Original article

Molecular typing of multidrug resistant bacteria isolated from health care professionals' mobile phone: A pilot study in Jashore, Bangladesh

Md. Shazid Hasan[1], Susmita Roy Chowdhury[2], Nigar Sultana Meghla[3], Najmuj Sakib[4], SM Tanjil Shah[5], Md. Tanvir Islam[6] , Ovinu Kibria Islam[7]

Abstract:

Background: Nowadays, Health Care Professionals' (HCPs) are increasingly using mobile phones which may act as reservoirs and vector for transmission of pathogens. The presence of multidrug resistant nosocomial microbes on the surface of mobile phones used by HCPs in hospitals can pose a great public health threat. So, this research was conducted to identify the concerned multi-drug resistant (MDR) bacteria and also to explore the recent status of bacterial contamination on mobile phones of HCPs in the Jashore region of Bangladesh and determine their antibiotic resistance pattern. ***Methods:*** Swab samples of mobile phones were collected between June and September 2019 from 24 different users (i.e., manager, worker, doctor and nurse) associated to four distinct hospitals of Jashore region, Bangladesh. After suitable morphological and biochemical identification, we determined their antimicrobial susceptibility by Kirby-Bauer disc diffusion method by using 18 antibiotics for Gram positive bacteria and 19 antibiotics for Gram negative bacteria. Later, the MDR isolates were grouped by amplified ribosomal DNA restriction analysis (ARDRA) and 16S rRNA sequencing with phylogeny were performed to confirm the bacteria at species level. ***Results:*** A total of 38 bacterial isolates were obtained from the sample. *Enterobacter* spp. isolates showed maximum resistance against Amoxicillin, followed by Ampicillin and Aztreonam (80% each) and one isolate showed highest antibiotic resistance (15 out of 19) among all the isolates. In addition, *Staphylococcus* spp. and *Exiguobacterium* spp. isolates showed 100% resistance against Penicillin, Ampicillin, Oxacillin, Erythromycin, Lincomycin and Cefotaxime. On the contrary, all of the isolates of *Escherichia* spp., *Bacillus* spp., *Proteus* spp. were sensitive to all tested antibiotics. Surprisingly, 20 MDR isolates were showing resistance to at least 2 antibiotics. Subsequently, three distinct genera of these MDR isolates were identified by ARDRA; the strains *Enterobacter cloacae* (75%), *Staphylococcus warneri* (15%) and *Exiguobacterium aurantiacum* (10%) were confirmed by the 16S rRNA phylogenetic analysis. ***Conclusion:*** We found that cell phones can act as reservoirs of multidrug-resistant pathogens, causative agents for Hospital-acquired infections. An effective hygiene practice for health care personnel should be introduced to prevent the cross-contamination by their cell phone

Keywords: Health Care Professional's; cell phones; multidrug-resistant bacteria.

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Introduction:

For convenient and faster communication, worldwide billions of people extensively use hand-held wireless mobile devices. The number of cellular phone subscribers in Bangladesh at the end of June, 2020 has reached 161.295 Million according to the Bangladesh Telecommunication Regulatory Commission (BTRC). However, because of constant handling and the heat generation, mobile phones can be a significant breeding ground for numerous

1. Md. Shazid Hasan, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh
2. Susmita Roy Chowdhury, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh
3. Nigar Sultana Meghla, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh.
4. Najmuj Sakib, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh.
5. SM Tanjil Shah, Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh.
6. Md. Tanvir Islam, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh.
7. Ovinu Kibria Islam, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh.

Correspondence: Md. Shazid Hasan, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh, E-mail: shazidmb@gmail.com, and Md. Tanvir Islam, Department of Microbiology, Jashore University of Science and Technology, Jashore-7408, Bangladesh, E-mail: mt.islam@just.edu.bd

microorganisms found usually on human skins.[1] Studies suggested that mobile phones also can act as a significant source of nosocomial pathogens affecting many hospitalized patients.[2,3] Nosocomial infections or Hospital acquired infections (HAI) refer to those appearing over 48 hours after enrollments in hospitals, which are not present or incubating at the time of admission.[4] According to World Health Organization (WHO), the HAIs contributes significantly to the global burden of morbidity and mortality of the patients, affecting approximately 15% of all hospitalized persons. In Bangladesh, this infection rate may even surpass 30% in some hospitals.[5] Since the incidence of HAIs is greater in developing countries with limited resources, the socio-economic burden to the added antimicrobial treatment and prolonged hospitalization is severe in these countries.[6]

