ORIGINAL ARTICLE

Microbial contamination on cell phones used by undergraduate students

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ABSTRACT

Background: Undergraduate students handle their cell phones in several places, getting them exposed and contaminated with a variety of microorganisms, which may include pathogenic and non-pathogenic microorganisms.

Objective: We investigated the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. and counts of microbial groups on the surface of undergraduate students' cell phones.

Methods: A total of 304 cell phones used by undergraduate students were sponge sampled to detect the presence of pathogenic bacteria and to enumerate yeasts and molds, aerobic plate count, *Enterobacteriaceae*, coliforms and *Escherichia coli*. A questionnaire was applied to users of the cell phones sampled to obtain information on phone usage habits.

Results: All undergraduate students use their cell phones at home, school, public and private sites. All sponge samples tested negative for the presence of the investigated pathogens. The intervals of counts (Log CFU/cell phone) were 1.7-6.7 for aerobic plate count, 1.7-5.4 coliforms, 1.7-5.2 yeasts and molds, 1.7-4.6 *Enterobacteriaceae*, and 1.7-3.3 *E. coli*. Conclusions: Cell phones used by undergraduate students are a source of microbial groups in variable levels. Despite the fact that bacterial pathogens were not isolated from tested samples, usage habits and presence of *E. coli* suggest that cell phones could be a potential source of enteric pathogenic bacteria.

KEY WORDS:
phones; microorganisms; pathogen; students; cross-contamination

INTRODUCTION

The number of cell phones used worldwide grew from fewer than 1 to around 6 billion between 2000 and 2012 (1). In Mexico, 77.7 million people used cell phones in 2015; 66% users have a smartphone, while the rest own a device enabled to make/receive calls or messages without internet access (2). The use of this mobile communication technology in healthcare and higher education (3) has increased and generated interest in evaluating their role as reservoir of pathogenic and opportunist bacteria, and as source of contamination to our foods or to ourselves (4,5). Several investigations in hospitals have demonstrated the presence of *Staphylococcus aureus*, *Bacillus* spp., *Enterococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Acinetobacter* and coliforms on mobile phones used by medical staff (4,6,7), students (7) and patients (8). In contrast, studies exploring the quantitative levels of microbial groups such as yeasts and molds, aerobic plate count, *Enterobacteriaceae* (9), coliforms and *E. coli* on cell phones are scarce. The enumeration of microbial groups could be useful in estimating the cell phones potential as reservoir of microorganisms including enteric bacteria, particularly when populations of pathogenic and opportunist microorganisms are below detectable levels. Populations of microbial groups may differ on cell phones according to their usage under different conditions and environments.

Cell phones are common among undergraduate students, which can be used to communicate for social or academic purposes, according to the technological features of device and Internet connection. Students related to health sciences majors use their cell phones while performing internships at hospitals or clinical laboratories, either to access information on their field of expertise, answer calls, text messages, or take pictures

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during their practices (10,11). On the other hand, students in social sciences majors use their cell phones when practicing or working at offices where a large number of people attend. The frequent use of cell phones in a diversity of sites raises the opportunity for cross-contamination, especially if no hygienic measures and safety practices are common among students (12). If pathogens are present on the surface of a cell phone, they could be transferred to the user skin, other surfaces, or foods, where survival and growth is possible. Two disease outbreaks were associated with exposure of students and employees after manipulating *Salmonella* Typhimurium in clinical and teaching microbiology laboratories in the United States (13). In this report, laboratory directors, managers, and faculty involved with clinical and teaching microbiology laboratories were advised to comply with biosafety guidelines that prohibits food, drinks or personal items like car keys, cell phones and music players use while working in the laboratory or placed on laboratory work surfaces as they may act as fomites.

The purpose of this study was to investigate the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp., and to enumerate yeasts and molds, aerobic plate count, *Enterobacteriaceae*, coliforms and *Escherichia coli* on the surface of cell phones used by undergraduate students in three University Campuses. In addition, a survey was conducted among the cell phone users to collect data on factors that might contribute to the microbial levels found.

