

## Research Article

# The prevalence of bacterial contamination on mobile phones of pharmacy university students

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## ABSTRACT

Mobile phones (MPs) become one of the most important tools in professional and social life. Bacteria-contaminated MPs were reported in healthcare and non-healthcare workers but no study has investigated among pharmacy university students. We aimed to examine the prevalence of bacterial contamination on MPs and personal behaviors of MP usage. Fifty-three participants whose MPs had been used at least 3 months were enrolled. Each MP (n=53) was swabbed and cultured on sheep blood agar. Isolated colonies were characterized by conventionally bacterial identification methods. All participants were asked to complete questionnaires about behaviors of MP usage. Bacterial contamination was found in 52 of 53 MPs (98.11%) and 44 of 52 devices (84.62%) composed of heavy growth colonies (more than 15 colonies). The most abundant colonies were coagulase-negative staphylococci (CoNS) (42.72%), following by *Bacillus* spp. (27.18%), *Staphylococcus aureus* (20.39%), *Micrococcus* spp. (7.77%), *Corynebacterium* spp. (0.97%) and viridans streptococci (0.97%). Co-contamination with CoNS and *Bacillus* spp. was commonly found (28.85%). Most participants had risks for bacteria-contaminated MPs, which were visiting hospitals in past 6 months (56.60%), sharing with others (92.45%) and using in toilets (98.11%). Additionally, 44 of 53 (83.02%) participants regularly cleaned their MPs and 20 of them (45.45%) used clean clothes or tissue papers. No correlation between MP-using or cleaning behaviors and bacterial contamination was observed ( $p>0.05$ ). Our findings emphasized that bacteria-contaminated MPs are commonly found among pharmacy university students. MP users should increase awareness of personal hygiene and perform effective cleaning procedures to reduce bacterial persistence and transmission.

## Keywords:

Bacteria, Contamination, Mobile phone, Pharmacy university student

## 1. INTRODUCTION

Mobile phones (MPs) become essential electronic devices in daily life of many people including university students for social, communication, entertainment and educational aspects. The use of MPs with non-hygienic conditions such as sharing with others and lacking of using proper protocols for cleaning may promote these devices as fomites for microbial contamination and colonization. Generally, microbe can survive on the surface of inanimate objects for a long period. Moreover, heat generated during MP usage can enhance the growth of microorganisms accumulating on MP surfaces<sup>1-3</sup>. Consequently, these objects may allow the microbial contami-

nation, persistence and transmission, especially via the surface contact between MPs and human areas such as skin, hands, mouth, nose or ears<sup>4</sup>.

Microbial contamination on MPs was widely investigated in healthcare workers (HCWs)<sup>5-11</sup> and non-HCWs, including in community<sup>1,7,12</sup>. Most common microbes include environmental germs, normal skin and mouth flora such as coagulase-negative staphylococci (CoNS), *Micrococcus* spp., Diphtheroids, *Bacillus* spp., *Staphylococcus aureus*, non-hemolytic streptococci, *Enterococcus* spp. and gram-negative bacilli (GNB) groups. Moreover, pathogenic and antimicrobial resistance microorganisms such as methicillin-resistant *S. aureus* (MRSA) and extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae

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(ESBL-producing *Enterobacteriaceae*) and other antimicrobial drug resistant microbes were isolated<sup>11,13-14</sup>.

There were previous studies focusing the microbial contamination on MPs of secondary school students<sup>15</sup>, healthcare and non-healthcare university students<sup>16-20</sup>. However, previous studies limited to medical, nursing and dental students and few studies have investigated in pharmacy university students. Moreover, additional details of MP contamination such as the pattern of isolated microbes and behaviors of using MPs of pharmacy students were not fully elucidated. Similar to other healthcare professional students, MPs have frequently been used and become the essential tools for pharmacy students in both preclinical and clinical courses. They have to contact with patients or others during their study or internship in healthcare centers or communities. Therefore, the investigation of microbial contamination on MPs may announce them to aware of using MPs and performing the effective cleaning procedures to reduce microbial contamination and transmission to others. Thus, this study aimed to determine the prevalence of microbial contamination on MPs and evaluate the personal behaviors of MPs usage in pharmacy university students.

