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Resistance to beta-lactams in *Staphylococcus aureus* isolated from cell phone screens of dentistry students based on an antibiogram and detection of *blaZ* and *mecA* genes

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ABSTRACT. Currently, the use of cell phones is booming within the health system. Since they are frequently used, they have become an important vehicle for nosocomial infections, with *Staphylococcus aureus* being the most commonly found pathogen. We tested for resistance to beta-lactams in *S. aureus* isolated from phone screens of senior dentistry students who were involved in pre-professional clinical practice. Out of a total of 220 students, 92 participated in the study, from which 16 *S. aureus* positive samples were obtained; these samples are relevant due to the fact that the participants were in contact with patients. The *S. aureus* genes were identified by means of a PCR and the antibiotic resistance by using the Kirby Bauer's diffusion technique. All 16 isolated strains contained the *blaZ* gene; in seven the *mecA* gene was identified. Phenotypic resistance to penicillin and oxacillin manifested in 12 and 7 strains, respectively. We conclude that dental professional cell phones have potential as a nosocomial risk for pathogenic bacterial contamination.

Key words: Beta-lactamic resistance; *Staphylococcus aureus;* Cell phone; Penicillin resistance; Methicillin resistance

INTRODUCTION

Within a hospital environment, health personnel are more likely to get microbial infections due to their daily contact with patients, which can cause serious morbidity and mortality problems. In the dental environment, the risk of cross-infection between the dentist and the patient is higher since the normal procedures require a close contact with the patient's mouth (Haun et al., 2016; Meng et al., 2020).

Currently, cell phones are among the most widely used devices by the population. Among health professionals, cell phones are necessary for communication and exchange of information. Despite its benefits, the use of cell phones generates concern because they have potential as a reservoir of microorganisms and can cause risks of nosocomial infections. Regardless of the surface of the mobile, one of the most frequently found microorganisms is *Staphylococcus aureus* (Chang et al., 2017; Kanayama et al., 2017; Galazzi et al., 2019).

S. aureus is a Gram-positive opportunistic pathogen that attains antibiotic resistance more easily than any other bacterium. It is located in the mucosa and skin of humans and animals. When these tissues are lacerated by various causes, *S. aureus* gets access to the tissue and generates mild or severe infections, such as soft tissue infection, abscesses, endocarditis, and sepsis. It can also remain symptom-free in approximately 30% of the population (Mehraj et al., 2016; Lee et al., 2018; Tam et al., 2019).

Beta-lactam antibiotics, such as penicillin and methicillin, can inhibit the proliferation of bacteria, but their indiscriminate use causes generation of resistance in *S. aureus*. The first antibiotic to which it acquired resistance was penicillin, through the *blaZ* gene that encodes the beta-lactamases, which hydrolyze the beta-lactam ring and inactivate the drug (Fuda et al., 2005; Castellano-González et al., 2010).

Since the first cases of resistance, modifications to the original molecule have been developed to create synthetic drugs such as methicillin, and thus, achieve a greater effect. Over time, the appearance of the *mecA* gene in *S.aureus* made it impossible for methicillin to exert its effect, adopting the name of methicillin-resistant *Staphylococcus aureus* (MRSA), which complicated the treatment options for *S. aureus* infections (Becerra et al., 2009; Foster, 2017; Lee et al., 2018).

Therefore, this study is focused on identifying resistance to beta-lactam in *S. aureus* isolated in cell screens from senior dentistry students by detecting the *blaZ* and *mecA* genes.

MATERIAL AND METHODS

An observational and cross-sectional investigation was carried out. In this study, out of a total of 220 students, 92 cell phones were analyzed and 16 positive samples were obtained for *S. aureus*. The participants that were included were those who voluntarily agreed to participate by signing an informed consent in order to take a sampling from their cell phones. It is necessary to mention that since a cell phone is an inanimate object, it was not necessary the approval of an institutional human research ethics committee to take samplings from the participants' phones.

The 16 strains of *S. aureus* were stored at minus 80º C in the Laboratory of Genetics and Molecular Biology of the Center for Research, Innovation and Technology Transfer (CIITT) of the Catholic University of Cuenca, further on, they were revived and

cultivated for 24 hours at 36º C in salted mannitol agar, before performing the DNA extraction with the lysis solution formed by SDS at 1% diluted in a 0.25 N sodium hydroxide solution.

A couple of colonies were taken with a bacteriological handle and placed in 1 ml of distilled water, homogenized, centrifuged at 3000 rpm for 10 minutes and the supernatant liquid was removed. We added 50 µl of the lysis solution, and boiled for 15 minutes; 450 µl of nuclease-free water was added. The sample was centrifuged for 30 seconds at 3000 rpm and preserved in an ARCTIKO-LFF270 freezer refrigerator at -20ºC up to the time of the polymerase chain reaction (PCR).

We identified the genes encoding penicillin resistance (*blaZ*) and methicillin (*mecA*) through a PCR that was carried out on an Agilent SureCycler thermocycler (Table 1) with a total volume of 20 µl, derived from: 10 µl of Mastermix GoTaq Green 2X of Promega, 1.5 µl of each initiator, 1.5 µl of white DNA and 5.5 µl of pure water (Andrade et al., 2019).