MATERIAL AND METHODS

Study context

A total of 304 cell phones belonging to undergraduate students attending three campuses of the University of Guadalajara (Jalisco State, Mexico) were sampled. Students in each campus are enrolled in different majors, and were divided into two groups depending on whether or not they were registered in courses that include visits to hospitals and/or clinical and microbiology laboratories. The first group named "health sciences" included students from medicine, nursing, pharmacy and biology or biology-related majors who attend different courses that include visits to hospitals and clinical or microbiology laboratories. The second group was called "non-health-related sciences", and included students from engineering and social sciences, who do not attend classes in hospitals or laboratories. All students were selected at different locations of each campus including main entrances, classrooms, laboratories, libraries, and restroom entrances. Each student was asked for his/her consent to respond a questionnaire about his/her cell phone characteristics and usage habits, and to allow the sampling of their device's surface. Personnel in charge of sampling visually verified that participants did not clean their phone before sampling. The questionnaire was filled out by each participant and inquired about age, gender, educational background, technical characteristics of the cell phone, usage habits, and cleaning and disinfection practices on the device. The protocol was previously approved by the Bioethics Committee of each campus.

TABLE 1: Characteristics and use of cell phones by undergraduate students at a university in Jalisco State, Mexico (n= 304)

Characteristic	No. students (%)
Type of cell phone	
Touch-screen phone	197 (65)
Keyboard phone	107 (35)
Use of cover protector	148 (49)
Location of usage	
Home	304 (100)
Public and private transportation	304 (100)
School	304 (100)
Other places (park, restaurants and supermarket)	133 (44)
Cell phone use	
Calls and texting	304 (100)
Surf the Internet	208 (68)
Play audios and/or videos	206 (68)
Take pictures and/or videos	178 (59)
View or download electronic documents	109 (36)
Other (access calendar, clock, Global Position System, play games)	304 (100)
Cleaning or disinfection of cell phone	183 (60)

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The whole surface of cell phone (including the front, back and lateral sides) was swabbed using a sterile sponge (3M™, St. Paul, MN, USA) aseptically hydrated with 50 ml of lactose broth (Becton, Dickinson and Company, Sparks, MD, USA). The sponge was then returned to the sterile bag and placed in an insulated cooler with refrigerant packs. Samples were transported to the laboratory and analyzed within 2 h.

Microbiological analysis

Cell phone sponge samples were homogenized using a peristaltic blender for 1 min; decimal dilutions in 0.1% peptone diluent (Becton, Dickinson de México, Estado de México, México) were prepared for enumeration of aerobic plate count (APC), yeasts and molds (Y/M), *Enterobacteriaceae*, coliforms and *Escherichia coli* on Petrifilm plates (3M™, St. Paul, MN, USA). *Enterobacteriaceae*, coliforms and *E. coli* plates were incubated at 35°C for 24 h, APC at 35°C for 72 h, and Y/M at 25°C for 120 h, before counting.

An aliquot of each sponge rinse liquid was streaked on trypticase soy agar supplemented with 5% sheep blood (Becton, Dickinson and Company) for isolation of *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. at 35°C for 24 h. Single typical colonies were selected and tested for Gram stain, catalase, mannitol fermentation, coagulase and esculin hydrolysis.

The remaining volume of the sponge rinse liquid was incubated at 35°C for 24 h for *Salmonella* spp. isolation (14). Aliquots of 0.5 and 0.1 ml were transferred to 10 ml of tetrathionate broth (TT, Becton Dickinson and Company) and 10 ml of Rappaport-Vassiliadis R10 broth (RV, Becton Dickinson and Company), respectively. The broths were incubated at 35°C and 42 ± 0.5°C for 18-24 h, respectively, in a water bath (Thermo Fisher Scientific Precision 2868, Marietta, OH). Aliquots of 10 µl from TT and RV were individually streaked onto xylose lysine deoxycholate agar (XLD), *Salmonella-Shigella* agar (SS), enteric Hektoen agar (HE) and bismuth sulfite agar (SB). XLD, SS and HE plates were incubated at 35±2°C for 18-24 h, and SB plates for 48 h. Two typical colonies from each plate were biochemically confirmed on triple sugar iron agar and lysine iron agar at 35±2°C for 24±2 h, and into urea broth at 35±2°C during 48 h.

Data Analysis

Data obtained from the questionnaires were used to perform descriptive statistics. Counts obtained for each microbial group were reported in Log CFU/cell phone prior to data analysis. The significance of differences among the counts of five microbial groups was assessed using an analysis of variance (Statgraphics Centurion XV ver.15.2.06; Statpoint Technologies, Inc., Warrenton, USA). When significant differences ($P<0.05$) were observed, separation of means was carried out using LSD (least difference statistical) multiple range test.