External sources like air, medical apparatus, hands of surgeons and staffs, and internal sources (e.g., the skin flora in the operative sites) can contaminate the healthcare workers' (HCW) mobile phones.[7,8] Since these small communication devices are rarely cleaned and repeatedly touched during or following the examination of patients, lack of good hygiene practices by the healthcare workers can turn them to potential nosocomial transmission media for patients in various hospital wards.[9] It was shown that approximately 40% and 20% of mobile phones handled by hospitalized patients and hospital staff, respectively, contained different pathogenic bacteria.[1] In the past, skin bacteria- like coagulase negative Staphylococci, a potent pathogen when present in the bloodstream, had been identified in large quantity from the blood cultures of the hospital patients.[10] Therefore, standard hygiene practices are recommended to prevent these pathogens from contaminating and growing on the cell phones.[11] Otherwise, they can lead to serious public health concerns (e.g., treatment failure of the patients due to persistent infections resistant to conventional antimicrobials).[12] And this scenario is of severe concern in the developing countries like Bangladesh for its higher nosocomial infection rate induced by poor investigations and inadequate knowledge and awareness among the health-care and non-health-care populations.[13,14]

Most of the earlier studies, to identify the bacterial species from mobile phones, relied primarily on conventional biochemical tests and Vitek-2 system, an automated microbial identification technique based on fluorogenic principle.[15–18] Nowadays, Amplified ribosomal DNA restriction analysis (ARDRA) has proven to be a more effective approach to identify and discriminate the bacterial isolates, which is mainly based on PCR amplification of the 16S rRNA gene, followed by restriction enzyme digestions of amplicons and agarose gel electrophoresis of fragments. The merit of this simple method is that it can be exploited universally for identification of the bacteria in pure cultures or in microbial communities.[19] On the other hand, it is a rapid and cost-effective bacterial profiling technique based on variation in the bacterial 16S rRNA gene sequence. Researchers can apply this technique in characterization, identification and phylogenetic analysis of closely bacterial species within varied complex environmental and biological samples. Also, to find out the genetic diversity and phylogenetic analysis of isolated bacteria from mobile phone samples, one study reported random Amplified Polymorphic DNA (RAPD) and 16S rRNA gene sequence analysis successfully.[20]

Though a handful of studies, depending on conventional microbial approaches entirely, have tried to evaluate the state of MDR organisms on the mobile phones of hospital personnel in Bangladesh, they are not enough to confirm the MDR bacteria at species level. Therefore, the current study focused to investigate the recent status of bacterial contamination of health care professional's mobile phones and identify the concerned multi drug resistant nosocomial pathogens by state-of-the-art molecular techniques in a pilot scale.

Methods and Materials:

Research design and Sampling site

Swab samples of mobile phones were collected from hospital personnel of four different hospitals (i.e., Uttora hospital, Bandhan hospital, Central hospital and diagnosis center and Genesis hospital) located in Jashore district. This study has been done according to the framework (Figure 1).

Sample collection and processing

Cellular phones of 11 different users (i.e, manager, worker, doctor and nurse) from Uttora hospital, 5 different users from Bandhan hospital, 3 different users from Central hospital and diagnosis center and 5 different users from Genesis hospital were chosen for this study during the study period (June 2019 to September 2019). Aseptic techniques were maintained during sample collection and sterile

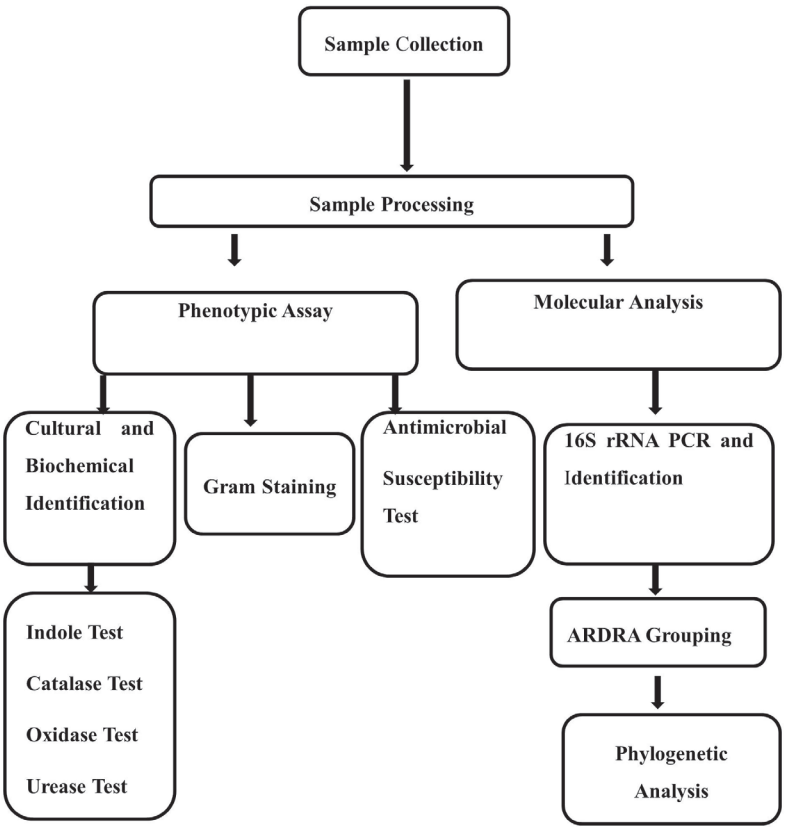

Figure 1: Complete design of the research work

cotton bud was swabbed onto both the sides of mobile phones. Peptone water was used as a transport media and samples were transported to laboratory within 2 hours for further processing.

