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Article

Isolation and identification of bacteria from mobile phones of students and employees of Hajee Mohammad Danesh Science and Technology University, Bangladesh

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Abstract: Microbes are capable to survive on mobile phone surface and serve as a potential transporter of microorganism amongst handlers. Thus, the study focused on isolation and identification of bacteria from mobile phones of academic and non-academic staffs (including students and cleaners) of Hajee Mohammad Danesh Science and Technology University Dinajpur, Bangladesh. A total of 32 swab samples of mobile phones were collected. The bacteria isolates were identified on the basis of morphological, cultural and biochemical characterization. The total viable count (TVC) of different swab samples of mobile phones in different categories were ranged from 73×10^{-6} CFU/ml to 260×10^{-6} CFU/ml. Analysis revealed that, among the samples 21 (25.6%) isolates were *Staphylococcus* spp, 17 (20.7%) were *Bacillus* spp, 16 (19.5%) were *Klebsiella* spp, 15 (18%) were *Pseudomonas* spp and 13 (15.85%) were *Salmonella* spp. Additionally, antibiotic sensitivity test revealed the bacteria isolates were resistant to Penicillin, Amoxicillin, Cefaclor, Ofloxacin and Ciprofloxacin. The findings suggest that all the samples under observation were highly susceptible to a number of microbes belongs to the natural flora of human body. Hence, it is encouraged to avoid mobile phone handling while eating. Last but not the least, personal hygiene is highly recommended, as mobile phone can be a potential source of disease transmission.

Keywords: mobile phones; transmission; microorganism; isolation; identification

1. Introduction

A mobile or cellular phone is now become an integral and indispensable part of daily life for communication. Considering the rate of cellular phone subscribers in the world, Asia has the fastest growth rate now a day (TRAI, 2009-10). Mobile phone is now considered as a potential source of infectious diseases due to frequent contact and handling (Kilic *et al*., 2009). In recent days, the mobile phone users are increasing at a significant rate both in academic and non-academic staffs of educational institutions. The multitasking facilities of mobile phone results in ease of life with better communication (Adetona *et al*., 2011). In turn, mobile phones become the potential channel for microbial transmission and health risk (Soto *et al*., 2006). The ecological findings directed towards the risk of communal infection those who are frequent users of mobile phone (Brady *et al*., 2006). It is now well documented that mobile phone can be contaminated by a wide range of vehicles and results in mild to chronic infections. The microorganisms isolated so far from mobile phones are not only the

source of contamination but also the reservoirs of infection, allowing their widespread migration in the environments (Brady *et al*., 2007). It's not surprising that on each square inch of the mobile phone thousands of microbes can exist, causing health hazards. *Staphylococci,* particularly *S. epidermidis,* are normal flora observed in human (Jayachandra *et al*., 2011). Studies suggest that 5-21% of healthcare workers using mobile phones provide a basin of bacteria causing nosocomial infections (Brady *et al*., 2006; Jeske *et al*., 2007; Brady *et al*., 2009). In other studies, healthcare organizations, domestic settings and other industries like food processing serve as a route of microbial transmission through human handling (Aiello *et al*., 2002; Brady *et al*., 2006). Due to the benefits of the mobile phone, its hazard to health is often overlooked. But, the continuous handling of cellular device seems to have a n impact on human health as it serves as a carrier of micro-organisms. In recent time, cells phone idenfied as a potential media for bacterial pathogen transmission *(*Mofolorunsho *et al*., 2013). It was observed that bacterial cells can adhere to mobile phone surfaces and form organized colonies (Beveridge *et al*., 1997; Vivekanandan *et al*., 2017), thus transmit macro-organisms between users (Ulger *et al*., 2009). Mobile phones have also been reported to be a reservoir of microorganisms. While waiting at restaurants after ordering food, people still in direct contact with mobile phones (texting, chatting, receiving calls) even after washing their hands and unconsciously transmit micro-organisms from phone to hands (Deepak *et al*., 2015). It is evidenced that mobile phones are more susceptible for bacterial transmission than lavatory, shoe or door handles (Brady *et al*., 2006). Additionally, mobile phone sharing makes it more vulnerable for spread of pathogenic organisms as well as serve as a vector of nosocomial and opportunistic infections (Rafferty *et al*., 1984; Brady *et al*., 2006; Brady *et al*., 2007; Soto *et al*., 2006) between users. Considering the above facts, the present study was aimed to isolate and identify bacteria from mobile phones of academic and non-academic staffs (including students and cleaners) of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