RESULTS

A total of 304 students participated in the study, 137 (45%) females and 167 (55%) males, ranging from 17 to 35 years old. One hundred and fifty-one students (49.7%) corresponded to the "health science" group and 153 students (50.3%) to the "non-health-related sciences" group. Sixty-five percent of students interviewed owned a cell phone with a touch screen and 35% had keyboard phones; 49% of cell phones had a protecting cover (Table 1).

All students (100%) reported using their cell phones at home, in places including their bedroom, bathroom and kitchen; also during their commute when using either public or private transportation, and at school. Forty-four percent said they use their cell phone at public sites such as parks, restaurants and supermarkets (Table 1). All students (100%) responded that they use their cell phone for making calls and send text messages, 68% use it to surf the Internet and to play audio and video, 59% to take pictures and/or videos, 36% to view or download electronic documents, and 100% to use software applications (Apps) like calendar, clock, Global Position System and/or games.

Approximately 72% (n=109/151) of students in the "health sciences" group said they use their phones in hospitals and/or laboratories. A 54% of these students said used to make phone calls and send text messages while providing health care for patients under professors' supervision in hospitals. In addition, 52% of students indicated that they have taken pictures in teaching laboratories during handling of *Salmonella* spp., *Shigella* spp., *Escherichia coli*, Gram positive cocci, *Listeria monocytogenes,* or gastrointestinal helminths and protozoans, despite the biosafety rules in place and the warning from professors and technicians about this hazardous practice. A 6% students said used it in hospitals and laboratories.

When students were asked if they perform cleaning or disinfection procedures to their cell phones, only 183 of 304 (60%) students answered that they clean or disinfect their device (Table 1). A variety of open responses on this topic were collected among students, who seem to be more familiar with the concept of cleaning than that of disinfection. Only 78% (n=142/183) of respondents said they clean their device and from those, 97% (n=138/142) described the cleaning procedure as rubbing the surface with damp clothes, personal clothes, hands, baby towels, toilet paper or cotton pads. An example of the lack of knowledge on proper cleaning practices is that 3% (n=4/142) of students said they clean their phones by breathing on the surface of the device and rubbing it on their clothes. Knowledge about disinfection procedures was also poor. Although 41 of 183 (22%) students said they disinfect their cell phones, only 27 of them (66%) use antibacterial substances (70% ethanol, isopropyl alcohol or sodium hypochlorite); 14 students (34%) said they use detergent or a cosmetic cream to disinfect the surface of their device. This illustrates the lack of information on cleaning and disinfection concepts among respondents.

All sampled cell phones tested negative for the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. The frequency of isolation of microbial groups was 99.8% for APC, 53% Y/M, 31% coliforms, 29% *Enterobacteriaceae*, and 5% *E. coli* (Table 2). Mean APC counts were significantly higher ($P<0.05$) that those of Y/M, coliforms, *Enterobacteriaceae* and *E. coli*. The APC counts ranging from 1.7 to 6.7 Log CFU/phone, from those, 87% of cell phones ranged from ≥3.0 to 5.0 Log CFU/phone, whereas 5% of

TABLE 2: Counts of microbial groups on the surface of cell phones used by undergraduate students at three university campuses

Microbial group	No. samples with enumerable levels (%)	Mean Log CFU/ cell phone[a] ± SD	Minimum-maximum count (Log CFU/cell phone)
Aerobic plate count	303 (99.8)	3.8 ± 0.64 A[b]	1.7 - 6.7
Yeasts and molds	161 (53)	2.2 ± 0.62 C	1.7 - 5.2
Coliforms	94 (31)	2.6 ± 0.80 B	1.7 - 5.4
Enterobacteriaceae	87 (29)	2.5 ± 0.70 B	1.7 - 4.6
Escherichia coli	16 (5)	2.2 ± 0.43 C	1.7 - 3.3

