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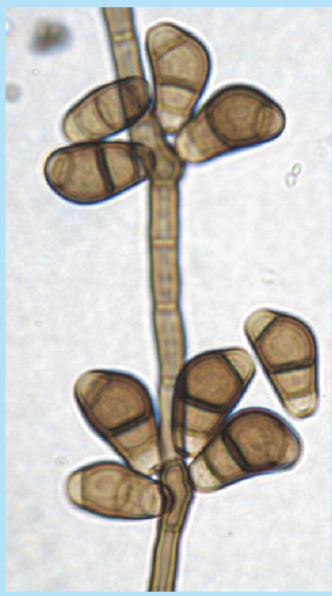
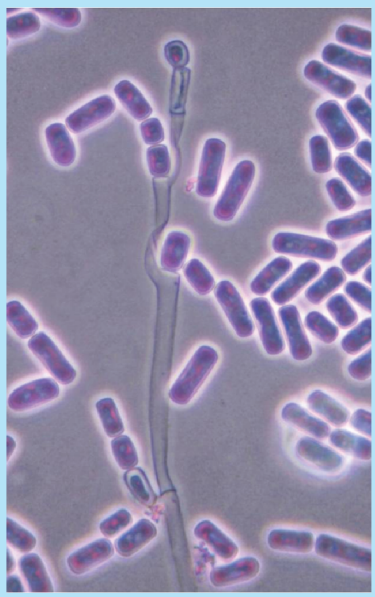

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Microbial Contamination of Yemeni Currencies and their Role as Potential Biological Hazard in Taiz City, Yemen

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Abstract: Yemeni banknotes (paper currencies and coins) of different values, resources and categories (including workers, sellers and school students) were microbially assessed. Samples were collected from different resources and examined for the microbial contamination. In general, the banknotes' samples were found to be of high loaded with a variety of bacterial and fungal species. The mean counts of aerobic mesophilic bacteria in the three banknotes were 172.55, 228.42 and 70.64 CFU/mL, respectively. The mean counts of molds in currencies collected from the three categories were 11.83, 7.06 and 3.94 CFU/mL, respectively. Bacterial assessments of Yemeni currencies exhibited that they were loaded with various bacterial species ranging from nonpathogenic bacteria to the potential pathogenic species such as *Staphylococcus* spp., *Salmonella* spp., *Pseudomonas* spp., and *Escherichia coli*. The results also showed that the most dominant fungal genera were *Aspergillus* and *Rhizopus*. In addition to the wide variety of species present, the load of microorganisms and the level of their spread were also shocking. The data reflect the potential role of banknotes as a potential source for microbial infections and pathogenicity. This necessitates the need to increase the awareness of their possible role in spreading diseases and the need to deal with them as a suspicious source of infection.

Key words: Banknotes, contamination, bacteria, fungi, pathogens, Taiz, Yemen.

Introduction

Banknotes are one of the items used in daily bases worldwide, although to variable extents, as they facilitate our economic and trade needs (El-Dars and Hassan 2005). In developing countries such as Yemen, they are of a special importance as using the credit or debit cards or online purchases are not common payment or shopping methods. Basically, any hands contaminants can end up on the currency, hence, they can be contaminated from wounds, droplets during coughing, sneezing, or just merely from hands touching dirty surfaces (Hassan *et al.* 2011). Accordingly, banknote can be contaminated and act as a potential environmental vehicle for the transmission of pathogenic microorganisms such as bacteria, fungi and viruses. Consequently, polluted currency can result in spreading risky diseases produced by pathogens which are of special concerns for immunocompromised people (Abrams and Waterman 1972, Snehalatha *et al.* 2016, Lamichhane *et al.* 2009). Generally, currency has been recognized as a potential public health hazard as pathogens distributing factor by circulating banknotes and were reported in many previously studies (Abram and Waterman 1972, Unekeand Ogbu 2007, El-Dars and Hassan 2005, Alwakeel and Nassar 2011). Some previous studies conclude that the currency notes collected from hospitals and open markets were contaminated with pathogenic microorganisms. The currency notes collected from hospitals were contaminated more with *S. aureus* than in the open market group (Sunil *et al.* 2020)

