

Bacterial contamination and their tolerance in banknotes and coins surrounding the area of Chiang Mai University Hospital in Chiang Mai Province

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ABSTRACT

Background: Currency including banknotes and coins are used in everyday life by everyone. It is, therefore, a universal medium for carrying potentially pathogenic bacteria which can spread and cause infection in the population. Thus, it is of interest to study the bacterial contamination in currency used daily in Chiang Mai province, of which there is scant information.

Objectives: To study the quantity and types of bacterial contamination on banknotes and coins circulating in the area surrounding Chiang Mai University Hospital in Chiang Mai province and examine the tolerance of detected bacteria on the currency.

Materials and methods: Banknotes and coins, totaling 343 samples, were collected from circulation in the area surrounding Chiang Mai University Hospital in Chiang Mai. The bacterial contamination was studied based on culture methods and identified by morphological and biochemical tests. To examine the bacterial tolerance, the contaminated bacteria recovered from the currency, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, were tested. These bacteria were inoculated on banknotes and coins and the viability of the bacteria at various time points was examined.

Results: The amount of bacterial contamination on banknotes, which are linen fibers, was higher than those on coins, which are opaque metals. The gram-positive bacteria were found in a higher amount than gram-negative bacteria, in both banknotes and coins. Human skin flora and pathogenic bacteria, especially *S. aureus* and pathogenic Enterobacteriaceae, were detected. The bacterial tolerance study revealed that *S. aureus*, the gram-positive cocci, showed a decrease of viability on banknotes and coins within 6 hours. Meanwhile, gram-negative bacilli presented a decrease of viability in 6 and 1 hours on banknotes and coins, respectively. The bacterial tolerance testing by used and unused currency showed the same results.

Conclusion: All banknotes and coins circulating in the area surrounding Chiang Mai University Hospital in Chiang Mai province were demonstrated to be contaminated with bacteria, some of which might be pathogens and dangerous to users. Gram-positive bacteria has higher tolerance than gram-negative bacteria on currency. Bacterial tolerance on banknotes is longer than on coins. The knowledge obtained is advantageous for people in general as an awareness of these harmful bacteria when using the currency.

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Introduction

Currency, including banknotes and coins, are fomites ubiquitously handled and transferred in the course of our daily lives. Therefore, they have a high chance of becoming contaminated with bacteria. Many reports showed that a large amount of bacterial contamination appeared on banknotes in circulation.¹⁻³ This may lead to the spread of bacterial infection in people and the environment.⁴⁻⁶

Generally, money is transferred from hand to hand between healthy and/or pathogen infected people with repetitive contact thereafter. Contamination of bacteria on currency may come from mucus, cough, sneeze and wound secretion. Moreover, someone who holds banknotes and coins temporarily between his/her lip can bring in contamination from the environment. Thus, banknotes and coins are presented as a universal medium that carries potentially pathogenic bacteria.^{4,7} Without the awareness of the dangers, there is a high chance of bacterial infection in people when using contaminated currency.

The currency used in Thailand is comprised of Thai banknotes and Thai coins that are issued under the supervision of and manufactured by the Bank of Thailand and The Treasury Department. In the year 2016, numerous Thai coins were used in circulation, mainly in 25, 50 Satang, 1, 2, 5 and 10 Baht denominations; around the country, there were approximately 3321, 2407, 14,991, 1614, 3118 and 1816 million coins, respectively.⁸ Moreover, a large amount of banknotes, including 20, 50, 100, 500 and 1000 Baht were used in circulation: 88.4, 7.3, 14.1, 0.6 and 1.3 million notes, respectively.⁹ The bacteria contamination on banknotes and coins may originate from the production, storage and usage.^{10,11} There is an unpredictable chance and rare opportunity of cleaning or disinfecting money,¹² therefore, this may enhance pathogenic bacteria transmission. Consequently, bacterial contamination of currency may be a risk factor in the development of a public health problem.

Most bacterial contamination on banknotes and coins were reported, including coagulase-negative staphylococci, commonly from human natural skin flora, and gram-positive spore-forming bacilli, especially *Bacillus* spp., which are environmental organisms.³ Moreover, other potential pathogen bacteria, including *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Salmonella* spp. are also commonly found.¹³ Some of these bacteria have been reported to survive well on the skin and can be transferred from hand to fabrics as well as from fabrics to hand.^{11,14} Thus, bacterial contamination related to currency is a significant topic which is reported from many parts of the world. However, the study of bacterial contamination of Thai banknotes and coins is scanty. In the present study, we determined the number and types of bacterial contamination of banknotes and coins circulating in the area surrounding Chiang Mai University Hospital in Chiang Mai province. The tolerance of bacteria on the currency was also examined.