## 2. MATERIALS AND METHODS

### 2.1. Participant enrollment

This was the cross-sectional study conducted at the Faculty of Pharmacy, Mahidol University, Thailand between August and September 2017. All participants had signed inform consents before enrolled in this project. The fifty-three pharmacy students whose MPs have been used for at least 3 months were enrolled in this study. They were asked for requesting sample collection from their MPs (1 phone per 1 person) and answering questionnaires about behavioral characteristics of MP usages. The exclusion criteria were participants who did not sign inform consent and had their own MPs less than 3 months. This study was approved by The Human Ethical Research Committee of Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, Thailand (COA.No. MU-DT/PY-IRB 2017/034.1606).

### 2.2. Sample collection

Aseptic techniques were applied during all processes of sample collection. A sterile cotton swab moistened with sterile distilled water was rolled around the top and bottom surfaces of each MP at least 30 seconds. Then, the swab was immediately streaked on sheep blood agar (SBA) following by incubating in aerobic condition at  $37\pm 2^\circ\text{C}$  for 18-24 hours. Hand gloves were routinely changed after finishing each sample collection to reduce cross contamination among MPs or from a collector to

experiments.

### 2.3. Characterization and identification of bacterial colonies

Semi-quantitative analysis of isolated colonies on SBA was explored to investigate the total microbial count by following the criteria described by Bhoonderowa et al<sup>12</sup>. Briefly, the presence of 1-5 colonies were reported as a few growth, 6-15 colonies as moderate growth and more than 15 colonies as heavy growth. In order to identify the different colony types, gross colony characteristics of each isolated colony such as hemolysis, size, form or margin and color were performed by blind identification from 2 scientists. Each of different colony type was furtherly subcultured in tryptic soy agar (TSA) and incubated at  $37\pm 2^\circ\text{C}$  for 18-24 hours prior performing bacterial identification with conventional techniques such as gram stain, colony morphology and biochemical tests. The selection of biochemical tests was performed based on gram stain results. For gram positive cocci, the biochemical tests were catalase, coagulase, 0.04 U bacitracin (Oxoid, Hampshire, England) susceptibility test, 5  $\mu\text{g}$  novobiocin (Oxoid, Hampshire, England) susceptibility test, 10  $\mu\text{g}$  optochin (Oxoid, Hampshire, England) susceptibility test, bile esculin hydrolysis test, 6.5% NaCl and the use of selective and differential media such as mannitol salt agar (MSA). Catalase, motility, bile esculin hydrolysis and triple sugar iron (TSI) agar were included in colonies with gram positive bacilli. In addition, oxidase, TSI, lysine iron agar (LIA), urease test, motility test, Simmons citrate agar, methyl red/Voges-Proskauer (MR/VP) broth, oxidative-fermentation glucose (OF-glucose) test and the use of selective and differential media such as MacConkey (MAC) agar and xylose lysine deoxycholate (XLD) agar. Due to the presence of gram-negative cocci (GNC), selected biochemical tests were oxidase, cystine tryptic agar (CTA) sugar and nitrate reduction test. All biochemical tests and selective-differential media were incubated at  $37\pm 2^\circ\text{C}$  for 18-24 hours with aerobic condition. However, the biochemical tests for GNC were incubated at  $37\pm 2^\circ\text{C}$  for 24-72 hours with 3%-7%  $\text{CO}_2$  condition. Results of biochemical tests were interpreted as referenced in the Bergey's manual of systematic bacteriology<sup>21</sup>.

### 2.4. Questionnaires related to behaviors of MP usage

Questionnaires related to behaviors of MP usage were performed in enrolled participants. All questions were adapted from and referenced to previous studies<sup>9,13,20,22</sup>. The questions included the history of visiting hospital in the last 6 months, sharing a MP with others, using a MP in a toilet and cleaning a MP as well as MP cleaning procedures. Moreover, the correlation between behaviors of MP usage and bacterial contamination was

investigated.

## 2.5. Statistical and data analysis

Data were analyzed as a descriptive study using GraphPad Prism version 9.3.0.463 (GraphPad Software Inc., San Diego, California, USA) and IBM SPSS version 23.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were shown as mean±standard error of mean (SEM) and percentage whereas qualitative data were illustrated as frequencies and percentages. The correlation between behaviors of MP usage or MP cleaning procedures and bacterial contamination was examined using Pearson's correlation coefficient<sup>13</sup>. The *p*-value <0.05 was considered as statistically significant.

