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Full Length Research Paper

Isolation and molecular characterization of some pathogenic mobile phone bacteria

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Mobile phones are indispensable accessories both professionally and socially but they are frequently used in environments of high bacteria presence. These can harbor various potential pathogens and become an exogenous source of nosocomial infections. A total of 91 mobile phones belonging to staff members in Taif University screened for bacterial isolates using bacteriological methods. Bacteriological analysis revealed that about (85.1 %) of mobile phone samples were contaminated with bacteria. Some bacterial species were isolated from mobile phone samples. They identified as *Gordonia*, *Pantoea*, *Ochrobactrum*, *Staphylococcus* and *Bacillus* spp. Genetic diversity of these bacteria was investigated by Random Amplified Polymorphic DNA (RAPD) analysis. The fingerprinting patterns revealed two main clusters of strains with a similarity level of approximately 55.8%. Phylogenetic analysis of the partial 16S rRNA sequences of bacterial strains were divided them into five species with similarity value ranged from 97 to 100 % comparing with bacterial sequences in NCBI database. These results recommend that mobile phones can be heavily colonized by high quantities of pathogenic bacteria and thus potential sources of disease transmission requiring application of sound personal hygiene as preventive methods.

Key words: Mobile phones, RAPD, 16S rRNA, bacterial contamination, human pathogenic bacteria.

INTRODUCTION

A number of studies have consistently reported that 5–21% of healthcare workers with mobile phones provide a reservoir of bacteria known to cause nosocomial infections (Brady et al., 2006; Jeske et al., 2007; Brady et al., 2009 and Sadat et al., 2010). Despite this knowledge, there exists a paucity of advice provided to either healthcare workers or inpatients on the use or decontamination of mobile phones in hospitals. This population handles infectious materials every day. Improper practice of hand hygiene and use of mobile phones both together play significant role in spread of some human harmful pathogens. So mobile phones may transmit more than just information in today's busy work places and other environments. They may also be involved in the transmission of infections. In spite that mobile phones are used in close contact with the body, the hygiene risk involved in using these instrument has not yet been determined. Previous studies demonstrated that the decolonized hands of healthcare workers can become contaminated by bacteria from their mobile phones (Khivsara et al., 2006; Jeske et al., 2007; Sadat et al., 2010). Such infections can be prevented by health care workers taking proper precautions when caring for patients. Source of infection may be exogenous such as from the air, medical equipment, hands of surgeons and other staff or endogenous such as the skin flora in the operative sites (Sham et al., 2011; Ducel et al., 2002).Thereality that many health care workers and patients were using the devices irrespective of restrictions and putative patient psychological advances in avoiding isolation from contacts (Aziz et al., 2003; Bell et al., 2009). In view of the previous restrictions, the investigators wished to characterize inpatient utilization of mobile phones and assess whether recent changes in policy had implications for infection prevention and control policies aimed at reducing healthcare associated infections.

A number of different methods have been used for typing bacteria species such as plasmid profile, analysis of cellular fatty acid content, SDS-PAGE protein pattern, Random

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Amplified Polymorphic DNA (RAPD) analysis and rRNA sequencing (Ash et al., 1991; Berber and Cokmus, 2001; Kim et al., 2010; Abelardo and Luís 2013). RAPD analysis is a genotyping system that has shown great specificity and sensitivity to characterize bacterial isolates. RAPD system uses short and arbitrary oligonucleotide random primers under low specificity conditions. It is efficient enough in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment (Shahaby et al., 2012). On the other hand, the 16S rRNA gene sequencing is now commonly used in the taxonomic purposes for bacteria (Tortoli, 2003; Lade et al*.,* 2014). The 16S rRNA gene, also designated as 16S rDNA, can be compared not only among all bacteria but also with the 16S rRNA gene of archeo bacteria.

The main objective of the present study was to isolate and identify on the molecular level of some pathogenic mobile phone bacteria from Taif university campus to avoid negative effects of improper practice of hand hygiene and use of mobile phones.

MATERIAL AND METHODS

Sampling: A total of 91 cell phones belonging to health care personals from both clinical and non-clinical departments of Taif University Campus and Hospital during the period from May 2013to Jun. 2014. Sterile swabs soaked in Nutrient broth were used for swabbing the front, the back and the sides of cell phones. This was followed by decontamination of cell phones with 70% isopropyl alcohol. After allowing it to dry for 10 minutes, repeat swabs were taken from the cell phones. These swabs were brought to the department of microbial genetics, Biotechnology and Genetic Engineering Research Unit, Scientific Research Center, where they were subjected to culture on Nutrient agar, blood agar and Mac Conkey agar. After incubation for 24 hours at 37 ° C, the growth obtained was identified on the basis of colonial characters, morphology by gram staining and various biochemical tests.

