IJAMBR 6 (2018) 87-94 ISSN 2053-1818

International Journal of Applied Microbiology and Biotechnology Research

www.bluepenjournals.org/ijambr

Bacterial profile of mobile phones used by college students in Kigali, Rwanda

Sujan Khadka[1], Jean Bosco Nshimiyimana[1,2]*, Alina Thapa[3], Vestine Akayezu[2], Esther Muhindo Mwizerwa[2] and Aberra Geyid Woldetsadik[2]

[1]Department of Biochemistry and Molecular Biology, School of Life Sciences, Central China Normal University, Wuhan, Hubei, P. R. China.
[2]Department of Biology, College of Science and Technology, University of Rwanda, Kigali, Rwanda.
[3]Department of Microbiology, Balkumari College, Tribhuvan University, Chitwan, Nepal.

Article History
Received 01 July, 2018
Received in revised form 19 July, 2018
Accepted 23 July, 2018

Keywords:
Hand hygiene, Extended-spectrum beta-lactamase, Methicillin-resistant *Staphylococcus aureus*.

Article Type:
Full Length Research Article

ABSTRACT

This study was aimed at determining the presence and level of microbial contamination associated with mobile phones. Fifty different samples of swabs from mobile phones were randomly collected from the students of College of Science and Technology, Kigali, Rwanda. Samples were collected from Muhabura and KIST II blocks from January to June, 2017 and were analyzed using standard microbiological techniques. Microbial analysis showed that 35 non-disinfected samples were contaminated by six different types of bacteria. The majority of the phones were contaminated by coagulase-negative Staphylococci (CONS). *Salmonella* spp. was the least isolated bacteria from mobile phones. The mobile phones of students from Muhabura block were found to be more contaminated (48.4×10^3 cfu/ml) than those from KIST II block (21.2×10^3 cfu/ml). On antibiotic susceptibility testing of the isolates, *S. aureus* (12.5%), *Escherichia coli* (21.1%) *and Klebsiella* spp. (20.0%) were found to be multi-drug resistant. *S. aureus* (33.3%) was found to be methicillin-resistant *S. aureus* (MRSA). Furthermore, *E. coli* (37.5%), *Klebsiella* spp. (37.5%) and *Enterobacter* spp. (25.0%) produced extended-spectrum beta-lactamase (ESBL). Significant associations were noted between the rate of incidence of bacteria with various aspects such as the gender and behavior of mobile phone users ($p<0.01$). This study indicates that mobile phones can carry potentially frightening bacteria which can cause severe health hazards to users. Alertness of regular disinfection of mobile phones, avoiding their use in contaminating areas like toilets and hand hygiene is recommended.

INTRODUCTION

Mobile phones are widely used in the present world; however, unhygienic ways of handling make them a leading reservoir of an array of pathogenic microorganisms (Zakai et al., 2016; Adhikari et al., 2018). Because of their abundant use in contaminated areas such as toilets, hospitals and kitchens, which are loaded with microorganisms, mobile phones might act as fomites to transmit various microorganisms (Bhoonderowa et al., 2014). Mobile phones which can be highly loaded with tens of thousands of microbes living on each square inch area represent an often overlooked reservoir for several enteric diseases (Ekrakene and Igeleke, 2007; Michaels, 2002). The combination of constant handling with the heat generated by the phones creates a prime breeding ground for many microorganisms that are normally found on the skin (Brady et al., 2006). Users of mobile phones

*Corresponding author. E-mail: boscomunyangeyo@gmail.com.

are found everywhere: in hospitals, market, at home, and schools. They could, therefore, be the cause of the spreading of several infections in the community. Contamination from the skin, anal region, wounds, nasal secretions and aerosols generated by sneezing and coughing are potential sources of transferring microorganisms to mobile phones during handling (Mackintosh and Hoffman, 1984). Due to their personal nature and proximity to a sensitive part of our bodies like faces, ears, lips, and hands of users, mobile phones could become absolute reservoirs of pathogens that might cause infections (Chawla et al., 2009). Mobile phones may get contaminated with different bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which cause hospital infections, and may serve as a vehicle for disseminating nosocomial pathogens (Karabay et al., 2007). In recent days increased antibiotic resistance has created higher morbidity and mortality associated with infections. Multi-drug resistant (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing microorganism strains were isolated from mobile phones. These pathogens can potentially cause terrible health hazards to the users (Adhikari et al., 2018; Bhat et al., 2011; Gashaw et al., 2014; Lee et al., 2014; Gudiol et al., 2008).

