



ORIGINAL ARTICLE

Dangerous passengers: multidrug-resistant bacteria on hands and mobile phones

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Keywords

Bacterial contamination • Mobile phones • Hand hygiene • Antibiotic resistance

Summary

Introduction. It is recognized that mobile phones may play a role in microorganism transmission and that hand hygiene, is considered the most important action for preventing infections and the spread of pathogens. The objective of this study was to determine presence and circulation bacteria on hands and mobile phones capable of causing infections in people and also determine if disinfection with gel-alcohol is useful to reduce the bacterial colonization.

Methods. The bacterial evaluation included 596 hands of participants and 256 mobile phones. Isolated colonies were identified by biochemical test and confirmed by gene 16S rRNA sequencing. Antimicrobial susceptibility was performed using the automated instrument Vitek®2-Compact and disk-diffusion-method.

Results. In total, 92.9% of mobile phones and 98.3% of participants in study demonstrated evidence of bacterial contamination with different types of bacteria. Surprisingly, we observed that 18.6% plaques inoculated with disinfected fingers showed bacterial growth. In general, Gram negative isolates showed resistance to a higher number of antibiotics tested than Gram positive isolates.

Conclusions. Our results could help to raise awareness in our society about the importance of hand hygiene, as well as frequently used devices, reducing bacterial contamination and limiting the possibility of transmission of resistant multi-drug bacteria.

Introduction

Hand hygiene, is considered the most important habit to prevent infections and the spread of microorganisms pathogens [1]. The common of people often believe that microbes are only present in rubbish and dumps, in research labs, in sick people, in hospitals and clinics and thus they have a misleading feeling of security in other places. Lack of knowledge about where germs occur and how are they transmitted could be the cause of health problems [2]. In fact, microorganisms are found almost everywhere in air, water, soil, food, plants and animals, including humans and may be transmitted, either directly, through hand-to-hand contact, or indirectly via food or other inanimate objects such as cell phones, money and coins [3]. Nowadays, mobile phones have become one of the most essential devices for professional and social life, and they can act as a vehicle for the spread of pathogenic bacteria and other microorganisms [2, 4]. One of the first studies reported that more than 90 % of cell phones of health-care workers were contaminated with microorganisms and 14 % of them carry pathogenic bacteria that commonly cause nosocomial infections [5]. Predominantly Gram-positive cocci (*Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Micrococcus* spp.), but also spore-forming rods (*Bacillus* spp.) or Gram-negative bacteria, can be transmitted through devices like mobile phones or computer keyboards [2, 6].

The purpose of this study was to determine presence and circulation of antibiotic-resistant bacteria on mobile phone and hands capable of causing systemic infections in healthy people and also determine if disinfection with gel-alcohol is useful to reduce the bacterial colonization.

Materials and methods

STUDY DESIGN

This cross-sectional study was conducted in a hand hygiene stand during massive exhibition Tecnopolis Federal, for a period of 2 week (May 2017), at the convention center of Posadas city (Misiones, Argentina). A total of 852 samples were collected from the touch surfaces of mobile phones (256 samples) and ventral surface of finger dominant (single hand) of apparently healthy volunteers (596 samples).

The protocol was approved by the ethical committee of Pediatric Hospital, and an oral informed consent was obtained from the participants or if they were minors, of their legal guardians.

SAMPLE COLLECTION

Samples of mobile phones were collected using the fingers previously disinfected with gel-alcohol or sterile swab, were immediately transferred into LB agar plates.

The fingers (two group: with or without disinfection) were supported onto plates for 5-7 seconds. Plates were incubated aerobically at 37°C for 48 h. Bacteria recovered from all plate were pooled and frozen in 20% medium glycerol.

BACTERIAL IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY

Colonies obtained in each processed plate were screened by their resistances, using broad spectrum antibiotics-10 µg ampicillin, 4 µg gentamicin and 5 µg chloramphenicol (Britania SA, Argentina) placed separately on Muller-Hinton agar plates and incubated aerobically at 37°C for 18 hours. The number of colony forming units (CFU) for the sample pool was estimated by plaque count.