Materials and methods

The currency used in this study

Currency, banknotes and coins used in this study were collected from those in common circulation in Chiang Mai. Different denominations of used banknotes and coins in a non-damaged state were randomly collected from people, patients, health care workers and students between April and September, 2016, in the hospital area and around the Faculty of Associated Medical Sciences, Chiang Mai University, as well as from the Treasury Department in Chiang Mai province. Meanwhile, unused new currency was obtained from a commercial bank and the Treasury Department in Chiang Mai.

Thai coins, including 25, 50 Satang and 2 Baht, consisting of 92% copper, 6% aluminum and 2% nickel were used to represent the commonly used copper alloy coin. One, 5 and 10 Baht, the commonly nickel alloy coins, consist of 75% copper and 25% nickel. Ten Baht coins also contain a ring inside comprised of 92% copper, 6% aluminum and 2% nickel.⁸ In addition, Thai banknotes used in this study were 20, 50, 100, 500 and 1000 Baht banknotes made from a cotton-linen blend.⁹

Between 20 and 30 samples were collected for each used denomination, totaling 343 samples. The new currency was collected in 27 samples per denomination for contamination study and tolerance test.

Sample processing

The banknotes or coins were placed individually into sterile polypropylene plastic bags, then transported to the Microbiology laboratory at the Faculty of Associated Medical Sciences, Chiang Mai University. Money was stored at room temperature until inoculation, which was carried out within 2 hours after collection.⁷

Extraction of bacteria from the banknotes and coins¹²

To study the number and types of bacterial contamination on banknotes and coins, each banknote and coin was aseptically placed individually into sterile tubes containing 20 and 10 ml of trypticase soy broth (TSB) (BD, Heidelberg, Germany), respectively. The samples were shaken for 30 minutes in an air conditioned room at 25 °C to remove the microorganisms from the currency into the medium. After that, the currency was aseptically removed with forceps.

Qualitative and quantitative bacterial analysis.⁷

The currency bacteria extracting media was diluted ten-fold and 0.1 ml of each dilution was spread onto sterile Petri dishes containing tryptic soy agar (BD), blood agar (Oxoid) and MacConkey (BD). The dishes were incubated aerobically for 24–48 hours at 35 °C.¹⁵ The colonies growing on media were counted using darkfield quebec colony counters (Reichert Technologies, USA). The total viable cells, referred to as colony forming units (CFU) per cm² and CFU per banknote and coin, were calculated. The isolated bacteria were identified by Gram staining, characterized on the basis of colony morphology and biochemical characteristics.¹⁰

The study of tolerance of bacteria on currency

Four bacterial species which were recovered in this study, including *Staphylococcus aureus* (gram-positive cocci bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (gram-negative bacilli bacteria), were used. All bacteria were subcultured individually at 37 °C in aerobic conditions and subsequently maintained on TSA (BD). Overnight cultures were prepared in tryptic soy broth (BD) at 37 °C. Broth cultures of each strain was prepared to equal the turbidity of a 0.5 McFarland standard (1×10^8 CFU/mL) using densitometer with the assistance of optical density followed by 1 in 100 dilution to obtain 1×10^6 CFU/ml. Unused or used banknotes and coins were sterilized using autoclaves at 121 °C, 15 lbs/in² for 15 minutes before use in the experiment. Then, bacteria at 10^6 CFU/ml 100 µl were spread onto unused new or used banknotes and 20 µl onto unused new or used coins, which then waited to dry in a biosafety cabinet. The bacteria from each sample were then extracted as described in qualitative and quantitative bacterial analysis. The survival of bacteria was studied¹⁶ at 0, 10, 30, 60 and 360 minutes after inoculation.

Results

Quantification and type of bacteria isolated from banknotes and coins circulating in Chiang Mai province

The mean number of viable bacteria isolated from circulating currency in Chiang Mai province were 58×10^2 CFU per cm² of banknote, $11,559 \times 10^2$ CFU per banknote, 41×10^2 CFU per cm² of coin and 466×10^2 CFU per coin, for all banknotes and coins studied. These results indicated that the amount of bacterial contamination on banknotes contained in linen fibers was much higher than that on coins, which are opaque metals.

As shown in Tables 1 and 2, gram-positive bacteria were found in a higher number than gram-negative bacteria, both in banknotes and coins. On banknotes, the recovery of gram-positive and gram-negative bacteria was $6,222 \times 10^2$ and $5,337 \times 10^2$ CFU/ banknotes, respectively (Table 1). The same pattern was observed in metal coins where the number of gram-positive and gram-negative bacteria was 242×10^2 and 224×10^2 CFU/coin, respectively (Table 2).