Isolation of microorganism

The specimen of mobile swabs was directly inoculated into 4 culture media (i.e., Nutrient agar, Mannitol salt agar, Blood agar and MacConkey agar) by spread plate method for the isolation of microorganisms and were incubated aerobically at 37˚C for 24 hours. Subculture technique was used to get the pure culture of the distinct microorganisms and identified through morphological, biochemical tests. The panel of biochemical tests included Catalase, Oxidase, Indole, Citrate, Urease activity, Kligler's Iron Agar (KIA), Motility Indole-Urease (MIU), Methyl-red (MR) and Voges Proskauer (VP).

Antimicrobial susceptibility test (AST)

All the isolates were subjected to antimicrobial susceptibility test (AST) by Kirby-Bauer agar disk diffusion method, to determine their multidrug resistant (MDR) pattern, following the Clinical and Laboratory Standard Institute (CLSI) guidelines.[21,22] Disk diffusion method is commonly chosen in routine laboratory tests because of its repeatability, feasibility, and low cost. A study showed disk diffusion method, compared to automated systems, had significant efficiency in determination of antibiotic susceptibility of gram-negative and gram-positive bacteria.[23] For Gram-negative bacteria 19 different types of antibiotics from 9 classes were cast-off for the study: β–lactamases (Ampicillin-10 µg, Aztreonam-30 µg, Amoxicillin-Clavulanic acid- 30 µg, Amoxicillin-30 µg), Carbapenem (Imepenem-10 µg, Meropenem-10 µg), Aminoglycosides (Streptomycin-10 µg, Kanamycin-5 µg, Gentamycin-10 µg, Amikacin-30 µg), Fluroquinolone (Levofloxacin-5 µg, Ciprofloxacin-5 µg), Phenicol (Chloramphenicol-30 µg), Tetracycline (Tetracycline-30 µg), Co-trimoxazole (Co- trimoxazole-25 µg), Cephalosporin (Cefepime-30 µg, ceftazidime-30 µg, Cefotaxime-30

µg), Macrolid (Azithromycin-10 µg) whereas for Gram-positive bacteria 18 different types of antibiotics from 8 classes were selected for the study: β–lactamases (Penicillin-10 µg, Ampicillin-10 µg, Oxacillin-30 µg, Amoxicillin-Clavulanic acid-30 µg), Carbapenem (Imepenem-10 µg, Meropenem-10 µg), Aminoglycosides (Gentamycin-10 µg), Fluroquinolone (Levofloxacin-5 µg, Norfloxacin-10 µg, Ciprofloxacin-5 µg), Cephalosporin (Cefotaxime-30 µg, Cefepime-30 µg, ceftazidime-30 µg), Tetracycline (Tetracycline-30 µg), Macrolid (Erythromycine-15 µg, Lincomycin-2 µg, Clindamycin-2 µg), Phenicol (Chloramphenicol-30 µg) against gram positive Staphylococcus spp.. Mueller-Hinton agar plate was used for antimicrobial susceptibility test (AST) and incubated at 37˚C for 18-24 hours and measured the inhibition zone diameters (IZDs). The IZDs were recorded according to the CLSI standard antibiotic breakpoints and interpreted as susceptible, intermediate and resistant.[22]

Genomic DNA extraction and molecular characterization of the isolates using Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Chromosomal DNA of the selected isolates were extracted through boiled DNA method.[24] The extracted DNA of the multidrug resistant isolates were quantified by using nano-drop (Implen NanoPhotometer®, Germany). The 16S rRNA gene of the isolates were amplified by polymerase chain reaction (PCR) using forward 27F (5'AGAGTTTGATCCTGGCTCAG-3') and reverse 1492R (5'-GGTTACCTTGTTACGACTT-3') primer. For ARDRA, PCR reaction were carried out in 15 µL volumes containing: 7.5 µL of commercial master mix (Taq DNA polymerase, dNTPs, MgCl2 and reaction buffer), 0.75 µL of forward primer, 0.75 µL of reverse primer, 1.5 µL of template DNA and nuclease free water up to the volume. Amplification was performed using PCR thermal cycler with condition: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 55 °C for 1 minute 30 seconds, extension at 72°C for 1 minute with a final extension at 72°C for 7 minutes. After amplification, the PCR product was analyzed using electrophoresis in 1% agarose gel with TAE buffer containing 0.5µg/mL of ethidium bromide and visualized by gel dock system using UV illuminator. In all cases the amplified product had a length of approximately 1500 bp.