## 3. RESULTS

### 3.1. Sample collection and demographic data of participants

The fifty-three MPs collected from 15 (28.30%) males and 38 (71.70%) females of pharmacy university

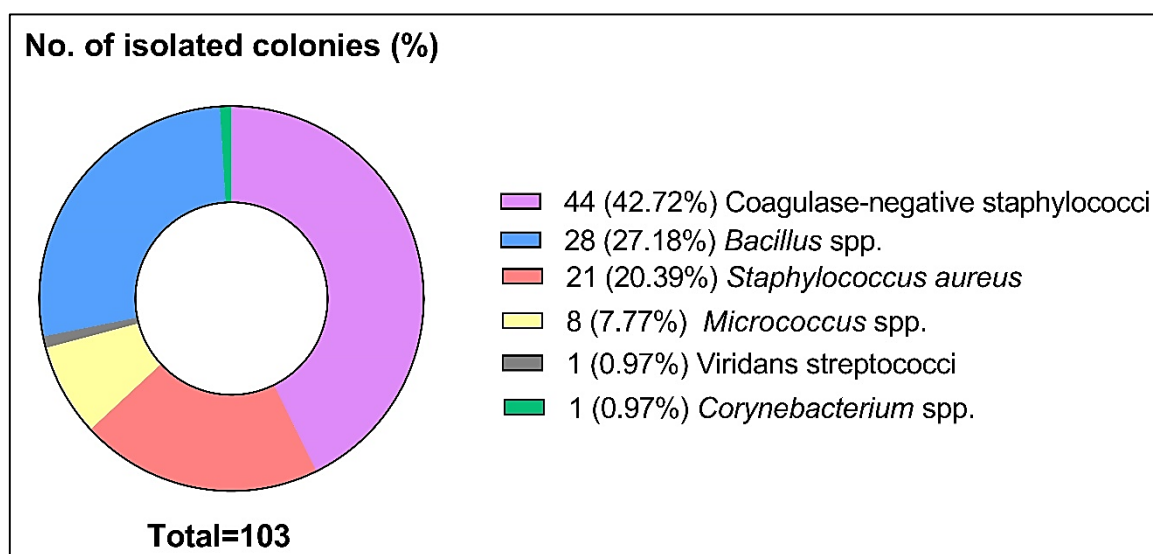
students were enrolled in this study (1 phone per 1 participant). The demographic data of all participants was illustrated in Table 1. Average ages (mean±SD) of participants were 21.58±1.22 and 21.22±1.21 years old for male and female, respectively. More than a half of participants were in 5<sup>th</sup>-year class (31 of 53, 58.49%) following by 3<sup>rd</sup>-year class (12 of 53, 22.65%) and 2<sup>nd</sup>-year class with equal to 4<sup>th</sup>-year class (5 of 53, 9.43%). Moreover, most participants had had own MPs for at least 12 months (31 of 53, 58.49%).

### 3.2. Prevalence and identification of bacteria detected from MPs

Bacterial contamination was observed in 52 of 53 MPs (98.11%) with different colony types and quantities. Semi-quantitative analysis of bacterial load revealed that 44 of 53 (83.02%) devices presented heavy growth colonies (more than 15 colonies) whereas 6 of 53 (11.32%) and 2 of 53 (3.77%) indicated few (1-5 colonies) and moderate colonies (6-15 colonies), respectively. However, no fungal colony was observed on SBA. There were 103 suspected colonies derived from 52 devices for

**Table 1.** The demographic data and questionnaire responses of participants in this study.

Characteristics	Numbers (%) (n=53)
Gender:	
Male (Age: 21.58±1.22 years old)	15 (28.30)
Female (Age: 21.22±1.21 years old)	38 (71.70)
Educational level:	
2 <sup>nd</sup> -year students	5 (9.43)
3 <sup>rd</sup> -year students	12 (22.65)
4 <sup>th</sup> -year students	5 (9.43)
5 <sup>th</sup> -year students	31 (58.49)
Duration of MP usage:	
3-6 months	12 (22.64)
7-12 months	10 (18.87)
> 12 months	31 (58.49)



**Figure 1.** The identification results of bacterial species isolated from MPs of pharmacy university students.

performing bacterial identification. We found that CoNS were the most abundant bacterial species (44 of 103, 42.72%), following by *Bacillus* spp. (28 of 103, 27.18%), *S. aureus* (21 of 103, 20.39%), *Micrococcus* spp. (8 of 103, 7.77%), *Corynebacterium* spp. (1 of 103, 0.97%) and viridans streptococci (1 of 103, 0.97%), respectively (Figure 1).

