ABCS HEALTH SCIENCES

ORIGINAL ARTICLE

OPEN ACCESS

Virulence, biofilm formation ability and antimicrobial resistance of *Staphylococcus aureus* isolated from cell phones of university students

Gabrielle Messias de Souza[1], Mariana Francelino Almeida de Jesus[1], Maria Vitória de Souza Ferreira[1], Karolinny Cristiny de Oliveira Vieira[1], Wilson Romero Nakagaki[1], Eliana Peresi Lordelo[1], Valéria Pereira Cataneli[1], Lizziane Kretli Winkelstroter[1]

[1]Faculdade de Ciências da Saúde, Universidade do Oeste de São Paulo (UNOESTE) – Presidente Prudente (SP), Brazil

ABSTRACT

Introduction: Contamination of cell phones can contribute to the dissemination of pathogens in the community and/or hospital environment. **Objective:** To characterize *Staphylococcus aureus* strains isolated from cell phones of university students. **Methods:** Samples were collected from 100 cell phones. Detection of genes associated with virulence factors such as biofilm formation (*icaA* and *icaD*), enterotoxins production (*SEA*, *SEB, SEC*, and *SED*), and resistance to methicillin (*mecA* and *mecC)* was performed in *S. aureus* isolates by PCR. Typing *mecA* gene performed by multiplex PCR. Susceptibility to antimicrobials and biofilm formation rate also evaluated by using disk diffusion test and crystal violet staining. **Results:** *S. aureus* was present in 40% of the total samples and about 70% of them belonged to Nursing students. Of the isolates, 85% presented resistance to penicillin and 50% were classified as moderate biofilm producers. In addition, 92.5% of isolates contained the gene *icaA* and 60% of the gene *icaD*. Approximately 25% of the isolates presented the *mecA* gene. Typing of the *mecA* gene showed the presence of staphylococcal chromosome cassette SCCmec I and c III respectively in 20% and 10% of the isolates. 70% of the samples could not be typed by the technique. Regarding the enterotoxins, the most prevalent gene was *SEA* (30%) followed by the *SEC* gene (2.5%). The presence of *SED* and *SEB* genes not observed in any of the isolates. **Conclusion:** The cleaning and periodic disinfection of cell phones can contribute to the reduction of the risk of nosocomial infection.

Keywords: Biofilms; cross infection; drug resistance, microbial; enterotoxins; Methicillin-resistant *Staphylococcus aureus*; virulence.

How to cite this article: Souza et al. Virulence, biofilm formation ability and antimicrobial resistance of Staphylococcus aureus isolated from cell phones of university students. ABCS Health Sci. 2022;47:e022203 https://doi.org/10.7322/abcshs.2020154.1608

Received: Sep 08, 2020
Revised: Jan 04, 2021
Approved: Feb 08, 2021

Corresponding author: Lizziane Kretli Winkelstroter - Universidade do Oeste de São Paulo - José Bongiovani, 700 – Cidade Universitária – CEP: 19050-920 - Presidente Prudente (SP), Brazil - E-mail: lizzianekretli@gmail.com

Declaration of interests: nothing to declare
Funding: FAPESP (2018/08097-7)

INTRODUCTION

Cell phones are devices necessary for both personal and professional life. For health professionals, they can improve communication, promoting collaboration and information sharing[1,2].

Cell phones have been frequently used in hospitals and healthcare settings. That fact raises major concerns about nosocomial infections as they may be involved in the

transmission of bacteria harboring genes of virulence and resistance, especially in areas requiring the highest standards of hygiene[2]. More than 50% of health professionals admit to the use of cell phones in the clinical setting, including during physical contact with patients, while bacterial contamination was observed in up to 25% of the devices[3].

Among the possible contaminants, the presence of *S. aureus* is highlighted. *S. aureus* is a common cause of hospital and community-based infections, and methicillin-resistant *S. aureus* (MRSA) is considered an important nosocomial pathogen[4]. The presence of these microorganisms on cell devices is a crucial point due to the possible dissemination of antimicrobial-resistant bacteria[5,6]. In addition, these bacteria can produce biofilm, a structure known as a set of bacteria adhered to a surface and which multiply surrounded by a matrix[7].

Students in the health field can be potential disseminators of contaminants since practical classes and clinical stages make possible direct contact with fomites and pathogens[8]. Several studies have already demonstrated that students in the health area are potential transmitters of pathogenic bacteria due to the use of contaminated objects and lack of hygiene. Garcia et al.[9] observed the presence of bacteria and fungi in ballpoint pens used by university students. Margarido et al.[10] demonstrated bacterial contamination on the coats of nursing students after their use in health care practices. Zadai et al.[11] reported the presence of pathogenic bacteria on the surfaces of cell phones of medical students.

