



# Microbiological Analysis and Identification of Pathogenic Microorganisms on Currency Notes (Congolese Francs) in Kinshasa, Democratic Republic of the Congo

Jeff Bekomo Iteku<sup>1\*</sup>, Donel Moswala Likabo<sup>1</sup>, Aaron Lelo Pambu<sup>1</sup>,  
Gédéon Ngiala Bongo<sup>1</sup>, Ruth Katunda<sup>1</sup>, Octavie Metila Lunguya<sup>2</sup>  
and Joseph Kasali Lumande<sup>1</sup>

<sup>1</sup>Department of Biology, Faculty of Science, University of Kinshasa, Kinshasa, Democratic Republic of the Congo.

<sup>2</sup>Department of Bacteriology, Laboratory of Bacteriology Research, National Institute of Biomedical Research, Democratic Republic of the Congo.

## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author JBI designed the study. Authors DML, ALP and GNB wrote the protocol and the first draft of the manuscript. Authors JKL, OML and GNB managed the analyses of the study. Authors RK and GNB managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** The incidence of infectious diseases is still a vital concern in developing countries. Recently, hygienists have focused on the risk of transmitted diseases through currency notes. This study aims at the determination of potential pathogenic microorganisms found on the Congolese Francs currency notes circulating in Kinshasa, Democratic Republic of the Congo.

**Place and Duration of the Study:** This study was carried out in Kinshasa city, in the Democratic Republic of the Congo between September 3 and 29, 2019 at the Bacteriology Laboratory of the National Institute for Biomedical Research.

\*Corresponding author: E-mail: [Jeffitekubekomo@gmail.com](mailto:Jeffitekubekomo@gmail.com);

**Methods:** During this study, 36 currency notes of different denominations have been used for microbiological analysis. Currency notes were collected from vendors of the Central market and currency note dealers at Kintambo Magasin market in Kinshasa. The identification of microorganisms (bacteria and fungi) was performed using gram staining and biochemical analyses.

**Results:** The findings reveal the presence of following microorganisms, namely *Bacillus* spp, *Staphylococcus aureus*, *Staphylococcus* spp., *Enterobacter* spp, *Escherichia coli*, *Serratia* spp, *Citrobacter* spp, *Salmonella enteritidis* as well as molds on the Congolese currency notes. It should be observed that circulated currency notes in Kinshasa are contaminated by bacteria and fungi. The contamination rate was based on the fact, that money is new, clean or dirty.

**Conclusion:** Congolese currency notes constitute the potential sources of infectious disease transmission if hygienic conditions are not respected. Molecular studies are required in order to determine the antibiotic resistance gene of these microorganisms. As the population does not know how to store these notes properly, their contamination would eventually become a major public health hazard. Therefore, a need of an awareness of the population in order to apply hygienic rules while handling currency notes. This is for the first time that such a study is being carried out in the Democratic Republic of the Congo.

**Keywords:** *Currency notes; microbiological contamination; hygiene; Kinshasa; Democratic Republic of the Congo.*

## 1. INTRODUCTION

Although the incidence of microbial diseases is not very high in developed countries, epidemics of infections are still a vital concern in most of developing countries [1-3]. In developing countries, microbial diseases have a large number of victims, both in terms of morbidity and mortality [3-4]. Every year, 3 to 5 billion episodes of infectious cases of diarrhoea (caused by some 30 possible pathogens) occur worldwide, resulting in between 5 and 10 million deaths, mainly among children. The pathogens that cause infections in humans cover a broad spectrum [5-7].

There are several means of infectious disease transmission, and currency notes are also among. Money whether in forms of coins or currency notes is handled by everybody throughout the world. It has promoted trade in communities since its first introduction in China approximately 1,000 AD [7]. Mostly used as a medium for exchange for goods and services; settlement of debts and for deferred payments in economic activities [8]. Current currency notes are made from a special blend of linen; cotton and gelatin from animals for the surface coating of currency paper note with few segments of fiber and the mixture of all the materials used make them strong. Recently, many nations adopted the plastic polymer made currency transferring from paper made [8-9]. However, the general hygiene levels of a community or society contribute to the amount of microbes found on currency notes, and thus increases chances of transmission of microbes during the handling of money.

Therefore, currency notes represent a universal medium for the transmission of bacteria in the environment and among humans. 'Dirty' money (money contaminated with pathogenic microorganisms) is always in circulation [10].

People and government are informed about spread of pathogens through food, air, water and have taken enough steps to control it [11]. Nevertheless, they are not aware about the possibility of acquiring infections while applying saliva on fingers while counting currency notes, which are widely exchanged from hand to hand [12]. During the counting, the notes may be contaminated with numerous pathogens from respiratory and digestive tracts [12]. Therefore, it represents a universal means of pathogen transmission in the environment and among humans [13]. There can be no doubt that advances in hygiene during the 19<sup>th</sup> and 20<sup>th</sup> centuries, along with other aspects of modern medicine, have combined to improve both the length and quality of our lives [14].