Isolated microorganisms were identified using Gram stain, colony morphology, standard biochemical tests and confirmed by the automated ID/AST instrument Vitek® 2 Compact (Biomerieux) both Gram positive (GP ID card) and Gram negative (GN ID card) cards (Biomerieux, France) were used. Minimal inhibitory concentration (MIC) was performed using the automated ID/AST instrument Vitek® 2 Compact (Biomerieux) and the Gram positive and Gram negative susceptibility test cards (AST-P577; AST-N117; Biomerieux, France). Diffusion method according to Kirby-Bauer was used for the antibiotics aztreonam, minocycline and levofloxacin. The breakpoints were interpreted following CLSI guidelines [7].

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Culture of each isolate was suspended in sarcosil (0.01%) and DNA extracted using a combination of heating and centrifuged. Universal 16S rRNA bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used to amplify this gene using 10 ng of genomic DNA isolated from each strain. PCR products were purified and sequencing using primers 27F and 1492R [8].

Results

Five hundred thirty-one (98.3%) out of 538 volunteers in study had hands contaminated with bacteria. Also, 238 (92.9%) out of 256 cell phones of volunteers were contaminated with bacteria. Surprisingly, we observed that 18.6% of the 58 plaques inoculated with disinfected fingers showed bacterial growth. Filamentous fungi were observed in 3% of the plates.

Twenty one different appearance colonies were recovered on plates with antibiotic. The organisms identified with 96-100% probability belonged to seven species of Gram positive bacteria (*Bacillus subtilis*, *B. pumilus*, *Lysinibacillus sphaericus*, *Staphylococcus cohnii* ssp *urealyticus*, *S. warneri*, *S. saprophyticus* and *Enterococcus durans*) and seven species of Gram negative bacteria (*Serratia marcescens*, *Pseudomonas putida*, *Sphingomonas paucimobilis*, *Acinetobacter baumannii* complex,

Stenotrophomonas maltophilia, *Klebsiella pneumoniae* ssp *pneumonia*, and *Ochrobactrum anthropi*).

Results of antibiotic resistance of Gram positive and Gram negative bacterial isolates are listed in Table I (Antibiotic susceptibility results for all tested antibiotics are shown in supplementary data Table SI and Table SII, respectively). A strain *S. cohnii* was resistant to ERI and intermediate to NIT. *S. warneri* was resistant to ERI and GEN. *S. saprophyticus* resistant to OXA, ERI, GEN and STX, plus positive cefoxitin screen. *E. durans* was resistant and intermediate to SXT and NIT, respectively. *Bacillus* sp evidenced intermediate resistance to CLI. In general, Gram negative bacteria exhibit increased resistance to antibiotics. *P. putida* strains were resistant to AMP, SAM, CTX, NAL, NIT, SXT and intermediate to TZP. *S. marcescens* strains were resistant to AMP, SAM, CF, COL and NIT. *S. paucimobilis* was resistant to NAL, GEN and NIT. *A. baumannii* complex was resistant to AMP, CTX, NIT. *S. maltophilia* was resistant to GEN, IPM and AZT. *K. pneumoniae* was resistant only to AMP. *O. anthropi* was resistant to AMP, SAM, NAL, NIT and SXT and intermediate to TZP and CTX. All the observed resistances respond to natural mechanisms of resistance.

Discussion

Our study – first of its kind in our region – carried out with aimed to determine presence and circulation of antibiotic-resistant bacteria on mobile phone and hands of healthy people. The most organisms recovered, do not typically cause infections in healthy people rather they have been known to cause significant infections in those with depressed immune systems, including those infected with HIV, patients undergoing cancer chemotherapy, or taking other medications that depress the immune system (transplanted) [9, 10]. However, other belong to species that have been protagonist in both nosocomial and community acquired infections [11, 12].

In recent years, dozens of publications report the presence of microorganisms on money, coins and mobile phones. While the studies can vary, due to the methods used, local community flora, environmental conditions, including the socio-cultural levels of the population, in general, Gram positive bacteria were the most predominant [13]. Staphylococci found in the mucous membranes and normal skin flora has recently got attention as a potential pathogen, specifically for hospital infections where are a major cause of septicemia and bacteremia, especially for the patients who have immune deficiency. Locally, *Staphylococcus aureus* (MRSA) emerged at the Pediatric Hospital, in 2003 as a cause of community-acquired (CA) infections [14]. In several studies, high resistance ratios against erythromycin, gentamicin and trimethoprim-sulfamethoxazole, which is an alternative medicine in the treatment of methicillin-resistant staphylococci infections were reported [15]. Some species of genus *Enterococcus* have currently a particular medical significance, considering their notable ability of acquire and disseminate antimicrobial resistance de-

Tab. I. Minimum inhibitory concentration in bacteria antibiotic resistant recovered in this study.

