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Research Article

Biofilm formation by *E. coli* and *S. aureus* on cellphone cover: sensitivity to commercially available sanitizers

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Abstract

Presence of pathogens on the cellphones and their accessories poses a significant risk for public health. This study aimed to determine the biofilm-forming capability of *S. aureus* and *E. coli* on pieces made from a different commercially available cell phone and aadditionally to test the effectiveness of the most common commercially available sanitizers. Therefore, bacterial biofilm biomasses were quantitatively determined on cellphone covers using crystal violet assay in the presence and absence of common sanitizers. This study revealed that *S. aureus* and *E. coli* could form biofilms on the surfaces of all cellphones covers. Additionally, the sanitizers that contain sodium hypochlorite 5.25% and those composed of 38.9% ethanol and 0.05% dodecyl dimethyl ammonium chloride showed the highest log reduction in the number of viable cells after 5 minutes of exposure against biofilms formed by both *E. coli* and *S. aureus* compared to other tested sanitizers (chloroxylenol 4.8%, 2-propanol 64%, and ethanol 70%). Moreover, 4.8% chloroxylenol and 70% ethanol-based sanitizers showed log reductions significantly higher than 2-propanol-based ones. In conclusion, cellphone covers were shown to be suitable surfaces for microbial biofilm formation produced by *S. aureus* and *E. coli*. The antimicrobial activity of commercially available sanitizers against these bacterial biofilms was variable, with sodium hypochlorite and ethanol/dodecyl dimethyl ammonium chloride sanitizer being the most effective.

Keywords

Biofilm formation, *E. coli*, *S. aureus*, Cellphone cover, Sanitizers

Introduction

Biofilm formation and its consequences on the economy and public health are considered serious global issues affecting various areas such as food processing and the medical field (Stewart and Costerton 2001; Hassett et al. 2003; Jahid and Ha 2014). Biofilms are highly regulated, complicated, cooperative, and coordinated communities

of microorganisms that adhere to biotic or abiotic surfaces (Giaouris et al. 2015). The bacterial population impacts the bacterial cell-to-cell communication density, a process termed quorum sensing. In fact, quorum sensing is mediated by small diffusible signaling molecules called autoinducers (AIs). Bacteria use quorum sensing to regulate diverse arrays of functions, including virulence and biofilm formation (Zhou et al. 2020). Microorganisms in a biofilm are enclosed in a self-produced extracellular matrix, which plays a crucial role in the stability of biofilm and protection against various environmental challenges (Stoodley et al. 2002; Hall-Stoodley et al. 2004). It has been reported that biofilms are implicated in approximately 80% of chronic human infections and around 65% of hospital-acquired infections in the USA (Mah and O'toole 2001; Kalmokoff et al. 2006; Francolini and Donelli 2010). Contaminated surfaces in clinical settings can be considered a reservoir of pathogens and contribute significantly to the cross-transmission of pathogens(Otter et al. 2013; Russotto et al. 2015).

Worldwide, there is a rising concern about the impact of contaminated cell phones on the spreading of pathogenic microorganisms. The use of cell phones has dramatically increased and become necessary for daily life communication. In the clinical setting, cell phones are frequently used for communication, web consultation, online communication, and downloaded applications such as medical dictionaries, drug information, and medical calculator, and access to patient's laboratories, and imaging results (Mosa et al. 2012; Ventola 2014). Cellphones are often used near patients, and inside patient zones; thus, the presence of pathogenic bacteria on cell phones poses a serious risk of cross-contamination. Several studies showed that a high percentage of mobile phones used by healthcare workers and trainees were contaminated by bacteria, and this act as a reservoir for pathogens (Heyba et al. 2015; Sedighi et al. 2015; Zakai et al. 2016; Asfaw and Genetu 2021; Mushabati et al. 2021).To date, the literature about the biofilm formation on cell phone covers is lacking as previous studies have focused mainly on the type of microorganisms isolated from mobile phones and the effectiveness of antimicrobial agents on the isolated microorganisms grown as planktonic rather than as biofilm (Badr et al. 2012; Corrin et al. 2016; Koscova et al. 2018; Loyola et al. 2018; Bodena et al. 2019). Therefore, this study was conducted to evaluate the ability of *S. aureus* and *E. coli*, frequently isolated from surfaces of cell phones (Srikanth et al. 2009; Koscova et al. 2018), to form biofilms on commercially available cellphones covers. In addition, the sensitivity of these biofilms to commercially available sanitizers was assessed.