Africa has one of the largest progress rates of cellular subscribers in the world with African markets mounting as fast as Asian markets regarding the use of mobile phones; however, constant handling and unhygienic practices of using mobile phones by different users make them vulnerable for arrays of microorganisms potential of causing several health hazards to users (Ekrakene and Igeleke, 2007). Probably, no reports regarding the contamination of mobile phones have been yet done elsewhere in the country. Therefore, this study was conducted to determine microbial contamination of mobile phones of some students of College of Science and Technology (CST) and to identify bacterial pathogens associated with these phones, possible risk factors along with their antibiotic resistance patterns against several widely used antibiotics in order to conclude and recommend the necessary remedial measures.

MATERIALS AND METHODS

Ethical considerations

This study was approved by the Department of Biology, College of Science and Technology, University of Rwanda. Informed consent was taken from the students prior to sample collection.

Study design and sample size

This cross-sectional study was carried out in the College of Science and Technology, University of Rwanda, from January to June 2017. During this period, 50 mobile phones of students were collected in sterilized containers and microbial analysis was carried out in the microbiology laboratory of the college. Data on age, gender and status of each participant were extracted by a semi-structured questionnaire which involved information regarding the type of mobile phones used, span of use by the users, the practice of handling the mobile phones such as disinfection, place of storing and their use in toilets.

Sample collection and processing

Fifty swab samples of mobile phones (25 from each block) were collected using sterile saline water and cotton swabs. The process used was cleaning the mobile phones by using sterile cotton swabs soaked in saline water and inoculated on media like Nutrient agar, MacConkey agar, Mannitol Salt agar, Eosin Methylene Blue agar, Selenite Cysteine agar and Salmonella-Shigella agar plates. The plates were incubated at 37°C for 24–48 h (Forbes et al., 2007). Fifteen mobile phones samples (8 from Muhabura block, 7 from KIST II block) were treated with disinfectant (70.0% ethanol) prior to swabbing.

Identification of isolates

Aerobic plate counts were performed to determine the loads of bacteria from the contaminated mobile samples of both blocks. Isolates were identified by colony morphology, arrangement, size and color (Barry, 2012). All the isolates were first differentiated by Gram staining reaction. The Gram-positive bacteria were further tested for oxidase, catalase, coagulase, DNase, oxidative/ fermentative tests, methyl red and Voges-Proskauer tests. For the identification of Gram-negative bacteria, colony morphology and various biochemical tests like indole, methyl red, Voges Proskauer, citrate, triple sugar Iron, catalase, oxidase, urease, oxidative/fermentative were performed (Forbes et al., 2007).

Antibiotic susceptibility test

Antibiotic susceptibility tests were performed by the disc diffusion method based on guidelines suggested by the Clinical and Laboratory Standards Institute using Mueller Hinton agar (CLSI, 2015). From the pure cultures of bacteria grown overnight on nutrient agar, a suspension matching 0.5 McFarland standard (1.5×10^8 cfu/ml) was made in nutrient broth. Mueller-Hinton agar plates were inoculated by lawn culture method using a sterile cotton swab. In addition, gentamicin (10 µg), ciprofloxacin (5 µg)

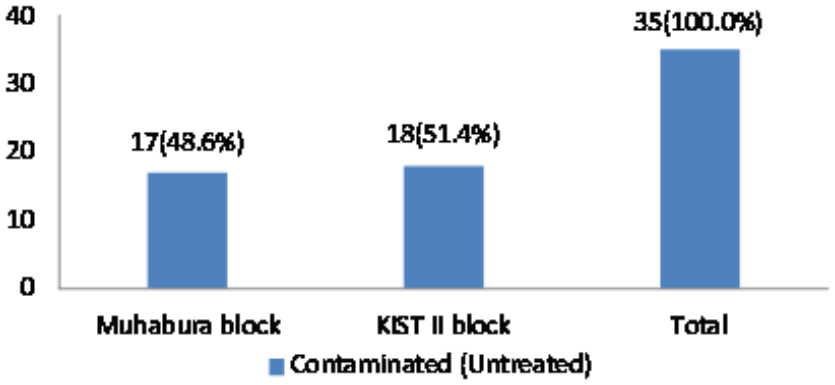

Figure 1. Contamination of mobile phones.

and ampicillin (10 µg) were tested against both Gram-positive as well as Gram-negative bacteria. Resistance to three or more classes of antimicrobials tested was considered as MDR (Simner et al., 2011; Magiorakos et al., 2012). The standard culture of *E. coli* ATCC 25922 was used as a reference strain for identification and standardization of the Kirby-Bauer test.

