INVESTIGATING CULTURABLE GRAM POSITIVE COCCI FROM THE SURFACE OF MOBILE PHONES WITH DIFFERENT FORM FACTORS

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ABSTRACT   A total of 99 mobiles phones were grouped according to their form factor that is flip phone and candy bar designs, and their contact surfaces were swabbed and cultured onto both R2A and SPCA media. We usually obtained bacterial abundance between $0 - 50$ cfu cm^{-2}, even though densities up to 490 cfu cm^{-2} on R2A and 212 cfu cm^{-2} on SPCA were also recorded. Our study showed that candy bar phones harbored more bacteria, and served as a more important bacterial reservoir as compared to flip phones. Our results also suggested the importance of using mobile phone covers to reduce bacterial abundance. We also found that the Gram positive cocci isolated were similar to the normal flora present on human skin. In this study, more bacterial colonies were consistently found on R2A. R2A was a more suitable isolation medium and performed better for surface microbes.

ABSTRAK   Sejumlah 99 telefon bimbit telefon telah dikumpulkan mengikut faktor reka bentuk iaitu telefon 'flip' dan kandi bar, dan permukaan sentuhan mereka telah dikesat dan dikultur di atas kedua-dua media R2A dan SPCA. Pada kebiasaannya, kepadatan bakteria diperolehi di antara $0 - 50$ cfu cm^{-2}, walaupun kepadatan bakteria sehingga 490 cfu cm^{-2} pada R2A dan 212 cfu cm^{-2} pada SPCA juga dicatat. Kajian kami menunjukkan bahawa telefon kandi bar mempunyai lebih bakteria, dan berpotensi sebagai takungan bakteria yang lebih penting berbanding telefon flip. Keputusan kami juga mencadangkan kepentingan menggunakan penutup telefon mudah alih untuk mengurangkan kepadatan bakteria. Kami juga mendapati bahawa Gram positif cocci yang dipencilkan adalah serupa dengan flora normal yang ada pada kulit manusia. Dalam kajian ini, secara konsisten lebih koloni bakteria telah dikultur pada R2A. R2A adalah medium pengasingan yang lebih sesuai dan berprestasi lebih baik untuk mikrob pada habitat permukaan.

INTRODUCTION

Environmental surfaces such as keyboards, phones, elevator buttons and desks serve as reservoirs for pathogens [1], and can be a potential source for cross transmission and community-acquired infection especially in hospitals [2]. Hospital-acquired or nosocomial infection is a growing problem and for example, the total number of healthcare-associated infections in the United States was more than 1.7 million for 2002 or 4.5 per 100 admissions [3] where the direct medical costs of these healthcare-associated infections range from USD28.4 to 33.8 billion [4].

The usage of mobile phones among medical personnel in hospitals is suggested to have contributed towards the increase in healthcare-associated infections because mobile phones may serve as bacterial reservoir and thus become a vehicle for the spread of nosocomial

pathogen [5]. Nosocomial pathogens reported include *Staphylococcus aureus*, Streptococcus sp., *Bacillus cereus*, *Acinetobacter* sp., coagulase negative *Staphylococcus* sp., *Enterococcus* sp., *Pseudomonas aeruginosa, Legionella* sp. and members of the *Enterobacteriaceae* family such as *Escherichia coli*, *Proteus mirabilis*, *Salmonella* sp., *Serratia marcescens* and *Klebsiella pneumonia* [6, 7]. However the spectrum of nosocomial pathogen has changed from Gram negative bacilli to Gram positive cocci [8], and at present Methicillin-resistant *S. aureus* (MRSA) has become the most common bacterial nosocomial pathogen [9].

In this study, we studied enumerated the culturable bacteria from mobile phones of different form factors i.e. flip phones and candy bar phones, and characterized

the Gram positive cocci to their genera. We found that most of the Gram positive cocci on the phone surface were normal flora of the skin, and that flip phones had a significantly lower bacterial count. Our results suggested covering mobile phones to reduce the bacterial numbers on mobile phone surface.

MATERIALS AND METHODS

A total of 99 samples were collected from mobile phones of students in the University of Malaya, Kuala Lumpur, Malaysia from July until September 2008. The model of the phone was recorded and surface or contact area was measured. The phones were divided into the flip phones (n = 17) and candy bar (n = 82) designs, and the surface area included both the numeric pad and the screen. A sterile cotton swab was soaked in 2 ml sterile Ringer's solution before sampling the surface area in a zigzag fashion. The cotton swab was then kept in sterile Ringer's solution before processing. In order to dislodge the bacteria from the cotton swab, we sonicated the cotton swab in Ringer's solution for 10 minutes (50/60 Hz, NEY, USA).

