of the slaughtered meat, the way the meat sold at the markets was displayed, and the personal opinion of the vendors on the quality of the meat offered for sale at the markets.

We conducted our survey among 114 meat vendors in the five slaughterhouses that we divided into different markets:



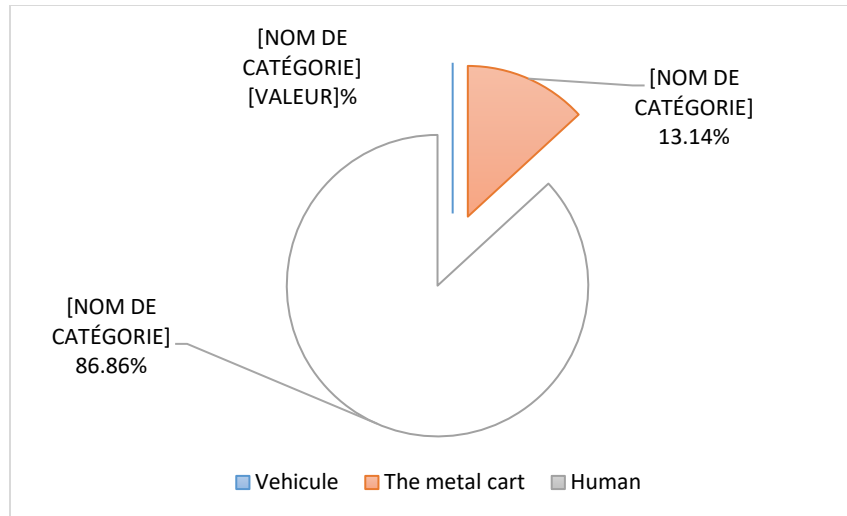
**Figure 1:** Education level of vendors at sampling sites

Secondary school students make up the highest percentage of vendors in the various markets at 80.69 percent, followed by primary school students at 19.31 percent. It should be noted that no cases of primary level and uneducated people were revealed (Figure 1).



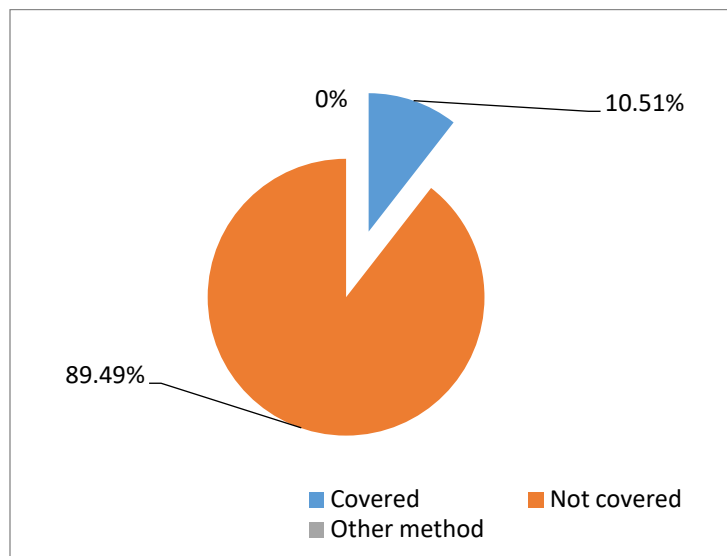
**Figure 2:** Sellers' personal opinion on meat quality

51.88% of the vendors believe that in a state of slow degradation, the meat offered for sale was still edible, 49.12% acknowledged that it is unhealthy and none of them find it safe (Figure 2).



**Figure 3:** Means of transporting meat from slaughterhouses to markets

None of the vendors **used** the vehicle to transport the meat to the market, from where the most commonly used means of transport **was** still human with 86.84%; only 13.16% **used** metal carts.



**Figure 4:** Method of displaying the meat sold at the different markets

The majority of vendors, 89.47%, **exposed** their meat to the open air, which **was** the real source of meat contamination, and only 10.53% cover their products.

### 3.2. Microbiological analyses of pig meat

The results obtained during the microbiological analyses of samples of meat produced in the five slaughterhouses focused on the microbial load involved in various food poisonings and contamination germs (diseases of dirty hands).

