Metagenomic Sequencing Revealed the Potential Pathogenic Threats of Banknotes

Jun Lin, Wenqian Jiang, Yang Shi, and Weiwen Cai*

ABSTRACT: Banknotes have long been suspected to be biologically “dirty” due to their frequent human contact, which may transmit human microbial pathogens. Still, it is an unsettled issue whether the microbes on banknotes pose a real threat to human health. In several previous studies, metagenomic sequencing was used to reveal the diversities of microbes on banknotes but live microorganism culture and functional verification were lacking. In this study, we collected banknotes of RMB in China as well as dollar bills in the United States and analyzed the microbial biodiversity and drug resistance genes carried by the identified microbes by metagenomic sequencing and in vitro culture methods. We identified eight major genera of drug-resistant bacteria through screening of 30 antibiotics, and the blood agar plate culture uncovered six pathogenic fungal species. Numerous phage and six dangerous viral sequences were also found. These results should substantiate our concern about the potential risk of banknotes to human health.

INTRODUCTION

Banknotes are paper currency. The earliest banknotes, called “Jiaozhi”, originated from the Northern Song Dynasty in China. Today, there are more than 200 types of banknotes circulating around the world. Paper banknotes are prone to contamination due to frequent human contact. Banknotes are not only the carrier but also the potential transmitter of many diseases.1 They have been news reports that bank employees suffered from Condyloma Acuminatum without washing their hands after counting money.2,3 Of particular concern are contagious microbial contaminants that pose serious health hazard. Some bad habits, such as touching food after using banknotes and not washing hands after counting money, have increased the chances of being infected with diseases.

Paper-based banknotes are excellent substrates for microbe attachment and absorption of various nutrients for microbial growth. It is likely that microbes on banknotes may carry antibiotic resistance genes and thus lead to the spread of antibiotic resistance to humans and other environments. Back in 1972, a JAMA published research paper4 reported that coins may carry human pathogens. There have been news reports that bank employees suffered from Condyloma Acuminatum without washing their hands after counting money.2,3 Of particular concern are contagious microbial contaminants that pose serious health hazard. Some bad habits, such as touching food after using banknotes and not washing hands after counting money, have increased the chances of being infected with diseases.

These results should substantiate our concern about the potential risk of banknotes to human health.

RESULTS

Species Found on the Banknotes of this Study. We randomly collected apparently heavily used 12 one-dollar bills in the United States and 12 one-yuan RMB bills in mainland China as two representative banknote groups. The classic STE method and a Mobio DNA extraction kit were used to obtain the DNA of microbes from the banknotes. The most common bacterial genera were Staphylococci, Enterococci, and E. coli, with significant pathogenicity on currencies of different countries. The most abundant bacteria genus was Staphylococci, comprising nearly 50% of the total genera in all the environmental samples. Shigella and Acinetobacter were the second and third most abundant antibiotic resistance gene. This study aims to provide a preliminary survey on the diversity of pathogenic microorganisms and their resistance genes on two widely circulated but little studied banknotes, the US dollar and the Chinese RMB, using a metagenomics approach and wet bench experimental validation.

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References:
1. Gabriel et al. 5 used the traditional microbial culture method to study the prevalence and drug resistance of Staphylococci on 155 European banknotes. Gedik et al. 6 used traditional microbial culture techniques to study the distribution and survival of methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus, extended spectrum beta-lactamase (+) Escherichia coli, and other microbes with significant pathogenicity on currencies of different countries. Jalali et al. 7 using a metagenomics approach in a study on Indian banknotes, identified 78 species of pathogens and 78 antibiotic resistance genes. However, the metagenomic DNA in this study was extracted using the traditional CTAB method, which only provides a biased representation of the microbial species. Heshiki et al. 8 found that the Hong Kong banknotes covered nearly 50% of the total genera in all the environmental samples. Some bad habits, such as touching food after using banknotes and not washing hands after counting money, have increased the chances of being infected with diseases.
the metagenomic DNA, resulting in four metagenomic DNA samples. The yield of DNA obtained by different extraction methods varied significantly; the DNA yields of SteD, SteR, KitD, and KitR were 14, 39, 18, and 52 μg, respectively. In the sample names of SteD, KitD, SteR, and KitR, the “STE” represents the STE extraction method and the “Kit” represents the Mobio kit extraction method; the “D” and “R” represent dollar and RMB, respectively. NGS sequencing was used to obtain sequencing reads from the metagenomic DNA samples. All NGS sequencing raw data was uploaded to the NCBI-SRA database under the accession number of SRP128023. For the four samples, we performed species annotation at the phylum and genus level.

