Short Research Article

Staphylococcus Isolated from Mobile Phones and Cheek and Ear Locales

ABSTRACT

Aims: To isolate and identify *Staphylococcus* and *Pseudomonas* from the cheek, ear, and mobile (cell) phones of college students.
Study design: A total of 150 samples were obtained from 50 randomly recruited college students who gave informed consent and answered a brief survey. Three swabs per student were obtained, one each from the cheek, the mid-ear, and from the mobile phone.
Place and Duration of Study: Microbiology Laboratory at the University of Central Missouri, United States from January 2011 until May 2011.
Methodology: Swabs were plated in duplicate onto Mannitol Salt Agar (MSA), Oxacillin Resistance Screening Agar Base (ORSAB) Chromagar containing Oxacillin supplement, and Cetrimide Agar. MSA positive colonies were subcultured onto ORSAB and Tryptic soy agar plates, incubated for 24 hours, and subjected to a confirmatory PBP2a assay. Statistical analysis was performed using a one-way Analysis of Variance (ANOVA) with significance set at (P = .05).
Results: Twenty-seven (54%) touch screen, 13 (26%) sliders, and 10 (20%) flip phones were swabbed. No *Pseudomonas aeruginosa* was detected. More *Staphylococcus* colonies were recovered from touch screen phones than other phone types (P = 0.028). Statistical analysis showed significance between the three locations of bacteria cultured from the phone, cheek, and ear (P = 0.034). One male tested positive for methicillin resistant *Staphylococcus aureus* (MRSA) at all three locations swabbed, and one female tested positive from a cheek swab. Two students (4%) tested positive for MRSA, and 10 (20%) harbored methicillin susceptible *Staphylococcus aureus* (MSSA). Most MSSA was on flip (10%) and touch screen (8%) phones. More *Staphylococcus* colonies were recovered from phones belonging to females than males (P = 0.0001).
Conclusion: Bacterial monitoring of mobile phones is important in identifying antibacterial resistance. Furthermore, reinforcing phone disinfection methods to individuals to keep from spreading bacteria with antibiotic resistance will be of global importance.

Keywords: bacteria; Staphylococcus; MRSA; methicillin; mobile; resistant; phone

1. INTRODUCTION

Mobile phones, also known as cell(ular) phones, have become utilized globally and are now accepted societally to keep in almost instant contact with others. The mobile phone has been investigated in clinical settings for the potential role of spreading bacteria to others such as *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Klebsiella*, *Escherichia*, and *Pseudomonas* [1-7]. Coagulase negative Staphylococci like *Staphylococus epidermidis* can cause skin and urinary tract infections. *Staphylococcus aureus* causes a wide range of illnesses from minor skin infections like impetigo and abscesses, to food poisoning, and even life-threatening diseases such as pneumonia, bacteremia, and sepsis [8]. With reported methicillin resistant *Staphylococcus aureus* (MRSA) cases increasing, identifying the bacterium for proper treatment is now of global importance [8]. The *mecA* gene binds the altered Penicillin Binding Protein (PBP) [9], which is how many of the confirmatory assays are designed to confirm the gene presence. *Pseudomonas aeruginosa* causes wound infections and pneumonia, as well as nosocomial infections in hospitalized

patients [10]. While studies have shown bacterial recovery from medical personnel such as surgeons, ICU nurses, pharmacists, medical laboratory scientists and their mobile phones [11], thus far a case report showing a direct link from a health care worker's mobile phone to a specific patient has been lacking.

Previous studies have investigated bacteria on mobile phones of people working in intensive care units and other areas of the hospital [11], types of cell phones [6,12], patient and visitor cell phones [13], veterinary students [14], and college students and community members [5]. Some of these studies have also included antibiograms of recovered bacteria [15], and have isolated microbes from public computer keyboards [16]. Thus far studies investigating current epidermal skin flora of an individual and their cell phone bacteria have also been lacking.