On the used banknotes, the highest to lowest number of bacteria was present in the following order

of denominations: 20, 100, 500, 1000 and 50 Baht banknote. Most microorganism recovered from banknotes were gram-positive bacteria, including coagulase-negative staphylococci, *Bacillus* spp., *Staphylococcus aureus*, *Enterococcus* spp., *Streptococcus* spp., *Micrococcus* spp., and other gram-positive bacteria (Table 1). However, gram-positive cocci catalase test positive *Micrococcus* spp., and gram-positive bacilli, including diphtheroid bacilli, were detected in low numbers in the tested currency (Table 1).

Various species of gram-negative bacteria in the Enterobacteriaceae family, comprised of *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter sakazakii*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Escherichia coli* were recovered from the tested banknotes. Moreover, non-fermenting bacteria, including *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were also detected (Table 1).

On the used coins, the highest to lowest number of bacteria was present in the following order of denominations: 1, 5, 10 Baht, 50 Satang, 2 Baht and 25 Satang coins (Table 2). Most bacteria recovered from coins were gram-positive including coagulase-negative staphylococci, *S. aureus*, *Streptococcus* spp., *Enterococcus* spp., *Bacillus* spp., Diphtheroid bacilli, *Micrococcus* spp., and other gram-positive bacteria (Table 2). In addition, a large number of gram-negative bacteria, including Enterobacteriaceae and non-fermenting bacteria, were detected on coins (Table 2).

It was found that in 100% of the samples in common circulation studied, both used banknotes and coins were contaminated with bacteria. However, no bacteria was found in unused new banknotes and coins obtained from the Treasury Office and the banks. It was found that gram-positive bacteria were detected at a higher rate than gram-negative bacteria. Interestingly, *E. coli*, the member of coliform enteric bacteria, was also detected in used banknotes and coins. Additionally, gram-negative bacilli non-fermented group, which may cause hospital acquired infection,¹⁷ comprised of *A. baumannii*, *P. aeruginosa*, *S. maltophilia* and *A. lwoffii*, were also detected in the tested currency. However, many of the bacterial contaminations in the tested currency were identified as commensalism bacteria, especially coagulase-negative *Staphylococcus* spp., *Bacillus* spp., *Micrococcus* spp., and *Neisseria* spp.

Table 1 Amount of bacterial isolation from banknotes in circulation in areas surrounding Chiang Mai University Hospital in Chiang Mai Province.

Microorganisms Bacteria	Frequency of isolation as total viable counts* (x 10 ² CFU) Banknotes (denominations; Baht)					Total viable countx10 ² CFU (%)
	20 Baht	50 Baht	100 Baht	500 Baht	1000 Baht	
Coagulase-negative staphylococci	668±0.14	143±1.1	810 ± 0.25	686 ± 0.23	254±0.32	2560±2.04 (22.1)
<i>Staphylococcus aureus</i>	146±0.41	1±0.71	103±0.21	129±0.43	28±0.33	422±2.09 (3.7)
<i>Streptococcus</i> spp.	15±0.32	16±0.43	23±0.22	17±0.75	28 ± 0.56	100±2.28 (0.9)
<i>Enterococcus faecalis</i>	23±0.87	9±0.98	18±0.23	7±0.11	5 ± 0.32	61±2.51 (0.5)
<i>Enterococcus</i> spp.	44±0.76	23±0.79	32±0.42	80±0.77	54±0.98	233±3.72 (2.0)
<i>Bacillus</i> spp.	697±0.11	302±0.23	389±0.55	337±1.02	366±0.45	2090±2.36 (18.1)
Diphtheroid bacilli	86±0.43	23±0.22	162±0.56	79±0.21	35 ± 0.13	385±1.55 (3.3)
<i>Micrococcus</i> spp.	23±0.54	9±0.33	19±0.87	22±0.43	23 ± 88	96±3.05 (0.8)

Table 1 Amount of bacterial isolation from banknotes in circulation in areas surrounding Chiang Mai University Hospital in Chiang Mai Province. (continues)