Pathogens can be transferred very efficiently via handling money and during the exchange of banknotes. Some microorganisms are removed, and others are

added to the banknote (Ukwuru 2012). Alabbasy (2019) explanted the microbial contamination of paper currency which came from contacting with different environments and different individuals from hand to hand everyday throughout the world. In this respect, potentially pathogenic organisms were isolated from 94% of one-dollar bills examined (Pope *et al.* 2002). Currency notes can be considered as efficient vehicles for microbe transmission as suggested for the first time in 1970s by Abrams and Waterman (1972) and can cause food-related illnesses. However, the degrees of contamination and the microbial types may be area-dependent because of the texture of banknotes, sanity condition, microbe endemism and the handlers hygiene levels (Pope *et al.* 2002).

Moreover, Jiang and Doyle (1999) proved that coins could serve as potential vehicles for transmitting both *Escherichia coli* O157:H7 and *Salmonella* sp. Also, several studies indicated that both banknotes and coins were contaminated by bacteria, fungi, nematodes, and protozoa (Abrams and Waterman 1972, El-Dars and Hassan 2005, Kalita *et al.* 2013). Emikpe and Oyero (2007) recognized *Enterobacter* spp., *Staphylococcus* spp., *Citrobacter* spp., *Klebsiella* spp. and *Proteus* spp. as pathogenic bacteria which found to be resistant to tetracycline and cotrimoxazole while they were sensitive to amoxoftine, gentamicin, nalidixic acid and ofloxacin (Emikpe and Oyero 2007). A survey carried out by Abirami *et al. (*2012) in Nanded city also revealed heavy contamination of currency notes and coins by important fungal species like *Aspergillus niger* (60.37%), *A. flavus* (3.98%), *A. nidulans* (0.2%), *Penicillium citrinum* (17.80%), *Alternaria tenuis* (0.20%), *Curvularia pallescens* (0.20%), *Cladosporium cladosporioides* (10.69%), *Rhizopus stolonifer* (1.04%), *Fusarium* sp. (0.20%) and *Trichoderma viride* (0.20%) (Isaac 1996, Jiang and Doyle 1999). In a study performed in India to estimate the microbial contamination of currencies found that, *S. aurous*, *Candida* spp. and *Aspergillus* spp. were isolated from Indian currency note samples (Snehalatha *et al*. 2016). Igumbor *et al.* (2007) showed that 90% of banknotes in South Africa were contaminated with bacteria and fungi.

A study in Nigeria revealed that the currency is a potential transmission method for pathogenic parasites and bacteria (Uneke and Ogbu 2007). Various bacterial genera including, *Enterobacter*, *Klebsiella, Cedecea, Yersinia, Bacillus, Brucillus, Clostridium, Corynebacterium, Acinetobacter, Staphylococcus, Listeria* and *Micrococcus* were isolated from banknotes (Han *et al.* 1989). In a study on Ghanian currency notes, it was revealed that 98.6% of the currency notes were contaminated with different genera of fungi including *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus* species (Kawo *et al.* 2007).

The objective of this study was to investigate the microbiological contamination of Yemeni currencies collected from different social categories including sellers, workers and school students in Taiz city, Republic of Yemen.

Materials and Methods:

Samples collection:

A total of 105 samples of currency were collected from three different social groups namely school students, workers and sale people (35 samples each). Samples were obtained from different locations in Taiz city, Yemen, using sterile techniques to rule out the collection procedure as a contamination resource. Both coins and paper banknotes were investigated for their microbial contamination. In addition, the currencies collected were at different stages (old, medium and new), different values (5, 10, 20, 50, 200, 500 and

1000 RY) and their issuance dates were ranged from 1993 to 2006, hence, they were handled for at least 12 years.

Microbial isolation and identification:

Bacterial isolation was done by initially swabbing the currencies and streaking on nutrient agar according to the method used by Baron (1990). The resulting isolates were subsequently used to inoculate EMB, S.S Agar and MacConkey Agar that were incubated at 37°C for 48 hrs. Identification of bacteria was carried out using different biochemical tests as described by Bjerring and Oberg (1986).