Screening and confirmation of extended-spectrum beta-lactamase (ESBL) production by phenotypic confirmatory test (PCT)

Ceftazidime and cefotaxime disks were placed on the Mueller Hinton agar plates and later on incubated at 35°C for 18–24 h. Organisms showing the zone of inhibition ≤22 mm for ceftazidime (CAZ)(30 µg) and ≤27 mm for cefotaxime (CTX)(30 µg) were considered to be probable ESBL producers. Phenotypic confirmatory test was done for suspected ESBL producing microorganisms for which antibiotics combinations of ceftazidime + clavulanic acid (CAZ/CAC) (30/10 µg) and cefotaxime + clavulanic acid (CTX/CTC) (30/10 µg) were used (CLSI, 2015). An increase in the zone of inhibition by ≥5 mm around the discs containing cephalosporin with clavulanate over the discs containing cephalosporin alone were ESBL producers (CLSI, 2015).

Data analysis

Tabulation and analysis of the collected data were performed using SPSS version 20. P-values less than 0.01 were considered to have significant associations.

RESULTS

Fifteen mobile phone samples treated with 70.0% ethanol from both blocks were free from contamination. All 35 untreated mobile phone samples were contaminated by bacteria. Mobile phone samples from KIST-II block (51.4%) were highly contaminated than Muhabura block (48.6%) (Figure 1).

Non-disinfected 35 mobile phones (17 from Muhabura and 18 from KIST II blocks) had shown growth among which the highest proportion of mobile phones from Muhabura block (94.1%) and KIST II block (72.2%) were contaminated by CONS. The mobile phones from Muhabura block were highly contaminated with *S. aureus*, *E. coli* (58.8%), *Enterobacter* spp. (52.9%), *Salmonella* spp. (29.4%), *Klebsiella* spp. (47.1%) than KIST II blocks which showed *S. aureus* (66.7%), *E. coli* (50.0%), *Enterobacter* spp. (44.4%), *Salmonella* spp. (11.1%), *Klebsiella* spp. (38.9%)(Table 1).

Aerobic plate count showed that total bacterial load observed from the mobile phones of Muhabura block (48.8×10^3) were more contaminated than the mobile phones of KIST II block (21.2×10^3)(Table 2).

Contamination was high in the mobile phones (57.1%) used by female students at both locations ($p<0.01$). Similarly, mobile phones used in toilets (65.7%) were found to be highly contaminated by bacteria ($p<0.01$). Users who did not cut their nails had highly contaminated mobile phones (71.4%) than those users who cut their nails ($p<0.01$)(Table 3).

Of the 111 total isolates, only one isolate of *E. coli* (5.3%) showed resistance to gentamicin. A large number of isolates showed high resistance to ciprofloxacin (16) and cotrimoxazole (16). No resistance was shown by tested organisms against vancomycin. Out of 24 *S. aureus,* 8(33.3%) were MRSA. Ten (9.0%) isolates were found to be MDR which included 3(12.5%) *S. aureus*, 4(21.1%) *E. coli* and 3(20.0%) *Klebsiella* spp. (Table 4).

All 51 isolates: *E. coli* (19), *Enterobacter* spp. (17) and *Klebsiella* spp. (15) belonging to Enterobacteriaceae family were only tested using third generation antibiotics,

Table 1. Frequency of isolates among contaminated mobile samples from Muhabura and KIST II blocks.

Isolates	Muhabura block	KIST II block
	Untreated samples (n=17)	Untreated samples (n=18)
S. aureus	12(70.6%)	12(66.7%)
CONS	16(94.1%)	13(72.2%)
E. coli	10(58.8%)	9(50.0%)
Enterobacter spp.	9(52.9%)	8(44.4%)
Salmonella spp.	5(29.4%)	2(11.1%)
Klebsiella spp.	8(47.1%)	7(38.9%)
Total (111)	60(54.1%)	51(45.9%)

Table 2. Overall aerobic plate count of untreated swabbed mobile phone samples from both Muhabura and KIST II blocks.