Identification of organisms: Pure isolated colonies were Gram differentiated and then biochemical identification using Catalase test, Oxidase test, motility and microscopic observation of bacteria color and shape. Identified and named based on the morphological, physiological and the biochemical characteristics presented in Bergey's Manual of Determinative Bacteriology (Christopher and Bruno, 2003).

Genetic Identification and Molecular Characterization

DNA extraction: The cell pellets from all isolates were used to extract genomic DNA using (Jena Bioscience, Germany) extraction kit following the manufacturer's instructions.

RAPD-PCR: RAPD analysis was performed according to Moschetti etal.(1998) using five primers Table (3). The RAPD-PCR amplification reactions were performed in Eppendorf® thermal cycler using the following PCR program: 1cycle at 94°C, 4 min; 35 additional cycles consisting of 94°C for 45 s, 36°C for 30 s and 72°C for 45 s. After the amplification, Amplified DNA products were analyzed by electrophoresis in 1.5% agarose gel run in TBE. The gels were stained with ethidium bromide (5 µg ml^{-1}). Gene Ruler^{TM} 100 pb. DNA Ladder (Fermentas) was used as a standard. DNA was visualized by UV illumination and were photographed by a Bio-Rad Gel Doc 2000 device.

PCR amplification of 16S-rRNA gene: DNA (2 µl), approximately 50 ng, was used as template for the polymerase chain reaction assays. The amplifications were carried out in thermo cycler as following. Amplified 16S-rDNA gene. Primers described by (Willems and Collins, 1993), which correspond to *Escherichia coli* 16S rRNA gene, were used for PCR amplification of 16S rDNA genes. 2X PCR Master Mix from (Fermentas®, Lithuania) was purchased and used for PCR reaction. Each reaction contains all necessary reagents (dNTPs 200 nm of each and 0.6 unit of Taq DNA polymerase) except primers and DNA template for performing 25 µl reaction. 50 ng of genomic DNA and 10 p.mol of each primer were added and conditions used for 16S rDNA gene amplification were done according to Willems and Collins, (1993). The presence of PCR products was ascertained by agarose (1.5% w/v) gel electrophoresis, at 100 V for 1 h.

Sequencing of 16S-rRNA gene: about 900 bp 16S rDNA fragments were purified using QIAquick PCR purification kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions and sequenced with the same primers using the sequencer (Gene analyzer 3121). The bacterial 16S-rDNA sequences obtained were then aligned with known 16S-rDNA sequences in Genbank using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information, and percent homology scores were generated to identify bacteria, and get accession numbers shown in table 4. The deduced sequence was aligned using Molecular Evolutionary Genetics Analysis (MEGA) version 5.10 and drawn phylogeny tree.

DATA ANALYSIS

In order to determination of genetic relationship among studied bacteria RAPD data were scored for presence (1) or absence (0) of the bands using Gene Tools software from Syngene. A simple matching coefficient was calculated to construct a similarity matrix and the UPGMA algorithm was used to perform hierarchical cluster analysis and to construct dendrogram using NTSYS-PC package (Rohlf, 2000).

Table 1. Types of bacteria isolated from mobile phones of health care personals.

Bacterial agents identified	Clinical	Non-Clinical	Total samples	Growth	Contamination rate (%)
Gordonia terrae	12	15	27	23	85.1
Pantoeaananatis	8	5	13	11	84.6
Pantoeaeucrina	4	3	7	4	57.1
Staphylococcus pasteuri	4	5	9	6	66.7
Staphylococcus warneri	6	3	9	5	55.6
Bacillus anthracis	7	6	13	9	69.2
O. pseudintermedium	2	4	6	4	66.7
Bacillus safensis	2	5	7	5	71.4

Table 2. Morphological and physiological characterization of mobile phone bacteria**.**

Strain Code	Proposed Name	Morphology	Gram Stain	Motility	Oxidase	Catalase
C-1	*Gordonia terrae*	Coryneform	+	+	+	+
C-2	*Pantoeaananatis*	Bacilli	-	+	-	+
C-3	*Pantoeaeucrina*	Bacilli	-	-	+	+
C-4	*Staphylococcus pasteuri*	Short coccui	+	-	-	+
C-5	*Staphylococcus warneri*	Short coccui	+	-	+	+
C-6	*Bacillus anthracis*	Long bacilli	+	+	+	+
C-7	*O. pseudintermedium*	Short rods	-	+	+	+
C-8	*Bacillus safensis*	Long bacilli	+	+	-	+