In this context, cell phones could harbor a diverse range of species of microorganisms including antibiotic-resistant organisms known to cause nosocomial infections.

This study aimed to evaluate the presence of *S aureus* on the cell phones of university students, as well as to evaluate the antimicrobial resistance, formation of biofilms, and presence of virulence and antimicrobial resistance genes of these microorganisms.

METHODS

Collection of samples and identification of microorganisms

Samples were collected from 100 cell phones of students from the Biomedicine (20), Pharmacy (20), Dentistry (20), Nutrition (20), and Nursing (20) courses of a private university from São Paulo State, Brazil, by friction with swabs moistened with sterile physiological saline. The swabs were placed in tubes containing 5 mL of Brain Heart Infusion (Oxoid, Basingstoke, Hampshire and England) broth, and incubated at 37°C for 24 h. Then, *S. aureus* identification tests were conducted through biochemical tests according to techniques already established for Gram-positive such as the catalase test and identification of *Staphylococcus* was tested for coagulase[12]. Also, sa442 DNA fragment amplification was used for genotypic identification of *S. aureus* species[13]. The project was approved by the Research Ethics Committee of Universidade do Oeste Paulista (CAAE: 49967115.8.0000.5515).

Antimicrobial disc-diffusion technique in Agar

The antimicrobial susceptibility test was performed using the agar diffusion technique, as recommended by the Clinical Laboratory Standards Institute-CLSI[14]. The disks used for the evaluation of antimicrobial resistance were oxacillin, cefoxitin, penicillin, clindamycin, erythromycin, and levofloxacin. For the assessment of multidrug resistance, the MAR index (multiple antibiotic resistance) was determined according to Magiorakos et al.[15].

Evaluation of biofilm formation

The isolates were cultured in BHI broth at 37°C for 24 hours. The cultures were adjusted by spectrophotometry at 600 nm to the value of 0.1. Aliquots of 20 µL of the cell suspension from each isolate added to 200 µL of BHI broth present in the wells of 96 well microplates (COSTAR, Corning Inc., Lowell, MA, United States) and then incubated at 37°C for 24 hours. The plates were washed three times with 0.9% (w/v) saline to remove the unbound cells. The adhered cells were stained with 200 µL of 0.1% (w/v) crystal violet (Sigma-Aldrich, St. Louis, MO, USA), for 5 minutes. The dye was removed, the microplate washed again three times and, after drying for 30 minutes in an oven, the dye solubilization was performed with alcohol/acetone solution (80:20) (Sigma-Aldrich, St. Louis, MO, USA), The optical densities of the solution were read at a wavelength of 590 nm. The value found is representative of bacterial cell adhesion[16]. The mean optical density (OD) of the negative control (ODc) was used as the cutoff point. The isolates classified as: Non-adherent (OD ≤ ODc); Weak adherence (ODc < OD ≤ 2xODc); Moderate adherence (2xODc < OD ≤ 4xODc); Strong adherence (OD > 4xODc).

Detection of virulence and resistance genes by PCR

The phenol-chloroform technique was used to extract DNA from microorganisms[17]. In brief, 1000 µl of bacterial pellet sediment were mixed to 500 µl of lysis buffer [100 mM Tris–HCl (pH=8.0), 50 mM EDTA (pH=8.0), SDS 10%] (Sigma-Aldrich, St. Louis, MO, USA) for one hour 60 °C. When incubation was completed, the supernatant was recovered, washed with 1 ml FCl solution (phenol-chloroform-isoamyl alcohol 25:24:1) (Sigma-Aldrich, St. Louis, MO, USA), and centrifuged at 9,000 rpm for 10 min at 4 °C. This procedure was repeated with the Cl solution (chloroform-isoamyl alcohol 24:1) and the supernatant incubated with RNAse (Takara Biotechnology Co. Ltd, Dalian, Liaoning China) for 30 min at 37 °C. It was then precipitated with cold isopropanol and 0.1 volumes of 3 M sodium acetate, and

incubated overnight at -20 °C. It was then centrifuged at 9,000 rpm for 10 min at 4 °C, the supernatant decanted, and the pellet washed with cold 70% ethanol. Finally, it centrifuged, the supernatant decanted, the remaining contents allowed to dry, and these resuspended in 50 µl TE buffer.

DNA concentration was evaluated with a nano-spectrophotometer (ND-1000 Nanodrop® Thermo Fisher Scientific, Waltham, MA, USA), using the 260/230 and 260/280 wavelength ranges. Quality was determined by electrophoresis in 1% gel to measure sample contamination and degradation. Subsequently, the DNA was quantified, evaluated for purity and quality and maintained at a temperature of -20˚C. The genotypic analysis of the strains was based on the genetic amplification from the PCR technique (polymerase chain reaction) by using the amplification protocol and the oligonucleotides (Table 1) used for the detection of genes *sa442, IcaA, IcaD, mecA, mecC, SEA, SEB, SEC,* and *SED as* described according to Martineau et al.[13].