Bacteria growth	Untreated mobile phones from Muhabura block (cfu/ml)	Untreated mobile phones from KIST II block (cfu/ml)
Total viable count	16.5×10^3	8.7×10^3
Coliform count (*E. coli, Enterobacter* spp. and *Klebsiella* spp.)	15.6×10^3	5.0×10^3
Salmonella spp. count	8.4×10^3	4.5×10^3
Staphylococcus spp. count	8.3×10^3	3×10^3
Total	48.8×10^3	21.2×10^3

Table 3. Association of various variables with the rate of bacterial contamination.

S/N	Attributes (users)		Distribution of contaminated mobile samples				Total (n=35)
			Muhabura block (N=17)	P-value	KIST II block (N=18)	P-value	
1	Gender	Male	7(41.2%)	**<0.01**	8(44.4%)	**<0.01**	15(42.8%)
		Female	10(58.8%)		10(55.6%)		20(57.1%)
2	Age of mobile phones	Less than 6 months	2(11.8%)		-		2(5.7%)
		6-12 months	4(23.5%)	0.1	8(44.4%)	0.09	12(34.3%)
		More than 12 months	11(64.7%)		10(55.6%)		21(60.0%)
3	Mobile phone types	Screen touch	5(29.4%)	0.08	6(33.3%)	0.1	11(31.4%)
		Keypad type	12(70.6%)		12(66.7%)		24(70.6%)
4	Toilet users	Non users	5(29.4%)	**<0.01**	7(38.9%)	**<0.01**	12(34.3%)
		users	12(70.6%)		11(61.1%)		23(65.7%)
5	Mobile phone storing place	Pockets	11(64.7%)	0.06	14(77.8%)	0.034	25(71.4%)
		Bags	6(35.3%)		4(22.2%)		10(28.6%)
7	Users cutting nails	Nail cutters	4(23.5%)	**<0.01**	6(33.3%)	**<0.01**	10(28.6%)
		Non-nail cutters	13(76.5%)		12(66.7%)		25(71.4%)

Table 4. Antibiotic resistance and MDR patterns of the isolates.

Isolates (n=111)	GEN	CIP	NIT	COT	TE	AZ	CX	VA	AMP	ERY	AMX	MDR
	R	R	R	R	R	R	R	R	R	R	R	
Staphylococcus aureus (n=24)	-	2(8.3)	NT	NT	NT	2(8.3)	8(33.3)	-	3(12.5)	2(8.3)	3(12.5)	3(12.5)
CONS (n=29)	-	1(3.4)	NT	NT	NT	3(10.3)	4(13.8)	-	3(10.3)	3(10.3)	2(6.9)	-
Escherichia coli (n=19)	1(5.3)	6(31.6)	5(26.3)	8(42.1)	4(21.1)	NT	NT	NT	2(10.5)	NT	NT	4(21.1)
Klebsiella spp.(n=15)	-	2(13.3)	2(13.3)	1(6.7)	-	NT	NT	NT	3(20.0)	NT	NT	3(20.0)
Enterobacter spp. (n=17)	-	2(11.8)	-	3(17.6)	1(5.9)	NT	NT	NT	1(5.9)	NT	NT	-
Salmonella spp.(n=7)	-	3(42.9)	5(71.4)	4(57.1)	2(28.6)	NT	NT	NT	2(28.6)	NT	NT	-
Total (111)	1(0.9)	16(14.4)	12(10.8)	16(14.4)	7(6.3)	5(4.5)	12(10.8)	-	14(12.6)	5(4.5)	5(4.5)	10(9.0)

Note: NT, Not tested; **R,** resistant; **GEN,** gentamicin; **CIP,** ciprofloxacin; **NIT,** nitrofurantoin; **COT,** cotrimoxazole; **TE,** tetracycline; **AZ,** azithromycin; **CX,** cefoxitin; **VA,** vancomycin; **AMP,** ampicillin; **ERY,** erythromycin; **AMX,** amoxicillin.

Table 5. Resistance pattern of ESBL producers.

Isolates	ESBL producers (AST by disc diffusion method)					
	Probable (n=21)			Confirmed (n=16)		
	CAZ	CTX	Both	CAZ/CAC	CTX/CTC	Both
E. coli	4(19.0%)	3(14.3%)	2(9.5%)	2(12.5%)	2(12.5%)	2(12.5%)
Enterobacter spp.	2(9.5%)	2(9.5%)	1(4.8%)	1(6.7%)	2(12.5%)	1(6.7%)
Klebsiella spp.	4(19.0%)	2(9.5%)	1(4.8%)	2(12.5%)	1(6.1%)	3(18.8%)
Total	10(47.6%)	7(33.3%)	4(19.0%)	5(30.0%)	5(31.3%)	6(37.5%)

Note: CAZ, Ceftazidime; **CTX,** cefotaxime; **CAZ/CAC,** ceftazidime + clavulinic acid; **CTX/CTC,** cefotaxime + clavulinic acid.