Microorganisms Bacteria	Frequency of isolation as total viable counts* (x 10 ² CFU) Banknotes (denominations; Baht)					Total viable countx10 ² CFU (%)
	20 Baht	50 Baht	100 Baht	500 Baht	1000 Baht	
Other gram-positive bacteria	78±1.02	28±1.03	63±0.46	70±0.43	38 ± 0.34	276±3.28 (2.4)
Gram-positive bacteria number/ denomination	1778±4.60	569±5.82	1618±3.77	1426±4.38	831±4.31	6222 (53.8)
<i>Enterobacter cloacae</i>	294±0.18	79±0.19	227±0.32	238±0.43	141±0.47	979±1.59 (8.5)
<i>Enterobacter aerogenes</i>	24±0.87	16±0.32	16±0.12	22±0.21	17±0.32	94±1.84 (0.8)
<i>Enterobacter sakazakii</i>	30±0.78	16±0.32	65±0.97	13±0.86	15±0.54	138±3.47 (1.2)
<i>Klebsiella ozaenae</i>	114±0.23	48±0.54	194±1.02	179±0.99	56±0.76	592±3.54 (5.1)
<i>Klebsiella pneumoniae</i>	89±0.78	48±0.46	97±0.43	119±0.14	35±0.43	388±2.24 (3.4)
<i>Citrobacter koseri</i>	68±0.33	32±0.12	32±0.89	99±0.53	35±0.77	267±2.64 (2.3)
<i>Escherichia coli</i>	23±0.39	14±1.12	45±0.39	50±0.88	10±0.88	142±3.66 (1.2)
<i>Acinetobacter baumannii</i>	254±0.87	111±0.57	162±0.34	269±0.33	113±0.34	909±2.45 (7.9)
<i>Acinetobacter lwoffii</i>	51±0.90	32±0.87	162±0.46	179±0.87	28±0.88	453±3.98 (3.9)
<i>Pseudomonas aeruginosa</i>	130±0.98	58±0.12	97±0.32	90±0.11	37±0.21	411±1.74 (3.6)
<i>Stenotrophomonas maltophilia</i>	23±0.33	48±0.78	65±0.98	90±0.76	15±0.34	240±3.19 (2.1)
<i>Pantoea agglomerans</i>	126±0.23	16±0.33	162±0.21	90±1.09	28±0.65	421±2.51 (3.6)
<i>Neisseria</i> spp.	33±0.43	35±0.22	32±0.54	47±0.78	13±0.34	161±2.31 (1.4)
Other gram-negative bacteria	43±0.33	16±0.78	32±0.45	16±0.33	35±0.24	141±2.13 (1.2)
Gram-negative bacteria number/denomination	1302±7.63	567±6.74	1389±7.44	1500±8.31	579±7.17	5337 (46.2)
Total bacterial number	3080	1136	3007	2927	1409	11559 (100)

* Values are means of three replicates for each denomination.

Table 2 Amount of bacterial isolation from Thai coins in circulation in areas surrounding Chiang Mai University Hospital in Chiang Mai Province.

Microorganisms Bacteria	Frequency of isolation as total viable counts (x 10 ² CFU) Coins (denominations; Baht)						Total viable countx10 ² CFU (%)
	25 Satang	50 Satang	1 Baht	2 Baht	5 Baht	10 Baht	
Coagulase-negative staphylococci	7±0.42	8±0.63	11±0.56	9±0.67	12±0.98	11±1.13	58±4.39 (12.4)
<i>Staphylococcus aureus</i>	4±0.12	5±0.44	7±0.73	5±1.01	9±0.78	8±0.23	38±3.31 (8.2)
<i>Streptococcus</i> spp.	3±0.22	4±0.21	4±0.22	4±0.31	3±0.32	4±0.07	22±1.35 (4.7)
<i>Enterococcus</i> spp.	3±0.23	3±0.43	4±1.01	3±0.32	3±0.33	3±0.23	19±2.55 (4.1)
<i>Bacillus</i> spp.	7±0.88	8±0.43	11±0.21	9±0.13	11±0.43	12±0.54	58±2.62 (12.4)
Diphtheroid bacilli	3±0.76	3±0.45	3±0.23	2±0.43	3±0.31	3±0.15	17±2.33 (3.6)
<i>Micrococcus</i> spp.	2±0.10	2±0.76	2±0.33	2±0.14	1±0.22	1±0.43	10±1.98 (2.1)
Other gram-positive bacteria	3±0.65	3±0.53	4±1.13	3±0.67	4±0.33	3±0.87	20±4.18 (4.3)
Gram-positive bacteria number/denomination	32±3.38	36±3.88	46±4.42	37±3.68	46±3.70	45±3.65	242 (51.9)
<i>Enterobacter cloacae</i>	4±0.18	5±0.24	7±0.32	6±0.43	6±0.14	4±0.56	32±1.87 (6.9)
<i>Enterobacter aerogenes</i>	4±0.5	4±0.33	5±0.70	4±0.23	4±0.87	5±0.32	26±2.95 (5.6)
<i>Klebsiella ozaenae</i>	4±0.64	4±0.53	5±0.71	4±0.23	4±0.78	4±1.01	25±3.90 (5.4)
<i>Klebsiella pneumoniae</i>	2±0.24	3±0.32	3±0.11	2±0.87	3±0.56	4±0.89	17±2.99 (3.6)
<i>Escherichia coli</i>	2±0.31	3±0.45	4±0.67	3±0.43	3±0.88	3±0.44	18±3.18 (3.9)
<i>Acinetobacter baumannii</i>	4±0.21	5±1.01	6±0.43	3±0.32	5±0.55	3±0.45	26±2.97 (5.6)
<i>Acinetobacter lwoffii</i>	4±0.89	4±0.77	5±0.64	2±0.98	4±0.35	3±1.1	22±4.73 (4.7)
<i>Pseudomonas aeruginosa</i>	4±0.24	5±0.31	7±0.23	5±0.22	6±0.12	5±0.21	32±1.33 (6.9)
<i>Neisseria</i> spp.	1±0.98	1±0.43	2±0.32	1±0.22	1±0.53	1±0.35	7±2.83 (1.5)
Other gram-negative bacteria	3±0.54	3±0.31	4±0.75	3±0.31	3±1.03	3±0.43	19±3.37 (4.1)

