Research Article

Isolation and Caracterization of Opportunistic Environmental Fungal Flora from Bank Notes Moving in Algiers. Algeria

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ABSTRACT

The potential role of paper banknotes in the spreading of pathogenic micro-organisms is reported in many countries. The aim of the study is to determine the presence, type and nature of fungal contamination on paper currency in circulation in Algiers. 200 samples of old unsanitary cash from different denomination and aseptically harvested, are analyzed in order to evaluate their fungal opportunistic load. Microbiological methods are used, followed by biochemical tests for yeast, in order to assess the contamination level of banknotes. The fungal identification based on morphocultural and biochemical characters revealed a frequent presence of *Penicillium* spp. (14.28% - 92.30%), *Aspergillus* spp.. (3.12% - 25.70%), *Rhizopus* spp. (2.85% - 15%), *Alternaria* spp. (2.85% - 3.12%), …. and yeasts such as *Candida dubliniensis* (0% - 8.57%), *Rhodotorula* spp. (5.71% - 50%), *Candida pararugosa* (0% - 28.12%)... are the least encountered. A real dependence between the denomination of banknotes, their physical condition (age) and the fungal population is raised. The number of isolated fungal colonies is 350 CFU/ticket of 200 DA and 320 CFU/ticket of 1000 DA. The screening for the pathogens of Algerian currency cuts reveals that these microbiomes are a source of microbial infection. Bank notes are a risk to public health especially when combined with the simultaneous handling of food and therefore could lead to a spread of human infections. A sensitization of the potential risks associated with improper handling of paper currency is essential at all levels.

Keywords: Micro-organisms, mycetes, bank notes, Algerian, contamination, security.

INTRODUCTION

At about the year 1000, paper currency has appeared in China. At the beginning of the XXth century, scientists reported that the circulation of paper currency has been the cause for a several diseases transmitted by pathogenic micro-organisms [12, 19, 8]. For a long time it was uncertain whether coins and banknotes, objects exchanged by hand all over the world can actually cause fungi, bacteria and infectious viruses. Microbiological tests confirmed this theory and showed that these organisms can be isolated on coins and banknotes surface.

At the beginning of the 1970s, the presence of pathogenic micro-organisms was found on different currencies [15, 13] and more recently, the risk of transmission of these pathogenic germs, bacteria, mushrooms, virus, nematodes, and protozoans [10, 13, 17, 1, 9, 14, 2, 7], is indicated all over the world. Worse still, coins and banknotes are considered as the "dirtiest" public objects well before the handrails of staircases, touches of payment terminal, computers, mobile phones and even books in libraries.

Thus, employees of food business who manipulate food and customer's money can contaminate a number of food stuff.

Indeed, the inter-human exchange money, within the reach of all social classes belongs to all circles and to all ages. The critical factor in the transmission of a microorganism from one person to the other is mainly its capacity to survive at the paper money surface and this obstinacy depends on physicochemical parameters, porosity and peppers' age, environmental conditions [16].

The opportunist mushrooms are usually little aggressive but can cause serious complications for people having a very weakened immune system. When the favorable conditions appear in the body host (modification of the ground), they are going to allow the parasitic adaptation of numerous mushrooms saprophytics and both yeasts and filamentous mushrooms. In such conditions, all the contaminants can become opportunist or pathogenic potential.

From then on, this mean of exchange through the diverse manipulations due to the users in a living environment where hygiene regulations are often insignificant, incites to question ourselves if banknotes could not potential vectors of optionally pathogenic or opportunist germs and correspond to a public danger of health in our country.

Worried thus about the existence of a contamination manu worn by our banknotes, which become risks factors because of their important use, we have suggested within the framework of this study, to isolate and characterize by means of several tests their cultivable mycoflore, in order to look for the opportunistic environmental fungi.

