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Detection of *blaZ* and *mecA* genes and antimicrobial susceptibility in *Staphylococcus aureus* colonizing multipurpose boxes of dentistry students

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ABSTRACT. Cross-contamination between patient and dentist is a real threat that has not been adequately studied. *Staphylococcus aureus*, through its characteristic genetic plasticity, has managed to develop multiple virulence and antibiotic resistance proteins. The antibiotic susceptibility profile and the presence of the *blaZ* and *mecA* genes that encode resistance to penicillin and methicillin, respectively, were analyzed in strains isolated from multipurpose boxes used by dental students at the Catholic University of Cuenca. These boxes are used to transport instruments and material. From the universe of study (249 boxes) 139 samples were obtained from boxes of the students who accepted and signed a consent to participate. Eight strains of *S. aureus* were identified, of which, through antibiogram analysis, it was found that seven were resistant to penicillin and two strains resistant to cefoxitin (MRSA strains). In molecular analysis, the *mecA* gene was identified in two strains, while the *blaZ* gene was found in all of them. It was concluded that the rate of *S. aureus* found in this study was low due to various factors, possibly including increased vigilance and cleanliness due to the COVID-19 pandemic during the study.

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

INTRODUCTION

Multipurpose boxes are small containers in which dental students transport instruments and materials needed for their pre-professional practices with patients. These boxes require permanent cleaning and disinfection that are not always carried out properly, thus favoring the contamination of their surfaces and therefore of all the content that they carry and transport.

Staphylococcus aureus, an opportunistic, ubiquitous, Gram-positive bacterium that grows in both, aerobic and anaerobic environments that are found at temperatures between 18 to 40º C. It is known to be one of the most frequent microorganisms, identified in up to 96% of these types of boxes according to a previous study, against a 36% frequency of *Moraxella catarrhalis*, 20% of *Staphylococcus epidermidis* and 10% of *Actynomyces* (Klevens et al., 2008; Xolo et al., 2012; Medina et al., 2021).

The nose, skin and oral cavity of a high percentage of human beings (around 30-50%) are reservoirs of *S. aureus* and cross-contamination could eventually be generated between patients and dentists through non-sterile dental instruments, this pathogen can easily contaminate and survive for a long period of time (months) on inanimate surfaces (Laica et al., 2021; Medina et al., 2021). Reyes et al. (2012), reported that after carrying out disinfection processes on handpieces with 2% glutaraldehyde, 5% sodium hypochlorite or 70% alcohol, it could still be isolated on blood agar, for which they recommend sterilization in autoclave to prevent spread.

The epidemiological importance of this nosocomial microorganism lies in its enormous genetic plasticity that has endowed it with a varied arsenal of virulence proteins. This, as a result, makes it one of the most worrying nosocomial pathogens (Laica et al., 2021; Pacheco et al., 2021).

The antimicrobial treatment of *S. aureus* infection is also difficult, owing the fact that it has a great capability of generating resistance to antibiotics, particularly beta-lactams through the expression of genes such as the *blaZ* gene that encodes resistance to penicillin and the *mecA* that codes for methicillin resistance through the production of proteins such as beta-lactamases and PBP2a, respectively (Pereira et al., 2014; Galarce et al., 2016). This characteristic has created it a very important health problem worldwide, so the goal of this study is to identify the microbiological and molecular antibiotic resistance in strains present in plastic boxes used to transport dental student instruments.

MATERIAL AND METHODS

The cross-sectional observational case that was carried out had a universe of study of 249 multipurpose boxes that were used to transport dental instruments of students from the last semesters of dentistry. From this universe, 139 students agreed to participate by signing an informed consent. Upon the authorization, the sampling was performed by swabbing the internal surface and handles of the boxes with a sterile swab moistened in trypticase soy broth, these were then placed in a tube with 5 ml of the same substance. After taking the samples, the tubes were immediately transported to the

Genetics and Molecular Biology Laboratory of the Center for Research, Innovation and Technology Transfer (CIITT) of the Catholic University of Cuenca for processing (Sanmartín et al., 2021; Villalta et al., 2021). This study was approved by the institutional ethics committee of the University.

