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Microbiological Assessment of Smartphone Surfaces Obtained from Final Year Students at Hezekiah University Umudi, Nkwerre, Imo State, Nigeria.

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Abstract

Smartphones of students in tertiary institutions have been described as an understudied and under-explored microcosm by leading public health experts. The microbial quality of smartphone surfaces has been a constant reason for several scientific discussions. Two hundred students were initially targeted for the study, ten students in the final year class at the Hezekiah University Umudi permitted access to their phones during the sampling. The study carried by swabbing the surface of their phones with a normal saline-moistened swab stick using a square quadrant. The content was dislodged and diluted using a 10-fold serial dilution technique. The samples were evaluated for total heterotrophic count, coliform, fungal and *Staphylococcal* count. The bacterial isolates were tentatively identified using biochemical reactions. The total heterotrophic count of the samples obtained from the male students ranged from Log 2.82 CFU/cm^2 to Log 3.21 CFU/cm^2 while the total coliform count was observed to range from Log 1.3 CFU/cm^2 to Log 1.6 CFU/cm^2. The total heterotrophic count ranged from Log 2.82 CFU/cm^2 to Log 3.21 CFU/cm^2 for male students and from Log 1.6 CFU/cm^2 to Log 2.73 CFU/cm^2 for female students. The isolates obtained from the study were *Acinetobacter* sp., *Escherichia coli, Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus* sp. There is a need for the University to educate students on the need to routinely use alcohol-based wipes to clean the surfaces of their phones as a precautionary measure to limit diseases associated with the use of mobile phones.

Keywords: *Smartphones, Microbial quality, Normal saline-moistened swab stick, Alcohol-based wipes*
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I. INTRODUCTION

Handsets, smartphones or mobile phones constitutes Frequently Touched Surfaces (FTS) as the name suggests are in constant contact with human hands, providing a microcosm for invasion, survival and cross-colonization of microbes (Akinyemi *et al*., 2009). Most of the frequently touched surfaces are touch-screen phones, laptop keyboards, automated Teller Machines and Post of Sale Machines. Investigating the indigenous microflora of phones is essential as they can act as fomites—objects capable of transmitting infectious agents (Sadiq *et al*., 2020). Smartphones make contact with several surfaces and enter almost every environment (Gashaw *et al*., 2014). The ease of cross-contamination of smartphones from unhygienic activities can in most cases be unexplainable. The need to identify a possible route of transmission of diseases through smartphone usage if identified can be controlled or source-tracked to stall the re-occurrence. The microflora of the mobile phones used by students at Hezekiah University needs to be explored. The potential of these smartphone surfaces to serve as a reservoir for invasive organisms has not been exhaustively reported from the mobile phones used by students among students; However, there are plethora of reports on healthcare professionals and transmission of nosocomial infections from frequently touched surfaces (Eboh *et al*. 2022).

Some of the pathogenic microbes can adapt to touchscreens for extended period of time, waiting to be transmitted to new hosts upon contact. Understanding the dynamics of these microcosms can elucidate the prevalence of potential pathogens, shed light on their survival mechanisms, and quantify the risks they pose to human health (Seal *et al*., 2021). Studies have shown that the surfaces of mobile phones

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can harbor a diverse array of pathogens. A report by Wang *et al*. (2018) observed that smartphones could inhabit organisms such as *Staphylococcus aureus and Escherichia coli*, underscoring the potential for these devices to serve as vehicles for transmission of enteric pathogens. Several screen-guarding devices have evolved in recent times, and a number of these screen-guards have been identified to several degrees of adsorption to a wide variety of microbes (Huang *et al*., 2024). Mobile phones have recently become the most touchable and serviceable object in the daily life especially when users can send text messages and email and access the internet, along with many other services. However, these achievements and benefits of the mobile phone place people at higher risk of overlooking health risks associated with its use due to contamination with different types of bacteria and other microbes. Microbiota of humans including several pathogens can be transferred onto the hands from contaminated surfaces with which they come into contact in daily life. Hands can easily transmit infectious diseases either to oneself or to others. Hand hygiene which involves washing with soap is one of the most effective and inexpensive approaches for the elimination of pathogens. In most communities as recognized by the World Health Organization (WHO, 2000). The proliferation of nosocomial infections has been identified to surge as a result of poor hand hygiene and cross-contamination of frequently touched surfaces by health personnel and caregivers. This has led to an increase in antimicrobial-resistant microbes.