We analyzed the diversity of microbes in the four samples from the “kingdom” to “species” level. We found many bacteria with significant abundance in the RMB samples but not in US
dollar samples. At the genus level, the microbes with the highest presentation in the KitR samples are *Pseudomonas*, but the microbes with the highest abundance in the KitD, SteR, and SteD samples are *Acinetobacter*. Species annotation analysis at the family level clearly showed the microbial diversity difference between the RMB and US dollar samples (Figure 1a). Clustering analysis of species at the genus level also showed the obvious difference between the dollar bills and RMB bills (Figure 1b). We also found that the presentation of species is dependent on the DNA extraction method (Figure 1b). However, regardless of the sample preparation methods, the diversity of microbes on RMB bills is significantly greater at all taxonomic levels than that on the US dollar bills (Table 1). A more detailed list of species annotation can be found in Tables S1–S7.

### Table 1. Comparison of Microbial Species of Each Level in Taxonomy per Sample

| taxonomy level | Ste dollar | Ste RMB | Kit dollar | Kit RMB |
|----------------|------------|---------|------------|---------|
| kingdom        | 3          | 4       | 3          | 4       |
| phylum         | 13         | 28      | 12         | 28      |
| class          | 23         | 41      | 21         | 41      |
| order          | 93         | 91      | 49         | 91      |
| family         | 86         | 184     | 84         | 184     |
| genus          | 170        | 452     | 180        | 449     |
| species        | 288        | 885     | 314        | 890     |

We found that on the RMB yuan bills, at the family level, about 65% of microbial species belonged to the family *Enterobacteriaceae*, *Pseudomonadaceae*, and *Moraxellaceae*. However, the abundance of *Moraxellaceae* and *Pseudomonadaceae* is different for the KitR and SteR samples. On the dollar bills, at the family level, about 35% of microbial species belonged to the family *Moraxellaceae* (Figure 1a). This may indicate that *Enterobacteriaceae*, *Pseudomonadaceae*, and *Moraxellaceae* are more likely to survive and/or reproduce on banknotes.

**Presence of Phages on Banknotes.** It is conceivable that wherever there are bacteria, there will be phages. We generated nonredundant de novo spliced nucleic acid sequences and searched them against the phage database containing 1534 phage genomic sequences. We screened nucleic acid sequences with identity greater than 85%, length greater than 200, gap less than 3, and *E* value less than 0.00001. Indeed, we found many kinds of phage sequences on banknotes. In the dataset of SteR and KitR, we found all 1534 kinds of phages. Interestingly, in the dataset of SteD and KitD, we detected only 23 kinds of phages. We counted the most abundant phage sequences in the four samples (Figure 2) and found that the most abundant phage on RMB samples was *Escherichia* phage and the most abundant phage on the US dollar samples was *Enterobacteria* phage. *Enterobacteria* are one of the most common Gram-negative bacteria, which can explain why *Enterobacteria* phage was found the most abundant phage on banknotes. On RMB samples, the most abundant phage found by two extraction methods is *Escherichia* phage EC1-UPM. However, on the US dollar samples, the most abundant phage found by the STE method is *Enterobacteria* phage ime09 and the most abundant phage found by the kit method is *Enterobacteria* phage Sf6. This suggests that the extraction method has little effect on the analysis results of phage sequences. A list of phages detected in the SteD and KitD sample could be found in Table S8. The huge difference in the number of phages between the dollar and RMB samples was unexpected and intriguing and could not be accounted for by the diversity of detected bacteria species.

**Pathogenic Prokaryotic Microorganisms on Banknotes.** Prokaryotic microbial infection is very common in clinics, but most infection can be effectively treated by proper use of antibiotics. The real threat to humans is microbial drug resistance. In this study, we attempted to identify known antibiotic resistance genes from the NGS data and verify the results using the standard blood agar culture method to grow bacteria to test drug resistance on plates containing antibiotics. We found 37 drug resistance mechanisms (Table S9) by mapping our NGS data to the ARDB and CARD database (version: 2015-February-10). These resistance mechanisms are divided into the following categories: (1) efflux pump conferring antibiotic resistance, (2) gene conferring antibiotic resistance via molecular bypass, (3) antibiotic resistance gene clusters, cassettes, or operons, (4) antibiotic inactivation enzymes, (5) antibiotic target modifying enzymes, (6) antibiotic target protection proteins, and (7) antibiotic target replacement proteins. By mapping our NGS data to the ARDB and CARD databases (Figure 3), we found seven types of drug resistance mechanisms. The efflux pump conferring antibiotic resistance and the antibiotic target modifying enzymes accounted for the highest proportion, accounting for 53% and 30%, respectively (Figure 3a). When compared with the CARD database, the efflux pump conferring antibiotic resistance and the antibiotic resistance gene clusters, cassettes, or operons accounted for the highest proportion, 43% and 45%, respectively (Figure 3b). The ARDB database has not been updated since 2009, so the mapping result is not