As described by Milheiriço et al.[18] strains positive for the *mec*A gene typed by the multiplex PCR technique. All assays were performed in a thermocycler. The optimal cycling conditions were the following: 94°C for 4 min; 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 4 min. Each PCR mixture, in a final volume of 50 µl, obtained 5 ng of chromosomal template; 1× PCR buffer with 1.5 mM $MgCl_2$ (Applied Biosystems, Foster City, California, EUA), 40 µM (each) deoxynucleoside triphosphate; 0.2 µM primers (IDT, Coralville, Iowa, USA) kdp F1 and kdp R1; 0.4 µM primers CIF2 F2, CIF2 R2, RIF5 F10, RIF5 R13, SCC*mec* III J1F, SCC*mec* III J1R, SCC*mec* V J1 F, and SCC*mec* V J1 R; 0.8 µM primers mecI P2, mecI P3, dcs F2, dcs R1, mecA P4, mecA P7, ccrB2 F2, ccrB2 R2, ccrC F2, and ccrC R2; and 1.25 U of Amplitaq DNA polymerase (Applied Biosystems, Foster City, California, EUA). Primers sequences shown in Table 2.

Table 1: Primers used in PCR amplifications.

Target gene	Sequence (5' – 3')	Amplicon (pb)
Sa442	$Sa442_F$: AAT CTT TGT CGG TAC ACG ATA TTC ACG $Sa442_R$: CGT AAT GAG ATT TCA GTA AAT ACA ACA	108
icaD	$icaD_F$: ATG GTC AAG CCC AGA CAG AG $icaD_R$: CGT GTT TTC AAC ATT TAA TG	198
icaA	$icaA_F$: ACA GTC GCT ACG AAA AGA AA $icaA_R$: GGA AAT GCC ATA ATG AGA AC	699
SEA	SEA_F: TTG GAA ACG GTT AAA ACG AA SEA_R: GAA CCT TCC CAT CAA AAA CA	120
SEB	SEB_F: TCG CAT CAA ACT GAC AAA CG SEB_R: GCA GGT ACT CTA TAA GTG CC	478
SEC	SEC_F: GAC ATA AAA GCT AGG AAT TT SEC_R: AAA TCG GAT TAA CAT TAT CC	257
SED	SED_F: CTA TGG TAA TAT CTC CT SED_R: TAA TCG TAT ATC TTA TAG GG	317

The evaluation of the obtained results was performed through visualization of electrophoresis in 3% agarose gel (Applied Biosystems, Foster City, California, EUA). stained with ethidium bromide. The controls used in the reaction were the *S. aureus* strains COL I, PER IA, HV25 III, and MW2 IV described by Milheiriço et al.[18] while the strain *S. aureus* ATCC 25923 used as a negative control for *mecA* and virulence factors genes. *Staphylococcus epidermidis* 12228 used as a negative control for the detection of gene *sa442*.

Data analysis

The experiments were performed in triplicate and the final results were submitted to statistical analysis. For statistical analysis, the chi-square test used to compare the results. The data analyzed with Prisma software, considering a level of significance of 0.05.

RESULTS

S. aureus identified in 40% of 100 samples. Among the courses evaluated, the samples obtained from the cell phones of students of the nursing course presented the highest percentage of contamination (65%), followed by Dentistry (50%), Biomedicine (40%). The courses Pharmacy (25%) and Nutrition (20%) presented a lower value compared to cell phones of students of the nursing course ($p<0.05$).

In the present work, the antimicrobial susceptibility of *S. aureus* isolated from the cell devices was also evaluated. A high percentage of resistance to Penicillin (85%) was observed. However, low levels of resistance ($p<0.05$) were found for Levofloxacin (2.5%) and Clindamycin (7.5%) Figure 1. The presence of multiresistant

Table 2: Primers used in multiplex PCR to classify MRSA strains.

Oligonucleotide	Sequence (5' - 3')	Amplicon (bp)
CIF2 F2 CIF2 R2	TTC GAG TTG CTG ATG AAG AAG G ATT TAC CAC AAG GAC TAC CAG C	495
ccrC F2 ccrC R2	GTA CTC GTT ACA ATG TTT GG ATA ATG GCT TCA TGC TTA CC	449
RIF5 F10 RIF5 R13	TTC TTA AGT ACA CGC TGA ATC G ATG GAG ATG AAT TAC AAG GG	414
SCCmec V J1 F SCCmec V J1 R	TTC TCC ATT CTT GTT CAT CC AGA GAC TAC TGA CTT AAG TGG	377
dcs F2 dcs R1	CAT CCT ATG ATA GCT TGG TC CTA AAT CAT AGC CAT GAC CG	342
ccrB2 F2 ccrB2 R2	AGT TTC TCA GAA TTC GAA CG CCG ATA TAG AAW GGG TTA GC	311
kdp F1 kdp R1	AAT CAT CTG CCA TTG GTG ATG C CGA ATG AAG TGA AAG AAA GTG G	284
SCCmec III J1 F SCCmec III J1 R	CAT TTG TGA AAC ACA GTA CG GTT ATT GAG ACT CCT AAA GC	243
mec I P2 mec I P3	ATC AAG ACT TGC ATT CAG GC GCG GTT TCA ATT CAC TTG TC	209
mec A P4 mec A P7	TCC AGA TTA CAA CTT CAC CAG G CCA CTT CAT ATC TTG TAA CG	162