Table 2 Amount of bacterial isolation from Thai coins in circulation in areas surrounding Chiang Mai University Hospital in Chiang Mai Province. (continues)

Microorganisms Bacteria	Frequency of isolation as total viable counts ($\times 10^2$ CFU) Coins (denominations; Baht)						Total viable count $\times 10^2$ CFU (%)
	25 Satang	50 Satang	1 Baht	2 Baht	5 Baht	10 Baht	
Gram-negative bacteria number/denomination	32 $\pm$ 4.73	37 $\pm$ 4.70	48 $\pm$ 4.88	33 $\pm$ 4.24	39 $\pm$ 5.81	35 $\pm$ 5.76	224 (48.1)
Total bacterial number	64	73	94	70	85	80	466 (100)

* Values are means of three replicates for each denomination.

Study of tolerance of bacteria on Thai currency

Four species of isolated bacteria from banknotes and coins, including *Staphylococcus aureus* (gram-positive cocci), *E. coli* (coliform gram-negative bacilli), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (non-fermenting gram-negative bacilli), were selected for the bacterial tolerance test. Three bacterial isolates of each species were randomly selected for the bacterial tolerance test. The currency, including 25 Satang coins (copper coin), 1 Baht coin (nickel alloy coin), 20 and 100 Baht banknotes were used in the bacterial tolerance study. We also compared the bacterial tolerance between unused and used denominations of coins and banknotes by detecting the viability of bacteria after inoculating bacteria for 0 to 360 minutes.

In the study of bacterial tolerance of banknotes, the decrease of viability of bacteria was observed in the same pattern as the test between used and unused 20 and 100 Baht banknotes (Figure 1). *Staphylococcus aureus* showed viability on used and unused banknote by persisting longer than 6 hours (Figure 1B). Moreover, *S. aureus* showed more tolerance than *E. coli*, *P. aeruginosa* and *A. baumannii* on

banknotes at 360 minutes (Figure 1B, 1F, 1H). In addition, these microorganisms showed more tolerance on banknotes than on coins at 360 minutes (Figure 1A, 1B).

Studying bacterial tolerance on coins, *Staphylococcus aureus* showed viability on 25 Satang copper coins test until 6 hours in unused and used coins and more tolerance on 1 Baht nickel coins test by persistence longer than 6 hours (Figure 1A). The gram-negative bacteria, *E. coli* and *P. aeruginosa* showed low viability at 1 hour on coins (Figure 1C, 1E), whereas *A. baumannii* showed low viability at 30 minutes on both unused and used 25 Satang copper coins (Figure 1G). The viability of *E. coli*, *P. aeruginosa* and *A. baumannii* on nickel coins indicated low viability at 60 minutes on both unused and used coins (Figure 1C, 1E, 1G).

These studies have illustrated that bacteria generally has better tolerance on banknotes than on coins. The decrease of viability of bacteria was observed in the same pattern when comparing unused and used coins and banknotes. The viability of all tested bacteria decreased considerably in the first hour on the surface of the currency (Figure 1).