Methods

Preparation of pieces

Eight different commercially available cellphone covers were purchased from the market. 4.5 mm circular diameter circular pieces were made from each cellphone cover. Pieces were rinsed with soap and sterile distilled water, and then they were immersed overnight in 70% ethanol and exposed to UV for 24 hours.

Biofilm development

S. aureus and *E. coli* were grown in LBA overnight in an orbital shaker at 37 °C. An overnight culture was adjusted to an optical density (OD_{550}) equivalent to 1×10^7 colony forming unit (CFU)/ ml. Three pieces made from the same cellphone cover were placed in each well of 24 well plates containing 1 ml of the standardized bacterial suspension and incubated at 37 °C for 24 h without agitation. Then, the pieces were gently rinsed in a circular motion with PBS three times to remove planktonic and loosely attached cells. The pieces were transferred to new 24-well plates for further studies.

Biofilm biomass quantification

The total biofilm biomasses of 24 h old biofilms were quantified using the crystal violet method described by Stepanovic and colleagues (Stepanović et al. 2000). Briefly, 24 h old biofilms grown on pieces were gently washed three times with sterile PBS to remove residual planktonic and loosely adhered cells. The pieces were air dried in a laminar flow cabinet for 45 min, and then 1 ml aliquots of 1% crystal violet solution were transferred to each well of 24-well plates, and the plates were incubated at room temperature for 45 min. After 45 min of incubation with crystal violet solutions, the pieces were washed with PBS to remove excess dye and then allowed to air dry overnight. Each piece was placed in a separate well of a 96-well plate, and 200 µL of 95% ethanol was added to each well to solubilize adherent cells. Then, 150 µL aliquot from each well was transferred to a new well of 96-well plate, and absorbance was measured at 595 nm.

Scanning electron microscopy (SEM)

Biofilm biomass structure was visualized using scanning electron microscopy (SEM) (FEI Quanta 450, USA). Briefly, the 24-hold biofilms grown on pieces were rinsed gently with PBS. Then the pieces were soaked with 25% glutaraldehyde and incubated for 24 h at 4 °C. After 24 h incubation, the pieces were transferred to a sterile plate and allowed to air dry for 48–72 hours. Then, the pieces were dried and mounted onto SEM stubs, and coated with gold. The SEM images were done for piece number 7 because of cost limitations.

Treatment of biofilms with sanitizers

The 24 h old biofilm grown on pieces was rinsed in PBS. Three pieces were transferred into a well containing 1 ml of sanitizer listed in Table 1. After treatment for 5 min, each piece was transferred into a new sterile well containing PBS.

Table 1. Antimicrobial composition of the sanitizers used.

Sanitizer	Composition
A	5.25%sodium hypochlorite
B	38.9 g / 100 ml ethanol and 0.05 g/100 ml dodecyl dimethyl ammonium chloride
C	Chloroxylenol B.P.4.8%w/v.
D	2 –propanol (63.14 g/100g)
E	70% ethanol

Recovery of biofilms after treatment with sanitizers

After exposure to sanitizers, the pieces were rinsed with PBS three times and then transferred to the new sterile well of a 96-well microtiter plate containing 200 µL D/E neutralizing broth in each well and sonicated for 10 min to dislodge and re-suspend the cells into the recovery medium. After sonication, the pieces were discarded, and the resultant bacterial suspensions were serially diluted using PBS in 96-well plates. Three aliquots (20 µl each) from each well were spotted on the agar surface and incubated at 37 °C. After 24 hours, the number of colonies was counted. The number of surviving cells was calculated as colony forming unit per ml (CFU/ml), and based on these values, the log reduction was calculated.