After which, 0.2 ml of the Ringer's solution was spread-plated on Reasoner's 2A (R2A) agar and Standard Plate Count agar (SPCA) (Difco, USA). The plates were then incubated at 37°C for 48 hours. The colony forming units (cfu) on the agar was counted, and Gram staining was performed on every cfu observed. Bacterial isolates that were Gram positive cocci were further characterized using catalase, modified oxidase [10], glycerol-erythromycin [11], furazolidone sensitivity [12] and coagulase tests. Table 1 shows the characterization of Gram positive cocci according to the tests carried out. In this study, bacterial density was calculated as the number of cfu over the surface area swabbed (cfu cm^{-2}). Student's t-test was carried out to determine whether the number of cfus obtained were different between R2A and SPCA, and between clam-shell and candy bar phones. Cfu data were log-transformed (log cfu + 1) and outliers removed before statistical testing.

Table 1. Characterization of Gram positive cocci. +, positive results; −, negative results; ND, tests not done

Gram positive cocci	Catalase	Modified oxidase	Glycerol-erythromycin	Furazolidone sensitivity	Coagulase
Streptococcus sp.	−	ND	ND	ND	ND
Micrococcus sp.	+	+	−	+	ND
Coagulase negative staphylococci	+	−	+	−	−
Coagulase positive staphylococci	+	−	+	−	+

RESULTS

In this study, we usually obtained bacterial abundance between 0 – 50 cfu cm^{-2}, even though densities up to 490 cfu cm^{-2} on R2A and 212 cfu cm^{-2} on SPCA were also recorded (Figure 1). We isolated bacteria on more occasions with R2A (88% of the sampling frequency) than with SPCA (76%), and the average density on R2A (17 ± 56 cfu cm^{-2}) was higher than on SPCA (9 ± 27 cfu cm^{-2}) (paired t_{98} = 3.30, p < 0.01).

When we compared the different form factors, our study showed that candy bar phones had higher cfu per surface area than flip phones on both R2A (t_{65} = 3.56, p < 0.001) and SPCA (t_{58}= 3.86, p < 0.001)