The purpose of this study was to determine the Staphylococcal and Pseudomonad presence on different cell phone types and compare the bacteria to the person's cheek and ear in direct contact with the cell phone, i.e. facial flora. This study focused on *Staphylococcus aureus* (both methicillin susceptible (MSSA) and methicillin resistant), *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* as the facial flora most likely to be recovered [1, 4, 5, 6, 7]. This study utilized three selective agars to initially isolate the desired bacteria – Mannitol Salt Agar (MSA), Oxacillin Resistance Screening Agar Base Chromagar (ORSAB) with Oxacillin supplement, and Cetrimide Agar. Suspected MSSA and MRSA colonies were then subjected to additional isolation, biochemical, and confirmatory tests.

2. MATERIAL AND METHODS

This project was approved by the Institutional Review Board Human Subjects Committee. Fifty college students who were at least eighteen years of age and possessed a cell phone were randomly recruited. Each person was asked to complete a short survey and to be swabbed. Survey questions asked cell phone type (flip, slider, or touch screen), participant gender, and participation in collegiate or intramural athletics. Surveys were assigned a random number, which was also placed on all corresponding plates and tests.

2.1 Sample Collection

A sterile swab was used to swab the subject's cell phone, the subject's cheek, and the outer mid-to-lower ear, both locations where phone contact occurred. Standardized 2 cm (length) × 2 cm (width) × 2.54 cm (height) squares made of cardstock served as templates to ensure equal areas were swabbed for each person. Swabs were rubbed on the dominant side of the face where the cell phone was usually held within the template area fifteen times left to right, then fifteen times up and down.

2.2 Culture Media and Growth Conditions

Swabs were then plated in duplicate onto Mannitol Salt Agar (MSA) (Oxoid, Basingstoke, UK) and Oxacillin Resistance Screening Agar Base (ORSAB) Chromagar containing Oxacillin supplement (Oxoid, Basingstoke, UK) for *Staphylococcus* recovery [17], and Cetrimide Agar (Difco, Detroit, MI) (Difco) for *Pseudomonas* recovery. Plates were incubated at 37°C for 24 to 48 hours and then results were recorded. Dark blue ORSAB positive colonies were subcultured onto Tryptic Soy Agar (TSA) (Thermofisher Remel, Overland Park, KS) slants, incubated for 24-48 hours at 37°C, and stored at 4°C. Colonies that were MSA positive were tested for catalase and coagulase positivity, and then subcultured onto ORSAB plates and incubated for 24 hours at 37°C. Colonies testing positive on both MSA and ORSAB were then subcultured onto TSA slants and stored at 4°C. Colonies grown on Cetrimide plates were subcultured onto TSA slants and stored at 4°C. Quality control strains (Hardy Diagnostics, Santa Maria, CA) of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were inoculated onto each media type to ensure proper reactions.

Staphylococcus aureus colonies testing positive on ORSAB were then checked for methicillin resistance using a PBP2a (Alere Wampole, Waltham, MA) latex agglutination assay for confirmation of the *mecA* gene.

2.3 Statistical Analysis

Statistical analysis was performed using a one-way Analysis of Variance (ANOVA) with the significance level set at ($P = .05$).

3. RESULTS AND DISCUSSION

3.1 Culture Results

Twenty-seven (54%) phones swabbed were touch screen, 13 (26%) were sliders, and 10 (20%) were flip phones. Fifteen (30%) subjects were male and 35 (70%) were female. None of the human subjects were student athletes, but eight people participated in intramural sports. None of the participants routinely disinfected their cell phone. The number of persons testing positive for MRSA, MSSA, and *Staphylococcus epidermidis* by gender, phone type, and involvement in intramurals is shown in Table 1.

Table 1. Individuals Testing Positive for Each Staphylococcal Species

Phone Type	#	Gender	Intramurals	MRSA	MSSA	MSSA per Phone Type	*S. epidermidis*
Flip	7	Female	0	0	5	10%	5
	3	Male	0	0	0		1
Slider	9	Female	1	0	1	2%	5
	4	Male	0	0	0		0
Touch	19	Female	6	1	3	8%	7
	8	Male	1	1	1		5
Totals	50		8 (16%)	2 (4%)	10 (20%)		23 (46%)

Non-participants in intramurals averaged 6.4 colony-forming units (CFUs) on cell phones, with growth on 19, or 45%. Non-participants averaged 34.9 CFUs per cheek sample, and 54.1 CFUs per ear sample, both with growth on 38 samples for 90.5%. Intramural participants averaged 9.5 CFUs on four (50%) cell phones and 28.5 CFUs per cheek sample on six (75%) cheek samples. Ear samples averaged 52.8 CFUs on seven (87.5%) ear samples.