Various patterns of bacterial species isolated from MPs were observed. Two different types of bacteria were the most abundant (37 of 52, 71.15%), following by a single bacterial type (11 of 52, 21.15%) and 3 types of bacteria (4 of 52, 7.70%), respectively. The most common pattern of multibacterial species was CoNS+*Bacillus* spp. (15 MPs, 28.85%), following by *Bacillus* spp.+*S. aureus* (10 MPs, 19.23%), CoNS+*S. aureus* (8 MPs, 15.38%) and CoNS (8 MPs, 15.38%), respectively. Other patterns included CoNS+*Micrococcus* spp.+*Bacillus* spp. (3 MPs, 5.77%), *S. aureus* (2 MPs, 3.85%), *Micrococcus* spp. (1 MP, 1.92%), CoNS+*Corynebacterium* spp. (1 MP, 1.92%), CoNS+*Micrococcus* spp. (1 MP, 1.92%), CoNS+viridans streptococci (1 MP, 1.92%), *Micrococcus* spp.+*S. aureus* (1 MP, 1.92%) and CoNS+*Micrococcus* spp.+*S. aureus* (1 MP, 1.92%), respectively (Figure 2).

### 3.3. The behaviors of using and cleaning MPs

All participants were asked for the survey about the behaviors of using MPs related to risks for microbial contamination and how to clean them. The results were presented in Table II. We found that more than half of participants (30 of 53, 56.60%) had been visited hospitals during the last 6 months. Moreover, more than 90% of participants had shared MPs with others (49 of 53, 92.45%) and had ever used MPs in toilets (52 of 53,

98.11%). No association between each behavior of MP usage and bacterial contamination was observed in this study ( $p$ -value>0.05) (Table 2). In addition, the bacterial load between pharmacy university students who had visited and had not visited hospitals during the last 6 months was not statistically different ( $p$ -value=0.823) (data not shown).

Most participants had ever cleaned their MPs, accounting for 44 of 53 (83.02%). Twenty of 44 MPs (45.45%) was cleaned with cleaned cloths or tissue papers, following by using 70% alcohol (15 of 44, 34.09%) and sterile water (6 of 44, 13.64%), respectively. The use of a cleaning solution or using an air blower was rarely performed by participants (2 of 44, 4.35% and 1 of 44, 2.17% respectively). In addition, different MP cleaning procedures were not correlated with bacterial persistence ( $p$ -value=0.386) (Figure 3 and Table 3) and the bacterial load among different cleaning methods was not statistically different ( $p$ -value=0.945) (data not shown).

## 4. DISCUSSION

The use of MPs for communication, entertainment and academic purposes has dramatically increased in healthcare university students. Furthermore, microbial contamination on MPs has been previously reported in those groups<sup>16-18,20</sup>. To our knowledge, this is the first study describing the prevalence of bacterial contamination on MPs of pharmacy university students, one discipline of healthcare professionals. Moreover, we also illustrate the patterns of bacterial species isolated from each contaminated MPs and present personal behaviors of MP usage. Due to the limited number of participants between male and female, and among educational levels,

**Table 2.** Personal behaviors of MP usage and the correlation with bacterial contamination on MPs of pharmacy university students.