Recently, hygienists have focused on the risks of transmission of infectious diseases through currency notes [12]. Several human's daily activities constitute different sources of currency note contamination. Numerous findings reported that it could be through coughing or sneezing droplets, using saliva when counting the notes, soil, dust, water, dirty wounds, handlers skin and hands micro-flora, contacting with preceding polluted hands or extra stuffs and emplacement on grubby work surfaces. Moreover, few manners of managing money including, maintaining denominations within brassiere,

pockets, socks, beneath the mattresses and pressing in the hands, consistently initiate microorganisms to the currency notes [12].

The placement of currency notes on dirty surface may allow microorganisms on the skin to be transferred from cashiers, salespeople to the public through currency notes that they handle [15]. Person-to-person contact and transfer through common contact surfaces are further routes that can lead to widespread infectious outbreaks of pathogens such as norovirus and methicillin resistant *S. aureus* [14, 16-19]. While the antimicrobial resistance has steadily been increasing, e.g. with Extended-Spectrum Beta Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella spp* contaminated currency notes and coins contribute to the transmission of these multi-drug resistant microorganisms in the community [20].

Contamination of objects by pathogenic microorganisms is of much public health concern as contaminated materials can be sources of transmitting pathogens. Studies of the contamination of money with microbial agents is lacking in developing countries. Investigations on the contamination of currency notes with microorganisms are deficient in many developing countries. Consequently, the shortage of information may contribute to the absence of public health policies regarding currency usage, handling and circulation [13, 15]. Nowadays, the carriage of pathogens on currency notes has been studied in Europe, America, Asia and many other countries of Africa with little data or none on the Democratic Republic of the Congo (DRC).

As all hygienic practices are not considered in developing countries like DRC, there is a reason to wonder whether the Congolese franc currency notes circulating might not also be vectors for the transmission of pathogenic microorganisms, causing a public health concern, as they pass from hand to hand?

Since each citizen keeps his currency notes in his own way and passes them on to another in the conditions in which they are found (dirty or clean), currency notes could be a vector for the transmission of pathogenic microorganisms causing illnesses related to the lack of hygiene.

This study aims at the determination of potential pathogenic microorganisms found on the

Congolese Francs currency notes circulating in Kinshasa, DRC. The acquired knowledge of the microbial diversity on Congolese currency notes in circulation can provide the basis to raise health awareness in people during currency handling and effective control of infection transmission.

In the context of clinical microbiology, this work contributes to an understanding of the pathogenic microorganisms that can live on currency notes, as well as to an awareness of the importance of hygienic practices in the handling of currency notes in order to reduce contamination. Furthermore, the findings of this research may be a template for further studies. This is for the first time that such a study is being carried out in DRC.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was conducted in two great markets of Kinshasa city, in DRC. The Central Market located in the intersection of Kinshasa, Barumbu and Gombe municipalities and the market of Kintambo Magasin located in Kintambo municipality (Fig. 1).