MATERIALS AND METHODS

Collecting samples

We had, for the sake of this study, a total of 200 tickets or banknotes dominations collected randomly in various places: supermarkets, gas stations, cafeterias, pizzerias, butcher's shop, fishmongers, restaurants, bus stations and post offices in the region of Algiers.

The random nature of the microbial contamination of these currencies and consequently the heterogeneous distribution of germs means that the sampling establishes a major problem. In the screening of this flora, it is thus important to create representative samples for its isolation.

This equipment is composed of 50 cuts of two hundred dinars' bills (200 DA), 50 of five hundred dinars bills (500 DA), 30 of one thousand dinars bills (1000 DA), and 30 of two thousand dinars bills (2000 DA) placed in sterile plastic bags protecting them against any factor of contamination and any change of the microbial flora, is directly forwarded in the laboratory to undergo the various microbiological analysis. We also tested 10 new witnesses for each cut.

Isolation of the mycoflora

The enumeration of the microbial germs is a collectively used method and several modalities of technique can be proposed; the most usual are the culture in Petri dishes.

The purpose of enumeration techniques is to determine the concentration micro-organisms contained in an initial preparation. Therefore, they require one or several decimal dilutions. We used the technique of suspension-dilutions developed by Pochon and Tardieux (1962) [22] and adopted by Kalita et al. (2013) [14], successful for the microbial analysis of bills and coins.

In order to isolate an important population of germs likely to contaminate our samples, we weighted at first every cut which we then put aseptically in suspension, in 10 mL of BHIB (Brain Heart Infusion) contained in a sterile erlenmeyer. Every bill is then strongly shaken in the whirpool during 30 minutes to dislodge micro-organisms and obtain their good dispersal in the environment. From this initial suspension (dilution 10^{-1}), we realize the second decimal dilution (10^{-2}).The enumeration on the surface of the agar media is made on 0.1 mL of dilution. The incubation is realized in the steam room at 37° C during 48 hours for yeasts and 27° C during 7 and 21 days for molds and dermathophytes. The microbial analysis is realized on the Petri disches and in which the number of colonies is between 10 and 100 for the filamentous fungi and 30 and 300 for yeasts. The numeration is carried out by determining germs represented by their frequency expressed in UFC (Units Forming Colonies)/bill. Knowing that the distribution of the microbial populations is heterogeneous, the analysis is made with 3 repetitions of every dilution on the selective medium of fungi.

Identification of fungi

It is based on macroscopic and microscopic characters of fungi obtained in pure culture. Where appropriate, by biochemical tests. Several cultural media were prepared: the Sabouraud medium (10 g of peptone; 20 g of glucose; 20 g of agar agar; 1000 mL of distilled water; pH 6 - 6.5), the medium with malt extract (20 g malt extract; 1 g peptone; 20 g glucose; 20 g agar agar; 1000 mL of distilled water; pH 5.8), the medium with rice agar tween (10 g of cream of rice, 10 mL of tween 80, 14 g of agar agar, 1000 mL of distilled water; pH 6.5), the serum of rabbit and galleries API 20 C AUX (AuxaColor 2) (Biomérieux, Ref 20 210).

Identification of filamentous fungal

For the filamentous fungi, the identification begins with a macroscopic examination of the isolates which is an essential act.

This characterization concretized by a microscopic examination by the technique of the flag or the technique of the adhesive tape.

Identification of yeasts

It is generally colonies of yeasts not presenting features with regards to the bacterial colonies except possibly pigmentation (*Rhodotorula* spp.). Their identification is based at the same time on their morphological characters determined on Sabouraud medium, PCB (Potato Carrot Bile for the test of filamentation) and RAT (Rice Agar Tween for the search for chlamydospores) as well as their biochemical characters determined by means of the microplate API 20 C AUX (AuxaColor 2) (Biomérieux, Ref 20 210).