The amplicons were taken to a horizontal electrophoresis chamber BIOSTEP-GELCO UNIT and separated into agarose gels (1.5% w/v with SYBR safe) immersed in TAE 1X buffer, with a protocol of 50 V, 90 A and 60 W for 60 minutes. The results of the migration were observed on a UV transilluminator and for the calculation of the size of the amplicons they were compared with the migrations of the Allelic Ladder Trackit of Invitrogen (1 Kb Plus DNA ladder). The ATCC® 11632 was used as a positive control of the *blaZ* gene, and the *mecA* gene ATCC® 43300 strain, both of *S. aureus.* The negative control was *Streptococcus pyogenes* ATCC® 12344 (Andrade et al., 2019).

Table 1. Primers, amplified product and protocol for the identification of *blaZ* and *mecA* genes of *Staphylococcus aureus* (Andrade et al., 2019).

Gene	Size (bp)	Primer sequence 5´- 3	Protocol
blaZ	674	Fw: GTTGCGAACTCTTGAATAGG Rv: GGAGAATAAGCAACTATATCATC	94 °C x 5 min. (Initial denaturation) 34 cycles: 94°C x 1 min. 54°C x 1 min. 72°C x 1 min. 72°C x 10 min. (Final elongation)
mecA	310	Fw: GTAGAAATGACTGAACGTCCGATGA Rv: CCAATTCCACATTGTTTCGGTCTAA	94 °C x 5 min. (Initial denaturation) 30 cycles: 94°C x 1 min. 62°C x 30 sec. 72°C x 35 sec. 72°C x 10 min. (Final elongation)

By means of the Kirby Bauer's disc diffusion technique it was possible to determine the phenotypical sensitivity to penicillin and methicillin of *S. aureus* isolates. To do this, spectrophotometrically standardized bacterial inoculums were prepared with the 0.5 pattern of the MacFarland nephelometer (625nm; OJ: 0.8-1.0) for each of the strains of *S. aureus* to be examined. From this suspension, the microorganisms were transferred to a Muller Hinton preparation, in which penicillin (10U) and oxacillin (1 µg) discs were placed and incubated in aerobiosis at 35ºC for 24 hours (Andrade et al., 2019).

The inhibition halos were then measured and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines (Ghanwate et al., 2016; Andrade et al., 2019; CLSI, 2021). It is considered that there is susceptibility to penicillin G when the halos are ≥ 29 mm, and there is resistance when the halos are ≤ 28 mm. In oxacillin, sensitivity and resistance occurs when the halos are ≥ 22 mm and ≤ 21 mm, respectively (CLSI, 2021).

The Microsoft office Excel program was used to record and report the results found.

The calculation of the absolute risk was carried out with a 95% confidence interval.

RESULTS

In the 16 strains of *Staphylococcus aureus* studied, we searched for resistance genes for penicillin and methicillin, finding the *blaZ* gene in all of them, while the *mecA* gene was identified in 7 isolates (Table 2; Figure 1; Figure 2).

Table 2. Resistance of *Staphylococcus aureus* by identifying the *blaZ* and *mecA* genes.

Gene	*S. aureus* positive N	Absolute Risk (95% CI)
blaZ	16	0 (0 – 0.10)
mecA	7	0.25 (0.19 – 0.68)

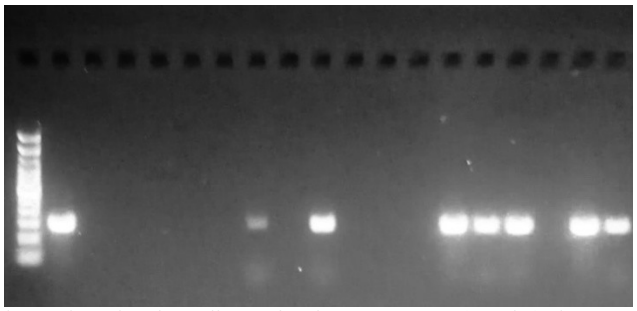

Figure 1. Result of electrophoresis of amplicons for the *mecA* gene (310 bp), lane 1: allelic ladder, lane 2: positive control, lane 3: negative control, Positive strains: rails 8, 10, 14-16, 18-19.

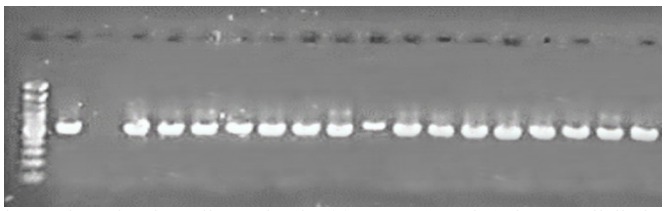

Figure 2. Result of electrophoresis of amplicons for the *blaZ* gene (674 bp), lane 1: allelic ladder, lane 2: positive control, lane 3: negative control, Positive strains: rails 4-19**.**

Among the 16 isolated strains of *S. aureus* that contained the *blaZ* gene, 12 were resistant to penicillin and 4 were sensitive. On the other hand, among the isolates that were positive for the *mecA* gene, 7 were resistant and 9 were sensitive to oxacillin (Figure 3).