Microbiological study

In the laboratory, samples were placed at 37°C in a Memmert incubator for 24 hours and then plated on mannitol salt agar. To carry out the Gram stain, the boxes with bacterial growth that had fermented and turned the mannitol to a yellow color were selected. In order to confirm microbiological identification, colonies with characteristics of Gram-positive cocci underwent DNAse and coagulase tests (CLSI, 2017; Sanmartín et al., 2021).

The strains of S. aureus, identified by microbiological procedures, were tested for antibiotic sensitivity using the Kirby Bauer method with the following antibiotic discs: erythromycin 15mcg, clindamycin 2mcg, rifampin 5mcg, linezolid 30mcg, penicillin 10U, chloramphenicol 30mcg, gentamicin 10mcg, trimethoprim/sulfamethaxazole 1.25/23.75mcg, tetracycline 30mcg, moxifloxacin 5mcg and cefoxitin 30mcg.

Molecular study

DNA extraction was performed on Gram-positive cocci colonies that were also positive for DNAse and coagulase, for which a sample was taken from the colony with a bacteriological loop and placed in an eppendorf tube with 1 mL of distilled water. It was later homogenized in a vortex shaker and centrifuged at 10,000 rpm for 5 minutes, the supernatant was discarded and 50 µL of a lysis solution consisting of Sodium Dodecyl sulfate (SDS) at 1% diluted in NaOH 0.25N was added to the pellet. It was homogenized again and in a thermal block, it was brought to 98°C for 15 minutes. Subsequently, 450 µL of nuclease-free water was added, centrifuged for 20 seconds at 5000 rpm and stored at -20°C until molecular identification of *S. aureus* by amplification of the *nucA* and *femB* genes (Villalta et al., 2021).

With the DNA extracted from the samples, the polymerase chain reaction (PCR) of the *nucA* and *femB* genes was performed in an Agilent SureCycler thermocycler at a final volume of 20 µL, for which 10 µL of Green master mix, Go Taq 2X, 5 µL of nuclease-free water, 1.5 µL of each primer, and 2 µL of DNA from each sample was used.

Obtained amplicons were migrated together with an Invitrogen Trackit allelic ladder (1 Kb Plus DNA ladder), in 50 mL of 2% w/v agarose gel in 1X TAE buffer with 2 µL of SYBR safe in a horizontal electrophoresis chamber BIOSTEP-GELCO UNIT, with a migration protocol of 50V, 60A and 50W for 70 minutes. Finally, it was taken to a UV transilluminator to develop the results and document them with a digital camera.

The protocols for the identification of the *nucA* and *femB* genes, used for molecular confirmation of *S. aureus* strains, as well as the *blaZ, mecA* and *vanA* genes,

which code for penicillin, methicillin and vancomycin resistance proteins, respectively, were developed according to the details of the publications referenced in Table 1.

Table 1. PCR data of the genes: *nucA, femB, blaZ, mecA* and *vanA* (Dutka et al., 1995; Olsen et al., 2006; Elhassan et al., 2015; Hamdan et al., 2016).

Gene	Primer sequence	Amplification protocol	Size	Reference
nucA	Fw: GCGGATGGTGBTAGGGTT Rv: AGCCAAGCCTTGACGAACTAAAGC	94°C x 5 min **10 cycles:** 94°C x 40 seg 68°C x 40 seg 72°C x 1 min **25 cycles** 94°C x 1 min 58°C x 1 min 72°C x 2 min **Final Elongation:** 72°C 10 min	270 pb	Hamdan et al., 2016
femB	Fw: TTACAGAGTTAACTGTTACC Rv: ATACAAATCCAGCACGCTCT	94°C x 5 min **35 cycles:** 94°C x 45 seg 50°C x 45 seg 72°C x 1 min **Final Elongation:** 72°C x 5 min	651pb	Hamdan et al., 2016
blaZ	Fw: GTTGCGAACTCTTGAATAGG Rv: GGAGAATAAGCAACTATATCATC	94 °C x 5 min **34 cycles:** 94°C x 1 min 54°C x 1 min 72°C x 1 min **Final Elongation:** 72°C x 10 min	674pb	Olsen et al., 2006
mecA	Fw: GTAGAAATGACTGAACGTCCGATGA Rv: CCAATTCCACATTGTTTCGGTCTAA	94 °C x 5 min **30 cycles:** 94°C x 1 min 62°C x 30 seg 72°C x 35 seg **Final Elongation:** 72°C x 10 min	310pb	Harrison et al., 2014; Elhassan et al., 2015
vanA	Fw: GGGAAAACGACAATTGC Rv: GTACAATGCGGCCGTTA	94 °C x 2 min **30 cycles:** 94°C x 1 min 54°C x 1 min 72°C x 1 min **Final Elongation:** 72°C x 10 min	732pb	Dutka S, Evers S., 1995

