



Investigation of *Staphylococcus* spp. and *Escherichia coli* Colonization and Biofilm Formation on University Students' Mobile Phones and Hands

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Abstract

Mobile phones, which became indispensable in our daily lives, are likely to be colonized by microorganisms found in the hands of people using them. In this study, a total of 30 mobile phones and owner hands (30) were screened for *Staphylococcus* and *E. coli* contamination in university students. Colonization was screened in these samples, and their susceptibility to 11 antimicrobials in different groups. And, oxacillin salt agar screening test was performed to detect methicillin resistance. Microplate (MP) method, Congo Red Agar (CRA) method and Standard Tube (ST) method were used to determine biofilm formation. According to our results, *E. coli* colonization was not found in any sample, while 31 samples were isolated as Coagulase-Negative Staphylococci (CoNS) and 2 samples as *Staphylococcus aureus*. All 33 Staphylococci isolates were found to be susceptible to vancomycin and rifampicin, while 27% were found to be resistant to oxacillin, 36% to ceftazidime, 70% to ampicillin, 48% to tetracycline, 76% to erythromycin, 70% to penicillin, 30% to gentamicin, 30% to ampicillin-clavulanic acid, 24% to ciprofloxacin, 27% to ciprofloxacin, 27% to trimethoprim/sulfamethoxazole and 27% to methicillin. It was determined that 9 (27.2 %) of the 33 Staphylococci isolates was resistant to methicillin. Staphylococci were 100% biofilm producers according to the microplate method. Especially hand hygiene should be carefully provided and mobile phones should be regularly cleaned in order to prevent bacterial colonization of mobile phones, and prevention strategies should be developed in terms of public health.

Key words- Antimicrobial resistance, Biofilm, College students, *Escherichia coli*, Mobile phones, Staphylococci.

1. Introduction

The rapid progress in modern technology has led to advances not only in medical fields, but also in technologies for individual use as well. As one of these technologies, mobile phones are an important tool used for faster communication and social purposes all over the world. Besides different benefits of the mobile phone, it is easy to overlook the health risk it might pose to many users [1]. The easy constant phone's contact with the hands, face, mouth and ears, the potential vehicle of spreading the pathogenic microorganisms mobile devices are obvious. The infection potential of mobile phones was first suggested by Borer in 2005, and many articles have been published since [2,3]. Most of the reports are also related to the potential role of spreading nosocomial pathogens like *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Klebsiella*, *Escherichia*, and *Pseudomonas* on clinical settings [4]. Metagenomic analysis has revealed that *Staphylococcus* and *Corynebacterium* spp. are the most abundant organisms colonizing moist areas on skin flora [5]. *S. aureus* and CoNS are the most common Gram-positive agents

isolated from the surface of mobile phones [6, 7, 8, 9, 10]. *S. aureus* is a global human pathogen and cause of different infections of the skin and other organs in immuno-competent patients, but CoNS is involved in the infectious processes in immuno-compromised patients or patients using catheters [11, 12]. Biofilm formation is one of the most important virulence factors responsible for pathogenicity in staphylococci. Bacteria in biofilm have been shown to be 100 to 10000 times more resistant to antibiotics than the planktonic forms [13]. Staphylococci are inherently susceptible to most antibiotics except those with purely anti-Gram-negative spectra [14].

In the view of these, this study was carried out to investigate colonized *Staphylococcus* spp., *E. coli*, and further characterize their antimicrobial susceptibility patterns and biofilm formation capability from mobile phones and hands of the college students.

2. Material and Methods

2.1 Study design and sample size

This cross-sectional study was carried out during a period of a half month from the beginning of March 2016 till the middle of March 2016. A total of 30 mobile phones of college students and their hands were tested for microbial contamination at the Art and Science Faculty Mustafa Kemal University, Hatay, Turkey. Volunteer students (male n=12, female n=18) were randomly selected from different departments and oral and signed consents were obtained from all.

A questionnaire was applied to collect information about mobile phone usage and hand washing habits of all participants. In the questionnaire, questions such as age, gender, department of study, duration of mobile phone usage, frequency of mobile phone usage, mobile phone cleaning habit and hand washing frequency were asked. Information gathered by the questionnaire were used to assess hand washing frequency and mobile phone cleaning habits.