Results and discussion

Biofilm formation on pieces made from commercially available cellphone covers

Biofilm-forming tendencies of two tested bacteria, *S. aureus* and *E. coli*, on pieces made from eight different commercially available cellphone covers made of plastic-based material were evaluated. Fig. 1 shows the extent of biofilm biomass formed after 24 hours. The quantitative biofilm biomass assay showed the ability of both tested bacteria to form biofilms. This indicates that cell phone cover provides a suitable environment for bacterial colonization and biofilm formation.

To further confirm biofilm formation on pieces, biofilms formed on piece number 7 were imaged using SEM. Representative SEM micrographs of biofilms produced by *S. aureus* and *E. coli* are shown in Fig. 2. Biofilm formation was observed on the selected piece.

Effectiveness of commercially available sanitizers against biofilm formed on pieces

The formed biofilm mass was challenged for 5 min with five commercially available sanitizers used daily (Table 1). The effectiveness of sanitizers against biofilm formation on the pieces is demonstrated in Fig. 3. Among the sanitizers tested and for both *E. coli* and *S. aureus*, sanitizer A (sodium hypochlorite 1.0%) and B (ethanol 38.9% and dodecyl dimethyl ammonium chloride 0.05%) showed

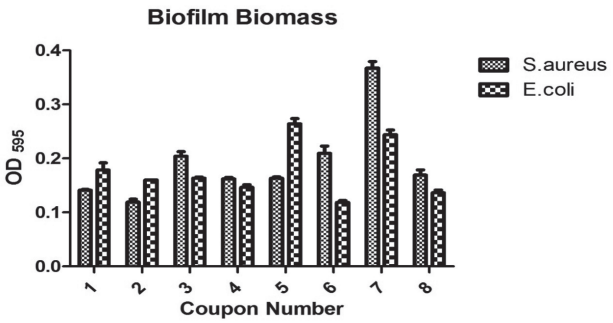

Figure 1. Biofilm biomass of *S. aureus* and *E. coli* grown on eight different pieces made of commercially available cell phone covers (n=3).

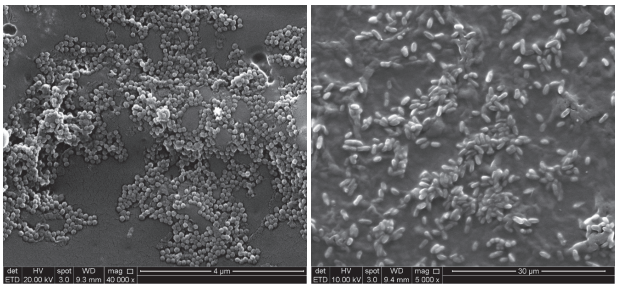

Figure 2. SEM images of biofilm formed by *S. aureus* (**A**) and *E. coli* (**B**) on piece number 7.

the highest log reduction in a number of viable cells after 5 min exposure compared to other tested sanitizers (sanitizers C: chloroxylenol 4.8%, D: 2-propanol 64%, and E: ethanol 70%). Moreover, sanitizers C (chloroxylenol 4.8%) and E (Ethanol 70%) showed log reductions significantly higher than those of sanitizer D (2-propanol 70%). It may be concluded that sanitizer D (2-propanol 70%) showed the lowest activity among tested sanitizers against biofilms formed by *E. coli* and *S. aureus*. However, sanitizer D was more effective against *E. coli* compared with *S. aureus*.