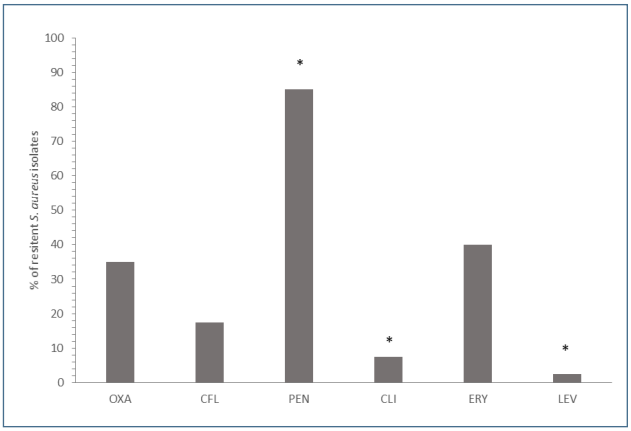

Figure 1: Antimicrobial resistance in *S. aureus* isolated from cell phones. OXA: oxacillin; CFL: cephalothin; PEN: penicillin; CLI: clindamycin; ERY: erythromycin; LEV: levofloxacin. *p<0,05

isolates was also evaluated. Approximately 57.5% of the isolates were considered multiresistant since they demonstrated resistance to two or more of the antimicrobials evaluated. It highlighted a particular sample, isolated from a student's cell phone in the nursing course, which presented resistance to 5 antimicrobials (Table 3), presenting a MAR index of 0.83.

The ability of *S. aureus* isolates to form biofilms was evaluated. Among the isolates, the following were observed: 10% non-adherent, 32.5% weak adherence, 50% moderate adherence, and 7.5% strong adherence (Figure 2). Despite the presence of microorganisms considered to be strong biofilm formers in the cell phones of students of the pharmacy and nursing students, the presence of microorganisms with weak adherence or non-adherence was also observed, demonstrating the great diversity of the samples obtained ($p<0.05$).

The sa442 DNA fragment was used for genotypic identification of *S. aureus* species. In the present study, the amplification of this fragment was evaluated by polymerase chain reaction (PCR) and positivity was observed in 100% of phenotypically identified *S. aureus* samples. It also investigated the presence of *IcaA* and *IcaD* genes and the isolates presented a frequency of 92.5% and 60%, respectively. Also, it observed that all samples that demonstrated a positive biofilm adhesion profile presented one of the analyzed genes.

Results demonstrated that 25% of the isolates presented the *mecA* gene, however none of the samples were positive for the *mecC* gene. Multiplex PCR assay for typing of Staphylococcal Cassette Chromosome Mec (SSCmec) showed that 20% SCCmec I, 10% SCCmec III, and 70% of the samples could not be typified by the technique, presenting distinct bands of evaluation. The most prevalent enterotoxin gene was *SEA* (30%) followed by the *SEC* gene (2.5%). The presence of *SED* and *SEB* genes was not observed in any of the isolates. The presence of *SEA* and *SEC* genes at the same time was observed in 2% of the samples.

DISCUSSION

The constant handling of cell devices favors the transmission of microorganisms, especially those associated with direct contamination through contact with the skin, saliva, and secretions[1]. Zakai et al.[19] identified *S. aureus* in 16% of samples from the cell phones of medical students. As demonstrated here, the nursing course presented the highest percentage of contamination. Nursing students are likely to become reservoirs of *S. aureus* since hospital practice is part of their education process and usually begins in the first years of the course. This fact could make the adhesion of pathogens easier on the surface of cell devices[20,21].

Biofilm plays a key role in the survival of bacterial species in diverse and hostile environments. It's believed that approximately 65% of human bacterial infections are associated with a biofilm. Bacteria associated with biofilms are generally resistant to antibiotics and present important virulence factors[22,23]. Marks et al.[24] demonstrated that microorganisms could survive the hostile environment and be spread through a hospital environment contaminated by biofilms. In this way, this work evidenced that mobile phones used in hospital or healthcare settings may be the focus of transmission of pathogenic bacteria due to their ability to form biofilms.