Species - isolated	OXA	GEN	ERI	NIT	SXT	AMP	SAM	TZP	CF	CTX	CAZ	CEF	IPM	MEM	NAL	COL	AZT
<i>S. cohnii</i> N132			≥ 8	64*													-
<i>S. warneri</i> G62		4	≥ 8														-
<i>S. saprophyticus</i> N4	≥ 4		≥ 8														-
<i>S. saprophyticus</i> N3	≥ 4				≥ 320												-
<i>E. durans</i> G152				64*	10												-
<i>E. durans</i> G153				64*	10												-
<i>S. paucimobilis</i> G61		≥ 16		256											≥ 32		≥ 16
<i>P. putida</i> Ch7				≥ 512	80	≥ 32	≥ 32	32*		16					≥ 32		-
<i>P. putida</i> Ch8				≥ 512	80	≥ 32	≥ 32	32*		16					≥ 32		-
<i>A. baumannii</i> Ch16				512		16				8							-
<i>A. baumannii</i> A10B				256		≥ 32		4		8	16						-
<i>S. marcescens</i> M2				256		≥ 32	16		≥ 64							≥ 16	-
<i>S. marcescens</i> A12				256		≥ 32	16		≥ 64							≥ 16	-
<i>S. maltophilia</i> G151		≥ 16											≥ 16	≥ 16			≥ 32
<i>K. pneumoniae</i> M11						2											-
<i>O. anthropi</i> N13				256		≥ 32	≥ 32	≥ 128*		≥ 64*	≥ 64	32					-

OXA, oxalin; GEN, gentamicin; ERI, erythromycin; NIT, nitrofurantoin; SXT, trimethoprim/sulfamethoxazole; AMP, ampicillin, SAM, Ampicillin/Sulbactam; TZP, Piperacillin/Tazobactam; CF, cefalotin; CTX, cefotaxime; CAZ, ceftazidime; CEF, cefepime; IPM, imipenem, MEM, meropenem, NAL, nalidic acid; COL, colistin; AZT, azteronam. (-) no tested; * Intermed according to CLSI [7].

terminants [16]. *Bacillus pumilus* and *B. subtilis* are environmental “non-pathogenic” bacteria that have rarely been associated with clinical infections [17]. *Lysinibacillus sphaericus* (best known as *Bacillus sphaericus*) is first bacteria with insecticidal activity against mosquito larvae were reported in the 1960s [18]. The majority of *Bacillus* species are susceptible to aminoglycosides, fluoroquinolones, vancomycin, clindamycin and carbapenems while penicillin and cephalosporin susceptibility is variable [19], unlike our isolates (*B. pumilus* - A10 and *B. subtilis* - A9) that evidenced intermediate resistance to clindamycin. Previous studies have shown that *Bacillus* species should be recognized as true pathogens, especially in neonates and other immunosuppressed host [9, 17, 19]. *In vitro* susceptibility testing has shown that strains of *S. paucimobilis* unlike our observed are susceptible to aminoglycosides and quinolones [20, 21]. In dissidence, one-third of *S. paucimobilis* strains recovered cell phone’s health worker were resistant to ampicillin, first and second generation cephalosporins, gentamicin and nitrofurantoin. Unfortunately, no antibiotic resistance mechanisms have yet been elucidated [22]. *S. marcescens* is natural sensitivity to aztreonam and naturally resistant to ampicillin, macrolides, and first-generation cephalosporins, expressing chromosomally-encoded AmpC β -lactamases [11]. *O. anthropi* was resistant to all β -lactams which is consistent with the reported expression of an AmpC β -lactamase [23]. *S. maltophilia* is naturally resistant to aminoglycosides, tetracycline, and quinolones due to the high level of expression of *smeA*

or *smeD*. β -lactam resistance is due to the expression of two β -lactamases that hydrolyzes all β -lactams with the exception of aztreonam [24]. Species of *Acinetobacter* exhibit mechanisms of resistance to all existing antibiotic classes as well as a prodigious capacity to acquire new determinants of resistance [12]. *P. putida* is usually susceptible to fluoroquinolones, aminoglycosides, monobactams, and extended-spectrum cephalosporins. However, acquisition metallo- β -lactamases that confer resistance to most β -lactams, including carbapenems, have been reported [25].