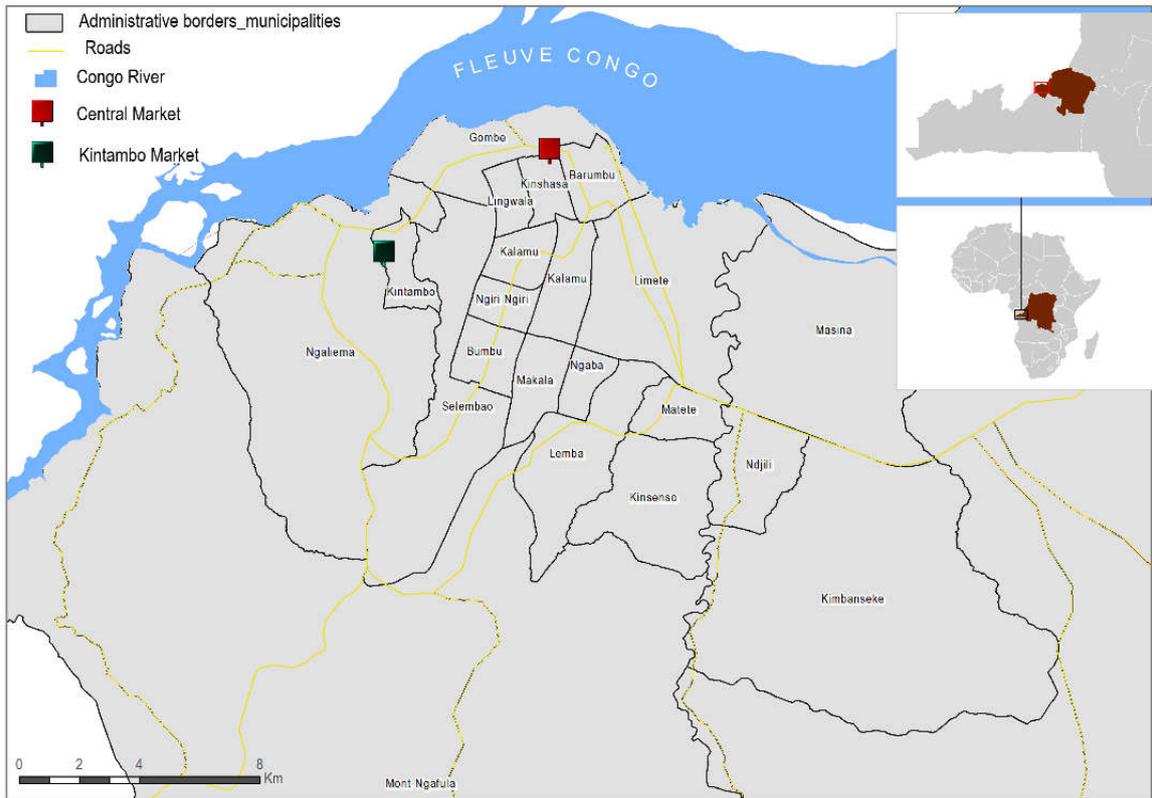
### 2.2 Materials

The biological material used was microorganisms found on different denominations of the Congolese currency notes.

### 2.3 Methods

#### 2.3.1 Sample collection

A total of 36 currency notes samples in circulation were collected from vendors in Kinshasa Central market and new currency notes were collected from foreign exchange dealers at Kintambo Magasin market in an aseptic manner by allowing individuals to deposit the currency notes inside sterile polyethylene bags. These plastic bags were sealed in order to avoid any other form of contamination. Samples were labelled and transported to the Bacteriology laboratory of the National Institute for Biomedical Research (INRB) for analysis. All the images (pictures) were captured using a Tecno CX Air brand mobile device with 100X magnification. The study was carried out between September 3 and 29, 2019.



**Fig. 1. Administrative map of Kinshasa city, DRC**

**Table 1. Details of different denominations selected for analysis**

Denominations	New currency notes	Clean currency notes	Dirty currency notes
50	<ul style="list-style-type: none"> <li>• KE2471567G</li> <li>• KE2471566G</li> </ul>	<ul style="list-style-type: none"> <li>• KC4379890B</li> <li>• KE8026644E</li> </ul>	<ul style="list-style-type: none"> <li>• KD2076309Y</li> <li>• KD7725804Y</li> <li>• KB2821265U</li> </ul>
100		<ul style="list-style-type: none"> <li>• MD9922017X</li> <li>• MD4780437K</li> </ul>	<ul style="list-style-type: none"> <li>• MC3006769T</li> <li>• MD01496393U</li> <li>• MD2602281X</li> <li>• MD3596451W</li> </ul>
200	<ul style="list-style-type: none"> <li>• NC3917040V</li> <li>• NC1255628H</li> </ul>	<ul style="list-style-type: none"> <li>• NC1065555R</li> <li>• NC8243792T</li> </ul>	<ul style="list-style-type: none"> <li>• NC9172677N</li> <li>• NB5363888T</li> <li>• NA2431486O</li> <li>• NC513155E</li> </ul>
500	<ul style="list-style-type: none"> <li>• PJ8402101T</li> <li>• PJ8402102T</li> </ul>	<ul style="list-style-type: none"> <li>• PH3795623T</li> <li>• PB8506774T</li> </ul>	<ul style="list-style-type: none"> <li>• PG0066301T</li> <li>• U4382552C</li> </ul>
1000	<ul style="list-style-type: none"> <li>• QC7002359K</li> <li>• QC7002360K</li> <li>• QC6836639A</li> </ul>	<ul style="list-style-type: none"> <li>• Q5677502L</li> <li>• Q3837790R</li> </ul>	<ul style="list-style-type: none"> <li>• Q3295057X</li> <li>• Q3341068V</li> </ul>
10000	<ul style="list-style-type: none"> <li>• S4180913F</li> </ul>	<ul style="list-style-type: none"> <li>• S3717823O</li> </ul>	

Table 1 provides the details of different denominations selected for the current study.

### 2.3.2 Physical condition of the currency notes

The currency notes selected had various physical conditions, and they were classified as : new, clean and dirty. The term "New" refers to

recently produced notes. "Clean" refers to notes that were clean but already in circulation, and "Dirty" refers to notes that were only half as clean as when they were originally produced and had lost the clarity of writing and were in the condition of being refused when purchased (Fig. 2) [21].