Prior to microbial isolation, each currency denomination was soaked in 100 mL broth wash. This was followed by transferring 0.1 mL of the washing liquid into sterilized appropriate media aseptically (Goktas and Oktay 1992, Han *et al.*1989). For total bacterial count, nutrient agar medium was used. The inoculated media were then incubated for 48 hours at 37°C and subsequently the growing bacterial colonies were counted. Pure colonies were isolated by using streaking technique and thereafter were characterized by colony morphology, growth patterns, colony color, etc. Bacterial pure colonies were then classified to gram positive and gram negative based on the reaction of gram-staining technique. The bacterial cell morphology and the arrangements in addition to gram staining reaction were observed microscopically. Biochemical tests were conducted to identify the bacterial isolates to the genus level. In addition, some selective and differential media were used for bacterial identification including Blood agar, *Salmonella Shigella* Agar (*S.S.* agar), MacConkey agar and EMB agar. The bacterial count was calculated as colony forming unit per mL (CFUs/mL)

Sabouraud's Dextrose Agar was used for fungal isolation (Baron 1990). The fungal plates were incubated at 28°C for one week and were examined microscopically and by colony characteristics (Baron 1990). The isolated fungi were identified morphologically based on macroscopic and microscopic feature according to the key of the following references: Bruge *et al*. (1977), Pitt (1979), Domsch *et al.* (2007).

Results and Discussion

Microbiological analysis of paper and coin currencies

In the current study, the microbiological analysis of the tested samples revealed that all currency samples were contaminated by diverse bacteria and fungi.

As shown in table (1), the aerobic mesophilic bacterial counts in banknotes (paper currencies and coins) from three different categories including workers, sellers and school students were 172.55, 228.42 and 70.64 CFUs/mL, respectively. The mean counts of molds in the currency samples of the three categories were 11.83, 7.06 and 3.94 CFUs/mL, respectively.

In general, there is no observable significant distinctive contamination pattern among different categories. The strongest trend was that the bacterial contamination levels were significantly higher than the fungal contamination levels (Table 1). We noticed a slight increase in the bacterial contamination in the category of sellers with denomination RY20 and the category workers with denomination RY 10.

Table 1: The total bacterial and mold counts isolated from currencies collected from three different social groups in Taiz city, Yemen.

Currency Denominations (RY)	Currency source					
	Workers		Sellers		School students	
	Bacteria (CFU/mL)	Molds (CFU/mL)	Bacteria (CFU/mL)	Molds (CFU/mL)	Bacteria (CFU/mL)	Molds (CFU/mL)
5	59.54	10.8	235.4	5.4	133.54	1.0
10	244.86	10.4	504	4.2	52.54	1.8
20	577.00	15.8	1.2	15.8	78.74	3.0
50	65.04	15.0	342	13.0	93.54	5.6
200	75.40	5.4	309.2	2.2	25.08	3.0
500	120.90	8.6	180.26	2.2	73.62	8.0
1000	65.12	16.8	26.86	6.6	37.40	5.2

The results in the present work showed that all currency samples were contaminated with various bacterial groups including Gram-positive Bacilli, Gram positive Cocci, hemolytic cocci, Gram-negative Cocci and Gram-negative Bacilli. Also, Lactose-fermenting bacteria were isolated from all samples (Tables 2, 3 & 4).

Interestingly, currency samples from worker category, especially the RY20, were highly contaminated with Gram-positive bacilli and Gram-positive cocci (except RY50). They were also loaded with the β-hemolytic bacteria except RY200 and RY50. It was found that the Y-hemolytic bacteria are present in the 500 and 200 RY whereas the α-hemolysis are present in the 5, 10, 500 and RY1000. The Gram-negative cocci and gram-negative bacilli were isolated from the 20 and 200 RY. Lactose-fermenting bacteria were isolated from this category (Table 3 & 4).