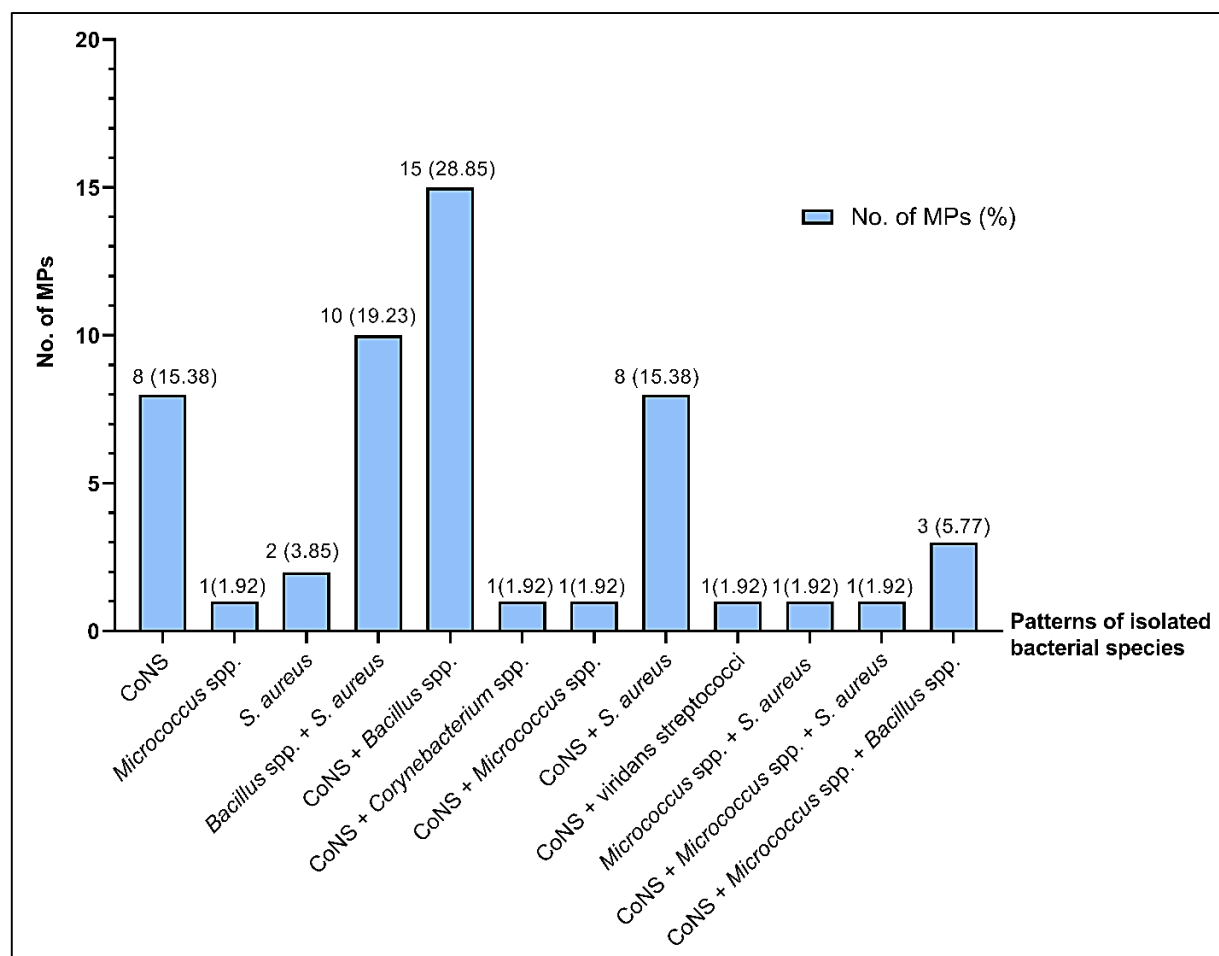
Factor	Total (%) (n=53)	Pearson's correlation coefficient value	$p$ -value*
1. Visiting a hospital in the past 6 months		-0.121	0.386
- Yes	30 (56.60)		
- No	23 (43.40)		
2. Sharing a MP with others		-0.040	0.778
- Yes	49 (92.45)		
- No	4 (7.55)		
3. Using a MP in a toilet		-0.019	0.891
- Yes	52 (98.11)		
- No	1 (1.89)		
4. Cleaning a MP		-0.063	0.655
- Yes	44 (83.02)		
- No	9 (16.98)		

\*The  $p$ -value was calculated based on Pearson's correlation coefficient.