Sequencing of 16S rRNA gene and Phylogenetic analysis

16S rRNA gene amplicons of the selected isolates representative of each genotype were sequenced using BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, USA) according to the manufacturer's instruction. The primers used for sequencing were 27F and 1492R in a concentration of 10 picomole. The 16S rDNA consensus sequence of selected isolates were submitted to NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for generation of accession number followed by phylogenetic analysis to find out their close relatives. The evolutionary history was inferred using the Neighbor-Joining method.[25] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.[26] The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA X.[27,28]

Result:

Biochemical characteristics of the isolated strains form antimicrobial Health Care Professional's (HCPs)

A total of 38 bacterial isolates from 24 mobile phone samples of HCPs were retrieved based on their morphological characteristics (Table I). Out of the 38 isolates, the presumptively identified candidates were *Enterobacter* spp. (n=15, 39.47%), *Staphylococcus* spp. (n=3, 7.8%), *Exiguibacterium* spp. (n=2, 5.2%), *Escherichia* spp. (n=9, 23.68%), *Bacillus* spp. (n=6, 15.78%) and, *Proteus* spp. (n=3, 7.8%) (Table II).

Table I: Classification of the isolates in different morph groups based on their culture

Source	Number of isolates	Growth characteristics on different media					Microscopic characteristics		
		Media	Appearance	Form	Elevation	Margin	Gram staining	Shape	Arrangement
Mobile phone	15	MacConkey agar	Pink	Circular	Raised	Entire	Gram negative	Rod	Single
	3	Manitol salt agar	Yellow	Circular	Convex	Entire	Gram positive	Coccus	Single
	2	Blood agar	Light orange	Circular	Raised	Entire	Gram positive	Rod	Single
	9	MacConkey agar	Dark pink	Circular	Convex	Regular	Gram negative	Small rod	Single
	6	Nutrient agar	fuzzy white	Circular	Flat	Irregular	Gram positive	Long rod	Single
	3	Nutrient agar	Pale color	Circular	Low convex	Smooth to wavy	Gram negative	Small rod	Single

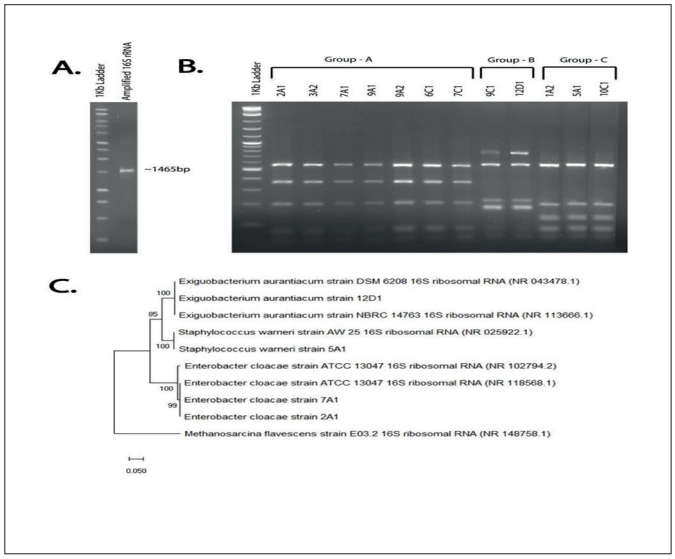

Figure 2: Phylogenetic analysis based on ARDRA genotyping. **(A)** Amplification of 16S rRNA. Visible band was found at approximately 1465bp for all the samples. 1Kb DNA ladder was used as marker. **(B)** ARDRA pattern of the MDR isolates. The isolated strains were divided into 3 groups based on their pattern. **(C)** The evolutionary history was inferred using the Neighbor-Joining method and conducted in MEGA X software. The evolutionary distances were computed using the Tamura 3-parameter method with 500 bootstrap test. *Methanosarcina flavescens* strain E03.2 16S rRNA (NR 148758.1) sequence was used as outer group during the phylogenetic study.

Table II: Biochemical characteristics of the bacterial isolates

Serial no.	In-house Isolate IDs	Hospital source	Hospital worker Source	Catalase	Oxidase	Indole	Citrate	VP	MR	Motility	Urease activity	KIA				Presumptive isolates
												Glucose fermenter	Lactose fermenter	H_2S production	Gas production	
1	2A1	Uttora hospital	Manager	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
2	3A2	Uttora hospital	Ward boy	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
3	7A1	Uttora hospital	Doctor	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
4	9A1	Uttora hospital	Ward boy	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
5	9A2	Uttora hospital	Ward boy	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
6	6C1	Uttora hospital	Ward boy	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
7	7C1	Uttora hospital	Doctor	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
8	12C1	Bandhan hospital	Nurse	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
9	12C2	Bandhan hospital	Nurse	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
10	NM9	Central hospital and diagnosis center	Nurse	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
11	NM10	Genesis hospital	Nurse	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
12	NM14	Genesis hospital	Nurse	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
13	NM12	Genesis hospital	Ward boy	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
14	WM15	Genesis hospital	Ward boy	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
15	DM16	Genesis hospital	Doctor	+	-	-	+	+	-	+	-	+	-	-	+	*Enterobacter* spp.
16	1A2	Uttora hospital	Manager	+	-	-	-	+	+	-	+	+	+	-	+	*Staphylcoccus* spp.
17	5A1	Uttora hospital	Ward boy	+	-	-	-	+	+	-	+	+	+	-	+	*Staphylcoccus* spp.
18	10C1	Bandhan hospital	Manager	+	-	-	-	+	+	-	+	+	+	-	+	*Staphylcoccus* spp.
19	9C1	Bandhan hospital	Manager	+	-	-	+	-	+	+	-	+	-	-	-	*Exiguobacterium* spp.
20	12D1	Bandhan hospital	Ward boy	+	-	-	+	-	+	+	-	+	-	-	-	*Exiguobacterium* spp.
21	2B1	Uttora hospital	Manager	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
22	3B1	Uttora hospital	Ward boy	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
23	6B1	Uttora hospital	Ward boy	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.