II. Materials And Methods

A. Study population

Swab samples were obtained from the touch-screen phones of the final year at the Hezekiah University Umudi. The sampling followed the simple random approach of collecting samples from the surface of smartphones from both male and female students.

B. Sample collection

Ten samples were collected using a constructed square quadrant and moist cotton swab sticks moistened with the physiological saline (Odoemelam *et al*., 2020). The samples were transported to the Microbiology laboratory of Hezekiah, University, Umudi, Nigeria.

C. Sample handling

The swabs were dislodged in peptone, and 1.0 ml were made to go through a 10-fold serial dilution using 9.0 ml physiological saline. Then 0.1 ml was made to go through spread plating. The plates were incubated at 37ºC (Odoemelam *et al*., 2020).

D. Determination of total heterotrophic bacterial count

The equivalent of 28g of nutrient agar was suspended in a litre of deionized water. The medium was swirled to mix and homogenize. Then it was boiled for 3 minutes to dissolve completely, then the media was sterilized by autoclaving under 121°C at 15 p.s.i for 15 minutes. and wait for the medium to solidify. The plates were incubated at 37ºC. The plates were counted after 24 hrs and the Average plate count was mathematically converted to CFU/cm^2.

E. Determination of total fungal count

This study was carried out to ascertain fungal composition on the surface of smartphones. The plates were inverted and incubated at 37ºC. Aliquots of the peptone carrying the peptone and the microbes obtained from the swab process were placed into the petri dish. Pure cultures of the fungal growth were subcultured on freshly prepared media. After three days the plates were counted and mathematically presented in CFU/cm^2 (Effiong *et al*., 2019).

F. Determination of Total Staphylococcal count

This procedure was done to ascertain the presence of halophytic or halotolerant microbes, especially members such as *Staphylococcus* spp. The Mannitol Salt Agar (MSA) is a selective and differential medium. The high concentration of salt (7.5%) is selected for members of the genus *Staphylococcus* since they can tolerate high saline levels. Then, 0.1 ml of the sample was obtained from the dilution tubes and spread plate technique was used in the study. The plates were inverted and incubated at 37ºC for 24 hrs. The count obtained was expressed in CFU/cm^2.

G. Determination of coliform count

This procedure was done to ascertain the presence of pathogens associated with the smartphone surfaces. This procedure employed Eosine Methylene Blue (EMB) agar to identify the presence of indicator organisms such as *Escherichia coli*. The plates were incubated at 37°C. Aliquots of the peptone carrying the peptone and the microbes obtained from the swab process were placed into the petri dish.

H. Biochemical identification of bacterial isolates

The isolates were tentatively identified using an 18-hr axenic culture of the isolates obtained during the study. Isolates were made to undergo Gram reaction, oxidase, catalase, Tripple sugar ion, citrate, Indole, Methyl Red Voges Proskauer and sugar fermentations. A dichotomous key was to ascertain the positive results. The entire results were compared to Bergey's Manual of Determinative Bacteriology (Asionye *et al.*, 2023; MacFaddin, 2000; Madigan *et al*., 2008).

III. Results

A. Details of the Samples obtained

The results presented in Table 1 show the details of samples obtained from population of the students sampled for the study did not have any restricted usage of their touchscreen phones in any environment. Furthermore, 100% of the students agreed not to routinely clean their phone surfaces with disinfectant-impregnated wipes as seen in the results presented below. The samples obtained from the female students were denoted with the alphabet "F" while the ones obtained from the male students were denoted with the letter

M. The duration of the usage of the touch-screen phones sampled from the students was identified to range between two (2) months to three years.

Table 1. Chart of Smart/Touch-screen phones obtained from final year students in Hezekiah University Umudi

Sample Code	Brand	Duration of Usage	Routine Cleaning	Restricted Usage
F1	Infinix Hot 6	3years	No	No
F2	iPhone 11 pro max	3 months	No	No
F3	iphone 13 pro max	4 months	No	No
F4	Tecno Spark	1 year	No	No
F5	Tecno	7months	No	No
M1	Oppo	2 years	No	No
M2	Nokia	8 months	No	No
M3	Oppo	2 months	No	No
M4	Redmi	1 year	No	No
M5	Itel	2 years	No	No