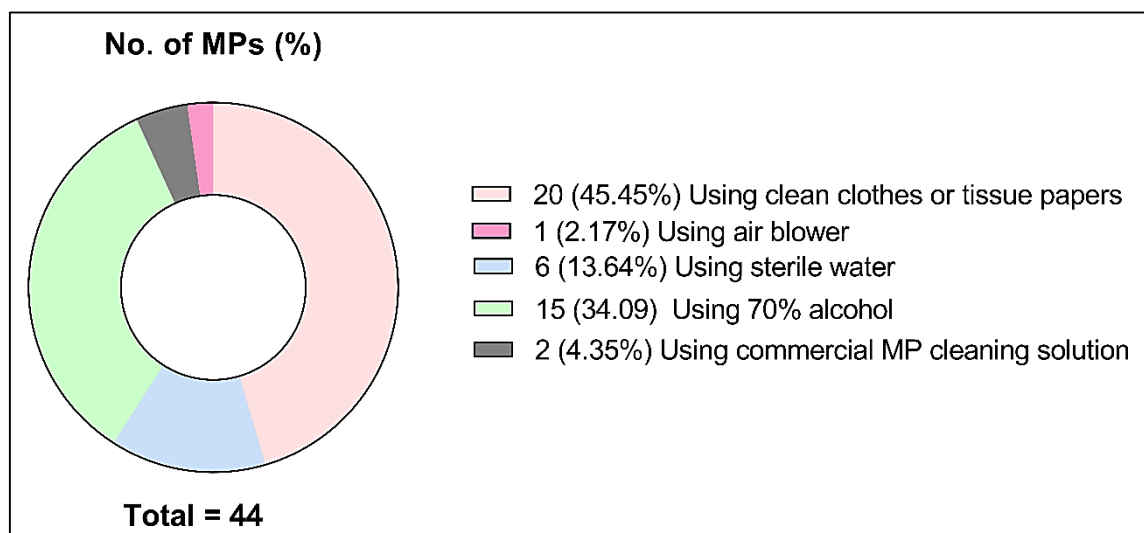
**Table 3.** The correlation between cleaning procedures and bacterial contamination on MPs of pharmacy university students (n= 44).

Factor	Pearson's correlation coefficient value	$p$ -value*
The 5 different cleaning procedures: (Using clean clothes or tissue paper, using air blower, using sterile water, using 70% alcohol and using commercial MP cleaning solution)	0.134	0.386

\*The  $p$ -value was calculated based on Pearson's correlation coefficient.



**Figure 2.** The patterns of isolated bacterial species from MPs of pharmacy university students (CoNS=coagulase-negative staphylococci).



**Figure 3.** The MP cleaning procedures performed by pharmacy university students.

the comparison between or among groups was not examined in this study.

The presence of bacterial contamination on MPs of pharmacy university students indicates that those devices may act as fomites harboring microbial agents and may be the potential source of microbial transmission. The high percentage (98.11%) of bacterial-contaminated MPs is similar to previous studies conducted in other health-

care-university students such as dental students in Iran<sup>18</sup> and medical students in Saudi Arabia<sup>20</sup>. We assessed the semi-quantitative of bacterial load by following the previous study of Bhoonderowa and his colleagues<sup>12</sup>. The high proportion of heavy growth in contaminated MPs (83.02%) indicates that most MPs had harbored numerous microbes and participants' MPs may be either lacking of cleaning MPs with proper procedures or



absence of MP cleaning for several times. These may increase risk of bacterial transmission during sharing MP with others. Our findings are similar to the previous study conducted in students, lecturers and other administration staffs of university in Malaysia<sup>23</sup>. However, the heavy growth of isolated microbes contradicts with previous study representing few growths of microorganisms on MPs of non-healthcare participants such as office staffs, technical and manual workers in community<sup>12</sup>. Moreover, our findings differ from previous cohort conducted in MPs of HCWs that represented few and moderate bacterial loads<sup>24</sup>. Controversial findings may be from different behaviors of MP usage among each participant group and different roles of their occupations. For example, HCWs rigorously follow the guidelines on infection prevention and control, which may present low prevalence of microbial contamination on MPs.