Colony growth from cell phones on Mannitol Salt plates ranged from zero to 65 CFUs, with a mean of 6.9 CFUs for all cell phones combined. Growth was observed on 23 of 50 (46%) cell phones and is shown in Table 2. The raw data of the MSA total and MSA positive CFUs differs substantially between phone type. Growth was obtained on 12 (44.4%) touch screen phones, five (38.5%) slider phones, and six (60%) flip phones, and not all plates had growth. A comparison of the location means per cell phone type can be seen in Figure 1.

Table 2. Mannitol Salt Agar (MSA) Growth by Location and Phone Type

Phone Type	Location	MSA Plates with Growth	MSA Total CFUs	MSA Mean CFUs	MSA Positive CFUs
Touch Screen	Phone	44%	237	8.8	8
	Cheek	85%	582	21.6	220
	Ear	85%	621	23	457
Slider	Phone	38%	24	1.9	2
	Cheek	85%	200	15.4	56
	Ear	92%	177	13.6	14
Flip	Phone	60%	85	8.5	17
	Cheek	100%	166	16.6	8
	Ear	100%	64	6.4	9

MSA Total CFUs = Both S. epidermidis and S. aureus are included.
MSA Positive CFUs = S. aureus.

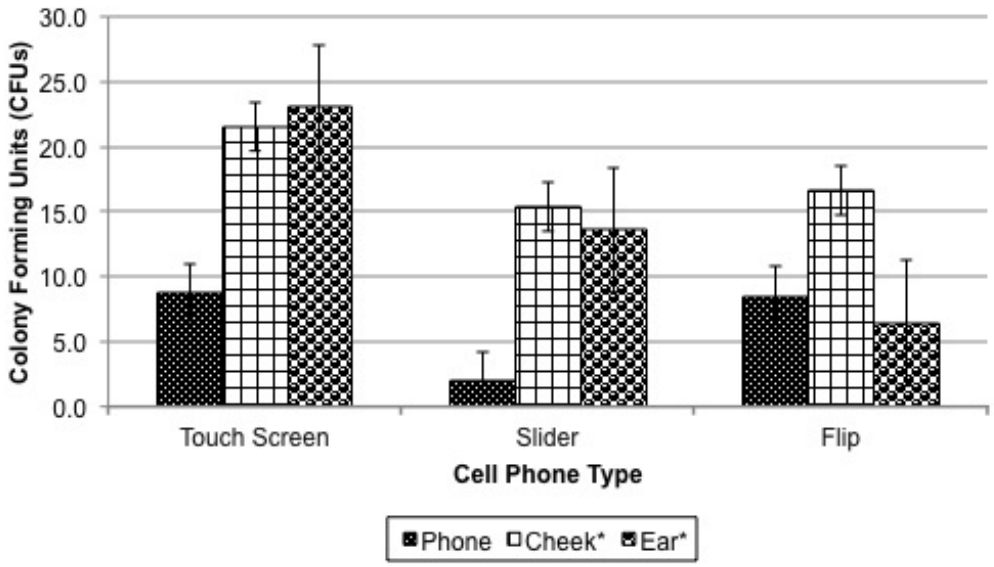

Fig 1. Mean Colony Forming Units (CFUs) recovered from swabs taken from each phone type, cheek, and ear
*Cheek and Ear CFUs: * P = .03*
Mean ± S.E.M = Mean values ± Standard error of means of colony counts of experiment performed in duplicate.

Based on location, a total of 23 (46%) cell phone samples, 44 (88%) cheek samples, and 45 (90%) ear samples yielded growth. The mean cell phone sample grew 6.9 CFUs, with cheek swabs growing 33.9 CFUs, and ear swabs growing 53.9 CFUs. By gender, males had a mean of 9.7 CFUs and females had a mean of 5.7 CFUs. For cheek samples, males averaged 47.3 CFUs and females averaged 28.1 CFUs. For ear samples, male averaged 50.1 CFUs and female averaged 55.5 CFUs.