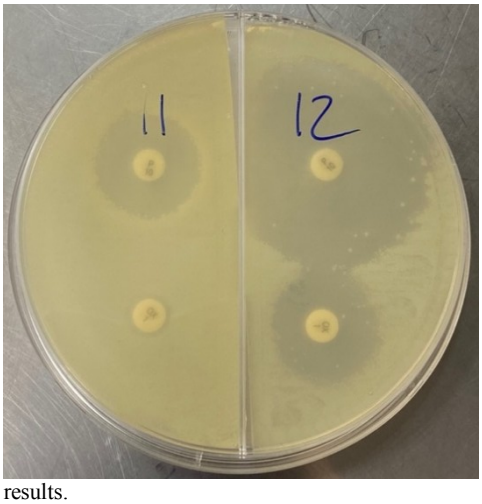

Figure 3. Typical antibiogram results.

DISCUSSION

About 77% of the world's population has a mobile phone, which has become the most common used accessory for communication. This accessory is widely used in various areas of health, which results in an equipment of easy contamination and a potential vehicle for the transmission of pathogenic bacteria for humans (Al Momani et al., 2019; Bodena et al., 2019; Noumi et al., 2020).

In Ecuador, no studies have been conducted on the genetic variability and antimicrobial resistance of *S. aureus* isolated in the dental health environment. Therefore, we investigated the phenotypic and genotypic resistance to beta-lactam of *S. aureus*, restricting sampling to the cell phone screens of dental students. The isolates of *S. aureus* were identified through the detection of the *mecA* and *blaZ* genes; resistance to penicillin and methicillin was subsequently determined.

The detection of resistance genes in the genome of *S. aureus* by PCR, showed the presence of the *mecA* gene in 7 of 16 isolated strains. This result differs from the one obtained in 2019 by Cave et al. (7.9%) in a London hospital. Other authors found higher percentages than those observed in this study. Thus, Ghanwate et al. (2016) in their research at a health center in India reported 78% of strains with the presence of the *mecA* gene. Similarly, Seng et al. (2017), reported the *mecA* gene in 70% of strains isolated in a hospital in Thailand. Probably, the low incidence found in the study is due to the fact that it was focused on samples of cell phone screens and not from hospital surfaces.

The 7 of the 16 isolates of *S. aureus* in this study were resistant to oxacillin according to the parameters of the CLSI. Similar percentages of MRSA were presented in 2015 by Sayed et al. (53%) and in 2017 were presented by Morubagal et al. (47%). On the other hand, Kanayama et al. (2017) reported 2.3%, Siqueira et al. (2019) 3.3%, Pal et al. (2015) 7.9% and Pathare et al. (2016) 9%, whose values were lower.

The ease that *S. aureus* acquires resistance to different antimicrobials is due to its genomic plasticity (Fuda et al., 2005). The prevalence of MRSA varies greatly from place to place, for example, in Europe and the United States the figures for MRSA are between

29% and 35%, while in underdeveloped countries it is between 30% and 70%. These data generate concern in the field of health because the impact of MRSA infections in low-income countries could be more severe (Phatare et al., 2016).

In the dentistry field MRSA could be transmitted directly (blood or saliva) or indirectly (instrumental). It is necessary to make the population aware that MRSA infections are not exclusive to hospital areas, but they are also common in the community environment (Khairalla et al., 2017). According to a study conducted in Egypt by Khairalla et al. (2017), there is a link between over-the-counter medicines, inappropriate consumption of antimicrobials, and antibiotic resistance.

In our study, all isolates had the *blaZ* gene. In contrast to our study, Cave et al. (2019) and Andrade et al. (2019), stated in their research that the gene occurred in 90% and 67%respectively.

The fraction of strains resistant to penicillin by antibiogram was 3/4, a figure similar to that was obtained by Cave et al. (2019) with 80%. The opposite was found in a study conducted by Al Momani et al. (2019) with 29%.

All strains isolated in our study manifested the *blaZ* gene, which is related to resistance to penicillin. However, there were strains found that contained the gene but did not manifest resistance to penicillin, probably because the gene was not expressed. This genotypic and phenotypic discordance is linked to point alterations at the site of gene acquisition (Jiménez et al., 2020). The complexity of genetic changes and mechanisms related to antibiotic resistance in strains of *Staphylococcu*s, has shown that the resistance or sensitivity to a particular antimicrobial does not only depend on the presence or lack of the resistance gene (Foster, 2017; Jiménez et al., 2020).

There is not much data that associate the contamination of mobile devices with contagion of the patient (Haun et al., 2016). However, to prevent possible spread of bacteria it is recommended to clean the surfaces of the cell phone, wash hands, or even use silicone covers for mobile phones (Ulger et al., 2015).

CONCLUSION

In conclusion, mobile phones can carry bacterial agents such as MRSA or other bacteria resistant to multiple antimicrobials. Our study reveals a high incidence of pathogens resistant to penicillin, and less so to methicillin. This shows that MRSA is a pathogen characteristic of the dental field**.**

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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