Serial no.	In-house Isolate IDs	Hospital source	Hospital worker Source	Catalase	Oxidase	Indole	Citrate	VP	MR	Motility	Urease activity	KIA				Presumptive isolates
												Glucose fermenter	Lactose fermenter	H_2S production	Gas production	
24	7B1	Uttora hospital	Doctor	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
25	8B1	Uttora hospital	Nurse	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
26	9B1	Bandhan hospital	Manager	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
27	11B1	Bandhan hospital	Nurse	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
28	12B1	Bandhan hospital	Nurse	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
29	13B1	Bandhan hospital	Nurse	+	-	+	-	-	+	+	-	+	+	-	+	*Escherichia* spp.
30	3A1	Uttora hospital	Ward boy	+	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* spp.
31	6A1	Uttora hospital	Ward boy	+	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* spp.
32	11A1	Bandhan hospital	Ward boy	+	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* spp.
33	12A2	Bandhan hospital	Nurse	+	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* spp.
34	14A2	Bandhan hospital	Nurse	+	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* spp.
35	15A1	Bandhan hospital	Ward boy	+	+	+	+	+	-	+	-	+	-	+	-	*Bacillus* spp.
36	NM1	Central hospital and diagnosis center	Nurse	+	-	-	+	-	+	+	+	+	-	+	+	*Proteus spp.*
37	NM11	Genesis hospital	Nurse	+	-	-	+	-	+	+	+	+	-	+	+	*Proteus spp.*
38	WM3	Central hospital and diagnosis center	Ward boy	+	-	-	+	-	+	+	+	+	-	+	+	*Proteus spp.*

VP: Voges-Proskauer test, MR: Methyl Red test, MIU: Motility Indole Urease test, KIA: Kliger's Iron Agar

Multidrug resistant (MDR) profile of the isolated strains

This study tried to reveal the efficacy of 19 commonly used antibiotics against Gram negative isolates (Table III) as well as 18 commonly used antibiotics against Gram positive isolates and the current resistant pattern of the organisms for those antibiotics (Table IV). The isolates of *Escherichia* spp., *Bacillus* spp., *Proteus spp.* were sensitive to all antibiotics. However, *Enterobacter* spp., *Staphylococcus* spp., *Exiguibacterium* spp. were found to be resistant to the most of the antibiotics used in this study. *Enterobacter* spp. which had the highest prevalence (39.47%) was resistant to Amoxicillin (100%), Ampicillin (80%), Aztreonam (80%), Kanamycin (67%), Azithromycin (67%), Amikacin (67%), Gentamycin (60%),

Streptomycin (47%), Imepenem (40%), Cefotaxime (40%), Chloramphenicol (33%), Ceftazidime (33%), Tetracycline (33%), Amoxicillin-Clavulanic acid (20%), Meropenem (20%), Co- trimoxazole (20%), Cefepime (20%), Ciprofloxacin (13%) (Table III). Another prevalent type *Staphylococcus* spp. was resistant with 7.8% prevalence rate, which showed resistance against Penecillin (100%), Ampicillin (100%), Oxacillin (100%), Cefotaxime (100%), Erythromycine (100%), Lincomycin (100%), Clindamycin (100%), Amoxicillin-Clavulanic acid (67%), Meropenem (67%), Ceftazidime (67%), Imepenem (33%), Tetracycline (33%), Ciprofloxacin (33%) (Table IV). The *Exiguibacterium* spp. showed the lowest prevalence with 5.2% and was resistant against Penecillin (100%), Ampicillin (100%), Oxacillin (100%), Amoxicillin-Clavulanic acid (100%), Cefotaxime (100%), Tetracycline (100%), Erythromycine (100%), Lincomycin (100%), Ceftazidime (50%) (Table IV). But, *Escherichia* spp., *Bacillus* spp. and *Proteus* spp. with 23.68%, 15.78%, 7.8% prevalence rate respectively showed no resistant pattern in our study.