![Figure 2. Most abundant phages of the four samples. The vertical axis represents the number of the phages; the horizontal axis represents the name of the sample; and the name of the phage corresponding to each color block is shown in the legend on the right. In the sample names of SteD, KitD, SteR, and KitR, the “STE” represents the STE extraction method and the “Kit” denotes the Mobio kit extraction method; the “D” and “R” represent dollar and RMB, respectively.](https://dx.doi.org/10.1021/acsomega.0c04546)
as complete as the CARD database. Our results indicate that the most common antibiotic resistance mechanism is the efflux of antibiotics (aminoglycoside and macrolide antibiotics, etc.) or generation of antibiotic resistance gene variants (aminoglycoside resistance gene, chloramphenicol resistance gene, etc.).

Based on the mechanisms of drug resistance, we selected 30 antibiotic drugs and designed the antibiotic resistance tests to isolate the corresponding drug-resistant microbes on blood agar plates. Taking tobramycin as an example, we designed four control groups and two experimental groups to screen drug-resistant bacteria on blood agar plates.

Blood agar medium is the most commonly used medium for laboratory testing to isolate and culture pathogenic microorganisms from clinical specimens. Due to the special growth factors provided by defibrinated sheep blood, most pathogenic microorganisms can grow on blood agar plates. Microorganisms that do not grow on blood agar plates are likely harmless to human health.

For microbes grown on blood agar plates with drugs, we chose single colonies of 660 microbes with different morphologies and colors and applied the Gram stain for classification. After classifying and removing redundancy, we took 350 single colonies to extract genomic DNA and performed PCR to amplify the 16s rDNA full-length sequences. Sanger sequencing and NCBI online Blast were performed to accurately taxonomic identification of each clone. We identified nine major categories of drug-resistant bacteria: Enterobacter sp., Enterococcus sp., Enhydrobacter sp., Klebsiella sp., Leclercia sp., Acinetobacter sp., Pantoea sp., Pseudomonas sp., and Moraxella sp. (Table S10). Among them, Bacteroides is the normal flora of the human intestine, oral cavity, upper respiratory tract, and the reproductive tract. However, it may cause endogenous infection only when the immune function of the body is compromised or the flora is imbalanced. Since banknotes are generally exposed to air, it is likely that most of these anaerobic microbes might not be viable on banknotes and thus we did not attempt to study the status of anaerobic microbes.

Pathogenic Viruses on Banknotes. We downloaded all nucleotide sequences of the 490 human infectious viruses from the NCBI data base (https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10239&host=human) and assembled a local Blast database to detect virus sequences in the banknote microbiome dataset. The banknote sequence reads were processed to remove low quality bases and adapter sequences to generate “clean reads”, which were then Blast searched against the 490 virus sequences. We found six viruses, Alphapapillomavirus 7 (human papillomavirus 18, HPV18), Human alphaherpesvirus 1 (herpes simplex virus type 1, HSV-1), Macaca mulatta polyomavirus 1 (SV40), Molluscum contagiosum virus subtype 1 (MCSV1), Orf virus, and Shamonda orthobunyavirus (SHAV), in the banknote dataset (Figure S5). These are all disease-causing or disease-associated virus in humans. The HPV is a spherical DNA virus that causes proliferation of human cutaneous and mucosal squamous