RESULTS AND DISCUSSION

Morphological Identification and Biochemical Characterization of the Bacterial Strains

All of health care personals sampled used their mobile phones at work at least once every day. 90% of them never washed their hands before using the device. The rate of routine cleaning of personal mobile phones was 25.2% which mean 74.8% of the health care personals never cleaned their mobile phones. Alcohol was used by 45% of those who clean their mobile phones; the rest used a dry wipe. Only 10.1% of health care personals cleaned their phones daily, 5.8% weekly and 76.4% cleaned their mobile phones occasionally (data not show). Out of 91 samples evaluated, growth was observed in the most of samples. Bacteriological analysis revealed that about (85.1 %) of mobile phone samples were contaminated with bacteria. About 38.2 % of bacterial species were isolated from the mobile phones samples and identified as *Gordoniaterrae, Pantoeaananatis,Pantoeaeucrina, Staphylococcus pasteuri, Staphylococcus warneri, Bacillus anthracis, Ochrobactrumpseudintermedium, and Bacillus safensis*.These strains are known to cause nosocomial infection. Details of the number and type of bacteria obtained from mobile phones are shown in (Table 1).

Eight isolates of about 91 mobile bacterial isolates were selected randomly for farther studies and named as C-1 to C-8. It found that five selected isolates were positive gram stain and positive catalase biochemical test with moderate motility. Two of them were found with long bacilli and identified as *Bacillus spp*, while other two were found with short coccui and were identified as *Staphylococcus spp*(Table 2). No bacterial growth was seen after decontamination with 70% Ethyl alcohol or 70% Isopropyl alcohol for 10 seconds. The negative control plates showed poor growth, (data not shown). Similar results were obtained with (Christopher and Bruno 2003; Ana et al., 2013) who suggested that colony morphology may be an indicator of phenotypic variation. This being an important adaptive process by bacteria to overcome environmental stressors. Furthermore, alterations in colony traits may reflect increased virulence and antimicrobial resistance.

As shown in Table 1 & 2, the main bacterial isolates frequently associated with mobile phone samples are *Staphylococcus* spp.,*Bacillus* spp. and *Pantoea* spp.

Table3. Polymorphic bands of each genetic primers and percentage of polymorphism in mobile phone bacteria.

Primers	Primers sequence	Amplified Bands	Monomorphic Bands	Polymorphic bands
OP-A1	5´CAGGCCCTTC3´	18	8	10
OP-A3	5´AGTCAGCCAC3´	12	6	6
OP-A4	5´AATCGGGCTG3´	17	10	7
OP-B5	5´TGCGCCCTTC3´	14	7	7
OP-B9	5´TGGGGGACTC3´	13	5	8
Total		74	36	38