M= Male; F= Female

B. Microbial population of smartphone surfaces of final year students in Hezekiah University, Umudi

The microbial population of the phone surfaces obtained from the male and female students in Hezekiah University was presented in Figures 1 and 2 respectively. The total heterotrophic count of the samples obtained from the male students ranged from Log 2.82 CFU/cm^2 to Log 3.21 CFU/cm^2 and was presented in Fig. 1. Samples obtained from M1 and M5 had the highest microbial population while samples obtained from the M2 had the lowest in terms of the total heterotrophic count which was observed to be Log2.82 CFU/cm^2. The results obtained for the total fungal count ranged from Log1.6 CFU/cm^2 for M2 while M4 and M5 had Log 2.6 CFU/cm^2 and Log 1.78 CFU/cm^2. Total coliform count was observed to range from Log 1.3 CFU/cm^2 to Log 1.6 CFU/cm^2. Samples obtained from M4 had the highest Total coliform and fungal counts respectively. The study conducted for total *Staphylococcal* count was observed to range from Log 1.30 CFU/cm^2 to Log 1.60 CFU/cm^2.

The result presented in Fig. 3.2 was observed to show the distribution of the microbial population on the surface of smartphones used by female students at Hezekiah University Umudi. The results obtained from the study showed the microbial population of heterotrophs varied from Log1.6 CFU/cm^2 to Log 2.73 CFU/cm^2. The samples obtained from F2 had the highest population of the total heterotrophic count while F3 had the least microbial population. The total fungal count of the swab samples from the female students was not identified for the F2 samples, but the samples obtained for F5 had a THC load of Log 2.3 CFU/cm^2. Total coliform count was identified to be Log 1.6, Log 1.5, 1.6, and Log 1.8 CFU/cm^2.

C. Tentative identification of the bacterial isolates

Six different isolates were selected for the biochemical characterization based on their morphological characterization. Isolate 1 was identified to have a Gram-positive reaction, its reaction to oxidase and catalase was identified to be positive. The triple sugar Ion test showed that the isolate was able to utilize the sugars which was identified to be acidic with the evolution of gas, although the isolate did not produce a black precipitate for hydrogen sulphide for the utilization of the ions. The isolate was also identified to be motile, with evident hydrolysis of starch. The biochemical outcome for Citrate, Urease, and Vogues Proskauer test was identified to be negative. The reaction for the isolate on MacConkey and Eosin-methylene blue agar (EMB) was positive for both golden-yellow and green metallic sheen. The isolate was tentatively identified to be *Escherichia coli.* Furthermore, Isolate had a similar biochemical reaction as isolate 1.0, it varied in the sense that during the TSI evaluation, the isolate was identified to produce hydrogen sulphide which is evident in the formation of black precipitation for the utilization of the ions and radicals. The urease and citrate tests conducted on the isolates were positive, but the citrate result showed that it was a slow utilizer of the citrate as a sole source of carbon. Isolate 2 was observed to be positive for the utilization of fermented sugars for glucose, fructose, lactose and sucrose with the evolution of gas. The isolates obtained were *Acinetobacter* sp., *Escherichia coli*, *Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus* sp as presented in Table 2 below.

.Table 2. Biochemical Identification of bacterial isolates

Biochemical	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Gram Reaction	-	-	-	-	-	+
Oxidase	-	-	-	+	-	+
Catalase	-	-	-	+	+	+
TSI Slant	A	A	A	A	A	A
Butt	K	K	A	A	A	K
Gas	+	+	+	+	+	+
H2S	-	+	+	+	-	-
MR	+	-	-	-	+	-
VP	-	-	-	-	+	-
Urease	-	+	-	-	-	-
Citrate	-	+	+	+	+	+
Motility	+	-	+	+	-	-
Starch hydrolysis	+	-	+	+	+	-
Glucose	A/G	A/G	A/G	A/G	A/G	A/G
Fructose	A/G	A/G	A/G	A/G	A/G	A/G
Lactose	A/G	A/G	A/G	K/-	K/-	A/G
Sucrose	A/G	A/G	A/G	A/G	A/G	A/G
Xylose	A/-	A/-	K/-	A/G	A/-	A/-
Maltose	A/G	K/-	A/-	A/G	A/G	A/G
Tentative Identity	*E. coli*	*Proteus* sp.	*Klebsiella* sp.	*Pseudomonas* sp.	*Acinetobacter* sp.	*Staphylococcus* sp.