Table III: Antimicrobial susceptibility test of presumptive Gram negative bacterial isolates

Bacterial isolates	Total no		Antimicrobial susceptibility																		
			ATM	AMP	AMX	AMC	IPM	MRP	S	K	CN	AK	LE	CIP	CTX	CPM	CAZ	TE	AZM	CHL	CoT
Enterobacter spp.	15	S	2	2	-	6	3	12	6	1	3	3	13	12	-	10	8	9	5	7	12
		I	1	1	-	6	6	-	2	4	3	2	2	1	9	2	2	1	-	3	-
		R	12	12	15	3	6	3	7	10	9	10	-	2	6	3	5	5	10	5	3
Escherichia spp.	9	S	9	9	9	7	5	3	7	8	6	5	6	2	8	3	3	8	3	4	6
		I	-	-	-	2	4	6	2	1	3	4	3	7	1	6	6	1	6	5	3
		R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteus spp.	3	S	2	3	3	1	1	3	3	2	1	-	-	1	1	2	2	3	-	1	1
		I	1	-	-	2	2	-	-	1	2	3	3	2	2	1	1	-	3	2	2
		R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (N)	27	S	13	14	12	14	9	18	16	11	10	8	19	15	9	15	13	20	8	12	19
		I	2	1	-	10	12	6	4	6	8	9	8	10	12	9	9	2	9	10	5
		R	12	12	15	3	6	3	7	10	9	10	-	2	6	3	5	5	10	5	3

ATM= Aztreonam, AMP= Ampicillin, AMX= Amoxicillin, AMC= Amoxicillin-Clavulanic acid, IPM= Imepenem, MRP= Meropenem, S= Streptomycn, K= Kanamycin, CN= Gentamycin, AK= Amikacin, LE= Levofloxacin, CIP= Ciprofloxacin, CTX= Cefotaxime, CPM= Cefepime, CAZ= ceftazidime, TE= Tetracycline, AZM= Azithromycin, CHL= Chloramphenicol, CoT= Co- trimoxazole; S: Sensitive, I: Intermediate, R: Resistant

Table IV: Antimicrobial susceptibility test of presumptive Gram positive bacterial isolates

Bacterial isolates	Total no	Antimicrobial susceptibility																		
			P	OX	AMP	AMC	IPM	MRP	CN	LE	NOR	CIP	CTX	CPM	CAZ	TE	ERY	L	CD	CHL
Staphylococci spp.	3	S	-	-	-	-	2	1	3	3	2	2	-	2	-	1	-	-	-	3
		I	-	-	3	1	-	-	-	-	1	-	-	1	1	1	-	-	-	-
		R	3	3	-	2	1	2	-	-	-	1	3	-	2	1	3	3	3	-
Exiguobacterium spp.	2	S	-	-	-	-	2	2	2	2	-	1	-	2	1	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	2	-
		R	2	2	2	2	-	-	-	-	-	-	2	-	1	2	2	2	-	2
Bacillus spp.	6	S	2	3	5	4	6	5	3	4	5	2	1	2	1	-	4	6	1	4
		I	4	3	1	2	-	1	3	2	1	4	5	4	5	6	2	-	5	2
		R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (N)	11	S	2	3	5	4	10	8	8	9	7	5	1	6	2	1	4	6	1	7
		I	4	3	4	3	-	1	3	2	4	5	5	5	6	7	2	-	7	2
		R	5	5	2	4	1	2	-	-	-	1	5	-	3	3	5	5	3	2

P= Penecillin, OX= Oxacillin, AMP= Ampicillin, AMC= Amoxicillin-Clavulanic acid, IMP= Imepenem, MRP= Meropenem, CN= Gentamycin, LE= Levofloxacin, NOR= Norfloxacin, CIP= Ciprofloxacin, CTX= Cefotaxime, CPM= Cefepime, CAZ= Ceftazidime, TE= Tetracycline, ERY= Erythromycine, L= Lincomycin, CD= Clindamycin, CHL= Chloramphenicol; S: Sensitive, I: Intermediate, R: Resistant

A total of 20 bacterial strains (52.3%) out of 38 presumptively identified bacterial isolates were found to be resistant against at least 2 antibiotics. Among the *Staphylococcus* spp. isolates, two and one isolates showed resistance against 9 and 10 antibiotics out of 18 antibiotics, respectively. In case of *Exiguibacterium* spp. isolates, one isolate showed resistance against 8 antibiotics and other one showed resistance against 9 antibiotics. Among the *Enterobacter* spp. isolates, one strain showed highest resistance against 15 antibiotics out of 19 whereas other isolates showed various number of antibiotic resistance (Table V).