2. Materials and Methods

2.1. Collection of samples

A total of 32 mobile phones swab samples were collected from academic and nonacademic staffs, students and cleaners of Hajee Mohammad Danesh Science and Technology University *Dinajpur, Bangladesh to isolate the bacteria.*

2.2. Transportation and preparation of samples

Samples were collected from participant's mobile phones during working hours. A sterile cotton swab was used to rolled over the surfaces of phone get exposed. Special care was taken to take samples from keypad and buttons as these areas get exposed frequently by tip of fingers. Mobile phones were decontaminated with 70% isopropyl alcohol and then sampled swabs to determine the total viable plate count, serial 10-fold dilutions of samples were prepared in physiological saline, and 0.05 ml of aliquot was spread plated on plate count agar (PCA). Plates were incubated for 24 hours at 37ºC before bacteriological counts were done. The number of colonies on each plate having 30–300 colonies were counted by using a digital colony counter. Plates with more than 300 colonies cannot be counted and are designated as too numerous to count-TNTC (Cappuccino, 2005) After that, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media such as MacConkey agar, mannitol salt agar, eosin methylene blue agar (EMB), *Salmonella Shigella* (SS) agar, blood agar, Cetrimide agar. Plates were incubated aerobically at 37°C, for 24 h. (Ekrakene *et al*., 2007). The number of samples collected on different categories and microbial growth are shown in Table no 1.

Table 1. Microbial growth in different samples.

Categories	Number of samples	Microbial growth	No growth
Academic staff	6	4	2
Nonacademic staff	8	6	2
Students	11	9	2
Cleaners	7	6	1
Total	32	25	7

2.3. Isolation and identification of bacteria

The cultural examination of mobile phone samples for bacteriological study was performed according to the standard method (ICMSF, 1986). Identification of bacteria was done on the basis of colony morphology; Gram's staining reaction and biochemical tests (Koch *et al*., 1984).

2.4. Antibiogram study

In this study, antimicrobial drug sensitivity test was initiated on freshly prepared and dried up Mueller Hinton agar (Oxoid). The test was conducted against 8 commonly used antibiotics using disc diffusion method or Kirby-Bauer method (Bauer *et al.*, 1966) following to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2015).

3. Results

3.1. Result of Total Viable Count (TVC)

The TVC of different samples (academic staff, nonacademic staff, students and cleaners) are given in Table 2 and the number of colonies in some dilution was over 300 and they were too numerous to count (TNTC).

Table 2. Result of total viable count.

Categories	Dilution	Number of colonies	Total viable count (TVC)
Academic staff	10^{-3}	100	1.00 CFU/ml
	10^{-4}	94	9.4 CFU/ml
	10^{-5}	85	8.5 CFU/ml
	10^{-6}	73	7.3 CFU/ml
Nonacademic staff	10^{-4}	210	2.10 CFU/ml
	10^{-5}	185	1.85 CFU/ml
	10^{-6}	130	1.30 CFU/ml
	10^{-7}	93	9.3 CFU/ml
	10^{-8}	88	8.8 CFU/ml
Cleaners	10^{-5}	255	2.55 CFU/ml
	10^{-6}	198	1.98 CFU/ml
	10^{-7}	152	1.52 CFU/ml
Students	10^{-6}	260	2.60 CFU/ml
	10^{-7}	200	2.00 CFU/ml
	10^{-8}	197	1.97 CFU/ml
	10^{-9}	154	1.54 CFU/ml
	10^{-10}	100	1.00 CFU/ml
	10^{-11}	91	9.1 CFU/ml

Table 3. Result of identification of bacteria by different bacteriological methods.

Name of media	Colony characteristics	Staining characteristic	Isolated bacteria
Nutrient Agar	Circular small yellowish colonies.	Gram positive cluster liked violet color.	*Staphylococcus* spp.
Mannitol Salt Agar	Yellowish color colonies.		
Blood agar	β-hemolytic colonies of *Staphylococcus* spp on Blood		
Nutrient agar	Thick grayish – white, or cream-colored colonies.	Gram positive rod-shaped purple color.	*Bacillus* spp.
Blood agar	Large cream colonies.		
Nutrient agar	Smooth. Opaque, translucent colonies.	Gram negative small rod-shaped pink color.	*Salmonella* spp.
Salmonella-Shigella Agar	Pale colour colony.		
Nutrient Agar	Large colony.	Gram negative rod-shaped pink colour.	*Klebsiella* spp.
Mac-Conkey Agar	Large, red, mucoid lactose fermented colony.		
Eosin Methylene Blue	produce pink color		
Nutrient Agar	Large, smooth, low convex and greenish pigment with fruity odor.	Gram negative small rod-shaped pink color.	*Pseudomonas* spp.
Cetrimide agar	Slant yellowish in color colonies		

Table 4. Result of cultural examination of different isolated organisms.