**Fig. 2. Currency notes of 50 (new), 100 (clean) and 200 (dirty) Congolese francs**

### **2.3.3 Isolation of microorganisms**

Each bag was carefully opened around the flame. The currency note, held fixed with an anatomical forceps previously flambéed in the Bunsen burner, was rubbed on both sides with a sterile swab moistened in sterile saline solution (0.9% NaCl). Then, the swab was inoculated with Heart-Brain Broth and Selenite Broth, which was incubated at 37°C for 18-24 hours.

After 24 hours, plates were observed for bacterial colonies in different media of which blood agar, Mannitol salt agar and Mac Conkey. Pure isolated colonies were Gram differentiated and then biochemically identified using Indole, Catalase, and metabolism of glucose.

### **2.3.4 Purification**

Thereafter the incubation, the reading was performed on different media used. The reading sequence was as follows: on Blood agar (the growth and shape of microorganisms); On Mac Conkey (for lactose fermentation before starting the biochemical gallery i.e. biochemical tests); on Mannitol Salt Agar (to determine if the mannitol tested positive or negative). The reading was to determine whether the mannitol is positive or negative); and on the Selenite Broth, it allowed to observe the growth of fungi.

### **2.3.5 Identification of microorganisms**

The identification of isolated strains involved a series of preliminary tests (macroscopic and

microscopic examination) which should enable us to group the identified microorganisms into different classes, and some biochemical tests were carried out to enable the identification at the species level.

#### **(i) Macroscopic examination**

##### **a. Bacteria**

The examination was based on the search of the following characteristics: size, color, pigmentation, shape, surface appearance, colony edge appearance, consistency and opacity [22].

##### **b. Fungi**

###### **- Yeast**

Yeast colonies do not present any great particularity compared to bacterial colonies.

###### **- Molds**

For filamentous fungi, the analyzed characteristics were: (i) the appearance of the colony on the surface and the shape; (ii) the color of the colony on both sides; (iii) the size (diameter of the colony in mm) and (iv) the pigmentation.

#### **(ii) Microscopic examination**

##### **1. Fresh condition**

It is a very simple examination of implementation and takes place under an optical microscope

(brand HITACHI) at 40X. This allows: (i) the observation of living bacteria and the determination of their morphology, their mode of grouping, their possible motility and the approximate quantity of bacteria. And (ii) to study the morphological characteristics of molds: appearance of the mycelium, hyphae partitioned or not, appearance of conidia and their mode of aggregation [22].

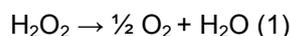
## 2. Gram staining

Examination of the Gram stained smear allows the observation of any bacteria present, differentiating them into Gram positive and Gram negative according to their morphology and dye affinity. It allows also to assess their abundance, grouping, homogeneity or morphological heterogeneity [22].

### 2.3.6 Biochemical tests

#### (i) Catalase test

Catalase is an enzyme present in most strict aerobic and facultative anaerobic bacteria. It breaks down hydrogen peroxide into water and oxygen, which is released in the following reaction [22].



The release of gas bubbles indicates the presence of catalase i.e. the test is positive.

#### (ii) Classical biochemical gallery

In order to identify Enterobacteriaceae, several traits need to be studied.

#### 1. Analysis of carbohydrates and energy metabolism

This analysis consisted on the use of lactose and sucrose, fermentation with or without glucose gas, production of H<sub>2</sub>S. Meanwhile, the use of citrate as a sole source of carbon and energy: the purpose of this test was to find out whether the bacteria use citrate as a source of carbon.

#### 2. Analysis of protein metabolism

Here, the focus was on the production of indole (Tryptophan metabolism) and the one of bacterial decarboxylases ODC (Ornithine decarboxylase), LDC (Lysine decarboxylase and ADH (amino acid metabolism). Table 2 presents different biochemical characteristics requested for Enterobacteriaceae.

## 3. RESULTS AND DISCUSSION

### 3.1 Identification and Isolation of Microorganism

The used method allowed the identification of bacteria before and after Gram staining.

#### 3.1.1 Bacteria

##### 3.1.1.1 Macroscopic study

After 24 hours of incubation at 37 °C, colony aspects on selective medium is presented below.

**Table 2. Biochemical characteristics requested for Enterobacteria**

Media	Researched test	Culture	Incubation	Positive result	Negative result
<b>Klidler</b>	Fermentation of Lactose and Glucose	Tight striae for the slope	24 h at 37°C	Slope and base are colored yellow.	Red-brown or pink color
	Production of gas	Simple prick for the base		Air Bubbles in Agar	Agar remains unchanged
	Production of H <sub>2</sub> S	Simple prick for the base		Darkening	No darkening
<b>Simmons' citrate</b>	Use of citrate as the sole source of carbon	Longitudinal slope stries	48 h at 37°C	From green to blue	Green
<b>MIL (Motility Indole Lysine)</b>	Motility	Piqure centrale	24 h at 37°C	Formation of a central axis veil	Absence of veil
<b>LDC ODC PGUA ONPG</b>	Hydrolysis of amino acid to amine	Bacterial suspension + paraffin oil	24 h at 37°C	From purple to dark orange	Yellow