Table V: Multiple antimicrobial resistance of bacterial isolates from the mobile phones of healthcare professionals' (n=20)

Bacterial isolates	Number of MDR isolates																			
	For 2 drugs	No.	For 4 drugs	No.	For 5 drugs	No.	For 6 drugs	No.	For 7 drugs	No.	For 8 drugs	No.	For 9 drugs	No.	For 10 drugs	No.	For 12 drugs	No.	For 13 drugs	No.	For 15 drugs	No.
Enterobacter spp. (n= 15)	1		1		1		2		2		1		2		1		1		2		1	
Staphylococcus spp. (n= 3)	-		-		-		-		-		-		2		1		-		-		-	
Exiguibacterium spp. (n= 2)	-		-		-		-		-		1		1		-		-		-		-	
Total N= 20	1		1		1		2		2		2		5		2		1		2		1	

Genotyping by Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Amplified product for 16S rRNA PCR was found at approximately 1465 base pair position in gel after visualization (Figure 2A). ARDRA grouping of the 20 MDR isolates was carried out based on their molecular size pattern. The isolates revealed 3 different molecular size patterns thus differentiated into 3 groups (Figure 2B).

Phylogenetic Analysis

Taking two of the isolates from group A and one of the isolates from group B and group C as a representative of the ARDRA groups, 16S rRNA sequencing was done. The typical phylogenetic tree exposed the position of the isolates on the evolutionary basis and represented the similar strains to the isolates (Figure 2C). *Enterobacter cloacae*, *Exigubacterium aurantiacum* and *Staphylococcus warneri* were found to be related to the ARDRA group's 1, 2 and 3, respectively (Figure 2). This phylogenetic and molecular evolutionary analysis was accorded with the presumptive biochemical identification results.

Discussion:

Nowadays, health care professionals like doctors, nurses and other stuff widely depend on mobile phones as these devices contain useful medical applications and facilitate emergency communication in the operation theatres and intensive care units of a hospital or clinic.[29]

However, antibacterial sensitivity test of the bacterial isolates was performed to the commonly used antibiotics as Bangladesh and its bordering countries have recently witnessed high prevalence of antimicrobial resistance, majorly because of their poor healthcare standards.[30] Furthermore, as the cellular phones generate constant heat and have numerous slits for accumulating dust particles and moisture, hence provide an ideal environment

for colonization of various non-pathogenic and pathogenic microbes, early detection of these isolates is beneficial to halt their transmission. Therefore, in the current study, we aimed to perform the isolation and molecular identification of multidrug resistant nosocomial pathogens at the species level on the mobile phones of the HCPs in the Jashore region of Bangladesh.

Staphylococcus aureus is the most common bacterial agent found from mobile phone surface in many countries[1,31]. In our study along with *Staphylococcus* spp., five other distinct genera of bacteria such as *Enterobacter* spp., *Exiguobacterium* spp., *Escherichia* spp., *Bacillus* spp., *Proteus spp.* were detected through standard biochemical analysis. It is reported that most of these bacteria are highly associated with nosocomial infection.[32–36] This diversified bacterial result has been consistent with relevant previous studies performed in our neighboring country India and among Bangladesh and Saudi Arabia where they detected more varied groups of bacteria on their mobile phone samples such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus arlettae*, *Pseudomonas aeruginosa*, *Neisseria sicca*, *Micrococcus luteus*, *Proteus mirabilis*, *Bacillus subtilis*, *Escherichia coli, Salmonella typhi*, and *Enterobacter aerogenes*.[15,37,38] However, other studies detected a mere 2 or 3 genera, including enteric coliforms (i.e., *E. coli*) or non-fermentative gram-negative bacteria (i.e., *S. typhi*).[39] We didn't find *Staphylococcus aureus* as the predominant one; this difference is owing to the high sensitivity of the method for the isolation of bacterial colonies followed by an enrichment step.[40]

In this study, the presumptively identified *Enterobacter* spp. isolates that had the highest prevalence of 39.47%, one of the isolates of them showed resistance to 15 drugs and 63.15% were resistant to more than 12 types of antibiotics. This rate was higher compared to the previous study, conducted in a medical ward, Northeast India, collected from mobile phone, was reported *Enterobacter cloacae* isolate resistance to 10 antibiotics.[17] This might be due to the extended-spectrum β-lactamase (ESBL) genes of *E. cloacae* complex that confer resistance to most β-lactam antibiotics, including extended spectrum (i.e., second and third-generation) cephalosporins (ESCs) and monobactams (i.e., aztreonam).[41]

However, in the present study, *Enterobacter* spp. showed sensitive against Levofloxacin (87%), Ciprofloxacin (80%), Co- trimoxazole (80%), Cefepime (67%), Meropenem (80%) which indicated these antibiotics might be helpful in treatment. All of the *Staphylococcus* spp. isolates showed resistance against five of the drugs used in this study. However, the antibiotic resistant rate is worse than the situations previously identified.[42] The unrestricted usage of antibiotics may make the pathogens more prone to be resistant and virulent day by day.[43] Gentamycin, Levofloxacin and Chloramphenicol were found to be sensitive in case of *Staphylococcus* spp. isolates. Prevalence of *Exiguibacterium* spp. was 5.2% and both of the isolates showed resistance to at least 8 drugs. We found *Exiguibacterium aurantiacum* was resistant to a higher number of antibiotics than a previous study showing 100% resistance Bacitracin, Erythromycin, Kanamycin, Norfloxacin and Vancomycin.[44] Therefore, this isolate with increased antibiotic resistance can be a matter of concern, as it can be found in the hospital environment and may easily transmitted through the mobile phones of HCPs and non- HCPs. However, *Exiguibacterium* spp. also showed sensitivity to Norfloxacin, Imipenem, Meropenem, Gentamycin, Levofloxacin, Chloramphenicol and Cefepime, thus suggesting these antibiotics as a preferred way of controlling the infection.