This determination is concluded by:

The test of blatese (test of germination or test of Tschadjian)

The *Candida albicans* tend to train germinal tubes, when we emulsify a pipette tip of this yeast in a tube containing 1 mL of calf or rabbit serum. The test is positive if approximately 50% of yeasts found after incubation to the steam room at 37° C for 3 to 4 hours, a "germination tube", which would be representative of the invasive capacity of *C. albicans*. Other yeasts do not do that.

The search for chlamydospores

The *Candida albicans* has the property to form chlamydospores and also well-developed pseudomycelium when he is cultivated on poor medium RAT (Rice Agar Tween) or PCB (Potato Carrot Bile). After incubation during 48 hours in 30° C, the presence of pseudomycelium, blastospores and chlamydospores (round, generally refractive spores) is in 95% of characteristic cases in *Candida albicans* species.

Biochemical tests

In order to identify the isolates yeasts, we have used the gallery API 20 C AUX (AuxaColor 2) (Biomérieux, Ref 20 210) miniaturized whose principle is based on the assimilation of sugar. The growth of yeasts is displayed by the bend of a pH indicator. 48 hours after the inoculation of cupules, the identification of strains is realized by the assistance of the identification software api Web TM.

Resistance to actidione

This property used for the identification of yeasts is sought on Sabouraud's universal medium associated with actidione. Cycloheximide is an antifungal agent that prevents mold growth on this support. Some yeasts are sensitive to it, while others that are resistant easily multiply at 37 ° C for 24 to 48 hours.

RESULTS AND DISCUSSION

Distribution of fungus on the analyzed banknotes

The purpose of this work is to estimate the frequency of isolation of micromycetes on various Algerian banknotes and to detect the potential cuts loaded in molds and yeasts.

The survey responses in UFC/bill of the cultivable total mycoflore, obtained in the dilution 10^{-1}, are recorded in table 1. In the dilution 10^{-2}, the found values are statistically not significant.

Indeed, the total number of fungal propagules on the mutilated cuts is situated between 200 and 350 UFC/bill, while on witnesses, it oscillates between 0 and 10 UFC/bill.

The analysis on the malt and Sabouraud media reveals that all the samples in circulation are contaminated by a diversified and considerably variable mycological population according to the cut. This heterogeneousness highlighted the presence of 30 species of molds belonging to 8 different genera: *Penicillia* spp., *Aspergilli* section *Flavi*, *Aspergilli* section *Nigri*, *Aspergillus glaucus*, *Rhizopus* spp., *Paecilomyces* spp., *Pseudobotrytis* spp… and of 21 strains of yeasts, linked to 3 different genera: *Candida dubliniensis*, *Candida glabrata*, *Geotrichum* spp., and *Rhodotorula* spp. among whom most of them are incriminated in human pathology.

Table 1. Distribution of fungus on the analyzed banknotes

Mushrooms		Isolation frequency (%)				Isolation frequency (%)			
		Used banknotes				New banknotes (witnesses)			
		200 DA	500 DA	1000 DA	2000 DA	200 DA	500 DA	1000 DA	2000 DA
Molds (MEA medium)	-*Acremonium* spp.	20	0	0	0	0	0	0	0
	-*Alternaria* spp.	2.85	0	3.12	0	0	0	0	0
	-*Aspergillus* section *Flavi*	20	0	0	0	0	0	0	0
	-*Aspergillus fumigatus*	0	0	0	0	100	0	0	0
	-*Aspergillus glaucus*	2.85	0	0	0	0	0	0	0
	-*Aspergillus* section *Nigri*	2.85	3.84	3.12	25	0	0	100	0
	-*Cladosporium* spp.	8.57	0	0	0	0	0	0	0
	-*Rhizopus* spp.	2.85	3.84	0	15	0	0	0	0
	-Sterile mycelium	0	0	3.12	0	0	0	0	0
	-*Paecilomyces* spp.	2.85	0	0	0	0	0	0	0
	-*Penicillium* spp.	14.28	92.30	0	60	0	0	0	0
	-*Pseudobotrytis* spp.	2.85	0	0	0	0	0	0	0
Yeasts (Sabouraud medium)	-*Candida dubliniensis*	8.57	0	0	0	0	0	0	0
	-*Candida glabrata*	0	0	3.12	0	0	0	0	0
	-*Candida krusei*	0	0	6.25	0	0	0	0	0
	-*Candida pararugosa*	0	0	28.12	0	0	0	0	0
	-*Candida tropicalis*	5.71	0	0	0	0	0	0	0
	-*Geotrichum* spp.	0	0	3.12	0	0	0	0	0
	-*Rhodotorula* spp.	5.71	0	50	0	0	0	0	0
Total number of fungus (UFC/bill)		350	260	320	200	10	0	10	0