(Figure 2). Of the 1816 cfu isolated from R2A, we found that Gram negative bacilli were the most frequently isolated (46%), followed by Gram positive bacilli (29%) and Gram positive cocci (13%), respectively. On SPCA, 839 cfu were isolated, and comprised of 38% Gram negative bacilli, 26% Gram positive bacilli and 18% Gram positive cocci. Further characterization of the Gram positive cocci isolated from R2A showed that most of the Gram positive cocci was coagulase negative staphylococci (40%), followed by micrococci (39%), coagulase positive staphylococci (21%) and streptococci (<1%).
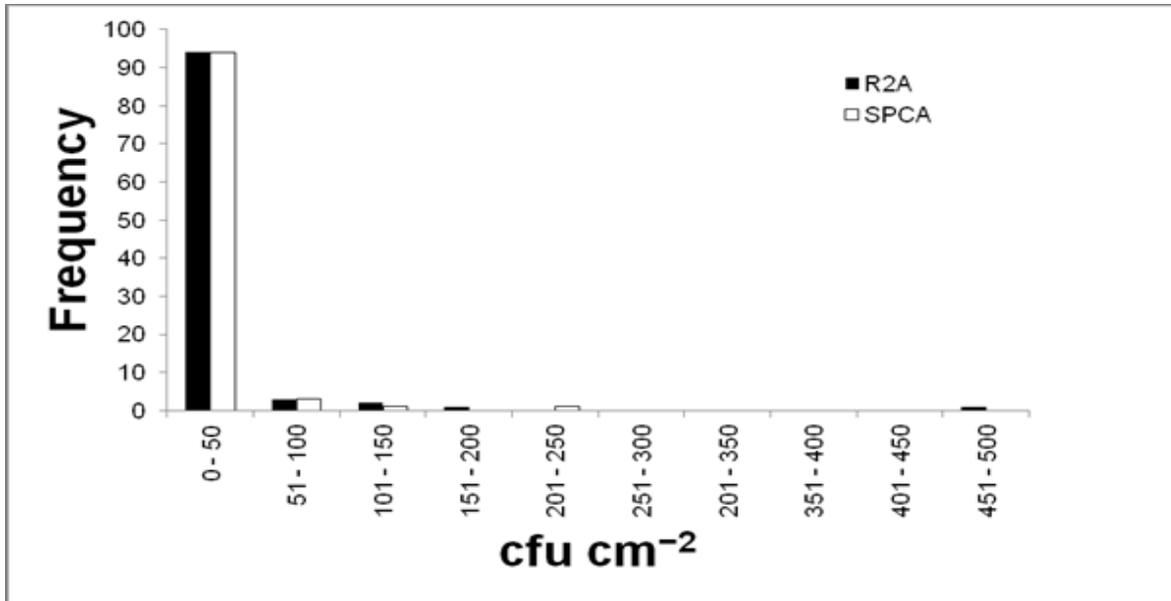
Figure 1. Frequency distribution of culturable bacterial abundance (cfu cm^{-2}) isolated from mobile phone surfaces on the bacteriological media R2A and SPCA.
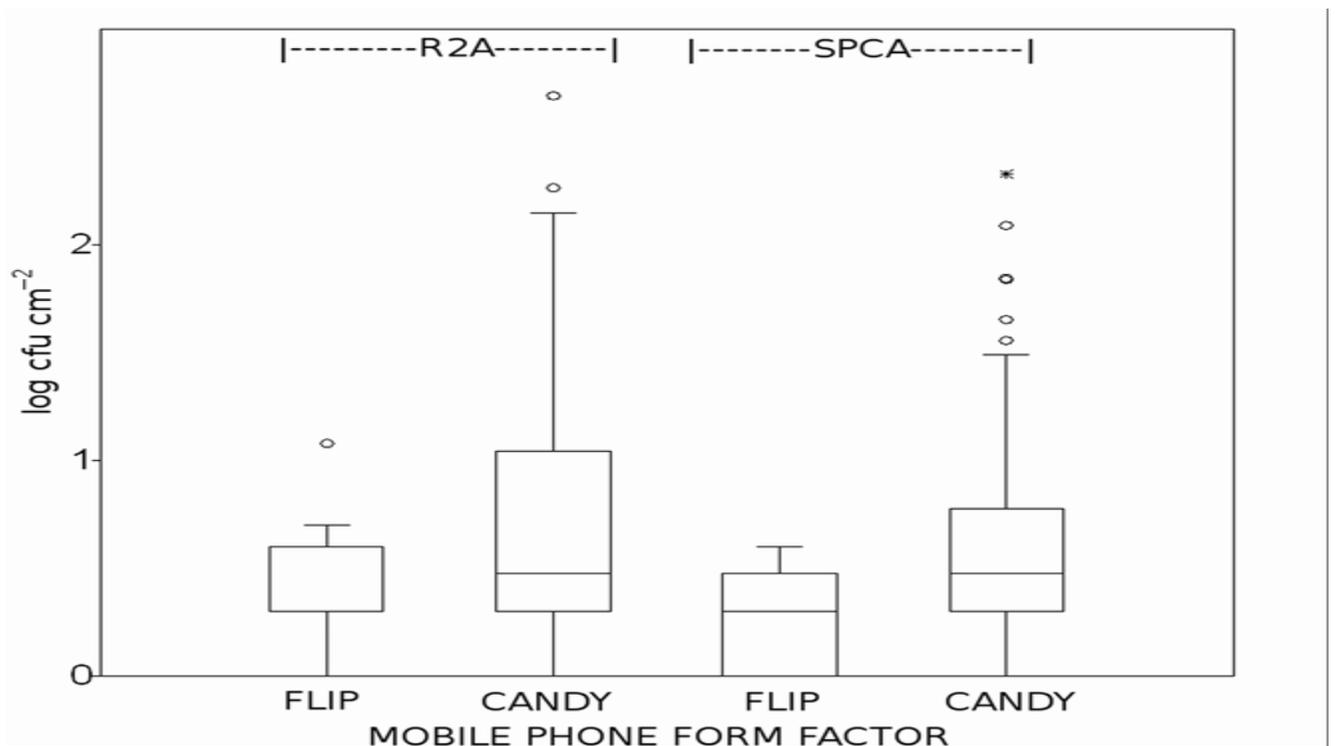

Figure 2. Box-and-whisker plots showing the range and the median of culturable bacterial abundance (log cfu cm^{-2}) on both bacteriological media R2A and SPCA. For each media, abundance from flip and candy bar phones are shown.