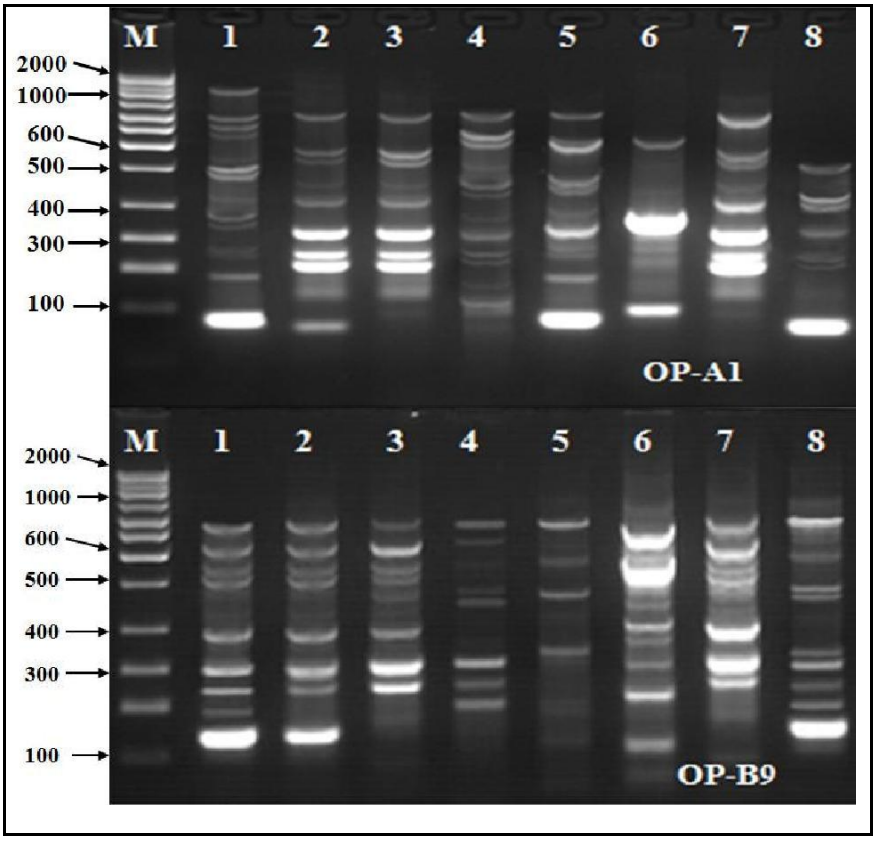

Figure 1. RAPD profile of mobile bacterial strains generated with five random primers; M = marker (1 kb DNA ladder; 1 to 8 = C-1 to C-8.

These organisms may probably have found their entry to the phone through the skin and hand to hand mechanism. This is because the isolated bacteria are subset of the normal microbiota of the skin as advanced by earlier researchers Roth and Jenner (1998). Many users of different hygiene profile having regular skin contact with the phones may have resulted in the frequency and the degree of population of the isolates (Sadat et al., 2010). This has a lot of health implication. *Staphylococcus* is known to cause illness ranging from pimples and boils to pneumonia and meningitis, which are not unlikely as corroborated by the high population of colonies even at low dilutions (Khivsara et al., 2006; Sadat et al., 2010).

Recently the gram-negative *Pantoea* was separated from the *Enterobacter* genus, which indicates the possibility of the presence of faecal contamination on these public handsets. Gram-negative sepsis is most commonly caused by *E coli*, *Klebsiellaspp, Enterobacters*pp and *Pseudomonas aeruginosa* (Sham et al., 2011; Ducel et al., 2002).

Bacillus sp. with a 25% frequency of occurrence in this study has been identified as an important organism in food spoilage. This, in no doubt, would contributeimmen-

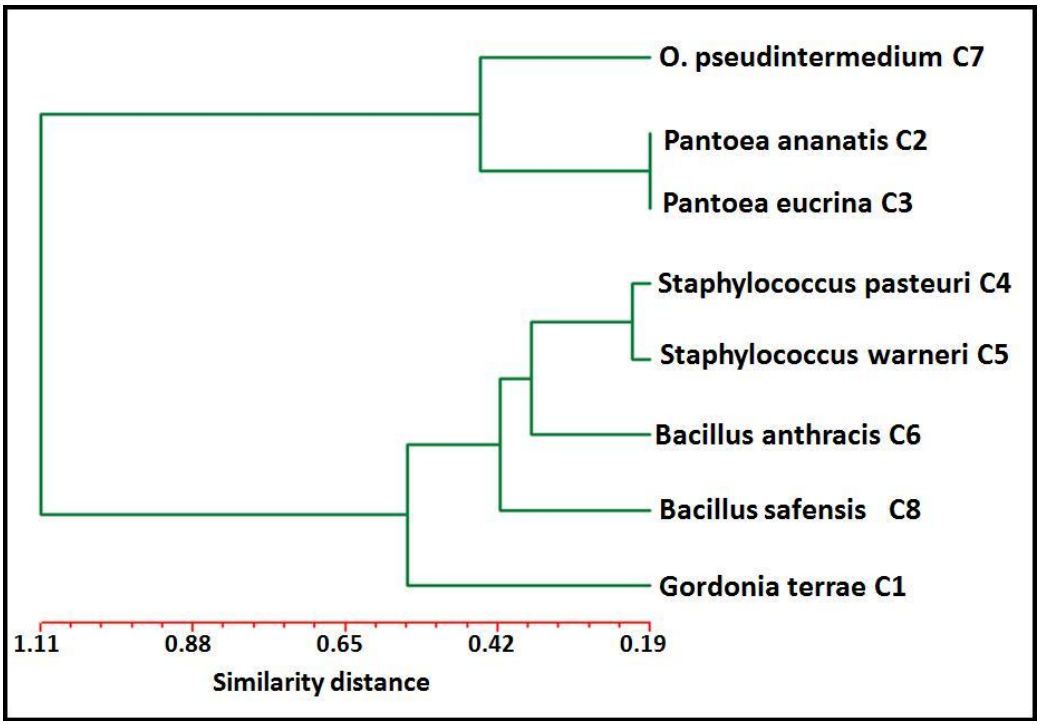

Figure 2. Dendrogram of eight mobile bacterial strains by RAPD-PCR with five primers.