2.2 Collection of samples and isolation

Swab cultures were collected from a 1 cm² area of both sides of mobile phones and thumbs and index fingers of dominant hands of 30 students, which were studying at various departments in Mustafa Kemal University, Faculty of Arts and Sciences. Samples were plated in duplicate onto selective medium Mannitol Salt Agar Base (MSA) (Hi Media, India) for *Staphylococcus* spp., and Endo Agar (HiMedia, India) for *Escherichia coli* recovery. Plates were incubated at 37 °C for 24-48 h with aerobically. Typical colonies were maintained by streaking on Nutrient agar slants. The pure isolated microorganisms were identified by conventional methods (Gram staining, haemolytic activity on 5% blood agar, catalase, tube coagulase tests). A tube coagulase test diversified staphylococcal isolates into *Staphylococcus aureus* and Coagulase-negative staphylococci (CoNS). All isolates were maintained at +4 °C in 20 % glycerol for further analysis.

2.3 Antibiotic susceptibility

Antibiotic susceptibility of *Staphylococci* spp. isolates were tested using disk diffusion method as recommended Clinical and Laboratory Standards Institute [15]. The following antibiotic disks (Bioanalyse, Turkey) were used : Ampicillin (10 µg), Amoxicillin/clavulanic acid (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Sulphamethoxazole/trimethoprim (1.25/23.75 µg), Vancomycin (30 µg), Erythromycin (15 µg), Rifampicin (5 µg), Penicillin (10 U). *S. aureus* ATCC 25923 was used as a positive control.

2.4 Methicillin-Resistance screening

All *Staphylococcus* spp. isolates were inoculated on to Mueller-Hinton agar (Oxoid) supplemented with 4% NaCl, from a 0.5 McFarland standard suspension, and 1 µg oxacillin disk placed cautiously. Methicillin

resistance also was tested phenotypically with cefoxitin (30 µg) (Bioanalyse, Turkey) disk. The isolates showing zone diameter of ≤21mm for *S. aureus* and ≤24 mm for CoNS were considered as methicillin resistant, for cefoxitin antibiotic. For oxacillin disk, ≤10 mm zone diameter also were considered as methicillin resistance for *Staphylococcus* spp. [15].

2.5 Biofilm production

The isolates were concurrently examined by three in vitro methods:

2.5.1 Congo Red Agar (CRA) method

The method defined by Freeman et al. [16] was used in this study. Inoculated cultures were incubated at 37 °C for 24 h aerobically. Black colonies with a rough, dry and crystalline consistency were regarded as positive producers, while red or smooth colonies were classified as negative strain.

2.5.2 Standart Tube (ST) method

The other qualitative assay for biofilm formation was performed according to the method described by Christensen et al. [17]. For this purpose, *Staphylococcus* spp. isolates were inoculated into five mL of Trypticase Soy Broth (TSB, Oxoid) and incubated at 37 °C for one day. Test tubes were poured and washed with sterile PBS. After then, empty test tubes were stained with 1% safranin dye for 7 min. Stained tubes were decanted gently and washed with sterile PBS again. The stained biofilm was dried at room temperature and then, the degree of biofilm formation was visually defined. The positive sum was considered by the existence of biofilm on the inner surface of the tube. The presence of stained material at the liquid-air interface alone was not regarded as indicative of slime production [18].

2.5.3 Microtitre Plate (MP) method

Quantitation biofilm formation was assessed using MP Method, based on formerly reported procedures by Christensen et al. [17]. For this assay, a volume of 200 µL (10⁵ CFU/ml) aliquots of overnight Trypticase Soy Broth (TSB) cultures of each strain was added to each well of 96-well microplates made of polystyrene (Nunc, USA) and kept for 24 h at 37 °C. After incubation, the contents were aspirated and the plates were washed twice with phosphate- buffered saline (PBS; pH: 7.2). The microplate wells were stained with Hucker crystal violet for 45 min at room temperature, then rinsed again and dried. The optic density (OD) was measured at 570 nm (OD540) using an enzyme linked immunosorbent assay (ELISA) reader (Thermo Scientific-Multiskan GO). As negative control, sterile TSB was used to the wells. All the experiments were repeated at least twice, and the values of optical density were then averaged. A 3- grade scale was used to evaluate the biofilm-forming ability of strains: (-): ODs < 0.120; (+): ODs 0.120–0.240; (++) : ODs > 0.240 [18].