In the schools' student category, the contamination with gram-positive bacilli almost appeared in all samples except in the 10, 20 and 1000 RY notes. Contamination with the cocci was also prevalent except in the 50 and 200 RY. β-hemolytic bacteria appeared in the 500 and 1000 RY categories. Y-hemolytic bacteria were isolated from all samples except 50 and 200 RY. Gram-negative cocci and Gram-negative bacilli were isolated from all samples except 10 and 50 RY. Lactose-fermenting bacteria were also isolated from denominations 5, 10 and 20 RY. In school' student category, *E. coli* appeared in denominations 5 and 10 RY (Table 3).

Prevalence of Enterobacteriaceae and Coliforms

According to tables (2 & 3), it is noticeable that all currency from the different categories were free from *E. coli*. In addition, it was found that currency denominations 5 and 10 were contaminated with coliforms whereas other denominations were not. This observation can be attributed to those 5 and 10 denominations are used frequently. Moreover, currency denominations from seller source were free from any contamination by coliforms (Table 4).

High load contamination by Enterobacteriaceae was found in all tested currency denominations from the three different categories (Tables 2, 3 & 4). This means that there are poor hygienic practices by people who

are dealing with currencies in addition to poor sanitary conditions.

Table 2: Bacterial counts of hemolytic bacteria, Enterobacteriaceae and Coliforms isolated from paper currencies collected from workers in Taiz city

Currency Denominations (YR)	Hemolytic bacteria				Enterobacteriaceae			Coliforms (CFU/mL)	
	Count (CFU/mL)	Alpha (%)	Beta (%)	Gamma (%)	Count (CFU/mL)	L.F (%)	Non L.F (%)	*E. coli* (CFU/mL)	Non (CFU/mL)
5	44.8	17.9	82.1	0	216	75	25	0	11.5
10	33.2	9.6	90.4	0	38.6	28	72	0	7.5
20	40	0	100	0	162.2	100	0	0	0
50	0	0	0	0	44	100	0	0	0
200	0	0	0	0	35	100	0	0	0
500	94	10	80	10	64.6	99.1	0.93	0	0
1000	75	52.1	21.4	26.5	33.4	93.5	6.59	0	0

Table 3: Bacterial counts of hemolytic bacteria, Enterobacteriaceae and Coliforms isolated from currencies collected from school students in Taiz city

Currency Denominations (YR)	Hemolytic bacteria				Enterobacteriaceae			Coliforms (CFU/mL)	
	Count (CFU/mL)	Alpha (%)	Beta (%)	Gamma (%)	Count (CFU/mL)	L.F (%)	Non L.F (%)	*E. coli* (CFU/mL)	Non (CFU/mL)
5	27.6	0	0	100	27.4	41.6	58.4	0	5.6
10	38.7	0	0	100	8.6	37.2	62.8	0	10.2
20	32.2	0	0	100	25	51.2	48.8	0	0
50	0	0	0	0	15.3	0	100	0	0
200	0	0	0	0	8.7	0	100	0	0
500	5.6	0	85.7	14.3	12.2	0	100	0	0
1000	187.8	0	100	0	10.2	0	100	0	0

Table 4: Bacterial counts of hemolytic bacteria, Enterobacteriaceae and Coliforms isolated from paper currencies collected from sellers in Taiz city

Currency Denominations (YR)	Hemolytic bacteria				Enterobacteriaceae			Coliforms (CFU/mL)	
	Count (CFU/mL)	Alpha (%)	Beta (%)	Gamma (%)	Count (CFU/mL)	L.F (%)	Non L.F (%)	*E. coli* (CFU/mL)	Non (CFU/mL)
5	188.4	0	17	83	20.4	12.3	87.7	0	0
10	41.6	0	100	0	22.4	1.8	98.2	0	0
20	117.6	29.1	53.1	17.9	93.2	40.99	59.01	0	0
50	188.6	0	90.1	9.9	62	35	65	0	0
200	115.2	0	0	100	3.2	100	0	0	0
500	119	20.2	38.7	41.1	65.4	51.7	48.3	0	0
1000	151	0	30.3	69.7	36.2	82.6	17.4	0	0

Low contamination with coliforms was observed in the examined samples especially 5 and 10 RY collected from workers and sellers. Whereas, *Escherichia coli* was not detectable in all currency samples. Similarly, Girma (2014) reported the presence of coliforms in banknotes papers is an indicator of inadequate hand washing practice after using toilet.