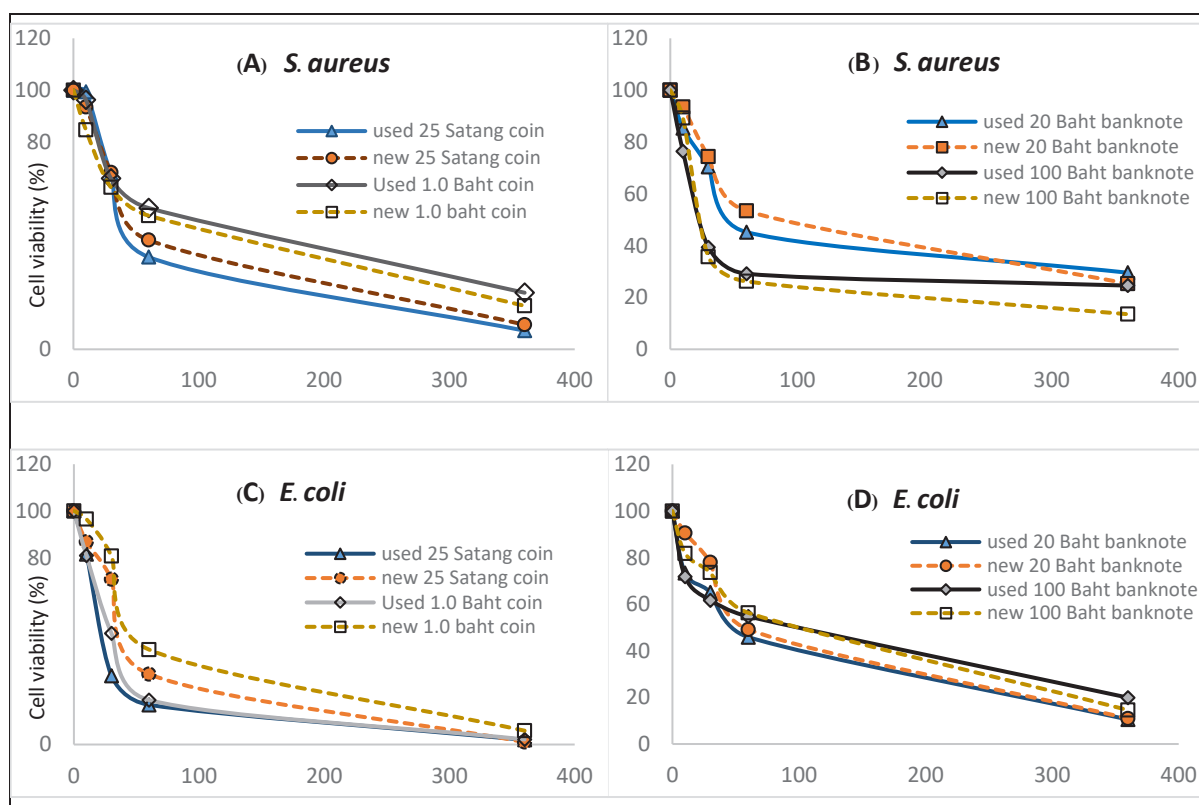


Figure 1 Cell viability of bacteria during exposure for 0 to 360 minutes on surface of new and used 25 Satang and 1 Baht coins, *Staphylococcus aureus* (A), *Escherichia coli* (C), *Pseudomonas aeruginosa* (E) and *Acinetobacter baumannii* (G). Cell viability of bacteria during exposure for 0 to 360 minutes on surface of new and used 20, 100 Baht banknotes, *Staphylococcus aureus* (B), *Escherichia coli* (D), *Pseudomonas aeruginosa* (F) and *Acinetobacter baumannii* (H).