CAZ and CTX. Twenty one isolates showed resistance in total including CAZ (47.6%), CTX (33.3%) and both (19.0%). Therefore, these 21 probable isolates were subjected to phenotypic confirmation by using combination of disc diffusion test using two combinations, CAZ/CAC and CTX/CTC, among which 5 isolates were resistant to (CAZ/CAC), 5 isolates were resistant to (CTX/CTC), and 6 isolates were resistant to both combinations. Hence, 16 isolates were found as the confirmed ESBL producers (Table 5).

Out of total 16 ESBL producers, *E. coli*-6(37.5%), *Enterobacter*-4(25.0%) and *Klebsiella*-6(37.5%) were found to be confirmed ESBL producers (Figure 2).

DISCUSSION

Dry objects such as mobile phones play a vital role in our daily life whilst the contribution of hands contaminated with pathogenic and non-pathogenic microorganisms to the spread of infectious disease has been recognized for many years (ICT, 2006). The rate of contamination of mobile phones (100.0%) of the students in the present study was found to be higher than that of health workers as shown by the research conducted in Palestine (71.6%) by Elmanama et al., (2015), in Turkey (94.5%) by Ulger et al., (2009), in Ethiopia (98.0%) by Gashaw et al., (2014), but similar to the result from Nigeria (100.0%) reported by Ilusanya et al., (2012). A similar study on mobile phones among college students and staffs done by Adhikari et al. (2018) in Nepal showed the lesser incidence of bacterial contamination (56.0%). Such differences might be due to the difference in the user's behavior and personal hygiene in different regions. Adhikari et al. (2018) from Nepal reported that mobile phones not disinfected were highly contaminated by harmful bacteria like MRSA and MDR-*S. aureus.* In our study, all of the 35(100.0%) untreated samples from both blocks were contaminated by bacteria whereas all the 15 treated samples were free from contamination. Routinely cleaning of mobile phones with effective disinfectant can prevent the microbial contamination of mobile phones (Ramesh et al., 2008).

A study conducted by Karabay et al. (2007) in Turkey isolated *S. aureus* (8.1%), CONS (68.4%), *E. coli* (26.0%), *Enterobacter faecalis* (1.8%) and *Klebsiella* (0.9%). However, no *Salmonella* spp. was isolated from mobile phones in their work. Tagoe et al. (2011) in Ghana reported that *Salmonella* spp. (3.0%) was isolated from mobile phones. Our study also reported *Salmonella* spp. from mobile samples of Muhabura block (29.4%) and KIST II block (11.1%). Presence of such detrimental

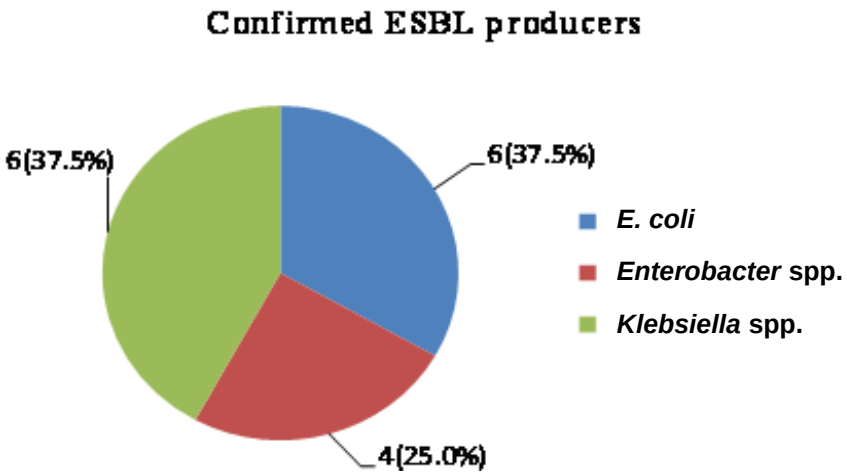

Figure 2. Distribution of confirmed ESBL producers.

bacteria on mobile phones was associated with poor handling and hygienic condition, sharing among multiple users, lack of disinfection and fecal contamination (Bhoonderowa et al., 2014; Auhim, 2013; Shahaby et al., 2012; Yusha'u et al., 2010). All of these pathogens can cause hazardous effects including opportunistic infections to the users (Cerdá et al., 2006).