RESULTS

In the 139 samples taken, the microbiological identification and molecular confirmation of eight strains of *S. aureus* was performed, which represents 5.75% of samples contaminated with this pathogen.

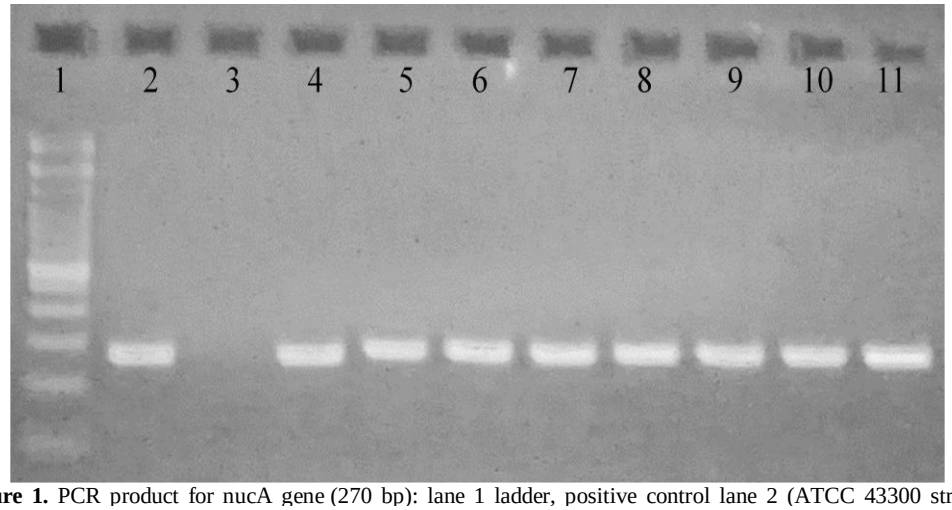

Figure 1. PCR product for nucA gene (270 bp): lane 1 ladder, positive control lane 2 (ATCC 43300 strain), negative control lane 3 (ATCC 12344 Streptococcus pyogenes strain), lanes 4 to 11 positive samples (06, 35, 36, 56, 88, 122, 133, 136) for the nucA gene.

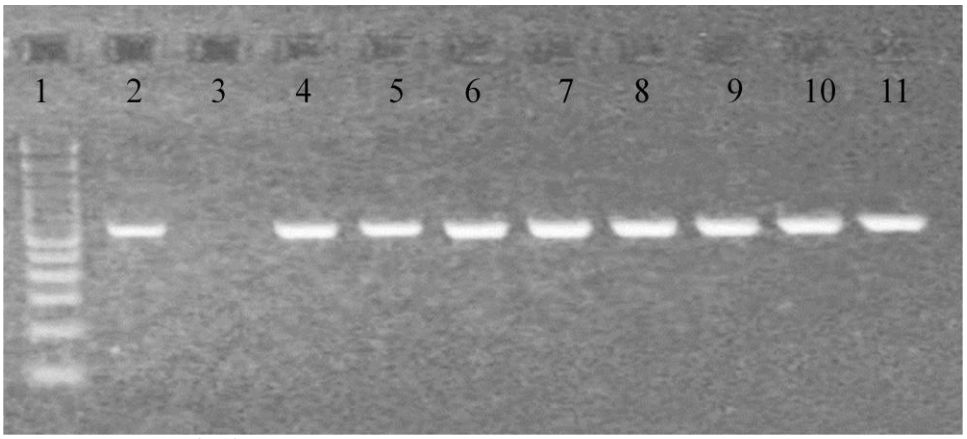

Figure 2. PCR product for femB gene (651 bp): lane 1 ladder, positive control lane 2 (ATCC 43300 strain), negative control lane 3 (ATCC 12344 Streptococcus pyogenes strain), lanes 4 to 11 positive samples (06, 35, 36, 56, 88, 122, 133, 136) for the nucA gene.