Our results revealed permanent colonization of bacteria on the mobile phones, which are very close to the hand of users. For this fact, contaminated phones can play a potential role in the spread of drug-resistant bacteria into the community. Food manipulators have been implicated in various outbreaks of food-borne diseases and human occupational activities could introduce the risk of food contamination. Food manipulators (workers) can be infected by pathogens from multiple sources and them in turn become potential sources of contamination in food processing and preparation facilities [26]. Equally important is way as parents tend to use their mobile phones at the bedside to communicate while touching, change diapers or holding their baby, increasing the risk of transmission [27].

Disinfection with gel-alcohol was effective in reducing bacterial colonization [4], however, it does not ensure complete disinfection, according to our results. We speculate the more likely explanation is related to the

inadequate application of gel-alcohol. Developing active preventive strategies like routine decontamination of mobile phones with gel-alcohol containing disinfectant materials might reduce cross-infection. Another way of reducing bacterial contaminations on mobile phones might be the use of antimicrobial additive materials, today available for medical applications [28]. We could easily avoid dispersion of bacterial infections just by using regular cleaning agents, such as gel-alcohol and rearranging our habits. This could include educating to children in schools and parents on the risk that a contaminated mobile phone poses for their baby, mobile phone hygiene and proper hand antimicrobial-gel application before and after mobile phone usage at the baby's bedside [27]. In the future mobile phones and devices could be produced by using protective material against the microbial contamination [29].

This activity was novel, Tecnopolis exhibition gave us the opportunity to interact with the community – especially students of initial and middle school levels to show the microscopic world we carry with itself. Highlighted the occurrence of pathogens bacteria on objects outside the health care environment in order to and raise awareness people on the necessity of improving the habit of washing their hands and employ appropriate disinfection methods to tactile electronic device in order to reduce microbial transmission. However, study presents some limitations. First of all we conducted a descriptive analysis, during an optional intervention, of sample obtained from non-probabilistic sampling thus no generalization of the results can be proposed. Questions to participants regarding the level of knowledge about microorganisms were not properly registered. Furthermore, the design not explore socio-demographic characteristics of the study sample. In future research, these variables should be considered for a more complete analysis of the thematic which allows identifying the determinants of health acting in hand washing adherence of the community to a more effectiveness intervention on specific population groups.

Conclusion

The present study shows that the mobile phones and hands of people even without symptoms of disease harbored a variety of pathogenic organisms with resistance to some of the therapeutic antibiotics used which can cause serious diseases. Our results could help to raise awareness in our society about the importance of hand hygiene and frequently used devices, decreasing bacterial contamination and limiting the transmission of pathogens.

Acknowledgements

The authors are grateful to the fellow-staff of Research Group in Applied Genetics (GIGA).

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

PFM and JF designed and coordinated the design and data acquisition. CKC, PFM and JF collected, analyzed and interpreted the results. MM and MVS were responsible for the microbiology analysis. PFM and MM drafted the manuscript. All authors read and approved the manuscript.

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Received on May 15, 2019. Accepted on October 24, 2019.

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How to cite this article: Martina PF, Martinez M, Centeno CK, Von Specht M, Ferreras J. Dangerous passengers: multidrug-resistant bacteria on hands and mobile phones. *J Prev Med Hyg* 2019;60:E293-E299. <https://doi.org/10.15167/2421-4248/jpmh2019.60.4.1283>

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Tab. S1. Determination of minimal inhibition concentration in Gram positive bacteria recovered in this study.