We used traditional bacterial identification techniques to examine bacterial species isolated from MPs. Although molecular assays and MALDI-TOF mass spectrometry have been implemented for bacterial identification, traditional identification with gram staining and biochemical tests are still widely used. Additionally, this technique is known as gold standard method for bacterial identification<sup>25</sup>. In this study, six bacterial species (CoNS, *S. aureus*, *Micrococcus* spp., viridans streptococci, *Bacillus* spp. and *Corynebacterium* spp.) were identified. All bacterial species are commonly found as normal flora on skin and in mouth as well as in environment<sup>26-28</sup>. The frequent use of MP may allow the transmission of these microbes persisting on a device. Moreover, prolonged bacterial species during MP usage may result from users forgetting to clean their MPs with proper techniques such as using disinfectant agents. Although these microorganisms do not generally cause diseases, they can involve in opportunistic infection<sup>12</sup>. We also presented CoNS as the most abundant bacterial species isolated from MPs. Our findings are similar to previous studies conducted in healthcare university students<sup>16</sup>, community participants<sup>12</sup>, medical students<sup>20</sup> and university students attending in basic science courses<sup>19</sup>. We hypothesize that high prevalence of CoNS-contaminated MPs results from the most abundant bacterial species found on human skin<sup>27</sup>. In this study, we did not identify some isolated microbes such as CoNS, *Micrococcus* spp., viridian streptococci, *Bacillus* spp. and *Corynebacterium* spp. into species due to the limitation of our identification assays. Additionally, gram-negative bacteria (GNB) were not isolated from MPs of pharmacy university students. The lacking of GNB isolated from MPs is contradicted with previous studies conducted in healthcare university students<sup>16,18,20</sup> and community volunteers<sup>12</sup>. Due to the lacking of isolated GNB from pharmacy students' MPs, we cannot actually conclude that MPs of pharmacy students do not harbor GNB. We ensure that the absence of GNB are

not from errors of identification assays because we had used SBA, the enrichment media supporting the growth of both gram-positive bacteria (GPB) and GNB.

Additionally, no growth of GNB may result from the more injured cells during direct inoculating the cotton swab on SBA. Moreover, there was the previous literature review mentioned that the most abundant bacterial species on palms and hands were GPB rather than GNB<sup>27</sup>. Therefore, this may support the predominant GPB isolated from MPs in our findings.

We also studied the patterns of isolated bacterial species. Twelve patterns with either single, double or triple species were observed in this study. The presence of multibacterial species reveals that contaminated MPs may flourish with different types and number of bacterial species. The co-contamination of CoNS and *Bacillus* spp. was the most abundant pattern in this study, which was correspondence with large proportions of CoNS and *Bacillus* spp. isolated from MPs. Moreover, both bacterial species are commonly found on human skin and in environment<sup>27-28</sup>.

Behaviors of MP usage had been observed by using questionnaires about risks for bacterial contamination on MPs and how to clean devices. More than 50% of participants experienced with visiting a hospital in the past 6 months, sharing a MP with others and using a MP in a toilet. These may imply that most participants have greater risks for bacterial contamination on their MPs unless they regularly clean devices or use them with good personal hygiene. Additionally, using MPs in toilets has the risk for microbial contamination because bioaerosols containing microbes are mostly generated during toilet flushing<sup>29</sup>. Aerosols contaminated with microbes may persist on hands and can be transmitted to colonize on MPs during device usage. Additionally, the microbial transmission to may occur when sharing devices with other people. The large proportions of participants sharing MPs with others and using MPs in toilets are similar to the previous study performed in HCWs<sup>13</sup> but our results represented higher percentage than that former study. In addition, the percentage of our participants using MPs in toilets is higher than previous study conducted in medical students<sup>20</sup>. The small proportions of participants sharing MPs with others and using MPs in toilets in medical students and HCWs may be from the strict policies of infectious disease prevention and control in their study and work places.

The correlation between MP using behaviors and bacterial contamination was investigated. Although previous studies mentioned this point in HCWs and healthcare university students<sup>13,20</sup>, this is the first study illustrating in pharmacy university students. Our findings revealed that all behaviors of MP usage were not related to bacterial contamination. These may imply that bacteria-persisted MPs independently occur and are generally found. Additionally, no correlation between

using a MP in a toilet and bacterial contamination contradicts with previous study of Banawas *et al.*<sup>13</sup> but is correspondence with the study of Zakai, *et al.*<sup>20</sup>. The controversial issues may result from different participants' groups. The former study examined in HCWs whereas the latter focused on medical university students, which is similar to our study. We also presented that sharing a MP with others did not correlate with bacterial persistence on MP. Our results are opposed to previous study conducted in HCWs<sup>13</sup> due to the different groups of study.