The sources of microbial contaminations are various as the currencies are in continues cycling and handling through all environments which contain various microbial groups (Pomperayer and Gaylarde 2000). Ejike (2017) revealed that, the demonstrated Nigeria currency notes in circulation are contaminated with both Gram-positive and gram- negative bacteria

It was reported that pathogenic bacteria such as *E. coli* can stay alive on surfaces for 11 days. Consequently, handling of banknotes currencies can be a risk factor for microbial contamination of ready to eat foods if not good hygienic practices are applied (Barro *et al.* 2006).

Prevalence of hemolytic bacteria and *Staphylococcus* spp.

Hemolytic bacteria are gram-positive, Cocci in shape and able to lysis the red blood cells. These are including two genera, Staphylococci and Streptococci. The genus *Streptococcus* contains many pathogenic species and the most pathogenic are *S. pyogenus* and *S. pneumonia*. The most pathogenic species of *Staphylococcus* are *S. aureus, S. epidermides* and *S. saprophyticus.* The presence of hemolytic bacteria in most of the tested currencies from three different categoriesis an indicator for poor hygiene practices.

One of the most medically important bacterial found in the tested currency are *Staphylococcus* spp. This genus is known to harbor many pathogens such as *Staphylococcus aureus* which is known to be resistant to many common antibiotics and was contaminating all of the currency samples collected (Figure 1).

The data in this study showed that all tested currencies were contaminated with *Staphylococcus* spp. (Figure 1). The prevalence of *Staphylococcus* spp. was dominant in currencies from seller's category (37.93%). The presence of *Staphylococcus* spp. in currencies collected from workers and school student's categories was 13.96% and 12.5%, respectively. These results are in agreement with the results obtained by Umeh *et al*. (2007) and Moosavy *et al.* (2013). Also, Sunil *et al.* (2020) concluded that, the bacterial contamination with *S. aureus, E. coli, Klebsiella,* and aerobic spore-bearing bacteria approximately >103 CFUs/plate. This similar to Ejike *et al.* (2017) who showed that *Bacillus* species had the highest occurrence, while *Staphylococcus* species and *Klebsiella* species had the least occurrence from different naira notes analyzed.

Staphylococcus aureus is a pathogenic bacterium presents in human nose, throat and skin which can contaminate hands, fingers and faces (Kumar *et al*. 2009). Hence, contamination of currencies with *Staphylococcus* spp could be attributed to rubbing off or may be surfing from a skin flake (Ahmed *et al.* 2010).

Prevalence of *Salmonella* spp., *Pseudomonas aeruginosa* and spore-forming bacteria

Salmonella spp., *Pseudomonas aeruginosa* and aerobic spore-forming bacteria were detected in tested currencies samples in this study. The results showed the prevalence of *Salmonella* spp., *Pseudomonas aeruginosa* and spore-forming bacteria and the percentages of each type of bacteria in all

denominations of currencies. The highest load of currencies with *Salmonella* spp. was in currencies collected from sellers category followed in currencies from school student's category and the lowest was found in currencies from workers category emerged in 37.93%, 13.96% and 12.50%, respectively (Figure 1). Presence of *Salmonella* spp. in currencies was reported earlier by Ahmed *et al.* (2010) who found that 15% of Bangladesh currencies were contaminated by *Salmonella* spp. The contamination of currencies by *Salmonella* spp. is an indicator of faecal-sourced contamination. In addition, *Salmonella* spp. is able to form biofilm on surfaces and survive for days (Kramer *et al.* 2006).

Ingestion of contaminated food or drinks by *Salmonella* spp., especially *S. typhimurium* and *S. enteritidis* can cause gastroenteritis (Salmonellosis). The symptoms of this disease are developed within 48 hours and characterized by non-bloody diarrhea, nausea vomiting, fever, and abdominal cramping.