[a] Minimum detection limit was 1.7 Log CFU/phone
[b] Means with the same letter within columns (A, B, C), are not significantly different (P > 0.05)

the sampled devices contained ≥5.0 to 6.7 Log CFU/phone, which belonged to students in the "health sciences" group. The samples with enumerable levels of Y/M showed counts ranging from 1.7 to 5.2 Log CFU/phone, from those, 91% had counts between 1.7 and 3 Log CFU/phone and 3% contained >4.0 to 5.2 Log CFU/phone corresponding to cell phones of students in the "health sciences" group. The distribution of *Enterobacteriaceae* and coliforms counts was similar, as these microbial groups were isolated from 100% and 99% phone samples respectively, with counts between ≥1.7 and 5.0 Log CFU/phone. *Enterobacteriaceae* counts >4.0 Log CFU/phone were observed on 3% the surface of cell phones; two of them belonging to students in the "health sciences" group. Similarly, high coliform counts from 4.3 to 5.4 Log CFU/phone were found on 6% of sampled devices, which corresponded to six samples, from those, five belonged to students in the "health sciences" group. *Escherichia coli* was present only in 5% of phone samples with counts from ≥1.7 to 3.3 Log CFU/phone (Table 3). Of those samples, 14 belonged to students of the "health sciences" group and two to not health sciences group.

No statistical differences (P>0.05) were observed for APC, Y/M, coliforms and *Enterobacteriaceae* mean counts between groups of students (Table 4). No statistical comparison was performed for *E. coli* mean counts because of the low number of samples showing enumerable levels of this indicator. Except for *Enterobacteriaceae*, the number of cell phone samples with enumerable levels of microbial groups was higher in the "health science" group when compared to the "non-health-related sciences" group.

DISCUSSION

Undergraduate students commonly use their cell phones for academic, recreation and/or communication activities, almost everywhere where they are. Our findings indicated that health sciences students use their phones in microbiology laboratories and while attending patients in clinics and hospitals. Usage of cell phone in these sites could lead to convert the devices as reservoir and source of pathogenic and non-pathogenic microorganisms and favor cross-contamination (15). The potential of cell phones to transfer microorganisms can be reduced through the use of cleaning and disinfecting practices (16). However, the students'

TABLE 3: Distribution of mean counts for microbial groups enumerated from the surface of cell phones used by undergraduate students at three university campuses

Mean Log CFU/ cell phone[b]	No. of samples (%)				
	Aerobic plate count (n= 303)[a]	Yeasts and molds (n=161)	Coliforms (n=94)	*Enterobacteriaceae* (n=87)	*Escherichia coli* (n=16)
≥ 1.7 - 2.0	3 (1)	90 (56)	34 (36)	26 (30)	8 (50)
≥ 2.0 - 3.0	22 (7)	57 (35)	36 (39)	40 (46)	7 (44)
≥ 3.0 - 4.0	190 (63)	10 (6)	18 (19)	18 (21)	1 (6)
≥ 4.0 - 5.0	72 (24)	3 (2)	5 (5)	3 (3)	0 (0)
≥ 5.0 - 6.0	12 (4)	1 (1)	1 (1)	0 (0)	0 (0)
≥ 6.0 - 7.0	4 (1)	0 (0)	0 (0)	0 (0)	0 (0)

[a] Number of samples with enumerable levels of microbial group
[b] Minimum detection limit was 1.7 Log CFU/phone

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TABLE 4: Counts of microbial groups on the surface of cell phones used by undergraduate students at three university campuses

Microbial group	No. samples with enumerable levels (%)		Mean count (Log CFU/phone)[a]		Minimum-maximum count (Log CFU/phone)	
	Health sciences group	Non-health-related sciences group	Health sciences group	Non-health-related sciences group	Health sciences group	Non-health-related sciences group
Aerobic plate count	152 (50.2)	151 (49.8)	3.9 ± 0.71 A[c]	3.9 ± 0.60 A	1.7-6.7	2.7-5.3
Yeasts and molds	96 (59.6)	65 (40.4)	2.2 ± 0.64 A	2.2 ± 0.62 A	1.7-5.2	1.7-3.7
Coliforms	62 (66)	32 (34)	2.7 ± 0.86 A	2.6 ± 0.81 A	1.7-5.4	1.7-4.3
Enterobacteriaceae	34 (39)	53 (61)	2.6 ± 0.71 A	2.5 ± 0.68 A	1.7-4.6	1.7-4.4
Escherichia coli	14 (87.5)	2 (12.5)	2.2 ± 0.43	1.7 ± 0.00	1.7-3.3	1.7[b]

[a] Minimum detection limit was 1.7 Log CFU/phone
[b] Both samples showed 1.7 Log CFU/phone
[c] Within rows, the means with the same letter are not significantly different ($P > 0.05$). No statistical comparison was performed for mean counts of *E. coli* because of the low number of samples showing enumerable levels of this bacterium

responses evidenced the lack of information on cleaning and disinfection concepts for cell phones. Therefore, we suggest involving to students in a program of sanitary education at early stage of academic training to increase their knowledge about transmission and control of microorganisms.