Table 4. Sequences producing significant alignments similarity and accession numbers.

Strain Code	Identified isolates	Accession No.	Similarity
C-1	*Gordonia terrae*	KF_971767	99 %
C-2	*Pantoeaananatis*	KF_971769	99 %
C-3	*Pantoeaeucrina*	KF_971770	100 %
C-4	*Staphylococcus pasteuri*	KF_971771	98 %
C-5	*Staphylococcus warneri*	KF_971772	97 %
C-6	*Bacillus anthracis*	KF_971768	98 %
C-7	*O. pseudintermedium*	KF_971774	98 %
C-8	*Bacillus safensis*	KF_971773	98 %

sely to food spoilage and food infection, if infected hands are used in the preparation or eating of food (Berber and Cokmus 2001).

Genetic Identification and Molecular Characterization

Genomic diversity of mobile bacterial strains was investigated by RAPD analysis. The RAPD results illustrated in table (3) and figure (1) showed polymorphic numbers of the genetic bands, which were the electrophoretic products of PCR for mobile bacterial strains. The results illustrate that the total numbers of amplified bands were 74. The highest number of polymorphic bands among strains was generated in reactions with the primers OPA-01 (18 bands) and OPA-04 (17 bands). Most primers used in this study produced unique patterns with strain number C-7 (Figure 2), indicating unequivocal differentiation of these strains. The RAPD fingerprinting patterns revealed two main clusters of strains with a similarity level of approximately 54%. Cluster I was formed by strains C-2, C-3 and C-7, with a similarity level of 46%. Interestingly, strain C-7 was located in a separate branch, closer to C-2 and C-3, but with a similarity level of 42%. Cluster II was formed by strains C-1, C-4, C-5, C-6 and C-8 with a similarity level of 52%. Strains C-1, C-6 and C-8 were located in a separate branch, closer to C-4 and C-5, but with a similarity level of 38%. Cluster analysis of RAPD profiles supported the differences noted by visual observation of the electrophoretic profiles. Furthermore, the profiles obtained by RAPD showed a higher level of variability and all strains could be distinguished by at least one band. Obtained unique fragments among eight isolates