For cell phones, a total of 319 colonies were *S. epidermidis*, and 27 colonies were MSA positive. For cheek samples, 1410 colonies were *S. epidermidis* and 284 colonies were MSA positive. For ear samples, a total of 2212 colonies were *S. epidermidis* and 483 colonies were MSA positive. Colonies testing MSA positive were scrutinized for proper colony color, size, shape and underwent catalase and coagulase screening before being subcultured to ORSAB media. A total of 44 samples were subcultured onto ORSAB Chromagar, and of those, 34 (77.3 %) tested negative and 10 (22.7%) tested positive. No growth was obtained on the Cetrimide agar for *Pseudomonas aeruginosa.*

3.2 MRSA Confirmation

Of the 10 ORSAB positive *Staphylococcus aureus* colonies, four (40%) samples (2.6% of total samples taken overall) tested positive on the PBP2a confirmatory assay test for MRSA. The results of the PBP2a confirmatory test showed one male subject, who was not involved in intramurals, tested positive on all three locations swabbed – the phone, cheek, and ear. In addition, one female also tested positive from a cheek swab. Thus, two individuals out of 50 (4%) were positive for MRSA and the gender distribution for this study was 50% male and 50% female. Figure 2 shows the number of positive samples, or plates with growth of each bacterial type, from the cheek, ear, and phone swabs taken.

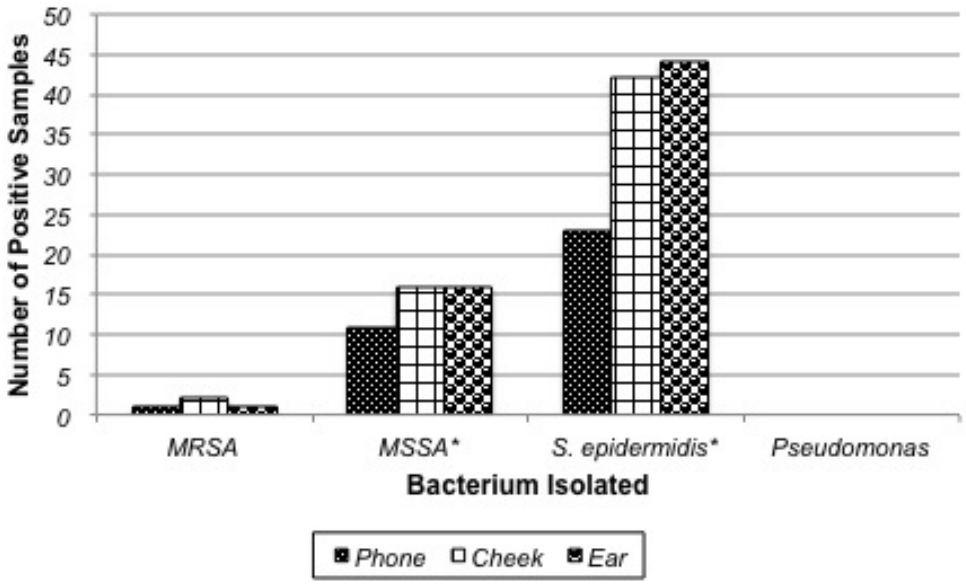

Fig 2. Number of plates with growth, out of the 50 swabs taken, that grew the specific bacterium from a phone, cheek, or ear swab
*Staphylococcus aureus (MSSA) and S. epidermidis: * P = .01*

3.3 Statistical Analysis

Statistical analysis showed significance between the three locations of bacteria grown on MSA cultured from the phone, cheek, and ear with $P = .03$. Phones belonging to females harbored more recovered microbes than males, with $P = .00$. Furthermore, touch screen phones appeared to harbor more *Staphylococcus* overall than the other two types of phones $P = .03$. However, when the means for each phone type were used in the ANOVA, $P = .4$.

When the total number of CFUs obtained on MSA were compared between the types of phones, $P = .01$. Because *S. epidermidis* is usually not associated with high mortality rates like *S. aureus*, the ANOVA was re-ran without *S. epidermidis* figures to see if significance still existed with *S. aureus* levels. Significance

was still seen with $P = .02$. Since no growth was seen for *Pseudomonas*, it was not used in any calculations.