Organisms	Nutrient agar	Mannitol Salt Agar	Mac Conkey agar	EMB	SS Agar	Blood agar	Simon Citrate Agar	Cetrimide agar
Staphylococcus spp.	Growth	+	-	-	-	+	-	-
Bacillus spp.	Growth	-	-	-	-	+	-	-
Salmonella spp.	Growth	-	+	+	+	-	-	-
Klebsiella spp.	Growth	-	+	+	-	-	-	-
Pseudomonas spp.	Growth	-	-	-	-	-	+	+

Legend: Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar

3.2. Types of bacteria isolated from different samples

Isolated bacteria using different bacteriological methods (Tables 3 and 4) from academic staff were *Staphylococcus* spp, *Pseudomonas* spp, *Bacillus* spp, *Klebsiella* spp *and Salmonella* spp (Table 5). From nonacademic staff, *Staphylococcus* spp, *Salmonella* spp, *Bacillus* spp, *Klebsiella* spp and *Pseudomonas* spp (Table 5). From students', organisms were *Staphylococcus* spp, *Salmonella* spp, *Bacillus* spp, *Klebsiella* spp and *Pseudomonas* spp and from cleaners, *Pseudomonas* spp, *Salmonella* spp, *Staphylococcus* spp, *Bacillus* spp *and Klebsiella* spp (Table 5).

Table 5. Types of bacteria isolated from different samples.

Categories	Number of collected samples	Isolated bacteria				
		***Staphylococcus* spp.**	***Bacillus* spp.**	***Klebsiella* spp.**	***Pseudomonas* spp.**	***Salmonella* spp.**
Academic staff	6	4	3	2	1	2
Nonacademic staff	8	5	4	4	4	5
Students	11	8	6	6	5	3
Cleaners	7	4	4	4	5	3
Total & %	32	21 (25.6%)	17 (20.7%)	16 (19.5%)	15 (18%)	13 (15.85%)

3.3. Results of Biochemical test

Table 6. Result of biochemical test for the isolated bacteria.

Organisms	MR	VP	TSI	CIRTARE	CATALASE	INDOLE
Staphylococcus spp.	-	-	+	+	+	-
Bacillus spp.	-	-	-	+	+	-
Salmonella spp.	+	+	+	+	+	+
Klebsiella spp.	+	+	-	+	+	-
Pseudomonas spp.	-	-	-	+	+	-

Legends
MR= Methyl Red, VP= Voges–Proskauer, TSI= Triple Super Iron

3.4. Results of antibiotic susceptibility pattern of isolated organisms from mobile phones

Table 7 shows the average zone of inhibition for those organisms tested against at least 15 different antibiotics that were available in the market.

Table 7. Result of antibiotic susceptibility pattern of isolated organisms from different mobile phones.

Name of the tested organism	Antibiotics	Disc concentration (µg/disc)	Zone of Inhibition (mm)	Result
Staphylococcus spp.	C	30	17	Intermediate
	P	10	0	Resistant
	MET	5	0	Resistant
	AMX	30	0	Resistant
	E	15	4	Resistant
Bacillus spp.	C	30	20	Susceptible
	AZM	30	14	Intermediate
	AMX	30	0	Resistant
	NX	10	5	Resistant
	COX	1	0	Resistant
Salmonella spp.	GEN	10	21	Intermediate
	E	15	0	Resistant
	CEC	30	0	Resistant
	AMX	30	0	Resistant
	COX	1	0	Resistant
Klebsiella spp.	GEN	10	18	Resistant
	AMX	30	15	Resistant
	CEC	30	0	Resistant
	OFX	5	17	Intermediate
	E	15	0	Resistant
Pseudomonas spp.	CN	30	0	Resistant
	P	10	0	Resistant
	CP	5	4	Resistant
	AMX	30	0	Resistant
	AK	30	3	Resistant

Chloramphenicol(C), Penicillin (P), Methicillin (MET), Amoxicillin (AMX), Erythromycin (E), Erythromycin (E), Chloramphenicol(C), Azithromycin(AZM), Amoxicillin (AMX), Norfloxacin (NX), Cloxacillin (COX), Gentamycin (GEN), Erythromycin (E), Cefaclor (CEC), Ofloxacin (OFX), Erythromycin (E), Cephalexin (CN), Penicillin (P), Ciprofloxacin (CP), Amikacin (AK)

The *Staphylococcus* spp had intermediate sensitivity to Chloramphenicol but was resistant to Erythromycin, Penicillin, Methicillin and Amoxicillin. *Bacillus* spp was susceptible to Chloramphenicol and intermediate to Azithromycin but was resistant to Norfloxacin, Amoxicillin and Cloxacillin. *Salmonella* spp had intermediate sensitivity to Gentamycin but was resistant to Erythromycin, Cefaclor, Amoxicillin and Cloxacillin. *Klebsiella* spp had intermediate sensitivity to Ofloxacin but was resistant to Gentamycin, Amoxicillin, Cefaclor and Erythromycin. *Pseudomonas* spp was resistant to Cephalexin, Penicillin, Ciprofloxacin, Amoxicillin and Amikacin.