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Statistical chart of seven drug resistance mechanisms obtained by mapping our NGS data to the ARDB and CARD databases. (a) Seven drug resistance mechanisms obtained by mapping our NGS data to the ARDB databases. (b) Seven drug resistance mechanisms obtained by mapping our NGS data to the CARD databases. The vertical axis represents the number of resistance mechanisms; the horizontal axis represents the categories of the drug resistance mechanisms; and the type of drug resistance mechanism corresponding to each color block is shown in the legend on the right.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Screening process of drug-resistant bacteria on banknotes.
cervical cancer, head and neck cancers, and carcinoma of the oropharynx and tonsils. HPV is a virus that causes herpes, which is categorized into two types: HSV-1 and HSV-2. HSV is widely distributed around the world. HSV-1 can cause oral herpes and genital herpes and its infections are life-long. SV40 is a small DNA tumor virus belonging to the Polyomaviridae family, which has transforming and tumorigenic properties. Whether SV40 can cause human cancer has been controversial. MCVI is a DNA virus, a member of the Poxviridae subfamily. Molluscum contagiosum is a human disease caused by MCVI, which has been the principal poxvirus cause of human disease since the eradication of smallpox. Orf virus is a DNA virus belonging to the Parapoxviridae genus, commonly known as sore mouth disease in sheep and cattle. It can be transmitted from animals to humans and cause orf, such as contagious pustular dermatitis, infectious pustular dermatitis, or Ecthyma contagiosum. However, human to human transmission is very rare. SHAV is an RNA virus that belongs to the genus Orthobunyavirus of the family Bunyaviridae. SHAV is an arthropod-borne virus (arboviruses), mainly transmitted by mosquitoes, and is teratogenic in ruminants. Interestingly, among the six viruses found, one is an RNA virus. This could indicate that the DNA produced in the life cycle might also be captured and detected by the high-throughput sequencing of this study. The virus DNA detected could either be the contaminating intermediate of the RNA virus’ life history or that the virus could still reproduce and produce DNA on banknotes. These findings suggest that other more common RNA viruses such as HIV and hepatitis C virus should also be present on banknotes in a more stable DNA form. At present, it is not known whether these viruses are infectious to humans. Banknotes as a medium for virus disease transmission are worth further study.

**Pathogenic Eukaryotic Microorganisms on Banknotes.** Fungi are an important category of human and animal pathogens. Previous studies were generally concerned with bacteria on banknotes. We analyzed the diversity of microbes in the four samples at the family level. We found that the number of fungi on RMB samples was slightly higher than that on the US dollar samples, but there was no significant difference in the diversity of fungi. Species annotation analysis at the family level clearly showed the eukaryotic diversity difference between the RMB and US dollar samples (Figure 6). At the family level, the fungi with the highest presentation in the KitR and SteR samples are Saccharomycetaceae, the fungi with the highest abundance in the KitD samples are Sordariaceae, but the fungi with the highest abundance in the SteD samples are unclassified. This shows that the DNA extraction method has a significant influence on the representation of fungi. The vertical axis represents the relative proportion of species annotated to a certain type; the horizontal axis represents the
name of the sample; and the species category corresponding to each color block is shown in the legend on the right. In the sample names of SteD, KitD, SteR, and KitR, the “STE” represents the STE extraction method and the “Kit” denotes the Mobio kit extraction method; the “D” and “R” represent dollar and RMB, respectively.

To survey the prevalence of viable fungi on banknotes, we used the classic PDA medium plate to enrich the fungi. We extracted genomic DNA from single colonies and amplified the 18s rDNA full-length region by PCR. We then used Sanger sequencing and NCBI online Blast analysis to identify fungal species. We found the following fungi: Pichia guilliermondii, Aureobasidium pullulans, Rhodotorula mucilaginosa, Meyerozyma guilliermondii, Debaryomyces Hansenii, Candida parapsilosis (C. parapsilosis) (Table S12). We found that the viable fungi on plates were mainly yeast and C. parapsilosis. Compared to those of the found bacteria, the number of fungus species and their abundance were much lower, which was consistent with the data obtained from metagenomic sequencing data. We noticed that C. parapsilosis is a pathogen that can cause recurrent skin diseases in humans. These findings suggest that banknotes could be an important medium to transmit fungal disease due to frequent contact with human hands.

**DISCUSSION**

We are taught to avoid microbial diseases by minimizing personal contact and sharing of personal items. However, banknotes seem to be the only exception. Banknotes are a daily used item that transferred among many people during their lifetime without sanitation. Ironically, rarely do we realize the fact that banknotes are a good carrier of microbes and banknote circulation could be an important medium of spreading contagious microbial diseases. All countries in the world have inspection and quarantine for the import of goods and banknotes to prevent species invasion and pathogen entry. However, there is no country where the inspection and quarantine departments would check banknotes carried by the passengers. This study showed that on banknotes, there are abundant viable and drug-resistant human pathogenic microbes that may pose potential threats to humans.