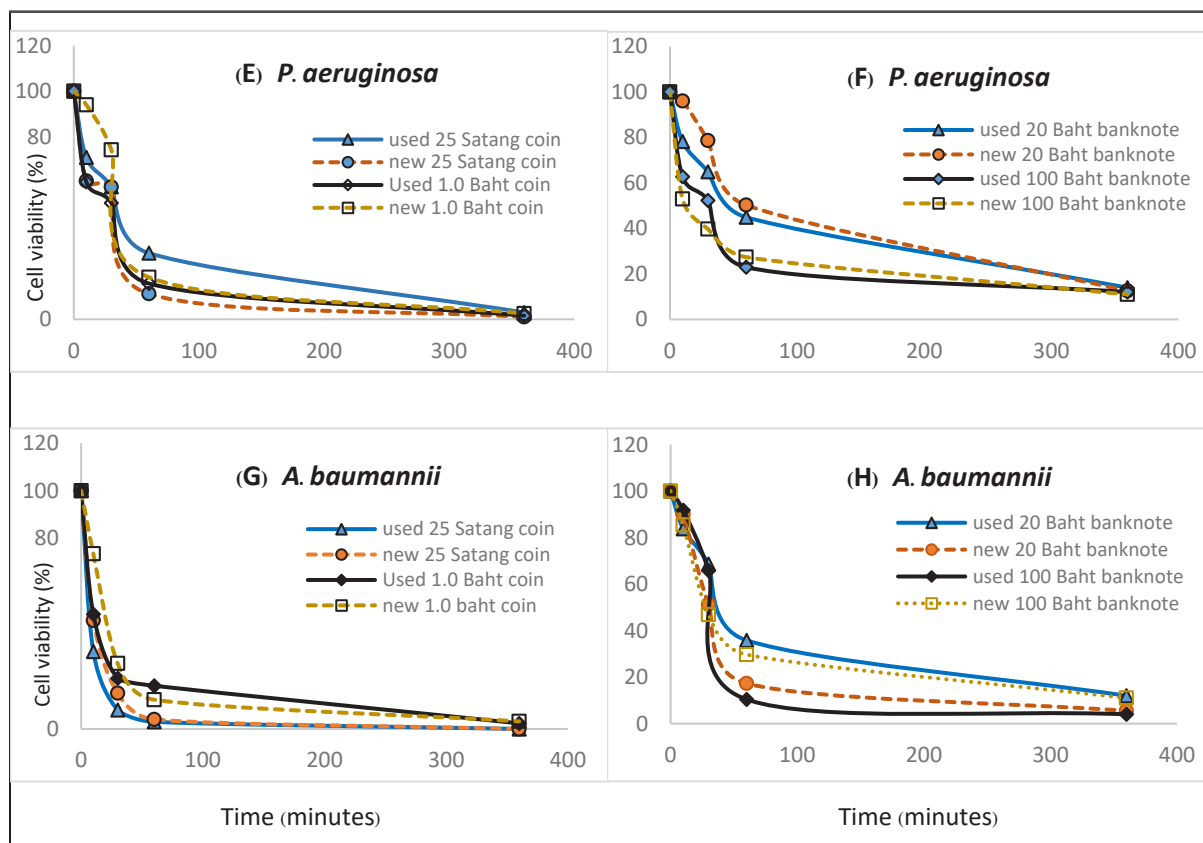


Figure 1 Cell viability of bacteria during exposure for 0 to 360 minutes on surface of new and used 25 Satang and 1 Baht coins, *Staphylococcus aureus* (A), *Escherichia coli* (C), *Pseudomonas aeruginosa* (E) and *Acinetobacter baumannii* (G). Cell viability of bacteria during exposure for 0 to 360 minutes on surface of new and used 20, 100 Baht banknotes, *Staphylococcus aureus* (B), *Escherichia coli* (D), *Pseudomonas aeruginosa* (F) and *Acinetobacter baumannii* (H).

Discussion

Banknotes and coins are used in everyday life by everybody; however, they might be a source of bacterial contamination and even a source of infections. In general, we usually use currency without the awareness of the danger from potential bacterial infections in different parts of the body. This may be due to a lack of information related to bacterial prevalence on the currency used. Therefore, studying the type, number and the persistence of bacteria on the currency can provide significant baseline data for the prevention of bacterial infection that may occur. In this study, the quantity and types of bacterial contamination on banknotes and coins circulating in the area surrounding Chiang Mai University Hospital in Chiang Mai province were disclosed. The tolerances of bacteria on the currency were also revealed.

In this study, the experiments were carried out by conventional culture methods which are related to many studies.^{13, 18} We found that 100% of used currency samples collected in the area surrounding Chiang Mai University Hospital in Chiang Mai province were contaminated with bacteria. In contrast, unused new banknotes and coins tested under the same conditions showed no presence of bacterial contamination. We speculated that new currency from the production line was subjected to a cleaning or sanitization process which can destroy bacterial contamination. The contamination found in the used currency might have come from bacteria deposited during daily use.

In this study, we found that bacterial contamination was higher in banknotes than in coins. This may be because banknotes contain many fibers that form a porous surface,¹⁹ which can easily trap bacteria. In contrast, the physical structure of coins is smooth and contains metals, such as copper, exhibiting anti-bacterial properties.²⁰ Moreover, a higher number of bacterial contamination on used currency corresponds to a lower value of the currency, such as 20 Baht banknotes or 1 Baht coins being the most contaminated; this may be due to a higher frequency of usage. Our results are compatible with a report from the Bank of Thailand that the denominations of banknote used most frequently, from highest to lowest, are 20, 100, 1000, 50 and 500 Baht, respectively.⁹ In agreement with the report from the Treasury Department that the large amount of coin currency used in circulation, the highest number was 1 Baht coins, and the number decreased respectively in 25 Satang, 5 Baht, 50 Satang, 10 and 2 Baht.

On both banknotes and coins, gram-positive bacteria were found in higher numbers than gram-negative bacteria. The predominate gram-positive bacterial contaminations were gram-positive bacilli, especially *Bacillus* spp. which is normally found in nature and human natural skin flora.¹³ Meanwhile, *Staphylococcus* spp. was the predominate gram-positive and catalase-positive cocci, including coagulase-negative staphylococci, which is normally detected in human skin flora,^{3, 15} and *Staphylococcus aureus* that can cause skin infection, abscess, furuncle, boil and

a number of diseases. *Bacillus* spp. and *Staphylococcus* spp. were predominately found on copper coins, in line with that report from Santo et al., 2012.