Polysaccharide intercellular adhesion (PIA) is an important aspect of biofilm production and is encoded by the chromosomal intercellular adhesion (*ica*) locus, consisting of the *icaADBC*. Among them, the *icaA* and *icaD* genes have been reported to play a significant role in biofilm production[25]. All samples that demonstrated a positive biofilm adhesion profile presented one of the analyzed genes. The strains that presented two genes simultaneously (53%) were classified with moderate to strong adherence, in this way, it is possible to correlate that the two genes together play a significant role in the formation of biofilm. A high percentage of non-adherent isolates with single ica locus genes was observed, generating great concern since these genes can be expressed under the effect of a stimulus and thus highlight the need for its expression by quantitative PCR[26].

Penicillin promotes the blockade of the synthesis of the peptidoglycan layer of the cell wall of the bacteria, thus inhibiting the synthesis of the cell wall. Resistance to this antibiotic had the highest percentage among the isolates of this study. Also, it highlighted the frequent presence of multidrug resistance[27]. Similarly, Silva et al.[28] evaluated the antimicrobial susceptibility of *S. aureus* isolated from nurses and found that resistance to penicillin was close to 100%.

An important mechanism of *S. aureus* resistance to antimicrobials is provided by the *mec*A gene, present in the mobile genetic element designated as the staphylococcal chromosome cassette (SCCmec). Methicillin resistance in *S. aureus* (MRSA) is due to

Table 3: Results obtained by antimicrobial susceptibility test, biofilm formation and PCR amplification from *S. aureus* isolates from cell phones of university students

Strain	Course	Resistance	Biofilm Formation	icaA	icaD	mecA	mecC	SCCmecI	SCCmecIII	SEA	SEB	SEC	SED
1	Biomedicine	PEN,	Weak	+	-	-	-	-	-	-	-	-	-
3	Biomedicine	PEN,OXA,	Moderate	+	+	-	-	-	-	-	-	-	-
4	Biomedicine	PEN, OXA,	Moderate	+	+	-	-	-	-	-	-	-	-
6	Biomedicine	PEN, ERY,	Moderate	+	-	+	-	-	-	-	-	-	-
7	Biomedicine	PEN, OXA,	Moderate	+	+	-	-	-	-	+	-	-	-
9	Biomedicine	PEN, ERY, OXA,	Moderate	+	+	+	-	-	-	+	-	-	-
14	Biomedicine	PEN, OXA,CFL,	Moderate	+	+	+	-	-	+	+	-	+	-
16	Biomedicine	CLI, LEV	Weak	+	-	+	-	-	-	+	-	-	-
21	Dentistry	PEN, OXA,	Moderate	+	+	-	-	-	-	+	-	-	-
22	Dentistry	PEN,	Moderate	+	+	+	-	-	-	+	-	-	-
23	Dentistry	PEN, OXA,	Weak	+	+	-	-	-	-	-	-	-	-
24	Dentistry	PEN,	Moderate	+	-	-	-	-	-	+	-	-	-
25	Dentistry	PEN,	Moderate	+	+	-	-	-	-	+	-	-	-
26	Dentistry	PEN, ERY,	Moderate	+	+	-	-	-	-	+	-	-	-
29	Dentistry	PEN,	Moderate	+	+	-	-	-	-	-	-	-	-
30	Dentistry	PEN,	Moderate	+	+	-	-	-	-	+	-	-	-
31	Dentistry	PEN,	Moderate	+	+	-	-	-	-	+	-	-	-
40	Dentistry	PEN,	Moderate	+	+	-	-	-	-	-	-	-	-
53	Nutrition	PEN, ERY,	Moderate	+	+	+	-	+	-	+	-	-	-
56	Nutrition	PEN,	Weak	+	-	-	-	-	-	-	-	-	-
57	Nutrition	PEN,	Not adherent	+	-	-	-	-	-	-	-	-	-
58	Nutrition	PEN,	Weak	+	-	+	-	-	-	-	-	-	-
62	Pharmacy	PEN, ERY, OXA, CFL,	Weak	+	-	+	-	+	-	-	-	-	-
66	Pharmacy	PEN, ERY,	Weak	+	-	-	-	-	-	-	-	-	-
67	Pharmacy	PEN, ERY, OXA, CFL,	Weak	+	-	+	-	-	-	-	-	-	-
71	Pharmacy	PEN, ERY, OXA, CFL,	Strong	+	+	-	-	-	-	-	-	-	-
80	Pharmacy	-	Strong	+	+	-	-	-	-	-	-	-	-
82	Nursing	PEN, ERY, OXA, CLI,	Moderate	+	+	-	-	-	-	-	-	-	-
83	Nursing	PEN, ERY,	Weak	+	-	-	-	-	-	-	-	-	-
85	Nursing	PEN, CFL,	Strong	+	+	-	-	-	-	-	-	-	-
86	Nursing	PEN, ERY, OXA,	Not adherent	+	-	-	-	-	-	-	-	-	-
87	Nursing	PEN,	Weak	+	-	-	-	-	-	-	-	-	-
88	Nursing	PEN, ERY,	Weak	-	+	-	-	-	-	-	-	-	-
90	Nursing	ERY,	Weak	-	+	-	-	-	-	-	-	-	-
93	Nursing	PEN,	Weak	+	-	-	-	-	-	-	-	-	-
94	Nursing	PEN, OXA, CFL,	Not adherent	+	-	-	-	-	-	-	-	-	-
95	Nursing	ERY,	Moderate	+	+	-	-	-	-	-	-	-	-
96	Nursing	PEN, ERY, OXA, CFL, CLI,	Weak	+	-	-	-	-	-	-	-	-	-
97	Nursing	-	Moderate	+	+	+	-	-	-	-	-	-	-
100	Nursing	PEN, ERY,	Not adherent	-	+	-	-	-	-	-	-	-	-