3.4 Cell Phone and Facial Flora Discussion

Based on our findings, there did not appear to be a correlation between the bacterial growth recovered from the cell phone, cheek and ear as correlation coefficients were 0.45, 0.46, and 0.18 respectively. When only *Staphylococcus aureus* growth was compared, the cheek and ear correlation coefficient increased to 0.7, and the other two decreased to -0.04 and -0.07, respectively. A large CFU recovered from a person's cheek did not mean a large CFU was recovered on the person's cell phone and vice versa. The cheek and ear plates had a higher colony count than cell phones on average, which was expected as *Staphylococcus spp.* is normal skin flora. The average colony count for cell phone samples on both touch screen and flip phones was very close. We hypothesized that touch screen cell phones would harbor more bacteria than the other two phone types. On average, slider phones had lower colony counts than the other two cell phone types. This could be due to less direct contact while talking on the phone, or less environmental exposure while not in use. Also, the keypad of slider phones may not get as much use as the one in flip or touch screen phones because many slider phones can be used without the keypad much of the time. Flip phones should have less exposure to the environment when not in use, so less bacteria would come in contact with the screen and keypad, but the bacteria deposited onto the keypad are protected for longer periods of time.

3.5 Staphylococcus Discussion

Recovery of coagulase negative Staphylococci in the highest concentration also supports the findings of Bhoonderowa et al. (2014). MRSA isolated from touch screen phones more than other types of phones supports Lee et al. (2013) and Pierson (2013), but differed from those of Pal et al. (2013). Our 4% MRSA finding is slightly higher than the estimated 2% listed by the Centers for Disease Control and Prevention (CDC) in the United States, but our 20% MSSA recovery is lower than the CDC's estimated 33% in the nares [9]. This could be attributed to the cheek area not being as favorable of an environment as the nares, or attributed to the immune system production of fatty acids [18] or other molecules.

No samples were knowingly taken from collegiate athletes; however, a small number of participants listed involvement in intramurals. Previous studies have shown a higher prevalence of *Staphylococcus spp*. on athletes [19], but our participants had lower means on their cheeks and ears compared to nonparticipants. However, they did have higher CFU means on their phone than nonparticipants. The higher phone CFUs might be that they more frequently contact transient bacteria. Potential reasons for the lower facial flora averages could include frequent showers lowering cheek and ear flora via pH changes or cell disruption from soaps and detergents, antibacterial inhibition or killing from soaps or body washes [20], and mechanical action dislodging bacteria from washing and drying off with towels.

Our findings of phones belonging to females harboring higher overall CFUs than males supported the findings of Bhoonderowa et al. (2014) but contradicted the findings of Amala and Ejikema (2015). These results could be caused by a variety of factors including personal hygiene, more females involved in intramurals, or more frequent use of earrings and makeup by females in the population studied. Because disproportionately more females participated in this study, a study involving more males would be beneficial to see if this pattern continues.

3.6 Pseudomonas Discussion

We were surprised no *Pseudomonas* was recovered on any plates as based on previous studies we expected approximately 1-6% [1, 3, 15]. Swabs were also plated onto TSA plates, and no characteristic odor, swarming, or fluorescent colonies were noted. Control organisms grew on the medium, and the age of the healthy population studied could explain the lack of results.

3.7 Summary

In summary, our findings did not show a correlation between cell phone, cheek and ear recovery of *Staphylococcus aureus*, but did find a difference in growth from touch screen phones ($P = .03$), between locations ($P = .03$), and phones owned by females ($P = .00$). The 4% MRSA rate was slightly higher than the CDC estimates, even though our 20% MSSA rates were below estimates.

3.8 Conclusion

In conclusion, bacterial monitoring of electronics such as mobile phones and tablets is important in identifying antibacterial resistance. Furthermore, reinforcing phone disinfection methods to individuals to keep from spreading bacteria with antibiotic resistance will be of global importance.

ETHICAL APPROVAL

This project was approved by the University Institutional Review Board human subjects committee.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

CFU: Colony Forming Unit
MRSA: Methicillin Resistant *Staphylococcus aureus*
MSSA: Methicillin Susceptible *Staphylococcus aureus*