Furthermore, the most contaminated samples are the 200 DA bills (350 UFC/bill) and 1000 DA bills (320 UFC/bill).

During this study, we have noticed a fast and plentiful proliferation of mycobiontes belonging to the *Penicillium* genus; they reveal a big prevalence in two samples (500 DA and 2000 DA), with a respective average of 92.30% and 60%; on the other samples they are almost rare or nonexistent.

Besides, the *Aspergilli* section *Flavi* and the *Acremonium* spp. found on the 200 DA currencies are isolated with 20% rate.

On the other hand, we notice the existence of the *Aspergilli* section *Nigri* on four cuts 200 DA, 500 DA, 1000 DA and 2000 DA, respectively with a 2.85%, 3.84%, 2.73%, 3.12%, and 25% values.

However, the micromycetes isolation of the *Cladosporium* genus, rare, was highlighted only on the 200 DA samples with an 8.57% frequency. It is absent on the remaining bills. The sterile mycelium (3.12 %) is only present on the 1000 DA samples.

The microflora hosting the species *Alternaria* spp. (2.85%), *Aspergillus glaucus* (2.85%), *Rhizopus* spp. (2.85%), and *Paecilomyces* spp. (2.85%) is the less dominant.

In the unmanipulated new samples, we have noted a single species of *Aspergillus fumigatus* on the 200 DA bills, and another one belonging to *Aspergillus* section *Nigri* on the 1000 DA bills with a 100% rate. Other new bills tested according to the same procedure indicate that they are free from micro-organisms (Table 1).

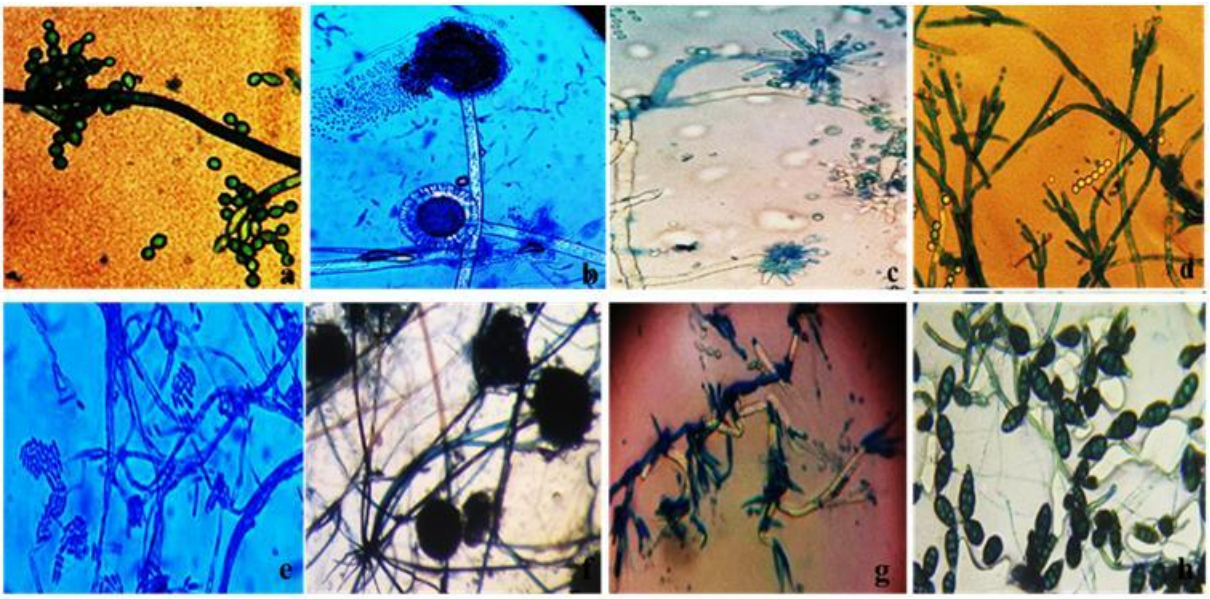
The analysis on the Sabouraud medium reveals the existence of a yeast population also heterogeneous with a frequency oscillating between 3.12% and 50%, only on the 200 DA and 1000 DA bills. We have noticed the presence of numerous *Candida* spp., *Geotrichum* spp., and *Rhodotorula* spp..