Legend: OXA: Oxacillin; CFL: Cephalothin; PEN: Penicillin; CLI: Clindamycin; ERY: Erythromycin; LEV: Levofloxacin. + positive - negative

https://doi.org/10.7322/abcshs.2020154.1608

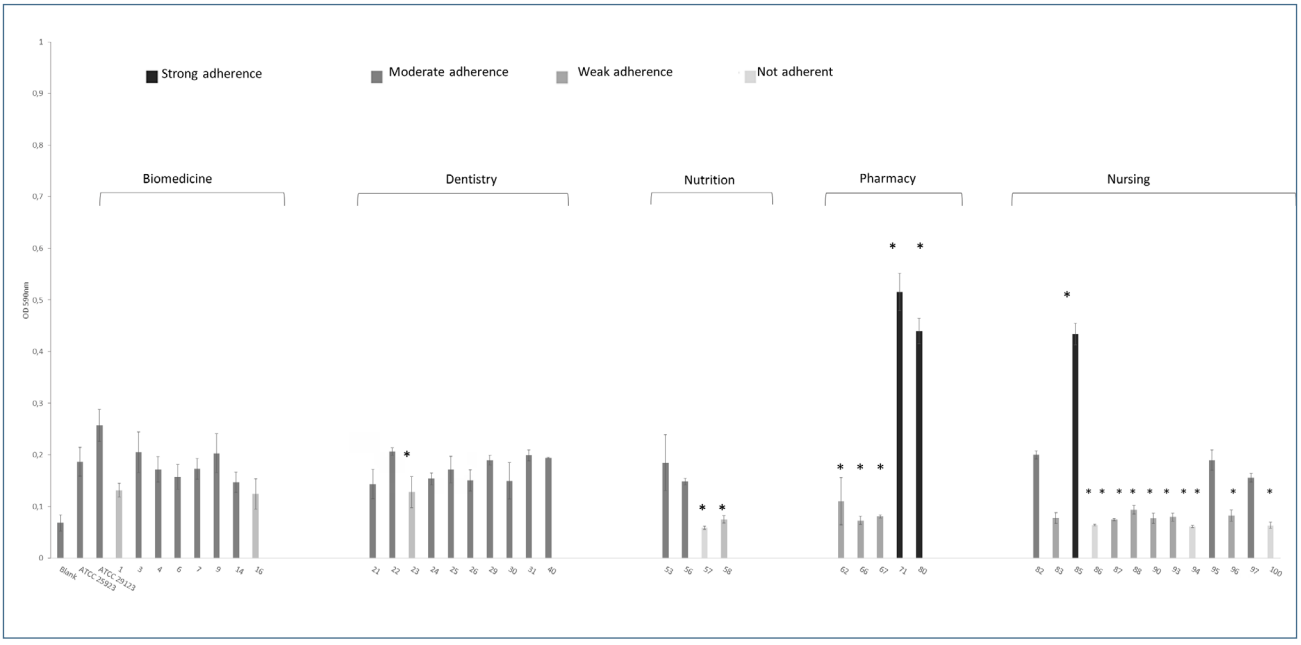

Figure 2: Evaluation of biofilm formation by *S. aureus* through the microplate adhesion crystal violet staining technique. *Statistically different from *S. aureus* ATCC 25923.

the presence of the *mecA* gene. Currently MRSA is an important pathogen that causes severe morbidity and mortality worldwide. MRSA strains are endemic in many countries and may be present in more than 50% of clinical isolates[29]. The homolog of the *mecA* gene, called the *mecC* gene, is also located in the SCCmec and produces a similar phenotypic profile of antimicrobial resistance[29]. In the present study, it was possible to observe that 25% of the isolates presented the *mecA* gene, however none of the samples were positive for the *mecC* gene. The low number of samples could justify the absence of *mecC* gene in samples, which was a major limitation in our study.