Discussion

It is practically difficult and not substantial to keep surroundings free from microorganisms; however, the growth of microorganisms can be reduced and controlled by compliance with hygiene practices. Thus, it is essential to regularly clean and disinfects surfaces and items in close contact with human, especially those in direct contact with patients. Cellphone is one of the devices used frequently in proximity with patients (Brady et al. 2011; Mosa et al. 2012; Ventola 2014). Thus, cellphone's role in transmitting infectious and communicable diseases has drawn global attention. A relevant body of evidence highlights the role of cell phones as a potential reservoir for pathogens responsible for hospital-acquired infections. Brady et al. (2011) conducted a study on the prevalence of bacterial colonization of the cell phones of healthcare workers. They found that 96.2% of tested phones were colonized with bacteria, and 14.3% were colonized with pathogens associated with hospital-acquired infections.

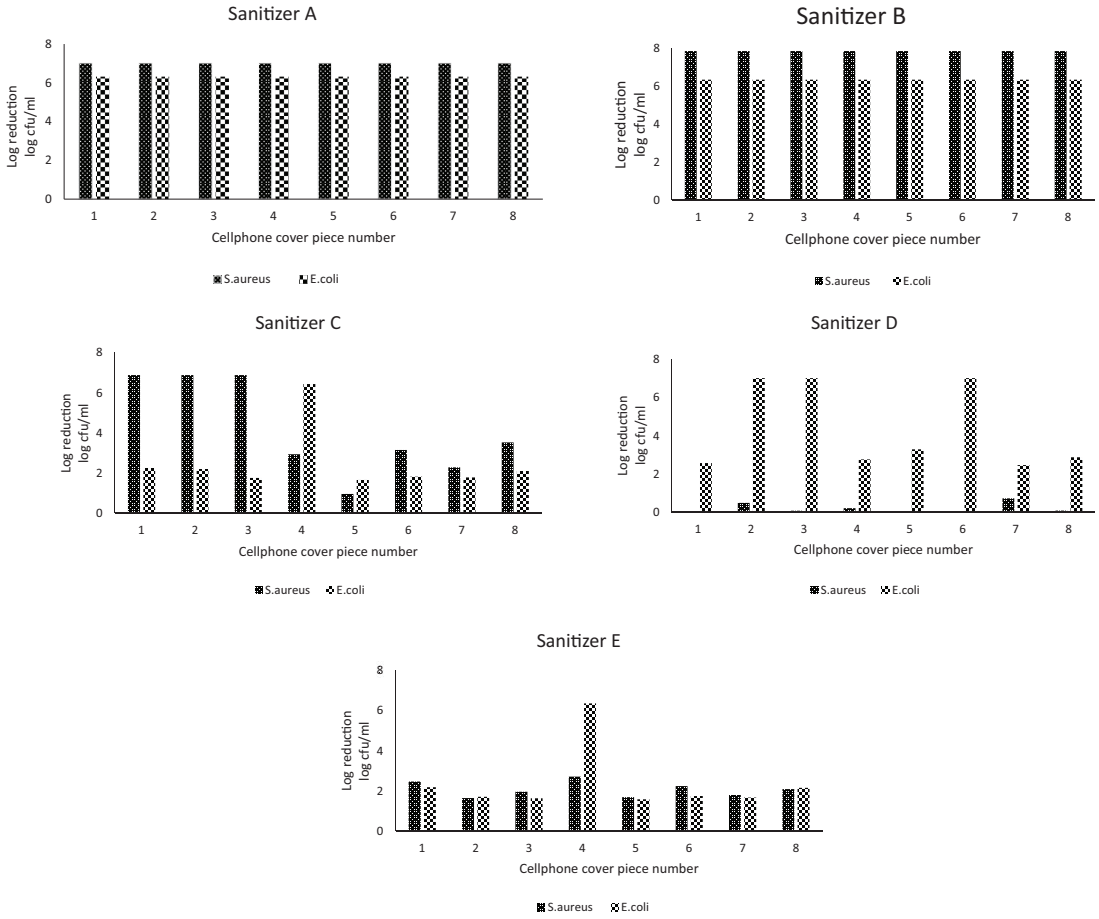

Figure 3. Viable cell number reduction of 24-hour old biofilms grown on cell phone covers pieces, expressed as $\log_{10}$ CFU/ml, after 5 minutes exposure to sanitizers tested (sanitizer A: sodium hypochlorite (5.25%), sanitizer B: Ethanol (38.9%) and dodecyl dimethyl ammonium chloride (0.05%), sanitizer C: chloroxylenol (4.8%), sanitizer D: 2-propanol (70%), and sanitizer E: ethanol (70%)