A previous study conducted in Bangladesh, identified 5 distinct MDR bacteria namely *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Escherichia coli*; *Salmonella typhi*, and *Staphylococcus epidermidis* from the cell phones of HCWs in different hospitals.[15] In this study MDR isolates were grouped into three through ARDRA and phylogenetic analysis by 16S rRNA sequencing identified three nosocomial strains *Enterobacter cloacae*, *Staphylococcus warneri* and *Exiguobacterium aurantiacum* from HCWs' mobile phones. These strains were reported as common multi-resistant bacterial pathogens found in the hospital wards during the last three decades.[44–46] Our findings were consistent with previous studies that stated 16srRNA sequencing was defined as a successful non-culture method, was used to identify bacterial phylogeny and taxonomy in the Diagnostic Laboratory.[47,48] Unlike our identified *Enterobacter cloacae*, a Gram negative agent, which contributed to most of the MDR isolates, Debnath et all found *Staphylococcus* spp. (55%) and Banawas et all found *Acinetobacter baumannii* and *Staphylococcus hominis* to dominate among their respective the MDR isolates.

It is to be discerned that the prevalence of antibiotic

resistance was high in our study. We found Gram-negative strains were dominant over gram-positive strains. The resistance of Gram-negative isolates is of high concern, as they cannot be treated with inexpensive antibiotics.[49] Resistance to β-lactam, Cephalosporin and Carbapenem among the clinical isolates of Gram-negative *Enterobacter* spp. are rising worldwide.[50,51] High resistance to Ampicillin, Aztreonam, Azithromycin, Amoxicillin, Kanamycin, Gentamycin, Amikacin was also found in the study. Studies suggest that any presence of antibiotic-resistant microbes on the surface of HCWs' mobile phones poses an enormous threat to public health worldwide.[39] Since the incidence of nosocomial infections is greater in developing countries with limited resources, the socio-economic burden because of added antimicrobial treatment and prolonged hospitalization is more severe in these countries.[6] The emergence of pre-antibiotic era has become the reality in countries throughout the world. Incomplete treatment and over-the-counter availability of antibiotics are two of the major reasons behind the increase of multidrug resistance in developing countries like Bangladesh.[52] Our study assumes that we are also walking through the same way. In today's world, mobile phones have become an integral part of telecommunication, therefore, stopping the usage of this device by the health care professionals is not pragmatic at all—above all, in urgent situations of operation theatre and intensive care units, mobile phones can become helpful for faster communication among the physicians, nurses and medical assistants. To mitigate the risk of cross contamination by the mobile phones of HCPs, ultrasonic cleansing or 70% isopropyl alcohol or antimicrobial additive materials have been recommended.[53-55]

Conclusion:

In summary, the present study identified that cell phones of HCWs are susceptible to be contaminated with harmful MDR pathogens such as *Enterobacter cloacae, Staphylococcus warneri, Exiguobacterium aurantiacum*. Majority of the isolates retrieved from their phones were Gram-negative bacteria. Among these isolates, *Staphylococcus spp. and Exiguobacterium* spp. isolates showed 100% resistance against Penicillin, Ampicillin, Oxacillin, Erythromycin, Lincomycin, and Cefotaxime. The isolated strains of *Staphylococcus* spp. *and Exiguobacterium* spp. were 100% resistance against Penicillin, Ampicillin, Oxacillin, Erythromycin, Lincomycin, and Cefotaxime whereas all *Enterobacter* spp. represented 100% resistance against amoxicillin. These nosocomial pathogens carried by HCPs' cell phones can be transmitted to patients during treatment procedures and cause serious illness among individuals with compromised immunity. However, this unexpected transmission of pathogens between HCPs and patients is not inevitable. That risk can be obviated effectively by taking proper initiatives like raising awareness among mobile phone users and promoting regular hand washing practices among healthcare personnel. Additional studies should be undertaken to evaluate the HCPs' cell phone capacity to reciprocally transmit nosocomial pathogens to the patients.

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Author Contributions:

MSH: Conceptualization, methodology, writing original draft, funding acquisition, supervision, project administration.

SRC: Methodology, investigation, resources, validation, formal analysis, writing original draft, data curation.

NSM: Formal analysis, data curation, investigation, funding acquisition.

NS: writing review and editing.

SMTS: writing review and editing.

MTI: Data curation, methodology, validation, writing review and editing, supervision.

OKI: writing review and editing.

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Conflicts of Interest:

All authors declare no competing interests in this study.

Informed Consent:

Required informed consent was obtained from the patients.

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