In this study, we found six viruses on banknotes, among which HPV18 and HSV-1 are known harmful agents. HPV has many subtypes, and HPV18 is considered high risk. HPV18 infection is closely related to cervical cancer in women. According to the World Health Organization (WHO), about 70% of cervical cancers are caused by HPV16 and HPV18. Cervical cancer is the most common gynecological malignancy, with an estimated 570,000 cases24 in 2018 (accounting for 84% of new cases worldwide). HSV infection is extremely common in the population with more latent and recurrent infections. Approximately 45—90% of the population carries HSV-1, which is more frequent in developing countries. According to WHO estimates, 3.7 billion people under the age of 50 (67%) are infected with the HSV-1 virus worldwide. Most oral and genital herpes infections are asymptomatic but can still be transmitted to others. The discovery of HPV18 and HSV1 on banknotes indicates that the virus may spread through the banknotes, endangering people’s lives and health.

WHO has published a list of “PRIORITY PATHOGENS”, listing 12 families of bacteria that are most threatening to human health. In this study, we found Acinetobacter baumannii (A. baumannii) and Shigella sp. on the banknotes of both RMB and US dollar. A. baumannii ranks first in the WHO’s list at the “Priority 1: CRITICAL” risk level. A. baumannii is a Gram-negative opportunistic nosocomial pathogen that causes 2—10% of all Gram-negative infections in hospitals. A. baumannii is one of the six most important worldwide multidrug-resistant microorganisms in hospitals. The found A. baumannii on banknotes was resistant to multiple antibiotics, including norfloxacin, ampicillin, fusidic acid, daptoycin, tetracycline, mupirocin, and vancomycin. A. baumannii can adhere to abiotic surfaces such as glass, plastics, etc. and form biofilms. A. baumannii has great vitality and can survive even long-term exposure to dry conditions and nutrient deficiencies. A. baumannii infection can cause skin and wound infection, pneumonia, soft tissue infections, endocarditis, urinary tract infection, meningitis, and septicemia and even lead to death and thus has raised considerable concerns as there are no antibiotics for the infection of this microbe. Shigella is a group of Gram-negative short bacilli, which is mainly prevalent in developing countries. It is the most common pathogen of human bacillary dysentery, which invades the human colon and can rapidly acquire antibiotic resistance. The presence of Shigella on banknotes is a potential risk factor for epidemic bacterial dysentery.

C. parapsilosis, an opportunistic human pathogen, is a yeast-like fungus of the genus Candida. It is an important cause of hospital-acquired infections that are commonly found on the skin of healthy people. C. parapsilosis is the main species causing infection of neonatal and adult people with low immunity in hospitals. The mortality rate of Candida infection in hospital-acquired infections is approximately 40%, of which approximately 15% is caused by C. parapsilosis. Even more frightening is that the species showed significant resistance to fungal drugs. The presence of C. parapsilosis on banknotes means that the probability of C. parapsilosis infection has increased.

A dollar bill is made of a mix of 75% cotton and 25% linen, while an RMB bill is made of short-staple cotton. Cotton-based banknotes provide fiber surfaces, which provide ample opportunities for bacteria to adhere. Linen has a rougher surface than cotton, and microbes are expected to adhere more easily on the dollar bill. However, our results show that the number of microorganisms on a US dollar bill is much smaller than that on an RMB bill, even without considering the fact that the size of the one dollar bill is about 20% larger than that of the one yuan RMB bill. This indicates that the types and quantities of microorganisms carried by banknotes are related not only to their materials but also to other factors, such as transmission frequency and environmental exposure, which are reflected by the economic situation of a country, the social and economic factors, and governmental inspection policies.
metagenomic DNA from dollar samples extracted using the KitD: metagenomic DNA from dollar samples using the Mobio Kit; SteR: metagenomic DNA from RMB samples using the Mobio Kit; SteD: metagenomic DNA from dollar samples using the STE method; KitR: metagenomic DNA from RMB samples using the Mobio Kit. The extracted DNA samples were sequenced and analyzed separately.

### Resistance Gene Annotation

The sequencing methods can be found in Scheme S1. The bioinformatics analysis method for NGS data of this study is described in Scheme S2. We Blast searched the Unigenes to ARDB database11 (http://ardb.cbcb.umd.edu/) and CARD database2,13,36 (https://card.mcmaster.ca/) with the default parameter setting blastp, -e 1e-5,37 then filtered the aligned result, and chose the identity value bigger than the lowest identity value from the aligned result of each sequence to obtain reliable results of resistance gene annotation. Based on the aligned result, the relative abundance of different resistance genes was calculated. Finally, based on the abundance of resistance genes, the abundance bar charts, the abundance cluster heatmap, and the resistance genes’ number difference between groups were displayed. In the same way, the resistance genes’ abundance distribution in each sample, the species attribution analysis of resistance genes, and the resistance mechanism of resistance genes analysis were also conducted.