DISCUSSION

In this study, the number of cfu isolated from the mobile phone surface was relatively higher than other surfaces e.g. 2.8 cfu cm^{-2} at trolley surfaces [13], from 2 to 8 cfu cm^{-2} for public telephones [14], 2.0 cfu cm^{-2} on telephone mouthpieces [1]. On the phone surface, the contamination was primarily due to skin contact where Rusin, Maxwell, & Gerba [15] have reported transfer efficiencies > 40%. In addition, the combination of constant handling and the heat generated by the phones creates a better environment for all sorts of microorganisms that are normally found on our skin to survive and grow [16].

When comparing the two types of bacteriological media for the isolation of bacteria from the surface of mobile phones, we showed that R2A was a more suitable isolation medium and performed better for surface microbes than SPCA. Both the frequency of isolation and average cfu density on R2A were higher than SPCA. The tendency for SPCA to select for copiotrophs could have suppressed the more predominant slow-growing bacteria [14] that are present on surfaces. Moreover R2A contains ingredients such as sodium pyruvate and soluble starch that help improve recovery of stressed bacteria [31].

Our study also showed that candy bar phones harbored more bacteria, and served as a more important bacterial reservoir than flip phones. A possible reason for this is that the screen and keypad surface of flip phones are normally protected and enclosed whereas candy bar phones are exposed by design, and could collect more bacteria from the environment. Although the owner's habit and frequency of usage could affect the amount of bacteria present on mobile phone surface, we assumed these were not factors in our experimental setup as all the mobile phones sampled belonged to undergraduates. Our study strongly suggested that the design or form factor of the phone was the main reason for the higher bacterial cfu on candy bar phones. Our results further suggested the importance of using a cover for your mobile phones in order to reduce the number of bacteria on the phone surface, which concurs with [17].

In this study, Gram negative bacilli formed 38 – 46% of the culturable bacteria, and were the major bacterial group isolated from the phone surface. Gram positive cocci were the least abundant. This contrasted with other studies [1,14,18] that found Gram positive cocci as the main bacterial group on phones and hospital paraphernalia. Of the Gram positive cocci, streptococci were the least abundant. The presence of *Streptococcus* sp. in low numbers is not surprising as they are transient colonizers of our skin [19]. The rest of the Gram positive cocci were catalase positive, and of these 79% were coagulase negative staphylococci and micrococci. Staphylococci have a symbiotic relationship with mammals, and are found on skin, skin glands and mucous membrane [20] whereas micrococci are widespread in nature and are also found on human skins [21].

Among the coagulase-negative staphylococci, the most prevalent is *Staphylococcus epidermidis* [22]. Previously, only coagulase-positive staphylococci were thought to be pathogenic. However coagulase-negative staphylococci are now emerging as pathogens especially in medical-device related infections, and in immuno-compromised patients [22,23]. Micrococci can also be an opportunistic pathogen in immuno-compromised patients [24,25]. About 21% of the Gram positive cocci were coagulase positive staphylococci or presumptive *S. aureus* [26]. *S. aureus* made up 34% of the total staphylococci population, in part due to its ability to survive long periods in the environment and hostile conditions to its development [27]. *S. aureus* is also part of the normal flora on skin, nose, mouth, throat, intestines and internal female genitals and is able to cause serious infections once it enters the host [28].

At present, *S. aureus* is a primary pathogen in both nosocomial and community-acquired blood stream, skin and soft tissue and lower respiratory infections [29,30]. In this study, up to 140 presumptive *S. aureus* were isolated, and averaged from 0.19 ± 0.4 to 0.64 ± 1.91 cfu of *S. aureus* cm^{-2}. From the characterization of Gram positive cocci isolated in this study, we found that they were mostly the normal flora present on human skin. Thus, the transmission most probably occurred through skin contact during usage. However further studies are needed to determine the pathogenicity of these isolates.

CONCLUSION

We used two types of bacteriological media and found that R2A generally performed better than SPCA. We also found more bacteria on candy bar phones in comparison to flip phones, and suggested that importance of using mobile phone covers to reduce bacterial abundance. From the types of Gram positive cocci found in this study, most were of normal skin flora and were probably transmitted through skin contact during use.

ACKNOWLEDGEMENT

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