In this study, the presence of *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. was investigated on the cell phone surface, which may be related to fecal and human contamination. None of these pathogenic bacteria were isolated from the students' cell phones, probably because the low levels in which pathogens are present on the surface of these devices. Also, it is likely that some devices were cleaned and/or disinfected sometime before our sampling; however, in our questionnaire we did not inquire when was the last time each device was cleaned and or disinfected, and we only made sure that it was not done immediately before sampling. Other researchers have reported a low isolation frequency of *Salmonella*, 1% on cell phones from university students in Nigeria (17) and 3% in Ghana (18). Most studies have included cell phones from medical science students in hospital setting, a site where the use of mobile phones raises the risk of cross-contamination, especially if effective disinfection are not enforced. A study performed at the Queen Elizabeth Hospital, in Barbados, showed that *Staphylococcus epidermidis* was isolated from 50% of mobile phones from medical staff, including students (6). Nwankwo et al. (7) reported the isolation of *S. epidermidis*, *S. aureus* and *Streptococcus* in 24, 14.8 and 11.1% of students' mobile phone swabs in Ghana. *Enterococcus* spp. was detected in 3% of cell phones belonging to food science students, but not in those devices from students on health and computer science in Slovenia (9).

The knowledge about quantitative levels of non-pathogenic organisms such as APC, Y/M, coliforms, *Enterobacteriaceae* and *E. coli* on the surface of cell phones was useful to estimate the potential of devices as reservoir of microorganisms, given the lack of isolation of pathogenic bacteria in our study. The counts of these microbial groups may be used to evaluate handling and hygiene practices, as well as exposure of the cell phones surface to contamination sources.

Studies on the distribution and enumeration of microbial groups on cell phones are scarce, and comparison of findings should be cautious due to differences in methods and reporting units. Most of reported studies include the enumeration of APC (9, 18, 19), *Enterobacteriaceae* (9, 19), Y/M (9, 19), and coliforms (19), with counts ranging from 0.9 CFU/100 cm^2 to 6.9 Log CFU/cm^2. Counts for these microbial groups in our study were higher than those reported in the previously cited studies (9, 18, 19). The high counts of microbial groups found on the surface of cell phones may be related to their constant handling in diverse sites, in which non-pathogenic and pathogenic microorganisms could be present. Cell phones do not possess conditions that favor microbial growth; therefore, high microbial counts may be originated from contact with heavily contaminated surfaces. The high variability observed for APC counts on cell phones of undergraduate students reflects a large diversity of contamination sources and handling conditions. Likewise, APC and Y/M are widely distributed in the environment and can contaminate the cell phones surface through contact with non-sanitized surfaces or as airborne contaminants. *Enterobacteriaceae* and coliforms enumeration could be useful to indicate general hygiene conditions of the devices, and the high counts found for these microbial groups could be a result of the direct or indirect exposition of cell phones to surfaces, persons, foods, and the environment, or could be related to the lack of proper hygienic measures of users. However, the presence of either of these groups does not necessarily imply fecal contamination or the presence of pathogens on the devices. Some *Enterobacteriaceae* and

coliform bacteria are common in human and animal feces, but others are commonly found in soil, water, and raw foods. From those sources, these microbial groups can be transferred to the surface of cell phones and their significance depends upon the conditions to which the device has been exposed (20). On the other hand, the presence of *E. coli* on a cell phone surface may indicate the possibility that fecal contamination has occurred and that other microorganisms of fecal origin, including pathogens, may be present. So, this bacterium may be used as an indicator of cell phone sanitation. The use of microbial groups as indicators of contamination of cell phones requires a thorough understanding of the handling and hygiene practices to which this device is subjected and the effect of these practices on microbial groups.

Results of this investigation show the potential of cell phones to participate as fomites and be a vehicle of different types of microorganisms. It is important to provide information not only to undergraduate students but also to general population on preventive strategies to reduce cross-contamination, as well as on hygiene measures to properly clean and disinfect these devices. We did not find evidence to support the hypothesis that these devices could be a reservoir for pathogens like *Salmonella*, *Staphylococcus*, *Streptococcus* or *Enterococcus*, however, information collected on usage habits evidences practices that increase the risk of microbial contamination of cell phones with pathogenic microorganisms.

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