### Screening of Antibiotic-Resistant Microorganisms

Blood agar medium is generally used for the isolation and culture of clinical specimens. First, the samples were subjected to serial dilution with dilution factors of 2, 4, 8, and 16. We used blood agar medium as well as 30 antibiotics to culture
pathogenic microorganisms in the diluted sample, using JM109 E. coli as a control. The list of antibiotics is shown in Table 2.

In this experiment, we picked a total of 660 single colonies and expanded culture in blood medium. Subsequently, Gram staining was performed, and single colonies were classified according to colony morphology, color, and Gram properties.

**Genome Amplification and Sanger Sequencing Verification.** According to the microscopic examination results, the resistant bacteria were classified into bacteria and fungi. Bacterial genome was amplified with 16S rRNA universal primers 27F, 1492R, and 2 × Taq Plus MasterMix (CWBio, China). Each reaction was carried out for 10 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 53 °C, and 90 s at 72 °C; and 10 min at 72 °C. Fungal genomes were amplified with 18S universal primers 18S+, 18S- or NS1, NS4 or NS1, NS8, and 2 × Taq Plus MasterMix (CWBio, China). Each reaction was carried out for 10 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 53 °C, and 2 min at 72 °C; and 10 min at 72 °C. PCR products were detected using 1% agarose gel and subjected to Sanger sequencing for accurate taxonomic identification. All primers used in this article are shown in Table S13.

■ ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04546.

STE DNA extraction method, sequencing, and infor- mational analysis of metagenomes of banknotes (PDF)

Tables of species annotations; phages, drug-resistant bacteria, anaerobic microbes, and fungi found; resistance mechanisms; and primers used (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Weiven Cai — Institute of Applied Genomics and College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China; orcid.org/0000-0001-5836-3486; Email: caiww@fzu.edu.cn

Authors

Jun Lin — Institute of Applied Genomics, College of Biological Science and Engineering, and Fujian Key Laboratory of Marine Enzyme Engineering, Fuzhou University, Fuzhou 350108, China; School of Basic Medical Sciences, Fuzhou Medical University, Fuzhou 350108, China; orcid.org/0000-0002-5971-9963

Wenqian Jiang — Institute of Applied Genomics and College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China

Yang Shi — Institute of Applied Genomics and College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c04546

Author Contributions

J.L. and W.J. contributed equally to the work.

Notes

The authors declare no competing financial interest. All raw data was uploaded to the NCBI-SRA database under the accession number of SRP128023.

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■ REFERENCES

(1) Angelakis, E.; Azhar, E. I.; Bibi, F.; Yasir, M.; Al-Ghamdi, A. K.; Ashshi, A. M.; Elshemi, A. G.; Raoult, D. Paper money and coins as potential vectors of transmissible disease. *Future Microbiol.* 2014, 9, 249–261.

(2) News, S. Bank clerks do not wash their hands after counting money and suffer from venereal diseases; http://sc.sina.com.cn/news/s/2015-06-22/detail-ifxeufrr0294649.shtml (accessed 8.17).

(3) Tuttle, B. *The Money in Your Wallet Might Be Covered With Poop, Mold, and Cocaine*; http://money.com/money/4621673/money-cash-currency-bacteria-disease-sickness/ (accessed 8.17).

(4) Abrams, B. L.; Waterman, N. G. Dirty Money. *JAMA, J. Am. Med. Assoc.* 1972, 219, 1202–1203.

(5) Gabriel, E. M.; Coffey, A.; O’Mahony, J. M. Investigation into the prevalence, persistence and antibiotic resistance profiles of staphylococci isolated from euro currency. *J. Appl. Microbiol.* 2013, 115, 565–571.

(6) Gedik, H.; Voss, T. A.; Voss, A. Money and transmission of bacteria. *Antimicrob. Resist. Infect. Control* 2013, 2, 22.

(7) Jalali, S.; Kohli, S.; Latka, C.; Bhatia, S.; Vellarikal, S. K.; Sivasubbu, S.; Scarra, V.; Ramachandran, S. Screening currency notes for microbial pathogens and antibiotic resistance genes using a shotgun metagenomic approach. *PLoS One* 2015, 10, e0128711.