**Table 3:** Isolation and Enumeration of Germs by Sampling Site

		Slaughterhouses				
		Gambela	Liberté	Marché Centrale	Matete	Bumbu
<b>GERMS</b>	<i>Candida albicans</i>	6.10 <sup>3</sup>	Sterile	36.10 <sup>4</sup>	48.10 <sup>4</sup>	Sterile
	<i>Citrobacter freundii</i>	20.10 <sup>4</sup>	12.10 <sup>4</sup>	Sterile	Sterile	Sterile
	<i>Escherichia coli</i>	Sterile	Sterile	135.10 <sup>4</sup>	Sterile	12.10 <sup>4</sup>
	<i>Enterobacter agglomerrans</i>	Sterile	Sterile	Sterile	45.10 <sup>4</sup>	Sterile
	<i>Klebsiella oxytoca</i>	Stérile	Sterile	81.10 <sup>4</sup>	Sterile	Sterile
	<i>Proteus mirabilis</i>	24.10 <sup>4</sup>	135.10 <sup>3</sup>	Sterile	Sterile	Sterile
	<i>Salmonella spp</i>	95.10 <sup>4</sup>	45.10 <sup>5</sup>	8.10 <sup>4</sup>	5.10 <sup>4</sup>	Sterile
	<i>Shigella dysenteriae</i>	Sterile	Sterile	Sterile	35.10 <sup>4</sup>	Sterile
	<i>Staphylococcus aureus</i>	7.10 <sup>4</sup>	Sterile	85.10 <sup>4</sup>	Sterile	9.10 <sup>4</sup>
	<i>Yersinia enterocolitica</i>	Stérile	45.10 <sup>3</sup>	Sterile	Sterile	Stérile

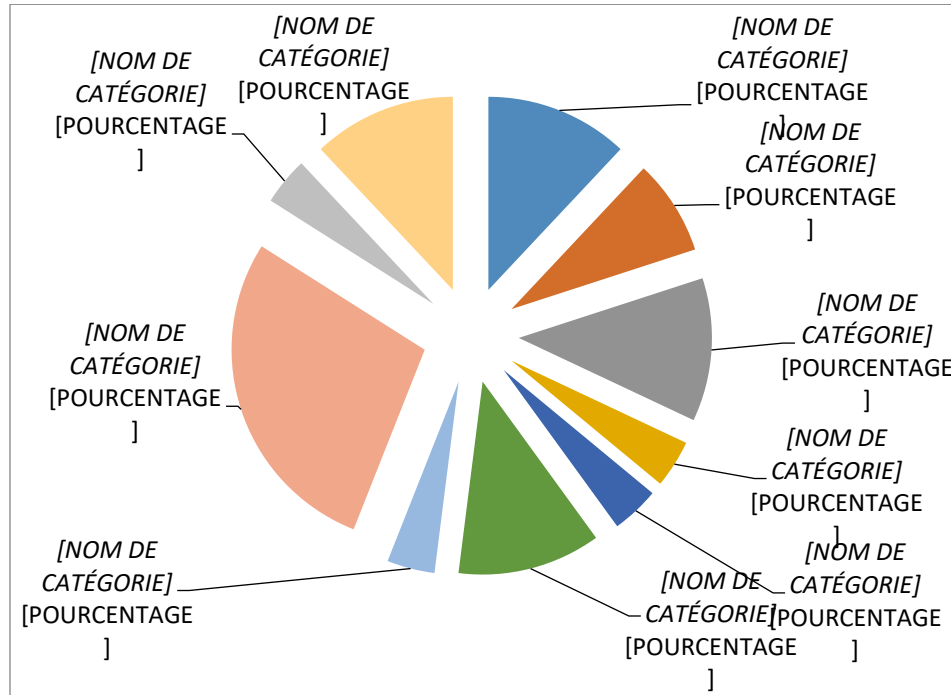
Of all the samples examined, two had a higher number of germs. One of the Liberté market samples with 135.10<sup>3</sup> germs of *Proteus mirabilis* and one of the Marché central market samples with 135.10<sup>4</sup> germs of *Escherichia coli*.

One of the Gambela samples had the lowest number of *Candida albicans* sprouts with 6.10<sup>3</sup>. The other samples had more than 6.10<sup>3</sup>.

**Table 4:** Degree of contamination of samples per slaughterhouse

Slaughterhouses	<i>Candida albicans</i>	<i>Citrobacter freundii</i>	<i>Escherichia coli</i>	<i>Enterobacter agglomerrans</i>	<i>Klebsiella oxytoca</i>	<i>Proteus mirabilis</i>	<i>Salmonella spp</i>	<i>Shigella dysenteriae</i>	<i>Staphylococcus aureus</i>	<i>Yersinia enterocolitica</i>	Total
Gambela	1	1	-	-	1	1	-	1	-	-	5
Liberté	-	1	-	-	2	1	-	-	1	-	5
Marché Central	1	-	1	1	-	1	-	1	-	-	5
Matete	1	-	-	-	-	-	1	3	-	1	6
Bumbu	-	-	2	-	-	-	-	2	-	-	4
<b>Total</b>	3	2	3	1	3	3	1	7	1	1	25

The slaughterhouse in Matete had the highest level of contamination with 6 contaminations out of 6 samples, the slaughterhouse in Bumbu was the least contaminated with 4 contaminations out of 6 samples. The others had 5 out of 6.