According to the combination of ccr allotypes with the mec gene complex, 11 types (I-XI) of SCCmec have already been reported[29]. The results demonstrated the presence of SCCmecI, SCCmecIII. SCCmec I is the chromosomal cassette that carries no transposons or plasmid that confer resistance to drugs other than methicillin and heavy metals. SCCmec III carries genes like *mecA* and *mecRI*, together with transposons and plasmids that form resistance to metals such as cadmium and mercury and also to antimicrobials such as tetracycline and oxacillin, being considered the oldest truly pandemic MRSA strain[30].

In the present work, staphylococcal enterotoxins (SEs) also investigated, since enterotoxigenic *S. aureus* is considered the second most prevalent pathogen in foodborne diseases in Brazil[31]. The most prevalent gene was the *SEA* toxin, commonly related to cases of intoxication, corresponding to 75% of outbreaks, and also correlated with human food contamination[32].

The data of this work emphasize that cell phones used in the healthcare environment allow the transmission of bacteria that harbor genes of virulence and resistance. The cleaning and periodic disinfection of cellular devices can contribute to reducing the risk of both nosocomial infection rates and those in the community as well as lowering the morbidity/mortality from these infections. In this way, it is necessary to raise awareness about the disinfection of mobile phones among health professionals since this is an extremely useful tool in the medical field.

REFERENCES

1. Selim HS, Abaza AF. Microbial contamination of mobile phones in a health care setting in Alexandria, Egypt. GMS Hyg Infect Control. 2015;10:1-9. http://doi.org/10.3205/dgkh000246

2. Chang CH, Chen SY, Lu JJ, Chang CJ, Chang Y, Hsieh PH. Nasal colonization and bacterial contamination of mobile phones carried by medical staff in the operating room. PLoS One. 2017;12(5):e0175811. http://doi.org/10.1371/journal.pone.0175811

3. Pillet S, Berthelot P, Gagneux-Brunon A, Mory O, Gay C, Viallon A, et al. Contamination of healthcare workers' mobile phones by epidemic viroses. Clin Microbiol Infect. 2016;22(5):456.e1-6. http://doi.org/10.1016/j.cmi.2015.12.008

4. Bamigboye BT, Olowe OA, Taiwo SS. Phenotypic and molecular identification of vancomycin resistance in clinical *Staphylococcus aureus* isolates in Osogbo, Nigeria. Eur J Microbiol Immunol. 2018;8(1):25-30. http://doi.org/10.1556/1886.2018.00003

5. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharm Therap. 2015;40(4):277-83.

6. Zaman SB, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain H. A review on antibiotic resistance: alarm bells are ringing. Cureus. 2017;9(6):e1403. http://doi.org/10.7759/cureus.1403

7. Costa KAD, Ferenz M, Silveira SM, Mellezi AF. Formação de biofilmes bacterianos em diferentes superfícies de indústrias de alimentos. Rev Inst Laticínios Cândido Tostes. 2016;71:72-85. https://doi.org/10.14295/2238-6416.v71i2.512

8. Graziano MU, Graziano KU, Pinto FMG, Bruna CQM, Souza RQ, Lascala CA. Eficácia da desinfecção com álcool 70% (p/v) de superfícies contaminadas sem limpeza prévia. Rev Latino-Am Enfermagem. 2013;21(2):618-23. https://doi.org/10.1590/S0104-11692013000200020

9. Garcia CTP, Saleh DMFVB, Sasagawa SM, Mimica LMI, Ueda SMY. Pesquisa de micro-organismos em canetas esferográficas utilizadas por estudantes universitários. Arq Med Hosp Fac Cienc. 2012;57(1):6-10.

10. Margarido CA, Boasi TMC, Mota VS, Silvai CKM, Poveda VN. Contaminação microbiana de punhos de jalecos durante a assistência à saúde. Rev Bras Enferm 2014;64(1):127-32. https://doi.org/10.5935/0034-7167.20140017

11. Zakai A, Mashat A, Abumohssin A, Samarkandi A, Almaghrab B, Barradah H, et al. Bacterial contamination of cell phones of medical students at King Abdulaziz University, Jeddah, Saudi Arabia. J Microsc Ultrastruct. 2016;4(3):143-6. https://doi.org/10.1016/j.jmau.2015.12.004

12. Mendoça RGM, Olival GS, Mimica LMJ, Navarini A, Paschoalotti MA, Chieffi PP, et al. Potencial infeccioso do transporte público de passageiros da cidade de São Paulo. Arq Med Hosp Fac Cienc Med Santa Casa São Paulo. 2008;53(2):53-7.

13. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG. Species-specific and ubiquitous-dna-based assays for rapid identification of *Staphylococcus aureus*. J Clin Microbiol. 1998;36(3):618-23. https://doi.org/10.1128/JCM.36.3.618-623.1998

14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI supplement M100. Pennsylvania: Wayne, PA: Clinical and Laboratory Standards Institute, 2019.

15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 2012;18(3):268-81. https://doi.org/10.1111/j.1469-0691.2011.03570.x

16. Tiba MR, Nogueira GP, Leite DS. Study on virulence factors associated with biofilm formation and phylogenetic groupings in *Escherichia coli* strains isolated from patients with cystitis. Rev Soc Bras Med Trop. 2009;42(1):58-62. https://doi.org/10.1590/S0037-86822009000100012

17. Sambrook J, Russell DW. Molecular cloning - a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory Press, 2001.

18. Milheiriço C, Oliveira DC, Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2007;51(9):3374-7. https://doi.org/10.1128/AAC.00275-07

19. Bodena D, Teklemarian Z, Balakrishnan S, Tesfa T. Bacterial contamination of mobile phones of health professionals in Eastern Ethiopia: antimicrobial susceptibility and associated factors. Trop Med Health. 2019;47:5. https://doi.org/10.1186/s41182-019-0144-y

20. Carvalho MSM, Andrade DFR, Sousa AFL, Valle ARMC, Freitas DRJ, Nascimento GC, et al. Nasal colonization with *Staphylococcus aureus* in nursing students: ground for monitoring. Rev Bras Enferm. 2016;69(9):1046-51. https://doi.org/10.1590/0034-7167-2016-0210

21. Silva ECBF, Samico TM, Cardoso RR, Rabelo MA, Bezerra Neto AM, Melo FL, et al. Colonization by *Staphylococcus aureus* among the nursing staff of a teaching hospital in Pernambuco. Rev Esc Enferm USP. 2012;46(1):132-7. https://doi.org/10.1590/S0080-62342012000100018

22. Iyamba JLM, Takaisi-Kikune NM, Dulanto S, Dehay JP. Study of the Adhesion of Clinical Strains of *Staphylococcus aureus* on an Abiotic Surface Using the Biofilm Ring Test. J Biomat Nanobiotechnol. 2012;3(4A):547-56. https://doi.org/10.4236/jbnb.2012.324057

23. Xu Z, Liang Y, Lin S, Chen D, Li B, Lin L, et al. Crystal Violet and XTT Assays on *Staphylococcus aureus* Biofilm Quantification, Curr Microbiol. 2016;73(4):474-82. https://doi.org/10.1007/s00284-016-1081-1

24. Marks LR, Reddinger RM, Hakasson AP. Biofilm formation enhances fomite survival of *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Infect Immun. 2014;82(3):1141-6. https://doi.org/10.1128/IAI.01310-13

25. Taha M, Kohnen C, Mallya S, Kou Y, Zapata A, Ramirez-Arcos S. Comparative characterization of the biofilm-production abilities of *Staphylococcus epidermidis* isolated from human skin and platelet concentrates. J Med Microbiol. 2018;67(2):190-7. https://doi.org/10.1099/jmm.0.000673

26. Alberts B, Johnson A, Walter P. Biologia molecular da célula. Porto Alegre: Artmed, 2017.

27. Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol. 2010;8(6):423-35. https://doi.org/10.1038/nrmicro2333

28. ECBF Silva, Samico TM, Cardoso RR, Rabelo MA, Bezerra Neto AM, Melo FL, et al. Colonization by *Staphylococcus aureus* among the nursing staff of a teaching hospital in Pernambuco, Rev Esc Enferm USP. 2012;46(1):128-32. https://doi.org/10.1590/S0080-62342012000100018

29. Moosavian M, Shahin M, Navidifar T, Torabipour M. Typing of staphylococcal cassette chromosome *mec* encoding methicillin resistance in *Staphylococcus aureus* isolates in Ahvaz, Iran. New Microbes New Infect. 2017;21:90-4. https://doi.org/10.1016/j.nmni.2017.11.006

30. Martins A, Cunha MLRS. Methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: epidemiological and molecular aspects. Microbiol Immunol. 2007;51(9):787-95. https://doi.org/10.1111/j.1348-0421.2007.tb03968.x

31. Lentz SAM, Rivas PM, Cardoso MRI, Morales DL, Centenaro FC, Martins AF. *Bacillus cereus* as the main casual agent of foodborne outbreaks in Southern Brazil: data from 11 years. Cad Saúde Pública. 2018;34:e00057417. https://doi.org/10.1590/0102-311x00057417

32. Chaves TF. Revisão teórica das técnicas utilizadas na detecção de enterotoxinas estafilocócicas. Cienc Equatorial. 2012;2(1):2-14.