Pseudomonas auroginosa was also present in a significant number in currency samples as well (Figure 1).The data in this study showed that the prevalence of *P. auroginosa* in currencies samples of school student category was high which reached 49.98%. *P. auroginosa* also contaminated 9.31% of workers category and 6.9% of sellers category (Figure 1a,b). Our results are in agreement with several previous studies. They reported that *P. aeroginosa* was found as a contaminant associated with banknotes currencies (Awe *et al.* 2010, Borah *et al.* 2012, Girma 2014). Also, *Pseudomonas* is commonly distributed in nature and it can be found in soil, water, animals and plants. *P. aeruginosa* is a causative pathogen of both localized and systemic infections and almost can infect any tissue or organ system. It is a significant opportunistic pathogen in hospital-acquired illness (Im *et al.* 1982).

P. aeruginosa is responsible for nosocomial pneumonia, nosocomial urinary tract infections, infections of severe burns, surgical site infections and infections of individuals undergoing either chemotherapy for neoplastic disease or antibiotic therapy. *P. aeruginosa* is resistance to many antibiotics due to its rapid development of resistance mutations against antibiotics and therefore, it is difficult to find antibiotics effective against *P. aeruginosa*. Presence of *P. aeruginosa* in currencies is a risk factor that should be taken in concern.

In addition, the spore-forming bacteria reached high contamination levels (53.53%) of currencies samples of workers category and the contamination level was 20.69% in currencies of sellers category (Figure 1a,b). The lowest prevalence of spore-forming bacteria was found in currencies of school student category as 6.25% (Figure 1c). The spore-forming bacteria are known to tolerate harsh environments including the normal sanitizing habits.

Many types of the isolated bacteria in this study have been concerned as infectious agents causing diseases such as celluliter, crysipselas, necrotizing facilities, scarlet fever, sore throat, pneumonia and others. Most of these diseases were leading causes of morbidity and mortality (Van Deuren *et al*. 2000).