In the antibiogram it was observed that:
7 of the 8 strains were resistant to penicillin.
2 of the 8 strains were resistant to cefoxitin (MRSA strains).
All of the strains were sensitive to the other antibiotics tested.

Molecular confirmation of antimicrobial resistance to Penicillin, Methicillin and Vancomycin was carried out by identifying the *blaZ*, *mecA* and *vanA* genes, respectively, identifying the *blaZ* gene in all the isolated strains.

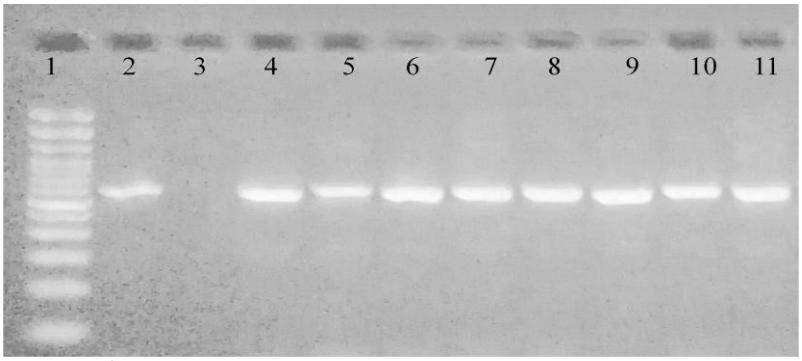

Figure 3. PCR product for *blaZ* gene (674 bp): lane 1 ladder, positive control lane 2 (ATCC 11632), negative control lane 3 (ATCC 12344 *Streptococcus pyogenes* strain), positive samples 06, 35, 36, 56, 88, 122, 133, 136.

The *mecA* gene was found in 2 of the 8 isolated strains.

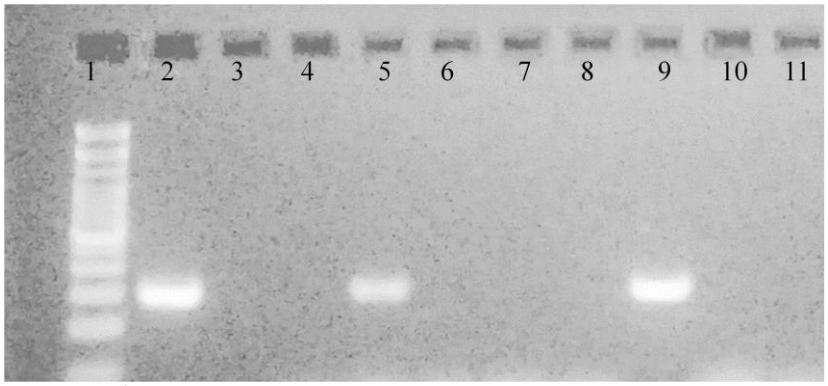

Figure 4. PCR product for *mecA* gene (310 bp): lane 1 ladder, positive control lane 2 (ATCC 43300), negative control lane 3 (*Streptococcus pyogenes* ATCC 12344), Positive samples: 35, 122 Negative samples: 06, 36, 56, 88, 133, 136 for the *mecA* gene.

For the *vanA* gene, all samples were negative.