Legend: LDC: Lysine decarboxylase; ODC: Ornithine decarboxylase, PGUA : Beta Glucuronidase, ONPG: Ortho-nitrophenyl beta galactosidase

**Table 3. Macroscopic aspect of isolated strains on BA and MSA**

Used media	Strains	Macroscopic aspects				
		Size (mm)	Form	Surface	Consistence	Color
BG	50/KB2	1-2	I	Mucous plate	Dry	Tern white
	50/KE27	1	R	Rounded	Mucous	Tern white
	100/MC3	2	I	Rough plate	Dry	Tern white
	100/MD2	2	I	Rough plate	Dry	Tern white
	100/MD4	1	R	smooth	Mucous	Tern white
	200/NC8	1-2	I	Rough plate	Dry	Tern white
	1000/QC7	2	R	Rounded smooth	Mucous	Tern white
	1000/Q38	2	I	Rough plate	Dry	Tern white
	1000/Q56	2	I	Rough plate	Mucous	Tern white
	10000/S37	2	R	Rounded	Dry	Tern white
MSA	50/KB2	1-2	I	Rough	Dry	Yellow
	50/KE27	1	R	Rounded	Mucous	Yellow
	100/MC3	2	I	Rough	Dry	Red
	100/MD2	2	I	Rough	Dry	Yellow
	100/MD4	1	R	Smooth	Mucous	Red
	200/NC8	1-2	I	Rough	Dry	Yellow
	1000/QC7	2	R	Rounded smooth	Mucous	Yellow
	1000/Q38	2	I	Rough	Dry	Yellow
	1000/Q56	2	I	Rough	Mucous	Red
	10000/S37	2	R	Rounded smooth	Dry	Yellow

Legend : I : Irregular form; R : Regular form; BA: Blood agar; MSA : Mannitol Salt Agar

From the above, it was observed that most of bacterial colonies have 1-2 mm of diameter, are regular, smooth domed, with a smooth appearance and rough in consistency. But it was also observed another aspect of colonies, which varied and became of rough, flat and of slightly irregular type having a dry aspect. All these characteristics represent *Staphylococcus spp.*

The difference between the bacteria on MSA medium and those on BA medium is the color, which is mostly yellow for those cultured on MSA.

Thereupon, a catalase test was necessary in order to confirm the presence of *staphylococci*. Then, the test was positive i.e. the observed characteristics were for *Staphylococci*.

Fig. 3 describes the evolution of the catalase + test confirming the presence of *Staphylococci*.

To establish the nature of *S. aureus*, the coagulase test was performed from rabbit plasma and the strains 50 / KE27, 200 / NC8 and 1000 / QC7 were tested coagulase +.

### 3.1.1.2 Microscopic analysis

We performed a gram staining in order to determine the type of microorganisms. .



**Fig. 3. Catalase positive test for *S. aureus***

Fig. 4 shows the *Escherichia coli* strain (100 / MC3) and *S. aureus* strain (200 / NC8).

### 3.1.1.3 Isolated strains from Mannitol Salt Agar and Blood agar media

Microscopic examination and enzymatic tests revealed that following strains 50/KB2, 100/MD2, 100/MD4, 200/NC8, 1000/QC7, 1000/Q38, 1000/Q56 and 10000/S37 are grouped together in bamboo and are a little thicker and colored purple (Gram positive) motile, having an enzyme,

catalase and these characteristics are quite similar to those of enterobacteria.

The set of preliminary and biochemical test results is presented in the Table 4:

According to the identification key used at INRB, the isolated strains represent the following species:

- **50/KB2** : *Serratia spp*
- **100/MC3** : *Escherichia coli*
- **200/NC8** : *Enterobacter spp.*
- **100/MD4** : *Klebsiella pneumoniae*

Enteric bacteria namely: *E.coli*, *Citrobacter sp*, *Enterobacter sp* and *Serratia sp* commonly called coliforms isolated from our analyzed samples are present in the intestinal flora of humans and other warm-blooded animals. So their presence indicates a faecal contamination by cross contamination with raw products or poor personal hygiene: improper washing of hands after using the toilet, handling of currency notes with bloody

hands or contaminated with animal faeces (case of a butcher).