Thus, it should be noted that not all bacteria that colonize the cell phones covers and other surfaces are pathogenic. Another study reported that 94.2% of swabbed mobile phones were contaminated with bacteria, where *S. aureus* and *E. coli* were among the primary isolates (Bodena et al. 2019). In this study, two bacterial strains frequently associated with hospital-acquired infections were selected as challenge organisms: *S.aureus* and *E. coli* (Haque et al. 2018; Poolman and Anderson 2018). Both *S. aureus* and *E. coli* have the propensity to colonize and form biofilms on surfaces. They are among the most common bacteria associated with biofilm formation on medical devices (Jacobsen et al. 2008; Khatoon et al. 2018b; Zheng et al. 2018). For example, on polypropylene mesh surface, 88.7% of isolates with *S. aureus* versus 54.3% of isolates with *E. coli* formed biofilm very strongly (Khatoon et al. 2018a). The present study aimed to assess the biofilm-forming tendency of *S. aureus* and *E. coli* on commercially available cellphone covers. The finding obtained indicated that the tested cellphone covers provide a suitable medium for microbial colonization and biofilm formation. However, considerable variations in the biofilm-forming ability of *S. aureus* and *E. coli* on cellphone covers were noticed. The adhesion of bacteria to abiotic surfaces is a highly regulated process that is strictly controlled by physiochemical characteristics of the bacterial cell, abiotic surfaces, and environmental factors (Gomes et al. 2015; Khelissa et al. 2017). Differences in chemical composition, charge, hydrophobicity, roughness, and texture of cellphone covers could contribute to the variability in biofilm biomass.

Previous studies have focused mainly on the type of microorganisms isolated from mobile phones and the effectiveness of antimicrobial agents on the isolated microorganisms grown as planktonic rather than as biofilm (Badr et al. 2012; Corrin et al. 2016; Koscova et al. 2018; Loyola et al. 2018; Bodena et al. 2019). Biofilm formation by pathogens on surfaces is a public health concern. Disinfectants and antisepsis are essential for controlling contamination and infection in clinical settings. Unfortunately, the development of tolerance to antimicrobial agents and sanitizer has been documented (Bridier et al. 2011; Sanchez-Vizuete et al. 2015; Meesilp and Mesil 2019). Disinfection and antiseptic approaches, which fail to eradicate biofilm entirely may result in nodes of persisting infection (Costerton et al. 1999; Donlan 2002). In some studies, resistance to sanitizers was associated with resistance to some antibiotics (Westfall et al. 2019; Amsalu et al. 2020; van Dijk et al. 2022). Thus, in the pres-

ent study, we evaluated the effectiveness of five commercially available sanitizers with distinct modes of action on the removal or inactivation of biofilm grown on the pieces made from commercially available cellphone covers. Sodium hypochlorite and ethanol (38.9%)/dodecyl dimethyl ammonium sanitizers showed the highest activity against the biofilm biomasses formed by *E. coli* and *S. aureus*. In contrast, and chloroxylenol, and ethanol (70%) sanitizers were seen acting in the middle. The lowest activity was found for the 2-propanol sanitizer.

Conclusion

Assessment of biofilm formation on pieces made from commercially available cellphone covers showed that these surfaces act as a suitable medium for microbial biofilm formation produced by *S. aureus* and *E. coli*. The antimicrobial activity of commercially available sanitizers against these bacterial biofilms was variable, as sodium hypochlorite and ethanol/dodecyl dimethyl ammonium chloride sanitizer being the most effective.

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