In this procession, yeasts connected to *Rhodotorula* spp and *Candida pararugosa* species expresses the biggest prevalence, with a respective average of 50% and 28.12% on the 1000 DA bills. Besides the co-contamination by *Candida dubliniensis* (close to the *Candida albicans*), *C. glabrata*, C. *krusei*, *C. tropicalis* and *Geotrichum* spp., is low and varies respectively of 8.57%, 3.12%, 6.25%, and 5.71% to 3.12% on the same packaging. On the other hand, the notes of 500 DA and 2000 DA are contaminated by no yeast (Table 1).

These globally significant results (Kruskal-wallis test) show clearly that the majority of the used samples are mainly infested by environmental opportunistic molds and yeasts.

During this screening we therefore isolated a batch of 51 strains, composed of 30 molds and 21 yeasts.

We were able to distribute 30 molds in 12 different species, which the main are showing in figure 1.



a- Blastospores of *Cladosporium* spp.
b- Radiaire vasculaire head of *Aspergillus* spp.
c- Star-shaped head of *Pseudobotrytis* spp.
d- Brush of *Penicillium* spp.
e- Conidies in cluster of the *Acremonium* spp.
f- Rhizoides and sporocystes of *Rhizopus* spp.
g- Brush of *Paecilomyces* spp.
h- Dyctiospores chain of *Alternaria*. spp.

Figure 1: Molds morphology observed in the photonique microscope (G x 40) after a simple coloring in the methylene blue.

In the prize counting 21 yeasts, we have selected by the means of suited standard tests 7 species distinguishing itself by their morpho-cultural and biochemical characters. Most of the isolates are indicated as the most frequently incriminated species in the human pathology. However, the *Candida krusei* species is saprophyte. The strain identified as *Candida dubliniensis*, close to the *Candida albicans* is the only one to produce germinal tubes and chlamydospores. The isolate which would be *Candida pararugosa* is indicated as rare yeast isolated from human excreta (Figure 2).

The risks related to the presence of the pathogenic agents on banknotes represent a major challenge and a threat for the public health in Algeria. We have analyzed a total of 200 samples of currency coming from various places. The isolation of fungus as shown in our study justifies that currency could play an important role in transmitting microbial agents within the population. However, no denomination is protected from the contamination: all the analyzed currency (100%) is infested. We can besides assert that small banknotes currency were the most contaminated. The highest contamination rate is observed on the

200 DA and 500 DA samples revealing an invasion composed of fungus (350 UFC/bill); generally their physical state is in a direct relation with the stain.