Cleaning MPs is one of good personal hygiene for reducing the microbial contamination. Our findings represented the high percentage of participants whose MPs had been cleaned. The high percentage of participants cleaning their MPs reveals that they pay more attention in the awareness of MP usages and realize in good personal hygiene. Our findings are similar to previous study conducted in HCWs, which represented the high ratio of participants cleaning MPs<sup>13</sup> but contradict with previous study presenting the high proportion of non-HCWs lacking of cleaning their MPs<sup>22</sup>. Although the large proportion of these participants cleaned MPs, the high prevalence of MP contamination still occurred in this study. We hypothesize that it may be from the different frequencies of MP cleaning and use of ineffective protocol for MP cleaning. However, our study did not ask participants whether they regularly had cleaned their MPs. Moreover, we observed that there was no correlation between MP cleaning behavior and bacterial contamination, which is similar to previous study conducted in HCWs<sup>13</sup>.

There were 5 protocols of MP cleaning assessed by 44 participants. Although the use of clean clothes or tissue paper was the most common technique in this study, it lacks of disinfectant. Therefore, microbial contamination may not be completely eliminated. In addition, some participants used 70% alcohol for cleaning MPs. Due to the property of 70% alcohol stated as disinfectant, using this reagent may reduce the bacterial persistence on MPs. However, we did not evaluate the different of bacterial load between pre and post cleaning with disinfectant. Besides, the use of 70% for MP cleaning was mentioned in the previous study but the authors did not describe the type of disinfectant<sup>20</sup>. Other cleaning methods such as using sterile water, MP cleaning solution and air blower had also been used by our participants in small proportions. These may imply that various methods had been used depending on user's satisfaction. Our findings may provide the important data to launch the campaign for announcing the effective protocol for cleaning MPs to reduce microbial contamination. Additionally, rarely using disinfectant may allow the microbial contamination on MPs. This assumption is supported by previous studies mentioning that using disinfectants for MP cleaning reduced microbial con-

tamination up to 60-100%<sup>3</sup>. Although there was no correlation between different protocols for MP cleaning and the bacterial persistence, the use of disinfectants should be suggested to MP users for their cleaning rather than other processes and other good personal hygiene such as cleaning hands with anti-microbial sanitizer should be performed simultaneously to reduced microbial contamination<sup>6</sup>.

Limitations of this study are mainly small sample size. Moreover, further studies for confirming our findings should perform the surface monitoring tests on MPs using standard swab method as control condition prior to conducting sample collection to ensure reliable results. In addition, we did not perform the antimicrobial susceptibility testing of isolated bacteria and the efficiency of each MP cleaning protocol on microbial contamination. Further studies need to be conducted in large cohorts and in other healthcare university students. Moreover, the antimicrobial susceptibility testing of isolated colony and the association between each MP cleaning and bacterial persistence should be included.

## 5. CONCLUSION

According to our pilot findings, we conclude that bacterial-contaminated MPs of pharmacy university students regularly occur. The high prevalence of bacterial species were CoNS, *Bacillus* spp. and *S. aureus*. Moreover, each contaminated MP contained either single or multi-bacterial species by representing co-persistence of CoNS and *Bacillus* spp. as the predominant pattern. Most isolated bacteria originate from not only environment but also personal skin microflora. Even though there was no correlation between MP-using behaviors or MP-cleaning practices and bacterial contamination, the past history of visiting a hospital, sharing with others and using in toilets may increase risk of microbial contamination and transmission via a MP. Additionally, good personal hygiene and effective cleaning procedures, especially the use of disinfectants should be recommended MP users to routinely perform in order to prevent and control bacterial transmission via MPs in a community.

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## Author contribution

All authors contributed to the study conception and

design, including manuscript preparation. MS designed the study, performed experiment, reviewed and analyzed data, and prepared the first draft of manuscript. OA and AP performed experiments, collected and analyzed data. All authors read and approved the final manuscript.

### Conflict of interest

None to declare

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### Ethics approval

This study was approved by The Human Ethical Research Committee of Faculty of Dentistry and Faculty of Pharmacy, Mahidol University, Thailand (COA.No. MU-DT/PY-IRB 2017/034.1606).

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