Antibiotic	<i>S. cohnii</i> N132		<i>S. cohnii</i> N13M		<i>S. warneri</i> G62		<i>S. saprophyticus</i> N3		<i>S. saprophyticus</i> IN4		<i>E. durans</i> G152		<i>E. durans</i> G153		<i>B. pumilus</i> A10		<i>B. subtilis</i> A9		<i>L. sphaericus</i> C14		<i>L. sphaericus</i> C15	
	0.5	S	0.5	S	0.25	S	4	R	4	R	2	S	2	S	-	-	-	-	-	-	-	-
Oxacilin	0.5	S	0.5	S	0.25	S	4	R	4	R	2	S	2	S	-	-	-	-	-	-	-	-
Centamicin	0.5	S	0.5	S	4	R	0.5	S	0.5	S	-	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-	-	-	-	-
Levofloxacin	1	S	0.5	S	0.12	S	0.5	S	1	S	0.5	S	0.5	S								
Moxifloxacin	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-
Erythromycin	≥8	R	0.5	S	8	R	0.5	S	8	R	0.25	S	0.25	S	-	-	-	-	-	-	-	-
Clindamycin	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	-	-	-	-	2	I	1	I	0.5	S	-	-
Quinupristin/ Dalbopristin	1	S	0.5	S	0.5	S	1	S	0.5	S	1	S	1	S	-	-	-	-	-	-	-	-
Linezolid	4	S	2	S	2	S	4	S	4	S	2	S	2	S	-	-	-	-	-	-	-	-
Teicoplanin	4	S	4	S	0.5	S	4	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-
Vancomycin	1	S	1	S	1	S	1	S	1	S	0.5	S	0.5	S	0.25	S	0.25	S	0.25	S	1	S
Minocycline	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-
Tetracycline	2	S	1	S	1	S	1	S	1	S	1	S	1	S	-	-	-	-	-	-	-	-
Nitrofurantoin	64	I	32	S	16	S	16	S	16	S	64	I	64	I	-	-	-	-	-	-	-	-
Rifampicin	0.5	S	0.5	S	0.5	S	0.5	S	0.5	S	-	-	-	-	-	-	-	-	-	-	-	-
Trimethoprim/ Sulfamethoxazole	10	S	10	S	10	S	320	R	10	S	10	R	10	R	-	-	-	-	-	-	-	-

(-) no tested. S, sensible. I, intermed. R, resistant according to CLSI [7].

Tab. SII. Determination of minimal inhibition concentration in Gram negative bacteria recovered in this study.

Antibiotic	<i>S. paucimobilis</i> G61		<i>P. putida</i> Ch7		<i>P. putida</i> Ch8		<i>A. baumannii</i> Ch16		<i>A. baumannii</i> A10B		<i>S. marcescens</i> M2		<i>S. marcescens</i> A12		<i>S. maltophilia</i> G151		<i>K. pneumoniae</i> M11		<i>O. anthropi</i> N13	
Ampicillin	2	S	≥ 32	R	≥ 32	R	16	R	16	R	32	R	32	R	-	-	2	R	≥ 32	R
Ampicillin/ Sulbactam	2	S	≥ 32	R	≥ 32	R	2	S	2	S	16	R	16	R	-	-	2	S	≥ 32	R
Piperacillin/ Tazobactam	4	S	32	I	32	I	4	S	4	S	4	S	4	S	-	-	4	S	≥ 128	I
Cefalotin											64	R	64	R	-	-	2	S		
Cefotaxime	1	S	16	R	16	R	8	R	8	R	1	S	1	S	-	-	1	S	≥ 64	I
Ceftazidime			4	S	4	S	4	S	4	S	1	S	1	S	4	S	1	S	≥ 64	S
Cefepime	4	S	1	S	1	S	2	S	2	S	1	S	1	S	-	-	1	S	32	S
Imipenem			1	S	1	S	0.25	S	0.25	S	0.5	S	0.5	S	≥ 16	R	0.25	S	1	S
Meropenem	4	S	1	S	1	S	0.25	S	0.25	S	0.25	S	0.25	S	≥ 16	R	0.25	S	1	S
Amikacim	2	S	2	S	2	S	2	S	2	S	2	S	2	S	-	-	2	S	16	S
Gentamicin	16	R	1	S	1	S	1	S	1	S	1	S	1	S	≥ 16	R	1	S	2	S
Nalidixic acid	32	R	≥ 32	R	≥ 32	R	2	S	2	S	2	S	2	S	-	-	2	S	4	R
Ciprofloxacin	1	S	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	0.25	S	-	-	0.25	S	0.25	S
Nitrofurantoin	256	R	512	R	512	R	512	R	512	R	256	R	128	R	-	-	32	S	256	R
Colistin	-	-	0.5	S	0.5	S	0.5	S	0.5	S	16	R	16	R	-	-	0.5	S		
Trimethoprim/ Sulfamethoxazole	-	-	80	R	80	R	20	S	20	S	20	S	20	S	20	S	20	S	≤ 20	S
Levofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	S	-	-	-	-
Minocycline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	≤ 4	S	-	-	-	-
Aztreonam	≥ 16	R	-	-	-	-	-	-	-	-	-	-	-	-	≥ 32	R	-	-	-	-

(-) no tested; () no detected. S, sensitive. I, intermed. R, resistant according to CLSI [7].