### 3.1.1.4 Strains isolated from BA, MSA and Dnase media

#### Strain 50/KE27:

- On BA : is a purple (gram positive), domed bacillus, grouped in regular clusters with catalase +.
- On MSA : Mannitol +
- On Dnase : Dnase +

#### Strain 200/NC8:

- On BA : is a flat shell colored in purple (gram positive), grouped in irregular clusters with catalase +.
- On MSA : Mannitol +
- On Dnase : Dnase +

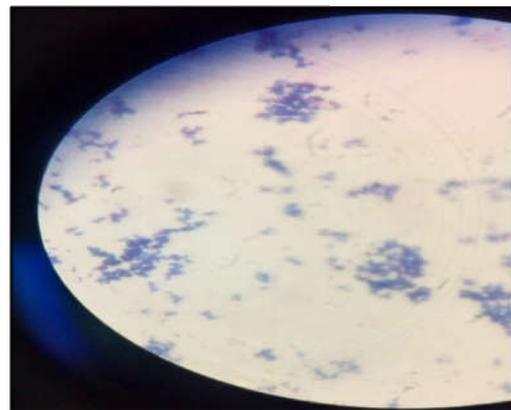


Fig. 4. Observation after gram staining of 100/MC3 strain (*E. coli*) and of 200/NC8 strain (*S. aureus*)

Table 4. Some identified samples

	Tests	100/MD3	100/MC3	200/NC8	100/MD4
Fresh	Motility	-	+	+	+
Gram coloration	Gram	+	-	+	+
	Form	Bacillus	Bacillus	Bacillus	Bacillus
Enzymatic test	Catalase	+	+	+	+
	Glucose	+	+	+	+
	Lactose	-	+	+	+
	H <sub>2</sub> S	-	-	-	-
Biochemical tests	Gase	-	+	+	+
	Citrate	+	-	+	+
	Motility	+	-	+	-
	Urea	-	-	+	+
	Indole	-	+	-	-
	LDC	-	+	-	+

**Strain 1000/QC7:**

- On BA : is a domed shell colored in purple (gram positive), grouped in regular clusters with catalase +.
- On MSA : Mannitol +
- On Dnase : Dnase +

**Strain 50/KE27:**

- On BA : is a rounded bacillus colored purple (gram positive), grouped in regular clusters with catalase +.
- On MSA : Mannitol +
- On Dnase : Dnase +

**Strain 200 / NC8:**

- On BA : is a flat shell colored purple (gram positive), grouped in irregular clusters with catalase +.
- On MSA : Mannitol +
- On Dnase : Dnase +

**Strain 1000/QC7:**

- On BA: is a domed shell colored purple (gram positive), grouped in regular clusters with catalase +.
- On MSA : Mannitol +

The global result is presented in Table 5 below.

Based on these results, we suspect that strains 50/KE27, 200/NC8, 1000/QC7 are *S. aureus*, by performing the coagulase test with rabbit serum.

*Staphylococci* (coagulase negative and *S. aureus*) isolated from currency notes are part of the normal flora of the skin and the human and animal mucous membranes. They are also found in the environment (water, soil, air, food, objects); their presence is justified by the handling. Some

*Staphylococci* (coagulated negative) are harmless with an important role in the pathogenicity. Unlike coagulase-negative staphylococci, *S. aureus* is recognized as a pathogen and can survive on currency notes for more than eight days. This lifetime allows a risk of high contamination.

**3.1.1.5 Isolated strains from Mueller Hinton**

After the gram staining of the used strains, we noticed that there were some strains that were bacillus gram +, grouped in bamboo and a little thicker. For a good identification, the following strains: 1000/Q33, 200/NB5, 50/KD7 and 500/PG0 were subjected to different tests in order to determine each bacillus species with precision.

These tests are namely: Motility after another gram staining; Citrate; Voges; Leukitinase. After analysis of these four tests, we have identified three different types of *Bacillus*, of which: *B. subtilis*, *B. anthracis* and *B. cereus*

**3.1.1.6 Seeding on Selenite Broth and Salmonella Shigella (SS) medium**

Selenite broth allows the enrichment of *Salmonella*.

Considering different aspects of this research, the culture was performed on Selenite broth in order to ensure the presence of *Salmonella spp.* After all the currency notes, all swabs were cultured in 1 mL of saline solution and then incubated for 15 minutes. Thereafter, subcultures were performed in tubes containing selenite broth, which were incubated for 24 hours at 37 ° C.

Fig. 5 illustrates the subculture performed on selenite broth

**Table 5. Specificity of *S. aureus* after gram staining and other tests**

Tests		50/KE27	200/NC8	1000/QC7
Mannitol		+	+	+
Fresh	Motility	-	+	-
Gram	Gram	+	+	+
	Form	Bacillus	Cocci	Cocci
Enzymatic test	Catalase	+	+	+
DNASE		+	+	+



**Fig. 5. Culture in the Selenite broth**

After the incubation, it was observed that there was a change in color only in five tubes out of thirty-six (these strains are therefore 200/NC1, 200/NA2, 500/PJ8, 500/U43 and 10000/S37). As there was a bacterial growth in these five tubes, we did other subcultures on the SS agar incubated for 24 hours.

Fig. 6 presents the five tubes in which there was microbial growth with change in color.