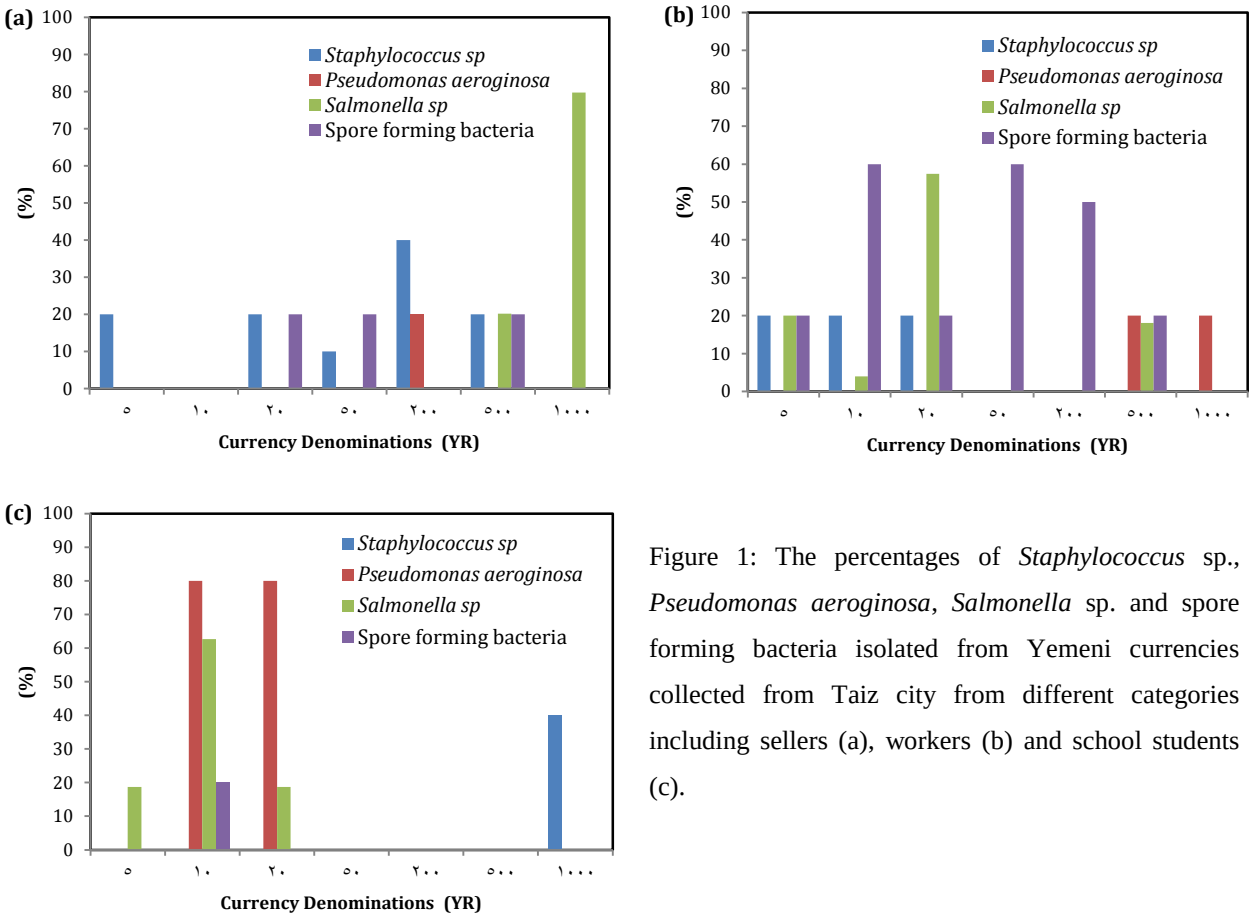

Figure 1: The percentages of *Staphylococcus* sp., *Pseudomonas aeroginosa*, *Salmonella* sp. and spore forming bacteria isolated from Yemeni currencies collected from Taiz city from different categories including sellers (a), workers (b) and school students (c).

Prevalence of fungi

As mentioned previously, the fungal contamination was less prevalent than the bacterial contamination in all currency' categories tested in this study (Figure 2). However, it was found that two species to be present in almost all categories namely: *Aspergillus niger* and *Rhizopus* spp. While, *A. niger* was the most dominant. Other fungal species such as *Mucor* spp., *Curvularia lunata* and some other species of *Aspergillus* were also identified in the tested currencies.

This study also revealed that all banknotes and coins currencies were found to obtain high fungal contamination (Figure 2). From the species isolated are *Rhizopus* spp., *Aspergillus flavus*, *A. flavus* var. *columonar*, *A. ochraceus*, *A. niger*, *A. versicolour*, *A. ustus*, *Pencillium* spp, *Curvularia lunata* and *Mucor* spp.

The presence of *Aspergillus* spp. was dominated in all tested currencies which might be due to its ubiquitous nature and adaptation to various environmental conditions. Some species belonging to genus *Aspergillus* are known as pathogens for human and can lead to a disease known as Aspergillosis (Anderson 1991, Dagenais and Keller 2009). The symptoms of Aspergillosis include allergic bronchopulmonary aspergillosis (ABPA), aspergilloma (AGM), and invasive aspergillosis (IA). The manifestations of ABPA range from asthma to fatal destruction of the lungs with defined clinical, serological, radiological, and pathological features. Aspergilloma symptoms include hemolysis, which

results from the disruption of blood vessels in the wall of the cavity occupied by the fungus. Most frequently, it leads to internal bleeding. Invasive aspergilllosis has become a leading cause of death, mainly among hematological patients. Its average incidence is estimated to be 15 to 25% in patients with acute leukemia. Invasive aspergillosis is recognized today as the main fungal infection in cancer patients and immunosuppressed individual (Segal and Walsh 2006). *Rhizopus* species can cause a group of diseases such as Zygomycosis. Zygomycosis involves mucocutaneous, rhinocerebral, genitourinary, gastrointestinal, pulmonary and disseminated infections (Anderson 1991).

Although, the results in this study revealed that high levels of contamination in almost all the currency samples collected and there is no observable significant contamination pattern based on the social categories chosen. This is due to the currencies cycling among these different groups in the same region. It will be interesting to obtain currency samples from different social categories such as rich versus poor communities, villages versus cities, universities, hospitals and homeless groups.