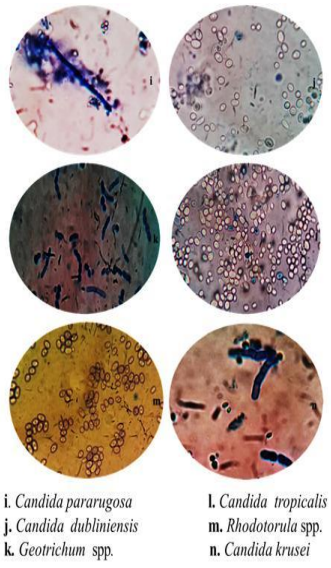


Figure 2: Yeasts morphology observed in the photonique microscope (G x 100) after simple coloring in methylene blue

Being older and exchange much more than the cuts of higher value, they shelter the major part of infectious agents manu carried. Besides these matrixes pass frequently through several hands for various daily transactions and consequently these operation predispose them to superior levels of contamination.

These results are even higher than those reported on the currencies of other developing countries such in Saudi Arabia: 72.3% [5]. These differences reflecting the practices and manipulation of currency indicate that this germ contamination is a world problem.

Indeed, during these last two decades we attend a very clear increase of mycosis incidence (candidiasis, cryptococcosis, aspergillosis). This resurgence is the result of the natural defenses failure and the evolution of the medical and surgical techniques, as well as the emergence of certain diseases promoting the occurrence of opportunistic mycosis, AIDS in particular. Three fungus are usually isolated from the samples made to the immunosupressed: *Candida* spp., *Aspergillus* spp., and *Cryptococcus* spp..

In practice, the opportunistic mycosis caused by yeasts *Candida* spp., *Cryptococcus* spp., *Trichosporon* spp., *Geotrichum* spp…. and of pathogenic fungus of filamentous aspect with clear mycelium (Hyphomycetes hyalins): *Rhizopus* spp., *Aspergilli* spp., *Penicillia* spp., or in darkened mycelium (Dematies): *Cladosporium* spp. (responsible for chromomycoses), *Alternaria* spp. (responsible for sinusitis, keratitis otomycosis and epidermal, dermal and nasal alternariosis) represent a real challenge today.

Thirty species of molds among which, *Penicillia* spp, *Aspergilli* section *Flavi*, *Aspergilli* section *Nigri*, *Aspergillus glaucus* (produces mycotoxins that can cause a fatal brain infection), *Rhizopus* spp. (cause rhinocerebral, cutaneous (in the burns) and visceral : pulmonary, digestive, renal affections), *Paecilomyces* spp., *Pseudobotrytis* spp. (Figure 1), and twenty one strains of yeasts: *Candida dubliniensis*, *Candida glabrata*… is isolated from our samples.

Several authors report the same sensibility of this currency by the colonization by molds [6, 20, 5, 9, 14, 2, 11, 23].

Penicillia spp. prevails in our isolation. To the immunosuppressed they can be pathogenic virulent. Only *Penicillium marneffei* species of south-east Asia can be considered pathogenic for the HIV-positive patients. *Penicillium griseofulvum* and *Penicillium expansum* can produce a dangerous mycotoxine: the patuline (clavacine).

The presence of *Aspergilli* spp. is not insignificant. Besides, the inhalation of their spores (*A. Fumigatus*) can cause the allergic broncho-pulmonary aspergillosis (the symptoms are similar to those of classic asthma), the aspergilloma (spores germinate in this cavity to form a mycelia ball) either an aspergillar sinusitis.

The presence of *Aspergilli* spp. is not negligible. In addition, inhalation of their spores (*A. fumigatus*) can cause allergic bronchopulmonary aspergillosis (the symptoms are similar to those of classic asthma), aspergilloma (the spores germinate in this cavity to form a mycelial ball) or an aspergillar sinusitis. Our samples are also colonized by *Acremonium* spp., which can be pathogenic for man (cause of the cutaneous of cervico-facial gums, of truck and members) and by *Rhizopus* spp.. *Rhizopus oryzae* is an agent of mortal infection at the immunosuppressed patients.

We have observed the existence of numerous *Candida* spp., *Geotrichum* spp., and *Rhodotorula* spp.