**Fig. 6. Bacterial growth in Selenite broth with orange color**

After 24 hours of incubation, we have lactose negative colonies with black center on one of these five boxes of SS agar. As a result, another biochemical identification gallery was

performed, namely: Kligler iron agar, Simmons citrate, MIL, urease on diatabs, ONPG, ODC.

This test allowed to determine that it was *Salmonella enteritidis*.

### 3.1.2 Fungi

As to fungi, which can be yeasts or molds, we transplanted onto the Sabouraud Chloramphenicol medium and we incubated all these tubes for 24 hours at 37 ° C.

Fig. 7 illustrates the growth of fungi on Sabouraud Chloramphenicol medium in five tubes.

The contamination by molds is inevitable because they are present everywhere in the environment (air, surface, etc.). Therefore, this increases the frequency of contamination of money in general and currency notes in particular due to their cottony texture and the moisture. Molds are harmless for the majority, but can cause different forms of disease in immunocompromised patients. Her infections are usually caused by inhaling spores.

### 3.2 Distribution of Different Microorganisms of Currency Notes Circulating in Kinshasa

Fig. 8 shows the distribution of microorganisms isolated in the laboratory on the 36 denominations selected.

As observed above, it was observed that *Bacillus spp* with 36.8% are the most abundant (36.8%) on the studied currency notes, followed by *Enterobacter spp* (17.5%); *Staphylococcus spp* (26.3%); *Klebsiella pneumoniae* (8.79%); *Serratia spp* (5.29%); *Citrobacter spp* (1.8%); *Escherichia coli* (1.8%) and *Salmonella enteritidis* (1.8%).

Afterwards, a slide serotyping was performed, which allowed to clearly clarify this genus.

**Table 6. Biochemical test for the determination of *Salmonella spp.***

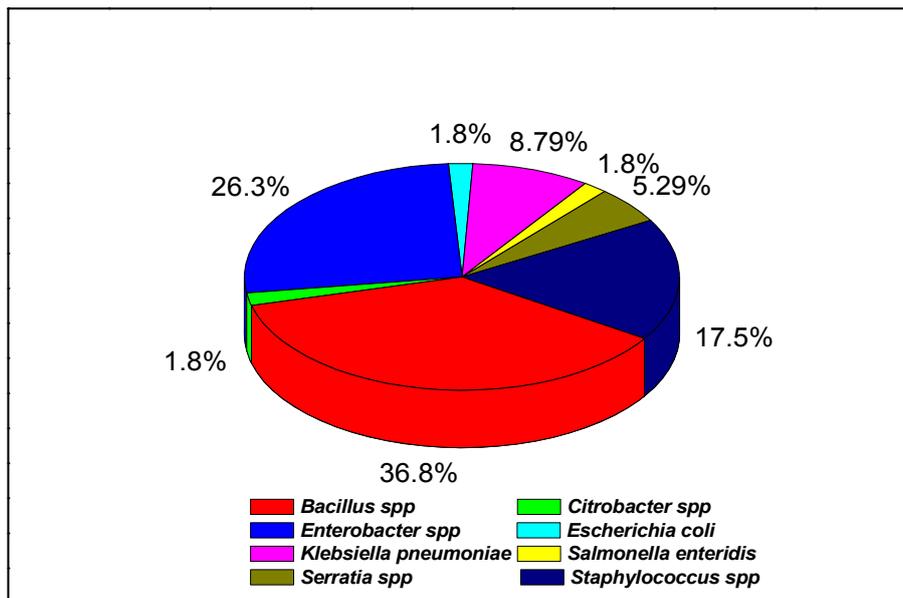
N° Isolate	Glucose	Lactose	Gas	H <sub>2</sub> S	Citrate	Urease	Motility	ONPG	LDC	ODC	Indole
10000/S37	+	-	-	+	+	-	+	-	+	+	-

**Table 7. Serotyping analysis**

N° Isolate	O	B	D	Hgm
10000/S37	+	-	+	+



**Fig. 7. Growth of fungi on Sabouraud Chloramphénicol tubes**



**Fig. 8. Distribution of microorganisms identified on different denominations of circulating currency notes in Kinshasa city**

### 3.3 Discussion

Since the nineteenth century, the hygienic status of currency notes was a speculative topic. While, *in vitro* cultures reports have demonstrated that microbial contamination of currency notes is widespread, and that money used as paper represents an important human-microbe interface [23-25]. That is due to the texture that

possess paper currency, which is rough surface and this texture provides a good niche for microorganisms and other particulates to settle and accumulate over long term and thereby constitute a potential source of infection [26].

It should be noted that the levels as well as the diversity of microbial contamination of currency notes depend on several factors like the period of

circulation, handling and its texture [26]. Yet, microbial contamination of paper money can occur by money counting machines, atmosphere, dust, soil, storage process, during usage or production process. Contamination during use is most often caused by handwashing after the toilet or false hand washing, by saliva counting, coughing and sneezing in hands [24]. As a result, paper money is contaminated with microorganisms from the human hand, mouth and even in the gastrointestinal tract microbiota. As a result of the exchange of these contaminated currency notes among people, microorganisms begin to spread, contributing to the spread of both antibiotic resistance and many virulence factors and they are constituting a risk to public health [24, 26].