3. Results

3.1 Prevalence of Staphylococci isolated from mobile phones and hands of college students

In this study, 18 female and 12 male students were included on a voluntary basis, and 60 samples were taken from their mobile phones and their dominant fingers (Table 1). The average age of the volunteers participating in the study was 23.16. The average age of female students was 21.88 and the average age of male students was 25.08. No *E. coli* was observed in any of the samples. Of the 60 samples, 55% was found to be contaminated with bacteria.

The percentages of Coagulase Negative Staphylococcus (CoNS) isolated from the mobile phones and hands of the students were 53.3% and 50%, and *S. aureus* isolated from the hands and mobile phones of the students were 3.3% and 3.3%, respectively. Of the 30 hand cultures analyzed, 16 had bacterial growth. In the hand cultures, there were 15 CoNS growths and 1 *S. aureus* growth. Similarly, 17 bacterial colonization were observed in 30 cultures from mobile phones. In the mobile phone cultures, there were 16 CoNS growths and 1 *S. aureus* growth. The source of the samples and the total *S. aureus* and CoNS frequency isolated are shown in Table 1.

Staphylococcus spp. colonization was detected in 56.6% of the mobile phones used by the students, whereas the rate of same colonization was 53.3% in their dominant hands. Of the mobile phones, 93.3% was found to be contaminated with bacteria. This rate was 90% for hands.

Table 1. Source of the sample and prevalence of isolated *Staphylococci* spp. isolates

Department	Student	CoNS number (%)		<i>S. aureus</i> number (%)	
		Mobile phones	Hand	Mobile phones	Hand
Anthropology	11	7(43.7)	4(26.6)	1(100)	1(100)
Biology	11	6(37.5)	5(33.3)	0	0
Geography	4	1(6.2)	4(26.6)	0	0

Table 2. Antimicrobial resistance profiles of *Staphylococci* isolates from mobile phone and hands of college students

Sample	Antimicrobial resistance (%)											
	OX	FOX	AM	RA	TE	E	P	CN	AMC	CIP	VA	SXT
Mobile	35.2	47.0	70.5	0.0	29.4	76.4	70.5	41.1	41.1	35.2	0.0	35.2
Hand	18.7	25.0	68.7	0.0	25.0	68.7	68.7	18.7	18.7	12.5	0.0	18.7

AM; Ampicillin, AMC; Amoxicillin /Clavulanic acid, CIP; Ciprofloxacin, CN; Gentamycin, E; Erythromycin, FOX; Cefoxitin, OX; Oxacillin, P; Penicillin, RA; Rifampin, SXT; Sulfamethoxazole trimethoprim, TE; Tetracycline VA; Vancomycin

3.3 Multiple antibiotic resistance profiles and biofilm formation of *Staphylococcus* spp. isolates

In this cross-section study, the multiple antibiotic resistance (MAR) phenotypes were observed in *Staphylococcus* spp. isolates (Table 3). The leading MAR phenotypes for methicilline resistant CoNS isolated from mobile phone isolates and hands of college students were 6 (18.1 %) and 3 (9 %), respectively.

Chemistry	2	1(6.2)	1(6.6)	0	0
History	1	1(6.2)	1(6.6)	0	0
Archaeology	1	0	0	0	0
Total	30	16	15	1	1

According to the results of the questionnaire, participants' routine mobile phone cleaning rate was 90%, daily cleaning rate was 26.6%, weekly cleaning rate was 46.6%, cleaning rate of 5 to 7 times a month was 16.6%, and 10% was never cleaning their mobile phone, respectively. Of the volunteers participated in the study, 6.6% was washing their hands 1-3 times a day, 20% was washing their hands 4-6 times a day, 36.6% was washing 7-9 times, and 46.6% was washing their hands 10 times or over a day.