In this study, yeasts belonging to the *Rhodotorula* spp. and *Candida pararugosa* species are majority. The co-contamination by *Candida dubliniensis*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *Geotrichum* spp. is insignificant. No yeast grown on actidione in 24 h. Numerous researchers corroborating the same data confirm the spread of these same opportunist yeasts through the currency.

Indeed, the fungal new and emergent pathogenic germs include mushrooms of opportunist yeast type such as *Rhodotorula* spp., and *Geotrichum capitatum. Rhodotorula* spp., is reported to cause a fatal endrocarditis and a meningitis and can probably cause a toxic shock [4]. *Candida dubliniensis*, pathogenic opportunist, is closely linked to *Candida albicans* (the most important yeast; it is a cause of death for the immunosuppressed patients such as patients reached by AIDS, the cancerous patients under the chemotherapy or after osseous marrow transplant) but differs with regard to the epidemiology, certain characteristics of virulence and the capacity to develop a resistance to the in vitro fluconazole.

It is necessary to indicate that approximately 95% of all the candidiasises are caused by four species: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis.* Considering that these species are common, they can emerge in the future as opportunist pathogenic [21].

Indeed, the presence of opportunist pathogenic fungus on these mutilated media is considered influenced:

-By the material (cotton-based) with which bills are made and which would play a role in the capacity to transport germs. This factor facilitates their colonization much more than the coins of metallic nature which are revealed to possess an antimicrobial activity.

-By the physical state of the currency which could serve as indication of its age because this one is suggested as another important factor which determines the presence of micro-organism [24, 3].

-And a series of economic indicators: the important tendency to manhandle the small cuts (200 DA and 500 DA) than the currency of higher value (1000 DA and 2000 DA).

Also fungus found on bills could be feed by the residues left by fingers that handle them.

Indeed, a precautionary measure requires:

-a rigorous awareness campaign on a national scale about the sanitary risks of bad hygiene during and after the manipulation of currency.

-the implementation of a hygiene control plan strengthened and accompanied by legislative and statutory texts.

Although the number of sampling places is limited, the result obtained in the study has supplied information on the presence and the distribution of a pathogenic and fecal opportunist microbial flora found on banknotes circulating in the inhabitant of Algiers. This contaminant distribution shows our big ignorance and indifference to this unhealthy currency.

CONCLUSION

Nowadays, banknotes caused a real sanitary worldwide issue. Being a part of objects which go from one hand to another, they are targeted for the presence of an important range of resistant opportunistic pathogenic agents. Hygienist and healthcare professionals are already worried about the fact that they represent a way of transmitting several diseases. In view of our results, the lack of health control in Algeria and the absence of strict regulations are likely to increase the danger of public contamination by our currency.

Without a good hygienic, micro-organism can be transferred by the currency to food. Thus, the simultaneous manipulation of food and money is one of the common practices of our food sellers: bakers, pasty cooks, grocers, pizzeria, butchers, owners of cheap restaurant, kebab sellers. Several other behaviors in our country can contribute to the contamination of currency: a bad hand-washing after the use of toilet, having wet fingers with some saliva during counting bills, cough or sneezes on hands.

As such, if none of these effective precautions can be implanted, it is highly recommended that the food staff service implement hand-washing procedures after touching currency and before having contact with food. Now that we know that the viable environmental opportunist pathogenic micro-organisms are present on our currency, it leads us to understand that food and currency must be physically separated in order to bring each task to a successful conclusion. It is even more advantageous, practical and reasonable to manipulate food only with a gloved-hand and currency with the other hand or better still to separate the manipulators.

A policy on the correct manipulation of currency should be introduced (improvement of personal hygiene standers, changing bills materials into polymers) or better still an electronic payment or bank cards that are requested to reduce risks of contamination manu carried. Finally, old mutilated currency must be removed from traffic because often it is the most contaminated.

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