The findings show that microorganisms can survive on currency notes and can cause serious illnesses related to the lack of hygiene of the people who use them. This outcome is consistent with the report of Yeo et al. [16], who stated that currency notes, as tools for exchange and integration of people, carry germs as a result of handling through dirty hands and also by airborne means due to our living environment". Several studies conducted in the United States have reported that the contamination of coins and currency notes reveals the presence of pathogenic microorganisms such as *S. aureus*, *E. coli* and *K. enterobacter*. A similar study reported the isolation of 93 types of the bacteria (including *Staphylococcus* spp, *Streptococcus* spp, *Enterobacter* spp, *Acinobacter* spp, *Pseudomonas* spp, *Bacillus* spp, *Klebsiella pneumoniae*, *Escherichia vulneris*, and Diptheroids) [27-28].

In 2014, Akoachere et al. [23] showed that bacteria and fungi contaminated most of the currency notes and coins. The isolation of these microorganisms from currency notes proves that currency can play an important role in the transmission of microbial agents in the community and present a public health problem. Other reports from countries such as Nigeria (52.5%), Nepal (75%), Saudi Arabia (72.3%), United States (94%), simply reflect the differences in hygiene practices and exchange of currency notes in different environments while showing that currency contamination is a global problem. Furthermore, other authors have shown that bacteria of following genera, namely: *Klebsiella*, *Salmonella*, *Escherichia*, *Bacillus* and *Staphylococcus* are the most common bacteria found on currency notes and for the most part

their pathogenicity is remarkable [16,21,29].

In this research, we also found the same genera of bacteria among our isolated strains as reported in the literature i.e. that our Congolese Franc currency notes contain pathogenic microorganisms which constitute a biological hazard to public health. Abrams and Waterman were the first people to declare that currency notes and coins can be counted among the objects serving as a source of microbial contamination [21]. Besides bacteria, in the current research the presence of yeasts and molds have been observed but they were not identified due to the lack of appropriate materials. Yet, it is quite possible to find the following genera: *Aspergillus* and *Penicillium* on currency notes. Some microorganisms are not pathogenic to humans (opportunistic) but are found on currency notes simply because they are found in our environment. [27]. The presence of fungi on the currency notes is from environmental contamination.

Enterobacteriaceae are generally non-pathogenic, but some strains can cause serious infections and food poisoning (*E. coli*). While others can cause primary pneumonia and peritonitis in patients with compromised immune systems, urinary tract infections (*Enterobacter agglomerans*) more and septicemia (*Serratia* sp) [22]. According to Basavarajappa et al. [30], *Citrobacter* spp, *Klebsiella* spp and *E. coli* are enteric microorganisms that are potentially pathogenic specifically when they change their living environment. In our study, we found these types of microorganisms and this explains the presence of the pathogens on our everyday currency notes. Singh et al. [31] and Oyero & Emikpe [32] showed that bacteria isolated from the currency notes were in contact with the mouth, nose, skin, and fecal contamination. This explains the lack of applying hygienic rules while handling money in the daily life. As we are living with this great pandemic of Coronavirus (Covid-19), it is compulsory for us to apply preventive measures while handling currency notes. Covid-19 is the latest string in the coronavirus family and the World Health Organization has stated that it is not certain how long Covid-19 survives on surfaces. While there is no conclusive scientific study that links the spread of the current strain of coronavirus to contaminated currency notes, the World Health Organization has advised barrier measures to maintain proper hygiene post handling of notes [33].

#### 4. CONCLUSION

This study aimed at the determination of pathogenic microorganisms found on the currency notes circulating in Kinshasa city whereby the focus was on two markets namely the Central Market and Kintambo Magasin market. The findings showed that most of microorganisms found on these currency notes are from bacterial origin.

Congolese franc currency notes constitute potential vectors capable of circulating different types of microorganisms when they pass through several hands. So, during their use, the population must avoid putting this currency in contact with food, glanders and saliva. As the population does not know how to store these currency notes properly, their contamination will eventually become a major public health hazard.

At from bacteria, fungi also were found, namely: yeasts and molds. But due to material constraints, we could not identify the species down to the specific level. Therefore, future work will allow the determination of all the microorganisms living on the currency notes. Further studies are required in order to determine if these microorganisms have antibiotic resistance genes. This issue is serious and constitutes a real challenge in the world currently.

Henceforth, washing hands after handling currency notes remains the most effective way of prevention to reduce the contamination of pathogenic microorganisms. We suggest, as in some countries in the world, that the manufacture of the Congolese currency may be made in plastic because on plastic the microorganisms have a shorter lifespan.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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