(8) Heshiki, Y.; Dissanayake, T.; Zeng, T.; Kang, K.; Yueqiong, N.; Xu, Z.; Sarkar, C.; Woo, P. C. Y.; Chow, B. B. C.; Baker, D.; Yan, A.; Webster, C. J.; Panagiotou, G.; Li, J. Toward a Metagenomic Understanding on the Bacterial Composition and Resistant in Hong Kong Banknotes. *Front. Microbiol.* 2017, 8, 632.

(9) Szafiranski, S. P.; Wos-Oxley, M. L.; Vilchez-Vargas, R.; Jauregui, R.; Plumeier, I.; Klawonn, F.; Tomash, J.; Meisinger, C.; Kuhnisch, J.; Sztajer, H.; Pieper, D. H.; Wagner-Dobler, I. High-resolution taxonomic profiling of the subgingival microbiome for biomarker discovery and periodontitis diagnosis. *Appl. Environ. Microbiol.* 2015, 81, 1047–1058.

(10) Jurtz, V. I.; Villarroel, J.; Lund, O.; Voldby Larsen, M.; Nielsen, M. MetaPhinder-Identifying Bacteriophage Sequences in Metagenomic Data Sets. *PLoS One* 2016, 11, No. e0163111.

(11) Liu, B.; Pop, M. ARDB–Antibiotic Resistance Genes Database. *Nucleic Acids Res.* 2009, 37, D443–D447.

(12) Jia, B.; Raphenya, A. R.; Alcock, B.; Waglechner, N.; Guo, P.; Tsang, K. K.; Lago, B. A.; Dave, B. M.; Pereira, S.; Sharma, A. N.; Doshi, S.; Courtot, M.; Lo, R.; Williams, L. E.; Frye, J. G.; Elsayegh, T.; Sardar, D.; Westman, E. L.; Pawlowski, A. C.; Johnson, T. A.; Brinkman, F. S.; Wright, G. D.; McArthur, A. G. CARD 2017: expansion and model-centric curating of the comprehensive antibiotic resistance database. *Nucleic Acids Res.* 2017, 45, D566–D573.

(13) McArthur, A. G.; Waglechner, N.; Nizam, F.; Yan, A.; Azad, M. A.; Baylay, A. J.; Bhullar, K.; Canova, M. J.; De Pascale, G.; Elsayegh, T.; Kalan, L.; King, A. M.; Koteva, K.; Morar, M.; Mulvey, M. R.; O’Brien, J. S.; Pawlowski, A. C.; Piddock, L. J.; Spanogiannopoulos, P.; Sutherland, A. D.; Tang, J.; Taylor, P. L.; Thaker, M.; Wang, W.; Yan, M.; Yu, T.; Wright, G. D. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* 2013, 57, 3348–3357.