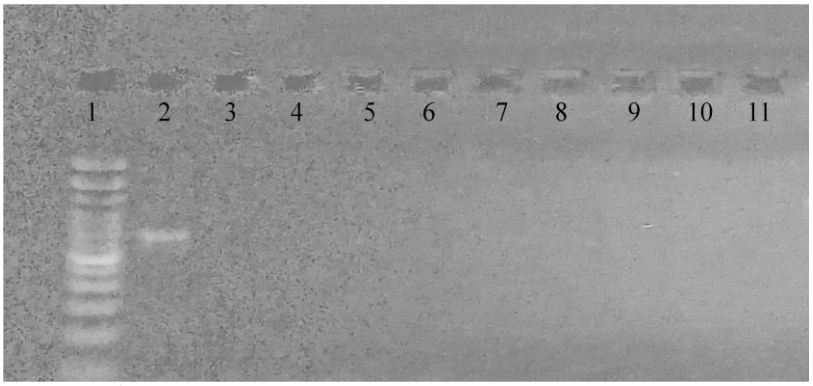

Figure 5. PCR product for *vanA* gene (732 bp): lane 1 ladder, positive control lane 2 (*Enterococcus faecium* ATCC 700221), negative control lane 3 (*Streptococcus pyogenes* ATCC 12344), lanes 4-11 negative samples.

DISCUSSION

It is essential to keep in mind that *S. aureus* is part of the nasal microflora and of the human skin, which is why cross-contamination is possible in two ways, dentist-patient and patient-dentist. De la Vega and Cava proved this by isolating it even after surgical hand washing, which generates a significant probability of postoperative complications due to infections caused by its dissemination, and may even lead to bacterial endocarditis (Badillo et al., 2019; De la Vega and Cava, 2019).

Gonçalves et al. (2020) report having worked with 354 samples taken from 6 surfaces of dental medical equipment, taken before and after the consultation, finding "55.6% of uncontaminated samples" which makes us understand that 44.4% of the samples were contaminated. This rate of presence of S. aureus is common worldwide, but it differs greatly from the results obtained in the present study of 5.75%. They also observed 17.5% contamination of the MRSA data, which is no far to our findings of 25%.

Likewise, Khairalla et al. (2017) conducted a study with 1,300 samples from patients, healthcare personnel, and environmental surfaces from a dental clinic in Egypt to detect MRSA. Of these, 1030 were environmental specimens, of which 1.3% were positive for MRSA, a percentage similar to the 1.4% found in our study, despite our study size being only 139 samples.

Health personnel, including dentists and their instruments, due to the generation of aerosols during their daily activities, are exposed to a wide variety of pathogens, among which *S. aureus* stands out. This has been reported in a limited studies carried out in the dental field, Zumba- Reino et al. (2022) isolated this pathogen in 8% of the samples taken from high-speed handpieces for dental use. Badillo et al. (2019) on the other hand, showed 4.5% presence of this pathogen in all his processed samples, however, statistics are shown that near 5.75% of the population of this present study, carried this pathogen in the surface of their multipurpose boxes, used by students to transport their instruments for pre-professional practices and that are not always given the most appropriate biosecurity measures and cleaning.

Laica et al. (2021), with a similar report, worked on cell phones of dental students, identifying the mecA gene that Methicillin-Resistant *S. aureus* (MRSA) strains possess in 43.75% of the isolated data, this, is comparable to a report of Orellana Bravo. (2021), where her study displayed 40% of MRSA found in dental clinics, likewise, reported by Pineda Higuita et al. (2020), isolated S. aureus in 67.7% of the samples collected, and found that 28.5% were resistant to cefoxitin and 35.7% to oxacillin.

Capozzi et al. (2015), through their study of 30 individuals from the nursing staff of a health center in Carabobo State, Venezuela, identified S. aureus in 50% of the samples, of which 47% resulted in MRSA. These values differ to some extent from what was found our study, in which, out of 139 samples, *S. aureus* was isolated in eight of the samples, of which only two presented resistance to cefoxitin and had the *mecA* gene (MRSA).

CONCLUSION

It was important to observe in the antibiogram the resistance to Cefoxitin (MRSA strains) and to confirm with the positive identification of the *mecA* gene in 2 of the 8 strains of *S. aureus* isolated, given that the difficulty of its treatment is added to its high pathogenicity, so it would be very important to carry out a further study of their virulence genes.

It was concluded that the low rate of *S. aureus* (5.75%) found in the present study could be related to the fact that it was carried out during the COVID-19 pandemic, during which time biosecurity measures were extreme worldwide.

It is also noteworthy that despite the fact that the eight samples have the *blaZ* gene, one sample was sensitive to penicillin in the antibiogram, which could be due to structural damage to said gene, or to a deficiency in its expression.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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