Furthermore, in this study, gram-negative bacteria were also recovered, especially in the Enterobacteriaceae group. The *Enterobacter* and *Klebsiella* that were found on the tested currency are related to enteric bacteria. Interestingly, coliform bacteria such as *Escherichia coli*, which can be detected in the gastrointestinal system of humans, were also found in the tested currency. This bacterial species is an indicator of fecal contamination.²¹ This is a useful data to raise awareness of personal hygiene related to fecal-oral route transmission and infection. Moreover, non-fermenting gram-negative bacteria were also recovered from the currency circulating in Chiang Mai province. Most contaminate non-fermenters are *Acinetobacter* spp. and *Pseudomonas* spp. Bacteria in these groups can be found in the environment and can cause hospital acquired infection and a high number of drug resistant bacteria were reported.¹⁷ Moreover, *Stenotrophomonas maltophilia*, an emerging multidrug-resistant, globally opportunistic bacteria, was also detected. These bacteria are normally found in the hospital,²² which is in concordance to the places of collected currency samples in this study, i.e. the area surrounding Chiang Mai University Hospital. Therefore, some types of bacteria recovered from this study include pathogenic bacteria and may cause disease in humans that the general population should be aware of. This detection showed the possibility of bacteria spreading from hospital to community via banknotes and coins.

In the tolerance test of bacteria, four bacterial species were selected in the study. *Staphylococcus aureus*, the gram-positive cocci that can cause skin infection in humans²³ were used as representative of gram-positive bacteria. *Escherichia coli*, the gram-negative bacilli normally found in the small and large intestines²⁴ were used as representative of gram-negative fecal coliform bacteria. In addition to *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, gram-negative bacilli that might cause hospital acquired infection and environmental infection, were selected as representative of non-fermenting gram-negative bacilli. We found that many bacteria can survive only a few days on coins and banknotes. This data, however, was different from the studies of Kramer et al., 2006, which showed that many bacteria including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter* spp., *E. coli* and *Pseudomonas aeruginosa* may survive for months on dry surfaces.²⁵ We speculated that the different climate in the studied area and the currency component of banknotes and coins studied may cause the different results. The survival viability of bacteria indicated that gram-positive bacteria such as *Staphylococcus* spp. can survive longer than gram-negative bacteria. Previous reports showed that gram-positive bacteria are comprised of thick cell walls, which made them tolerant to the environment.²⁰ On the other hand, gram-negative bacilli contain thinner cell walls, causing a shorter time of survival in a non-optimal environment. While the results showed that gram-positive and gram-negative bacterial persistence was short lived,

we still detected bacteria; this might be because bacteria were being transferred from hand or from the environment onto money continuously. Therefore, some of the bacteria detected may have been a transient bacterial contamination. In order to prevent and control infection from the use of cash, the currency should be cleaned, as reported in Japan, by heat-sterilization of money or replacement with polymer banknotes.¹⁸

Used and unused currency may show different structural changes and differences in chemical contamination, which may affect the survival of bacteria on them. Thus, the study of tolerance of bacteria on currency was conducted with used and unused, sterile currency. However, the results of the tolerance study of bacteria in used and unused currency showed no difference. This might be because bacteria used in this study, including *Staphylococcus aureus*, a gram-positive cocci, has a thick cell wall that provides durability on the surface of used and unused currency.²⁰ However, gram-negative bacilli studied, including *P. aeruginosa*, *A. baumannii* and *E. coli*, are able to adapt themselves to the surface of currency.²⁶ In addition, time used in this study of was limited to 6 hours. Some bacteria were still alive. The result of tolerance of bacteria on used and unused cash, therefore, showed no difference.

Furthermore, this study has some limitations. Firstly, bacterial contamination on Thai currency in this study was cultured in simple media. Therefore, it was possible that fastidious bacteria (bacteria that have a complex nutritional requirement) was not detected in this study. Secondly, it was conducted to study only bacterial contamination on currency and not other microorganisms, including fungus, virus and parasite. Consequently, other microorganism should be identified in further studies. Thirdly, currency samples studied were collected in a limited area surrounding the university hospital and the Faculty of Associated Medical Sciences. However, samples should be collected in many places in order to get more information of bacterial contamination on currency. Fourthly, in the study of tolerance of bacteria on currency, there were four bacteria species tested. However, these bacteria might not be representative of all bacteria species. For additional benefits, the inclusion of other bacteria species is needed for further studies. Finally, time used in the study of tolerance of bacteria ranged from 0 to 6 hours; the result was that some bacteria were still alive. Therefore, tolerance should be studied for longer than 6 hours.

Taken together, in this study we presented information about bacteria contamination on banknotes and coins in Chiang Mai province. We, however, believe that the same situation might be occurring in other provinces of Thailand. The information obtained from this study might have some advantages, including: i) the baseline information of the amount and types of bacteria on the currency used in Chiang Mai province; ii) the prevalence of the contaminated bacteria on new and used coins and banknotes; and iii) the tolerance of gram-positive and gram-negative bacteria on new and used coins and banknotes. This study demonstrated that banknotes and coins in circulation have much bacterial contamination, some of which might contain pathogens

and are dangerous to human users. This knowledge is beneficial for people in general, as awareness of these harmful bacteria points to the necessity of cleaning and washing hands after using banknotes and coins for preventing bacterial contamination on coins and banknotes.

Conflict of interest:

The authors declare no conflict of interest.

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