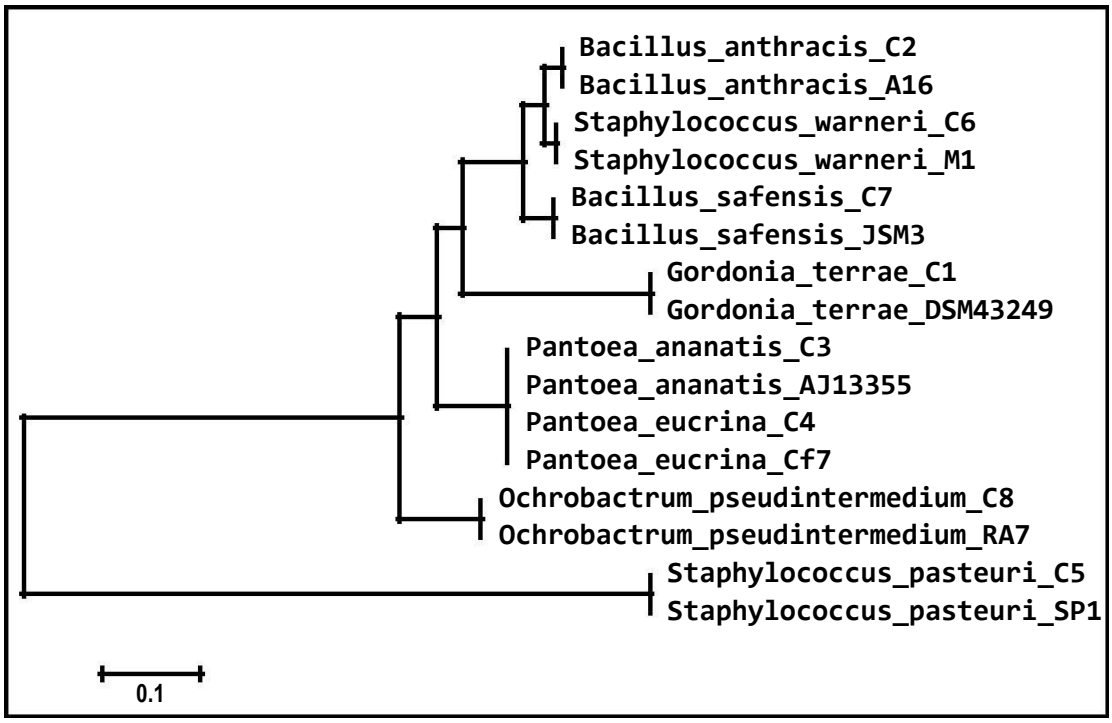

Figure 3. Phylogenetic relationship of the mobile bacterial strains and related genera based on full size16S rRNA or 16SrDNA (you should uniform) sequences. The tree was constructed using neighbor joining algorithm with Kimura 2 parameter distances in MEGA 5.1 software. The bar indicates the Juke-Cantor evolutionary distance.

using random primers were different, thus indicating that the studied isolates contained more diverse sequence and it is might be considered as specific markers for the bacterial isolates (Alok et al., 2009). The broad spectra of bacteria isolated here is indicative of the potential of the mobile phone to act as a fomite, which is similar to other fomites such as paper currency, which has been extensively researched on (Zarei et al., 2009; Tagoe et al., 2010 and Shahaby et al., 2012). The genetic proximity between mobile bacterial strains of the species was also observed by (Haiwen et al., 2005 ; Shahaby et al. 2012 ; Lade et al. 2014) using RAPD and sequencing of approximately 1200 bp of the 16S rDNA, thereby highlighting the low level of variability of these organisms.

Our results show that RAPD is the most sensitive and convenient method tested to unequivocally identifying bacterial strains using whole genomic DNA. However, since low reproducibility has been attributed to RAPD profiling, this may reduce its potential application (Juliana et al., 2003 and Shahaby et al., 2012). To minimize this effect, we used the same procedure for DNA purification from all strains and a commercially available RAPD system with standardized components and conditions, which was reliable and reproducible in this study. Using RAPD by Alok et al., (2009) it was possible were able to obtain different and discriminative fingerprint patterns for all strain of bacterial tested. These results are agreement with (Haiwen et al., 2005; Abelardo and Luís 2013), who suggested that oligonucleotides sequence analysis has been successfully used to identify various types of bacteria, including *Gordonia*,*Pantoea*, *Ochrobactrum*, *Staphylococcus* and *Bacillus* species, and it has an adequate level of taxonomic resolution at the species and subspecies levels (Lade et al., 2014).

The partial sequences of the 16S rRNA genes of the mobile bacterial strains deposited in the GenBank database (Kirchhof et al., 2001; Haiwen et al., 2005) were differed somehow from the sequence obtained in this study. The 16S rDNA sequence of the mobile bacterial strains deposited in GenBank was identical to that determined in this work. Ribosomal operons are of great relevance for the study of bacterial evolution and phylogeny (Zarei et al., 2009). Sequencing of 16S rDNA has been widely used to re-construct phylogenetic relationships of microorganisms (Shahaby et al., 2012). Phylogenetic analysis of the partial 16S rDNA sequences from the mobile bacterial strains studied here, together with related sequences deposited in GenBank (Figure 2 and table 4) were positioned the mobile bacterial species into *Gordonia*,*Pantoea*, *Ochrobactrum*, *Staphylococcus* and *Bacillus* spp. The strain C-1 with accession no. KF_971767 has 99 % similarities value with *Gordoniaterrae* DSM 43249 strain. Strains C-2 and C-3with accession no. KF_971769 and KF_971770 has 99 and 100% similarities value with*Pantoea*ananatis AJ 13355 and *Pantoea*eucrina Cf7 strains, respectively.

Moreover, strains C-4 and C-5 with accession no. KF_971771 and KF_971772 has 98 and 97% similarities value with *Staphylococcuspasteuri* SP1 and *Staphylococcuswarneri* M1 starins. Strains C-6 and C-8 with accession no. KF_971768 and KF_971773 has 98% similarities value with *Bacillusanthracis* A 16 and *Bacillussafensis* JSM 3 starins. On the other hand, strain C-7 with accession no. KF_971774 has 98 % similarities value with *Ochrobactrumpseudinter medium* RA 7 strain.

CONCLUSION

These results showed that health care workers hands and their mobile phones were contaminated with various types of microorganisms. Mobile phones used by health care workers in daily practice may be a source of nosocomial infections in hospitals.

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