Samples collected from Muhabura block showed the aerobic plate count of 48.4×10^3 microorganisms in aggregate which was higher than those obtained from KIST II block. This can be explained by reason that Muhabura block is huge and occupied by many students from different places than KIST II block. Contamination of mobile phones was aided by personal hygiene and lifestyles of those students using mobile phones in Muhabura block.

In our study, mobile phones (57.1%) used by female students in both Muhabura and KIST blocks were highly contaminated by bacteria ($p<0.01$). In addition to this, users who did not cut their nails had highly contaminated mobile phones (71.4%) than those users who cut their nails ($p<0.01$). This might be because females usually keep longer nails which can directly transmit bacteria to the mobile phones. In a meeting of the Infectious Disease Society of America in San Francisco, researchers showed that artificial and natural nails longer than 3 millimeters (beyond the tip of the finger or the length of a pencil tip), transport more harmful bacteria and yeast under them as compared to the short nails (Puleo, 2017). Likewise, mobile phones used in toilets (65.7%) were found to be highly contaminated by bacteria ($p<0.01$). This finding is in agreement with Adhikari et al. (2018) in Nepal who also found higher bacterial contamination among toilet users (59.8%) than non-users (40.2%). Our study revealed MRSA (33.3%) which was comparatively higher than 26.8% reported by Adhikari et al. (2018) in Nepal; 21.0% by Pal et al. (2015) in India but lesser than 52.0% reported by Ulger et al. (2009) in Turkey. No MRSA was reported from the study conducted by Akinyemi et al. (2009) in Nigeria. Different geographic areas or different time period showed the difference in antibiotic resistance patterns which might depend on antibiotic policy at that specific time. Adhikari et al. (2018) reported 21.4% of *S. aureus* were MDR whereas in the present study only 12.5% of *S. aureus* were MDR. Similarly, no resistance was shown by any isolate against vancomycin in their study which is consistent with our study. Gashaw et al. (2014) in Ethiopia showed that all the *E. coli* was sensitive to gentamicin and ciprofloxacin unlike in the current study where 5.3% *E. coli* was resistant to gentamicin and 31.6% were resistant to ciprofloxacin.

Our study found large numbers of 16(14.4%) isolates showing high resistance to both cotrimoxazole and ciprofloxacin which is lesser than the figures reached by Pal et al. (2015) in India who found 34(51.5%) and 27(40.9%) isolates resistant to cotrimoxazole and ciprofloxacin respectively. Ten (9.0%) isolates were found to be MDR in our study but Bhat et al. (2011) in India showed 37.9% of isolates as MDR. *E. coli* (21.1%) and *Klebsiella* spp. (20.0%) were MDR in our study while no MDR *E. coli* and *Klebsiella* spp. were detected in the work done by Gashaw et al. (2014).

Furthermore, a research conducted in Peru by Loyola et al. (2016) among mobile phones of health care workers working in intensive care unit reported that *E. coli* (55.9%), *Enterobacter* spp. (18.8%) and *K. pneumoniae* (30.8%) was found to be ESBL producers which might be associated with poor hygienic practices of handling mobile phones. However, the present study revealed *E. coli* (37.5%), *Enterobacter* spp. (25.0%) and *Klebsiella* spp. (37.5%) as ESBL producers. These

microorganisms containing ESBL enzymes can cause enormous morbidity and mortality and escalating treatment expenses have become progressively challenging in the community as well as healthcare settings (Lee et al., 2014). Thus, the presence of such harmful and potent multiple drug resistant and ESBL producing pathogens on mobile phones ensure the need for serious attention and awareness.

Conclusion

This study points that mobile phones from Muhabura block are more contaminated than those from KIST II. Detection of MRSA, MDR and ESBL producing isolates from mobile phones which are the essential gadget of every day's life is an alarming issue. This study also reveals the ability of mobile phones to serve as a source of transmission of an array of pathogens potential of causing severe health implications. Therefore, awareness programs regarding hand hygiene, regular disinfection of mobile phones, discouraging their uses in toilets and using antibiotics properly and judiciously can be suggested.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Sanjib Adhikari for drafting this manuscript. We are also indebted to all the study participants for their support and co-operation during the study.

CONFLICT OF INTEREST

All authors declare there is no conflict of interests.

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