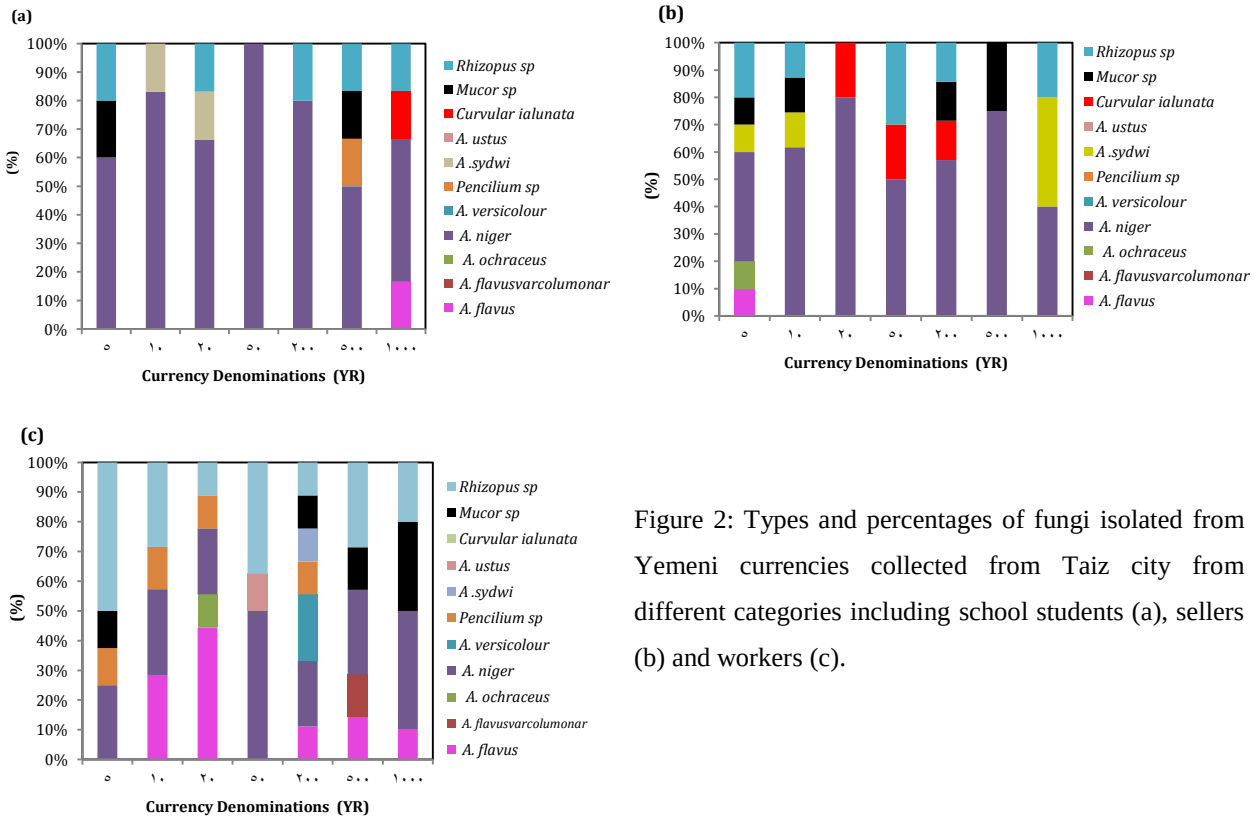

Figure 2: Types and percentages of fungi isolated from Yemeni currencies collected from Taiz city from different categories including school students (a), sellers (b) and workers (c).

Conclusions

Banknotes and coins currencies collected from Taiz city and from different social groups were loaded with different microbial contaminants. This suggested that currency notes are potential source of disease transmittance and necessitates the need for increasing the awareness of their danger. Emphasizing the importance of a good personal hygiene in schools, TVs, and homes can help in decreasing the risks.

Efforts can also be made to decrease the needs for handling cash currency very often through establishing online or cards payments.

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