Key: TSI= Triple Sugar Iron, MR= Methyl Red; A=Acid; K=Alkaline; G= Gas; H2S; -= Negative reaction; + = positive reaction

IV. DISCUSSIONS

Mobile phones have been described as one of the dirtiest gadgets we use in our day-to-day lives. Their microbial load might be seven times higher than most conventional fomites (Brady *et al.* 2018; Gunasekara, 2009). The usage of mobile phones by the youths has been identified as a poorly regulated part of their social life, some have replaced the use of wristwatches by use of phones thereby increasing the frequency of contact to the phones. Most phones due to unrestricted usage have been reportedly been kept on surfaces laden with microbial contaminants; this might be predicated on unregulated use of mobile phones in places like toilets and laboratories. Smartphones have been reportedly involved in cross-contamination as a result of frequent contact with surfaces and skin microflora. This research further identified that the male final-year students at the Hezekiah University were more unrestricted to use their phones in the lavatories and were more likely to drop their phones on tables without minding the sanitary status of the surface. These findings were in tandem with the report of Nwankwo *et al.* (2014) whose report indicated the microbial load in samples obtained from the male subjects was significantly higher due to cross-contamination from unhygienic practices. This was also observed in the microbial load of their touch-screen phones. These findings also agree with the report of Auhim (2013) whose study fully identified that microflora of the body either skin or nasal nares were constantly reported in samples obtained from male students.

The microbial population of any smartphone may vary from one phone type to another. Elmanama *et al.* (2015) reported that the nature of screen guards may also impact the microbial population of smartphones. The rough ones may accumulate more of the microbes than the smooth ones. In this study, the total heterotrophic count of the samples obtained from the male students ranged from Log 2.82 CFU/cm^2 to Log 3.21CFU/cm^2 while Log 1.6 CFU/cm^2 to Log 2.73 CFU/cm^2 was observed for the female students. This study further buttresses the microbial load from male students to female students. This corroborates the report of Sadiq *et al.* (2020) whose investigation observed a population of heterotrophs within 120 CFU/ml and 210 CFU/ml after and before swiping with alcohol-based wipes. These findings were in tandem with the report by Bodena *et al.* (2019) whose investigation revealed an over growth of microbes obtained from the surface of smartphones in 80% of healthcare professionals at 92.8 CFU/ml. These microbial counts were linked directly to the microbiome of the users of the phones (Maier *et al.*, 2009).

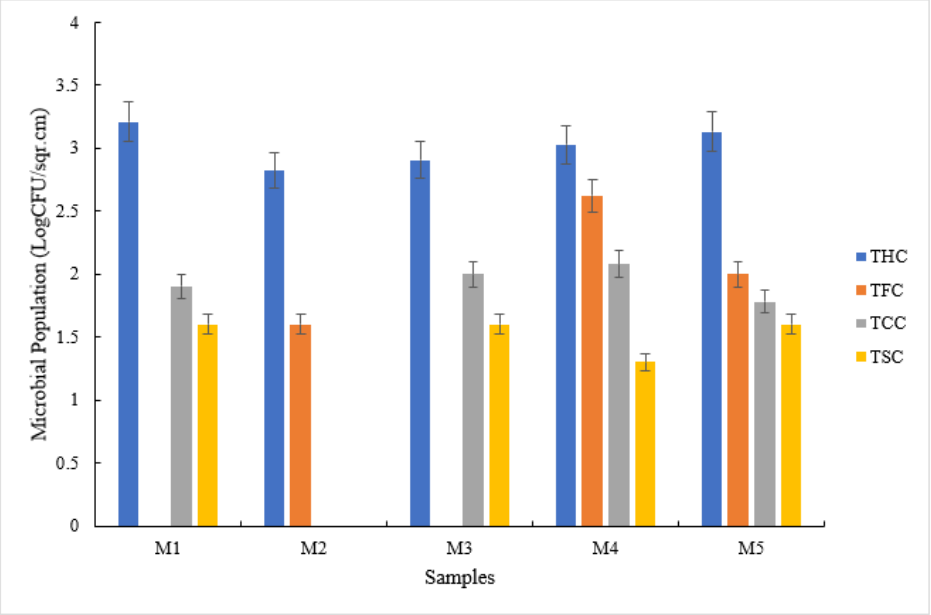

Figure 1. Microbial population of the surface of smartphones obtained from Male students in Hezekiah University, Umudi.
THC=Total Heterotrophic Count, TFC=Total Fungal Count, TCC- Total Coliform Count, TSC= Total Staphylococcal Count. CFU= Colony Forming Unit.