3.2 Antibiotic susceptibility

Two *S. aureus* and 31 CoNS isolates from student's mobile phone and their hands were subjected to antibiotic susceptibility tests. Twelve antibiotic disks, from different antibiotic classes were used. It was determined that there was various rate of resistance to a number of tested antimicrobials (Table 2). All *Staphylococci* isolates were sensitive to vankomisin and rifampicine. However, the isolates were found highly resistant to Penicillin G, Ampicillin and Erythromycin in both mobile phone and hands of college student's. There were less resistant groups for SXT, CIP and TE antibiotics. Oxacillin and Cefoxitin disk diffusion tests were used to determine the methicillin resistance of the isolates. The isolates resistant to both antibiotics were considered methicillin resistant. Two isolates of *S. aureus* were susceptible to methicillin, whereas only 2 out of 31 isolates of CoNS were susceptible to methicillin. Nine coagulase-negative *Staphylococci* (CoNS) isolates were determined to be methicillin-resistant by both the oxacillin and cefoxitin disk diffusion method. These were also resistant to other several antibiotics.

The MAR phenotypes OXA-FOX-AM-TE-E-P-CN-AMC-CIP-SXT were 5.8% in mobile phone sources. Besides, OXA-FOX-AM-E-P-CN-AMC-CIP MAR phenotype was observed only mobile phone samples (Table 3). However, among the isolates from mobile phone were 70.1 % and from hands of college student's were 56.1% isolates developing MAR. Among all MAR phenotypes of *Staphylococci* isolates, 58.4% of them were resistance to more than four different antibiotics in

mobile phone isolates and 43.6% were from hands of college student's. In this study, Ampicillin, Penicillin and Erythromycin were effective than other antimicrobials. All tested *Staphylococcus* spp. isolates were resistant to ampicillin more 70%, to penisillin more 70% and more 76% to erythromycin in both sample sources.

Among 33 *Staphylococci* spp. isolates, CRA method detected none of them as a biofilm producers. Otherwise,

MP assay findings demonstrated that all isolates produced biofilms. And as a result of the ST assay, 63.6% isolates were biofilm producers. According to ST assay, 33.3% of *Staphylococci* spp. were a positive producer in mobile phone isolates, and 30.3 % were from hands of college student's. In the study, the biofilm formation capabilities of *Staphylococci* spp. isolates from mobile and hands of student's at 37 °C for 24 h in TSB were given in (Table 4).

Table 3. The multiple antibiotic resistant phenotypes for *Staphylococcus* spp. isolates

Phenotype	Mobile phone (n=17) No.(%)	Hands of college student's (n=16) No.(%)
AM-E-P	2(11.7)	2(12.5)
AM-TE- E-P	2(11.7)	3(18.7)
FOX-AM-TE-E-P	1(5.8)	-
OX-FOX-AM-TE-E-P	-	1(6.2)
OX-FOX-AM-E-P-CN-AMC-CIP	1(5.8)	-
OX-FOX-AM-E-P-CN-AMC-SXT	1(5.8)	16.2
OX-FOX-AM-E-P-CN-AMC-CIP-SXT	4(23.5)	2(12.5)
OX-FOX-AM-TE-E-P-CN-AMC-CIP-SXT	1(5.8)	-

AM; Ampicillin, AMC; Amoxicillin /Clavulanic acid, CIP; Ciprofloxacin, CN; Gentamycin, E; Erythromycin, FOX; Cefoxitin, OX; Oxacillin, P; Penicillin, SXT; Sulfamethoxazole trimethoprim, TE; Tetracycline

Table 4. Biofilm formation capacities of *Staphylococci* spp. isolates

Test	<i>S. aureus</i> (n=2)								CoNS (n=31)							
	Mobile (n=1)				Hands of student's (n=1)				Mobile (n=16)				Hands of student's (n=15)			
	Biofilm Positive		Biofilm Negative		Biofilm Positive		Biofilm Negative		Biofilm Positive		Biofilm Negative		Biofilm Positive		Biofilm Negative	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
MP	1	100	0	0	1	100	0	0	16	100	0	0	15	100	0	0
CRA	0	0	1	100	0	0	1	100	0	0	16	100	0	0	15	100
ST	1	100	0	0	1	100	0	0	10	62.5	6	37.5	9	60	6	40

N (n): Number, MP: Mikrotitre Plate, CRA: Congo Red Agar, ST: Standart tube

4. Discussion

Mobile phones are likely to be in contact with bacteria in the hand flora. This study represented the isolation, antibiotic susceptibility pattern and biofilm formation of *Staphylococcus* spp. and *E. coli* from university student's mobile phones and hands. The absence of *E. coli* in any of the samples indicated that there was no fecal contamination. In some studies, mobile phones have been shown to be contaminated with *E. coli* at different rates [1, 10, 19, 20, 21].