**Figure 5:** Frequency of germs isolated and identified in 5 markets of study

Of all the germs isolated and identified, *Staphylococcus aureus* presents the highest frequency (7) or 28% contamination, *Enterobacter agglomerans*, *Klebsiella xyloca*, *Shigella dysenteriae* and *Yersinia enterocolitica* were the least frequent with 4% contamination; *Citrobacter* occurs twice, or 8%, and the others 3 times out of 25 or 12% contamination (**Figure 5**).

## DISCUSSION

The vendors surveyed were of average educational level, with 80.69% at the secondary level and 18.42% at primary level.

A very small proportion (0.88%) of the vendors surveyed considered the meat offered for sale in private slaughterhouses in Kinshasa to be fit for consumption. But for 48.15% of the sellers, the meat put on sale at the market was unfit for consumption; for 50.88% it was edible.

Almost all the meat put on the market **was** transported by humans (86.86%). Human contact with food is said to be a source of contamination. The metal carts used (13.14%) **were** not exclusive **to the transport** of meat.

**The vendors exposed the meat to the open air represented 89.49% hence** the high risk of microbial contamination by different vectors; only 10.53% of the vendors **covered** their products.

The results of the microbiological analyses carried out throughout our research corroborate with those of Samba (2004), Masungi (2003), Kunga (2004) and Tshibanda (2006), which allowed the successive identification and isolation of bacteria of the *Enterobacteriaceae* family : *Citrobacter freundii*, *Escherichia coli*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Salmonella spp*, *Shigella dysenteriae*, *Yersinia enterocolitica*; the family *Micrococcaceae*: *Staphylococcus aureus*, and the fungus *Candida albicans*. These germs are responsible for food poisoning resulting in diseases such as **diarrheal**, respiratory, urogenital, etc.

Our results show that 12 % of the contaminated samples **were** due to ***Salmonella spp*** whose presence makes the sample declared unfit for human consumption, 28 % of the contaminated samples **were** due to ***Staphylococcus aureus*** whose number of samples **were** well above 102 and therefore considered unfit for human consumption. All other samples contaminated by other *Enterobacteriaceae* representing 48% **were** below the threshold required by the FAO to declare the sample clean or unfit for human consumption, three samples are contaminated by *Candida albicans* representing 12%.

In addition to the Matete market where 42.8% of the samples **were** contaminated with ***Staphylococci aureus***, three out of six samples **may** be declared unfit for consumption; the samples from the other sites could also be declared unfit for consumption **because the bacterial count reveal were above the threshold recommended by the FAO**. These **were**: 33.3% (2 cases/6) of samples contaminated with ***Staphylococcus aureus***; Central Market 33.3% (*Staphylococcus* and *Salmonella*); Gambela 33.3% (*Staphylococcus* and *Salmonella*), Liberté Market 20% of samples contaminated with *Salmonella spp*.

## CONCLUSION

The results of our study may complement the scarce public health data on pork-borne zoonoses in Kinshasa (The Democratic Republic of the Congo, DRC) toward a better understanding of their relative impact and importance. Of the work carried out and found on the search for microorganisms from fresh meat sold in Kinshasa's markets, most **were** based on beef.

Given the importance and the number of **the slaughter** of small ruminants and pigs in slaughterhouses as well as the eating habits of consumers with regard to these species, it requires special attention from experts in the field as well as from the competent authorities so that there can be significant improvements in order to help slaughterers to do better and make consumers safer. A survey of 114 meat sellers in the targeted slaughterhouses shows that the meat offered for sale does not meet the required standards.

Indeed, the quality of the meat to be consumed is not only limited to its organoleptic characteristics, but also to the conditions under which it is preserved, **transported, and** sold at the various distribution points. The organoleptic and nutritional potential of **pig meat** in particular means that it is both attractive (appealing) and perishable, which means that it must be handled and stored with care so as not to damage the health of consumers. Research on the enumeration, **isolation,** and identification of microbes on meat from pigs slaughtered and sold on the markets in Kinshasa has enabled us to discover the germs responsible for meat spoilage and the various diseases caused by them in consumers of spoilt meat.

This analysis gave us two groups of germs: bacteria and fungi. Bacteria are made up of two families: *Enterobacteriaceae* and *Micrococcaceae*, the second group, that of fungi, precisely *Candida albicans* which is a yeast.

We are not responsible for stating that the germs identified are the only ones that can be found in samples taken from private slaughterhouses in Kinshasa, the media used **were** mostly selective and other techniques or methods could certainly reveal other germs.

Given the clear relevance of pork as a possible source of human illness in Kinshasa (The Democratic Republic of the Congo, DRC), further efforts should be undertaken to monitor the related animal and human disease burdens and to strengthen food safety throughout the pork production chain.

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