The isolates obtained from the study were *Acinetobacter* sp. *Escherichia coli*, *Proteus* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus* sp. The results were in tandem with the report of Sadiq *et al.* (2020) coagulase-negative *Staphylococci* (CoNS), *Staphylococcus aureus*, *Micrococcus* spp., spore-forming *Bacillus* spp. and Gram-negative bacteria predominantly *Escherichia coli*, *Proteus* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* sp. This was also in agreement with He *et al*. (2022) *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*. This study further identified the presence of coliforms on the surface of smartphones of male students. Akinyemi *et al*. (2009) conducted a similar study and isolated *Pseudomonas aeruginosa*, Staphylococcus sp. and *E. coli*. They also identified *Staphylococcus aureus* as a predominant isolate, followed by *E. coli*, *Pseudomonas* sp. and *Salmonella* sp. Our results also agreed strongly with Bhat *et al*. (2011) with the isolation of *S. aureus* and *E. coli*. In addition, Elmanama *et al*. (2016) also confirmed that the overall percentage of positive cultures was 71.6% most predominant isolate (with 27%). This did not tally strongly with the current findings of the present study; as a significant number of Gram-Negative isolates were observed in the study. The *Staphylococcus* sp. has been identified as having been reported to originate from the nasal nares while *E. coli* have been associated with faecal contamination or cross-contamination. In some other studies, *Klebsiella* sp. and *Proteus* sp. have been widely reported in most cases of nosocomial infections and wound sepsis this was represented in Table 2 above.

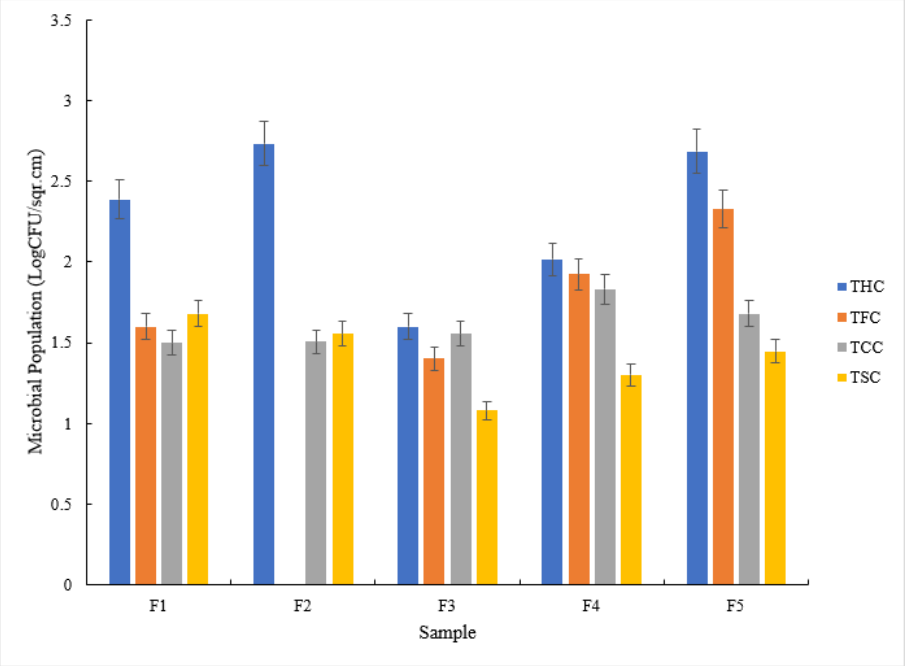

Figure 2. Microbial population of the surface of smartphones obtained from female students in Hezekiah University, Umudi.
Key: THC=Total Heterotrophic Count, TFC=Total Fungal Count, TCC- Total Coliform Count, TSC= Total Staphylococcal Count. CFU= Colony Forming Unit.

V. Conclusions

The surfaces of smartphones are critical source trackers of the microbiomes of their users. The high microbial population of microbes in the male used smartphones unlike the female used alternatives also indicate the incidences of poor hand hygiene by the male students sampled in the Hezekiah University Umudi. The presence of coliforms in the samples obtained by both males and females also are indicative of the role of possible cross-contamination or the microbiota of the lavatories and poor hand-washing. The study carried out observed that the microbial population of the smartphones used by male students had a significantly higher counts than the ones used by the female students as the counts showed the counts almost double the trend observed in the female students.

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