The results of this study showed that 56.6 % of the samples were colonized for *Staphylococcus* spp. from mobile phone and 53.3 % of the samples were colonize for *Staphylococcus* spp. from owner hands. None of the 2 strains of *S. aureus* isolated in this study were MRSA, like as Furuhta et al. [22] results. In contrast to this study, the reports of Seuli et al. [23], Tekerekoglu et al. [10], Yusha et al. [24], Jayalakshmi et al. [7] and Brady et al. [25] found that MRSA was isolated from mobile phones at rates of 84 %, 5.5 %, 76%, 2.7 % and 1.9 %, respectively. These mentioned reports, the objects of the

studies were focused on medical treatment. It is possible that these study results are differed from those of previous reports because we selected healthy students. Many studies have been also carried out on microbial contamination [6, 19, 22, 25, 26, 27]. The results of this study were found to be higher than in other literature. [19, 22, 26]. This can be explained by the fact that mobile phone cleaning is not performed routinely and that hand washing technique is not applied correctly and regularly. However, Coagulase Negative Staphylococci (CoNS) isolates were more frequently isolated, being 93.9 % of the total isolates. Most of the studies reported that the most commonly found bacterial isolate was Coagulase-Negative Staphylococci (CoNS) as a member of normal skin flora [10, 19, 28, 29, 30]. Bacteria known to potentially be associated with hospital infections were isolated in 60 samples, including methicillin-resistant CoNS (27.2%). Methicillin-resistant CoNS (MRCoNS) have emerged as a major pathogen among the university students. Tekerekoglu et al. also reported high level MRCoNS [10].



In this study, antimicrobial susceptibility testing was carried with the *S. aureus* and Coagulase-Negative Staphylococci (CoNS) isolates from mobile phones and owner's hands. Ampicillin, Penicillin and Erythromycin were the antimicrobials to which an extend proportion of the isolates were resistant, in this study as similar to previous reports in this area [26]. However, Trimethoprim/sulfamethoxazole, Ciprofloxacin and Tetracycline were detected less effective antimicrobials. In one study, conducted in Ethiopia, the Ciprofloxacin resistance of Staphylococci isolates from the mobile phone and owner's hands of healthcare workers (HCWs) was reported as 31.7 % and 30.9 %, respectively [26]. This can be explained by the healthcare professionals' more frequent encounter with nosocomial infection agents in the hospital. In the study, the percentage of MAR phenotypes were also found to be antibiotic resistant. It would be appropriate to genotypically detect these antibiotic resistance determinants using molecular methods.

The rate of routine cleaning of student's mobile phones and hand washing habits were high. But, we have not carried out any experiment about the mobile phone's disinfection. The training of personal hygiene control procedure and optimal disinfection methods are of great significance for reduce to contamination [31].

The other purpose of this study was to detect the biofilm formation of the isolated strains. So far, there was no study in the literature about the biofilm formation of bacteria isolated from mobile phones and hands. In this respect, our study is the first in Turkey. According to qualitative methods ST and MP experimental results, isolates were found to be 63.6% and 100% biofilm producers, respectively. Bacterial adhesion is a prevalent event in nature and is an important factor for biofilm formation. MDR biofilm formation was reported by Vickery et al. [32] somewhere else from surfaces and furnishings in a 16-bed insentive care unit. Biofilm may enhance bacterial survival capacity on dry surfaces and may confer resistance to against physical and chemical agents [33]. It can be assumed that inanimate surfaces such as mobile phones are also potential reservoirs for biofilm formation due to microbial colonization.

Concerning about the total 2,232 students in our faculty, as a matter of fact, comparatively few healthy students were included in the study. Besides, lightly of the number of students grouping, antimicrobial resistance patterns of Staphylococci isolates were very remarkable. We also intend to examine resistance genes with regard to these isolates.

5. Conclusion

In conclusion, all sampled mobile phones and owner hands were highly contaminated with *Staphylococci* spp. MDR bacteria were also found, but reverse to expectations, MRSA were not. And high level biofilm production in isolates caused great concern for students'

mobile phones. To reduce the colonization, students should be encouraged to clean their phones regularly and wash their hands frequently.

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