4. Discussion

To get a healthy life it is important to adapt microbiological standards and proper hygiene practices, rather than create a micro-organisms free environment. This study was aimed to isolate and identify bacteria and create awareness that mobile phones could serve as vectors for the transfer of bacteria from one individual to another. This study suggest that there are a variety of microbes on mobile phones belonging to five genera- *Staphylococcus* spp, *Bacillus* spp, *Klebsiella* spp, *Pseudomonas* spp and *Salmonella* spp. It is obvious that pathogens remain infectious on affected surfaces for several days, if get favorable environment. As for example, in humid environment pathogens can colonize surfaces and transform a passive reservoir to an active one. Mobile phone become the potential reservoir of pathogens and results in infections due to their close contact with sensitive body parts such as faces, ears, lips and hands of users. The prevalence of bacteria isolated from different samples were *Staphylococcus* spp 21 (25.6%), *Bacillus* spp, 17 (20.7%), *Klebsiella* spp 16 (19.5%), *Pseudomonas* spp 15 (18%) and *Salmonella* spp 13 (15.85%). Findings of our research work are nearly similar with findings of Oguz Karabay *et al.* (2007). The prevalence of *Klebsiella* spp is also in agreement with the finding of (Famurewa *et al*., 2009). The current findings were observed in earlier studies of (Amira *et al*., 2010).

In the present investigation, *Bacillus* spp were found in a lower proportion than reported by Haider *et al.* (2016). Our research work is nearly similar with the findings of (Bone *et al.*, 1993). The presence of Gram-negative rod such as *Klebsiella* spp and *Pseudomonas aeruginosa* spp indicates the possibility of the presence of the faecal contamination on the mobile phones by users. The organisms were consistently isolated from the environment and humans. The roles of these organisms in both nosocomial and community acquired infections have been stressed (Topley *et al*., 1990; Walther *et al*., 2004). According to the present study, *Staphylococcus* spp were found in most of the phones and thus correlate with previous finding of (Vivekanandan *et al*., 2017; Shahaby *et al*., 2012). Commercial phones had different types of bacteria. This might be due to long-term exposure to the open surface. Most importantly, mobile phone surfaces were more susceptible than the user's earpiece. On the other hand, medical professionals such as nurses are less vulnerable to pathogenic bacteria. This result shows the frequency of the use and exposure of cell phones to environmental microbes on the hand and skin of the users, which was in agreement the findings of (Rusin *et al*., 2000). The *in vitro* antibiotic sensitivity test of isolated bacteria *Staphylococcus* spp, *Bacillus* spp, *Klebsiella* spp, *Pseudomonas* spp *and Salmonella* spp showed resistance to Penicillin, *Amoxicillin,* Cefaclor, Ofloxacin and Ciprofloxacin. Through this study, use of mobile phones by academic and non-academic staffs in the laboratories may have serious hygienic consequences, because unlike fixed phones, mobile phones are often carried about within and outside the classrooms and laboratories. The laboratory environment plays a critical role in the transmission of organisms associated with infections. Micro-organisms can be transferred from person to person from inanimate objects such as (microscopes, fixed telephones, autoclaves, ovens, incubators fridges etc.).

5. Conclusions

In this experiment, different types of bacterial pathogens such as *Staphylococcus* spp, *Bacillus* spp, *Salmonella* spp, *Klebsiella* spp and *Pseudomonas* spp. were collected. Isolated microorganisms were identified on the basis of morphological characteristics, cultural characteristics and biochemical characterization. The results of the present study are conclusive evidence for the prevalence of different bacterial pathogens, due to the sharing of mobile phones and sensitive parts of our bodies in contact with it such as faces, hands and ears. Personal hygienic sanitation, such as cleaning and washing hands when mobile phones is used, is required for decontamination of mobile phones. Regular cleaning of mobile phones with a suitable cleaning fluid as well as frequent hand washing should be encouraged as means of curtailing any potential disease transmission from mobile phones.

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Conflict of interest

None to declare.

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