(14) Li, Y.; Xu, C. Human Papillomavirus-Related Cancers. *Adv. Exp. Med. Biol.* 2017, 1018, 23–34.
(15) Bansal, A.; Singh, M. P.; Rai, B. Human papillomavirus-associated cancers: A growing global problem. Int. J. Appl. Basic Med. Res. 2016, 6, 84–89.
(16) Rotondo, J. C.; Mazzoni, E.; Bononi, I.; Tegnon, M.; Martini, F. Association Between Simian Virus 40 and Human Tumors. Front. Oncol. 2019, 9, 670.
(17) Poulin, D. L.; DeCaprio, J. A. Is there a role for SV40 in human cancer? J. Clin. Oncol. 2006, 24, 4356–4365.
(18) Chen, X.; Anstey, A. V.; Bugert, J. J. Molluscum contagiosum virus infection. Lancet Infect. Dis. 2013, 13, 877–888.
(19) Demiralslan, H.; Dinc, G.; Doganay, M. An Overview of ORF Virus Infection in Humans and Animals. Recent Pat. Anti-Infect. Drug Discovery 2017, 12, 21–30.
(20) Bergqvist, C.; Kurban, M.; Abbas, O. ORF virus infection. Rev. Med. Virol. 2017, 27, No. e1932.
(21) Yanase, T.; Maeda, K.; Kato, T.; Nyuta, S.; Kamata, H.; Yamakawa, M.; Tsuda, T. The resurgence of Shamonda virus, an African Simbu group virus of the genus Orthobunyavirus, in Japan. Arch. Virol. 2005, 150, 361–369.
(22) Yanase, T.; Kato, T.; Aizawa, M.; Shuto, Y.; Shirafuji, H.; Yamakawa, M.; Tsuda, T. Genetic reassortment between Sathuperi and Shamonda viruses of the genus Orthobunyavirus in nature: implications for their genetic relationship to Schmallenberg virus. Arch. Virol. 2012, 157, 1611–1616.
(23) Alemu, A. Microbial Contamination of Currency Notes and Coins in Circulation: A Potential Public Health Hazard. Biomed. Biotechnol. 2014, 2, 46–53.
(24) Ferlay, J.; Ervik, M.; Lam, F.; Colombo, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today, https://gco.iarc.fr/today (accessed 8.6).
(25) Everett, R. D. HSV-1 biology and life cycle. Methods Mol. Biol. 2014, 1144, 1–17.
(26) Joly-Guillou, M. L. Clinical impact and pathogenicity of Acinetobacter. Clin. Microbiol. Infect. 2005, 11, 868–873.
(27) Antunes, L. C.; Visca, P.; Towner, K. J. Acinetobacter baumannii: evolution of a global pathogen. Pathog. Dis. 2014, 71, 292–301.
(28) Rocca, I.; Espinal, P.; Vila-Farres, X.; Vila, J. The Acinetobacter baumannii Oxymonor: Commensal Hospital Dweller Turned Pan-Drug-Resistant Menace. Front. Microbiol. 2012, 3, 148.
(29) Bergogne-Bérézin, E.; Towner, K. J. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. 1996, 9, 148–165.
(30) Anderson, M.; Sansonetti, P. J.; Marteyn, B. S. Shigella Diversity and Changing Landscape: Insights for the Twenty-First Century. Front. Cell Infect. Microbiol. 2016, 6, 45.
(31) Toth, R.; Toth, A.; Vagvolgyi, C.; Gacser, A. Candida parapsilosis Secreted Lipase as an Important Virulence Factor. Curr. Protein Pept. Sci. 2017, 18, 1043–1049.
(32) Kuhn, D. M.; Mikherjee, P. K.; Clark, T. A.; Pujol, C.; Chandra, J.; Hajjeh, R. A.; Warnock, D. W.; Soil, D. R.; Ghannom, M. A. Candida parapsilosis characterization in an outbreak setting. Emerg Infect. Dis. 2004, 10, 1074–1081.
(33) Quindos, G. Epidemiology of candidiasis and invasive candidiasis. A changing face. Rev. Iberoam. Micol. 2014, 31, 42–48.
(34) El-Dars, F. M.; Hassan, W. M. A preliminary bacterial study of Egyptian paper money. Int. J. Environ. Health Res. 2005, 15, 235–240.
(35) Vriesekoop, F.; Russell, C.; Alvarez-Mayorga, B.; Aidoo, K.; Yuan, Q.; Scannell, A.; Beumer, R. R.; Jiang, X.; Barro, N.; Otokunefor, K.; Smith-Arnold, C.; Heap, A.; Chen, J.; Iturriage, M. H.; Hazeleger, W.; DeSlandes, J.; Kinley, B.; Wilson, K.; Menz, G. Dirty money: an investigation into the hygiene status of some of the world’s currencies as obtained from food outlets. Foodborne Pathog. Dis. 2010, 7, 1497–1502.
(36) McArthur, A. G.; Wright, G. D. Bioinformatics of antimicrobial resistance in the age of molecular epidemiology. Curr. Opin. Microbiol. 2015, 27, 45–50.
(37) Yang, Y.; Li, B.; Ju, F.; Zhang, T. Exploring Variation of Antibiotic Resistance Genes in Activated Sludge over a Four-Year Period through a Metagenomic Approach. Environ. Sci. Technol. 2013, 47, 10197–10205.
(38) Forsberg, K. J.; Patel, S.; Gibson, M. K.; Lauber, C. L.; Knight, R.; Fierer, N.; Dantas, G. Bacterial phylogeny structures soil resistomes across habitats. Nature 2014, 509, 612–616.
(39) Fang, H.; Wang, H. F.; Cai, L.; Yu, Y. L. Prevalence of Antibiotic Resistance Genes and Bacterial Pathogens in Long-Term Manured Greenhouse Soils As Revealed by Metagenomic Survey. Environ. Sci. Technol. 2015, 49, 1095–1104.
(40) Martinez, J. L.; Coque, T. M.; Baquero, F. What is a resistance gene? Ranking risk in resistomes. Nat. Rev. Microbiol. 2015, 13, 116–123.